Rapid Development of Small-Molecule producing Microorganisms based on Metabolite Sensors

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2. Schendzielorz G, Binder S, Bott M, Eggeling L: Verfahren zur Herstellung von Vektoren enthaltend ein für in seiner feedback-Inhibierung gemindertes oder ausgeschaltetes Enzym kodierendes Gen und deren Verwendung für die Herstellung von Aminosäuren und Nukleotiden. DPA 102012 016 716.4, Anmeldetag 22.08.2012.

3. Siedler S, Bringer S, Schendzielorz G, Binder S, Eggeling L, Bott M: Sensor für NADP(H) und Entwicklung von Alkoholdehydrogenasen. DPA 10 2012 017 026.2, Anmeldetag 28.8.2012.

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Content

1.1. Summary

Small-molecules made by microorganisms, such as amino acids, vitamins, organic acids or antibiotics are industrially important substances. However, there are two major limitations in microbial strain development. First, laborious plasmid constructions are usually involved in strain development. Second, no general high-throughput screening methodology exists to identify a producer at the single-cell level. In the present doctoral thesis these problems are addressed and applied to *Corynebacterium glutamicum*.

The metabolite sensor pSenLys was constructed. It uses the transcriptional regulator LysG of *C. glutamicum,* as well as the promotor of its target gene *lysE*. Fusion with *eyfp* resulted in a graded fluorescence output in response to the cytosolic L-lysine concentration, which increases in strains with higher productivity. Turning the inconspicuous metabolite L-lysine into a conspicuous one enabled the high-throughput screening of producing cells via fluorescent activated cell sorting (FACS). A screening accuracy exceeding 91 % was determined by isolation of fluorescent cells out of a population consisting of non-producing cells in a 10.000 fold excess over producing cells. Furthermore, metabolite sensors were developed for the detection of L-serine and O-acetyl- L-serine in *C. glutamicum* and L-arginine in *E. coli*. Single-cell analysis using metabolite sensors and FACS of L-lysine producing strains is demonstrated, opening up a number of different possibilities for microbial population analysis.

The established screening routine was used to isolate 270 fluorescent cells from a randomly mutagenized population of *C. glutamicum*. 185 clones accumulated L-lysine in the range of 0.2 to 37 mM. Targeted sequencing of six genes from 40 of the 185 mutants resulted in 24 strains carrying known mutations, or mutations in known genes, whereas in 16 mutants no known gene was mutated. Sequencing the genomes of 10 mutants revealed that they carry between 36 and 268 SNPs. In one strain the UDP-MurNac-tripeptide synthetase was mutated resulting in MurE-G81E. Introduction of this, so far unknown, mutation into the genome of the wild type, and also into defined L-lysine producers, caused an increased L-lysine production in all strains. Consequently, *murE* is now the third gene in addition to *lysC* and *hom* which, when mutated alone, causes an increased L-lysine production. Thus, the principle of the use of a metabolite sensor for high-throughput isolation of new producers and identification of new targets has been demonstrated successfully.

Driven by the wish for rapid manipulation of the *C. glutamicum* genome, recombination-mediated genetic engineering was developed. Recombineering is a highly efficient technology for rapid *in vivo* genome engineering and does not rely on laborious vector constructions, but the use of ssDNA oligonucleotides (oligos) or PCR-generated dsDNA fragments. A tester strain was constructed containing a defective Kan^R gene integrated in its genome. Five different recombinases were expressed and tested for functionality in *C. glutamicum* using a 100-mer oligo recovering the Kan^R

gene. RecT of the *E. coli* prophage Rac was identified as a very efficient recombinase yielding $2x10^6$ kanamycin resistant cells. This means that the frequency of recombination in *C. glutamicum* under optimized conditions is higher than standard transformation frequencies obtained with a plasmid.

Combination of both, recombineering and metabolite sensing using pSenLys, enabled the simple and fast introduction of the *lysC*-T311I mutation and screening for producers carrying this mutation. This mutation is known to cause L-lysine overproduction and it was efficiently introduced into the genome using an appropriate oligo. In another experiment codon 81 of *murE* was targeted for *in vivo* site directed saturation mutagenesis. In this case 20 different oligos were used at once to introduce genetic diversity at this locus causing L-lysine overproduction. 21 recombinants were isolated, where G in MurE position 81 was replaced by C, F, L, D, S, V, W, or Y, leading to increased productivities. These experiments demonstrate the successful use and enormous potential of recombineering in *C. glutamicum*, when used together with metabolite sensor-based producer isolation. The procedure developed might represent a general and highly efficient strategy for rapid development of microbial metabolite producers.

1.2. Zusammenfassung

Durch Mikroorganismen hergestellte Metabolite, wie z.B. Aminosäuren, Vitamine, organische Säuren oder auch Antibiotika, stellen wichtige industrielle Produkte dar. Allerdings ist die Entwicklung der mikrobiellen Produzenten dadurch beschränkt, dass in der Regel aufwendig konstruierte Plasmide benötigt werden und in vielen Fällen noch keine Methoden zum Hochdurchsatzscreening von Produktionsstämmen auf Ebene von Einzelzellen existieren. In der vorliegenden Dissertation werden diese Probleme angegangen und die resultierenden Entwicklungen auf *Corynebacterium glutamicum* angewandt.

Zunächst wurde der Metabolitsensor pSenLys konstruiert, der den Transkriptionsregulator LysG und die Promotorsequenz des Zielgens *lysE* nutzt. Durch Fusion von *eyfp* mit dieser Promotorsequenz zeigen Zellen als Antwort auf graduell erhöhte cytosolische L-Lysinkonzentration – dies ist bei Stämmen mit erhöhter Produktivität der Fall ist – ein graduell erhöhtes Fluoreszenzsignal. Dies ermöglicht das Hochdurchsatzscreening unterschiedlich produzierender Einzellzellen mittels *fluorescent activated cell sorting* (FACS). Die Isolation von Produzenten aus einer Population mit Nicht-Produzenten, die in 10.000 fachem Überschuss vorlagen, ergab eine Sortiergenauigkeit von mehr als 91 %. Weitere Metabolitsensoren für die Detektion von L-Serin und O-Acetyl- L-Serin in *C. glutamicum* und L-Arginin in *E. coli* wurden entwickelt. Hiermit wurde die Nutzbarkeit von Metabolitsensoren für die Einzellzell-Analytik demonstriert und eröffnet eine Vielzahl neuer Möglichkeiten für die mikrobielle Populationsanalyse. Die etablierte Screeningroutine wurde erfolgreich eingesetzt, um 270 fluoreszierende Zellen aus einer mutagenisierten *C. glutamicum*

Population zu isolieren. 185 Klone akkumulierten L-Lysin in Konzentrationen von 0,2 bis 37 mM. Die Sequenzierung von sechs Genen aus 40 der 185 Mutanten führte zur Identifikation von 24 Stämmen, welche bekannte, aber auch neue Mutationen in diesen Genen aufwiesen. In 16 Stämmen war keines dieser Gene mutiert. Von insgesamt zehn dieser Stämme wurden die Genome sequenziert und pro Genom zwischen 36 und 268 SNPs ermittelt. In einem dieser Stämme war die UDP-MurNac-Tripeptid Synthetase an Position 81 mutiert, resultierend in MurE-G81E. Einführen dieser bislang unbekannten Mutation in das Genom des Wildtyps, aber auch in definierte Lysinproduktionsstämme, führte in allen Fällen zu einer deutlichen Produktivitätssteigerung. Es sind zwar verschiedene Gene bekannt, die zu erhöhter L-Lysinbildung führen, allerdings ist die hier beschriebene *murE*-Mutation, neben Mutationen in *lysC* und *hom*, bislang die erste neue Mutation, die als alleinige Mutation im Wildtyphintergrund zu erhöhter L-Lysinbildung führt.

Zur schnellen Manipulation des *C. glutamicum* Genoms wurde anschließend *recombination-mediated genetic engineering (recombineering)* entwickelt. *Recombineering* ist eine hoch effiziente Technologie zur schnellen *in vivo* Manipulation von Genomen. Diese unterliegt im Gegensatz zu herkömmlichen Methoden nicht der Nutzung aufwendiger Vektorkonstruktionen, sondern benötigt lediglich den Einsatz von Oligonukleotiden (Oligos) oder auch PCR-Fragmenten. Ein Teststamm wurde konstruiert, bei dem ein defektes Kan^R Gen im Genom integriert wurde. In diesem Stamm wurden fünf unterschiedliche Rekombinasen exprimiert und mit Hilfe von 100-mer Oligonukleotiden, welche die defekte Kan-Sequenz bei erfolgreicher Integration reparieren, auf Funktionalität hin überprüft. RecT aus dem *E. coli* Prophagen Rac wurde als außerordentlich effiziente Rekombinase identifiziert. Insgesamt konnten in einem Ansatz $2x10^6$ Kan-resistente Zellen erzeugt werden. Dies bedeutet, dass die erreichte Rekombinationsfrequenz in *C. glutamicum* höher ist, als die mit Plasmiden erreichten Transformationseffizienzen.

Darüber hinaus ermöglichte *recombineering* in Kombination mit dem Metabolitsensor pSenLys und der FACS Routine die Generierung und direkte Isolation produzierender Zellen, welche die *lysC*-T311I Mutation tragen. Von dieser Mutation ist bekannt, dass sie zur L-Lysinüberproduktion führt und sie wurde mittels Oligos in das Genom eingefügt. In einem weiteren Experiment wurde das Codon 81 von *murE in vivo* durch *site directed saturation mutagenesis* modifiziert. Hierfür wurden 20 unterschiedliche Oligos genutzt, um an diesem Lokus, in einem einzigen Ansatz, genetische Diversität zu erzeugen, welche zur L-Lysinproduktion führt. Insgesamt konnten 21 rekombinante Stämme mit erhöhter L-Lysinbildung isoliert werden, bei denen G in MurE an Position 81 durch C, F, L, D, S, V, W oder Y ausgetauscht wurde. Diese Experimente demonstrieren den erfolgreichen Einsatz und das enorme Potential von *recombineering* in *C. glutamicum* in Kombination mit sensor-basierter Produzentenisolierung. Die entwickelte Methode könnte zukünftig eine universelle und effiziente Strategie darstellen, um mikrobielle Metabolitproduzenten in hoher Geschwindigkeit zur entwickeln.

2. Introduction

2.1. Fermentative production of small-molecules

Small-molecules made by microorganisms, such as amino acids, vitamins, organic acids or antibiotics are industrially important substances. According to the "New Report by Global Industry Analysts, Inc. 2012" the global market for "fermentation chemicals" is projected to exceed US $48 billion by 2015 with a compound annual growth rate of 6 % [2, 3].

Aside from small-molecules, which are naturally synthesized as building blocks for growth of microorganisms and which can be produced on an industrial scale, the biosynthetic capacity of microorganisms is also increasingly used for the production of small-molecules not naturally made by them. Examples are pharmaceuticals normally synthesized in plants. Furthermore, the production of biofuels not made by microorganisms, or at least not made in high concentrations, is a theme in modern microbial biotechnology [4-6]. Examples for production of pharmaceuticals are strains of *Escherichia coli*, which have been developed for the production of amorpha-4,11-diene. This is a precursor of artemisinin, effective for the treatment of malaria [4], or for taxadiene production, an intermediate of the anticancer compound taxol [5]. The combination of the successful application of microbial synthesis, progress in synthetic biology and changes in global economy, which necessitate intensified use of renewable raw materials, indicate that microbial metabolite production will continue to expand. In the following thesis, the focus will be on amino acid production by microorganisms and in particular L-lysine production. Annually about 1,800,00 tons of L-lysine are produced, mainly with *Corynebacterium glutamicum* [2].

2.1.1. Industrial relevance of L-lysine and other amino acids

Amino acids represent major industrial products derived by fermentation of bacteria. For 2015, the global market for amino acids is predicted to reach US $ 11.6 billion [2]. Growing demand for amino acids as feed additives, health food, dietary supplements, artificial sweeteners, and cosmetics is expected to further fuel market growth in the coming years.

Animal feed additives represent a large market for amino acids. Generally, feed additives are used to provide poultry, swine, and other animals with adequate nutrients to offer an optimal balanced diet. For this purpose, amino acids such as L-lysine, L-methionine, L-tryptophane, and L-threonine are used. Usually L-methionine is the first limiting amino acid in feed, followed by L-lysine, L-threonine, and L-tryptophan. The particular use and dose of amino acids in the feed composition depends on several factors, like for instance, on the source of the protein content in the diet (corn or soja), from the age of the animal, and the type of animal, as well.

With an annual production of 2,300,000 tons sodium glutamate represents the largest product segment within the amino acid market. Opposed to the amino acids L-lysine, L-methionine, L-tryptophan, and L-threonine, L-glutamate is a non-essential amino acid. However, monosodium glutamate is responsible for the specific "umami" taste [7]. Particularly in Asia it is used as flavor enhancer, where this flavor is highly appreciated. But it is also used for roasted peanuts, corn flakes, and similar products to increase the specific "umami" taste.

L-lysine represents the fastest growing amino acid segment with a compound annual growth rate of 5.4 %. The reason is the increasing demand for meat, in particular in China and South America. Moreover, L-lysine, as well as all of the other proteinogenic amino acids, is also used for pharmaceutical products and amino acid-based elemental diets for humans.

2.1.2. Biosynthesis and production of L-lysine by *Corynebacterium glutamicum*

Corynebacterium glutamicum was isolated in 1957 as an L-glutamate producing microorganism [8]. Soon after its discovery, experiments were done to produce also other amino acids with this bacterium. In 1961, it was reported that a homoserine auxotrophic mutant of *C. glutamicum* [9] and a methionine-threonine auxotrophic mutant [10] produced L-lysine. From the 1960s to the 1970s, the biosynthesis of L-lysine in *C. glutamicum* and the mechanism of L-lysine overproduction were investigated intensively in the wild type, as well as in mutants obtained by undirected mutagenesis. With the subsequent advent of recombinant technology available to *C. glutamicum,* a deeper understanding of L-lysine synthesis and on the relevance of mutations for increased L-lysine synthesis was obtained.

Strain improvement for L-lysine synthesis with *C. glutamicum* has been achieved by overcoming limiting reactions within the L-lysine biosynthesis pathway [11, 12], reducing competing reactions, increasing the supply of NADPH [13, 14] or of precursor metabolites [15], and increase in sugar uptake [16], as well. The most relevant mutations known for L-lysine-producing strains are associated with L-aspartate kinase (*lysC*) and homoserine dehydrogenase (*hom*). Aspartate kinase is the key enzyme that regulates the metabolic flux to the L-aspartate family of L-amino acids through feedback inhibition of the enzyme activity by L-lysine together with L-threonine, while the homoserine dehydrogenase directs the flux to L-lysine or to L-threonine, L-methionine, and L-isoleucine. A number of point mutations in *lysC* are known leading to a deregulated, feedback-resistant L-aspartate kinase and therefore to L-lysine overproduction. A single mutation in *hom* is known, V59A, decreasing the dehydrogenase activity, which leads to reduced L-threonine synthesis. Therefore, the flux is directed towards L-lysine and additionally feed-back inhibition of the kinase by L-threonine is reduced [17, 18]. Amplification of *dapA* [11, 12], encoding dihydrodipicolinate synthase (DDPS), or *dapB* [19], encoding dihydrodipicolinate reductase, also leads to L-lysine overproduction.

For L-lysine synthesis oxaloacetate and pyruvate are important precursors. Therefore, the availability of these precursors effect the efficiency of L-lysine production. An increased expression of *pyc,* encoding for pyruvate carboxylase, in an L-lysine-producing *C. glutamicum* strain increases the L-lysine production due to higher pyruvate availability [15]. Deletion of *pck* prevents oxaloacetate from beeing decarboxylated by phosphoenolpyruvate carboxykinase, and therefore leading to an increase in the L-lysine production in a producer [20]. Another way to improve precursor supply and influencing the flux towards oxaloacetate is by downregulating the TCA cycle. This can be performed by downregulation of the *icd* gene, encoding isocitrate dehydrogenase [19], or of the *gltA* gene, encoding citrate synthase [21]. These results indicate that the enzymes at the anaplerotic node are important for metabolic engineering of L-lysine producing strains.

NADPH is an important reduction equivalent of anabolism and is highly required for L-lysine biosynthesis. Four moles of NADPH are required for the biosynthesis of one mole of L-lysine from oxaloacetate and pyruvate [22]. Using mutated alleles of *gnd*, encoding glucose-6-phosphate dehydrogenase [23], and *zwf,* encoding 6-phosphogluconate dehydrogenase [23], increases the activity of the pentose-phosphate pathway and therefore higher amounts of NADPH are generated in the cell. Overexpression of the gluconeogenic enzyme fructose 1,6-bisphoshatase (*fbp*), together with the entire transketolase (*tkt*) operon, has been proven to be successful [19] for redirecting the carbon flux into the pentose phosphate pathway

In a recent approach, twelf individual targets, known to be of relevance for increased L-lysine formation of *C. glutamicum* (Fig 1), were combined by molecular methods to create a defined producer [19]. Four point mutations, *lysC*T311I, *hom* V59A, *pyc*P458S and a silent mutation in *icd,* leading to an altered start codon, were introduced and the genes *lysC*T311I, *dapB*, *pyc*P458S, *fbp* and the *tkt* operon were upregulated by exchanging their native promotor with the strong *sod* promotor. Futhermore, *pck* was deleted and a second copy of the *ddh* gene, encoding diaminopimelate dehydrogenase, as well as *lysA*, encoding diaminopimelate decarboxylase, was integrated into the genome. The resulting strain *C. glutamicum* Lys-12 accumulated about 120 g l^{-1} L-Lysine [19]. However, with industrial strains still far higher concentrations of L-lysine are achieved [24].

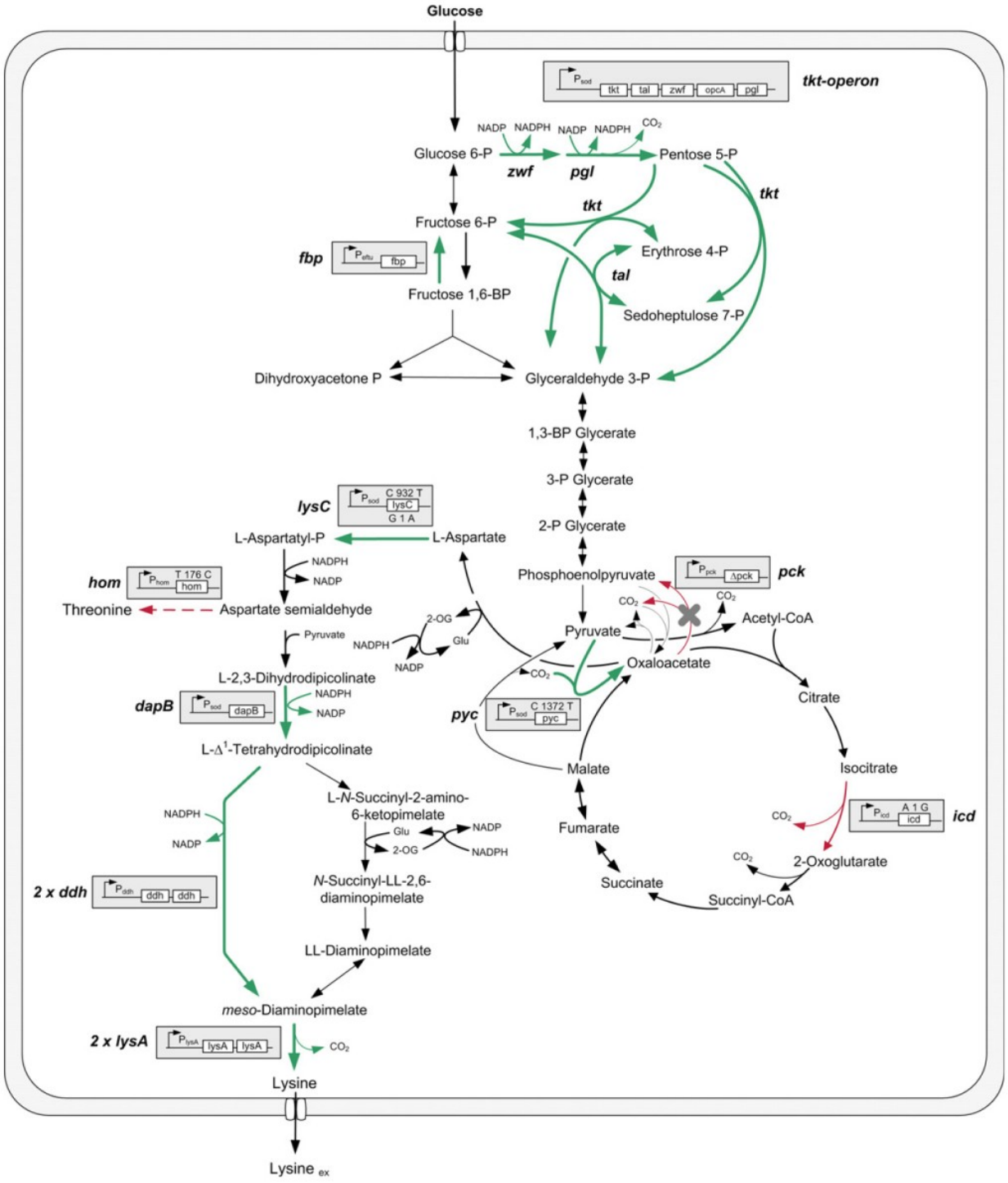

Figure 1: L-lysine biosynthesis and metabolic engineering for the design and creation of a genetically defined *C. glutamicum* strain for L-lysine production. Strain engineering started from the non-producing wild-type *C. glutamicum* ATCC 13032. All modifications were implemented into the genome. Gray boxes represent the targeted modifications of the genes. The symbol ''X'' indicates gene deletion. Green arrows indicate amplification, red arrows attenuation or deletion [19].

2.2. Development of small-molecule producing bacteria

Usually the metabolism of native bacteria is carefully controled in order to avoid waste of energy and to prevent excessive formation of building blocks, not needed for growth. Therefore, developing small-molecule producing bacteria requires genetic manipulations leading to overproduction of the desired metabolite. Traditionally, such manipulations have been achieved through undirected mutagenesis and selection, frequently used in several rounds. Also protoplast fusion techniques have been used to combine advantageous mutations of strains, derived by undirected mutagenesis [25]. Such approaches yielded a number of mutant alleles, which still form the basis in a number of approaches used in recombinant DNA technology. In addition, recombinant DNA technology offers a variety of techniques, which, together with traditional methods, provide powerful tools for the development of small-molecule producing strains.

2.2.1. Diversity generation

2.2.1.1. Classical approach: random mutagenesis

Production strains used in industrial fermentation processes have traditionally been developed by repeated random mutagenesis and selection [26, 27]. For this purpose, N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) has been the mutagen of choice [28]. Further mutagens to mention are methylmethane sulfonate (MMS), ethylmethane sulfonate (EMS), hydroxylamine (HA), and ultraviolet light. For introducing mutations into a strain the population is treated with a mutagenic agent until a certain “desired” kill is obtained. Survivors from the population are randomly picked and tested for their ability to produce the metabolite of interest. Two examples for such classically derived L-lysine producers are *C. glutamicum* B-6 [29] and *C. glutamicum* MH22-B [30].

This method offers a significant advantage over molecular engineering. No knowledge of the biosynthetic pathway or genetics of the producing microbe is required. Furthermore, this kind of mutagenesis is whole-genome directed. The current knowledge is always predetermined by the current status of research and might still miss unknown targets important for production. An example might be the recently discovered fact, that a mutation in ribonuclease G of *E. coli* results in a marked improvement of the production of L-lysine and L-threonine [31]. The major drawback of this method is the accumulation of secondary, unnecessary mutations in the genome over the course of repeated cycles of mutagenesis. Because of this, production strains generally have a weak performance with respect to growth, sugar uptake, and genetic stability.

2.2.1.2. Recombinant DNA techniques and metabolic engineering

Advances in genetic engineering have enabled a far more rational approach for strain improvement than the classical approach via mutagenesis and screening, namely introduction of directed genetic changes through recombinant DNA technology. In recent years, additional new techniques, which increase the options available to improve the production of microbial metabolites, have been developed including systemic approaches like proteomics, transcriptome analysis, and whole genome sequencing, as well as further techniques. However, in many cases the basis for a very good producer strain still is a classical producer, or even a pool of classically derived producers, derived by undirected mutagenesis. This is very well documented in case of L-lysine synthesis, where systemic approaches were used to analyse classical strains and in conjunction with recombinant DNA technology excellent producer strains were generated.

In this approach, called "genome based strain reconstruction", strains were generated only carrying mutations responsible for overproduction. These strains are devoid of the many unknown mutations accumulated by undirected mutagenesis and screening, causing poor growth. The industrial L-lysine producer *C. glutamicum* B-6 was sequenced to identify mutations leading to L-lysine production [32]. Sequence comparison of alleles in the relevant terminal pathways from L-aspartate with their wild-type counterparts revealed that point mutations occurred in *hom* and *lysC*. The mutations were introduced separately into a wild type by allelic replacement. Strain HD-1, carrying *hom*V59A, accumulated about 10 g l^{-1} and strain AK-1, carrying *lysC*T311I, accumulated about 50 g l^{-1}. Combining these mutations leads to 70 g l^{-1} L-lysine (strain AHD-2). Three specific mutations, *pyc*P458S in the anaplerotic pathway, *gnd*S361F in the pentose phosphate pathway, and *mqo*W224opal in the TCA cycle, were defined as positive mutations for improved production. They were then introduced, one by one, into the AHD-2 genome, resulting in a step-wise increase of final L-lysine titers. The resulting strain AGM-5 produced nearly 100 g l^{-1} L-lysine and showed several advantages in comparison to the industrial producer, such as a high rate of sugar consumption, or improved stress tolerance. But, strain AGM-5 still could not attain similar L-lysine production levels as the classical producer B-6. Transcriptome analysis of B-6 and AGM-5 indicated that global regulatory effects, not directly linked to the biosynthesis of L-lysine, are present in the classically derived strain, which have a positive influence on L-lysine synthesis [32].

Another example of strain construction, as already mentioned above, was the implementation of twelve defined changes in genes, encoding central metabolic enzymes, redirected major carbon fluxes as desired towards the optimal pathway usage. The final engineered strain was able to produce L-lysine with a yield of 0.55 g per gram of glucose (120 g l^{-1}) in fed-batch culture [19]. This again illustrates the benefits of combining classical and modern techniques in strain construction, but also the fact that modern technologies still cannot replace classical methods.

2.2.2. Screening for small-molecules

Whereas methods for diversity generation of plasmids or strains allow for a large number of variants to be created [33], this is not the case for technologies directly generating small-molecule producers. The reason is that always a screening step is required. In most cases laborious cultivations of individual clones, followd by determination of product formation using low-throughput technologies, such as chromatography, mass spectrometry, or NMR is necessary decreasing a possible throughput dramatically. There is no general technology for the direct screening of small-molecule producers. One reason is that small-molecules, such as amino acids, usually do not confer a phenotype, thus preventing the use of high-throughput colorimetric and fluorometric assays. For that reason, screening and selection processes for small-molecule producers are still lagging behind those for diversity generation. Screening and selection represent the most significant bottlenecks in strain development [33].

2.2.2.1. Plate-based screens

Plate-based screens are traditionally those, where petri dishes are used, but also include those performed in microtiter plates in liquid media. The throughput for assays in microtiter plates is moderate, approximately 10^4 cells per experiment. Screening of colonies on solid media provides a higher throughput, in particular, when selection for growth is possible. With modern robotics up to 10^5 variants can be screened per experiment [33]. One of the very rare examples for a plate-based assay is the screening of lycopene producing bacteria. Lycopene colors cells red and it allows visual inspection of colonies for detection of clones, which are colored more intensely [34]. However, most metabolites, including amino acids, are not natural chromophores and cannot be detected directly. Screening for strains with increased amino-acid production requires the cultivation and analysis of the cultivation-broth for amino acid formation. In microtiter plate assays inconspicuous small-molecules may be visualized by using enzyme-coupled assays. Such photometric assays have been described for quantification of many enzyme cofactors, like ATP and ADP [35-37], reduced and oxidized states of NAD and NAPD [38, 39], and free coenzyme A [40].

2.2.2.2. Auxotrophic strains

Strains, auxotrophic for essential small-molecules, are natural biosensors and have long been used to build selection assays [41]. For example, an engineered producer strain expressing the target pathway is co-cultivated with a reporter strain. This strain constitutively expresses an autofluorescent protein and is auxotrophic for an essential metabolite, found in the target pathway. When the reporter strain is able to grow, the fluorescence of the culture can be quantified, which is coupled to the metabolite yield, achieved by the producer strain. This strategy was successfully employed in the development of a whole-cell biosensor for mevalonate [42].

2.2.2.3. Fluorescence-activated cell sorting (FACS) for screening

The technique offering the highest throughput is flow cytometry, which in combination with cell sorting allows the direct isolation of cells with altered fluorescence. Fluorescence-activated cell sorting (FACS) is a method, which enables the simultaneous measurement of multiple physical and chemical/biological characteristics of single cells. The flow cytometer fluidics system transports particles in a fluid stream, one cell at a time at high speed, to a laser beam for the interrogation of particle properties. Cell properties measured include the relative size, internal complexity, and relative fluorescence intensity of the specific fluorescent molecule being interrogated. Fluorescent dyes can be used to label cellular components directly or indirectly (e.g. using antibodies), such as DNA, surface proteins/receptors, intracellular structural proteins and enzymes, specific nucleic acids, membrane properties and ion fluxes, secreted proteins, or small-molecules. While not all of these have been widely applied to bacteria, the potential of such applications is becoming increasingly apparent [33].

While throughput is restricted to 10^5 cells using other approaches, FACS-based screening increases the throughput to more than 10^9 variants per experiment [33]. The major pre-requisite for a FACS-based screen is that the producing cell has to produce an optical output in response to the concentration of the small-molecule of interest. Many small-molecules are not natively fluorescent or chromophoric. Thus, a universal high-throughput screening platform for small-molecules does not exist. Genetically encoded biosensors can transform inconspicuous small-molecule inputs into optical outputs by expressing an autofluorescent protein as reporter. For this purpose, i.e. aptamers [43] and FRET biosensors [44] can be engineered for small-molecule detection. Another promising strategy is the use of transcription factors. These proteins regulate the transcriptional output of their target promoter in response to ligand binding, which in many cases is a small-molecule. While the use of transcription factors, to construct whole-cell biosensors for the detection of environmental small-molecule pollutants, have long been used [45], this same approach has remained largely untranslated with respect to single-cell analysis and library screening. In one example, whole cultures were enriched for a mutated transcription factor, exhibiting fluorescence in response to mevalonate [46]. In this manner, a transcription factor with new specificity was generated. The generated mevalonate sensor was not used in FACS screenings for the isolation of mutants with increased mevalonate synthesis. Instead, a petri dish based assay was used to visually inspect colony colors in response to mevalonate-induced *lacZ* expression [47].

In a recent approach, engineered RNA switches were used to link metabolite concentrations and GFP expression levels in *Sacharomyces cerevisiae* [48]. Using such a sensor, large enzyme libraries in cells were screened to increase caffeine demethylase enzyme activity *in vivo* 33 fold using fluorescence.

2.3. Genetic tools for genome engineering

2.3.1. Homologous recombination using non-replicative integration vectors

For industrial production processes it is not desirable that strains carry plasmids. As a consequence, advantageous mutations and genes have to be integrated into the genome. In the case of *C. glutamicum* and many other bacteria this is achieved by the use of non-replicative vectors, carrying sequences homologous to chromosomal sequences [49]. This can be used for allelic exchange (deletion, substitution, integration). The basic features of this method are that the vector requires two rounds of positive selection for homologous recombination [50], and that it carries an insert with chromosomal sequences upstream and downstream of the sequences to be deleted or exchanged.

For example, the non-replicative integration vector pK19mobsacB in use for *C. glutamicum* carries a Tn5-derived kanamycin resistance cassette (KanR) and a modified *sacB* gene of *Bacillus subtilis*. Since this vector does not possess a replicon for *C. glutamicum,* and homologous recombination is a rare event, transformation efficiencies are very low. The KanR gene enables positive selection of recombinant cells carrying the integrated plasmid after electroporation. The *sacB* gene encodes levansucrase, whose activity is lethal for *C. glutamicum* in the presence of sucrose. Therefore, *sacB* enables selection for the loss of vector sequences from the recombinant strain. For allelic exchange, the part to be exchanged should be flanked by sequences of about equal size of 300 to 500 bp each corresponding to genomic sequences [24].

The major drawback of this method is that it is extremely laborious and ineffective. First, the integration vector has to be constructed and second, integration via selection and counter-selection demands manual picking of many hundreds of clones. Unfortunately, for *C. glutamicum* this method is currently the only available method for genome engineering.

2.3.2. Recombination-mediated genetic engineering (Recombineering)

In recent years, recombineering as a surprisingly simple technique to manipulate the genome of *E. coli* was developed. It is indisputable that recombineering can be regarded as a breakthrough technology to promote efficient homologous recombination. It is a highly efficient technology for rapid *in vivo* genome engineering. It does not rely on vectors and therefore not on the laborious *in vitro* use of restriction enzymes and DNA ligases for vector construction [51, 52]. It allows for genetic modifications of the genome, such as that described before, using non-replicative vectors. It only requires the expression of specific phage-derived genes in the host and can be performed by electroporation of PCR products or even synthetic single-strand DNA oligonucleotides (oligos).

In *E. coli* the proteins required for recombination are either the Red proteins of phage λ [51-53] or RecET from the prophage Rac [54]. RecET functions are encoded by two adjacent genes, *recE* and *recT*, present on the cryptic Rac prophage, found in the genome of many *E. coli* K-12 strains [55]. RecE is a 5'-3' exonuclease, which degrades the 5' ends of linear duplex DNA, creating 3' single-strand DNA overhangs [56, 57]. RecT is a single-strand annealing protein (SSAP), which binds to these ssDNA overhangs, pairs them with complementary ssDNA [58-60] and then induces homologous recombination. The phage λ derived Red proteins, Exo and Beta, are homologs of RecE and RecT. Beta and RecT, as well as Exo and RecE, have similar functions (Fig. 2). An additional third protein of the λ system is Gam, which increases recombineering frequencies by preventing RecBCD nuclease from degrading double-stranded linear DNA fragments. A Gam homolog is not present in the prophage Rac recombineering system, but Gam is also not necessary in recombineering applications using oligos as DNA substrates. In contrast to RecA dependent homologous recombination, RecT does not require the RecA protein [61]. This proves useful, as after recombineering functions have been briefly expressed to allow recombination, expression can be shut off to prevent further homology-dependent rearrangements.

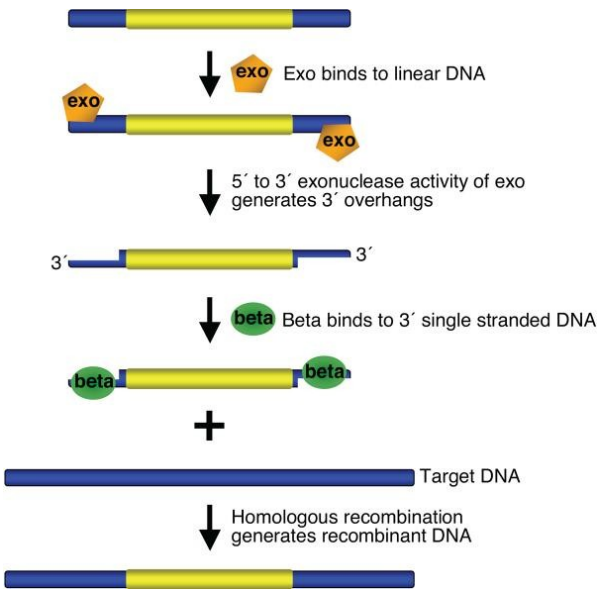

Figure 2: Overview of bacteriophage recombination system used for recombineering [1]. Exo has a 5' to 3' dsDNA exonuclease activity, which can generate 3' overhangs on linear DNA. Beta binds the single stranded DNA (3' overhangs), promotes single strand-annealing and generates recombinant DNA. An additional protein, Gam (not shown here), which prevents RecBCD nuclease from degrading double-strand linear DNA fragments, increases recombineering with dsDNA in *E. coli*. For ssDNA recombineering only Beta, respectively RecT, is required.

For recombineering, either a double-stranded DNA (dsDNA), such as a PCR product [62-64], or a single-stranded DNA (ssDNA), such as an oligonucleotide can be used. [61, 65-67]. The ssDNA must carry short ~50 bp segments homologous to the target sequences. These linear ssDNA substrates are recombined precisely *in vivo* by the phage proteins with the target genomic DNA. When linear dsDNA is used for recombination, both, the exonuclease RecE or Exo, and the SSAP RecT or Beta, are

required. In contrast, recombineering with an oligo just requires the expression of an SSAP [61, 67, 68]. In general, ssDNA oligo recombination is mechanistically simpler and more efficient than dsDNA recombination. Due to the power of recombineering, Red and λ related proteins were also searched in other bacteria. This led to the demonstration of recombineering in selected bacteria, such as *Mycobacterium tuberculosis* [69], *Pantoea ananatis* [70], *Bacillus subtilis* [71], *Lactobacillus reuteri* and *Lactococcus lactis* [72].

2.4. Aims of this work

In strain development, screening and selection processes still represent the most significant bottleneck. Moreover, the introduction of specific mutations in the chromosome of *C. glutamicum* is cumbersome.

Development of metabolite sensors based on transcriptional regulators

The major goal of the work was the development of a small-molecule sensor, based on a transcription factor, and the demonstration of its use in strain development. The sensor should be based on the transcriptional regulator LysG, sensing cytosolic levels of L-lysine in *C. glutamicum,* and in response to increased concentrations drives transcription of its target gene *lysE*. Fusion of the *lysE* promotor with *eyfp* should transmit the concentration into fluorescence, as an optical output. Subsequently, the L-lysine sensor should be assayed in high-throughput screenings of a *C. glutamicum* population using FACS for isolation of new L-lysine producing mutants, after chemical mutagenesis of the wild type. This should be followed by further analyses, such as whole-genome sequencing, identification of new mutations, and strain reconstructions.

New applications for strain development

Another goal of this work was to establish recombineering in *C. glutamicum* to introduce rapidly genomic mutations. Furthermore, the use of recombineering together with metabolite sensors and FACS-based high-throughput screening should be assayed as a combination of new technologies to investigate, whether the link of rapid introduction of target specific mutations in the chromosome with the rapid selection of producer cells from a large library is possible. This would open up entirely new perspectives for engineering bacterial strains, producing small-molecules of industrial relevance.

3. Results

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METHOD Open Access

A high-throughput approach to identify genomic variants of bacterial metabolite producers at the single-cell level

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Abstract

We present a novel method for visualizing intracellular metabolite concentrations within single cells of *Escherichia coli* and *Corynebacterium glutamicum* that expedites the screening process of producers. It is based on transcription factors and we used it to isolate new L-lysine producing mutants of *C. glutamicum* from a large library of mutagenized cells using fluorescence-activated cell sorting (FACS). This high-throughput method fills the gap between existing high-throughput methods for mutant generation and genome analysis. The technology has diverse applications in the analysis of producer populations and screening of mutant libraries that carry mutations in plasmids or genomes.

Background

Since the first demonstration of microbial product formation more than a century ago [1], vitamins, antibiotics, nucleotides, amino acids and organic acids have been produced in ever increasing quantities. For example, about three million tonnes of sodium glutamate are produced each year as a small microbial molecule. Bacterial synthesis is increasingly also used for the production of small molecules not naturally made by bacteria, such as pharmaceutical intermediates [2,3] or biofuels [4]. The combination of the successful application of microbial synthesis, progress in synthetic biology and changes in the global economy that necessitate intensified use of renewable raw materials indicates that microbial metabolite production will continue to expand.

Microorganisms are not naturally designed for profitable metabolite formation, however, and there is an unrelenting need to optimize strains and pathways. Current strain improvement strategies make use of a variety of methods for engineering and isolating microbial variants with the desired traits. These techniques fall into two major categories: 'rational' methods, which involve the targeted alteration of known genetic information; and 'random' approaches, which are typically based on the creation of mutant libraries containing nondirected changes in genotype with subsequent screening for phenotypes of interest. Both approaches have been successful but the use of mutant libraries has proven to have distinct advantages. The reason is that the exact genomic mutations necessary to adapt the cellular metabolism for increased product synthesis are often difficult to predict, and that 'rational' methods are restricted to known targets. Random approaches with subsequent screening for the phenotype of interest enable us to overcome these difficulties. They have made possible the commercial-scale production of a variety of compounds, such as the unrivaled formation of succinate by *Escherichia coli* [5] or riboflavin by *Bacillus subtilis* [6]. Random and combinatorial approaches were also profitably used for the development of plasmid-encoded targets for the optimization of pathway flux in *E. coli*. This has been demonstrated with amorpha-4,11-diene production [2], which is an artemisinin precursor that is effective for the treatment of malaria, or with taxadiene production [3], an intermediate of the anticancer compound taxol.

However, with few exceptions, the evaluation of methods that utilize random approaches currently requires the cultivation of individual clones to determine production properties. This presents an obstacle. While high-throughput

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(HT) techniques for introducing genetic diversity and for product analysis or sequencing are well developed [7], comparable strategies for the identification and isolation of high-producer bacterial cells are still lacking. The opportunity to directly monitor product formation within single cells *in vivo* would add a new dimension to the characterization and development of microbial producers.

Here, we present examples of the monitoring of intracellular metabolite concentrations in single bacterial cells and demonstrate in an HT screen the isolation of new bacterial producer cells, as well as the identification of novel mutations based on whole-genome sequencing. The sensors we use are based on transcription factors (TFs) that regulate the transcriptional output of a target promoter in response to a cytosolic metabolite. Whereas the use of TFs to construct whole-culture biosensors for the detection of environmental small-molecule pollutants has long been established [8], this same approach has remained largely untranslated with respect to single-cell analysis and library screening. TFs are naturally targeted to a variety of small ligands, ranging from amino acids to sugars, sugar phosphates, vitamins, antibiotics, oxoacids and lipids [9]. They can also be engineered to obtain altered specificity [10,11], as recently summarized in a comprehensive review [12]. Coupling transcription of the target gene to a reporter protein provides a molecular device for recognition. This has already been successfully applied for screening in plate-based assays using colony color or colony size [10,13], for instance. Here we make full use of intracellular recognition of a specific metabolite in single cells by applying an autofluorescent protein as reporter and also fluorescence-activated cell sorting (FACS). This enables the isolation in HT screens of new bacterial small-molecule producers with random mutations introduced into the genome that enhance production of the molecule of interest, and we present an example of this.

Results

Schematic of approach

The workflow for HT selection of genomic variants of metabolite producers consists of the following steps: a) design of a suitable metabolite sensor, b) generation of genetic diversity in genomes of cells carrying the sensor, c) screening of the mutant library and selection of single producer cells via FACS, d) verification and characterization of mutants, and e) sequencing for target identification. We developed sensors for intracellular detection of basic amino acids, as well as of L-serine and O-acetyl-serine, and demonstrated the feasibility of the approach by isolating bacteria producing L-lysine from a library of randomly mutagenized wild-type (WT) cells, culminating in the identification of new useful mutations by whole-genome sequencing.

Design of L-lysine sensor

To develop a sensor suitable for intracellular L-lysine detection, we focused on the LysR-type transcriptional regulator (LTTR) LysG of *Corynebacterium glutamicum*. This protein senses elevated concentrations of basic amino acids, causing transcription of its target gene *lysE*, which encodes a basic amino acid exporter [14]. To explore the application of this native regulatory device to the conversion of an intracellular metabolite concentration into an optical output, we characterized the interaction of LysG with its target region upstream of *lysE* in a series of electrophoretic mobility shift assays (Figure S1 in Additional file 1). These data were used to construct the metabolite sensor pSenLys shown in Figure S2 in Additional file 1. It contained *lysG*, together with the LysG-binding site in front of the *lysE* promoter driving transcription of *eyfp* coding for enhanced yellow fluorescent protein (EYFP). In addition, P_{tac}-driven *crimson* was incorporated as a second fluorescence protein in the vector backbone. All strains and plasmids are listed in Table S1 in Additional file 1.

Characterization of L-lysine sensor

The WT of *C. glutamicum* does not excrete L-lysine but there is a genealogy of defined mutants that exhibit increased L-lysine productivity [15]. We determined the cytosolic L-lysine concentration by silicone oil centrifugation and the response of pSenLys in these strains. The WT had a cytosolic L-lysine concentration below 5 mM, while the defined producers had steady-state concentrations ranging from roughly 8 to 25 mM (Figure 1a). A clear increase in specific EYFP fluorescence in cultures is seen with increasing cytosolic L-lysine concentration. As evident from microscopic inspection (insets in Figure 1a), pSenLys is a tool for visualizing cytosolic L-lysine concentrations also within single cells. The range of the cytosolic L-lysine concentration covered translates into a dynamic range of signal output of 8.3-fold.

The fluorescence signal from the pSenLys sensor also correlates with the extracellular L-lysine concentration that accumulates after glucose is consumed (Figure 1b). One instructive exception to this is strain DM1920, which accumulates extracellular L-lysine at concentrations comparable to that of DM1919 despite displaying lower fluorescence due to lower cytosolic concentrations of L-lysine. This is due to altered L-lysine export: Strain DM1919 has one copy of the exporter gene *lysE* and an export rate of 10.1 ± 0.4 nmol $minute^{-1}$ mg(dry weight) $^{-1}$ (Figure 1c), whereas strain DM1920 has two copies of *lysE* (Table S1 in Additional file 1), which results in an increased rate of export of L-lysine of 12.1 ± 0.6 nmol $minute^{-1}$ mg(dry weight) $^{-1}$, and thus reduced cytosolic concentration. This observation provides an opportunity of influencing the read-out properties of a sensor that

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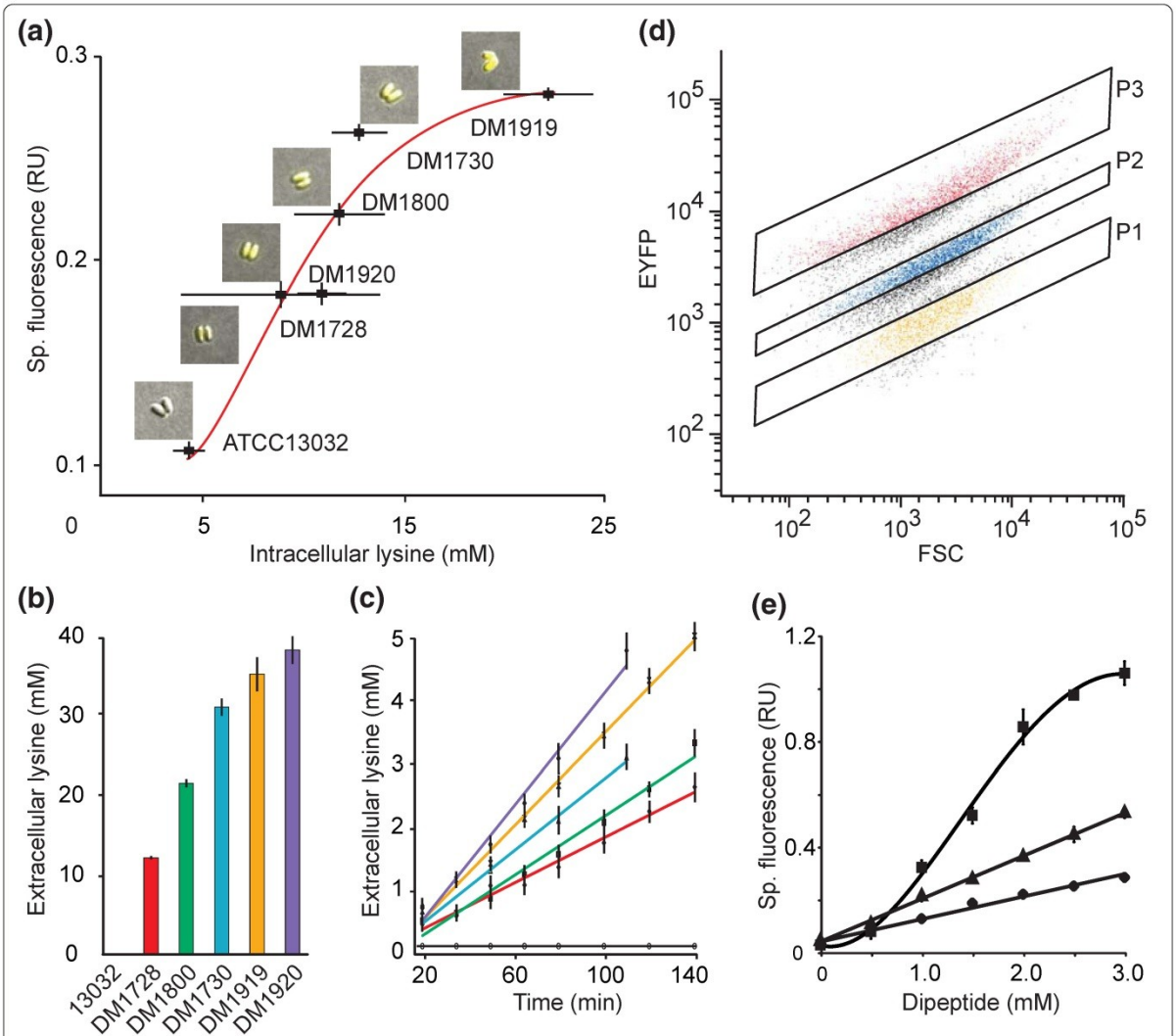

Figure 1 Characterization of lysine sensor and lysine-producing recombinant cells. (**a**) Cytosolic L-lysine concentration in *C. glutamicum* WT and five defined lysine producer strains, all carrying pSenLys, and specific fluorescence (Sp. fluorescence) of the cultures. Error bars give the means of three independent cultures for each strain. Fitting the data to the Hill equation describes the signal transfer function by an n_{app} of 3.19 ± 1.45 and this is shown as the red curve. (**b**) Extracellular accumulation of lysine after 48 h by the strains used in (a). (**c**) Lysine excretion rates of the same strains showing that strains with increased final lysine accumulation have increased excretion rates. Strain DM1920 has two copies of the lysine exporter gene *lysE* present in its chromosome and shows the highest excretion rate, but an intermediate cytosolic lysine concentration (color code as in (b)). (**d**) Differentiation of an equal mixture of cells of ATCC13032, DM1728 and DM1919 each carrying pSenLys by flow cytometry. The success of strain-specific sorting using gates P1 to P3 was over 90%. (**e**) Influence of dipeptide addition on specific fluorescence of *C. glutamicum* WT carrying pSenLys. The peptides Lys-Ala (circle), Arg-Ala (triangle) and His-Ala (square) were added to the cultures at the indicated concentrations. Additionally, Ala-Ala was added to give a total dipeptide concentration of 3 mM. The specific fluorescence was measured 1.5 h after dipeptide addition. FSC, forward scatter; RU, relative units.

had not previously been taken into consideration. As demonstrated for the production of antibiotics [16], amino acids [17], and biofuels [4], small-molecule production depends on export proteins. The ability to manipulate export activities permits cytosolic concentration of the substrate to be increased or decreased, which may be helpful, for example, when using strains that display high productivity and high cytosolic concentrations of substrate.

To isolate single cell producers by HT flow cytometry, it is essential that the cells can be separated according to their fluorescent properties. To demonstrate that this is the case with pSenLys, sensor-carrying cells of WT, DM1728, and DM1919 grown in glucose minimal medium

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were mixed in a 1:1:1 ratio to give a total of 4×10^7 cells ml^{-1}. This cell population was analyzed via FACS at a rate of 10^3 events per second. Clear differentiation of the population was achieved on the basis of intensity of the EYFP signal (Figure 1d). Three further qualities were assessed (Table S2 in Additional file 1): 1) the sorting specificity achieved using gates P1 to P3 resulted in the selection of ≥ 89% of the L-lysine producer expected within the respective gate; 2) the recovery of viable single cells for each gate was ≥ 84%; 3) when DM1728 was mixed with a 10,000-fold excess of WT cells and then re-isolated, 92% of the prepared cells were DM1728.

LysG recognizes L-arginine and L-histidine in addition to L-lysine [14]. Peptide addition is a proven method to increase the cytosolic pool of a specific amino acid in *C. glutamicum* or *E. coli* [18,19]. The fluorescence response of *C. glutamicum* carrying pSenLys to exogenously applied Lys-Ala is similar to endogenously synthesized L-lysine (Figure 1e). The fluorescence response due to Arg-Ala supply was substantially greater, and that to His-Ala was greater still, which may indicate a higher affinity of these ligands for LysG.

Further metabolite sensors

To assess the general utility of TFs for reporting on small-molecules at the single-cell level, we constructed pSenArg based on ArgP of *E. coli* controlling *argO* transcription as a function of cytosolic L-arginine [20]. When pSenArg was assayed for dipeptide responsiveness, the addition of Arg-Ala resulted in fluorescent *E. coli* cells (Figure 2, left), with fluorescence increasing in proportion to the dose of dipeptide (Figure S3 in Additional file 1). As additional metabolite sensors, pSenSer and pSenOAS were constructed (Table S1 in Additional file 1), suitable for L-serine (Figure 2, middle), and *O*-acetyl-L-serine detection in *C. glutamicum* (Figure 2, right), respectively.

Generation of genetic diversity and library screening

To demonstrate the feasibility of metabolite sensors for HT screening of mutant libraries, we introduced chromosomal mutations into the WT of *C. glutamicum* carrying pSenLys by treatment with MNNG (N-methyl-N'-nitro-N-nitrosoguanidine), one of the most effective chemical mutagens for creating genetic diversity [21]. While the separation of mixtures of defined producers could be achieved using FACS (Figure 1d), direct processing of the mutagenized library with FACS was not successful. We used a liquid culture recovery and enrichment step taking into account that metabolically active cells require 2 h for Crimson synthesis (Figure S4 in Additional file 1), and that cells derived from the glycerol stock have to be incubated for 6 h on minimal medium to establish host-specific EYFP fluorescence. The suspension of mutagenized cells from glycerol stock (200 µl) was diluted into minimal medium containing IPTG (isopropyl-β-D-thiogalactopyranoside and, after 2 h, 6.5×10^6 Crimson-positive cells were sorted in minimal medium. After cultivation for a further 22 h, 350 EYFP positive cells were spotted onto minimal medium plates. Of these, 270 grew into colonies within 48 h.

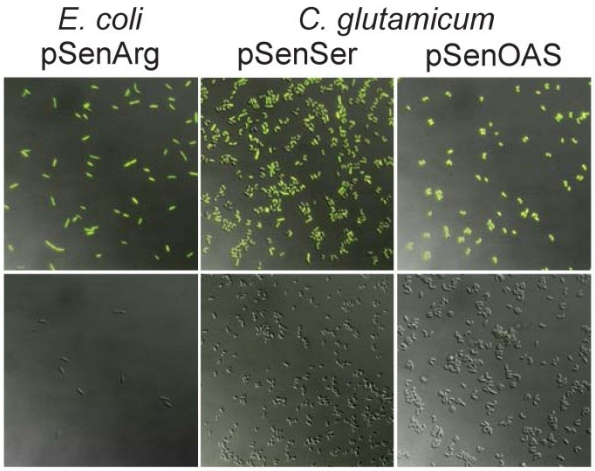

Figure 2 Metabolite sensors reporting on cytosolic arginine, serine, and *O*-acetyl-serine. Cells of *E. coli* DH5α (left) carrying pSenArg fluoresce when Arg-Ala was added (top), but not following supplementation with Ala-Ala (bottom). The peptide-dose response curves for Arg-Ala, Lys-Ala and His-Ala are shown in Figure S3 of Additional file 1. The serine-producing strain *C. glutamicum*-Ser4 (middle) [45] with pSenSer is fluorescent (top), but the WT carrying pSenSer is not (bottom). (**c**) Fluorescence is seen with the L-cysteine producer *C. glutamicum*-Cys3 carrying pSenOAS (top right), but not with the control strain (bottom right). Epifluorescence microscopic analysis was done at λ_{ex} = 490 to 510 nm and λ_{em} = 520 to 550 nm.

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Producer verification

The 270 colonies were used to inoculate microtiter plates, to enable HT screening of cultures using 0.75 ml minimal medium. L-lysine was detected in culture supernatants of 185 clones. Re-cultivation of 120 clones (Figure 3a) revealed that the L-lysine concentration ranged from 0.2 to 37 mM. Four clones accumulated 3.6 to 5 mM L-lysine plus 0.6 to 0.8 mM L-arginine and one clone 24.9 mM L-lysine plus 0.6 mM L-arginine. None of the mutants accumulated L-histidine, possibly due to the length of this pathway and its tight regulation.

Of the 120 mutants, 40 were selected randomly, and their culture fluorescence and growth recorded (Figure S5 in Additional file 1). Using an expectation maximization algorithm [22], two clusters relating specific fluorescence to L-lysine accumulation were apparent (Figure 3b). The cluster represented by the lower curve showed characteristics similar to the defined recombinant strains used in Figure 1a, which are included in Figure 3b as gray dots and numbered in parentheses. This cluster includes the mutants of main interest. Mutants in the other cluster show in part extreme fluorescence at a comparatively low extracellular L-lysine accumulation. Since we screened for high fluorescence, it is possible that mutants with reduced L-lysine export activity - and therefore increased intracellular L-lysine concentration - accumulated. This finding warrants further exploration. Cellular export activity is influenced by a number of parameters, including the lipid environment of carriers and the composition of the outer membrane [4,16], which may cause mutants to excrete metabolites at different rates than the WT does.

Gene analysis in 40 mutants

We sequenced *lysC*, which encodes aspartate kinase in the 40 mutants described above (Figures 4 and 5a). To date, all L-lysine producers described have a mutation in *lysC*, preventing feedback inhibition of aspartate kinase activity by the concerted action of L-lysine plus L-threonine [23]. In 15 of the mutants that we found, *lysC* was mutated, including seven cases of the known mutation *lysC*-T308I, which is located in the regulatory β-subunit of the aspartate kinase [23]. New mutations - *lysC*-H357Y, *lysC*-T313I, *lysC*-G277D, and *lysC*-G277S - that also affect the regulatory subunit (Figure S6 in additional file 1) were

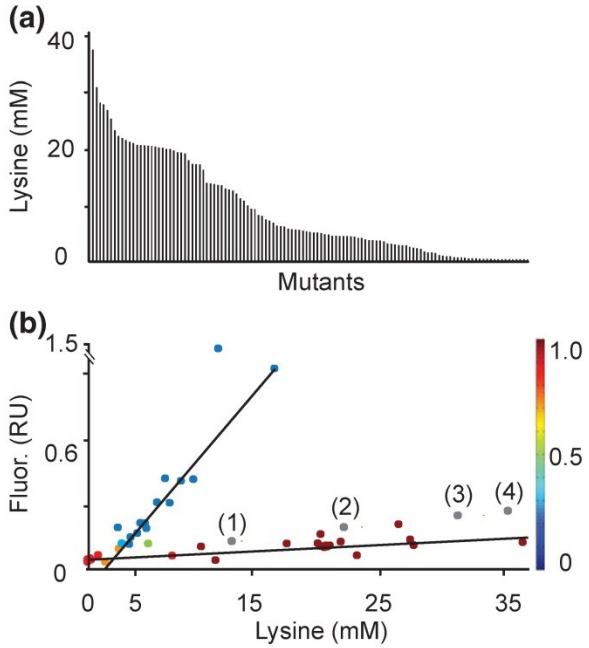

Figure 3 Characterization of L-lysine-producing mutants isolated by FACS from a library of chemically mutagenized wild type cells. (**a**) The spectrum of L-lysine accumulation in the culture supernatant of 120 mutants obtained using FACS selection. Mutants were grown in minimal medium with 4% (w/v) glucose and lysine concentrations determined after 48 h. (**b**) Specific culture fluorescence was determined for 40 arbitrarily chosen mutants. Two clusters are apparent after applying an expectation maximization (EM) algorithm to construct a distribution containing maximum likelihood estimates of the parameters in a Gaussian mixture model with two components for data in the 40-by-2 data matrix [22]. The heat bar on the right gives the probability of clones belonging to cluster one, which corresponds to that with the flat curve. The probability of clones belonging to cluster two, corresponding to that with the steep curve, uses the same heat bar with the highest probability in blue and lowest in red. The four gray circles marked (1) to (4) give lysine accumulation and specific fluorescence for the defined recombinants DM1728 (1), DM1800 (2), DM1730 (3), and DM1919 (4) used in Figure 1a. RU, relative units.

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Strain	Lys(mM)	μ(h^{-1})	Mutation	Strain	Lys(mM)	μ(h^{-1})	Mutation
K015	36.9	0.34	*lysC* A279T	K042	4.2	0.12	*hom* T233I
K037	27.4	0.33	*lysC* A279T	K039	10.9	0.24	*hom* V211F
K053	19.8	0.39	*lysC* A279V	K016	0.1	0.11	*thrB* S102F
K008	7.2	0.36	*lysC* G277D	K055	2.7	0.18	*murE* L121F
K106	19.6	0.39	*lysC* G277S	K051	15.9	0.23	*murE* G81E
K096	0.9	0.25	*lysC* H357Y				
K035	21.5	0.35	*lysC* T308I				
K100	20.6	0.29	*lysC* T308I				
K047	20.3	0.27	*lysC* T308I	K117	7.0	0.34	
K065	20.2	0.30	*lysC* T308I	K005	6.6	0.38	
K019	20.1	0.23	*lysC* T308I	K021	5.9	0.37	
K090	19.8	0.34	*lysC* T308I	K079	5.2	0.30	
K078	0.3	0.18	*lysC* T308I	K093	5.0	0.39	
K101	26.4	0.23	*lysC* T311I	K013	4.9	0.38	
K115	1.5	0.25	*lysC* T313I	K048	4.5	0.38	
K002	27.7	0.30	*hom* A328V	K062	3.7	0.26	
K049	9.6	0.15	*hom* A364V	K107	3.6	0.36	
K074	2.9	0.14	*hom* G241S	K023	16.9	0.17	
			thrC A372V	K120	2.6	0.37	
K052	0.2	0.18	*hom* G241S	K046	0.1	0.39	
			thrC A372V	K118	10.5	0.36	
K032	22.9	0.26	*hom* R158C	K063	9.0	0.37	
			hom T351I	K112	7.9	0.38	

Figure 4 Genetic characterization of mutants. In 40 mutants, targeted sequencing revealed mutations in *lysC*, *hom*, *thrC* and *thrB*, with some of the mutations already known from prior work (see text). Mutations that resulted in amino acid exchanges are indicated, along with growth rates and final external lysine titers. The mutation *murE*-G81E in strain K051 was identified by whole-genome sequencing. The second *murE* mutation, *murE*-L121F, was identified by subsequent targeted sequencing. In 15 mutants (plus K051), it was not possible to identify any mutation by site-directed mutagenesis.

found. We also sequenced *hom* in all 40 mutants, since reduced homoserine dehydrogenase activity reduces L-threonine availability in cells and thus also reduces kinase activity (Figure 5a). Current approaches to engineering L-lysine synthesis rely on just a single *hom* mutation [15]. Seven of the mutants isolated in this study carry novel mutations in *hom* (Figure 4).

In the remaining 18 mutants, neither *lysC* nor *hom* was mutated. We therefore sequenced *thrB* and *thrC* as further genes of L-threonine synthesis (Figure 5a). In strain K016 the mutation *thrB*-S102F was identified and in strain K074 the mutation *thrC*-A342V was found (Figure 4). The introduction of four selected mutations into the WT chromosome resulted in significant L-lysine concentrations (Table S3 in Additional file 1), demonstrating that these new mutations cause increased L-lysine formation.

Genome sequence of mutant K051

We performed whole-genome sequencing on strain K051, which has no mutation in *lysC*, *hom*, *thrB*, or *thrC* yet accumulates L-lysine up to a concentration of 15.9 mM. Paired-end sequencing on an Illumina HiSeq 2000 provided more than 20 million reads. Trimming and mapping to the WT genome (NC_000913) [15] resulted in a 260-fold coverage (Table S4 in Additional file 1). The genome sequence of strain K051 has been deposited at the European Nucleotide Archive under accession number HE802067. Within K051, 268 SNPs are manifest. They are unevenly distributed in the genome (Figure 5b). The number of SNPs is within the range observed for *E. coli* treated with MNNG [24]. All of the SNPs identified are transitions, as expected with this mutagen, the majority of them resulting in amino acid exchanges (Figure 5b; Table S1 in Additional file 2). In addition, NCgl0863, which carries the amino acid exchange G54D, was partially duplicated, with the variant copy placed 6,108 bp distant from NCgl0863 in an intergenic region.

We searched the mutations in K051 for genes known to increase L-lysine production and to participate in the pathway from glucose uptake up to L-lysine excretion

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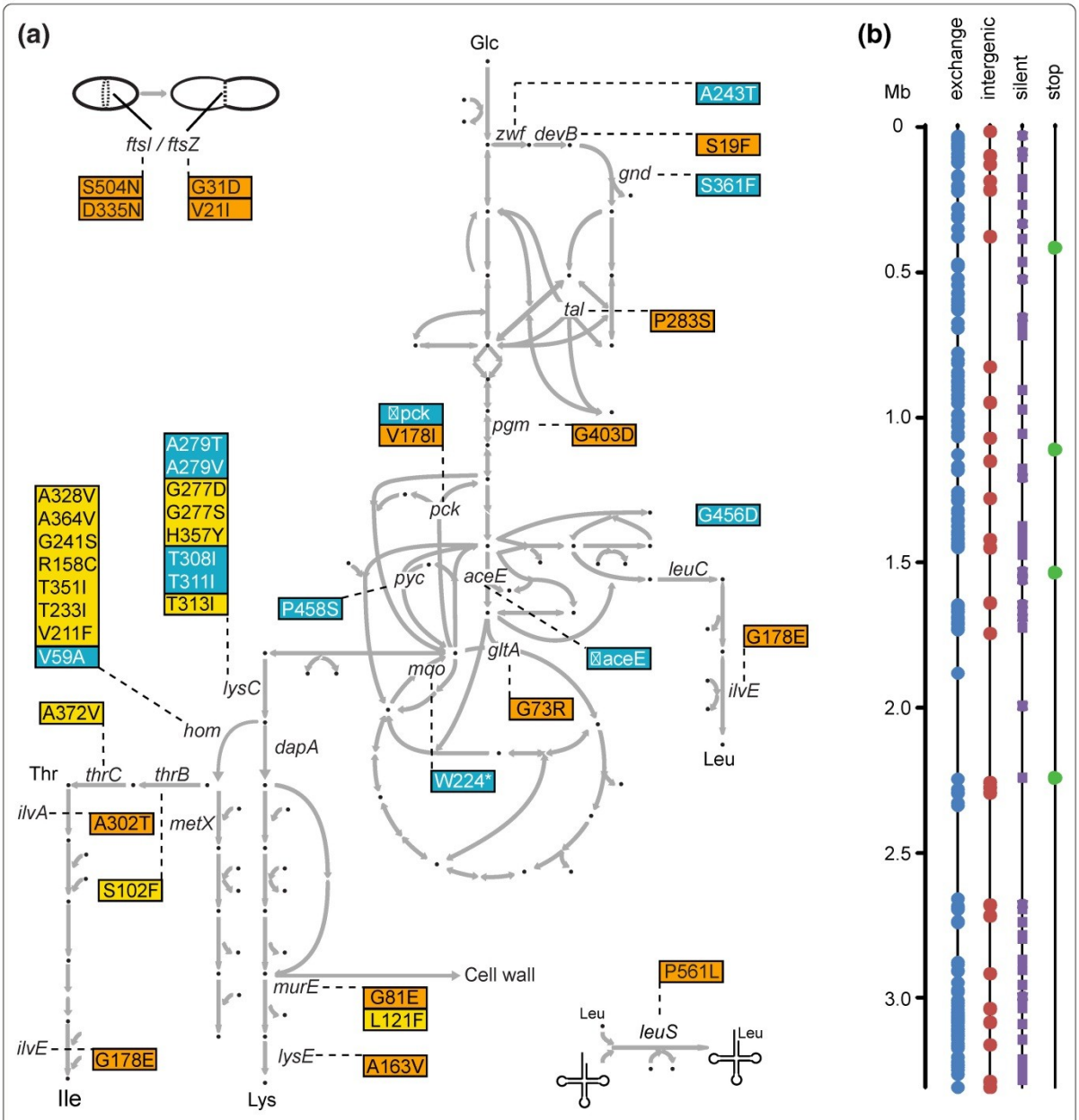

Figure 5 Sketch of the central metabolism and localization of mutations in *C. glutamicum* together with whole-genome sequencing results of the mutant K051. (**a**) Pathways and reactions required for and related to lysine synthesis. Genes that carry mutations leading to amino acid exchanges are indicated as follows: blue box, mutations known previously; yellow box, new mutations identified by targeted sequencing in 40 mutants; orange box, new mutations derived from the K051 genome. (**b**) Localization of the 268 SNPs in the genome of strain K051 determined by whole-genome sequencing. The size of the genome is 3.28 Mb, scale on the left. The mutations are classified into those causing an amino acid exchange, those that are silent, those leading to a stop codon, and those located in non-coding regions. The genome sequence of strain K051 is deposited at the European Nucleotide Archive under the accession number HE802067.

(Figure 5a). Specific mutations in *zwf* and *gnd* in the pentose phosphate pathway are known to increase L-lysine formation due to an increased supply of NADPH [25]; K051 has mutations in *devB* and *tal* that could also be effective. K051 also has mutations in *pck* and *gltA*, genes encoding phosphoenolpyruvate carboxykinase and citrate synthase, where reduced activities are known to increase the supply of pyruvate and oxaloacetate for L-lysine

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synthesis [26,27]. Also, mutations of branched-chain amino acid metabolism have been demonstrated to increase lysine formation, and K051 carries a mutation in *ilvE*, as well as in the Leu-tRNA synthetase LeuS. Of particular interest was the *murE* mutation (*murE*-G81E) in K051. This gene encodes UDP-N-acetylmuramyl-tripeptide synthetase, an enzyme that utilizes D, L-diaminopimelate as a substrate, as does the D, L-diaminopimelate decarboxylase, in L-lysine synthesis.

Influence of *murE* mutations on L-lysine synthesis

To determine whether the *murE*-G81E mutation identified could generate increased L-lysine formation, we introduced it by allelic replacement into DM1132, DM1728, DM1730, DM1800, and DM1933. The new strains were cultivated in parallel to their ancestor strains in shake flask cultivations and final L-lysine concentrations were determined after 48 h. As shown in Figure 6, the mutation caused strong L-lysine accumulation when introduced into the WT DM1132 and also DM1728, the strains that have few mutations and which form comparatively little L-lysine. Yet even with the best producer available, strain DM1933, a significant increase in L-lysine accumulation was determined. Given this finding, we sequenced *murE* in the remaining mutants isolated by our HT technology that had no identified mutation (Figure 4), and found *murE*-L121F in strain K055. Introduction of this specific mutation into the five defined L-lysine producers yielded increased L-lysine accumulation, too (Figure 6). Whether the increases with the two *murE* mutations identified were due to increased availability of D, L-diaminopimelate for L-lysine synthesis, or whether a global regulatory effect pushes synthesis of D, L-diaminopimelate remains to be studied.

Discussion

The key requirement for visualization of single cells with elevated concentrations of a small molecule of interest is the availability of suitable *in vivo* sensor systems with sufficient sensitivity and specificity. There are a large number of options for developing customized reporters sensing intracellular metabolites. They are based on natural molecular recognition, allosteric switching, and gene regulation behavior of proteins and RNA. Every system has its own specific advantages and disadvantages, and the reader is referred to recent reviews on the numerous ideas and ongoing developments in the field [12,28-33]. Whereas protein sensors based on periplasmic binding proteins and Förster resonance energy transfer (FRET) in principle enable concentration determinations in real time, use of TFs relies on expression of the reporter gene. This delay between ligand binding and the corresponding phenotypic change is not a disadvantage in developing or characterizing recombinant cells since stable genetically encoded genotypes are sought. With respect to the use of TFs in metabolite sensing for screening purposes, the present work based on LysR of *C. glutamicum* is the first example where the responsiveness of the optical output to an existing intracellular metabolite concentration is given, and where a TF-based sensor is used in an HT screen applying FACS for the isolation of new bacterial small-molecule producers.

The responsiveness of TFs previously characterized is deduced from the external addition of the effector molecule and whole culture response. Although this may only be of limited significance for screening, it is disadvantageous for precise characterization since various processes such as active uptake, active export, diffusion and degradation of effector might result in a different cytosolic concentration than that present extracellularly. In the case of LysG-based pSenLys, we determined a detection range of 4 to 25 mM intracellular L-lysine. Sensor responsiveness is characterized by an analog-like response that, when fitted to the Hill equation, is described by n_{app} of 3.19 ± 1.45. It enables the differentiation of WT from medium- and high-level producer cells (Table S2 in Additional file 1). As our intracellular determinations and the comparison of the isogenic strains with one copy and two copies of *lysE* revealed, the effective range of detection may be extended by altering export activity. This could be of relevance for further improvement of good producers. Sensor response and its usefulness will depend on the interplay between the cytosolic concentration of the small-molecule and export activity, as well as on the affinity of the sensor to the effector and target promoter site.

Three of the small-molecule sensors described in the present work are based on a LysR-type TF, and one on a ROK-type TF. Fortunately, the range of small molecules detectable by TFs is large. *E. coli* has more than 230 TFs, with many of them detecting small molecules. In bacteria, TFs have been found to sense sugars, sugar phosphates, vitamins, 2-oxoacids, ions, antibiotics, and acyl-CoA derivatives [9]. Moreover, TFs with new specificities can be generated [11]. An example is AraC, which has been switched from a natural L-arabinose sensor to a sensor detecting D-arabinose [34] or mevalonate [10], and the latter effector specificity has been used in a plate-based assay to screen for improved mevalonate producers. Other sensors that were given new specificities were developed from NahR or XylR for the detection of benzoic acid-related compounds [35], or TetR for structural derivatives of tetracycline [36]. Advances in the design of microbial-based molecular reporters and customizing ligand dependence derived from natural TFs have recently been reviewed [12].

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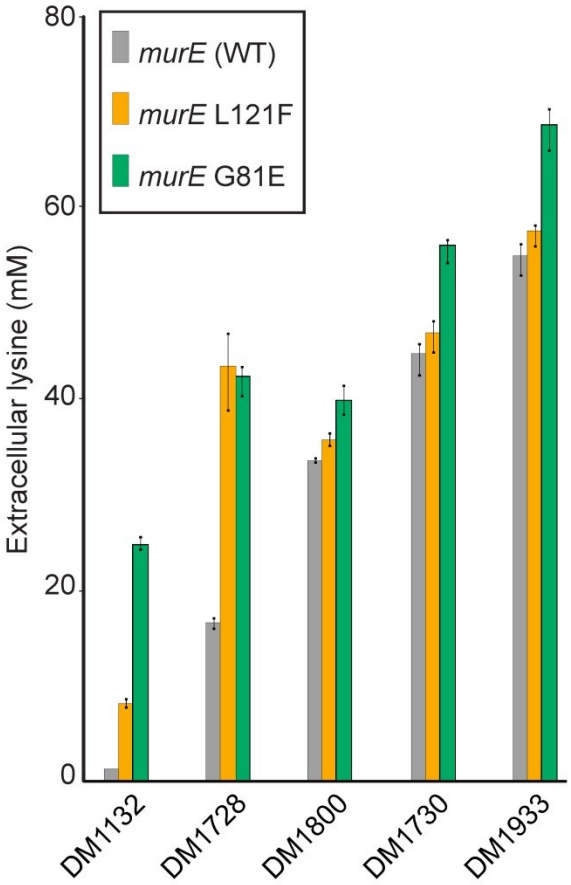

Figure 6 Effect of *murE* mutations on lysine accumulation. Lysine production by different strains modified to carry a chromosomal *murE* mutation. Color code: gray, ancestor strains; orange, strains carrying the amino acid exchange L121F in MurE; green, strains carrying the amino acid exchange G81E in MurE.

Thus, sensors for a significant number of small molecules of biotechnological or pharmaceutical importance are within reach.

Whereas the WT of *C. glutamicum* does not excrete L-lysine, cytosolic sensing and FACS as an efficient screen enabled the rapid isolation of 185 new mutants accumulating L-lysine in the culture supernatant. The current number of genes where mutations cause increased L-lysine synthesis is about 12 [37,38]. These mutations serve to increase flux through the L-lysine pathway itself, or to increase the pyruvate and oxaloacetate pool, or the NADPH supply. However, there are still unknown mutations to be discovered, since it is known that in an L-lysine-producing mutant developed over decades in classical screenings, many genes of biosynthesis pathways exhibit increased expression [39], and in a similarly derived L-arginine producer, arginine biosynthesis genes are highly expressed in a manner not achievable by plasmid-encoded expression [40]. Our approach provided alleles of known genes, and this is very useful for genomic reconstruction of producers where advantageous mutations are combined, and alleles may result in different productivity [2,41]. The number of 268 SNPs present in K051 is too great to study their individual impact on product formation, but new possibilities might be offered when more genome sequences become available. Striking was the *murE* mutation present in K051. We suggest that the catalytic activity of UDP-N-acetylmuramoyl-L-alanyl-D-glutamate:meso-diaminopimelate ligase in MurE-G81E is reduced, with the consequence that more D, L-diaminopimelate is available for L-lysine synthesis. MurE of *C. glutamicum* is similar to MurE of *Mycobacterium tuberculosis* and *E. coli*, the crystal structures of which are known [42]. From these, it can be deduced that G81E is close to the nucleoside part of UDP-MurNAc-L-Ala-D-Glu, and L121F in the second mutant identified is close to the ATP-binding site. Thus, a reduced activity is meaningful, and in line with the increased L-lysine

formation obtained with all strains when the *murE* mutations were introduced in their genomes. It is also in line with the reduced growth rates of these new recombinants (Table S5 in Additional file 1), since less D, L-diaminopimelate is channeled towards cell wall synthesis. An alternative to simple mass balance effects is that a lack of cell wall building blocks initiates a global response that has a positive effect on biosynthesis.

We applied one of our transcriptional sensors for HT screening of a mutant library with chromosomal mutations, but the same principle may also be explored for HT screening of cells carrying plasmid libraries. This is attractive, since many pharmaceuticals currently produced microbially, such as amorpha-4,11-diene, taxadiene and lycopene, use plasmid-encoded biosynthesis pathways, for example, in *E. coli* [2,3,13]. Use of an appropriate sensor combined with FACS-assisted screening may significantly accelerate the development of producers for such small molecules, too. The HT selection routine for mutant isolation closes the gap between HT generation of mutant libraries and HT sequencing technologies, and further applications of sensing small molecules in single cells are in progress, such as the verification of producer population homogeneity and time-lapse microscopy of *C. glutamicum* in microfluidic chips [43].

Conclusions

This work examines visualization of the intracellular concentration of small molecules at the single cell level by the use of specific TFs. It opens up various possibilities to characterize and analyze single cells in populations with respect to their cytosolic small molecule concentration. We have demonstrated that the visualization of L-lysine combined with HT sorting of genomic mutant libraries via FACS enables the isolation of new mutants. Together with whole-genome sequencing, this therefore establishes rapid access to new mutations to achieve more efficient product formation. In addition to the screening of cells with genomic mutations, the system is also suitable for screening cells with plasmid libraries to identify more efficient product accumulation.

Materials and methods

Sensor plasmid construction

The regulatory units were synthesized (LifeTechnologies GmbH, 64293 Darmstadt, Germany) and cloned into pJC1 using the restriction sites *Bam*HI and *Sal*I. An overview of the sensor plasmids is shown in Figure S2 in Additional file 1. The entire plasmid sequences were deposited at EMBL under the accession numbers HE583184 (pSenLys), HE583185 (pSenArg), HE583186 (pSenSer), and HE583187 (pSenOAS1).

FACS analysis and cell sorting

Cells were diluted to an optical density below 0.1 and immediately analyzed by a FACS ARIA II high-speed cell sorter (BD Biosciences, Franklin Lakes, NJ USA 07417) using excitation lines at 488 and 633 nm and detecting fluorescence at 530 ± 15 nm and 660 ± 10 nm at a sample pressure of 70 psi. Data were analyzed using BD DIVA 6.1.3 software. The sheath fluid was sterile filtered phosphate-buffered saline. Electronic gating was set to exclude non-bacterial particles on the basis of forward versus side scatter area. For sorting of Crimson- or EYFP-positive cells the next level of electronic gating was set to exclude non-fluorescent cells. Background was estimated using non-induced *C. glutamicum* for sorting of Crimson-positive cells. When sorting EYFP-positive cells, non-producing *C. glutamicum* cells were used.

Mutagenesis and library screening

C. glutamicum ATCC13032 carrying pSenLys was grown in 5 ml BHI complex medium (Difco Laboratories Inc., Detroit, MI 48201, USA) containing 25 μg ml^{-1} kanamycin to an optical density of 5 to ensure exponential growth. Whole-cell mutagenesis was done by the addition of MNNG dissolved in dimethyl sulfoxide (DMSO) to a final concentration of 0.1 mg ml^{-1} and incubation for 15 mintes at 30°C. The treated cells were washed twice with 45 ml NaCl, 0.9% (w/v), resuspended in 10 ml BHI and regenerated for 3 h at 30°C and 180 rpm. Mutant cells were stored at -30°C as cryostocks in BHI containing 40% glycerol (w/v). Of the initial cells, 46.2% survived the MNNG treatment and among the surviving cells approximately 16% were auxotrophs. For FACS screening, the mutant stock population containing 7.5×10^8 viable cells per ml was diluted 1:100 in 20 ml minimal medium containing 0.1 mM IPTG to induce expression of the far-red fluorescent protein Crimson, which was taken as an indicator of metabolically active cells. After 2 h of cultivation, 6.5×10^6 cells were analyzed by FACS and 2×10^6 Crimson-positive cells collected in fresh 20 ml minimal medium without IPTG. After cultivation for a further 22 h, 1.8×10^7 cells were screened and 350 EYFP-positive cells spotted on Petri dishes containing minimal medium. Colonies grown after 48 h at 30°C were further analyzed.

HT cultivation and culture fluorescence analysis

HT cultivation was done in 48-well Flowerplates (FPs; m2p-labs GmbH, 52499 Baesweiler, Germany) at 30°C, 990 rpm and a throw of ø 3 mm. The specific geometry of the FPs ensures high mass transfer performance and can be used together with the microcultivation system BioLector [44], allowing online monitoring of growth

and fluorescence. The medium used for FP cultivations was the MOPS-buffered salt medium CGXII [45], with 4% glucose as substrate and 25 μg ml^{-1} kanamycin to select for maintenance of pSenLys. For offline cultivations, FPs were cultivated on a Microtron high-capacity microplate incubator operating at a shaker speed of 990 rpm, throw ø 3 mm (Infors AG, CH-4103 Bottmingen, Switzerland). Shake flask cultivations were used to compare the consequences of the *murE* mutations for L-lysine accumulation (Figure 4b); these were done in 500 ml baffled Erlenmeyer flasks with 50 ml medium. The medium was the same as used in FP cultivations, except that the phosphate concentration was reduced by half. Cells pregrown in CGXII medium were used as inocula for all cultivations.

Amino acid quantification

Amino acids were quantified as their *o*-phthaldialdehyde derivatives via high-pressure liquid chromatography using a uHPLC 1290 Infinity system (Agilent, Santa Clara, CA 95051, USA) equipped with a Zorbax Eclipse AAA C18 3.5 micron 4.6 × 75 mm and a fluorescence detector. As eluent, a gradient of 0.01 M Na-borate buffer pH 8.2 with increasing concentrations of methanol was used, and detection of the fluorescent isoindole derivatives was at λ_{ex} = 230 nm and λ_{em} = 450 nm.

Determination of cytosolic amino acid concentrations and amino acid export rates

Cells were pregrown as for FP cultivations for 24 h. They were washed once with fresh CGXII medium at room temperature and transferred into new medium in FPs to give an initial optical density of 10, which corresponds to 3.0 mg (dry weight) ml^{-1}. Cultures were incubated at 30°C on the Microtron high-capacity microplate incubator as above. Samples were processed at regular intervals to separate extra- and intracellular fluid by silicone oil centrifugation [46]. For the resulting fractions, amino acids were quantified as described above. The intracellular volume used to calculate the internal amino acid concentration was 1.6 μl mg (dry weight)$^{-1}$. When peptides were added (Figure 1e; Figure S3 in Additional file 1) mixtures of dipeptides at a final concentration of 3 mM were used, such as 1 mM Arg-Ala plus 2 mM Ala-Ala, to ensure that a constant supply of Arg-Ala-derived Arg is present over time in the cytosol at the lower Arg-Ala concentrations.

Epifluorescence microscopic analysis

Fluorescence imaging was performed using a fully motorized inverted microscope (Nikon Eclipse Ti) equipped with a focus assistant (Nikon PFS), Apo TIRF 100× Oil DIC N objective, NIKON DS-Vi1 color camera, ANDOR LUCA R DL604 camera, Xenon fluorescence light source and standard filters for EYFP detection (λ_{ex} = 490 to 510 nm; λ_{em} = 520 to 550 nm). Differential interference contrast (DIC) microscopy images as well as fluorescence images were captured and analyzed using the Nikon NIS Elements AR software package. Prior to analysis, cells were fixed on soft agarose-covered glass slides.

Additional material

Additional file 1: Supplementary Tables S1 to S4 and Figures S1 to S6. Table S1: strains used. Table S2: quality assessment of sorting cells carrying pSenLys. Table S3: L-lysine formation with mutations introduced by reverse engineering. Table S4: statistics on whole-genome sequencing of strain K051. Table S5: growth rates of *murE* mutants. Figure S1: isolation of LysG and characterization of the LysG binding site. Figure S2: the vector pSenLys and general configuration of sensor plasmids. Figure S3: peptide-dose response curves with sensor-carrying *E. coli* and *C. glutamicum*. Figure S4: development of Crimson and EYFP signals in mixtures of ATCC13032 with DM1728 over time. Figure S5: growth curves and fluorescence of 40 mutant cultures. Figure S6: structural presentation of LysC and localization of mutations identified.

Additional file 2: All mutations of *C. glutamicum* strain K051.

Abbreviations

bp: base pair; EYFP: enhanced yellow fluorescent protein; FACS: fluorescence-activated cell sorting; FP: Flowerplate; HT: high throughput; IPTG: isopropyl-β-D-thiogalactopyranoside; MNNG: N-methyl-N'-nitro-N-nitrosoguanidine; SNP: single nucleotide polymorphism; TF: transcription factor; WT: wild type.

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Authors' contributions

SB performed experimental studies and the FACS analyses. GS constructed sensors and did the graphic work. NS and KH contributed to sensor construction. The determination of pool concentrations and export rates was done by KK, MB contributed to manuscript writing, and LE designed the project and wrote the paper. All authors have read and approved the manuscript for publication.

Competing interests

The authors declare that they have no competing interests.

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Recombineering in *Corynebacterium glutamicum* combined with optical nanosensors: A general strategy for fast producer strain generation

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ABSTRACT

Recombineering in bacteria is a powerful technique for genome reconstruction, but until now not generally applicable for development of small-molecule producers due to the inconspicuous phenotype of most compounds of biotechnological relevance. Here we establish recombineering for *Corynebacterium glutamicum* using RecT of prophage Rac and combine this with our recently developed nanosensor technology, which enables the detection and isolation of productive mutants at the single-cell level via FACS. We call this new technology RecFACS which we use for genomic site-directed saturation mutagenesis without relying on pre-constructed libraries to directly isolate L-lysine producing cells. A mixture of 19 different oligonucleotides was used targeting codon 81 in *murE* of the wild type, at a locus where one single mutation is known to cause L-lysine production. Using RecFACS, productive mutants were screened and isolated. Sequencing revealed 12 different amino acid exchanges in the targeted *murE* codon which caused different L-lysine production titers. Apart from introducing a rapid genome construction technology for *C. glutamicum*, the present work demonstrates that RecFACS is suitable to simply create producers as well as genetic diversity in one single step thus establishing a new general concept in synthetic biology.

INTRODUCTION

For the development of microbial producer strains fast methodologies are required for introducing genomic mutations. They inevitably have to go beyond serial and tedious introduction of single DNA constructs into cells and identification of the desired mutations. Recombineering offers such a methodology and enables the rapid introduction of a single mutation into the genome (1-3), or even numbers of mutations in one experiment such as exemplified in a multiplex automated engineering approach for *E. coli* (4). However, to make full use of recombineering in producer strain generation, also a rapid detection of those recombinants that cause improved synthesis of the desired product is required. In case the product is colored, a visual inspection of colonies is possible (5), but even in this case the library size that can be screened is limited. Even worse, most small molecules of interest are not colored. The problem of ultrahigh-throughput detection and isolation of productive recombinants has recently been solved by the development of optical nanosensors based on transcription factors. They allow the detection of intracellularly synthesized small-molecules at the single cell level (6-8), and together with fluorescence-activated cell sorting (FACS) enable the ultrahigh-throughput screening of large libraries. We have developed an L-lysine sensor for *Corynebacterium glutamicum* which transmits the cytosolic L-lysine concentration of a single cell into a graded optical output. Using this sensor we isolated new L-lysine producers via FACS from a library of 10^7 randomly mutagenized wild-type cells and identified novel relevant mutations causing increased L-lysine synthesis by whole-genome sequencing (6).

The bacterium *C. glutamicum* is of particular interest, since it is one of the most important metabolite producers used in industry (9). Mutants of *Corynebacterium* are used to produce about 2,500,000 tons L-glutamate, 1,800,000 tons L-lysine, and 15,000 tons nucleotides annually. Driven by the need for the rapid introduction of chromosomal mutations we asked whether recombineering in *C. glutamicum* would be possible. Recombineering takes advantage of phage-encoded recombination proteins and has greatly improved genetic manipulation in *E. coli* in a simple and efficient manner (2). The phage λ recombination proteins Exo, Beta and Gam – as well as RecE and RecT encoded by the *E. coli* Rac prophage – promote homologous recombination at a high frequency requiring only short stretches of homologous DNA-sequences (10,11). The Exo and RecE proteins are double-stranded DNA (dsDNA)-dependent 5′−3′ exonucleases (12), Beta and RecT are single-stranded DNA (ssDNA) annealing proteins (SSAPs). They are capable of annealing homologous DNA (13) to perform strand exchange and strand invasion, and are therefore also called recombinases. Exonuclease and recombinase together facilitate homologous exchange of dsDNA substrates (14). Synthetic ssDNA substrates (oligos) recombine efficiently to generate point mutations, deletions and insertions, and require only recombinase activity (15). Beta and RecT facilitate recombineering in *Salmonella enterica*, *Lactobacillus, Bacillus subtilis*, and other

bacteria (1,16-19). However, they work less efficiently in *Mycobacterium smegmatis* (20). More recently, the RecE and RecT homologs, Gp60 and Gp61, from mycobacteriophage Che9c were demonstrated to encode recombination proteins and used to establish allelic exchange in *M. smegmatis* and *Mycobacterium tuberculosis* (21). Also the more distantly related two recombineering proteins of mycobacteriophage Halo were used for gene replacement and introduction of point mutations into mycobacterial genomes (3).

Recombineering as demonstrated for a few species stimulated us to ask whether this technology can be established for *C. glutamicum*. Our metabolite sensors are of extraordinary profit in this context. The reason is that recombineering has been almost exclusively used to date in plate assays for selectable and visible phenotypes, such as resistance to a compound or colony color. Our metabolite sensor technology is based on the expression of *eyfp* in response to elevated small-molecule concentrations within cells. This enables FACS screening of single cells which in conjunction with recombineering establishes a new general concept for genome engineering in synthetic biology.

MATERIALS AND METHODS

Bacterial strains

For recombinant DNA work *Escherichia coli* DH5α was used. The *Corynebacterium glutamicum* strain used was the type strain ATCC13032 and its derivatives which were grown at 30°C as previously described (22). The *C. glutamicum* test strain used to establish recombineering in *C. glutamicum* was derived from DM1728. The test strain contained at nucleotide 1.404.651 (Acc.-no. NC_006958) a defective kanamycin resistance gene with an additional cytosine inserted at position 234 introducing a frame-shift mutation. This strain was constructed by plasmid-based homologous recombination using pK19mobsacB-Kan(-) containing the defective gene, as well as genomic sequences adjacent to the insertion site (23). The resulting strain DM1728-Kan(-) served as a tester strain for the development of recombineering, whereas RecT-aided producer strain development was based on *C. glutamicum* ATCC13032. All recombinant strains generated were transformed by electroporation.

Plasmid constructions and oligonucleotides

The genes for the recombinases Gp43, Gp61 and RCau were synthesized (LifeTechnologies GmbH, Darmstadt, Germany) and cloned into pCLTON2 which confers spectinomycin resistance to *C. glutamicum*. Fragments were generated with BglII and EcoRI, made blunt and cloned into the SmaI site of pCLTON2 to generate pCLTON2-gp43, pCLTON2-gp61, and pCLTON2-rCau, respectively. To construct pCLTON2-bet, bet was amplified from plasmid pRSFRedkan (24) using primer pair bet-F/bet-R and cloned blunt-end into pCLTON2. For the construction of pCLTON2-recT the gene was amplified from the genome of *E. coli* MG1655 using primer pair recT-F/recT-R and cloned blunt-end into pCLTON2. Plasmid pEKEx3-recT was constructed using BglII-RBS-recT-F and EcoRI-recT-R for amplification and cloned using BglII and EcoRI into pEKEx3 resulting in pEKEx3-recT. Primers used for cloning and oligos for recombineering were purchased from Eurofins MWG Operon (Ebersberg, Germany). They were salt free, without a 5' phosphate, and are listed in Supplemental Table S1 and Table S2, respectively.

Preparation of cells for recombineering

Strains of *C. glutamicum* DM1728-Kan(-) carrying pCLTON2 or pEKEx3 derivatives encoding recombinases were inoculated from a fresh BHIS-Spec100 petri dish (22) into 50 ml BHIS-Spec100 and grown for 16 hours at 30 °C and 120 rpm. 10 ml of this preculture were used to inoculate 500 ml BHIS-Spec100. In addition, cultures containing pEKEx3 derivatives were supplemented with 0.5 mM IPTG, and cultures containing pCLTON2 derivatives received 250 ng/ml anhydrotetracyclin, which served for induction of the recombinases. Five hours later cells were harvested and made electrocompetent. They were chilled on ice for 20 min and then harvested at 4000 g and 4 °C for 20 min, washed twice in 50 ml TG-buffer (1 mM Tris-HCl pH 8, 10 % glycerol) and twice in 50 ml glycerol 10 %. The competent cells were then re-suspended in 1-ml 10 % glycerol and 150 µl aliquots stored at -70 °C before use.

To prepare ATCC13032 pSenLys pEKEx3-recT for recombineering, media additionally contained kanamycin to select for pSenLys (plates 15 µg ml^{-1}, liquid media 25 µg ml^{-1}).

Recombineering assay for repair of defective KanR

Electrocompetent cells of DM1728-KanR(-) carrying the plasmid with the recombinase to be assayed were thawed on ice and mixed with 0.1-100 µg of ssDNA oligos and transferred into 4 °C precooled electroporation cuvettes. Electroporation was performed at 25 µF, 200 Ω and 2.5 kV. Subsequently cells were immediately transferred into 4 ml pre-warmed BHIS medium containing 100 µg/ml spectinomycin and heat shocked for 6 min at 46 °C in a water-bath. They were allowed to regenerate and segregate for up to 5 hours at 30 °C and 170 rpm. Cells were plated on BHIS-Spec100-Kan15 and incubated at 30 °C for 2 days for cfu determination. As a negative control an oligonucleotide with no sequence similarity to the *C. glutamicum* genome was added to one aliquot of electrocompetent cells. In addition, one aliquot was transformed with 100 ng pJC1 conferring kanamycin resistance to determine competence and transformation efficiency.

Recombineering assay for generating L-lysine producers

Electrocompetent cells of *C. glutamicum* ATCC13032 pSenLys pEKEx3-recT were prepared as described above. For the generation of recombinant strains carrying the *lysC*-T311I mutation, 20 µg of the lysC-60-EcoRV*-oligo was used. For *in vivo* site directed saturation mutagenesis of *murE*-81 a mixture of 20 oligos (1 µg of each 100-mer, 20 µg in total) was used for electroporation. After electroporation and regeneration for 5 hours, 100 µl of the cell suspension was centrifuged (5 min, 4000 g, 4 °C) washed once with CGXII, re-suspended in 800 µl of CGXII-Spec100-Kan25 and transferred into a flowerplate, FP, (m2p-labs GmbH, Baesweiler, Germany) for further cultivation for 48 hours at 30 °C, 900 rpm and a throw of 3 mm.

FACS analysis and two-step HT-screening

For FACS analysis all samples were diluted to an optical density below 0.1 and immediately analyzed by a FACS ARIA II high-speed cell sorter (BD Biosciences, Franklin Lakes, NJ USA 07417) using excitation lines at 488 nm and 633 nm and detecting fluorescence at 530 ± 15 nm and 660 ± 10 nm at a sample pressure of 70 psi and a processing rate of about 10,000 cells per second. Data were analyzed using the BD DIVA 6.1.3 software. As sheath fluid sterile filtered phosphate-buffered saline was used. Electronic gating was set to exclude non-bacterial particles on the basis of forward versus side scatter area. For sorting of EYFP-positive cells the next level of electronic gating was set to exclude non-fluorescent cells. The background was estimated using a negative control of the corresponding recombineering experiment. As a positive control, the L-lysine producing strain *C. glutamicum* DM1728 pSenLys pEKEx3-recT was used.

Before FACS selection, 8 µl of the cells cultivated for 48 hours were inoculated into fresh 800 µl of CGXII-Spec100-Kan25 and grown for 2-5 hours as above. The two-step screening routine consisted of an enrichment step where 10,000 fluorescent cells were spotted into 800 µl CGXII-Spec100-Kan15 followed by 48 hours of cultivation in FPs at 30 °C and 900 rpm. 8 µl of this culture served to inoculate 800 µl of fresh CGXII-Spec100-Kan25 which was grown for 2-5 hours. This was followed by FACS selection and spotting of cells onto BHIS-Spec100-Kan15 plates. After incubation for 2 days at 30 °C, clones were further analyzed in terms of fluorescence and product formation.

HT cultivation and culture fluorescence analysis

HT cultivation was done in 48-well FPs at 30°C, 900 rpm and a throw of 3 mm. The specific geometry of the FPs ensures a high mass-transfer performance and can be used together with the microcultivation system BioLector (25), allowing online monitoring of growth and fluorescence. For offline cultivations, FPs were cultivated on a Microtron high-capacity microplate incubator operating at a shaker speed of 900 rpm, throw of 3 mm (Infors AG, CH-4103 Bottmingen, Switzerland) for 48 hours until all cultures reached the stationary phase. Offline fluorescence determinations were done by mixing 5 µl of the culture with 195 µl H_2O and using a Tecan microplate reader. The cultures were excited at 500 nm and emission quantified at 530 nm.

RESULTS

Selection of recombinases

Single-stranded DNA (ssDNA) annealing proteins (SSAPs) play critical roles in recombination-dependent DNA replication in any organism, with specific subclasses typical for bacteriophages and prophages (26,27). Using RecT of the Rac prophage as a query sequence, we screened in a BLAST search for homologs within 25 genomes of *Corynebacterium* species. The sole homolog identified was cauri_1962 of *Corynebacterium aurimucosum* (Figure 1), which has 61% similarity with RecT. Adjacent and co-transcribed to cauri_1962 is a gene encoding a viral exonuclease domain (PF09588). This protein has no significant similarity to the exconuclease RecE. For our initial studies to assay on the functionality of recombinases in *C. glutamicum* we focussed on RecT and cauri_1962. In addition, we chose the well-studied lambda red gene *bet*, as well as the SSAPs of the mycobacteriophage Che9c and Halo. The reason for this is that both *Mycobacterium* and *Corynebacterium* belong to the order *Corynebacteriales,* and genes of *M. tuberculosis* show functionality in *C. glutamicum* (28,29). Cauri_1962 is also closely related to Gp61 of Che9c and Bet, but has no similarity to Gp43 of Halo. Cauri_1963, the putative exonuclease, has a very weak similarity to Gp60 of Che9c and Exo of lambda, but no similarity to RecE or the Halo protein Gp42. Thus, the exonucleases appear to be more species-specific, whereas the recombinases tend to be conserved. This agrees with functional studies on recombinase/exonuclease pairs from diverse bacteria in *E. coli*: selected pairs displayed good recombination activity with ssDNA but were less efficient with dsDNA, the latter requiring both activities (30).

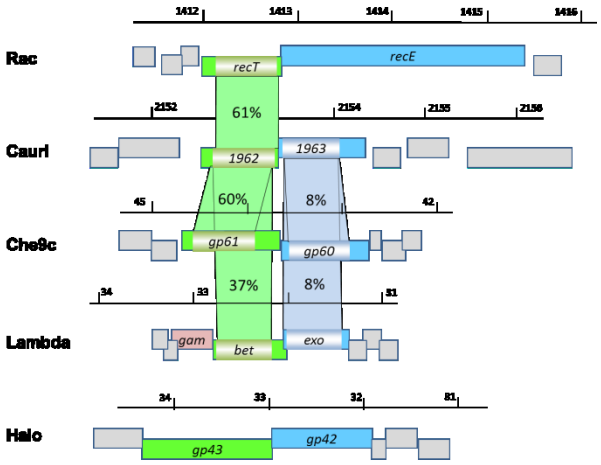


Figure 1. Comparison of the *Corynebacterium aurimucosum* recombination proteins. The cauri_1962 gene product contains a RecT domain, as does gp61 and Bet, with highest homology of cauri_1962 to the prophage Rac protein and gp61. Halo gp43 is not related to these ssDNA-binding proteins. The putative exonuclease cauri_1963 belongs to the YqaJ family of exonucleases. cauri_1963 is only distantly related to the gp60 protein and Exo, and unrelated to RecE and gp42. The genome of *C. aurimucosum* does not contain a λ Gam homologue (red).

Functionality of recombinases

We used the expression vector pCLTON2 to clone the five selected recombinases, which is based on the tetracycline inducible Tet repressor system derived from *E. coli* (31). The five constructs pCLTON2-bet, pCLTON2-recT, pCLTON2-gp43, pCLTON2-gp61, and pCLTON2-rCau - where rCau stands for the recombinase cauri_1962 – were introduced into *C. glutamicum* DM1728 Kan(-). This latter strain was the tester strain. It contained a defective kanamycin resistance gene integrated into its genome. It was constructed by using pK19mobsacB-Kan(-) encoding the Kan(-) gene with an additional cytosine inserted at position 234 introducing a frame-shift and ultimately a truncated non-functional protein. Using this non-replicative vector, the defective Kan gene was placed in a non-coding region of the chromosome by two rounds of homologous recombination. This tester strain was transformed with the pCLTON2 derivatives and the resulting strains were used in recombination assays.

Table 1. Comparison of recombinase efficiencies

Vector	cfu[a] (spont)	cfu (rec)	cfu (pJC1)	cfu (rec/pJC1)
pCLTON2-bet	0	8	2.2×10^{5}	3.7×10^{-5}
pCLTON2-recT	31	12513	5.0×10^{5}	2.5×10^{-2}
pCLTON2-gp43	57	97	4.3×10^{5}	2.3×10^{-4}
pCLTON2-gp61	1	306	3.4×10^{5}	9.1×10^{-4}
pCLTON2-rCau	7	2475	2.9×10^{5}	8.5×10^{-3}

[a]cfu (spont) is the number of spontaneous Kan^R clones, cfu (rec) the number of clones in electroporation assays which received the Kan* oligo, and cfu (pJC1) the number in assays which received 1 µg of the replicative vector pJC1. cfu (rec/pJC1) gives the number of Kan* oligo-dependend cfu in relation to the transformation efficiency of the expression culture.

In one early experiment, heterologous expression of the recombinase genes was induced with 250 ng ml^{-1} anhydrotetracyclin. After 4 hours of induction cells were made electrocompetent and frozen. Electroporation was done with 1 µg of oligo Kan50*, which is a 50-mer with the correct sequence part of KanR and homology to the leading strand. Cells were plated after regeneration for 5 hours on BHIS-Kan15-Spec100. With pCLTON2-recT about 12,500 and with pCLTON2-rCau 2,500 KanR colonies per transformation assay were obtained (Table 1). This shows that RecT is functional in *C. glutamicum* and rCau from *C. auromicosum* encodes a functional protein that is also active in *C. glutamicum*. A weak activity was also obtained with gp61 from *M. smegatis*. In controls where no oligo was added, at best 57 cfus were observed on BHI-Kan15-Spec100 plates. Each transformation assay was done with about 10^9 cells surviving electroporation. Such assays yielded 2.2×10^5 - 5.0×10^5 Kan^R cfus when transformed with the replicative plasmid pJC1 (Table 1), illustrating that the competence for uptake of DNA after expression of the recombinases, is comparable to standard transformation conditions without protein expression (32). When the oligo-specific number of transformants was put in relation to that derived by the replicative plasmid, the recombinase RecT yielded 4.7 chromosomal recombinants per 100 cells capable of taking up plasmid. In these experiments, approximately 40,000 oligo molecules per cell or 300 plasmid molecules, respectively, were present in the electroporation assay.

Optimization of recombineering

Since RecT was the most efficient recombinase in *C. glutamicum*, we next varied the induction time and used pEKEx3 as another vector background for recombinase expression (31). Cells of the tester strain *C. glutamicum* DM1728 Kan(-) carrying either pCLTON2-recT or pEKEx3-recT were induced for 0, 1, and 4 hours. Transformation with 1 µg of the healing oligo Kan50* and regeneration was performed as described before. There was a clear increase in the number of recombinants with increasing induction time (Figure 2A). The highest numbers of recombinants were obtained with pEKEx3-recT. This is probably due to the known stronger expression of target genes in pEKEx3 compared to pCLTON2 (31). In all subsequent experiments, we therefore used pEKEx3-recT with the recombinase expression induced for 4 hours and cell regeneration and segregation for 5 hours.

With *E. coli*, concentrations of 0.2 µg oligos are routinely used in recombineering assays (10), although in special applications such as multiplex automated genome engineering, concentrations exceeding 20 µg are recommended (4). We assayed various oligonucleotide concentrations to optimize recombineering efficiency in *C. glutamicum* (Figure 2B). There was a strong increase in the number of recombinants with increasing oligo concentration, with as many as 9.5×10^5 recombinants when using 10 µg Kan*. This means that 1 of approximately 1.000 cells surviving electroporation is also recombinant. Higher concentrations did not improve the recombineering

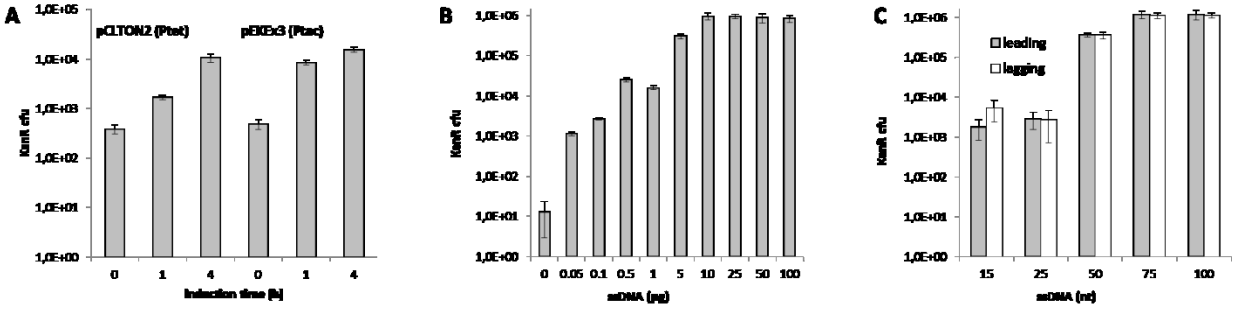


Figure 2. Optimization of recombineering efficiency. (**A**) Recombination efficiency of recT induced for 0, 1, and 4 hours using either pCLTON2-recT and 0.25 mg/L anhydrotetracyclin or pEKEx3-rect and 0.24 mg/L IPTG. (**B**) Recombination efficiency in dependence on the amount of oligonucleotide added. (**C**) Recombination efficiency in dependence on length and strand homology.

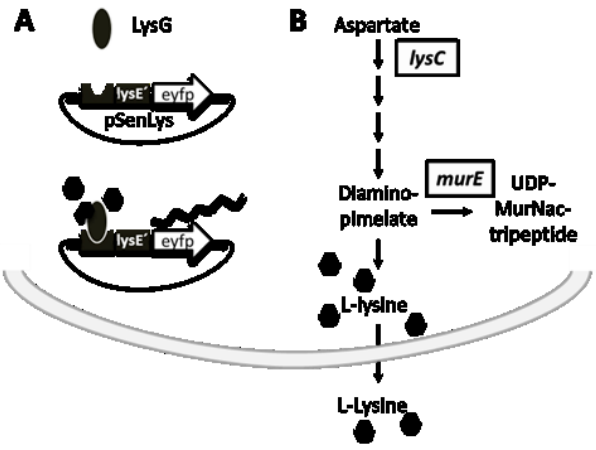


Figure 3. (**A**) Principle of L-lysine sensor pSenLys. The transcriptional regulator LysG senses L-lysine (35) to naturally drive transcription of its target genes *lysE*. When this is fused to *eyfp*, cells exhibit fluorescence at an increased cytosolic L-lysine concentration. (**B**) *lysC* and *murE* are two targets known in *C. glutamicum* which upon specific mutations provoke an excess L-lysine synthesis.

efficiency, but rather led to a slight decrease in the number of recombinants.

For a given target, there are two complementary ssDNA oligos, either one of which can be used for recombineering. The corresponding oligonucleotide that is complementary to the template strand for discontinuous DNA synthesis (i.e. the lagging strand) recombines approximately 15-fold to 20-fold more efficiently in *E. coli* than the oligonucleotide complementary to the leading strand due to activity of the recombinase Beta (10,16). As further parameters for recombineering efficiency in *C. glutamicum,* we therefore assayed both complementary oligonucleotides and we varied the lengths of the oligos. In these experiments, equimolar concentrations of 32.5 µmol were used, which corresponds to 10 µg for the 100-mer. As can be seen in Figure 2C, the use of a 75-mer yields more than 10^6 recombinants per assay, while a further increase in the length of the oligo did not increase recombination frequency. This largely agrees with the situation in *E. coli* where the highest level of recombinants is generated with a 60-mer (10). Comparison of the two complementary oligo pairs yielded a slightly increased number of recombinants in *C. glutamicum* with oligos annealing to the lagging strand (Figure 2C). Thus, there is a strand bias, but this is less pronounced than in *E. coli*. With phage Che9c gp61-mediated recombination in *M. tuberculosis*, there is an even stronger strand bias and an oligonucleotide targeting lagging strand DNA synthesis can recombine more than 10,000-fold more efficiently than its complementary oligonucleotide (20). Thus, for ssDNA recombineering in *C. glutamicum*, there is no need to differentiate between the two complementary ssDNA oligos possible, and with the oligo length of choice, approximately 10^6 recombinants are obtained per assay.

We also performed experiments to reduce a possible mismatch repair during recombination, and we used C·C mismatches or mismatches at consecutive wobble positions nearby to the correcting base making silent mutations (Supplementary Figure S1). An up to 1.3-fold increase in recombineering frequency was obtained demonstrating the importance of sequence context. This is rather low compared to the comparable experiments on *E. coli* and it could indicate a difference in the mismatch repair system between these organisms, as indicated by comparisons of the repertoire of DNA-repairing enzymes in *Corynebacterium* species (33). However, this aspect warrants further studies.

Recombineering and direct producer isolation by product sensing

Whereas selection for antibiotic resistance is useful to establish recombineering, it is of limited use for producer strain development. To overcome this problem, screening methods for nonselectable recombinants such as hybridization of colonies, and other techniques were established (16). We developed optical sensors which respond to increased product formation in single cells by emitting fluorescence (6,34). These sensors provide a direct signal when a "productive mutation" is introduced. It enables the selection of productive mutants in ultrahigh-throughput screens using fluorescence activated cell sorting (FACS).

One metabolite sensor we have developed is pSenLys. It is based on the transcriptional regulator LysG of *C. glutamicum* (Figure 3), which recognizes increased L-lysine concentrations in the cytosol to drive transcription of its target gene *lysE* (35). The fusion of *lysE* with *eyfp* results in cells emitting increased fluorescence at elevated L-lysine concentrations (6). To assay for the use of this metabolite sensor and FACS screening of recombinants, we chose *lysC* as a recombineering target in the chromosome of *C. glutamicum*. *lysC* encodes the aspartate kinase, which controls the entry of L-aspartate into the L-lysine synthesis pathway (Figure 3). When the codon ACC at position 931 to 933 of *lysC* is changed to ATC a threonine is replaced by isoleucine in the protein sequence. As a consequence, the kinase is no longer feedback inhibited by L-lysine and the wild type is converted into an L-lysine producer (36).

Cells of *C. glutamicum* pEKEx3-recT pSenLys were induced for expression of RecT and electroporated with 10 µg of the oligo lysC-60-EcoRV*. This 60-mer carries in the middle the sequence GTGAAG**A**T*GAT**A**TC*GG, with the nucleotides in bold being exchanged. The underlined codon introduces *lysC*-T311I, and the nucleotides in italics an adjacent *Eco*RV site. After regeneration and segregation, cells were grown in minimal medium CGXII-glucose, and cells with increased fluorescence enriched via FACS. The gate for cell selection was chosen according to the fluorescence of the L-lysine producer SBK052 carrying pSenLys (Figure 4). The enrichment culture derived from the recombination assay was then analyzed again with 2.4% of the cells exhibiting the increased fluorescence as expected for an L-lysine producer. This number was 0.05% for the negative control which received water instead of the oligo (Figure 4). Cells with increased fluorescence were spotted onto petri dishes, and from 12

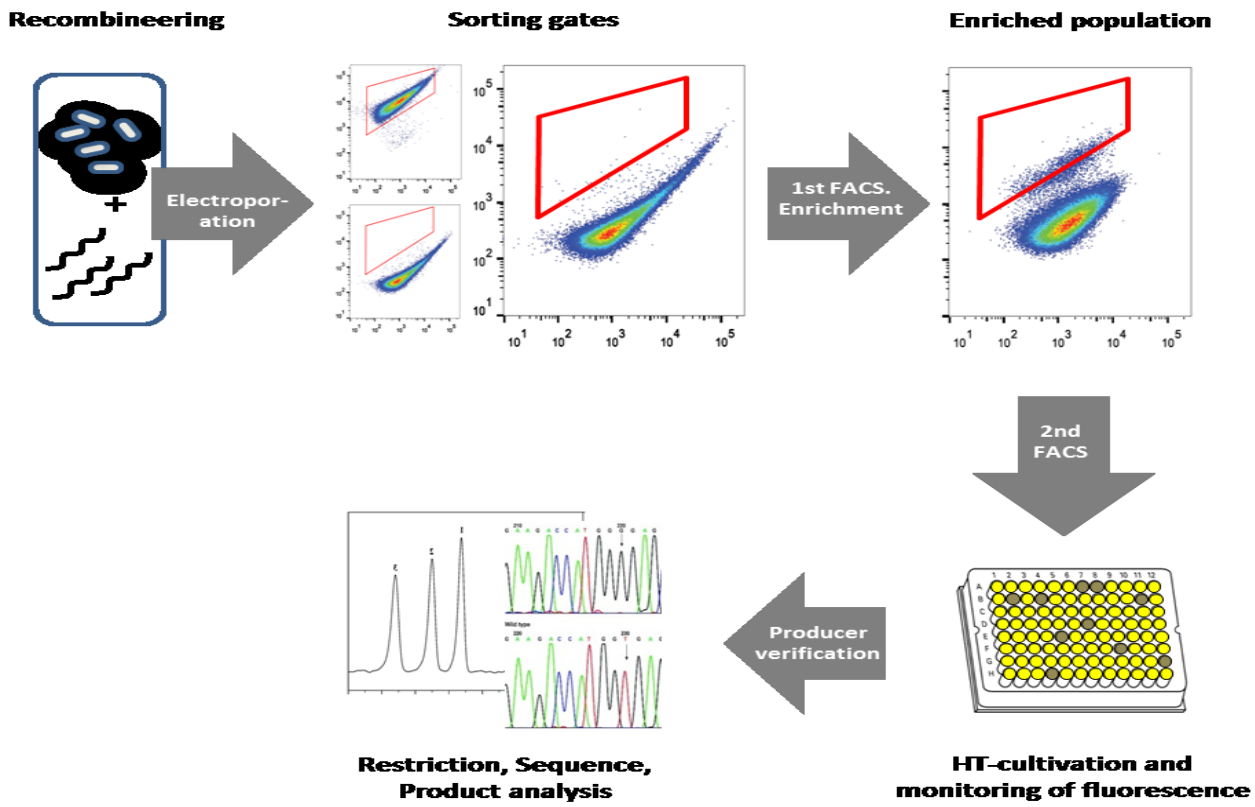


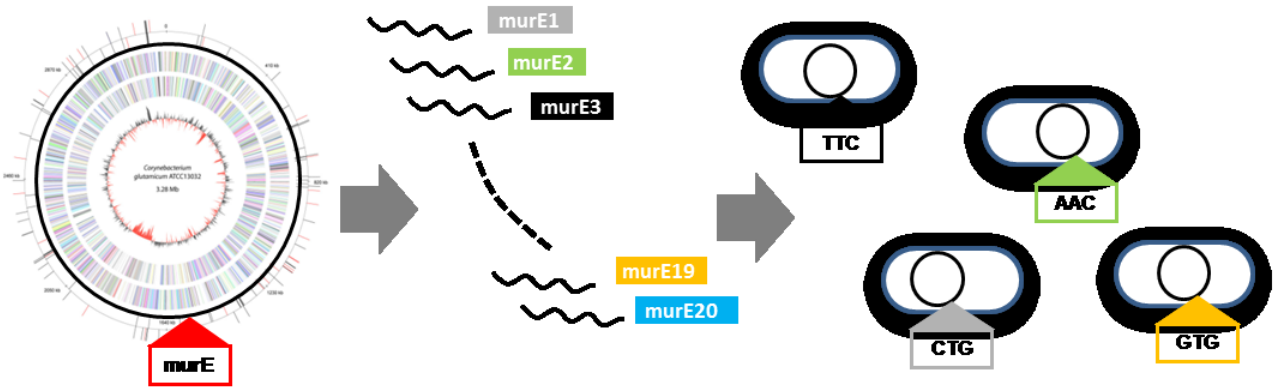
Figure 4. Principle of RecFACS as demonstrated for introduction of the productive lysC-T311I mutation. After recombineering, cells were grown in liquid culture on minimal medium, and subjected to FACS selection for an enrichment of positive clones. The setting of the gates was done with negative and positive controls electroporated and cultivated in parallel. The enrichment cultures were used for the 2nd FACS selection, where cells were spotted on petri dishes and subsequently cultivated in a microtiter plate to follow growth and fluorescence. The correct genomic integration and product formation of fluorescent clones was finally verified by sequencing and L-lysine quantification in culture supernatants.

clones the *lysC* target amplified via PCR. The diagnostic restriction analysis revealed that the *Evo*RV restriction site was present in five clones resulting from the recombineering assay, whereas this was not the case for any clone of the negative control.

One-step diversity generation and producer selection

To go even further with respect to the possibilities offered by the single-cell metabolite sensor and high-frequency recombineering, we aimed to generate genetic diversity at a given locus across a population in one single experiment. A related attempt has been successfully demonstrated for *E. coli* and *lacZ* as a target via multiplex automated genome engineering (MAGE) (4). Combining diversity generation by recombineering with our sensor technology should allow the direct selection of diverse productive cells. The target gene chosen for this experiment was *murE* (Figure 3). In previous work we identified this gene by whole genome sequencing as an attractive target to engineer L-lysine synthesis, and found that the specific mutation *murE*-G81E results in increased L-lysine titers (6).

Twenty different 100-mer oligos were designed, each with 2-3 nts exchanged in the middle of the sequence to introduce the respective codons for any of the 19 amino acids in position 81 of *murE*, except the original codon and plus one oligo with stop codon (Supplementary Table S2). A mixture of these oligos, 1 µg each, was added to recombineering competent *C. glutamicum* pEKEx3-recT pSenLys (Figure 5). Regenerated cells were inoculated as above on minimal medium and cells were screened via FACS without an enrichment step. We set a sorting gate based on positive and negative controls, which would collect all 10.000 cells analyzed from DM1728 pEKEx3-recT pSenLys but only 8 cells from WT pSenLys. Using this gate 10^5 cells of the recombineering assay were analyzed and 220 positive cells spotted onto a petri dish. Using tooth picks 132 clones were inoculated into 0.8 ml CGXII-glucose in flower plate wells. 126 cultures grew, of which 53 exhibited increased fluorescence. The oligos were designed in such a way that a chromosomal PvuII restriction site would disappear upon successful recombineering. A diagnostic restriction analysis revealed that this was the case in 21 clones, which were subsequently sequenced. One clone was obtained in which G in position 81 of MurE was replaced by V, and two or more clones where a C, F, L, N, S, W, or Y was present in this position. The experiment was repeated starting from new recombineering proficient cells and yielded a comparable result.



No. of Mutants	Codon 241-243	Amino acid 81		Fluorescence (AU)	L-Lysine (mM)
WT	GGA	Glycin	G	0.07	0.1
4	TCC	Serin	S	0.19	0.1
2	CAC	Histidine	H	0.59	2.1
4	AAC	Asparagine	N	0.65	3.6
3	ACC	Threonine	T	0.90	5.0
6	TGC	Cysteine	C	0.96	6.0
6	TAC	Tyrosine	Y	1.01	7.0
4	TTC	Phenylalanine	F	1.34	8.9
3	GTG	Valine	V	1.51	8.9
1	ATC	Isoleucine	I	1.85	10.2
3	CGC	Arginine	R	2.11	11.6
5	CTG	Leucine	L	1.89	12.0
3	TGG	Tryptophan	W	1.96	12.5

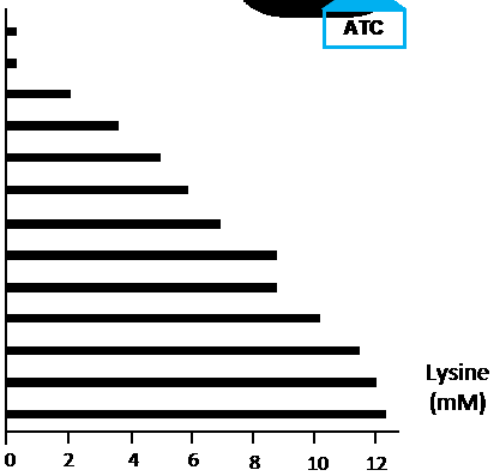


Figure 5. RecFACS to create producers with genetic diversity at codon 241-243 of murE in the genome of *C. glutamicum* wild type. A mixture of oligonucleotides murE1 to murE20 was used for ssDNA recombineering and recombinants with productive mutations were selected via FACS. Thirteen of the twenty possible recombinants were obtained (table), exhibiting L-lysine formation to different degrees (black bar).

All data and analyses of individual clones are given in Supplementary Table S3. The combined data are given in the table included in Figure 5. In total, 44 recombinants were derived, a broad range of substitutions were obtained and G81 was replaced by 12 different amino acids. The mutations were ranked in the table according to the fluorescence of the respective cultures, and this largely agrees as expected with L-lysine accumulation. With the exception of the G81S mutation, all mutations produced L-lysine. Furthermore, the production of individual clones carrying the same mutation is largely consistent (Supplementary Table S3). Thus, RecFACS, the combined use of metabolite sensors in conjunction with recombineering, allows in one single step the selection of productive mutants exhibiting a range of genetic diversity at a defined genomic locus.

DISCUSSION

Recombineering with ssDNA is easy and rapid to perform requiring just the presence of an SSAP. It has already been realized for different bacteria. Gammaproteobacteria clearly represent the largest group for which recombineering has been demonstrated (1,2), and its application is best developed for *E. coli* (4,37). Recombineering has additionally been demonstrated for a few other bacteria including *Bacilli* and *Mycobacterium* species (3,18,19). In the present work, we assayed five different SSAPs for functionality in *C. glutamicum*, among them cauri_1962. This is present in the genome of *C. aurimucosum* at a locus where phage-related proteins occur (38), and our work demonstrates that the protein encodes a functional recombinase.

The highest recombineering activity in *C. glutamicum* is obtained with RecT enabling frequencies exceeding 10^6 recombinants per assay. This is only about one order of magnitude away from that obtained with Bet in *E. coli* with a proper genetic background like with a decreased activity of the methyl-directed mismatch repair system (10). Apart from the possible use of such mutations in *C. glutamicum*, it is evident from the variation of vector use and induction time that there is still room for further optimization of the recombineering system in *C. glutamicum*. Signficant activities were also obtained with gp43 and gp61. In these latter cases, the formation of the corresponding SSAPs might be limiting because the codon adaption index is less than 0.29 for both genes, suggesting weak protein formation.

While selectable phenotypes, e.g. drug resistance or auxotrophy, are useful for the development of recombineering, this is less so the case if the production quality for a small molecule is to be improved. The reason is twofold: first, small molecules usually have an inconspicuous phenotype, and second, plate assays for phenotypic selections are limited with respect to the number of clones which can be analyzed. To overcome limitations in the absence of a direct selectable phenotype, a two-step protocol for *E. coli* can be used to modify a region of interest, where in the first step the target region is replaced by *cat-sacB* as a dual selection cassette (16). A recent technique developed for *E. coli* is the simultaneous use of two oligos with one of them introducing a selectable marker within 500 kb of the second target (37). Since the frequency of co-selection of the second target is up to three-fold higher than without selection, the numbers of clones to be characterized by subsequent colony PCR are reduced, for instance.

Our previously developed sensor technology provides an effective screen for single cells producing small molecules, such as amino acids (6). In contrast to plate assays, it is an ultrahigh-throughput technology. Its use together with recombineering - a procedure we call RecFACS - opens up a number of exciting possibilities to engineer genomes with the direct selection of producers. As the first example, we demonstrated this for the nucleotide exchange C→T at position 932 of the aspartate kinase of *C. glutamicum* resulting in LysC-T311I. The mutation reduces feedback control of the kinase, making the strain an L-lysine producer. Since kinase mutations are known to control the enzyme activity to different degrees (39), these – or other mutations known to increase L-lysine productivity - can now easily be introduced in strains to assay for their consequences on L-lysine formation.

RecFACS enables the direct selection of productive mutations. Even better, it allows the introduction of genetic diversity during producer creation. This is an additional quality of RecFACS as we have demonstrated for *murE* when we performed target-specific random mutagenesis. In one single experiment, a range of substitutions of MurE-G81 were obtained. In addition, the correlation between fluorescence of the isolates and their L-lysine accumulation is given, as we expected from the increasing cytosolic steady-state concentrations of producers, which correlate with sensor responsiveness (6). A few clones of the same mutation were potential outliers. This can simply be counteracted by analyzing a larger number of clones.

Some of the 20 mutagenic oligos did not result in productive isolates by RecFACS, and there are various explanations for this. Among them is recombineering itself. The pool of 100-mers we used differed over a sequence of up to 4 nts and it has been demonstrated that oligos with more homology to the target will be incorporated into the chromosome at a higher frequency than those with less homology (4). Moreover, although the DNA repair system of *C. glutamicum* is poorly defined (33), methyl-directed mismatch repair of *E. coli* is very sensitive to sequence context which can lead to an about 100-fold variation in oligo recombination frequency (40). Another reason for the absence of some amino acid substitutions is that they are either non-productive or that they result in poor or even absent UDP-N-acetylmuramoyl-L-alanyl-D-glutamate:meso-diaminopimelate ligase activity. This particular activity is required for cell-wall synthesis and is essential for *E. coli* (41). Enzymes with poor activity will result in poorer growth and might be outgrown before the FACS selection step of RecFACS.

In summary, extensive genome recombineering as achieved in *E. coli* is now accessible for *C. glutamicum*. Even more importantly, the potential of recombineering is significantly increased due to its alliance with metabolite sensors and establishment of RecFACS. Genetic diversity of productive mutants can be readily created. The technology can easily be extended and is expected to boost microbial strain development for small-molecule production in general.

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online

ACKNOWLEDGEMENTS

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Conflict of interest statement. None declared.

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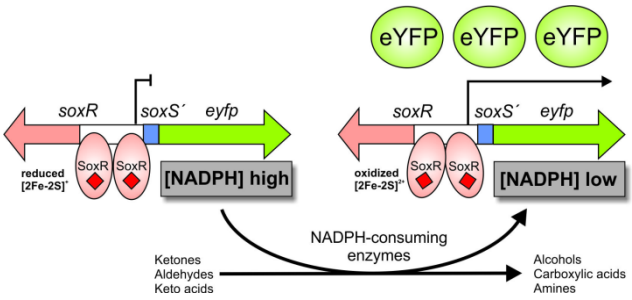
NADPH biosensor

SoxR as Single-Cell Biosensor for NADPH-Consuming Enzymes in *Escherichia coli*

*Solvej Siedler, Georg Schendzielorz, Stephan Binder, Lothar Eggeling, Stephanie Bringer, and Michael Bott**

Redox reactions are at the core of cellular metabolic processes and about one quarter of the known enzymes are oxidoreductases.[73] A feature of many of these enzymes is their stereo- and regiospecificity. Pharmaceutical industry takes advantage of these properties, as well as from the mild and environmentally friendly conditions at which enzyme-catalyzed reactions proceed. Among such processes, alcohol dehydrogenases are of particular interest.[74-76] They are employed in the reduction of various ketones to produce enantiopure secondary alcohols. These enzymes are frequently NADPH-dependent, and there is a need for continuous supply of the reduced cofactor for the reaction to proceed.[74, 77] This applies both for isolated enzymes and for whole-cell processes.[78, 79]



Scheme 1. NADPH biosensor based on the transcriptional regulator SoxR of *E. coli*. Dimeric SoxR with two Fe–S clusters binds to the *soxR-soxS* intergenic region. At sufficient NADPH levels, the Fe-S clusters are kept in the reduced state and SoxR is inactive. Enhanced activity of NADPH-consuming enzymes impedes SoxR reduction and the oxidized Fe-S clusters trigger a conformational change of SoxR, causing transcription of its target gene *soxS*. In the NADPH biosensor pSenSox, *soxS* has been replaced by *eyfp* coding for an auto fluorescent protein (eYFP), which allows identifying cells with a low NADPH level by their increased fluorescence.

In our studies with *Escherichia coli* on the reductive biotransformation of methyl acetoacetate (MAA) to (*R*)-methyl 3-hydroxybutyrate (MHB) by an NADPH-dependent alcohol dehydrogenase from *Lactobacillus brevis*,[78, 80] we noticed an increased mRNA level of the *soxS* gene at high NADPH demand. SoxS and SoxR are transcription factors involved in the oxidative stress response of *E. coli*.[81, 82] The genes of the SoxRS regulon mediate the cellular response to superoxide, to diverse redox-cycling drugs like paraquat, or to nitric oxide. SoxR is a homodimer with two [2Fe-2S] clusters. Only when oxidized to $[2Fe–2S]^{2+}$ they confer transcriptional activity to SoxR, which in turn results in expression of *soxS*. SoxS then activates expression of the SoxRS regulon, which includes e.g. *sodA*, *fumC* and *zwf*. Inactivation of SoxR involves NADPH-dependent reduction catalyzed by the *rsxABCDGE* and *rseC* products.[83] Following the original idea of the Fridovich laboratory in 1992 that the SoxRS regulon "perhaps does respond to some consequence of a decrease of the ratio of NADPH to $NADP^+$"[84], which was supported by recent studies[85], we here provide further evidence that SoxR senses the NADPH availability. We use this finding to develop a sensor for the *in vivo* analysis of NADPH-dependent reactions offering a number of interesting possibilities for high-throughput analysis and development of NADPH-dependent enzymes.

To construct the NADPH sensor plasmid pSenSox, *soxR* of *E. coli* DH5α together with the *soxR-soxS* intergenic region and 63 bp of the *soxS* coding region were cloned in front of *eyfp*, thereby placing synthesis of the autofluorescent protein eYFP under transcriptional control of the *soxS* promoter (Scheme 1; see Supplementary Information for details). The sensor plasmid also encodes the NADPH-dependent alcohol dehydrogenase of *L. brevis*, *Lb*Adh, under the control of an isopropyl β-D-thiogalactoside-inducible promoter. *Lb*Adh was previously shown by us and others to efficiently convert MAA stoichiometrically to MHB.[78, 80, 86, 87]. *E. coli* BL21(DE3) was transformed with plasmid pSenSox and whole-cell biotransformation assays were performed using up to 70 mM MAA.[86, 87] Fluorescence of the cultures was recorded online using a BioLector system (Figure 1a, see Supplementary Information for details).

Upon addition of MAA the cultures started to emit fluorescence with the initial slope of fluorescence increase being independent of the MAA concentration added (Figure 1a). For the lowest MAA concentration of 10 mM, the specific fluorescence maximum was achieved already after 1 hour, when MAA reduction to MHB was complete.[86] When increasing concentrations of MAA were used, the fluorescence maxima also increased and were reached at later time points (Figure 1a). This correlates with the increased time required for MAA reduction to MHB, during which the NADPH demand is increased. Importantly, the maximal fluorescence intensity obtained for different initial MAA concentrations remained constant for several hours, due to the high stability of eYFP. When the specific fluorescence achieved after 10 h was plotted against the initial MAA concentration, an almost linear relationship was obtained up to 60 mM MAA (Figure 1b). In the absence of MAA, constant background fluorescence was observed. Similarly, in biotransformation experiments with cells carrying plasmid pSenNeg encoding an inactive *Lb*Adh fragment only background fluorescence was detectable independent of the MAA concentration added. These controls confirm that the fluorescence increase was strictly dependent on the NADPH-dependent reduction of MAA to MHB. Also at the

single cell level the increased fluorescence at increasing initial MAA concentrations was visible by epifluorescence microscopic analysis (Figure 1c) and also by flow cytometry (see Supporting Information for details).

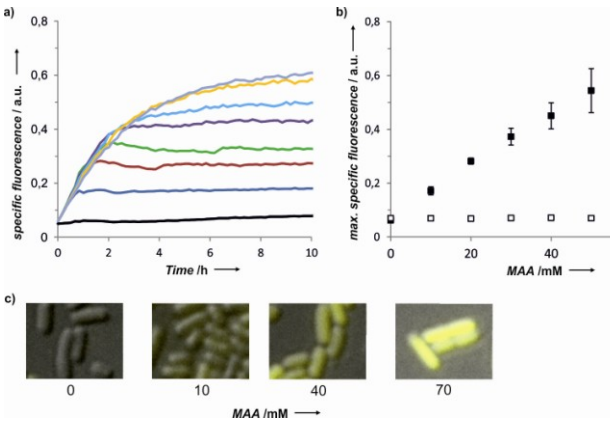


Figure 1. a) Specific fluorescence of *E. coli* carrying the NADPH biosensor pSenSox during biotransformation of 10 mM (dark blue), 20 mM (brown), 30 mM (green), 40 mM (purple), 50 mM (light blue), 60 mM (yellow) and 70 mM (grey) MAA to MHB via the NADPH-dependent alcohol dehydrogenase *Lb*Adh. In black a control without MAA is shown. b) The specific fluorescence obtained after 10 h of biotransformation was plotted against the initial MAA concentration (■). The empty squares (□) show the values obtained with the control plasmid pSenNeg encoding an inactive *Lb*Adh fragment. c) Epifluorescence of cells from biotransformations with 0, 10, 40, or 70 mM MAA.

Table 1. Dependence of the maximal specific fluorescence from the specific *Lb*Adh activity of cells.

E. coli BL21(DE3) strains	IPTG[a]	*Lb*Adh activity[b]	Maximal specific fluorescence[c]
pSenNeg	-	0.03 ± 0.01	0.067 ± 0.005
pSenSox + pET28a	-	0.5 ± 0.1	0.09 ± 0.01
pSen-L194S	-	0.7 ± 0.3	0.11 ± 0.01
pSen-L194A	-	2.7 ± 0.6	0.18 ± 0.04
pSenSox	-	6.3 ± 0.6	0.38 ± 0.02
pSenSox	+	15.2 ±2.0	0.46 ± 0.04

[a]IPTG was added to 1 mM. [b]*Lb*Adh activity of cell-free extracts is given in µmol min^{-1} (mg protein)$^{-1}$. [c]Values were measured after 19 h of biotransformation with 40 mM MAA.

In a next series of experiments we tested the influence of varying *Lb*Adh activities on fluorescence output at a constant MAA concentration (Table 1). For this purpose, strains with a specific *Lb*Adh activity between 0.03 U (mg protein)$^{-1}$ (background activity) and 15.2 U (mg protein)$^{-1}$ were used in biotransformations with 40 mM MAA. The different Adh activities were achieved either by varying the expression level of the wild-type enzyme by induction or repression or by using mutant *Lb*Adh proteins with the amino acid exchanges Leu194Ser and Leu194Ala. In the biotransformation assays, constant fluorescence was achieved after 10 hrs. As shown in Table 1, higher *Lb*Adh activities led to higher maximal specific fluorescence. This suggests that increasing specific Adh activities lead to an increased NADPH consumption rate which in turn reduces the rate of NADPH-dependent reduction of oxidized SoxR, leading to an increased SoxR activity. Thus, the pSenSox system offers the possibility to distinguish NADPH-dependent enzymes with varying specific activity.

Recently, metabolite-activated transcription factors controlling *eypf* expression were used to monitor the cytosolic concentration of the respective metabolites in single bacterial cells, which allowed high-throughput screening and isolation of single producer cells using fluorescence-activated cell sorting (FACS).[88-90] Based on these results we tested if also single cells differing in their specific Adh activity can be analysed and sorted via FACS. For this purpose, *E. coli* cells carrying either pSenSox (6.2 U mg^{-1}), pSen-L194A (2.7 U mg^{-1}), or pSenNeg (0.03 U mg-1) were used for biotransformation of 70 mM MAA. The resulting combined histogram (Figure 2a) showed three well resolved peaks of eYFP fluorescence indicating that the three strains differing in their specific Adh activity form homogeneous populations. Using an appropriate gate (P1), where 0% of the cells with background fluorescence (carrying pSenNeg) would be selected, still 80.8% of the population of cells with high fluorescence (carrying pSenSox) and 1.5% of the cells with lower fluorescence (carrying pSen-L194A) could be isolated.

These results encouraged us to test the suitability of the NADPH sensor for HT-screening of alcohol dehydrogenase mutant libraries. To do this we introduced mutations in *Lb*Adh by saturation mutagenesis at positions Ala93, Leu152, and Val195 and randomly by error-prone PCR. The cells of the mutant library were then used for reductive biotransformation of 20 mM 4-methyl-2-pentanone (4M2P). This prochiral ketone was chosen since the reduced product (*R*)-4-methyl-2-pentanol is of economic interest, and wild-type *Lb*Adh has only ~12% activity with this substrate as compared to MAA (15.5 µmol min^{-1} (mg protein)$^{-1}$). After 3 h of biotransformation, the cells were subjected to FACS. We performed a sort on the library, in which the lower bound of the sorting gate was set by cells with wild-type *Lb*adh after biotransformation of 4M2P and and the higher bound by the same strain after biotransformation of MAA instead of 4MP (Figure 2b). 10^6 cells of the *Lb*Adh mutant library were analysed and 250 showing high fluorescence were selected and spotted on plates, of which 123 grew up to colonies. 96 of them were analyzed in a microtiter plate for fluorescence intensity after 2 h incubation with 4M2P. From 6 selected clones with high specific fluorescence the specific *Lb*Adh activity was determined and one clone was identified having a 36% increased activity with the substrate 4M2P accompanied by an 8-fold increased K_M value (Table 2). The plasmid of this mutant clone was fully sequenced and found to contain a single mutation in the *Lbadh* gene leading to an Ala93Met exchange.

The present study supports the view that SoxR of *E. coli* is activated under conditions causing an increased NADPH demand, such as reductive biotranformation with *Lb*Adh. Such conditions impede the NADPH-dependent reduction of oxidized SoxR by the *rsxABCDGE* and *rseC* products, which ensure inactivity of SoxR despite its presumably permanent oxidization under aerobic conditions. It is thus likely that all conditions leading to activation of SoxR are associated with an increased NADPH demand. By coupling SoxR activity to the synthesis of an autofluorescent protein, SoxR can be used to monitor cellular NADPH consumption. The correlation between NADPH

consumption and fluorescence is visible at the single-cell level and suitable for FACS analysis, allowing the highest throughput assays currently possible. Therefore, the SoxR sensor provides a generalized high-throughput screening system for NADPH-consuming enzymes. As long as educts and products can enter and leave the cytoplasm of the sensor-containing cells, libraries of dehydrogenases requiring NADPH, or P450-monooxygenases which are also dependent on NADPH, can now be assayed in a high-throughput format without development of specific assays. This novel technology is expected to expedite the availability of new enzymes for the synthesis of chiral compounds significantly.

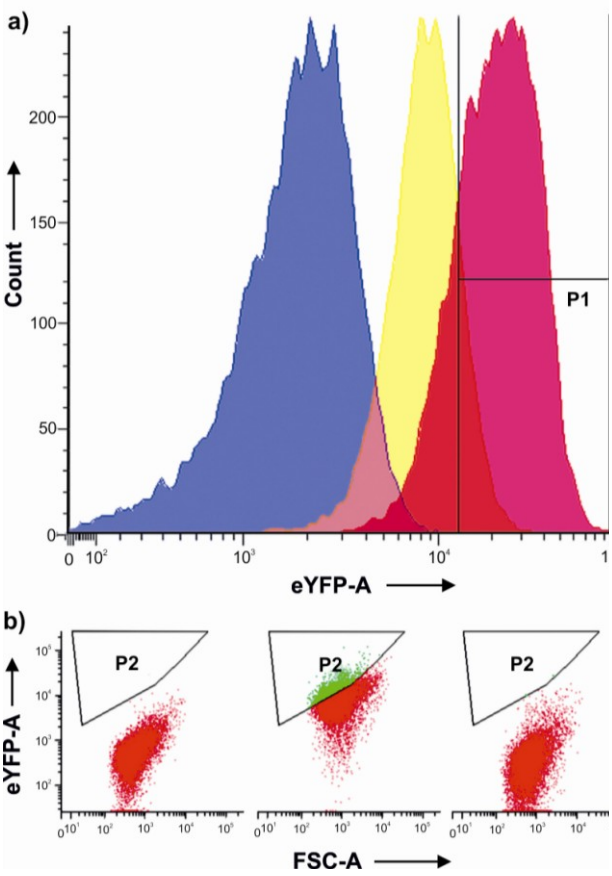


Figure 2. Flow cytometric analysis of *E. coli* cells with the *soxR-soxS*-based NADPH sensor and different NADPH-dependent *Lb*Adh activities. a) Combined fluorescence histograms of three *E. coli* strains carrying either pSenNeg (blue, 0.03 U mg^{-1} *Lb*Adh acitivity), pSen-L194S (yellow, 0.70 U mg^{-1} *Lb*Adh activity), or pSenSox (red, 6.28 U mg^{-1} *Lb*Adh activity) after biotransformation for 19 h with 70 mM MAA. Gate P1 was used for differentiation of pSenNeg and mutant or wild type populations. b) FACS-generated dot plots displaying the FSC signal (forward scatter) and the eYFP signal of *E. coli* cells carrying pSenSox after reductive biotransformation of 20 mM 4M2P (left side) or of 20 mM MAA (middle). On the right side, the library of mutant *Lb*Adhs was used for biotransformation of 20 mM 4M2P and then subjected to FACS. Gate P2 was used for mutant screening.

Table 2. Properties of wild-type *Lb*Adh and the variant *Lb*Adh-A93M for the substrate 4-methyl-2-pentanone

Enzyme[a]	v_{max}[b] µmol min^{-1} mg^{-1}	K_M[b] (mM)
*Lb*Adh	1.94 ± 0.02	0.11 ± 0.01
*Lb*Adh-A93M	2.62 ± 0.03	0.88 ± 0.07

[a] Enzyme activities were determined with crude extracts of the respective strains carrying either pSenSox or pSen-LA93M. [b] Mean values and standard deviation from three replicates are given.

Keywords: NADPH biosensor · alcohol dehydrogenases · enzyme evolution · single-cell analysis · FACS

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NADPH biosensor

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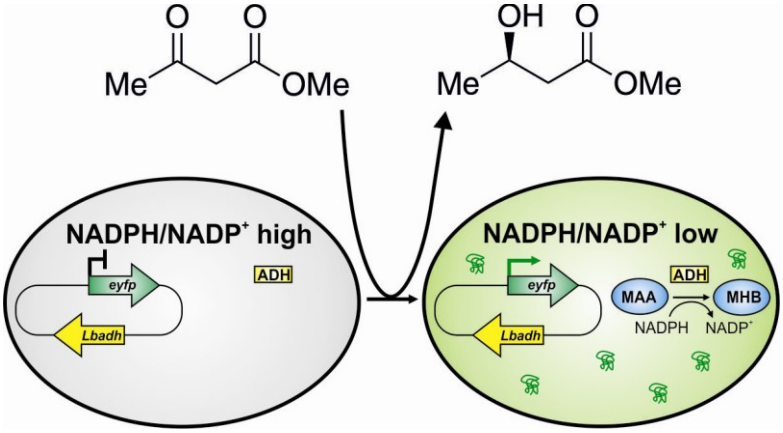
SoxR-SoxS as single-cell biosensor for NADPH-consuming enzymes in *Escherichia coli*



One sensor for many enzymes: NADPH is an important reducing agent in enzymatic and cellular synthesis of chiral building blocks and pharmaceuticals. We use the transcription factor SoxR to construct a single-cell biosensor which couples increased NADPH oxidation rate to the synthesis of an auto fluorescent protein in *E. coli*. This tool enables to monitor NADPH-dependent enzymatic conversions by whole cells, and the screening of large libraries of NADPH-dependent enzymes via FACS.

4. Discussion

4.1. Nanosensors for the detection of intracellular metabolite concentrations

The key requirement for visualization of a single cell with an elevated concentration of the small-molecule of interest is a suitable *in vivo* sensor with sufficient sensitivity and specificity. There are basically three different types of devices to monitor cytosolic concentrations as fluorescence output. These are FRET sensors, RNA devices and transcription factors. Each with their own advantages, disadvantages, and developed to varying degree.

FRET sensors are based on *Förster resonance energy transfer* between two different chromophores. When these chromophores are connected by a protein that alters its structure in response to the analyte concentration, this alters the output signal of the chromophore, which was not excited with light. A variety of such sensors based on bacterial periplasmic binding proteins have been developed [91]. FRET sensors are available to detect sugars, amino acids, nucleotides, ions, and other molecules. However, the sensitivity of the signal is low and there is only one single report, where such a sensor in *E. coli* has been used to monitor the tryptophane concentration [92]. However, this was only possible in cultures not in single cells, due to the low sensitivity. An advantage of FRET sensors is that they provide temporally resolved quantitative information.

The second type of sensors is based on RNA devices. These are currently developed and used mostly for *Saccharomyces cerevisiae*. RNA aptamers bind to a specific target molecule, the analyte, and they can be combined with ribozymes [93]. Constructions are possible where in absence of analyte the transcript of the target gene, usually *gfp*, is self-cleaved. Therefore, in response to the analyte no cleavage occurs. Such a construct has been applied to monitor theophylline in *S. cerevisiae* and to select via FACS for increased activity of a caffeine demethylase [48]. Prototypes of a number of RNA-based sensors have been developed [94].

The third option is to use transcription factors. In such a system proteins are used, which regulate a promoter's transcriptional output in response to a small-molecule ligand, to report on the concentration of the small-molecule *in vivo*. Transcription factors have long been used to construct whole-cell biosensors for the monitoring of environmental small-molecule pollutants by culture fluorescence [95]. However, this same approach has remained largely untranslated towards single cell analysis and library screening. An advantage of transcription factors is that many of them recognize small-molecules and a large number of proteins with different specificity naturally exist. Moreover, the specificity of transcription factors can be engineered [96]. In one example the transcription factor AraC, whose natural ligand is arabinose, was engineered to exhibit fluorescence in response to mevalonate [46]. However, this mevalonate sensor was not used in FACS screenings

for the isolation of mutants with increased mevalonate synthesis. Instead, it was used to visually inspect colony color on petri dishes for response to mevalonate-induced *lacZ* expression [47].

In the present work the metabolite sensor pSenLys (Fig. 3) was constructed and used. It is based on the transcriptional regulator LysG of *C. glutamicum,* which senses elevated concentrations of basic amino acids, causing transcription of its target gene *lysE.* In pSenLys *lysE* is replaced by *eyfp.* In addition, pSenLys encodes for Ptac-driven *crimson*, leading to red fluorescence after induction with IPTG. Crimson is a far-red protein, derived from DsRed–Express2 [97], and shows ideal fluorescent properties in flow cytometric experiments. It proved useful in the present work in mutant screens as inducible marker for living cells.

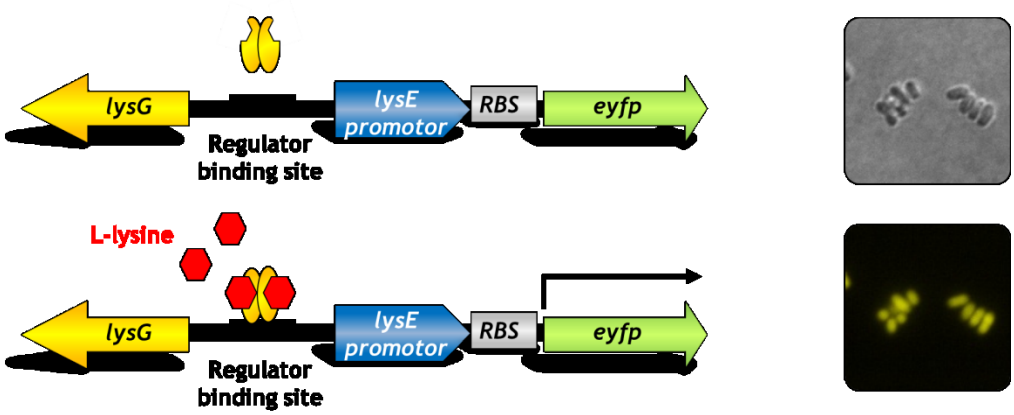


Figure 3: Metabolite sensor principle. The Transcriptional regulator LysG senses elevated concentrations of L-lysine to control transcription of its target gene *lysE*. Replacing the target gene sequence by *eyfp*, converts the cytosolic metabolite concentration into a graded optical output. This enables quantification of metabolite concentrations in a single cell.

Whereas in previous examples the responsiveness of sensor proteins, such as LacI, is deduced from the external addition of the effector molecule and whole culture response [98], the responsiveness of pSenLys is quantified in relation to the intracellular L-lysine concentration in *C. glutamicum*. There is a graded response in fluorescence in the range of 4 to 25 mM intracellular L-lysine. This enables FACS separation of cells exhibiting different productivity. In case the range should be extended, several options exist to do this. One option is to reduce the specificity of LysG towards L-lysine by mutation of the protein, or by altering the target sequence. This could be done using the newly developed recombineering routine for *C. glutamicum*. Another option is to increase the export rate. Indeed, this has already been achieved with strain DM1920. This strain is isogenic to DM1919, but contains two copies of the L-lysine exporter gene instead of one. DM1920 exhibits about half the fluorescence of DM1919 and it contains about half of the cytosolic L-lysine concentration compared to DM1919 (Fig. 1a in Binder *et al.* Genome Biology 2012, 13:R40).

This instructive example also clearly demonstrates that the transport step for the conversion of sugar to L-lysine by the catalyst *C. glutamicum* is a protein catalyzed reaction. This reaction can be limiting, as any other step within L-lysine synthesis can be. This limitation also represents a prerequisite for the sensor technology, since otherwise single cells with increased productivity would not necessarily have an increased intracellular concentration of the metabolite of interest. In general, the influence of export in microbial production processes is only rarely considered. One example is a recent work on biofuel production with *E. coli* [6]. 43 efflux pumps from different microorganisms were assayed for tolerance of *E. coli* towards the toxic biofuel. The intrinsic assumption was that they actively export the biofuel to reduce its cytosolic concentration. Whereas no carrier could be identified to improve tolerance towards *n*-butanol and isopentanol, carriers could be identified originating from *Alcanivorax borkumensis* or *Marinobacter aqueolei*, which enhanced tolerance to geranyl acetate. In another example the impact of the export carrier RhtC of *E. coli* was shown for L-threonine production with *C. glutamicum* [99]. Expression of *rhtC* in *C. glutamicum* resulted in an increased export rate from 2.3 nmol min^{-1} mg^{-1} to 11.2 nmol min^{-1} mg^{-1}, and an increase in extracellular L-threonine accumulation from 21 mM L-threonine to 54 mM.

The sensor pSenLys also reacts upon addition of the dipeptides LysAla, ArgAla and HisAla (Fig. 1e in Binder *et al.* Genome Biology 2012, 13:R40). This was expected, because LysG is able to recognize L-arginine and L-histidine in addition to L-lysine [100]. Dipeptides offer the possibility to trigger the cytosolic concentrations of amino acids in *C. glutamicum* and *E. coli* [100]. This has been used to assay for functionality of additional sensors constructed in this work. The higher fluorescence response to HisAla and even ArgAla of pSenLys, in contrast to LysAla could indicate a higher import and/or hydrolysis rate of the peptides. However, competition experiments with mixtures of ArgAla and LysAla and determinations of cytosolic concentrations have shown that LysG has comparable affinity towards L-lysine and L-arginine [100].

4.2. Screening and isolation of new L-lysine producing strains after mutagenesis

Use of fluorescence activated cell sorting (FACS) for screening purposes enables a throughput of more than 10^9 cells per experiment. Therefore FACS is the optimal choice for screening of huge libraries [33]. In the present work, the applicability of pSenLys was demonstrated by separating a mixture of cells of the WT, DM1728, and DM1919 (Fig. 1d in Binder *et al.* Genome Biology 2012, 13:R40). This cell mixture consisted of a non-producer, an intermediate producer, and a good producer and was grown on minimal medium. FACS differentiation was easily achieved. However, the situation is totally different when a mutant population has to be screened amongst others, since steps with complex medium are involved.

The mutagenesis itself is performed in complex medium BHI. The mutagen chosen was N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), which is one of the most effective chemical mutagens for creating genetic diversity [28]. It has already successfully been used in previous applications to isolate mutants of *C. glutamicum*. For instance, auxotrophic mutants of *C. glutamicum* were isolated [101], as well as mutants with aspartate kinase inhibition abolished and therefore producing L-lysine [30]. Due to mutagen treatment a number of cells die, and those surviving have to be recovered on complex medium. This makes a subsequent screening impossible, due to strong fluorescence of cells. Fluorescence in this case is most probably due to an elevated concentration of peptide-derived amino acids within the cell. In addition, a high amount of cell debris is present in the sample where cells recover.

Therefore, a two-step enrichment strategy was developed to enable screening after mutagenesis. After mutagenesis and recovery cells are grown on minimal medium and applied to a dual color screen. The first color, which is Crimson, serves to enrich for viable cells. EYFP, as second color, indicates the intracellular L-lysine concentration. The development of the respective time points used for screening of cells is demonstrated for a mixture of ATCC13032 and DM1728 in figure 4. The cell mixture was transferred from complex medium to minimal medium, and IPTG was added (Fig. 4, top). The Crimson signal appeared after 2 hours, and was stable for more than eight hours. Therefore, it could be expected that viable cells in a mutagenized population would exhibit a strong Crimson signal after two hours. Next the L-lysine specific EYFP-fluorescence was followed in the cell mixture (Fig. 4, bottom). Here it can be seen that initially all cells, which originated from a BHI-grown culture, exhibited strong fluorescence and only after 8 hours the population of the non-producer and the producer can be distinguished by their EYFP signal.

This result was the basis for the actual screening of the mutant population. In a first screening round a population exhibiting Crimson- together with EYFP-fluorescence - was enriched, representing metabolically active mutants with a functional L-lysine sensor. In the second screening single-cells showing increased cytosolic L-lysine concentrations were sorted with a high efficiency due to a suitable signal-to-noise ratio. Smolke *et al.* have chosen a related type of enrichment for the selection of *S. cerevisiae* mutants [48]. These authors called it dual-color screening and also used two different fluorescent markers on one plasmid to improve the signal-to-noise ratio in selections.

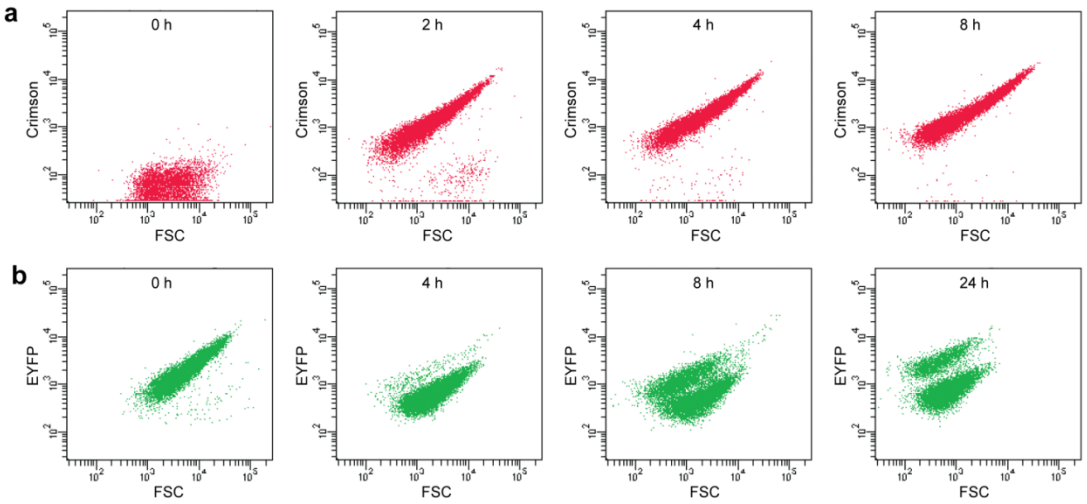


Figure 4: Development of Crimson and EYFP signals in mixtures of equal numbers of ATCC13032 and DM1728. To simulate the transfer of the mutant glycerol stock into minimal medium, the two cell types grown on BHI were mixed, glycerol was added and cells frozen. (**a**) Cells from the stock were diluted in minimal medium plus 0.1 mM IPTG. At the beginning of cultivation and 2, 4, and 8 h later, cells were assayed by flow cytometry for development of the Crimson signal. After 2 h, the majority of cells expressed Crimson, indicating active protein synthesis and thus living cells. (**b**) Cells were diluted as above and assayed at the beginning of culture and 4, 8, and 24 h later by flow cytometry for the EYFP signal. All cells derived from the complex medium exhibited high levels of fluorescence. After 4 h the majority showed reduced fluorescence and after 8 h the signals specific for the two populations were apparent.

4.3. Identification of new mutations by whole-genome sequencing

The current number of genes known in *C. glutamicum*, where mutations cause increased L-lysine synthesis, is about 12 [19, 24]. These mutations serve to increase flux through the L-lysine pathway itself, or to increase the pyruvate and oxaloacetate pool, or to increase the NADPH supply. Genome based strain reconstruction has been proven to be a useful strategy to identify mutations and to reconstruct L-lysine producing strains. Ikeda *et al*. [32] partially reconstructed the industrial producer *C. glutamicum* B-6 resulting in a strain accumulating a high concentration of L-lysine. However, the reconstructed strain could not compete with the productivity of the classically derived strain [32]. Another example is the use of 12 defined genome alterations. Among them, three mutant alleles derived from a classical strain, together with promoter mutations and gene duplications, resulted in a defined strain producing L-lysine with a yield of 0.55 g per gram of glucose (120 g l^{-1}) in fed-batch culture [19]. Both approaches have in common that a feedback resistant allele of *lysC* and a less active *hom* gene were the starting points for strain reconstruction.

The results of the present work support the importance of aspartate kinase in L-lysine overproduction, due to the high number of isolated strains carrying mutations in *lysC*. Among these are known and also new mutations (Fig. 4 in Binder *et al.* Genome Biology 2012, 13:R40). While 15 strains of the 40 mutants analysed carry mutations in *lysC*, seven contain a mutated *hom* gene. Moreover, also one *thrB* and two *thrC* mutations were identified (Fig. 4 in Binder *et al.* Genome

Biology 2012, 13:R40). To assay for the impact of these identified mutations, they were introduced individually into the WT background. Four new strains were constructed, carrying either a *lysC*, two *hom* or the *thrB* mutation. The resulting strains were able to produce small concentrations of L-lysine, but in no case the reconstructed strain was able to reach L-lysine concentrations comparable to the isolated strain (Tab. S3).

These data verify the impact of these genes on L-lysine synthesis. At the same time they illustrate that there are still unknown mutations improving L-lysine synthesis and which are of importance for a further increase of L-lysine formation. Even more important, 16 of the 40 mutants analyzed in the present work did not exhibit any mutation in *lysC* or *hom*. All together, these facts indicate that there are still treats of importance for L-lysine synthesis, but which are still unknown. Thus, genes could be affected in producer strains directly being related to the biosynthesis of the product. Also indirect effects are imaginable, such as those influencing osmotic effects, RNA stability, or regulation. From Japanese work it is known that in the classically derived strain *C. glutamicum* B-6 genes of different amino acid biosynthesis pathways exhibit increased expression due to still unknown mechanisms [102]. Moreover, the analysis of a classically derived L-arginine producer showed increased expression of arginine biosynthesis genes in a manner not achievable by plasmid-encoded expression [103].

For a further investigation of the mutants isolated in this work, the genome sequence for 10 strains without *lysC* or *hom* mutation was derived. A complete list of all single nucleotide polymorphisms (SNPs) identified is given in supplementary material 6.3. The strains chosen exhibited significant L-lysine accumulation between 2.6 and 15.9 mM and differences with respect to growth rate and specific fluorescence. In total 1329 SNPs were identified in the ten genomes. All SNPs were transitions causing a single nucleotide exchange. This is in accordance with the bias introduced by MNNG treatment [104]. Despite the difference in the phenotypic characteristics of the strains chosen, some of them exhibited a strong relation with respect to the number of mutations shared. For instance strain K012 has 267 mutations, of which 257 are identical to that in strain K093. Also strains K005, K063 and K112 share 170 mutations and differ only in two genes. This means, that due to the selection procedure, involving growth for more than 24 h on minimal medium and where duplication of the mutants occurred, almost identical strains were isolated. The minor difference in the number of SNPs could be due to MNNG molecules still present in some cells after the washing step during mutagenesis. For practical purposes, it means that in new approaches the entire mutagenesis/selection procedure should be reconsidered.

The number of SNPs in the individual mutants was between 36 and 268. Also from the entire number of 1329 SNPs, and a comparison of them, currently no reliable conclusion can be drawn. Unfortunately, this number is too large to study their individual impact on product formation. The mutations introduced may present a treasure, which is waiting to be uncovered. The reason is that in several mutants neither *lysC*, nor *hom* or *murE* are mutated. Of course, further genes of the biosynthesis pathways of threonine and peptidoglycan could be mutated, thus causing a limitation of metabolites branching off from the L-lysine biosynthesis pathway. Indeed, this is the case for mutants K051, K118 and K120. The relevance of these mutations has to be assayed. K120 shows a mutation in *gltA*, which may down-regulate TCA-cycle flux, as described by van Ooyen *et al.* [21]. Furthermore, it is conspicuous that mutants K013 and K093 accumulate L-lysine and L-arginine as well. The biosynthesis of these amino acid shares common mechanisms and is evolutionary related. Therefore, a regulatory connection, still to be discovered, might exist. In fact in *C. glutamicum* one transcript encodes *lysA* as a gene specific for L-lysine synthesis and *argS* specific for L-arginine synthesis [105]. Other examples of similar organization between genes for aminoacyl-tRNA synthases and amino acid biosynthetic enzymes have been described. In this context it is noticeable that the strains K051 and K120 show SNPs in *leuS*, encoding for Leu-tRNA synthetase.

Striking is strain K051, showing the highest productivity and also carrying the largest number of mutations. Among them are mutations in genes known already to be involved in increased L-lysine formation. The genes *pck* and *gltA*, encoding phophoenolpyruvate carboxykinase and citrate synthase, are known to increase the supply of pyruvate and oxaloacetate for L-lysine synthesis when mutations reduce their activities [21, 106]. Specific mutations in *zwf* and *gnd* of the pentose phosphate pathway are also known to increase L-lysine formation due to an increased supply of NADPH [32]. K051 carries mutations in all of these genes. Also, mutations in branched-chain amino acid metabolism have been demonstrated to increase lysine formation [107], and K051 carries a mutation in *ilvE*, as well as in the Leu-tRNA synthetase *leuS*.

Of particular interest was the *murE* mutation (*murE*-G81E) in K051, coding for UDP-N-acetylmuramyl-tripeptide synthetase. When *murE*-G81E is introduced into the genome of a *C. glutamicum* producer strain, but also into the wild type DM1132, L-lysine formation is increased. It can be hypothesized that catalytic activity of this enzyme is reduced with the consequence, that more D,L-diaminopimelate is available for L-lysine synthesis. The mutation G81E is located close to the nucleoside part of the enzyme recognizing UDP-MurNAc-L-Ala-D-Glu, as deduced from the crystal structure of MurE from *Mycobacterium tuberculosis* and *E. coli* [108]. Therefore, *murE*-G81E and also *murE*-S121F, which is located in the ATP-binding site, are the first mutations leading to increased L-

lysine production uncoupled from a *lysC* or *hom* mutation. With the *murE*-G81E and also the *murE*-S121F mutation increased L-lysine formation was obtained in different defined producer strains, developed to various degrees (Fig. 6 in Binder *et al.* Genome Biology 2012, 13:R40). This makes *murE* an interesting new target to be assayed in industrial producer strains for increased productivity of L-lysine formation. In addition to the idea that, due to the *murE* mutation more diaminopimelate is available for L-lysine synthesis, the limitation of diaminopimelate for cell wall synthesis could also have regulatory consequences leading to a further flux of aspartate and pyruvate into the diaminopimelate/ L-lysine biosynthesis pathway. This deserves further investigations. It is known that a disbalance of diaminopimelate/ L-lysine synthesis as achieved by *ddh* deletion and ammonium limitation causes a strong effect on growth and cell wall integrity of *C. glutamicum* [109].

4.4. Recombineering in *Corynebacterium glutamicum*

The ssDNA recombineering system developed for *C. glutamicum* provides a simple and fast method to efficiently introduce defined mutations into the chromosome. Recombineering has been described so far for a limited number of organisms (Tab. 1). This is the first report of successful genome editing in the industrial relevant strain *C. glutamicum* by ssDNA recombineering.

Table 1: Recombineering in different hosts.

Bacterium	Reference	Bacterium	Reference
Bacillus subtilis	[71]	*Pseudomonas aeruginosa*	[110]
Enterohemorrhagic E. coli	[111]	*Pseudomonas syringae*	[112]
Enteropathogenic E. coli	[111, 113]	*Salmonella enterica*	[114-116]
Klebsiella aerogenes	[117]	*Salmonella typhimurium*	[118-120]
Lactobacillus reuteri	[72]	*Serratia marcescens*	[121]
Lactococcus lactis	[72]	*Shigella flexneri*	[122, 123]
Mycobacterium abscessus	[124, 125]	*Uropathogenic E. coli*	[126, 127]
Mycobacterium smegmatis	[69, 128]	*Yersinia pestis*	[129, 130]
Mycobacterium tuberculosis	[69, 128]		
Pantoea ananatis	[70]	*Corynebacterium glutamicum*	this work

Use of RecT from the *E. coli* prophage Rac enables recombineering efficiencies of up to $2x10^6$ recombinant cells out of approximately 10^9 surviving cells, when 10 µg of an 75-mer oligo is used. In *Corynebacterium* the single strand annealing protein (SSAP) has to be expressed for 4 hours before competent cells are prepared to achieve optimal results. In addition to RecT, also with the SSAP of *C. aurimucosum* high activities were obtained, indicating that the protein could be active – or can become activated – in *C. aurimucosum* under special conditions. Use of Gp61 and Gp43 only led to a

low number of Kan^R recombinants, Bet from the λ Red system was apparently inactive. Bet works in a number of Gammaproteobacteria including *E. coli, Salmonella, Yersinia, Pantoea,* and others [131]. Apparently, the replication machinery of these organisms is sufficiently related to allow expression and interaction of proteins involved, while that of *Corynebacteria* is much more distant. Interestingly, Che9c gp61 and Halo gp43 derived from *Mycobacterium* species function substantially worse in *C. glutamicum* than RecT, although *Mycobacteria* and *Corynebacteria* are more closely related, than *Corynebacteria* and *E. coli*. Both belong to the suborder *Corynebacterineae* and this group of bacteria share many similarities, such as synthesis and structure of their cell wall. This makes *Corynebacterium* a model organism of the human pathogen *M. tuberculosis* [132]. Nevertheless, the genome of *C. glutamicum* has a much lower GC-content of 53.8 % [24] compared to *M. tuberculosis* with 65.6 % [133]. Furthermore, the genes gp61 and gp43 only show codon adaption index values (CAI) of about 0.289 and 0.247 for *C. glutamicum* ATCC13032, and GC contents of 65.6 and 67.6 %. In both cases the CAI for *M. tuberculosis* is above 0.63. Therefore, it is possible that *gp61* and *gp43* are not or only weakly expressed in *C. glutamicum*. It is an attractive option to test codon optimized variants of these genes in *C. glutamicum*, which offers the possibility to further increase recombination frequencies in *C. glutamicum*. These considerations and the results obtained emphasize the need to examine more than one possible candidate SSAP for recombineering, as their activities in a specific host vary considerably.

Electroporation of the test strain with 5 µg of the Kan50*-oligo, designed to create a 2-bp change in the defective Kan^R gene, generated $3x10^5$ colonies per 10^9 survivors. If only 5-fold lower amounts were used, the rate of oligo-mediated mutagenesis fell 20-fold. In contrast, using a 2-fold higher concentration of the oligo led to a 3-fold increase in recombinant cells. More than 10 µg of oligo did not result in more recombinant cells. The reason could be that an excess of DNA saturates a ssDNA exonuclease present in the host. This is plausible since such enzymes are present in any organism required for repair processes [134] and in *C. glutamicum* three candidate enzymes are annotated as exonuclease. Furthermore, use of a 75- and 100-mer increased the number of colonies again to up to $2x10^6$ colonies per 10^9 survivors, which is in comparison to the use of a 50-mer a 4-fold increase. This largely agrees with the situation in *E. coli*, where at an oligo length of 60nt the highest level of recombinants is generated [135]. A surprising result for *C. glutamicum* is the small difference in recombination efficiency obtained between use of an oligo annealing to the leading strand or one to the lagging strand (indicated by *). This bias is much stronger in *E. coli,* where the recombineering frequency with an oligo annealing to the lagging strand is 5- to 10-fold increased [61]. In *M. tuberculosis* this effect is even more dramatic with a 1000-fold increased frequency [128]. These

large differences in the bias observed for *Corynebacteria, Mycobacteria,* and *E. coli* likely reflect differences in the repertoire of DNA interacting enzymes between these organisms, as indicated by genome comparisons of different *Corynebacterium* species with that of *E. coli* [136].

4.5. Recombineering and nanosensors: Rapid development of small-molecule producing strains

Genomic diversity is difficult to generate in the laboratory and directed evolution assays are generally performed *in vitro*. These methods are limited to laborious and serial manipulations of single genes and in most cases, when the metabolite of interest is inconspicuous, not suitable for high-throughput screening systems. Recombineering has enabled many new applications and the list is continuously expanded [134]. It allows performing manipulations of bacterial chromosomes and artificial chromosomes (BACs) beyond the capabilities of procedures that involve the use of individual plasmid constructions. Recombineering also played an important role in the construction of multiple gene fusions in the chromosome of *E. coli* [137]. Other examples where recombineering has expanded the capabilities of genomic studies in *E. coli* include the development of mutagenic procedures for *E. coli* genes [138], the ability to generate strains with reduced genomes [139, 140], *multiplex automated genome engineering* (MAGE) for accelerated evolution of *E. coli* [141], and genome wide codon replacement [142]. Whereas for several other organisms recombineering is demonstrated (Table 1), this technology is seldom applied and by far not developed to a degree as achieved for *E. coli*.

Recombineering, as genetic tool for genetic diversity generation, in combination with metabolite sensors offers novel applications for metabolic engineering. Since an inconspicuous molecule is turned into an optical output, producers can be directly isolated. Thus with an appropriate nanosensor in hand, producer isolation is in principle as simple as isolating Kan^R clones, and an additional advantage is the suitability of the system for high-throughput applications. One application demonstrated in this work is the rapid development of a strain producing L-lysine by removing feedback inhibition of the aspartate kinase. For L-lysine it is known that one single mutation in *lysC* [32], *hom* [17, 18] or *murE* [this work] leads to L-lysine overproduction. L-lysine is an inconspicuous small-molecule, which when overproduced does not exhibit a visible phenotype. A number of recombinant clones carrying the *lysC*-T311I mutation, leading to L-lysine overproduction, were isolated by FACS after electroporation of the WT, carrying pSenLys and pRecT, with the lysC-60-EcoRV* oligo. After a first enrichment step of 10,000 single cells exhibiting increased fluorescence and further cultivation, sorting gates were set that 2.468 % (Fig. 5b, left) of the enriched population, but only 0.057 % (Fig. 5b, right) of the negative control matched the sorting criteria. The fact that in this procedure about half of the cells, obtained after the second screening, carried the *lysC*-T311I

mutation, although the others exhibited also the increased fluorescence, could have several reasons. Maybe during the enrichment process cells with increased fluorescence, due to mutations in *lysG* for instance, were also enriched in this particular assay. Indeed, in the first part of the present work, where pSenLys was used for producer isolation obtained by undirected mutagenesis, it was observed that about half of the cells exhibited an unexpected high fluorescence, higher than expected from defined producers (Fig. 3b in Binder *et al.* Genome Biology 2012, 13:R40). In four cases mutations occurred in *lysG* encoded by the sensor plasmid.

The combination of recombineering with FACS offers a number of advantages compared to conventional strain construction. First, no integrative plasmid has to be constructed. Second, no laborious steps for integration of the gene into the genome of *C. glutamicum* involving two rounds of selection are necessary. This method is time consuming and inefficient due to the fact that up to 10^2 clones have to be screened manually for homologous-recombination events by selection and counter-selection. Third, due to the use of the metabolite sensor positive recombination events are linked to fluorescence, as far as the integrated SNP leads to an increased productivity. Therefore recombinant cells can be isolated using high-throughput screening methods like FACS.

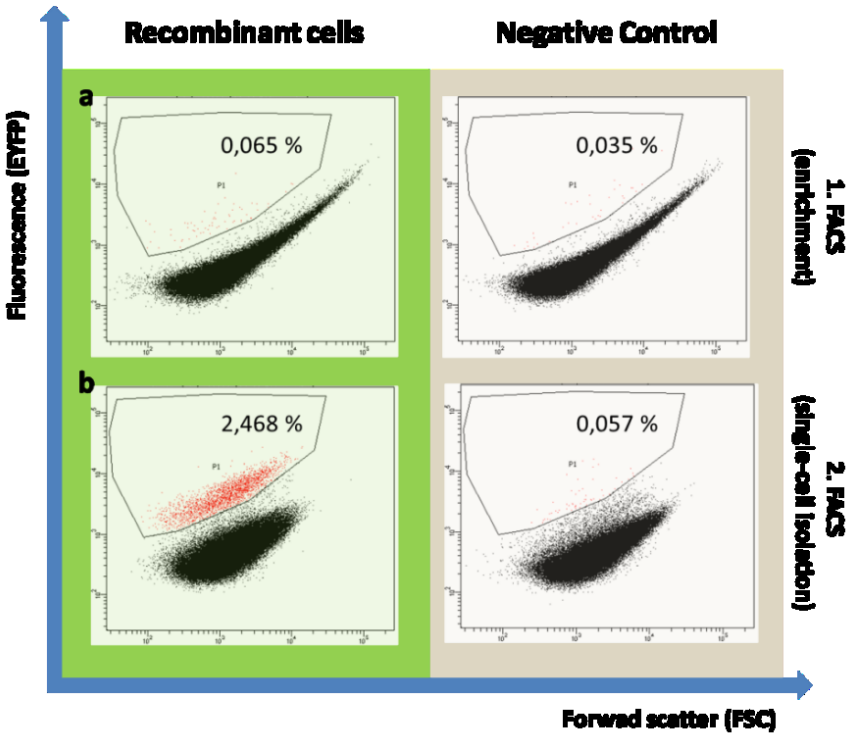


Figure 5: Dotblots obtained for cultures after recombineering with lysC-60-EcoRV* by flow cytometry. (a) The dotblots show regenerated cells after electroporation and 1st cultivation (left) and the corresponding negative control (right). 10,000 cells matching the fluorescence criteria set by the indicated gate were enriched and subjected to a 2nd cultivation. (b) Cells of this cultivation, and matching the criteria of the gate, were directly sorted on agar plates for further characterization. Each dotblot represents the analysis of 100,000 single cells.

To take this new technology to the next level, with respect to the possibilities offered by the nanosensor and high frequency recombineering, we wanted to generate genetic diversity at a given genomic locus across a population. In strain K051, which has been isolated after chemical mutagenesis from a WT population of *C. glutamicum*, *murE*-G81E was identified as an SNP leading to strong overproduction of L-lysine when introduced into the genome of the WT and also in different L-lysine producing strains. For the treatment of *C. glutamicum* with MNNG it is reported that it solely induces G•C → A•T transitions [104], and therefore the amino acid glycine at position 81 could only be modified to alanine, arginine, aspartate or glutamate. We were interested to answer the question, whether the developed recombineering and screening procedure can be applied to introduce other amino acid substitutions in this position, also leading to an increased L-lysine production.

The *in vivo* site-directed saturation mutagenesis method applied, using the 20 different oligos in one single electroporation assay, yielded 44 recombinants with a broad range of substitutions obtained, and G81 replaced by 12 different amino acids. As expected, the specific fluorescent property of each strain correlated with its productivity (6.2. Table S3). Only a few recombinants carrying the same mutation accumulated different L-lysine concentrations. It is possible that the genotypes of these strains differ at other positions in the genome resulting in secondary effects decreasing productivity. One explanation for this is that the strains used for recombineering still express *recT* although it is under control of the inducible Ptac promotor. A disadvantage of Ptac is the leaky expression of the target gene in the absence of inducer [143]. This may induce additional unwanted recombineering events in the cell and deserves further studies. It is also possible that secondary mutations occur due to selective pressure and not introduced by RecT. Moreover, the high mortality rate between 90 and 95 % during electroporation indicates that during electroporation also stress, and thus an increased susceptibility to mutagenesis might be present. In general, it would be an advantage to remove the plasmid encoding the recombinase RecT after recombineering from the cell, which may be achieved with plasmids carrying a temperature sensitive replicon. Okibe *et al.* describes a *Corynebacterium glutamicum* - *E. coli* shuttle vector with the two amino acid substitutions G109D and E180K within the replicase RepA resulting in such a plasmid [144].

The developed *in vivo* site-directed mutagenesis and screening routine avoids a number of time consuming steps in strain construction and offers new possibilities not yet attained so far, and increases the throughput to a maximum. It enabled rapid *in vivo* diversity generation of *murE* avoiding inefficient construction of 20 different pK19mobsacB integration vectors and separate integration of these alleles into the genome of *C. glutamium*. A major improvement is that *in vivo* diversity generation is no longer limited to a certain bias of a chemical mutagen, because unbiased oligos can be used to introduce a defined set or random mutations. Another application could be the

screening for mutations responsible for L-lysine formation in the strains with their entire genome sequenced. Strain K117 could be an interesting first candidate, because it produced about 7 mM of L-lysine and only carries 36 mutations which could have an impact on L-lysine overproduction. In case that only one mutation is responsible for the L-lysine production in the WT of *C. glutamicum*, this method could be applied for systematic screening. The system described here may add a basic key tool necessary for simple construction of improved strains for the industrial production of small-molecules.

4.6. A new class of nanosensors: detection of cytosolic NADPH/NADP$^+$ levels

For the production of chiral alcohols and amino acids it has been shown, that biotransformation has become an important method in chemo-enzymatic synthesis approaches [145]. These processes are dependent on the specificity and activity of the enzymes used, and thus for the establishment of new processes desired enzyme activities have to be screened. For the screening of NADPH dependent enzymes, there is the possibility to monitor substrates or products, but this requires physical assays, such as gas chromatography or HPLC. Also assays where cofactor consumption during substrate oxidation is monitored were developed [146, 147]. Unfortunately, all of these assays normally are done *in vitro*. Therefore, they cannot be regarded as high-throughput assays to enable rapid enzyme evolution or screening.

In this work a sensor was developed monitoring the *in vivo* NADPH/NADP$^+$ ratio in *E. coli*. The stability of the fluorescence signal is dependent on the ratio of NADPH/NADP$^+$. The possibility of mutant library screening via FACS makes this set-up an ideal and universal high-throughput screening method for the isolation of NADPH-dependent enzymes, showing altered activity or specificity, which occurs in this case with single cells. The sensor is based on the SoxRS regulatory system which is known to respond to the redox state of the cell. The current view is that two different SoxR reductases [148, 149], both using NADPH as electron donor, keep SoxR in the reduced inactive state. When NADPH is limiting, SoxR is less reduced and as a consequence oxidized SoxR activates expression of *soxS*, encoding a transcriptional regulator of the AraC family. This in turn increases the transcription of a large number of genes [150-153]. The genes of the SoxRS regulon include a number of genes responding to oxidative stress and to NADPH limitation. For instance, expression of *zwf*, encoding glucose-6-phosphate dehydrogenase, is increased. This enzyme catalyzes the first step of the pentose phosphate pathway and therefore reverses NADPH limitation [150]. In this work a sensor was constructed where *soxS* was replaced by *eyfp*. The fluorescence output correlates with the NADPH/NADP$^+$ ratio as demonstrated for cells with an NADPH-dependent alcohol dehydrogenase (ADH) of *Lactococcus brevis* with varying activity, and for the enzyme with different substrate

concentrations available for reduction. The intracellular NADPH/$NADP^+$ ratios in *E. coli* were determined in prior work in almost identical assays [154]. After analyzing the system in detail the sensor was applied for high-throughput screening of mutagenized ADHs. ADH is able to convert 4-methyl-2-pentanone (MP) as a substrate, but with only 10 % activity as compared to methyl-acetoacetate (MAA. Applying the sensor in the developed FACS routine for the screening of a mutated ADH library resulted in 123 mutants showing increased fluorescence in contrast to the strain expressing the WT enzyme. Six mutants were analyzed in detail and in one case a mutant showing 36 % higher maximal velocity was identified, demonstrating the suitability of the sensor for the screening of enzyme variants with increased activity by increased NADPH consumption.

As long as educts and products are diffusible through the cytoplasmic membrane, libraries of dehydrogenases requiring NADPH, or P450-monooxygenases where NADPH is part of the catalytic cycle can now be assayed in our established HT screening routine avoiding the development of specific assays. Together with recombineering the developed redox sensor offers a completely new and generalizable strategy for rapid enzyme evolution of NADPH dependent dehydrogenases like P450-monooxygenases.

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6. Appendix

6.1. Supplementary Material "A high-throughput approach to identify genomic variants of bacterial metabolite producers at the single-cell level"

Tables and Figures

The file contains detailed data on strains and sorting performance as follows:

Table S1. Strains and plasmids

Strain or plasmid	Description	Reference
WT *C. glutamicum*	WT strain ATCC 13032, biotin-auxotroph	This laboratory
C. glutamicum DM1132	WT strain ATCC 13032, biotin-auxotroph	Evonik laboratory
C. glutamicum DM1728	*pyc*(P458S), *hom*(V59A)	[155]
C. glutamicum DM1730	*pyc*(P458S), *hom*(V59A), *lysC*(T311I), Δ*pck*	Evonik laboratory
C. glutamicum DM1800	*pyc*(P458S), *lysC*(T311I)	[155]
C. glutamicum DM1919	*pyc*(P458S), *hom*(V59A), 2 copies of *lysC*(T311I), Δ*pck*	Evonik laboratory
C. glutamicum DM1920	*pyc*(P458S), *hom*(V59A), 2 copies of *lysC*(T311I), Δ*pck*, 2 copies of *lysE* derived from WT *C. glutamicum*	Evonik laboratory
C. glutamicum DM1933	Δ*pck, pyc*(P458S), *hom*(V59A), 2 copies of *lysC*(T311I), 2 copies of *asd*, 2 copies of *dapA*, 2 copies of *dapB*, 2 copies of *ddh*, 2 copies of *lysA*, 2 copies of *lysE* derived from WT *C. glutamicum*	[156]
C. glutamicum-Ser4	ATCC13032 Δ*sda*AΔ*pab*ABC pserAfbrCB	[156]
C. glutamicum-Cys3	ATCC13032 Δ*sda*AΔ*pab*ABCΔ*aec*D pserAfbrCB	This laboratory
E. coli DH5α	F- endA1 glnV44 thi-1 recA1 relA1 gyrA96 deoR nupG Φ80dlacZΔM15 Δ(lacZYA-argF)U169, hsdR17(rK- mK+), λ–	[157]
pK19mobsacB	Km^r, Suc^r, mobilizable (*oriT*), *oriV*	[50]
pSenLys	Encodes *C. glutamicum* LysG, and its target promoter of *lysE* with a transcriptional fusion to *eyfp*	HE583184
pSenArg	Encodes *E. coli* ArgP, and its target promoter of *argO* with a transcriptional fusion to *eyfp*	HE583185
pSenSer	Encodes *C. glutamicum* NCgl0581, and its target promoter of NCgl0580 with a transcriptional fusion to *eyfp*	HE583186
pSenOAS	Encodes *C. glutamicum* CysR, and its target promoter of *cysI* with a transcriptional fusion to *eyfp*	HE583187

The regulatory units of the pSen series of vectors were synthesized (LifeTechnologies GmbH, Frankfurter-Str.129b, 64293 Darmstadt, Germany) and cloned into pJC1. Full sequences have been deposited at EMBL.

Table S2. Quality assessment of sorting cells carrying pSenLys

Sorting criteria	Viability			Verified strain			Sorting specificity
	Sorted in total	Grown		WT	DM1728	DM1919	
P1 (ATCC13032)	432	386	89.4 (%)	99.4	0.6	0	99.4 (%)
P2 (DM1728)	288	257	89.2 (%)	3.1	94.8	2.1	94.8 (%)
P3 (DM1919)	288	244	84.7 (%)	4.2	6.2	89.6	89.6 (%)
Average			87.8 (%)				94.6 (%)

Sorting criteria	Viability			Verified strain		Sorting specificity
	Sorted in total	Grown (%)		WT	DM1728	
P2 (DM1728)	200	184	92.0 (%)	7.3	92.7	92.7 (%)

The upper part of the Table shows the result of sorting the mixture of three strains using gates P1-P3. Viability of sorted cells was determined by counting cfus grown up after spotting single cells on BHI petri dishes and incubating for 48 hrs at 30°C. Strains were verified by cultivating 96 clones in a microtiter plate containing minimal medium CGXII with 4% (w/v) glucose and quantification of their L-lysine forming capability after 48 hrs.

The lower part of the Table shows the result of sorting DM1728 out of 10,000 wild type cells, both carrying pSenLys. Determination of viability and L-lysine formation was as above.

Table S3. L-lysine formation with mutations introduced by reverse engineering

Mutation	Recombinant strain	Lysine (mM)	Isolated mutant	Lysine (mM)
thrB S102F	Lys016	0.4	K016	2.1
hom V211F	Lys039	6.3	K039	10.9
hom A364V	Lys049	1.1	K049	9.6
lysC H357Y	Lys096	0.9	K096	2.3

In the recombinant strains the single mutation given in the left column was introduced in the wild type genome ATCC13032 by allelic exchange as described [50]. L-lysine accumulations were determined in SK cultivations and the L-lysine formed determined after 48 hrs. For comparison L-lysine accumulations are shown for the original clones from which the specific mutations were derived by targeted sequencing.

Table S4. Statistical analysis of whole-genome sequencing

	Strain K051	
Description	**value**	
Number of sequenced reads	20,156,524	
Avg. length of sequenced read [bp]	51.0	
Number of reads after trimming	19,908,254	
Avg. length of reads after trimming [bp]	48.1	
Number of paired reads after trimming used for mapping	19,664,448	
Number of reads mapped to reference	17,877,215	
Coverage (# mapped reads * avg. length/3301500)	260.5	
SNPs in total	268	100 %
Transitions	268	100 %
SNPs leading to amino acid exchange	171	63.8 %
Silent mutations	65	24.3 %
Intergenic SNPs	28	10.4 %
Introduced stop-codons	4	1.5 %

Sequence reads were generated on an Illumina HiSeq 2000 and performed at GATC (GATC Biotech AG, Jakob-Stadler-Platz 7, 78467 Konstanz, Germany). Trimming and mapping was done using the CLC Genomics Workbench Version 4.7.2 software of CLC bio (Finlandsgade 10-12, Katrinebjerg , 8200 Aarhus N, Denmark).

Table S5. Growth rates of *murE* mutants

Strain	μ h^{-1}
DM1132 (WT)	0.49 ± 0.11
DM1728	0.46 ± 0.16
DM1730	0.43 ± 0.11
DM1800	0.43 ± 0.16
DM1933	0.37 ± 0.13
DM1132 (L121F)	0.45 ± 0.09
DM1728 (L121F)	0.43 ± 0.20
DM1730 (L121F)	0.40 ± 0.11
DM1800 (L121F)	0.43 ± 0.19
DM1933 (L121F)	0.35 ± 0.14
DM1132 (G81E)	0.39 ± 0.12
DM1728 (G81E)	0.36 ± 0.16
DM1730 (G81E)	0.41 ± 0.10
DM1800 (G81E)	0.45 ± 0.18
DM1933 (G81E)	0.31 ± 0.21

Strains were pregrown for 8 hrs on complex medium BHI, followed by growth overnight in minimal medium CGXII-glucose, and this used to inoculate cultures in a new CGXII-glucose to determine growth rates. Cultivations were done in microtiter plates and growth was recorded from three parallel cultures.

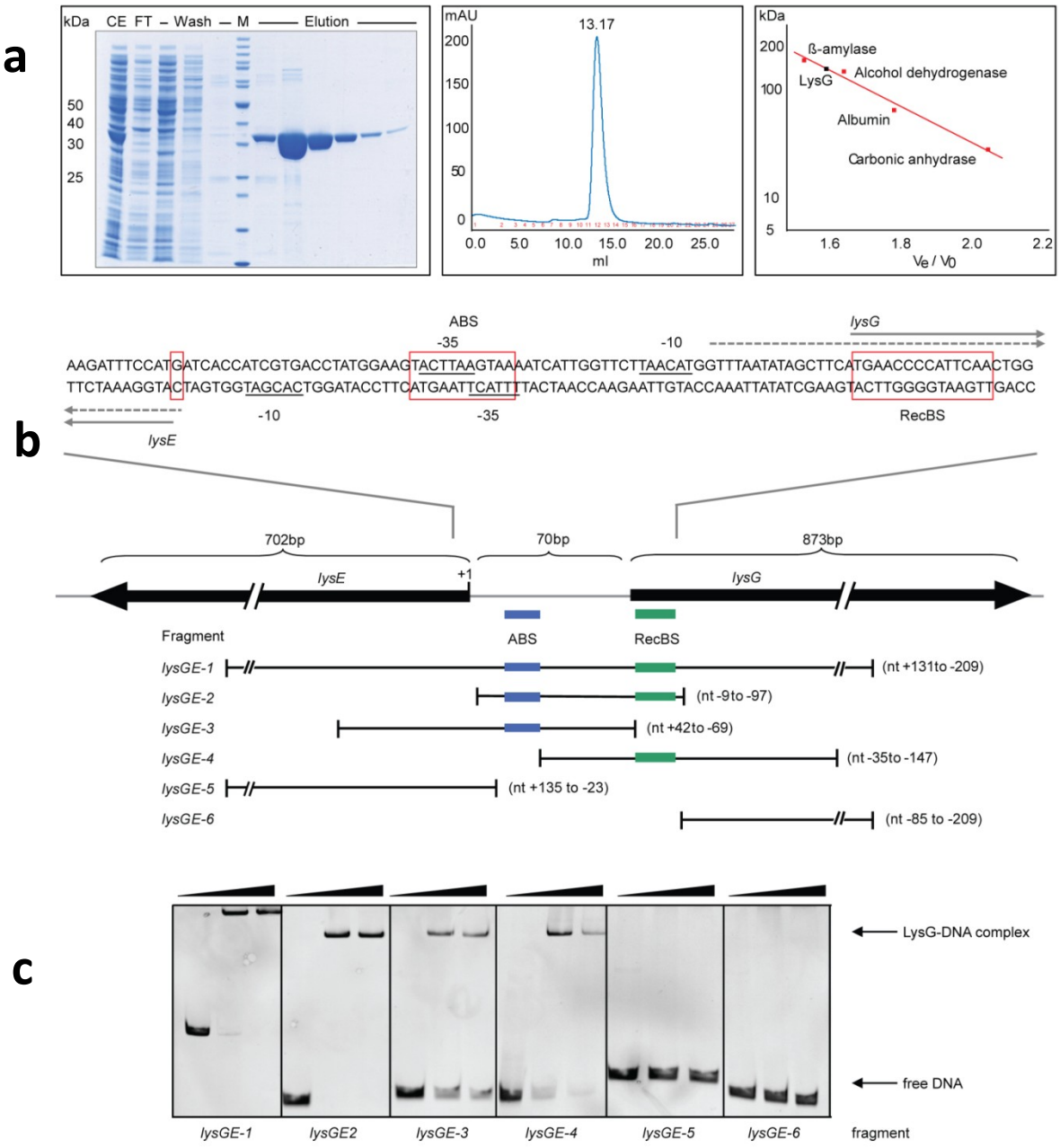


Figure S1.

Isolation of LysG and characterization of LysG binding site. (a) Isolation of His-LysG and gel filtration analysis of LysG devoid of tag together with the calibration curve used for molecular mass determination. LysG eluted with an apparent molecular mass of ~140 kDa. Since the LysG monomer has a molecular mass of 32 kDa, LysG in solution forms a homotetramer similar to other LTTR-type regulators such as CbnR of Ralstonia eutropha [158] or CysB of Escherichia coli [159]. (b) Overview on lysGE organization with the intergenic region on top. The relative position of DNA fragments lysGE-1 to lysGE-6 are given and the location of the activation binding site (ABS) in blue and the recognition binding site (RecBS) in green. (c) Electrophoretic mobility shift assays showing binding of fragments 1-4 to LysG. In each panel (from left to right) no LysG was added, added in a 20-fold excess, and 50-fold excess.

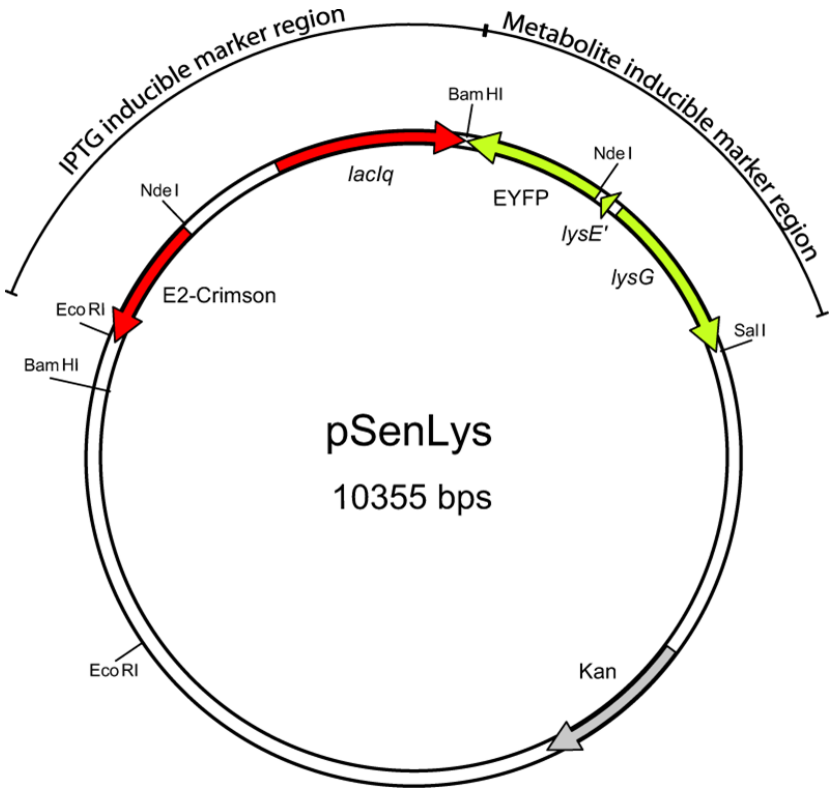


Figure S2.

Vector pSenLys and general configuration of sensor plasmid. The vector pSenLys is a shuttle vector replicating in both *C. glutamicum* and *E. coli*. It carries the metabolite inducible marker region encoding the L-lysine sensing transcriptional regulator LysG. In presence of L-lysine LysG drives transcription of *lysE*, which is fused with *eyfp,* resulting in green fluorescence. pSenLys also carries the IPTG inducible marker region encoding the LacIq repressor which, in the presence of IPTG, diffuses from the tac promoter region in front of E2-Crimson, resulting in red fluorescence. The system permits the selection of viable cells that are capable of protein synthesis.

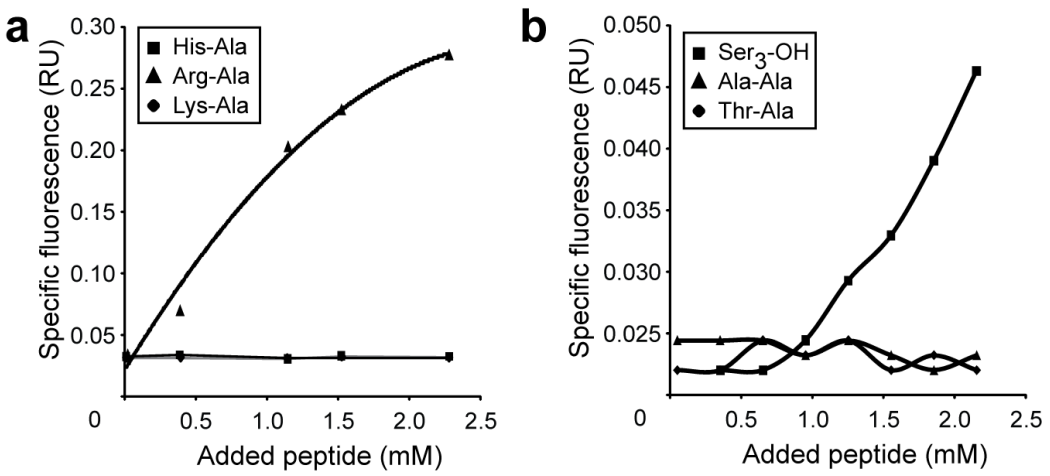


Figure S3.

Peptide-dose response with *E. coli* pSenArg and *C. glutamicum* pSenSer. (**a**) *E. coli* pSenArg was cultivated in minimal medium and the specific peptide added at the given concentration. Addition of Arg-Ala results in fluorescent cultures, but this is not the case with His-Ala or Lys-Ala. (**b**) *C. glutamicum* pSenSer was cultivated in minimal medium and peptides added as shown. Ser-Ser-Ser results in fluorescent cultures, but this is not the case with Ala-Ala or Thr-Ala. In all cases Ala-Ala was included to give a total peptide concentration of 3 mM.

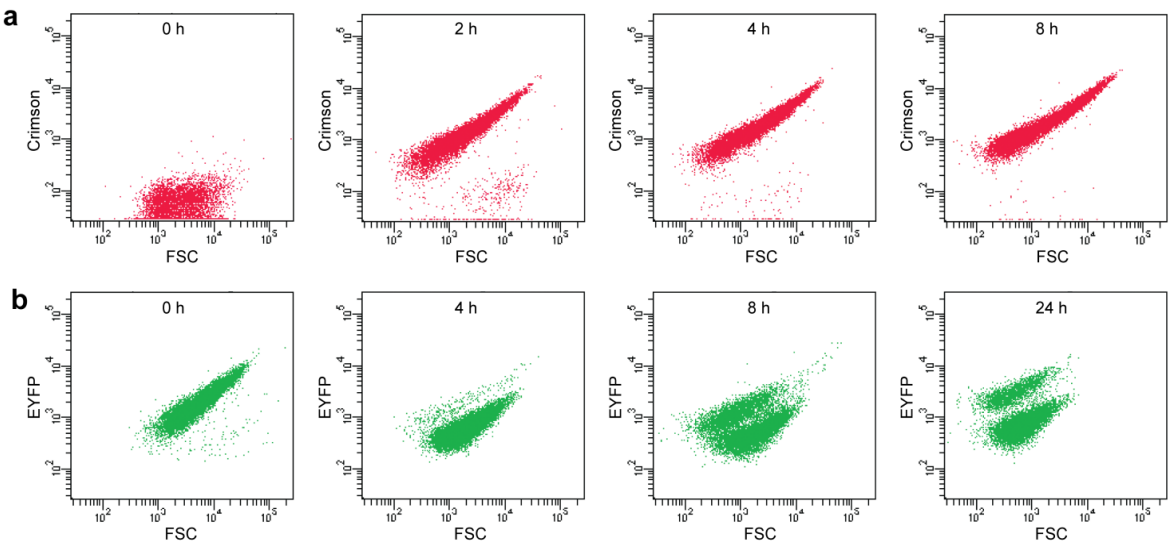
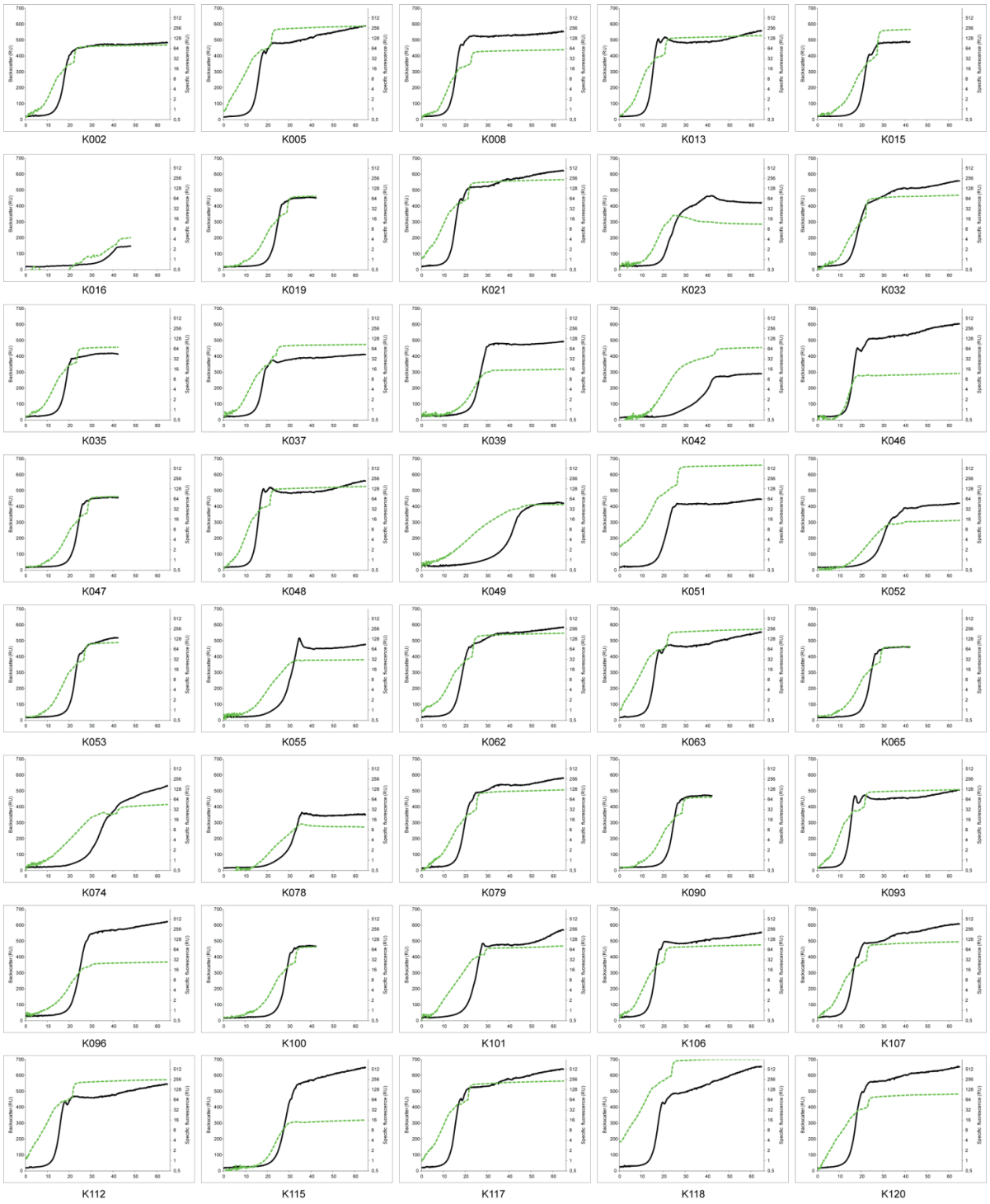


Figure S4.

Development of Crimson and EYFP signals in mixtures of equal numbers of ATCC13032 and DM1728. To simulate the transfer of the mutant glycerol stock into minimal medium, the two cell types grown on BHI were mixed, glycerol was added and cells frozen. (**a**) Cells from the stock were diluted in minimal medium plus 0.1 mM IPTG. At the beginning of cultivation and 2, 4, and 8 hrs later, cells were assayed by flow cytometry for development of the Crimson signal. After 2 hrs, the majority of cells expressed Crimson, indicating active protein synthesis and thus living cells. (**b**) Cells were diluted as above and assayed at the beginning of culture and 4, 8, and 24 hrs later by flow cytometry for the EYFP signal. All cells derived from the complex medium exhibited high levels of fluorescence. After 4 hrs the majority showed reduced fluorescence, and after 8 hrs the signals specific for the two populations are apparent.



6.1.10 Figure S5.

Growth curves and fluorescence for 40 mutant cultures. The BioLektor cultivation system was used (see Methods section) to follow the growth of 40 mutants in 0.75 ml cultures as measured by the backward scatter (black curve) and fluorescence at λ_{ex} 485 nm and λ_{em} 520 nm (green curve). Numbering of the different mutants is as given in Figure 4 of the main text.

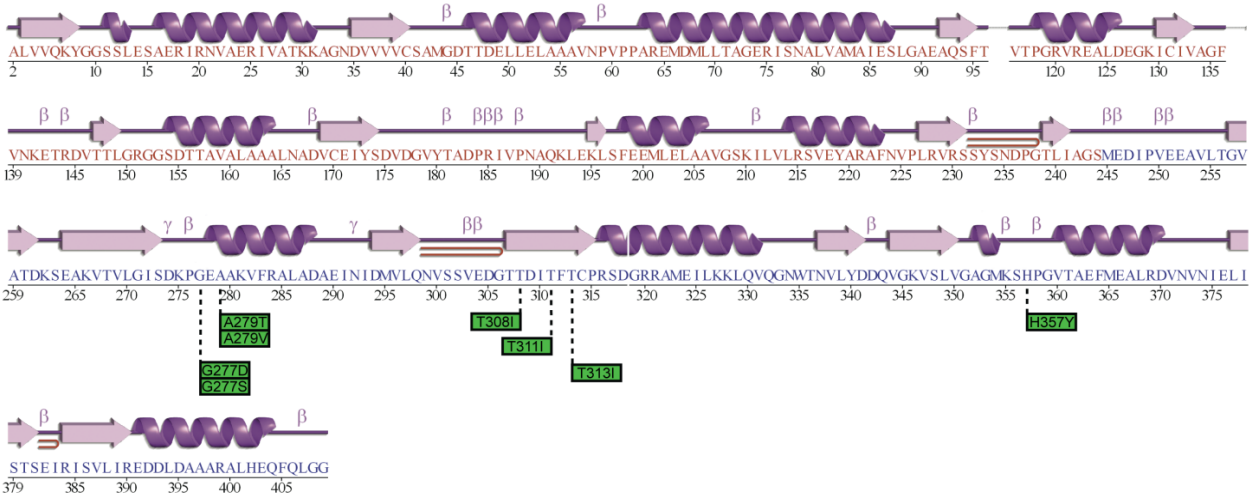


Figure S6.
Structural presentation of LysC with the localization of mutations identified.

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6.2. Supplementary Material "Recombineering in *Corynebacterium glutamicum* combined with optical nanosensors: A general strategy for fast producer strain generation"

Table S1: List of primers used for plasmid constructions

Primer	Sequence	Construction of
Bet-F	AAGGAGATATAGATATGAGTACTGCACTCGCAAC	pCLTON2-bet
Bet-R	TCATGCTGCCACCTTCTGCTC	
recT-F	AAGGAGATATAGATATGACTAAGCAACCACCAATC	pCLTON3-recT
recT-R	CGGTTATTCCTCTGAATTATCG	
BglII-RBS-recT-F	GCAGATCTAAGGAGATATACATATGACTAAGCAACCACCAATCG	pEKEx3-recT
EcoRI-recT-R	GCGCGAATTCCAGGCTGAATTATTCCTC	

Table S2: List of oligonucleotides used

Lagging strand oligos for establishment of recombineering in *C. glutamicum*	
Kan100*	ATGCATCATCAGGAGTACGGATAAAATGCTTGATGGTCGGAAGAGGCATAAATTCCGTCAGCCAGTTTAGTCTGACCATCTCATCTGTAACATCATTGGC
Kan75*	AGTACGGATAAAATGCTTGATGGTCGGAAGAGGCATAAATTCCGTCAGCCAGTTTAGTCTGACCATCTCATCTGT
Kan50*	ATGCTTGATGGTCGGAAGAGGCATAAATTCCGTCAGCCAGTTTAGTCTGA
Kan25*	GGAAGAGGCATAAATTCCGTCAGCC
Kan15*	GAGGCATAAATTCCG
Leading strand oligos for establishment of recombineering in *C. glutamicum*	
Kan100	GCCAATGATGTTACAGATGAGATGGTCAGACTAAACTGGCTGACGGAATTTATGCCTCTTCCGACCATCAAGCATTTTATCCGTACTCCTGATGATGCAT
Kan75	ACAGATGAGATGGTCAGACTAAACTGGCTGACGGAATTTATGCCTCTTCCGACCATCAAGCATTTTATCCGTACT
Kan50	TCAGACTAAACTGGCTGACGGAATTTATGCCTCTTCCGACCATCAAGCAT
Kan25	GGCTGACGGAATTTATGCCTCTTCC
Kan15	CGGAATTTATGCCTC

Oligos to assay for mismatch repair	
Kan100	GCCAATGATGTTACAGATGAGATGGTCAGACTAAACTGGCTGACGGAATTTATGCCTCTTCCGACCATCAAGCAT TTTATCCGTACTCCTGATGATGCAT
Kan100-a48g	GCCAATGATGTTACAGATGAGATGGTCAGACTAAACTGGCTGACGGAGTTTATGCCTCTTCCGACCATCAAGCAT TTTATCCGTACTCCTGATGATGCAT
Kan100-g45c	GCCAATGATGTTACAGATGAGATGGTCAGACTAAACTGGCTGACCGAATTTATGCCTCTTCCGACCATCAAGCAT TTTATCCGTACTCCTGATGATGCAT
Kan100-g42c	GCCAATGATGTTACAGATGAGATGGTCAGACTAAACTGGCTCACGGAATTTATGCCTCTTCCGACCATCAAGCAT TTTATCCGTACTCCTGATGATGCAT
Kan100-t51c	GCCAATGATGTTACAGATGAGATGGTCAGACTAAACTGGCTGACGGAATTCATGCCTCTTCCGACCATCAAGCAT TTTATCCGTACTCCTGATGATGCAT
Kan100-t57c	GCCAATGATGTTACAGATGAGATGGTCAGACTAAACTGGCTGACGGAATTTATGCCCCTTCCGACCATCAAGCAT TTTATCCGTACTCCTGATGATGCAT
Kan100-all	GCCAATGATGTTACAGATGAGATGGTCAGACTAAACTGGCTcACcGAgTTcATGCCcCTTCCGACCATCAAGCATTT TATCCGTACTCCTGATGATGCAT
Kan100*	ATGCATCATCAGGAGTACGGATAAAATGCTTGATGGTCGGAAGAGGCATAAATTCCGTCAGCCAGTTTAGTCTGA CCATCTCATCTGTAACATCATTGGC
Kan100*-a48g	ATGCATCATCAGGAGTACGGATAAAATGCTTGATGGTCGGAAGAGGCATAAACTCCGTCAGCCAGTTTAGTCTG ACCATCTCATCTGTAACATCATTGGC
Kan100*-g45c	ATGCATCATCAGGAGTACGGATAAAATGCTTGATGGTCGGAAGAGGCATAAATTCGGTCAGCCAGTTTAGTCTG ACCATCTCATCTGTAACATCATTGGC
Kan100*-g42c	ATGCATCATCAGGAGTACGGATAAAATGCTTGATGGTCGGAAGAGGCATAAATTCCGTGAGCCAGTTTAGTCTG ACCATCTCATCTGTAACATCATTGGC
Kan100*-t51c	ATGCATCATCAGGAGTACGGATAAAATGCTTGATGGTCGGAAGAGGCATGAATTCCGTCAGCCAGTTTAGTCTG ACCATCTCATCTGTAACATCATTGGC
Kan100*-t57c	ATGCATCATCAGGAGTACGGATAAAATGCTTGATGGTCGGAAGGGGCATAAATTCCGTCAGCCAGTTTAGTCTG ACCATCTCATCTGTAACATCATTGGC
Kan100*-all	ATGCATCATCAGGAGTACGGATAAAATGCTTGATGGTCGGAAGgGGCATgAAcTCgGTgAGCCAGTTTAGTCTGA CCATCTCATCTGTAACATCATTGGC
Oligo for engineering of *C. glutamicum lysC*	
lysC_60_EcoRV*	CGGCGGCCGTCGGAACGAGGGCAGGTGAAGATGATATCGGTGGTGCCGTCTTCTACAGAA
Oligos for engineering of *C. glutamicum murE*	
G81amb	AACAACGATGACTGGGCGGGTCTCTCCTGCTTCGTTGAGCACCTCAAGTCAAGCTGCGTCAGTCAAAATGGCCAC AGCTTTCGCAGCGTTATCCGTACCT
G81A	AACAACGATGACTGGGCGGGTCTCTCCTGCTTCGTTGAGCACCTCAAGTGCAGCGGCGTCAGTCAAAATGGCCA CAGCTTTCGCAGCGTTATCCGTACCT

G81C	AACAACGATGACTGGGCGGGTCTCTCCTGCTTCGTTGAGCACCTCAAGGCAAGCTGCGTCAGTCAAAATGGCCAC AGCTTTCGCAGCGTTATCCGTACCT
G81D	AACAACGATGACTGGGCGGGTCTCTCCTGCTTCGTTGAGCACCTCAAGGTCAGCGGCGTCAGTCAAAATGGCCA CAGCTTTCGCAGCGTTATCCGTACCT
G81E	AACAACGATGACTGGGCGGGTCTCTCCTGCTTCGTTGAGCACCTCAAGTTCAGCGGCGTCAGTCAAAATGGCCAC AGCTTTCGCAGCGTTATCCGTACCT
G81F	AACAACGATGACTGGGCGGGTCTCTCCTGCTTCGTTGAGCACCTCAAGGAAAGCTGCGTCAGTCAAAATGGCCA CAGCTTTCGCAGCGTTATCCGTACCT
G81H	AACAACGATGACTGGGCGGGTCTCTCCTGCTTCGTTGAGCACCTCAAGGTGAGCTGCGTCAGTCAAAATGGCCAC AGCTTTCGCAGCGTTATCCGTACCT
G81I	AACAACGATGACTGGGCGGGTCTCTCCTGCTTCGTTGAGCACCTCAAGGATAGCTGCGTCAGTCAAAATGGCCAC AGCTTTCGCAGCGTTATCCGTACCT
G81K	AACAACGATGACTGGGCGGGTCTCTCCTGCTTCGTTGAGCACCTCAAGCTTAGCTGCGTCAGTCAAAATGGCCAC AGCTTTCGCAGCGTTATCCGTACCT
G81L	AACAACGATGACTGGGCGGGTCTCTCCTGCTTCGTTGAGCACCTCAAGCAGAGCTGCGTCAGTCAAAATGGCCAC AGCTTTCGCAGCGTTATCCGTACCT
G81M	AACAACGATGACTGGGCGGGTCTCTCCTGCTTCGTTGAGCACCTCAAGCATAGCTGCGTCAGTCAAAATGGCCAC AGCTTTCGCAGCGTTATCCGTACCT
G81N	AACAACGATGACTGGGCGGGTCTCTCCTGCTTCGTTGAGCACCTCAAGGTTAGCTGCGTCAGTCAAAATGGCCAC AGCTTTCGCAGCGTTATCCGTACCT
G81P	AACAACGATGACTGGGCGGGTCTCTCCTGCTTCGTTGAGCACCTCAAGTGGAGCTGCGTCAGTCAAAATGGCCAC AGCTTTCGCAGCGTTATCCGTACCT
G81Q	AACAACGATGACTGGGCGGGTCTCTCCTGCTTCGTTGAGCACCTCAAGCTGAGCTGCGTCAGTCAAAATGGCCAC AGCTTTCGCAGCGTTATCCGTACCT
G81R	AACAACGATGACTGGGCGGGTCTCTCCTGCTTCGTTGAGCACCTCAAGGCGAGCTGCGTCAGTCAAAATGGCCA CAGCTTTCGCAGCGTTATCCGTACCT
G81S	AACAACGATGACTGGGCGGGTCTCTCCTGCTTCGTTGAGCACCTCAAGGGAAGCTGCGTCAGTCAAAATGGCCA CAGCTTTCGCAGCGTTATCCGTACCT
G81T	AACAACGATGACTGGGCGGGTCTCTCCTGCTTCGTTGAGCACCTCAAGGGTAGCTGCGTCAGTCAAAATGGCCAC AGCTTTCGCAGCGTTATCCGTACCT
G81V	AACAACGATGACTGGGCGGGTCTCTCCTGCTTCGTTGAGCACCTCAAGCACAGCGGCGTCAGTCAAAATGGCCA CAGCTTTCGCAGCGTTATCCGTACCT
G81W	AACAACGATGACTGGGCGGGTCTCTCCTGCTTCGTTGAGCACCTCAAGCCAAGCTGCGTCAGTCAAAATGGCCAC AGCTTTCGCAGCGTTATCCGTACCT
G81Y	AACAACGATGACTGGGCGGGTCTCTCCTGCTTCGTTGAGCACCTCAAGGTAAGCTGCGTCAGTCAAAATGGCCAC AGCTTTCGCAGCGTTATCCGTACCT

Table S3: Generated strains carrying a *murE*-G81 mutation and their specific L-lysine production and fluorescence

Codon 241-243	Amino acid 81	Fluorescence (AU)	L-Lysine (mM)	Codon 241-243	Amino acid 81	Fluorescence (AU)	L-Lysine (mM)
TGC	Cysteine (C)	0.16	0.00	CGC	Arginine (R)	2.05	12.26
		1.08	7.29			2.23	12.85
		1.08	7.40			2.05	9.55
		1.12	8.77	TCC	Serine (S)	0.18	0.00
		1.13	7.68			0.19	0.00
		1.22	4.59			0.20	0.00
TTC	Phenylalanine (P)	1.36	8.19			0.20	0.04
		1.21	9.39	TGG	Threonine (T)	0.47	1.46
		1.36	9.52			1.12	5.92
		1.41	8.46			1.11	7.70
CAC	Histidine (H)	0.46	0.63	GTG	Valine (V)	1.47	9.49
		0.72	3.50			1.49	9.12
ATC	Isoleucine (I)	1.85	10.18			1.56	8.00
CTG	Leucine (L)	1.80	12.03	TGG	Tryptophan (W)	2.39	17.16
		1.83	12.06			1.68	9.15
		1.92	12.09			1.80	11.30
		1.97	11.76	TAC	Tyrosine (Y)	1.10	7.10
		1.93	12.23			1.11	9.22
AAC	Asparagine (N)	0.67	4.17			1.10	9.13
		0.73	4.62			1.15	6.77
		0.48	1.29			0.45	0.87
		0.71	4.35			1.17	8.86

```
KanR (-)      TGG CTG ACG GAA TTC TAT GCC TCT T 3'
              ACC GAC TGC CTT AAG ATA CGG AGA A 5'

Oligo         5'-3' Sequence                          KanR cfu

Kan100        TGG CTG ACG GAA TTT ATG CCT CTT          2.7x10^6
Kan100-a48g   --- --- --- --G --- --- --- ---          7.5x10^4
Kan100-g45c   --- --- --C --- --- --- --- ---          2.7x10^6
Kan100-g42c   --- --C --- --- --- --- --- ---          3.7x10^6
Kan100-t51c   --- --- --- --- --C --- --- ---          4.0x10^6
Kan100-t57c   --- --- --- --- --- --- --C ---          3.5x10^6
Kan100-all    --- --C --C --G --C --- --C ---          9.2x10^5
```

Figure S1: Effect of multiple nucleotide changes on oligo recombination frequencies. The double-strand sequence of the region around the KanR mutation leading to a frame-shift (yellow) is shown on top. All oligos are 60 bases in length, and only relevant changes are shown. Recombination values obtained from these oligos are normalized per 10^9 viable cells.

6.3. Whole-genome sequencing: Statistical data and SNP list for ten selected *C. glutamicum* strains

Table S1: Technical data

Description / Strain	M90	M95	M97	M117	M131	M98	M102	M121	M140	M144
Number of sequenced reads	15.203.442	22.920.864	24.364.120	18.087.088	23.125.014	21.079.778	20.156.524	23.369.104	25.053.240	21.443.130
Avg. length of sequenced read [bp]	51,0	51,0	51,0	51,0	51,0	51,0	51,0	51,0	51,0	51,0
Number of reads after trimming	15.044.318	22.651.003	23.154.212	17.893.878	22.809.808	20.801.468	19.908.254	23.017.432	24.335.760	21.012.303
Avg. length of reads after trimming [bp]	48,1	48,1	47,8	48,1	48,0	48,1	48,1	48,1	47,9	48,0
Number of paired reads after trimming used for mapping	14.888.144	22.386.002	21.951.810	17.704.208	22.499.708	20.527.654	19.664.448	22.670.366	23.625.048	20.586.576
Number of reads mapped to reference	13.672.499	21.484.643	19.845.607	16.879.657	21.515.398	19.601.164	17.877.215	22.057.335	22.189.555	19.619.473
Coverage (# mapped reads * avg. length/3301500)	199,2	313,0	287,3	245,9	312,8	285,6	260,5	321,4	321,9	285,2

Table S2: Statistical data

SNP effect	K118	K117	K063	K005	K112	K120	K051	K079	K093	K013
aa exchange	71	20	99	100	99	115	171	150	134	136
intergenic	18	5	24	23	23	23	28	30	35	37
silent	47	11	47	47	47	68	65	73	65	66
ribosomal	0	0	0	0	0	0	0	2	1	1
stop codon	3	0	0	0	0	3	4	2	4	4
total	139	39	170	170	169	209	268	257	239	244
L-Lysine (mM)	10.5	7	9	6.6	7.9	2.6	15.9	5.2	5	4.9
Growth rate (μ)	0.36	0.34	0.37	0.38	0.38	0.37	0.23	0.3	0.39	0.38

Table S3: List of SNPs identified in ten isolated mutants

SNP	TYPE	GENES	NAME	AA-ex.	FUNCTION	K118	K117	K063	K005	K112	K120	K051	K079	K093	K013
C1732T	intergenic	NCgl0001, NCgl0002				wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G12367A	silent	NCgl0012	gyrA	A179A	DNA gyrase subunit A	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G15154A	intergenic	NCgl0013, NCgl0014				wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G21832A	exchange	NCgl0021		G79D	5'-nucleotidase/2',3'-cyclic phosphodiesterase or related esterase	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G24328A	exchange	NCgl0023		A12T	stress-induced protein	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G28805A	silent	NCgl0026		L105L	ABC-type transporter, periplasmic component	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G30080A	exchange	NCgl0028		G29E	ABC-type transporter, ATPase component	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G30860A	exchange	NCgl0029		G55D	ABC-type transporter, periplasmic component	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C32345T	silent	NCgl0030		R223R	ABC-type transporter, permease component	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C33504T	silent	NCgl0032		K257K	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C34070T	exchange	NCgl0032		A69T	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G35666A	exchange	NCgl0034		V229M	uncharacterized membrane protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C35974T	intergenic	NCgl0034				wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G37335A	exchange	NCgl0036		A31T	ABC-type transporter, permease component	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G37731A	exchange	NCgl0036		G163S	ABC-type transporter, permease component	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G38195A	silent	NCgl0036		E317E	ABC-type transporter, permease component	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
G39143A	exchange	NCgl0038		A56T	siderophore-interacting protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G42509A	exchange	NCgl0040	pknB	T2I	serine/threonine protein kinase	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C44248T	exchange	NCgl0042	pbpA	G367D	cell division protein FtsI	wt	mut	wt	wt	wt	wt	wt	wt	wt	wt
G45154A	exchange	NCgl0042	pbpA	S65F	cell division protein FtsI	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C55918T	exchange	NCgl0052		G152D	hypothetical membrane permease protein	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G55970A	exchange	NCgl0052		L135F	hypothetical membrane permease protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
A56627G	exchange	NCgl0053		I17T	rhodanese-related sulfurtransferase	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C58823T	exchange	NCgl0056		A246V	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C60800T	silent	NCgl0059		I44I	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G61812A	exchange	NCgl0059		D382N	hypothetical protein	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt

SNP	TYPE	GENES	NAME	AA-ex.	FUNCTION	K118	K117	K063	K005	K112	K120	K051	K079	K093	K013
G61903A	exchange	NCgl0059		G412D	hypothetical protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G62832A	exchange	NCgl0060		A226V	Mg2+ and Co2+ transporter protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G67440A	exchange	NCgl0064	phoH1	S281N	ATPase related to phosphate starvation-inducible protein PhoH	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G73161A	exchange	NCgl0069		L190F	phosphoglycerate dehydrogenase or related dehydrogenase	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C82101T	intergenic	NCgl0073,NCgl0074				wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C83444T	exchange	NCgl0074		G42E	permease	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C84058T	exchange	NCgl0075	codA	G293D	cytosine deaminase or related metal-dependent hydrolase	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G85705A	silent	NCgl0077		Y172Y	hypothetical protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G86071A	silent	NCgl0077		S50S	hypothetical protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G87011A	exchange	NCgl0078		A119T	NAD-dependent protein deacetylase, SIR2 family	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C89186T	exchange	NCgl0080		E87K	predicted acetyltransferase or hydrolase	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G90274A	silent	NCgl0081		E220E	hypothetical protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G91563A	exchange	NCgl0084	ureB	A21T	urea amidohydrolase (urease) beta subunit	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
T94111C	exchange	NCgl0086	ureE	V128A	urease accessory protein UreE	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
C96511T	exchange	NCgl0090		V212I	predicted hydrolase or acyltransferase	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G98597A	intergenic	NCgl0091,NCgl0092				wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G102198A	exchange	NCgl0093		T413I	molecular chaperone, HSP90 family	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G103097A	silent	NCgl0093		I113I	molecular chaperone, HSP90 family	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G103614A	exchange	NCgl0094	amn	D38N	AMP nucleosidase	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C116219T	exchange	NCgl0103		L69F	hypothetical membrane protein	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C117097T	silent	NCgl0104		R331R	acyl-CoA synthetase (AMP-forming)/AMP-acid ligase II	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G117672A	exchange	NCgl0104		P140S	acyl-CoA synthetase (AMP-forming)/AMP-acid ligase II	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G119171A	exchange	NCgl0105		P140L	deoR family transcriptional regulator of sugar metabolism	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G121801A	exchange	NCgl0108	mtlD	S220F	mannitol-1-phosphate/altronate dehydrogenase	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C122038T	exchange	NCgl0108	mtlD	G141E	mannitol-1-phosphate/altronate dehydrogenase	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C123771T	exchange	NCgl0109	rbtT	G24D	hypothetical membrane permease protein	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut

SNP	TYPE	GENES	NAME	AA-ex.	FUNCTION	K118	K117	K063	K005	K112	K120	K051	K079	K093	K013
C124999T	exchange	NCgl0111	xylB	S10F	sugar (pentulose and hexulose) kinase	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C126008T	silent	NCgl0111	xylB	A346A	sugar (pentulose and hexulose) kinase	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C128084T	intergenic	NCgl0113, NCgl0114	panB			wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G129149A	intergenic	NCgl0114, NCgl0115	mag			wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C129392T	intergenic	NCgl0114, NCgl0115	mag			wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C149734T	exchange	NCgl0134		S538F	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C151067T	exchange	NCgl0136		V130I	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C152330T	silent	NCgl0137		V247V	predicted permease	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C154644T	exchange	NCgl0139	hrpB	G324S	HrpA-like helicase	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C158032T	exchange	NCgl0142	tagA2	A98V	3-methyladenine DNA glycosylase	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C161536T	exchange	NCgl0148		A245T	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G162106A	exchange	NCgl0148		P55S	hypothetical protein	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C164714T	exchange	NCgl0151	pepO	E301K	predicted metalloendopeptidase	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G166276A	exchange	NCgl0152		S187N	hypothetical protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G172031A	exchange	NCgl0157	msmA	G367S	NAD-dependent aldehyde dehydrogenase	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C172162T	silent	NCgl0157	msmA	N410N	NAD-dependent aldehyde dehydrogenase	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C176810T	exchange	NCgl0161	iolG	S168F	predicted dehydrogenase	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C177581T	exchange	NCgl0162	iolH	T83I	sugar phosphate isomerase/epimerase	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G178917A	silent	NCgl0163		L211L	permease of the major facilitator superfamily	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C179198T	exchange	NCgl0163		A305V	permease of the major facilitator superfamily	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C181746T	exchange	NCgl0167		E312K	transcriptional regulator	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C182551T	silent	NCgl0167		A43A	transcriptional regulator	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C184526T	silent	NCgl0169		F150F	sugar phosphate isomerase/epimerase	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G186158A	intergenic	NCgl0170, NCgl0171	cspA			wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C187372T	exchange	NCgl0173		A24V	transcriptional regulator	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt

SNP	TYPE	GENES	NAME	AA-ex.	FUNCTION	K118	K117	K063	K005	K112	K120	K051	K079	K093	K013
G194451A	exchange	NCgl0179		G532D	transposase	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
C195802T	exchange	NCgl0181	gltB	T188I	glutamine 2-oxoglutarate aminotransferase large subunit	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C196857T	exchange	NCgl0181	gltB	L540F	glutamine 2-oxoglutarate aminotransferase large subunit	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G201117A	exchange	NCgl0182	gltD	R449H	Glutamine 2-oxoglutarate aminotransferase small subunit	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G201272A	exchange	NCgl0182	gltD	D501N	Glutamine 2-oxoglutarate aminotransferase small subunit	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G204261A	silent	NCgl0184	embC	D906D	putative arabinosyl transferase	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G205139A	exchange	NCgl0184	embC	L614F	putative arabinosyl transferase	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C206636T	exchange	NCgl0184	embC	G115S	putative arabinosyl transferase	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G207123A	silent	NCgl0185	aftA	L639L	arabinofuranosyltransferase	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G207220A	silent	NCgl0185	aftA	A606A	arabinofuranosyltransferase	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G216838A	intergenic	NCgl0196,NCgl0197				wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G219307A	exchange	NCgl0199		R120H	selenocysteine lyase	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G220948A	exchange	NCgl0200		P62S	NADPH:quinone reductase or related Zn-dependent oxidoreductase	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C223244T	exchange	NCgl0203		V151I	Na+/alanine symporter	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C230124T	exchange	NCgl0211	modA	V131M	ABC-type transporter, periplasmic component	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
A232640G	exchange	NCgl0215	pat	Y215H	aromatic amino acid aminotransferase	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C235047T	exchange	NCgl0217		V49M	hypothetical protein	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C235232T	intergenic	NCgl0217, NCgl0218				wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C237327T	silent	NCgl0219		V334V	Zn-dependent alcohol dehydrogenase	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G246297A	exchange	NCgl0228		V466I	predicted drug exporter of the RND superfamily	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C246787T	exchange	NCgl0228		A629V	predicted drug exporter of the RND superfamily	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C250321T	silent	NCgl0231		A298A	hypothetical malic enzyme protein	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C252565T	exchange	NCgl0233	gltX	S205F	glutamyl-tRNA synthetase	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C253379T	silent	NCgl0234		E147E	hypothetical dioxygenase protein	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C255394T	silent	NCgl0235		K15K	putative transposase	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C256592T	intergenic	NCgl0236, NCgl0237	aspB			wt	wt	wt	wt	wt	wt	wt	wt	mut	mut

SNP	TYPE	GENES	NAME	AA-ex.	FUNCTION	K118	K117	K063	K005	K112	K120	K051	K079	K093	K013
C266128T	intergenic	NCgl0244, NCgl0245	leuA			wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G267507A	silent	NCgl0245	leuA	N165N	2-isopropylmalate synthase	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C268945T	exchange	NCgl0246		M60I	hypothetical membrane protein	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G278967A	exchange	NCgl0258	arsC1	E37K	arsenite efflux pump ACR3	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G280246A	silent	NCgl0259	arsX	Q118Q	protein-tyrosine-phosphatase	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C281460T	exchange	NCgl0263	mnhD	A492T	NADH dehydrogenase subunit N	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C287240T	exchange	NCgl0266		P287S	predicted membrane protein	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C287581T	silent	NCgl0266		A400A	predicted membrane protein	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G288092A	silent	NCgl0267		V246V	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C289594T	exchange	NCgl0268	cgtR1	R68K	two-component system, response regulator	wt	mut	wt	wt	wt	wt	wt	wt	wt	wt
C290007T	exchange	NCgl0269	cgtS1	D413N	two-component system, sensory transduction histidine kinase	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C295358T	exchange	NCgl0274	ponA	G344D	membrane carboxypeptidase	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C299581T	exchange	NCgl0278		G35D	permease of the major facilitator superfamily	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G302609A	exchange	NCgl0281	fabG1	G148D	dehydrogenase	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G303340A	exchange	NCgl0282		A70T	predicted metal-dependent hydrolase of the TIM-barrel fold	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G312608A	exchange	NCgl0292		A95V	predicted hydrolase or acyltransferase	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C315100T	exchange	NCgl0295		L53F	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G324113A	exchange	NCgl0304	topA	G402S	DNA topoisomerase I	wt	wt	wt	wt	wt	wt	wt	wt	mut	wt
G333545A	silent	NCgl0311	bglS(N-Term)	E209E	beta-glucosidase-related glycosidase	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G337140A	silent	NCgl0315		G120G	dehydrogenase	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G339653A	silent	NCgl0317	rmlB2	E287E	nucleoside-diphosphate-sugar epimerase	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G340255A	silent	NCgl0318		A105A	hypothetical membrane protein	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G341938A	exchange	NCgl0320		A195T	hypothetical protein	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G349652A	exchange	NCgl0326	rmlCD	A220V	dTDP-4-dehydrorhamnose 3,5-epimerase or related enzyme	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G352149A	intergenic	NCgl0328, NCgl0329				wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G356314A	silent	NCgl0332		L305L	aminopeptidase N	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt

SNP	TYPE	GENES	NAME	AA-ex.	FUNCTION	K118	K117	K063	K005	K112	K120	K051	K079	K093	K013
G365567A	silent	NCgl0338	ptpA2	K106K	protein-tyrosine-phosphatase	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
G371429A	exchange	NCgl0343		G180R	predicted glycosyltransferase	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G376953A	intergenic	NCgl0347, NCgl0348	tnp17a (ISCg17a)			wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G377831A	exchange	NCgl0348	tnp17a (ISCg17a)	G50D	putative transposase	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G384696A	silent	NCgl0352		F165F	hypothetical membrane protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G386758A	exchange	NCgl0354		G68E	acetyltransferase	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G388811A	exchange	NCgl0355	lpd	D374N	dihydrolipoamide dehydrogenase	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
T399017C	exchange	NCgl0365		V230A	uncharacterized membrane protein	wt	wt	wt	wt	wt	wt	wt	wt	mut	wt
G401161A	silent	NCgl0369		Q4Q	permease of the major facilitator superfamily	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G405333A	silent	NCgl0371	purU	D29D	formyltetrahydrofolate deformylase	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G405999A	exchange	NCgl0372	deoC	V176M	deoxyribose-phosphate aldolase	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G415971A	stop	NCgl0381		W110*	hypothetical membrane protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G418886A	exchange	NCgl0384		A123V	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C422500T	exchange	NCgl0388	fadD5	A432T	acyl-CoA synthase	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C433412T	silent	NCgl0396	ppx1	F89F	exopolyphosphatase	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G433759A	exchange	NCgl0396	ppx1	R205Q	exopolyphosphatase	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G435588A	exchange	NCgl0398	proC	G238S	pyrroline-5-carboxylate reductase	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G444367A	silent	NCgl0407		Q61Q	sugar phosphate isomerase/epimerase	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C446342T	exchange	NCgl0408	aroD	L86F	3-dehydroquinate dehydratase	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G450911A	exchange	NCgl0413		L335F	ABC-type transporter periplasmic component	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G451072A	exchange	NCgl0413		A281V	ABC-type transporter periplasmic component	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G451225A	exchange	NCgl0413		A230V	ABC-type transporter periplasmic component	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C453289T	exchange	NCgl0414	hemD	T218I	uroporphyrinogen-III synthase/methylase	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C456430T	silent	NCgl0417		L139L	hypothetical membrane protein	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C460823T	silent	NCgl0420	hemE	V284V	uroporphyrinogen decarboxylase	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C461122T	exchange	NCgl0421	hemY	A4V	protoporphyrinogen oxidase	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut

SNP	TYPE	GENES	NAME	AA-ex.	FUNCTION	K118	K117	K063	K005	K112	K120	K051	K079	K093	K013
C463395T	exchange	NCgl0422	hemL	A277V	glutamate-1-semialdehyde aminotransferase	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C463983T	silent	NCgl0423		A39A	phosphoglycerate mutase/fructose-2,6-bisphosphatase	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C464176T	exchange	NCgl0423		P104S	phosphoglycerate mutase/fructose-2,6-bisphosphatase	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C465706T	silent	NCgl0425	ccsA	L201L	cytochrome C biogenesis protein	wt	mut	wt	wt	wt	wt	wt	wt	wt	wt
C467004T	silent	NCgl0426		R352R	cytochrome C biosynthesis protein ResB	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C467391T	silent	NCgl0426		I481I	cytochrome C biosynthesis protein ResB	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G467569A	exchange	NCgl0426		D541N	cytochrome C biosynthesis protein ResB	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G468933A	intergenic	NCgl0427, NCgl0428	ccsB			wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C469776T	exchange	NCgl0428		S136F	hypothetical protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C472448T	exchange	NCgl0433	menA	A121T	1,4-dihydroxy-2-naphthoate octaprenyltransferase.	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C474655T	exchange	NCgl0435	menE	G96D	O-succinylbenzoic acid--CoA ligase	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C475929T	exchange	NCgl0437		V374M	ketoglutarate semialdehyde dehydrogenase	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C478365T	silent	NCgl0439		K203K	transcriptional regulator	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C479829T	exchange	NCgl0441		G125S	hypothetical protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G482200A	exchange	NCgl0445	pitA	L193F	phosphate/sulphate permease	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G485606A	exchange	NCgl0448		V85I	peptidase E	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G485727A	exchange	NCgl0448		S125N	peptidase E	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G489057A	exchange	NCgl0451		G133D	hypothetical membrane protein	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G490928A	exchange	NCgl0453		G117S	gamma-aminobutyrate permease or related permease	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G491317A	exchange	NCgl0453		M246I	gamma-aminobutyrate permease or related permease	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G491396A	exchange	NCgl0453		A273T	gamma-aminobutyrate permease or related permease	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C492034T	silent	NCgl0454	ubiE	N23N	ubiquinone/menaquinone biosynthesis methyltransferase	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
C505619T	intergenic	NCgl0464, NCgl0465	tyrP			wt	mut	wt	wt	wt	wt	wt	wt	wt	wt
G513927A	exchange	NCgl0471	rpoB	G333S	DNA-directed RNA polymerase beta subunit	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C520212T	exchange	NCgl0472	rpoC	S1240F	DNA-directed RNA polymerase beta' subunit	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C523087T	exchange	NCgl0477	rpsG	P7S	30S ribosomal protein S7	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt

SNP	TYPE	GENES	NAME	AA-ex.	FUNCTION	K118	K117	K063	K005	K112	K120	K051	K079	K093	K013
C523129T	exchange	NCgl0477	rpsG	L21F	30S ribosomal protein S7	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C523479T	silent	NCgl0477	rpsG	R137R	30S ribosomal protein S7	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G528686A	exchange	NCgl0481		G206E	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C529373T	silent	NCgl0482		R66R	ABC-type transporter, ATPase component	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
G529502A	silent	NCgl0482		L23L	ABC-type transporter, ATPase component	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G538107A	exchange	NCgl0496	rpsQ	G44D	30S ribosomal protein S17	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G540528A	exchange	NCgl0501	rplE	G35S	50S ribosomal protein L5	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C542680T	exchange	NCgl0503	dkg	A73T	aldo/keto reductase	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
C543172T	exchange	NCgl0504		A81T	hypothetical protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C544846T	exchange	NCgl0507	fdhF	V682I	putative formate dehydrogenase	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
G547557A	exchange	NCgl0508		D34N	hypothetical protein	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
C550533T	exchange	NCgl0510		G55E	ABC-type cobalt transport system, ATPase component	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
C551027T	exchange	NCgl0511		G261D	ABC-type cobalt transport system, permease component	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
	intergenic					wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C556568T	silent	NCgl0517	rplR	L94L	50S ribosomal protein L18	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G560724A	silent	NCgl0524		I215I	hypothetical protein	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C570455T	exchange	NCgl0532	secY	S335F	preprotein translocase SecY	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C572664T	exchange	NCgl0535		R79C	hypothetical protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G584639A	silent	NCgl0548	cma	Q108Q	putative cyclopropane-fatty-acyl-phospholipid synthase	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C585183T	exchange	NCgl0548	cma	P290S	putative cyclopropane-fatty-acyl-phospholipid synthase	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
G586709A	exchange	NCgl0550		A311V	subtilisin-like serine protease	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G589803A	exchange	NCgl0552		G185S	DNA segregation ATPase FtsK/SpoIIIE family protein	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G592737A	exchange	NCgl0552		A1163T	DNA segregation ATPase FtsK/SpoIIIE family protein	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C592785T	exchange	NCgl0552		P1179S	DNA segregation ATPase FtsK/SpoIIIE family protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C596701T	exchange	NCgl0558	mrsA	A198V	phosphomannomutase	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
C600156T	exchange	NCgl0562		A241T	hypothetical protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C600279T	exchange	NCgl0562		G200S	hypothetical protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt

SNP	TYPE	GENES	NAME	AA-ex.	FUNCTION	K118	K117	K063	K005	K112	K120	K051	K079	K093	K013
G602881A	exchange	NCgl0565		G24D	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C604264T	exchange	NCgl0565		A485V	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G611157A	exchange	NCgl2621	groEL	V167I	chaperonin GroEL	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G613465A	exchange	NCgl0575	sigD	E104K	RNA polymerase sigma-70 factor	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C622837T	exchange	NCgl0584		G267S	hypothetical membrane protein	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C623058T	exchange	NCgl0584		R193Q	hypothetical membrane protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C625717T	exchange	NCgl0587		A14V	hypothetical protein	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
C626560T	intergenic	NCgl0588, NCgl0589				wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
C626666T	exchange	NCgl0589		G244S	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C628520T	exchange	NCgl0590		P253L	hypothetical protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G650219A	intergenic	NCgl0609,NCgl0610				wt	wt	wt	wt	wt	wt	wt	wt	wt	mut
G655577A	silent	NCgl0613		E152E	predicted membrane protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C657885T	exchange	NCgl0615		G40S	hypothetical protein	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
C659201T	exchange	NCgl0617		A90V	cytosine/adenosine deaminase	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
C659619T	exchange	NCgl0618		S26F	ABC-type Fe3+-siderophores transport system, periplasmic component	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
C660052T	silent	NCgl0618		N170N	ABC-type Fe3+-siderophores transport system, periplasmic component	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
C660682T	exchange	NCgl0619	spoU	A147T	predicted SpoU class rRNA methylase	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
C661404T	exchange	NCgl0620	folD	A80V	5,10-methylene-tetrahydrofolate dehydrogenase	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
C662344T	silent	NCgl0621		F106F	hypothetical membrane protein	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
C664870T	silent	NCgl0623		A26A	hypothetical protein	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
C665154T	intergenic	NCgl0623, NCgl0624	metX			wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
C669771T	exchange	NCgl0626	cstA	T528I	carbon starvation protein, predicted membrane protein	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C670087T	silent	NCgl0626	cstA	F633F	carbon starvation protein, predicted membrane protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
A672597G	exchange	NCgl0628		I260V	hypothetical protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C678766T	exchange	NCgl0634	icd	A427T	monomeric isocitrate dehydrogenase (NADP+)	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt

SNP	TYPE	GENES	NAME	AA-ex.	FUNCTION	K118	K117	K063	K005	K112	K120	K051	K079	K093	K013
C683618T	silent	NCgl0638		R82R	ABC-type transporter, permease component	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C686069T	exchange	NCgl0640		L321F	arabinose efflux permease	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C687662T	silent	NCgl0642		G104G	predicted membrane protein	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
C691557T	exchange	NCgl0646		A56T	ABC-type transporter, permease component	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
C692120T	exchange	NCgl0647	trpS	A80V	tryptophanyl-tRNA synthetase	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C696963T	exchange	NCgl0651		A345T	hypothetical protein	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
C699141T	silent	NCgl0653		E14E	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C712001T	exchange	NCgl0663		A93V	thioredoxin reductase	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C712149T	silent	NCgl0663		A142A	thioredoxin reductase	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C717440T	silent	NCgl0668		Q190Q	predicted transcriptional regulator	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C720123T	exchange	NCgl0670	accBC	R77H	acyl-CoA carboxylase	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
C722757T	silent	NCgl0672		F327F	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
A723378G	intergenic	NCgl0673, NCgl0674	wbpC			wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C724607T	exchange	NCgl0674	wbpC	A399V	predicted acyltransferase	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C728899T	exchange	NCgl0678	dtsR1	G476S	detergent sensitivity rescuer dtsR1	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
C731288T	exchange	NCgl0679	birA	L266F	biotin--protein ligase	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
G739360A	silent	NCgl0688		T252T	putative transposase	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
C745320T	exchange	NCgl0696		G60D	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C745729T	exchange	NCgl0697		A389T	ABC-type transporter, periplasmic component	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
C745759T	exchange	NCgl0697		A379T	ABC-type transporter, periplasmic component	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
C747978T	exchange	NCgl0698	msiK2	E17K	ABC-type transporter, ATPase component	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C748807T	intergenic	NCgl0699, NCgl0700				wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G754108A	silent	NCgl0701		D1096D	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	wt	wt	mut
C757312T	silent	NCgl0701		R28R	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C770467T	exchange	NCgl0706		G1226R	type II restriction enzyme, methylase subunits	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
C776608T	exchange	NCgl0707		G143E	SNF2 family helicase	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt

SNP	TYPE	GENES	NAME	AA-ex.	FUNCTION	K118	K117	K063	K005	K112	K120	K051	K079	K093	K013
C781053T	exchange	NCgl0710	rmlA2	S323F	nucleoside-diphosphate-sugar pyrophosphorylase	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C788467T	exchange	NCgl0718		A91V	hypothetical protein	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
C789549T	exchange	NCgl0719	sahH	A297V	S-adenosyl-L-homocysteine hydrolase	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
C792887T	exchange	NCgl0722	mtrB	A459V	two-component system sensory transduction histidine kinase	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
C793801T	exchange	NCgl0723		A265V	hypothetical protein	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
C797789T	exchange	NCgl0726	secA	P514S	translocase	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C798403T	silent	NCgl0726	secA	V718V	translocase	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G801296A	exchange	NCgl0730	aroA	A395V	3-phosphoshikimate 1-carboxyvinyltransferase	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C804488T	silent	NCgl0733	sigH	D27D	RNA polymerase sigma-70 factor	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
C806442T	exchange	NCgl0735		T42M	hypothetical membrane protein	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
G810069A	exchange	NCgl0739		G181E	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G812865A	exchange	NCgl0741		A566T	putative helicase	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C819543T	exchange	NCgl0745		P97L	putative helicase	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
C822913T	exchange	NCgl0748		V398I	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C824829T	exchange	NCgl0749		P214S	predicted secreted protein	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C825405T	exchange	NCgl0750		G85D	hypothetical protein	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
G825887A	intergenic	NCgl0750, NCgl0751				wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G825970A	intergenic	NCgl0750, NCgl0751				wt	wt	wt	wt	wt	wt	wt	wt	wt	mut
C827637T	exchange	NCgl0752		T341I	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G830394A	exchange	NCgl0753	pdxR	P198S	transcriptional regulator	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
C831688T	exchange	NCgl0754		A225V	pyridoxine biosynthesis protein	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
C832947T	exchange	NCgl0756		V71I	hypothetical membrane protein	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C835404T	intergenic	NCgl0758, NCgl0759				wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
C837573T	exchange	NCgl0760		P88S	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G841749A	exchange	NCgl0765	hisN	T183I	L-histidinol-phophate phosphatase	wt	wt	wt	wt	wt	wt	wt	wt	wt	mut

SNP	TYPE	GENES	NAME	AA-ex.	FUNCTION	K118	K117	K063	K005	K112	K120	K051	K079	K093	K013
G843617A	exchange	NCgl0767	prfB	A121T	peptide chain release factor 2	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C848472T	intergenic	NCgl0772, NCgl0773	tnp18a (ISCg18a)			wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C849478T	exchange	NCgl0774		D256N	ABC-type cobalamin/Fe3+-siderophore transport system. periplasmic component	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G855057A	exchange	NCgl0779		D112N	ABC-type cobalamin/Fe3+-siderophore transport system. ATPase component	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C864293T	silent	NCgl0782		E258E	putative helicase	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C864505T	exchange	NCgl0782		D188N	putative helicase	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C864574T	exchange	NCgl0782		A165T	putative helicase	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C865336T	exchange	NCgl0783		G661E	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G873093A	exchange	NCgl0790		M414I	permease	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C873661T	exchange	NCgl0791		A150V	rRNA methylase	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C874070T	exchange	NCgl0792		R290H	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C874829T	exchange	NCgl0792		G37D	hypothetical protein	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C877204T	intergenic	NCgl0794, NCgl0795	serC, gltA			wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G878054A	exchange	NCgl0795	gltA	G73R	citrate synthase	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C880847T	exchange	NCgl0797	accDA	G83S	acetyl-CoA carboxylase beta subunit	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C890852T	silent	NCgl0802	fas-IB	R1727R	fatty-acid synthase	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G892287A	exchange	NCgl0802	fas-IB	G2206S	fatty-acid synthase	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G903908A	exchange	NCgl0812		R1289H	Lhr-like helicase	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G905244A	silent	NCgl0813	nei	G210G	formamidopyrimidine-DNA glycosylase	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C906010T	exchange	NCgl0815		A128T	hypothetical membrane-associated protein	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C912964T	exchange	NCgl0820	pcrA	A580V	hypothetical helicase	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C915925T	exchange	NCgl0822		G147D	ABC-type transporter, ATPase component	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C921633T	exchange	NCgl0828	citE	P14S	citrate lyase beta subunit	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G922278A	exchange	NCgl0828	citE	A229T	citrate lyase beta subunit	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C924676T	exchange	NCgl0832	rpsN	A18T	30S ribosomal protein S14	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut

SNP	TYPE	GENES	NAME	AA-ex.	FUNCTION	K118	K117	K063	K005	K112	K120	K051	K079	K093	K013
C926061T	exchange	NCgl0835		G273D	sulfate permease	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C931246T	silent	NCgl0841		N307N	trypsin-like serine protease	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C931481T	exchange	NCgl0841		L386F	trypsin-like serine protease	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C931757T	exchange	NCgl0842		P18S	molybdopterin biosynthesis enzyme	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G933932A	exchange	NCgl0845		P124L	5-formyltetrahydrofolate cyclo-ligase	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C935858T	exchange	NCgl0847	moeA2	L170F	molybdopterin biosynthesis enzyme	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C942323T	intergenic	NCgl0853, NCgl0854				wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C945507T	exchange	NCgl0856	betP	P160L	choline-glycine betaine transporter	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G948748A	intergenic	NCgl0857, NCgl0858	metS			wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G950355A	exchange	NCgl0858		G522E	putative helicase	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C952243T	exchange	NCgl0860		R250H	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G954440A	exchange	NCgl0863		G54D	hypothetical protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C955210T	silent	NCgl0864	tnp7a (ISCg7a)	G90G	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C957074T	exchange	NCgl0865	dld	V109M	FAD/FMN-containing dehydrogenase	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C959521T	intergenic	NCgl0866, NCgl0867				wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C961572T	silent	NCgl0869		I92I	predicted arsR family transcriptional regulator	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C963884T	silent	NCgl0872	rpf2	T24T	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C967712T	silent	NCgl0875		T295T	ABC-type transporter	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C968115T	exchange	NCgl0875		L430F	ABC-type transporter	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G971784A	silent	NCgl0880	pvdS1	Q307Q	hypothetical protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C972104T	intergenic	NCgl0880, NCgl0881	pvdS1			wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G988774A	exchange	NCgl0894		G251E	ABC-type transporter, permease component	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G989597A	exchange	NCgl0895		D232N	ABC-type transporter, permease component	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G990379A	exchange	NCgl0896		V134M	ABC-type transporter, ATPase component	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G1004489A	silent	NCgl0910		Q179Q	hypothetical protein	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt

SNP	TYPE	GENES	NAME	AA-ex.	FUNCTION	K118	K117	K063	K005	K112	K120	K051	K079	K093	K013
C1006486T	exchange	NCgl0912		A133V	two-component system, response regulator	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G1007790A	exchange	NCgl0913		A248T	hypothetical membrane protein	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G1009921A	exchange	NCgl0914		D420N	putative ABC transporter ATPase component	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G1011314A	exchange	NCgl0915		V372I	ABC-type transporter, ATPase component and permease component	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G1029949A	exchange	NCgl0928		A112V	hypothetical membrane protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G1037601A	exchange	NCgl0938	ppx2	A52T	exopolyphosphatase	wt	wt	wt	wt	wt	wt	wt	wt	wt	mut
G1053320A	exchange	NCgl0955	pabAB	E441K	anthranilate/para-aminobenzoate synthase component I	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G1056739A	silent	NCgl0960		D154D	allophanate hydrolase subunit 2	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G1057127A	exchange	NCgl0960		A25V	allophanate hydrolase subunit 2	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G1058846A	silent	NCgl0963		G362G	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G1059338A	silent	NCgl0963		S198S	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G1059567A	exchange	NCgl0963, NCgl0964		A70T; A122V		wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G1065395A	exchange	NCgl0971		S158F	hypothetical protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C1070002T	exchange	NCgl0975	ssuD2	T15I	coenzyme F420-dependent N5,N10-methylene tetrahydromethanopterin reductase	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G1071321A	intergenic	NCgl0975, NCgl0976	ssuD2,glpX			wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G1072443A	exchange	NCgl0976	glpX	A14V	putative fructose-1,6-bisphosphatase/sedoheptulose 1.7-bisphosphatase	wt	wt	wt	wt	wt	wt	wt	wt	wt	mut
G1075987A	silent	NCgl0981	xseA	I399I	exodeoxyribonuclease VII large subunit	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
C1078293T	intergenic	NCgl0982, NCgl0983	lytB			wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G1079261A	exchange	NCgl0984		T477M	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G1085385A	exchange	NCgl0988		A336T	predicted GTPase	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G1088545A	exchange	NCgl0993	tnp9a (ISCg9a)	A399V	transposase	wt	wt	wt	wt	wt	wt	wt	wt	wt	mut
G1090758A	exchange	NCgl0994		G199D	GGDEF family protein	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G1091700A	exchange	NCgl0994		G513E	GGDEF family protein	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C1098118T	silent	NCgl1003		N109N	aldo/keto reductase	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt

SNP	TYPE	GENES	NAME	AA-ex.	FUNCTION	K118	K117	K063	K005	K112	K120	K051	K079	K093	K013
C1098543T	exchange	NCgl1003		T251I	aldo/keto reductase	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G1107972A	exchange	NCgl1013		G157E	phosphoglycerate mutase	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C1111076T	stop	NCgl1016		Q429*	ABC-type transporter, duplicated ATPase component	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G1114526A	exchange	NCgl1021		R14H	transposase	wt	wt	wt	wt	wt	wt	wt	wt	wt	mut
G1123383A	silent	NCgl1030	phnB2	K111K	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G1124099A	exchange	NCgl1031	benK3	P243L	permease of the major facilitator superfamily	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G1127220A	exchange	NCgl1034		G70S	ABC-type transporter, duplicated ATPase component	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G1127588A	silent	NCgl1034		V192V	ABC-type transporter, duplicated ATPase component	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
G1127999A	silent	NCgl1034		A329A	ABC-type transporter, duplicated ATPase component	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G1131144A	exchange	NCgl1038		A142T	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G1132367A	silent	NCgl1040		G702G	excinuclease ATPase subunit	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G1133399A	silent	NCgl1040		R358R	excinuclease ATPase subunit	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
G1136659A	exchange	NCgl1042		L59F	hypothetical membrane protein	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
G1139416A	exchange	NCgl1046		G74D	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
T1142670C	exchange	NCgl1051		I116V	hypothetical protein	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
G1147438A	exchange	NCgl1054		D448N	ABC-type transporter, periplasmic component	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G1150998A	silent	NCgl1059		K186K	hypothetical membrane protein	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G1151164A	intergenic	NCgl1059, NCgl1060				wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G1153084A	silent	NCgl1061	dapD	A60A	tetrahydrodipicolinate N-succinyltransferase	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G1158732A	exchange	NCgl1067		G70D	glycosyltransferase	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G1164341A	exchange	NCgl1073	glgC	D214N	glucose-1-phosphate adenylyltransferase	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G1165101A	exchange	NCgl1074		A171V	hypothetical protein	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G1165475A	silent	NCgl1074		T46T	hypothetical protein	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G1166765A	exchange	NCgl1076		A64T	hypothetical protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G1175231A	silent	NCgl1084	kgd	T347T	alpha-ketoglutarate decarboxylase	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G1177157A	silent	NCgl1085		P964P	ABC-type transporter, duplicated ATPase component	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt

SNP	TYPE	GENES	NAME	AA-ex.	FUNCTION	K118	K117	K063	K005	K112	K120	K051	K079	K093	K013
G1178622A	exchange	NCgl1085		A476V	ABC-type transporter, duplicated ATPase component	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G1182503A	exchange	NCgl1088	lipT	A171T	carboxylesterase type B	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G1182514A	silent	NCgl1088	lipT	G174G	carboxylesterase type B	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
G1205525A	exchange	NCgl1105		T435I	putative helicase	wt	wt	wt	wt	wt	wt	wt	wt	wt	mut
G1206394A	silent	NCgl1105		N145N	putative helicase	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C1206772T	silent	NCgl1105		K19K	putative helicase	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G1223232A	exchange	NCgl1118		G81S	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G1227894A	exchange	NCgl1121	lipP	G2E	esterase/lipase	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G1245504A	exchange	NCgl1138		A10V	Aerobic repressor of nitrate reductase R	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G1246476A	silent	NCgl1139	narI	F7F	nitrate reductase gamma subunit	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G1256595A	exchange	NCgl1145		P27L	serine protease	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G1261788A	exchange	NCgl1150	moaA	A344V	molybdenum cofactor biosynthesis protein A	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G1262622A	exchange	NCgl1150	moaA	T66I	molybdenum cofactor biosynthesis protein A	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G1267745A	exchange	NCgl1153	prfA	D106N	peptide chain release factor 1	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G1268187A	exchange	NCgl1153	prfA	G253D	peptide chain release factor 1	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G1268521A	silent	NCgl1154		G5G	predicted rRNA or tRNA methylase	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G1272376A	exchange	NCgl1159	atpB	E13K	ATP synthase subunit A	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G1279584A	intergenic	NCgl1166, NCgl1167	atpC			wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G1279748A	intergenic	NCgl1166, NCgl1167	atpC			wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G1283092A	exchange	NCgl1172		G300E	thioredoxin domain-containing protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C1284480T	intergenic	NCgl1173, NCgl1174	ssuD1,ssuC			wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G1285133A	exchange	NCgl1174	ssuC	R206H	ABC-type transporter, permease component	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G1285304A	exchange	NCgl1175	ssuB	M1I	ABC-type transporter, ATPase component	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
G1286671A	exchange	NCgl1176	ssuA	G210E	ABC-type transporter, periplasmic component	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C1290985T	exchange	NCgl1178	glgE	G185D	glycosidase	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G1293767A	silent	NCgl1180		G180G	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt

SNP	TYPE	GENES	NAME	AA-ex.	FUNCTION	K118	K117	K063	K005	K112	K120	K051	K079	K093	K013
G1297397A	silent	NCgl1184	nifS1	E62E	cysteine sulfinate desulfinase	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C1299071T	exchange	NCgl1186		G359R	uncharacterized protein related to capsule biosynthesis enzyme	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G1310516A	silent	NCgl1197		R194R	hypothetical protein	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G1318422A	exchange	NCgl1204		G330D	ABC-type transporter, duplicated ATPase component	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G1318519A	silent	NCgl1204		L362L	ABC-type transporter, duplicated ATPase component	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C1318718T	exchange	NCgl1204		L429F	ABC-type transporter, duplicated ATPase component	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G1321544A	stop	NCgl1208		W23*	acetyltransferase	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C1323363T	exchange	NCgl1209		A324V	ABC-type transport system, periplasmic component	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G1328466A	exchange	NCgl1214	lysE	A163V	lysine efflux permease	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C1328522T	silent	NCgl1214	lysE	K144K	lysine efflux permease	wt	wt	wt	wt	wt	wt	wt	wt	mut	wt
G1343545A	exchange	NCgl1228		P44L	ABC-type transport system periplasmic component	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G1345024A	silent	NCgl1231		G30G	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C1345651T	exchange	NCgl1232		L56F	Co/Zn/Cd efflux system component	wt	wt	wt	wt	wt	wt	wt	wt	mut	wt
G1347031A	exchange	NCgl1233		G192R	hypothetical protein	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G1349367A	exchange	NCgl1234		G345D	hypothetical protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C1352516T	intergenic	NCgl1235, NCgl1236	serA			wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G1354906A	silent	NCgl1238		D221D	ABC-type transport system periplasmic component	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G1355456A	exchange	NCgl1238		S38F	ABC-type transport system periplasmic component	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G1363055A	silent	NCgl1245		T28T	predicted transcriptional regulator	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G1367538A	intergenic	NCgl1250, NCgl1251				wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G1367906A	intergenic	NCgl1250, NCgl1251				wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G1369070A	exchange	NCgl1251		R224H	hypothetical protein	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G1370174A	silent	NCgl1253	thiC	F488F	thiamine biosynthesis protein ThiC	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C1370778T	exchange	NCgl1253	thiC	R287H	thiamine biosynthesis protein ThiC	wt	wt	wt	wt	wt	wt	wt	wt	mut	wt
G1374411A	exchange	NCgl1255	glgP1	R442C	glucan phosphorylase	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G1374931A	silent	NCgl1255	glgP1	S268S	glucan phosphorylase	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt

SNP	TYPE	GENES	NAME	AA-ex.	FUNCTION	K118	K117	K063	K005	K112	K120	K051	K079	K093	K013
G1378312A	exchange	NCgl1258		S35F	predicted permeases	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G1379428A	stop	NCgl1260		W142*	guanosine polyphosphate pyrophosphohydrolase/synthetase	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G1386827A	silent	NCgl1268		N148N	hypothetical protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G1387673A	silent	NCgl1269	thiL	E112E	thiamine monophosphate kinase	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G1387839A	exchange	NCgl1269	thiL	G168R	thiamine monophosphate kinase	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G1393418A	exchange	NCgl1274		D89N	N6-adenine-specific methylase	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G1395578A	exchange	NCgl1277		S313F	ABC-type transporter, permease component	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C1396615T	stop	NCgl1278		W316*	ABC-type transporter, periplasmic component	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G1402578A	exchange	NCgl1284		G103R	hypothetical protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G1403503A	exchange	NCgl1285		G126R	hypothetical protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G1407700A	silent	NCgl1291		D58D	hypothetical protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G1416131A	exchange	NCgl1299	polA	G771E	DNA polymerase I	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G1420201A	exchange	NCgl1303		A216V	SAM-dependent methyltransferase	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G1420334A	exchange	NCgl1303		P172S	SAM-dependent methyltransferase	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G1420999A	intergenic	NCgl1303, NCgl1304	rpsA			wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G1423078A	intergenic	NCgl1304, NCgl1305	rpsA,ptsG			wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G1428919A	exchange	NCgl1309	iunH3	H81Y	inosine-uridine nucleoside N-ribohydrolase	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C1429573T	exchange	NCgl1310		G357E	permease of the major facilitator superfamily	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
T1431020C	exchange	NCgl1311	rbsK1	H187R	ribokinase family sugar kinase	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
G1431501A	exchange	NCgl1311	rbsK1	P27S	ribokinase family sugar kinase	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G1431544A	silent	NCgl1311	rbsK1	V12V	ribokinase family sugar kinase	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G1431702A	exchange	NCgl1312	ccpA1	S304F	transcriptional regulator	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G1435360A	exchange	NCgl1315	uvrB	G440D	excinuclease ABC subunit B	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G1437375A	exchange	NCgl1318		G11E	predicted nucleoside-diphosphate-sugar epimerase	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G1438402A	exchange	NCgl1319		T692I	hypothetical protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G1439894A	exchange	NCgl1319		H195Y	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt

SNP	TYPE	GENES	NAME	AA-ex.	FUNCTION	K118	K117	K063	K005	K112	K120	K051	K079	K093	K013
C1440180T	silent	NCgl1319		E99E	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G1441278A	silent	NCgl1320		T103T	hypothetical protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G1442674A	exchange	NCgl1322	uvrA	R63H	excinuclease ABC subunit A	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G1445871A	exchange	NCgl1323		L526F	hypothetical membrane protein	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C1445978T	exchange	NCgl1323		G490D	hypothetical membrane protein	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G1448312A	exchange	NCgl1324	infC	G174E	translation initiation factor IF3	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G1449082A	intergenic	NCgl1326, NCgl1327	rplT			wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
T1451127C	silent	NCgl1329	ugpA	R70R	ABC-type transporter, permease component	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G1453362A	exchange	NCgl1331	ugpB	G202D	ABC-type transporter, periplasmic component	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G1454580A	exchange	NCgl1332	ugpC	V156M	ABC-type transporter, ATPase component	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G1456213A	intergenic	NCgl1333, NCgl1334	glpQ2,tsnR			wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C1457672T	exchange	NCgl1335	pheS	T214I	phenylalanyl-tRNA synthetase alpha subunit	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C1458045T	silent	NCgl1335	pheS	T338T	phenylalanyl-tRNA synthetase alpha subunit	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G1461670A	exchange	NCgl1337		A172T	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C1473477T	silent	NCgl1348		T167T	hypothetical protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G1480784A	ribosomal	rRNA:rRNA ,NCg1r08				wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C1492673T	exchange	NCgl1361		A176V	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G1494048A	exchange	NCgl1362	pyrG	R179H	CTP synthetase	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G1504008A	exchange	NCgl1371		A244T	16S rRNA uridine-516 pseudouridylate synthase	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C1504872T	exchange	NCgl1372	cmk	S212F	cytidylate kinase	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G1505393A	exchange	NCgl1373		G150D	GTP-binding protein EngA	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C1505434T	exchange	NCgl1373		P164S	GTP-binding protein EngA	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C1513001T	silent	NCgl1380		R509R	NhaP-type Na+/H+ and K+/H+ antiporter	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C1515009T	exchange	NCgl1381		A51T	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G1515360A	intergenic	NCgl1381, NCgl1382				wt	wt	wt	wt	wt	wt	wt	mut	wt	wt

SNP	TYPE	GENES	NAME	AA-ex.	FUNCTION	K118	K117	K063	K005	K112	K120	K051	K079	K093	K013
G1529861A	exchange	NCgl1395		A126T	siderophore-interacting protein	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G1530722A	silent	NCgl1396	gnd	N357N	6-phosphogluconate dehydrogenase	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G1531326A	exchange	NCgl1396	gnd	S156F	6-phosphogluconate dehydrogenase	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G1533565A	silent	NCgl1399		R175R	ABC-type transporter, ATPase component	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
T1533818C	exchange	NCgl1400		V13A	ABC-type transporter, permease component	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G1534335A	silent	NCgl1400		R185R	ABC-type transporter, permease component	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G1535335A	stop	NCgl1401		Q23*	transcriptional regulator	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G1537192A	silent	NCgl1404	pctB	N214N	ABC-type transporter, ATPase component	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G1540026A	exchange	NCgl1407	thiD1	P460S	thiamine-phosphate pyrophosphorylase	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G1540176A	exchange	NCgl1407	thiD1	P410S	thiamine-phosphate pyrophosphorylase	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
G1542250A	exchange	NCgl1408	thiM	H225Y	hydroxyethylthiazole kinase	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G1547166A	exchange	NCgl1411		P176L	permease of the major facilitator superfamily	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
G1552445A	intergenic	NCgl1416, NCgl1417				wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C1552508T	intergenic	NCgl1416, NCgl1417				wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
C1554098T	exchange	NCgl1418		G196E	hypothetical protein	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G1554224A	exchange	NCgl1418		T154I	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C1556970T	intergenic	NCgl1422, NCgl1423	ppmC			wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
G1560037A	silent	NCgl1425		I11I	hypothetical membrane protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C1560079T	intergenic	NCgl1425, NCgl1426	lip			wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
G1560126A	intergenic	NCgl1425, NCgl1426	lip			wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G1563568A	exchange	NCgl1428	cobL	P79S	precorrin-6B methylase 1	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
C1563777T	exchange	NCgl1428	cobL	G9E	precorrin-6B methylase 1	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G1564218A	intergenic	NCgl1428, NCgl1429	cobL			wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G1564255A	intergenic	NCgl1428, NCgl1429	cobL			wt	wt	wt	wt	wt	wt	wt	wt	mut	mut

SNP	TYPE	GENES	NAME	AA-ex.	FUNCTION	K118	K117	K063	K005	K112	K120	K051	K079	K093	K013
C1572545T	exchange	NCgl1436		D307N	predicted transcriptional regulator	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G1573545A	silent	NCgl1437		S461S	hypothetical protein	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G1574083A	exchange	NCgl1437		T282I	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G1578776A	exchange	NCgl1441	pimT	P209S	predicted SAM-dependent methyltransferase involved in tRNA-Met maturation	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C1582098T	exchange	NCgl1444		A83V	plasmid maintenance system antidote protein	wt	mut	wt	wt	wt	wt	wt	wt	wt	wt
G1582969A	silent	NCgl1445		I171I	permease of the major facilitator superfamily	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C1583741T	intergenic	NCgl1445, NCgl1446	aspA			wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
C1584724T	exchange	NCgl1446	aspA	R256Q	aspartate ammonia-lyase	wt	mut	wt	wt	wt	wt	wt	wt	wt	wt
G1588709A	exchange	NCgl1450	metH	P843S	Methionine synthase I, cobalamin-binding domain	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C1591786T	silent	NCgl1451		G148G	hypothetical protein	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C1592041T	exchange	NCgl1452		G309E	predicted flavoprotein involved in K+ transport	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G1598760A	exchange	NCgl1459		A32T	predicted oxidoreductase	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C1600250T	exchange	NCgl1460	lppL	T191I	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C1602783T	exchange	NCgl1463		P42S	hypothetical protein	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
C1608887T	exchange	NCgl1468	tetA	G121S	ABC-type transporter, ATPase component and permease component	wt	mut	wt	wt	wt	wt	wt	wt	wt	wt
C1612737T	exchange	NCgl1471	mcmB	D570N	methylmalonyl-CoA mutase	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
G1616902A	exchange	NCgl1473		A126T	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C1617158T	exchange	NCgl1473		P211L	hypothetical protein	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
G1617319A	intergenic	NCgl1473, NCgl1474				wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C1617585T	exchange	NCgl1474		A63V	hypothetical protein	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
G1618332A	exchange	NCgl1475		P429S	membrane protease subunit	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C1618634T	exchange	NCgl1475		R328H	membrane protease subunit	wt	mut	wt	wt	wt	wt	wt	wt	wt	wt
C1620538T	exchange	NCgl1477		E158K	hypothetical protein	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
G1620605A	silent	NCgl1477		D135D	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C1620612T	exchange	NCgl1477		G133E	hypothetical protein	wt	mut	wt	wt	wt	wt	wt	wt	wt	wt

SNP	TYPE	GENES	NAME	AA-ex.	FUNCTION	K118	K117	K063	K005	K112	K120	K051	K079	K093	K013
C1624940T	intergenic	NCgl1480, NCgl1481				wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
G1625121A	intergenic	NCgl1480, NCgl1481				wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C1626696T	exchange	NCgl1482	acn	P140S	aconitate hydratase	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
C1629531T	silent	NCgl1483		G78G	transcriptional regulator	wt	mut	wt	wt	wt	wt	wt	wt	wt	wt
G1629541A	exchange	NCgl1483		V82M	transcriptional regulator	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C1629624T	silent	NCgl1483		D109D	transcriptional regulator	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
G1630046A	exchange	NCgl1484		R45Q	glutamine amidotransferase	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
G1631097A	exchange	NCgl1485		A81V	predicted nucleoside-diphosphate-sugar epimerase	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C1633282T	silent	NCgl1487		H448H	hypothetical protein	wt	mut	wt	wt	wt	wt	wt	wt	wt	wt
C1634384T	silent	NCgl1488	pacL	H273H	cation transport ATPase	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
G1636261A	exchange	NCgl1489		L153F	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C1637037T	intergenic	NCgl1489, NCgl1490				mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
G1639535A	intergenic	NCgl1490, NCgl1491				wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C1639595T	intergenic	NCgl1490, NCgl1491				wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G1642112A	exchange	NCgl1493		T188I	ABC-type transporter, duplicated ATPase component	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G1642735A	intergenic	NCgl1493, NCgl1494				wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C1643173T	exchange	NCgl1494		G349E	hypothetical protein	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
C1644956T	exchange	NCgl1495	nanH	A187T	hypothetical protein	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
C1645012T	exchange	NCgl1495	nanH	G168E	hypothetical protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C1645026T	silent	NCgl1495	nanH	K163K	hypothetical protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G1647007A	silent	NCgl1497		Q153Q	hypothetical protein	wt	mut	wt	wt	wt	wt	wt	wt	wt	wt
G1647240A	exchange	NCgl1498		A128V	putative aromatic ring hydroxylating enzyme	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G1647576A	exchange	NCgl1498		A16V	putative aromatic ring hydroxylating enzyme	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C1647592T	exchange	NCgl1498		V11I	putative aromatic ring hydroxylating enzyme	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt

SNP	TYPE	GENES	NAME	AA-ex.	FUNCTION	K118	K117	K063	K005	K112	K120	K051	K079	K093	K013
C1650274T	exchange	NCgl1502	sufD	S384N	predicted iron-regulated ABC-type transporter SufB	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
G1650388A	exchange	NCgl1502	sufD	A346V	predicted iron-regulated ABC-type transporter SufB	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C1651765T	exchange	NCgl1503	sufB	A371T	predicted iron-regulated ABC-type transporter SufB	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G1652298A	exchange	NCgl1503	sufB	S193F	predicted iron-regulated ABC-type transporter SufB	wt	mut	wt	wt	wt	wt	wt	wt	wt	wt
G1652871A	exchange	NCgl1503	sufB	T2I	predicted iron-regulated ABC-type transporter SufB	wt	mut	wt	wt	wt	wt	wt	wt	wt	wt
G1654052A	exchange	NCgl1505	mptB	E19K	a(1->6) mannopyranosyltransferase	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
C1654801T	silent	NCgl1505	mptB	Y268Y	a(1->6) mannopyranosyltransferase	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G1657453A	exchange	NCgl1507		V248M	ABC-type transporter, permease component	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C1657814T	silent	NCgl1508	ctaA	T59T	cytochrome oxidase assembly protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C1658012T	silent	NCgl1508	ctaA	I125I	cytochrome oxidase assembly protein	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C1661018T	exchange	NCgl1509		A744V	putative helicase	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G1662448A	exchange	NCgl1510	qor	V294I	NADPH:quinone reductase	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G1666562A	intergenic	NCgl1512, NCgl1513	tkt,tal			wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
C1667519T	exchange	NCgl1513	tal	P283S	transaldolase	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G1667641A	silent	NCgl1513	tal	E323E	transaldolase	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C1670450T	exchange	NCgl1516	devB	S19F	6-phosphogluconolactonase	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G1672392A	exchange	NCgl1518		D214N	hypothetical protein	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
G1680915A	exchange	NCgl1524	tpi	T65I	triosephosphate isomerase	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G1682073A	exchange	NCgl1525	pgk	T111I	phosphoglycerate kinase	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C1682090T	silent	NCgl1525	pgk	E105E	phosphoglycerate kinase	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
G1682277A	exchange	NCgl1525	pgk	A43V	phosphoglycerate kinase	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
G1683071A	silent	NCgl1526	gap	D185D	glyceraldehyde-3-phosphate dehydrogenase	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C1686307T	exchange	NCgl1529		G258R	hypothetical protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C1687698T	exchange	NCgl1530	uvrC	G498E	excinuclease ABC subunit C	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G1687733A	silent	NCgl1530	uvrC	Y486Y	excinuclease ABC subunit C	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G1688309A	silent	NCgl1530	uvrC	D294D	excinuclease ABC subunit C	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut

SNP	TYPE	GENES	NAME	AA-ex.	FUNCTION	K118	K117	K063	K005	K112	K120	K051	K079	K093	K013
G1688544A	exchange	NCgl1530	uvrC	A216V	excinuclease ABC subunit C	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C1689714T	silent	NCgl1531	ribX	G22G	hypothetical membrane protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C1691313T	exchange	NCgl1533	ribA	G105S	bifunctional 3,4-dihydroxy-2-butanone 4-phosphate synthase/GTP cyclohydrolase II protein	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
C1691804T	silent	NCgl1534	ribC	E156E	riboflavin synthase subunit alpha	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
C1692105T	exchange	NCgl1534	ribC	G56E	riboflavin synthase subunit alpha	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G1693323A	exchange	NCgl1536	rpe	S199F	ribulose-phosphate 3-epimerase	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
C1694535T	exchange	NCgl1537	fmu	G255D	tRNA and rRNA cytosine-C5-methylase	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G1696076A	exchange	NCgl1538	fmt	A123V	methionyl-tRNA formyltransferase	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C1698877T	exchange	NCgl1540	priA	A91T	primosome assembly protein PriA	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C1699889T	exchange	NCgl1541	metK	G170D	S-adenosylmethionine synthetase	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
G1700233A	silent	NCgl1541	metK	V55V	S-adenosylmethionine synthetase	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C1704442T	exchange	NCgl1547	carB	A1086T	carbamoyl-phosphate synthase large subunit	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G1705574A	silent	NCgl1547	carB	F708F	carbamoyl-phosphate synthase large subunit	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G1707987A	exchange	NCgl1548	carA	P300S	carbamoyl-phosphate synthase small subunit	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C1710081T	exchange	NCgl1549	pyrC	A93T	dihydroorotase	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C1712806T	exchange	NCgl1552		P71S	predicted SulA family nucleoside-diphosphate sugar epimerase	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C1712913T	silent	NCgl1552		R106R	predicted SulA family nucleoside-diphosphate sugar epimerase	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
C1713697T	exchange	NCgl1552		P368S	predicted SulA family nucleoside-diphosphate sugar epimerase	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
G1714426A	exchange	NCgl1554		G43E	hypothetical protein	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
G1715754A	silent	NCgl1556	nusB	S103S	transcription antitermination protein NusB	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G1715974A	exchange	NCgl1556	nusB	S30F	transcription antitermination protein NusB	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G1719879A	silent	NCgl1561	aroC	G340G	chorismate synthase	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G1723657A	exchange	NCgl1565		G263D	ABC-type transporter, periplasmic component	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C1724848T	exchange	NCgl1567	aroE3	A198T	shikimate 5-dehydrogenase	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C1724897T	silent	NCgl1567	aroE3	E181E	shikimate 5-dehydrogenase	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G1725657A	silent	NCgl1568		A323A	predicted periplasmic solute-binding protein	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt

SNP	TYPE	GENES	NAME	AA-ex.	FUNCTION	K118	K117	K063	K005	K112	K120	K051	K079	K093	K013
G1727443A	exchange	NCgl1570	alaS	A869V	alanyl-tRNA synthetase	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
G1727530A	exchange	NCgl1570	alaS	A840V	alanyl-tRNA synthetase	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
G1727901A	silent	NCgl1570	alaS	Y716Y	alanyl-tRNA synthetase	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
C1730210T	exchange	NCgl1571		A445T	uncharacterized ATPase related to the helicase subunit of the Holliday junction resolvase	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C1731672T	exchange	NCgl1572		R375H	hypothetical protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G1732049A	silent	NCgl1572		T249T	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C1735654T	exchange	NCgl1574		A204V	predicted metalloprotease	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
G1736818A	exchange	NCgl1575		T621I	hypothetical helicase	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G1736914A	exchange	NCgl1575		A589V	hypothetical helicase	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C1741348T	silent	NCgl1578		I12I	transcriptional regulator	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
G1742682A	intergenic	NCgl1579, NCgl1580				wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
C1744594T	intergenic	NCgl1582, NCgl1583	sdaA			wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C1745519T	silent	NCgl1583	sdaA	I212I	L-serine deaminase	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
G1749703A	silent	NCgl1586		V87V	putative Zn-dependent hydrolase	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
C1750063T	intergenic	NCgl1586, NCgl1587	ppiB			wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C1751537T	exchange	NCgl1588		T14I	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C1752077T	intergenic	NCgl1588, NCgl1589				wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G1753269A	silent	NCgl1590	rel	S542S	guanosine polyphosphate pyrophosphohydrolase/synthetase	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
C1759022T	silent	NCgl1594	secD	L571L	protein export protein SecD	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
G1760378A	silent	NCgl1594	secD	S119S	protein export protein SecD	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
G1762022A	silent	NCgl1596	ruvB	V162V	Holliday junction DNA helicase RuvB	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
G1766619A	silent	NCgl1602		A110A	hypothetical protein	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
C1766632T	exchange	NCgl1602		R106H	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G1768410A	exchange	NCgl1604		S196F	lipid A biosynthesis lauroyl acyltransferase	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
G1769709A	exchange	NCgl1606		A171V	diadenosine tetraphosphate (Ap4A) hydrolase	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt

SNP	TYPE	GENES	NAME	AA-ex.	FUNCTION	K118	K117	K063	K005	K112	K120	K051	K079	K093	K013
C1777109T	intergenic	NCgl1610, NCgl1611				wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G1778164A	exchange	NCgl1611		R449C	hypothetical protein	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
G1782794A	intergenic	NCgl1615, NCgl1616				wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C1783673T	exchange	NCgl1616		G601S	hypothetical membrane protein	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
G1787513A	silent	NCgl1618		R439R	hypothetical protein	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
C1788008T	silent	NCgl1618		G274G	hypothetical protein	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
C1789256T	silent	NCgl1619		R59R	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C1790469T	exchange	NCgl1622		V112M	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C1790560T	stop	NCgl1622		W81*	hypothetical protein	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
C1791082T	intergenic	NCgl1622, NCgl1623				mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
C1792408T	silent	NCgl1623		I189I	ABC-type transporter, ATPase component	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
C1793449T	intergenic	NCgl1624, NCgl1625				mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
G1793618A	intergenic	NCgl1624, NCgl1625				wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
C1796297T	exchange	NCgl1627		P30S	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C1799883T	silent	NCgl1631		S60S	hypothetical protein	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
G1803523A	intergenic	NCgl1635, NCgl1636				wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
G1811241A	exchange	NCgl1646		S202N	hypothetical protein	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
G1811882A	exchange	NCgl1647		D107N	hypothetical protein	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
G1820939A	exchange	NCgl1658		L485F	hypothetical protein	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt

SNP	TYPE	GENES	NAME	AA-ex.	FUNCTION	K118	K117	K063	K005	K112	K120	K051	K079	K093	K013
G1825335A	exchange	NCgl1661		S91F	hypothetical protein	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C1827925T	intergenic	NCgl1664, NCgl1665	tnp14a(ISCg14a)			wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C1827974A	intergenic	NCgl1664, NCgl1665	tnp14a(ISCg14a)			wt	wt	mut	wt	wt	wt	wt	wt	wt	wt
G1827996A	intergenic	NCgl1664, NCgl1665	tnp14a(ISCg14a)			wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
C1828567T	intergenic	NCgl1664, NCgl1665	tnp14a(ISCg14a)			mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
C1829744T	exchange	NCgl1665		D53N	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G1833560A	exchange	NCgl1667	recJ	R459H	single-stranded DNA-specific exonuclease	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C1835615T	intergenic	NCgl1668, NCgl1669	priP			wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C1836162T	intergenic	NCgl1668, NCgl1669	priP			wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C1837258T	exchange	NCgl1669	priP	P236L	predicted ATPase	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C1839005T	silent	NCgl1670		L219L	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C1842239T	exchange	NCgl1671		S2F	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G1843142A	silent	NCgl1672		E74E	hypothetical membrane protein	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
G1844865A	exchange	NCgl1673		V450I	hypothetical helicase	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
C1847653T	silent	NCgl1677		V109V	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C1849478T	exchange	NCgl1680		A147V	hypothetical protein	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
C1852529T	intergenic	NCgl1683, NCgl1684				mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
C1853129T	silent	NCgl1684		T178T	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C1857205T	silent	NCgl1688		N97N	hypothetical protein	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
C1861563T	intergenic	NCgl1691, NCgl1692				wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C1862146T	intergenic	NCgl1691, NCgl1692				wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C1865603T	intergenic	NCgl1692, NCgl1693				wt	wt	mut	mut	mut	wt	wt	wt	wt	wt

SNP	TYPE	GENES	NAME	AA-ex.	FUNCTION	K118	K117	K063	K005	K112	K120	K051	K079	K093	K013
C1866003T	silent	NCgl1694		I54I	hypothetical protein	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C1867289T	silent	NCgl1697		H64H	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C1873741T	silent	NCgl1702		E1382E	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C1875927T	exchange	NCgl1702		D654N	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C1876995T	exchange	NCgl1702		G298S	hypothetical protein	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
C1879591T	silent	NCgl1704	cglIR	T60T	stress-sensitive restriction system protein 1	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C1880982T	exchange	NCgl1705		L165F	stress-sensitive restriction system protein 2	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C1883645T	exchange	NCgl1706		G116S	hypothetical protein	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
C1886837T	silent	NCgl1708		T536T	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C1887890T	stop	NCgl1710		W38*	hypothetical helicase	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C1889168T	intergenic	NCgl1711, NCgl1712				wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C1889288T	intergenic	NCgl1711, NCgl1712				wt	mut	wt	wt	wt	wt	wt	wt	wt	wt
C1889489T	intergenic	NCgl1711, NCgl1712				wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C1891168T	silent	NCgl1712		E150E	hypothetical protein	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C1891246T	silent	NCgl1712		Q124Q	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C1891770T	intergenic	NCgl1712, NCgl1713				mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
C1894458T	exchange	NCgl1714		A59T	hypothetical membrane protein	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
C1895503T	exchange	NCgl1715		A577T	hypothetical protein	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
C1898816T	exchange	NCgl1716		E115K	ATPase with chaperone activity, ATP-binding subunit	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C1900541T	exchange	NCgl1718		E79K	hypothetical membrane protein	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
C1901734T	exchange	NCgl1719		G60S	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C1901908T	exchange	NCgl1719		A2T	hypothetical protein	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
C1902341T	silent	NCgl1720		K181K	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C1903357T	exchange	NCgl1721		R841H	hypothetical protein	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
C1904322T	silent	NCgl1721		E519E	hypothetical protein	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt

SNP	TYPE	GENES	NAME	AA-ex.	FUNCTION	K118	K117	K063	K005	K112	K120	K051	K079	K093	K013
C1904798T	exchange	NCgl1721		D361N	hypothetical protein	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
C1906184T	exchange	NCgl1722		G130E	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C1910011T	silent	NCgl1726		Q166Q	hypothetical protein	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
C1910205T	exchange	NCgl1726		A102T	hypothetical protein	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
C1917918T	silent	NCgl1734		E51E	hypothetical protein	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C1921174T	exchange	NCgl1736		V35I	hypothetical protein	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C1923291T	exchange	NCgl1737		G907D	hypothetical membrane protein	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
C1923297T	exchange	NCgl1737		G905D	hypothetical membrane protein	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
C1925528T	silent	NCgl1737		R161R	hypothetical membrane protein	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
C1927974T	silent	NCgl1739		A72A	hypothetical protein	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
C1931229T	intergenic	NCgl1743, NCgl1744				wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C1932326T	intergenic	NCgl1745, NCgl1746				wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
C1933043T	intergenic	NCgl1746, NCgl1747				mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
C1939480T	exchange	NCgl1753		T112I	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C1940815T	exchange	NCgl1754		H187Y	hypothetical protein	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C1941749T	exchange	NCgl1756		V228M	hypothetical protein	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C1941974T	exchange	NCgl1756		G153R	hypothetical protein	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C1942727T	exchange	NCgl1757		T70I	hypothetical membrane protein	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C1946661T	exchange	NCgl1765		G126E	hypothetical protein	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
C1949047T	exchange	NCgl1767		V802M	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C1961512T	exchange	NCgl1774	psp1	G486R	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C1962509T	silent	NCgl1774	psp1	Q153Q	hypothetical protein	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
C1963366T	exchange	NCgl1775		A22T	hypothetical protein	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
C1963531T	exchange	NCgl1776		A405T	hypothetical protein	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C1964597T	silent	NCgl1776		K49K	hypothetical protein	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
C1966680T	exchange	NCgl1779		T111I	micrococcal nuclease-like protein	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt

SNP	TYPE	GENES	NAME	AA-ex.	FUNCTION	K118	K117	K063	K005	K112	K120	K051	K079	K093	K013
C1966818T	exchange	NCgl1779		A157V	micrococcal nuclease-like protein	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
C1967404T	intergenic	NCgl1779, NCgl1780				wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C1967807T	silent	NCgl1780		D68D	hypothetical protein	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
C1969178T	silent	NCgl1781		H305H	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C1971433T	exchange	NCgl1783		H367Y	hypothetical protein	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C1975213T	exchange	NCgl1788		L15F	single-stranded DNA-binding protein	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C1975642T	exchange	NCgl1788		P158S	single-stranded DNA-binding protein	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C1981496T	exchange	NCgl1797		P178S	hypothetical protein	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
C1985280T	silent	NCgl1805		C63C	hypothetical protein	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
C1986937T	intergenic	NCgl1806, NCgl1807				wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C1988732T	intergenic	NCgl1810, NCgl1811				wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C1989916T	silent	NCgl1812		T283T	hypothetical protein	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C1993579T	silent	NCgl1815		E181E	hypothetical protein	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
A1993809G	silent	NCgl1815		L105L	hypothetical protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C1993912T	silent	NCgl1815		P70P	hypothetical protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G1993984A	silent	NCgl1815		G46G	hypothetical protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C1993987T	silent	NCgl1815		T45T	hypothetical protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
T1994098C	silent	NCgl1815		L8L	hypothetical protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C1994235T	intergenic	NCgl1815, NCgl1816	int2(N-Term)			mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
C1994352T	intergenic	NCgl1815, NCgl1816	int2(N-Term)			wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C1995055T	silent	NCgl1816	int2(N-Term)	R80R	hypothetical protein	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
C1998171T	silent	NCgl1821	ribD	R209R	hypothetical protein	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C1999122T	silent	NCgl1822		V246V	hypothetical membrane protein	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
C1999613T	silent	NCgl1823		L24L	hypothetical protein	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt

SNP	TYPE	GENES	NAME	AA-ex.	FUNCTION	K118	K117	K063	K005	K112	K120	K051	K079	K093	K013
C1999973T	intergenic	NCgl1823, NCgl1824				wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
C2006506T	silent	NCgl1828		K64K	SAM-dependent methyltransferase	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C2008590T	exchange	NCgl1831		P114L	hypothetical membrane protein	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
C2010164T	exchange	NCgl1834	suhB	A134T	archaeal fructose-1,6-bisphosphatase	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
C2010483T	silent	NCgl1834	suhB	K27K	archaeal fructose-1,6-bisphosphatase	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
C2013217T	exchange	NCgl1836	sigA	T452I	RNA polymerase sigma factor	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C2013945T	intergenic	NCgl1836, NCgl1837	sigA			mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
C2015774T	silent	NCgl1838		K116K	hypothetical membrane protein	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C2017634T	silent	NCgl1839		Q111Q	hypothetical protein	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C2028478T	exchange	NCgl1849		A662V	putative helicase	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
C2034120T	silent	NCgl1852	hrpA	V881V	HrpA-like helicase	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G2035455A	silent	NCgl1853		R142R	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	wt	wt	mut
C2035844T	exchange	NCgl1853		V13I	hypothetical protein	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C2037188T	exchange	NCgl1855	lexA	T147I	LexA repressor	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
C2037446T	exchange	NCgl1855	lexA	A233V	LexA repressor	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C2037474T	silent	NCgl1855	lexA	I242I	LexA repressor	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C2037724T	intergenic	NCgl1855, NCgl1856	lexA,sugR			mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
C2038948T	silent	NCgl1857		L120L	fructose-1-phosphate kinase	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C2051744T	exchange	NCgl1867		L147F	hypothetical protein	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
G2055446A	silent	NCgl1871		A348A	hypothetical protein	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
C2059418T	silent	NCgl1874	miaB	L5L	2-methylthioadenine synthetase	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
G2062419A	intergenic	NCgl1877, NCgl1878	gluC,gluD			wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
C2068688T	silent	NCgl1886		Q232Q	phage shock protein A (IM30)	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
C2074829T	exchange	NCgl1893	ftsK	G448S	DNA translocase spoIIIE-like protein	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
C2077865T	exchange	NCgl1895		D471N	predicted hydrolase of the metallo-beta-lactamase superfamily	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt

SNP	TYPE	GENES	NAME	AA-ex.	FUNCTION	K118	K117	K063	K005	K112	K120	K051	K079	K093	K013
C2082391T	exchange	NCgl1899		P48S	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G2083590A	exchange	NCgl1900	gpsI	A534V	polyribonucleotide nucleotidyltransferase	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
C2084500T	exchange	NCgl1900	gpsI	V231I	polyribonucleotide nucleotidyltransferase	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
G2086719A	silent	NCgl1902	iunH2	T36T	inosine-uridine nucleoside N-ribohydrolase	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C2094120T	silent	NCgl1910	infB	K868K	translation initiation factor IF-2	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G2094212A	exchange	NCgl1910	infB	R838C	translation initiation factor IF-2	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
G2094641A	exchange	NCgl1910	infB	P695S	translation initiation factor IF-2	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
C2095416T	silent	NCgl1910	infB	Q436Q	translation initiation factor IF-2	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C2105435T	exchange	NCgl1918		S488F	ABC-type transporter, duplicated ATPase component	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
G2115364A	intergenic	NCgl1926, NCgl1927	mqo			wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G2125335A	exchange	NCgl1936		G83S	ABC-type transporter, permease component	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C2126370T	silent	NCgl1937		L103L	ABC-type transporter, ATPase component	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G2130781A	exchange	NCgl1940	dxr	L100F	1-deoxy-D-xylulose 5-phosphate reductoisomerase	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G2135304A	exchange	NCgl1944		P83L	predicted Fe-S-cluster redox enzyme	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C2138988T	intergenic	NCgl1948, NCgl1949	pyrH,tsf			mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
C2143937T	exchange	NCgl1953		E44K	predicted Rossmann-fold nucleotide-binding protein	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C2148623T	silent	NCgl1959		A131A	ABC-type transport systems, periplasmic component	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
G2155303A	silent	NCgl1966		V477V	predicted RNA binding protein,	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G2156669A	exchange	NCgl1966		A22V	predicted RNA binding protein,	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G2158572A	exchange	NCgl1968		P204S	di- and tricarboxylate transporters	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G2161345A	silent	NCgl1970		L48L	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C2164932T	silent	NCgl1976	rpsP	K126K	30S ribosomal protein S16	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
C2165851T	exchange	NCgl1977		P76L	ankyrin repeat containing protein	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
G2174239A	silent	NCgl1984	ftsY	F422F	signal recognition particle GTPase	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G2175228A	exchange	NCgl1984	ftsY	P93S	signal recognition particle GTPase	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G2180225A	silent	NCgl1986	smc	A956A	chromosome segregation ATPase	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt

SNP	TYPE	GENES	NAME	AA-ex.	FUNCTION	K118	K117	K063	K005	K112	K120	K051	K079	K093	K013
G2181606A	exchange	NCgl1986	smc	S496F	chromosome segregation ATPase	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G2183234A	exchange	NCgl1987		T53I	acylphosphatase	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G2191239A	silent	NCgl1996		P30P	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G2195208A	exchange	NCgl1999	gdh	S292F	glutamate dehydrogenase	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G2195618A	silent	NCgl1999	gdh	H155H	glutamate dehydrogenase	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G2196608A	intergenic	NCgl1999, NCgl2000	gdh,glxK			wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G2201677A	exchange	NCgl2005		P65S	hypothetical membrane protein	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G2207250A	intergenic	NCgl2008, NCgl2009	pyk,lgt			wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C2209092T	exchange	NCgl2010		E20K	indole-3-glycerol phosphate synthase	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
C2213051T	exchange	NCgl2016	hisH	G75R	imidazole glycerol phosphate synthase subunit HisH	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
G2213846A	intergenic	NCgl2016, NCgl2017	hisH			wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G2214530A	exchange	NCgl2017		L369F	permease of the major facilitator superfamily	wt	wt	wt	wt	wt	wt	wt	mut	mut	mut
G2215453A	exchange	NCgl2017		T61I	permease of the major facilitator superfamily	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G2222279A	exchange	NCgl2025		E112K	transcriptional regulator	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G2223430A	silent	NCgl2026	glgX	E301E	pullulanase	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G2225151A	exchange	NCgl2027		M1?	SAM-dependent methyltransferase	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C2227921T	exchange	NCgl2030		P6S	transcriptional regulator	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
G2228110A	exchange	NCgl2030		A69T	transcriptional regulator	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G2230696A	exchange	NCgl2032		A81V	ABC-type transporter, permease component	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G2232703A	intergenic	NCgl2034, NCgl2035				wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G2235274A	exchange	NCgl2037	treY	G141D	maltooligosyl trehalose synthase	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G2237360A	exchange	NCgl2038		G10D	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C2241044T	silent	NCgl2041		F121F	coenzyme F420-dependent N5,N10-methylene tetrahydromethanopterin reductase	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C2242412T	stop	NCgl2044		W211*	hypothetical protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C2245269T	exchange	NCgl2046	ilvA	A302T	threonine dehydratase	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt

SNP	TYPE	GENES	NAME	AA-ex.	FUNCTION	K118	K117	K063	K005	K112	K120	K051	K079	K093	K013
G2245811A	exchange	NCgl2046	ilvA	T121I	threonine dehydratase	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G2252719A	silent	NCgl2050		L234L	predicted permease	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G2253829A	exchange	NCgl2052		S57N	predicted Co/Zn/Cd cation transporter	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C2257514T	intergenic	NCgl2054, NCgl2055	cysY			wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G2259781A	silent	NCgl2056		L74L	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C2268738T	exchange	NCgl2065		G170D	permease of the drug/metabolite transporter (DMT) superfamily	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C2269306T	silent	NCgl2066		T319T	predicted transcriptional regulator	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G2271755A	exchange	NCgl2068	ileS	T799I	isoleucyl-tRNA synthetase	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C2274470T	intergenic	NCgl2068, NCgl2069	ileS			wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C2275040T	exchange	NCgl2070		A275T	cell division initiation protein	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
C2276166T	intergenic	NCgl2070, NCgl2071				wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C2280125T	exchange	NCgl2075	ftsZ	G31D	cell division protein FtsZ	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C2280156T	exchange	NCgl2075	ftsZ	V21I	cell division protein FtsZ	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C2285714T	silent	NCgl2080	murD	K380K	UDP-N-acetylmuramoyl-L-alanyl-D-glutamate synthetase	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
C2288795T	silent	NCgl2082	murF	K239K	UDP-N-acetylmuramyl pentapeptide synthase	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
G2289280A	exchange	NCgl2082	murF	P78S	UDP-N-acetylmuramyl pentapeptide synthase	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C2290845T	exchange	NCgl2083	murE	G81E	UDP-N-acetylmuramoylalanyl-D-glutamate--2, 6-diaminopimelate ligase	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C2291655T	exchange	NCgl2084	ftsI	S504N	cell division protein FtsI	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C2292163T	exchange	NCgl2084	ftsI	D335N	cell division protein FtsI	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G2292307A	silent	NCgl2084	ftsI	L287L	cell division protein FtsI	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C2293174T	intergenic	NCgl2084, NCgl2085	ftsI			wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C2294660T	exchange	NCgl2086	mraW	D157N	S-adenosyl-methyltransferase	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G2296797A	silent	NCgl2088		L35L	hypothetical membrane protein	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G2324518A	exchange	NCgl2111	qcrC	P227S	cytochrome C	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C2324800T	exchange	NCgl2111	qcrC	A133T	cytochrome C	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt

SNP	TYPE	GENES	NAME	AA-ex.	FUNCTION	K118	K117	K063	K005	K112	K120	K051	K079	K093	K013
G2324951A	silent	NCgl2111	qcrC	C82C	cytochrome C	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C2327508T	exchange	NCgl2115	ctaC	G164D	cytochrome C oxidase subunit II	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C2336484T	exchange	NCgl2123	ilvE	G178E	branched-chain amino acid aminotransferase	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G2337267A	exchange	NCgl2124	pepB	G11E	leucyl aminopeptidase	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C2338635T	exchange	NCgl2124	pepB	P467L	leucyl aminopeptidase	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
C2339072T	exchange	NCgl2125		V24M	hypothetical protein	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
C2343581T	silent	NCgl2129		V34V	hypothetical membrane protein	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
C2349336T	silent	NCgl2133	glnA	N169N	glutamine synthase	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
A2352892G	exchange	NCgl2138		D20G	putative translation initiation inhibitor	wt	wt	wt	mut	wt	wt	wt	wt	wt	wt
G2355889A	exchange	NCgl2141		G123D	permease of the major facilitator superfamily	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G2359203A	exchange	NCgl2146	hmuO	A72V	hypothetical protein	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G2370371A	silent	NCgl2153		V57V	exoribonuclease	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G2378244A	silent	NCgl2163		E159E	transcriptional regulator	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G2378868A	intergenic	NCgl2164, NCgl2165				wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G2383655A	exchange	NCgl2169		P286S	ABC-type transporter, permease component	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G2396171A	exchange	NCgl2185	phoD	E319K	phosphodiesterase/alkaline phosphatase D	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G2399436A	silent	NCgl2188	dnaG	R623R	DNA primase	wt	mut	wt	wt	wt	wt	wt	wt	wt	wt
C2400209T	exchange	NCgl2188	dnaG	A366T	DNA primase	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
G2405210A	silent	NCgl2193	dgt	H350H	deoxyguanosinetriphosphate triphosphohydrolase-like protein	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G2406787A	exchange	NCgl2194		P51S	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G2411337A	silent	NCgl2198	glyS	L335L	glycyl-tRNA synthetase	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G2412097A	silent	NCgl2198	glyS	H81H	glycyl-tRNA synthetase	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G2419719A	silent	NCgl2206		L198L	uncharacterized CBS domain-containing protein	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G2421329A	exchange	NCgl2208	phoH2	P208S	phosphate starvation-inducible protein PhoH	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C2421424T	exchange	NCgl2208	phoH2	S176N	phosphate starvation-inducible protein PhoH	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
C2421836T	exchange	NCgl2208	phoH2	G39R	phosphate starvation-inducible protein PhoH	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut

SNP	TYPE	GENES	NAME	AA-ex.	FUNCTION	K118	K117	K063	K005	K112	K120	K051	K079	K093	K013
G2431734A	exchange	NCgl2217	malQ	G480S	4-alpha-glucanotransferase	wt	mut	wt	wt	wt	wt	wt	wt	wt	wt
G2435935A	exchange	NCgl2219	dcp	A302V	Zn-dependent oligopeptidase	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G2438015A	exchange	NCgl2220		V382M	carboxylesterase type B	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G2441664A	exchange	NCgl2224		A565V	hypothetical protein	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G2449758A	exchange	NCgl2230	ectP	S139F	EctP protein	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G2458038A	exchange	NCgl2238		A59T	ABC-type transporter, periplasmic component	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
G2465988A	intergenic	NCgl2244, NCgl2245				wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G2467058A	intergenic	NCgl2245, NCgl2246				wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G2469212A	exchange	NCgl2247	aceB	L311F	malate synthase	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G2471279A	exchange	NCgl2248	aceA	G180D	isocitrate lyase	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G2475800A	intergenic	NCgl2251, NCgl2252	lcoP			mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
G2477813A	exchange	NCgl2254		E55K	hypothetical protein	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
G2478410A	exchange	NCgl2254		E254K	hypothetical protein	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
G2482364A	silent	NCgl2258	dctP	L40L	dicarboxylate-binding periplasmic protein	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G2483691A	silent	NCgl2259	lepA	L235L	GTP-binding protein LepA	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
G2491619A	exchange	NCgl2268		L81F	fructose-2,6-bisphosphatase	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G2491965A	exchange	NCgl2269		S127F	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G2505572A	exchange	NCgl2281	rne	R775C	ribonuclease E	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
G2505980A	exchange	NCgl2281	rne	L639F	ribonuclease E	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
G2507275A	exchange	NCgl2281	rne	T207I	ribonuclease E	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
C2510895T	intergenic	NCgl2284, NCgl2285				wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
C2511277T	silent	NCgl2285		A77A	pirin-related protein	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
G2514280A	silent	NCgl2292	folC	I403I	folylpolyglutamate synthase	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C2515892T	exchange	NCgl2293	valS	E819K	valyl-tRNA synthetase	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
C2518326T	silent	NCgl2293	valS	Q7Q	valyl-tRNA synthetase	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt

SNP	TYPE	GENES	NAME	AA-ex.	FUNCTION	K118	K117	K063	K005	K112	K120	K051	K079	K093	K013
G2522003A	exchange	NCgl2296		H84Y	predicted Rossmann fold nucleotide-binding protein	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
G2524198A	exchange	NCgl2298		V213I	transcriptional regulator	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C2526141T	exchange	NCgl2300	vanA	R348C	ferredoxin subunits of nitrite reductase and ring-hydroxylating dioxygenase	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
C2527942T	silent	NCgl2302	vanK	L240L	permease of the major facilitator superfamily	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
G2529823A	exchange	NCgl2304	clpX	P314S	ATP-dependent protease ATP-binding subunit	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
G2529875A	silent	NCgl2304	clpX	L296L	ATP-dependent protease ATP-binding subunit	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C2530631T	silent	NCgl2304	clpX	E44E	ATP-dependent protease ATP-binding subunit	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
C2533817T	silent	NCgl2308	pcaR	T133T	transcriptional regulator	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
G2536724A	stop	NCgl2311		W176*	DNA-binding HTH domain-containing protein	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C2537471T	silent	NCgl2311		V425V	DNA-binding HTH domain-containing protein	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
G2539326A	exchange	NCgl2313	pcaB1	T136I	putative 3-carboxy-cis,cis-muconate cycloisomerase	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
C2540538T	exchange	NCgl2315	pcaH	R163H	protocatechuate 3,4-dioxygenase beta subunit	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
G2541250A	silent	NCgl2316		L368L	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G2546339A	exchange	NCgl2320	benA	G349S	benzoate dioxygenase large subunit	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G2546514A	exchange	NCgl2320	benA	G407E	benzoate dioxygenase large subunit	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
G2550792A	exchange	NCgl2324		E319K	DNA-binding HTH domain containing protein	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
G2550867A	exchange	NCgl2324		G344S	DNA-binding HTH domain containing protein	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G2551266A	exchange	NCgl2324		E477K	DNA-binding HTH domain containing protein	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G2553663A	exchange	NCgl2325	benK1	G353D	putative benzoate transporter	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G2554121A	exchange	NCgl2326	benE	G32D	putative benzoate membrane transport protein	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
G2561430A	intergenic	NCgl2333, NCgl2334	tnp12a(ISCg12a)			wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G2563720A	exchange	NCgl2336		G253S	hypothetical protein	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
G2565174A	silent	NCgl2338		L25L	uncharacterized membrane protein	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G2571111A	silent	NCgl2342		C79C	hypothetical protein	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
G2571436A	intergenic	NCgl2342, NCgl2343				wt	wt	wt	wt	wt	wt	wt	wt	mut	mut

SNP	TYPE	GENES	NAME	AA-ex.	FUNCTION	K118	K117	K063	K005	K112	K120	K051	K079	K093	K013
G2571506A	intergenic	NCgl2342, NCgl2343				wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
G2573349A	silent	NCgl2346	crtI(N-Term)	L173L	hypothetical protein	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
G2573467A	silent	NCgl2346	crtI(N-Term)	D133D	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G2573667A	exchange	NCgl2346	crtI(N-Term)	P67S	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G2574925A	silent	NCgl2348		F325F	permease of the major facilitator superfamily	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G2576634A	exchange	NCgl2349		P194L	hypothetical protein	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
G2578833A	exchange	NCgl2350		S14F	ABC-type transporter, duplicated ATPase component	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
G2588346A	exchange	NCgl2357		D124N	uncharacterized membrane-associated protein	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G2589868A	silent	NCgl2359		R101R	transcriptional regulator	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
G2591423A	silent	NCgl2360	metB	H16H	cystathionine gamma-synthase	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G2594137A	silent	NCgl2364		I153I	hypothetical protein	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
G2594339A	exchange	NCgl2364		S86F	hypothetical protein	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
G2598455A	silent	NCgl2369		G10G	single-stranded DNA-binding protein	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G2600842A	intergenic	NCgl2370, NCgl2371	phoB			wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G2605340A	exchange	NCgl2373		S61F	ABC-type transporter, permease component	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
G2605368A	exchange	NCgl2373		L52F	ABC-type transporter, permease component	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G2606648A	stop	NCgl2375	amyE	Q415*	ABC-type transporter, periplasmic component	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G2610113A	silent	NCgl2377	msiK1	T176T	ABC-type transporter, ATPase component	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G2610237A	exchange	NCgl2377	msiK1	A135V	ABC-type transporter, ATPase component	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G2612123A	stop	NCgl2379	int3	W197*	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G2612818A	intergenic	NCgl2379, NCgl2380	int3			wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G2616388A	exchange	NCgl2383		S245F	hypothetical protein	wt	mut	wt	wt	wt	wt	wt	wt	wt	wt
G2616391A	exchange	NCgl2383		T244I	hypothetical protein	wt	mut	wt	wt	wt	wt	wt	wt	wt	wt
G2619401A	silent	NCgl2386	ornA	A173A	oligoribonuclease	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut

SNP	TYPE	GENES	NAME	AA-ex.	FUNCTION	K118	K117	K063	K005	K112	K120	K051	K079	K093	K013
G2620207A	exchange	NCgl2387		P175S	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G2620416A	exchange	NCgl2387		S105F	hypothetical protein	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
G2620844A	intergenic	NCgl2387, NCgl2388	lppS			wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G2621460A	silent	NCgl2388	lppS	D241D	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G2622719A	ribosomal	NCg1t50,tRNA:NCg1t50				wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G2624441A	silent	NCgl2392		R307R	putative transposase	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
G2626275A	silent	NCgl2394		G53G	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G2626387A	exchange	NCgl2394		S16F	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
T2626549C	exchange	NCgl2395	glsK	D395G	glutaminase	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
G2626908A	silent	NCgl2395	glsK	I275I	glutaminase	wt	mut	wt	wt	wt	wt	wt	wt	wt	wt
G2627095A	exchange	NCgl2395	glsK	A213V	glutaminase	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G2630079A	exchange	NCgl2398	uxaC	A392T	uronate isomerase	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
G2637501A	exchange	NCgl2408		P52S	hypothetical protein	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
G2638531A	intergenic	NCgl2408, NCgl2409	fas-IA			wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G2640704A	silent	NCgl2409	fas-IA	L2312L	3-oxoacyl-(acyl-carrier-protein) synthase	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
G2643399A	silent	NCgl2409	fas-IA	Y1413Y	3-oxoacyl-(acyl-carrier-protein) synthase	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G2645389A	exchange	NCgl2409	fas-IA	A750V	3-oxoacyl-(acyl-carrier-protein) synthase	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G2656548A	stop	NCgl2420		W32*	putative transposase	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
G2656595A	exchange	NCgl2420		G48D	putative transposase	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
C2657871T	exchange	NCgl2422		A211T	metal-dependent hydrolase	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G2661290A	silent	NCgl2426		S43S	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G2662306A	silent	NCgl2427		F8F	L-aminopeptidase/D-esterase	wt	mut	wt	wt	wt	wt	wt	wt	wt	wt
G2664823A	exchange	NCgl2431		A255T	nicotinate phosphoribosyltransferase	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
G2666014A	exchange	NCgl2433	dinG	E52K	Rad3-related DNA helicase	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
G2667756A	silent	NCgl2433	dinG	T632T	Rad3-related DNA helicase	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
G2668591A	silent	NCgl2434		I57I	hypothetical membrane protein	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt

SNP	TYPE	GENES	NAME	AA-ex.	FUNCTION	K118	K117	K063	K005	K112	K120	K051	K079	K093	K013
G2673578A	exchange	NCgl2438	nrdF	L255F	ribonucleotide-diphosphate reductase beta subunit	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G2676630A	exchange	NCgl2441		L83F	Mn-dependent transcriptional regulator	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G2676742A	silent	NCgl2441		G45G	Mn-dependent transcriptional regulator	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G2677263A	intergenic	NCgl2442, NCgl2443	nrdE			wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C2677797T	silent	NCgl2443	nrdE	K609K	ribonucleotide-diphosphate reductase alpha subunit	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G2679447A	silent	NCgl2443	nrdE	N59N	ribonucleotide-diphosphate reductase alpha subunit	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
G2679604A	exchange	NCgl2443	nrdE	T7I	ribonucleotide-diphosphate reductase alpha subunit	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C2680715T	intergenic	NCgl2445, NCgl2446	nrdH,nadE			wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G2680788A	intergenic	NCgl2445, NCgl2446	nrdH,nadE			mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
C2683383T	exchange	NCgl2448		G54S	hypothetical protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C2683851T	exchange	NCgl2449		G266E	putative Zn-NADPH:quinone dehydrogenase	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C2684300T	silent	NCgl2449		L116L	putative Zn-NADPH:quinone dehydrogenase	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C2688844T	exchange	NCgl2453	pgm	G403D	phosphoglucomutase	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G2689576A	exchange	NCgl2453	pgm	P159L	phosphoglucomutase	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G2691252A	exchange	NCgl2456		G160E	hypothetical protein	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
G2693396A	exchange	NCgl2458	psp4	V33I	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G2693672A	exchange	NCgl2458	psp4	A125T	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C2693887T	silent	NCgl2458	psp4	N196N	hypothetical protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C2695069T	exchange	NCgl2459		A48V	hypothetical membrane protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G2698679A	exchange	NCgl2463		T301I	Na+/H+-dicarboxylate symporter	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G2699233A	silent	NCgl2463		I116I	Na+/H+-dicarboxylate symporter	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
G2700633A	exchange	NCgl2464		A612V	ABC-type transporter, permease component	wt	mut	wt	wt	wt	wt	wt	wt	wt	wt
G2702163A	exchange	NCgl2464		S102F	ABC-type transporter, permease component	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G2708963A	intergenic	NCgl2465, NCgl2466				wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
G2709594A	intergenic	NCgl2465, NCgl2466				wt	wt	wt	wt	wt	wt	wt	wt	mut	mut

SNP	TYPE	GENES	NAME	AA-ex.	FUNCTION	K118	K117	K063	K005	K112	K120	K051	K079	K093	K013
G2709619A	intergenic	NCgl2465, NCgl2466				wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G2710101A	exchange	NCgl2466		E75K	hypothetical protein	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
G2710218A	exchange	NCgl2466		A114T	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G2710635A	intergenic	NCgl2466, NCgl2467				wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G2711192A	silent	NCgl2467		K185K	predicted dehydrogenase	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C2711664T	intergenic	NCgl2467, NCgl2468				mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
C2718364T	intergenic	NCgl2469, NCgl2470	murA2			wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G2721880A	intergenic	NCgl2472, NCgl2473	ramA,cysK			wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G2721930A	exchange	NCgl2473	cysK	G9D	cysteine synthase	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G2727702A	exchange	NCgl2478		L145F	predicted dithiol-disulfide isomerase	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G2729122A	intergenic	NCgl2479, NCgl2480	actA			wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C2731048T	intergenic	NCgl2480, NCgl2481	actA			mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
C2733132T	exchange	NCgl2482	phoU	R166C	phosphate uptake regulator	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
C2735439T	exchange	NCgl2485	pstC	G260S	ABC-type transporter, permease component	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C2738462T	exchange	NCgl2487		R84K	histone acetyltransferase HPA2-like protein	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
C2739917T	silent	NCgl2489		Q252Q	hypothetical protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C2740305T	exchange	NCgl2489		G123D	hypothetical protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G2747582A	exchange	NCgl2495	purF	T16I	amidophosphoribosyltransferase	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G2751399A	silent	NCgl2499	purL	R305R	phosphoribosylformylglycinamidine synthase subunit II	wt	mut	wt	wt	wt	wt	wt	wt	wt	wt
G2755841A	exchange	NCgl2503	nuc	D617N	predicted extracellular nuclease	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
G2756473A	silent	NCgl2503	nuc	E827E	predicted extracellular nuclease	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G2760555A	exchange	NCgl2507	ptrB	L366F	protease II	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
C2768281T	intergenic	NCgl2512, NCgl2513				wt	wt	wt	wt	wt	mut	wt	wt	wt	wt

SNP	TYPE	GENES	NAME	AA-ex.	FUNCTION	K118	K117	K063	K005	K112	K120	K051	K079	K093	K013
C2770531T	silent	NCgl2514		E3E	dipeptide/tripeptide permease	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
G2772952A	exchange	NCgl2517	cgtS3	T383I	two-component system, sensory transduction histidine kinase	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
G2777608A	exchange	NCgl2521	poxB	R300C	pyruvate dehydrogenase	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G2777695A	silent	NCgl2521	poxB	L271L	pyruvate dehydrogenase	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C2779475T	silent	NCgl2522		F170F	permease of the major facilitator superfamily	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G2780863A	exchange	NCgl2523		A142T	transcriptional regulator	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G2782588A	exchange	NCgl2525		A628V	hypothetical membrane protein	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
G2783074A	exchange	NCgl2525		P466L	hypothetical membrane protein	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
G2785137A	silent	NCgl2526		Y160Y	succinate dehydrogenase/fumarate reductase, flavoprotein subunit	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G2786907A	silent	NCgl2528	ddh	N270N	D-2-hydroxyisocaproate dehydrogenase	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
G2787305A	exchange	NCgl2528	ddh	H138Y	D-2-hydroxyisocaproate dehydrogenase	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
G2789663A	exchange	NCgl2531		R92C	hypothetical protein	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
G2792154A	exchange	NCgl2533	thrE	G391D	hypothetical protein	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G2793799A	exchange	NCgl2535	otsA	G309D	trehalose-6-phosphate synthase	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
C2794215T	exchange	NCgl2535	otsA	P448S	trehalose-6-phosphate synthase	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
G2796310A	silent	NCgl2538		A149A	transcriptional regulator	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C2797279T	silent	NCgl2539		D141D	ABC-type transporter, periplasmic component	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
C2797342T	silent	NCgl2539		Y162Y	ABC-type transporter, periplasmic component	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
G2797865A	silent	NCgl2540		R15R	ABC-type transporter, ATPase component	wt	mut	wt	wt	wt	wt	wt	wt	wt	wt
G2798637A	exchange	NCgl2541		V42M	ABC-type transporter, permease component	wt	mut	wt	wt	wt	wt	wt	wt	wt	wt
G2803177A	exchange	NCgl2544	ksdD	A24V	3-ketosteroid 1-dehydrogenase	wt	mut	wt	wt	wt	wt	wt	wt	wt	wt
G2803804A	exchange	NCgl2545		A65V	hypothetical protein	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
C2804698T	silent	NCgl2547		E138E	hypothetical protein	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
G2812628A	exchange	NCgl2554	scrB	S211F	beta-fructosidase	wt	mut	wt	wt	wt	wt	wt	wt	wt	wt
G2819328A	exchange	NCgl2560	nanP	H80Y	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G2822176A	exchange	NCgl2562		G534D	ABC-type transporter, periplasmic component	wt	mut	wt	wt	wt	wt	wt	wt	wt	wt
G2825599A	silent	NCgl2565		K86K	ABC-type transporter, ATPase component	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt

SNP	TYPE	GENES	NAME	AA-ex.	FUNCTION	K118	K117	K063	K005	K112	K120	K051	K079	K093	K013
G2829934A	intergenic	NCgl2571, NCgl2572	cgtR5			wt	mut	wt	wt	wt	wt	wt	wt	wt	wt
G2836332A	exchange	NCgl2578	xylC	P390L	NAD-dependent aldehyde dehydrogenase	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G2840416A	exchange	NCgl2581		G285E	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G2841651A	stop	NCgl2582	butA	W192*	L-2.3-butanediol dehydrogenase	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G2845057A	silent	NCgl2585	clpC	L629L	ATPase with chaperone activity, ATP-binding subunit	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G2847822A	exchange	NCgl2586	guaB1	G198D	inositol-5-monophosphate dehydrogenase	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G2849983A	exchange	NCgl2588		M16I	phenol 2-monooxygenase	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G2853879A	exchange	NCgl2590		G37E	hypothetical protein	wt	mut	wt	wt	wt	wt	wt	wt	wt	wt
G2855714A	intergenic	NCgl2590, NCgl2591				wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
G2859821A	exchange	NCgl2593		S109F	hypothetical protein	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C2860477T	intergenic	NCgl2593, NCgl2594	lysS			wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C2866205T	exchange	NCgl2601	folP1	A122T	dihydropteroate synthase	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
G2866804A	exchange	NCgl2602	folE	P129L	GTP cyclohydrolase I	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C2867200T	silent	NCgl2603	ftsH	E843E	cell division protein	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
G2867723A	exchange	NCgl2603	ftsH	A669V	cell division protein	wt	mut	wt	wt	wt	wt	wt	wt	wt	wt
G2868294A	silent	NCgl2603	ftsH	L479L	cell division protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C2870322T	exchange	NCgl2604	hpt	E48K	hypoxanthine-guanine phosphoribosyltransferase	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
G2874101A	silent	NCgl2608	speE	F448F	spermidine synthase	wt	mut	wt	wt	wt	wt	wt	wt	wt	wt
G2874853A	exchange	NCgl2608	speE	L198F	spermidine synthase	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G2874946A	exchange	NCgl2608	speE	P167S	spermidine synthase	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C2875378T	exchange	NCgl2608	speE	G23R	spermidine synthase	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
G2876902A	exchange	NCgl2612		A162V	hypothetical protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G2877241A	exchange	NCgl2612		T49I	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G2878739A	exchange	NCgl2615		P325S	uncharacterized NAD(FAD)-dependent dehydrogenase	wt	mut	wt	wt	wt	wt	wt	wt	wt	wt
C2880362T	intergenic	NCgl2616, NCgl2617				wt	wt	mut	mut	mut	wt	wt	wt	wt	wt

SNP	TYPE	GENES	NAME	AA-ex.	FUNCTION	K118	K117	K063	K005	K112	K120	K051	K079	K093	K013
C2882366T	silent	NCgl2618	cps	A456A	non-ribosomal peptide synthetase	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C2882461T	exchange	NCgl2618	cps	S488F	non-ribosomal peptide synthetase	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
G2884236A	exchange	NCgl2618	cps	E1080K	non-ribosomal peptide synthetase	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C2884517T	silent	NCgl2618	cps	F1173F	non-ribosomal peptide synthetase	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
G2886324A	exchange	NCgl0463	gabD2	L59F	NAD-dependent aldehyde dehydrogenase	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C2889764T	exchange	NCgl2621	groEL	E260K	chaperonin GroEL	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C2890158T	silent	NCgl2621	groEL	E128E	chaperonin GroEL	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
C2890178T	exchange	NCgl2621	groEL	A122T	chaperonin GroEL	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C2898739T	exchange	NCgl2627		G530E	hypothetical protein	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
C2900663T	exchange	NCgl2628		V1087I	hypothetical protein	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
C2902730T	exchange	NCgl2628		D398N	hypothetical protein	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
G2903688A	silent	NCgl2628		S78S	hypothetical protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G2905416A	exchange	NCgl2629		P410S	hypothetical membrane protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G2906942A	exchange	NCgl2630		L104F	hypothetical membrane protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G2907703A	exchange	NCgl2631		S63N	hypothetical protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C2907855T	exchange	NCgl2631		R114C	hypothetical protein	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
C2910388T	silent	NCgl2633	mrpA	F72F	probable NADH dehydrogenase	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
G2912611A	silent	NCgl2633	mrpA	V813V	probable NADH dehydrogenase	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C2912894T	exchange	NCgl2633	mrpA	P908S	probable NADH dehydrogenase	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
G2913155A	exchange	NCgl2633	mrpA	D995N	probable NADH dehydrogenase	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C2915527T	silent	NCgl2636	mrpE	F37F	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G2916734A	intergenic	NCgl2638, NCgl2639	mrpG			wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G2918197A	silent	NCgl2640		L188L	hypothetical protein	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
C2918645T	silent	NCgl2640		E38E	hypothetical protein	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
C2922207T	exchange	NCgl2646	cls	P30L	cardiolipin synthase	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C2925055T	exchange	NCgl2647		A32T	permeases of the major facilitator superfamily	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt

SNP	TYPE	GENES	NAME	AA-ex.	FUNCTION	K118	K117	K063	K005	K112	K120	K051	K079	K093	K013
C2926050T	exchange	NCgl2648		T170I	Na+/phosphate symporter	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
C2926556T	silent	NCgl2648		L339L	Na+/phosphate symporter	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
G2929100A	exchange	NCgl2651		R45W	ABC-type transporter, ATPase component	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G2929516A	exchange	NCgl2652		P81L	NTP pyrophosphohydrolase	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G2934666A	exchange	NCgl2655	pknG	A768T	serine/threonine protein kinase	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C2937704T	exchange	NCgl2657	pta	G63E	phosphate acetyltransferase	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G2941890A	silent	NCgl2661		F193F	hypothetical protein	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C2944473T	intergenic	NCgl2663, NCgl2664	purT			mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
G2946706A	exchange	NCgl2665	tnp8b (ISCg8a)	S296F	putative transposase	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C2947935T	intergenic	NCgl2666, NCgl2667	tnp8a(ISCg8a),cgtS6			mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
G2950546A	silent	NCgl2669	purA	A393A	adenylosuccinate synthetase	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C2952193T	exchange	NCgl2670		P87L	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G2955818A	silent	NCgl2674		N273N	hypothetical protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G2957881A	exchange	NCgl2676	pyrE	R53C	orotate phosphoribosyltransferase	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C2958081T	intergenic	NCgl2676, NCgl2677	pyrE			wt	mut	wt	wt	wt	wt	wt	wt	wt	wt
G2960089A	exchange	NCgl2678	sseA1	T85I	thiosulfate sulfurtransferase	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G2960603A	exchange	NCgl2679		P196S	hypothetical protein	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G2961311A	intergenic	NCgl2679, NCgl2680	cmr			wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G2961625A	exchange	NCgl2680	cmr	D95N	multidrug resistance protein	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G2962953A	intergenic	NCgl2680, NCgl2681	cmr			wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G2964571A	exchange	NCgl2682	clpB	T531I	ATPase with chaperone activity, ATP-binding subunit	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C2967242T	silent	NCgl2683		K188K	sodium coupled L-glutamate uptake system	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
C2969905T	exchange	NCgl2686		S24F	predicted flavoprotein involved in K+ transport	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
G2970699A	exchange	NCgl2686		A289T	predicted flavoprotein involved in K+ transport	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut

SNP	TYPE	GENES	NAME	AA-ex.	FUNCTION	K118	K117	K063	K005	K112	K120	K051	K079	K093	K013
C2971806T	silent	NCgl2687		G263G	alkanal monooxygenase	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G2972579A	exchange	NCgl2688	metZ	L210F	cystathionine gamma-synthase	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G2979508A	exchange	NCgl2697		A176T	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G2979512A	exchange	NCgl2697		G177D	hypothetical protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G2979855A	silent	NCgl2697		E291E	hypothetical protein	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G2982758A	exchange	NCgl2700	dnaJ	A308V	molecular chaperone	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C2987190T	silent	NCgl2703		F151F	predicted permease	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G2987209A	exchange	NCgl2703		V158I	predicted permease	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C2989741T	stop	NCgl2705		W70*	hypothetical protein	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
G2995223A	silent	NCgl2708		L62L	hypothetical protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
T2996352C	exchange	NCgl2709	adhA	D144G	Zn-dependent alcohol dehydrogenase	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G2999063A	silent	NCgl2713		R131R	predicted permease	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C2999699T	exchange	NCgl2714		G168D	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G3000977A	silent	NCgl2715	cysN	T188T	sulfate adenylate transferase subunit 1	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G3000980A	silent	NCgl2715	cysN	D187D	sulfate adenylate transferase subunit 1	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C3002086T	silent	NCgl2716	cysD	E123E	sulfate adenylyltransferase subunit 2	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
C3002621T	exchange	NCgl2717	cysH	E176K	phosphoadenosine phosphosulfate reductase	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G3002657A	exchange	NCgl2717	cysH	L164F	phosphoadenosine phosphosulfate reductase	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G3004669A	silent	NCgl2718	cysI	T165T	putative nitrite reductase	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G3005567A	exchange	NCgl2719	cysJ	A8T	putative ferredoxin/ferredoxin-NADP reductase	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G3005638A	silent	NCgl2719	cysJ	V31V	putative ferredoxin/ferredoxin-NADP reductase	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G3008034A	exchange	NCgl2720		R340K	hypothetical protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C3008106T	exchange	NCgl2720		A364V	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G3008324A	exchange	NCgl2720		D437N	hypothetical protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C3009261T	exchange	NCgl2723		P7S	hypothetical protein	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
C3013114T	exchange	NCgl2727		G242E	ABC-type transporter, ATPase component	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
G3016868A	exchange	NCgl2730		P95S	putative peptidase	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt

SNP	TYPE	GENES	NAME	AA-ex.	FUNCTION	K118	K117	K063	K005	K112	K120	K051	K079	K093	K013
G3022641A	exchange	NCgl2735		P97S	nitroreductase	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G3023363A	exchange	NCgl2736	iunH1	S180F	inosine-uridine nucleoside N-ribohydrolase	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G3023938A	intergenic	NCgl2736, NCgl2737	iunH1			wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G3025883A	exchange	NCgl2739	tagA1	A111T	3-methyladenine DNA glycosylase	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G3026643A	exchange	NCgl2740	hmp	L220F	hemoglobin-like flavoprotein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G3026800A	silent	NCgl2740	hmp	V167V	hemoglobin-like flavoprotein	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C3028724T	silent	NCgl2742		A152A	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G3029778A	exchange	NCgl2745	bglY2	D92N	hypothetical protein	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G3031690A	exchange	NCgl2747		E341K	aspartate aminotransferase	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C3031836T	silent	NCgl2747		L389L	aspartate aminotransferase	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
G3035768A	silent	NCgl2750	udgA2	N330N	predicted UDP-glucose 6-dehydrogenase	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G3037596A	ribosomal	NCg1t60,tRNA:NCg1t60				wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G3037650A	intergenic	NCgl2751, NCgl2752	dcd			wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G3037872A	intergenic	NCgl2751, NCgl2752	dcd			wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G3040043A	exchange	NCgl2753		L214F	hypothetical protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G3047401A	silent	NCgl2760		V56V	hypothetical membrane protein	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C3047931T	exchange	NCgl2760		A233V	hypothetical membrane protein	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C3048344T	exchange	NCgl2761		P96S	hypothetical membrane protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G3052831A	silent	NCgl2765	pck	I354I	phosphoenolpyruvate carboxykinase (GTP)	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C3053361T	exchange	NCgl2765	pck	V178I	phosphoenolpyruvate carboxykinase (GTP)	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G3056165A	exchange	trmB		A100T		wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G3059609A	exchange	NCgl2769	mmpL1	A761T	putative integral membrane protein	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G3060094A	exchange	NCgl2770		G148D	predicted integral membrane protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C3060272T	silent	NCgl2770		R207R	predicted integral membrane protein	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G3063208A	exchange	NCgl2773	pks	A1525V	putative polyketide synthase	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G3063297A	silent	NCgl2773	pks	F1495F	putative polyketide synthase	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut

SNP	TYPE	GENES	NAME	AA-ex.	FUNCTION	K118	K117	K063	K005	K112	K120	K051	K079	K093	K013
G3063470A	silent	NCgl2773	pks	L1438L	putative polyketide synthase	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G3063823A	exchange	NCgl2773	pks	S1320F	putative polyketide synthase	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C3065182T	exchange	NCgl2773	pks	S867N	putative polyketide synthase	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G3065749A	exchange	NCgl2773	pks	S678F	putative polyketide synthase	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G3066171A	silent	NCgl2773	pks	D537D	putative polyketide synthase	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G3069783A	exchange	NCgl2774	fadD2	A74V	acyl-CoA synthase	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G3071750A	silent	NCgl2777		F624F	putative esterase	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G3075108A	exchange	NCgl2778		R344H	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G3075176A	exchange	NCgl2778		E367K	hypothetical protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G3076274A	exchange	NCgl2779	cmt2	A97V	putative esterase	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G3077751A	silent	NCgl2780	aftB	H341H	arabinofuranosyltransferase	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C3080412T	exchange	NCgl2783		R641H	hypothetical protein	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
G3081330A	exchange	NCgl2783		T335I	hypothetical protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G3085221A	intergenic	NCgl2786, NCgl2787				wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G3085581A	exchange	NCgl2787		G107D	predicted flavoprotein involved in K+ transport	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G3086263A	silent	NCgl2787		K334K	predicted flavoprotein involved in K+ transport	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G3087887A	exchange	NCgl2788	glf	L140F	UDP-galactopyranose mutase	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G3088982A	silent	NCgl2789	psp5	K131K	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G3090962A	silent	NCgl2790	glpK	V442V	glycerol kinase	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G3090977A	silent	NCgl2790	glpK	F437F	glycerol kinase	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C3093150T	silent	NCgl2791		K9K	hypothetical protein	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
C3093700T	exchange	NCgl2792		A118T	1-acyl-sn-glycerol-3-phosphate acyltransferase	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
G3094685A	silent	NCgl2793	serS	D220D	seryl-tRNA synthetase	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G3095719A	exchange	NCgl2794		A77T	transcriptional regulator	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G3098517A	silent	NCgl2798		H19H	putative phosphoglycerate mutase	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G3104941A	exchange	NCgl2805		D169N	hypothetical protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt

SNP	TYPE	GENES	NAME	AA-ex.	FUNCTION	K118	K117	K063	K005	K112	K120	K051	K079	K093	K013
C3106546T	silent	NCgl2806		K126K	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G3106598A	exchange	NCgl2806		A109V	hypothetical protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G3108041A	intergenic	NCgl2807 NCgl2808	glpQ1,gntP			wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G3108733A	exchange	NCgl2808	gntP	G201D	putative gluconate permease	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C3109222T	exchange	NCgl2808	gntP	A364V	putative gluconate permease	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
G3110256A	intergenic	NCgl2808, NCgl2809	gntP			wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G3112121A	silent	NCgl2809		S67S	pyruvate kinase	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C3112225T	exchange	NCgl2809		A33T	pyruvate kinase	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G3114913A	exchange	NCgl2811		G432S	hypothetical protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C3116572T	exchange	NCgl2813		P165S	predicted flavoprotein	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
G3119389A	exchange	NCgl2816		V369I	putative integral membrane transport protein	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G3123292A	silent	NCgl2821		I294I	hypothetical protein	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G3123722A	exchange	NCgl2821		S151F	hypothetical protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G3123872A	exchange	NCgl2821		A101V	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G3123899A	exchange	NCgl2821		T92I	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G3125127A	exchange	NCgl2823		S58L	putative metal-dependent hydrolase/carboxypeptidase	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G3125667A	silent	NCgl2825	msrA	A160A	peptide methionine sulfoxide reductase	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C3125672T	exchange	NCgl2825	msrA	D159N	peptide methionine sulfoxide reductase	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G3128919A	exchange	NCgl2828		A143T	putative transport protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G3128954A	silent	NCgl2828		K154K	putative transport protein	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G3129771A	intergenic	NCgl2828, NCgl2829				wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G3131636A	exchange	NCgl2830		A429V	hypothetical protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C3134933T	exchange	NCgl2832		D104N	putative membrane transport protein	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G3136480A	silent	NCgl2834		L5L	two-component system, response regulator	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C3137632T	silent	NCgl2835		L98L	two-component system, sensory transduction histidine kinase	wt	mut	wt	wt	wt	wt	wt	wt	wt	wt
G3141256A	exchange	NCgl2840	mcbR	T90I	transcriptional regulator	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt

SNP	TYPE	GENES	NAME	AA-ex.	FUNCTION	K118	K117	K063	K005	K112	K120	K051	K079	K093	K013
G3143310A	silent	NCgl2842	uspA3	A16A	hypothetical protein	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G3144423A	silent	NCgl2843		L21L	alkanal monooxygenase-like protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G3153232A	silent	NCgl2847		E273E	hypothetical protein	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
T3155212C	intergenic	NCgl2849, NCgl2850				wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
G3156111A	silent	NCgl2850		V47V	predicted transposase	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G3156500A	exchange	NCgl2851		T250I	hypothetical protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G3159398A	exchange	NCgl2854		A135V	uncharacterized membrane protein	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C3161507T	intergenic	NCgl2856, NCgl2857	fdxA			wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C3162334T	intergenic	NCgl2857, NCgl2858				wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G3162349A	intergenic	NCgl2857, NCgl2858				wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G3162637A	intergenic	NCgl2857, NCgl2858				wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G3163858A	exchange	NCgl2859		P717S	cation transport ATPase	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C3164422T	exchange	NCgl2859		V529M	cation transport ATPase	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
G3164911A	exchange	NCgl2859		P366S	cation transport ATPase	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G3165294A	exchange	NCgl2859		A238V	cation transport ATPase	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G3166184A	exchange	NCgl2860		P37L	hypothetical protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G3166574A	silent	NCgl2862	cgtS9	V358V	two-component system, sensory transduction histidine kinase	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G3167625A	exchange	NCgl2862	cgtS9	T8I	two-component system, sensory transduction histidine kinase	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C3169140T	silent	NCgl2864		I124I	hypothetical protein	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
C3171278T	silent	NCgl2866		S8S	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G3173077A	exchange	NCgl2868		A28T	hypothetical protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C3173466T	silent	NCgl2868		L157L	hypothetical protein	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
G3177607A	intergenic	NCgl2873, NCgl2874	tnp19b(ISCg19a),trxB1			wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G3179527A	exchange	NCgl2876		A160T	putative transmembrane transport protein	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut

SNP	TYPE	GENES	NAME	AA-ex.	FUNCTION	K118	K117	K063	K005	K112	K120	K051	K079	K093	K013
G3180547A	intergenic	NCgl2876, NCgl2877				wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C3181458T	silent	NCgl2878	dnaB	K470K	replicative DNA helicase	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G3185954A	silent	NCgl2883		I347I	hypothetical membrane protein	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C3187438T	exchange	NCgl2884	mrcB	A589T	penicillin-binding protein	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C3188170T	exchange	NCgl2884	mrcB	V345I	penicillin-binding protein	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
T3188395C	exchange	NCgl2884	mrcB	T270A	penicillin-binding protein	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G3190111A	silent	NCgl2886		E78E	transcriptional regulator	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C3193608T	exchange	NCgl2891		G303D	ABC-type transporter, permease component	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G3195629A	exchange	NCgl2893		T473I	efflux system protein	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C3197469T	silent	NCgl2894		S19S	myo-inositol-1-phosphate synthase	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C3200279T	exchange	NCgl2896		V137I	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C3200966T	intergenic	NCgl2896, NCgl2897	dps			wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C3201801T	intergenic	NCgl2897, NCgl2898	dps, mutM2			wt	mut	wt	wt	wt	wt	wt	wt	wt	wt
C3203355T	exchange	NCgl2899		P135S	hypothetical protein	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
G3203736A	exchange	NCgl2899		E262K	hypothetical protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C3203895T	exchange	NCgl2899		R315W	hypothetical protein	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
C3205788T	exchange	NCgl2902		R149Q	hypothetical protein	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C3207817T	exchange	NCgl2903		T323I	sugar transporter family protein	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G3207876A	exchange	NCgl2903		V343I	sugar transporter family protein	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C3208233T	intergenic	NCgl2903, NCgl2904	mez			mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
G3208967A	exchange	NCgl2904	mez	D230N	malic enzyme	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C3211649T	silent	NCgl2906		P63P	hypothetical membrane protein	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
G3212130A	silent	NCgl2907		T128T	hypothetical membrane protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C3212236T	exchange	NCgl2907		G93E	hypothetical membrane protein	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C3214571T	silent	NCgl2909	dadA	E198E	D-amino acid dehydrogenase subunit	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut

SNP	TYPE	GENES	NAME	AA-ex.	FUNCTION	K118	K117	K063	K005	K112	K120	K051	K079	K093	K013
C3216591T	exchange	NCgl2910		C57Y	hypothetical protein	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
C3218247T	exchange	NCgl2913		A85V	hypothetical protein	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
C3218564T	silent	NCgl2913		L191L	hypothetical protein	mut	wt	wt	wt	wt	wt	wt	wt	wt	wt
G3219744A	intergenic	NCgl2914, NCgl2915	leuS			wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G3219808A	exchange	NCgl2915	leuS	P943S	leucyl-tRNA synthetase	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G3220682A	silent	NCgl2915	leuS	L651L	leucyl-tRNA synthetase	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G3220953A	exchange	NCgl2915	leuS	P561L	leucyl-tRNA synthetase	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C3224356T	stop	NCgl2918		W120*	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G3226025A	exchange	NCgl2920	nagI	L222F	gentisate 1,2-dioxygenase	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G3228470A	exchange	NCgl2922	benK2	G241D	putative benzoate transporter	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G3228994A	exchange	NCgl2922	benK2	A416T	putative benzoate transporter	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
G3229486A	exchange	NCgl2923		V123I	putative hydroxylase/monooxygenase	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G3232660A	exchange	NCgl2925	trpP	V22I	hypothetical membrane protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G3235550A	silent	NCgl2928	trpG	L198L	anthranilate synthase component II	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G3238536A	exchange	NCgl2931	trpB	A152T	tryptophan synthase subunit beta	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G3238845A	exchange	NCgl2931	trpB	D255N	tryptophan synthase subunit beta	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C3239288T	silent	NCgl2931	trpB	R402R	tryptophan synthase subunit beta	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C3240021T	exchange	NCgl2932	trpA	S230F	tryptophan synthase subunit alpha	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G3240160A	silent	NCgl2932	trpA	A276A	tryptophan synthase subunit alpha	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C3241396T	exchange	ulaA		G153S		wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G3242438A	silent	NCgl2934	rmpA	R84R	hypothetical protein	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C3242472T	exchange	NCgl2934	rmpA	R73K	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G3247580A	exchange	NCgl2939		V118M	hypothetical protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C3251461T	intergenic	NCgl2943, NCgl2944				wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C3252838T	exchange	NCgl2947		T37I	short chain dehydrogenase	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G3255175A	silent	NCgl2949		S58S	dipeptide/tripeptide permease	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt

SNP	TYPE	GENES	NAME	AA-ex.	FUNCTION	K118	K117	K063	K005	K112	K120	K051	K079	K093	K013
G3255473A	intergenic	NCgl2949, NCgl2950				wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C3260591T	intergenic	NCgl2953, NCgl2954	iolT2			wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C3261824T	exchange	NCgl2954		S232F	transcriptional regulator	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G3263607A	exchange	NCgl2956		V124I	hypothetical protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C3264103T	exchange	NCgl2956		A289V	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
C3266867T	silent	NCgl2959		V88V	hypothetical membrane protein	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C3275641T	exchange	NCgl2963		D311N	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G3278249A	exchange	NCgl2964		L1118F	putative helicase	wt	wt	wt	wt	wt	mut	wt	wt	wt	wt
C3279748T	exchange	NCgl2964		G618D	putative helicase	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G3281100A	silent	NCgl2964		N167N	putative helicase	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G3283612A	silent	NCgl2969		S209S	putative membrane transport protein	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G3288620A	intergenic	NCgl2974, NCgl2975				wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
C3301487T	silent	NCgl2985	trxC	D26D	thioredoxin	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C3301619T	silent	NCgl2985	trxC	F70F	thioredoxin	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
C3303363T	silent	NCgl2987		K85K	hypothetical protein	wt	wt	wt	wt	wt	wt	wt	mut	wt	wt
G3304711A	silent	NCgl2988	parB	R21R	putative cell division protein ParB	wt	wt	mut	mut	mut	wt	wt	wt	wt	wt
C3308055T	exchange	NCgl2992	rnpA	G106R	ribonuclease P	wt	wt	wt	wt	wt	wt	wt	wt	mut	mut
G3308139A	exchange	NCgl2992	rnpA	L78F	ribonuclease P	wt	wt	wt	wt	wt	wt	mut	wt	wt	wt
G3308995A	intergenic	NCgl2993, NCgl0001	dnaA			wt	wt	wt	wt	wt	wt	mut	wt	wt	wt

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Erklärung

Die vorgelegte Dissertation habe ich eigenständig und ohne unerlaubte Hilfe angefertigt. Die Dissertation wurde in der vorgelegten oder ähnlichen Form noch bei keiner anderen Institution eingereicht. Ich habe bisher keine erfolglosen Promotionsversuche unternommen.

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Stephan Binder

Düsseldorf, den 20.12.2012