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The Use of Macroarray as a Simple Tool to Follow the Metabolic Profile of *Lactobacillus plantarum* during Fermentation

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Abstract

This study focused on defining the differences in *L. plantarum* gene expression levels in different media and in different growth phases using an easy and cost-efficient monitoring of gene expression. A macroarray based on a group of selected *L. plantarum* genes, 178 genes belonging to 18 main groups, printed onto a nitrocellulose filter was designed in this work. Using the macrofilters designed, the expression of a selected set of *L. plantarum* genes was assayed in synthetic MRS medium and in extracted carrot juice. To compare the potential differences of starter gene expression in hygienic and contaminated cultivation media, the *L. plantarum* strain was cultivated in both sterile and contaminated (yeast and *Escherichia coli*) MRS and carrot juice. The number of genes found to be regulated as a function of growth was clearly higher in MRS-based growth medium than in carrot juice, In carrot juice, expression of the gene encoding malolactic enzyme (MLE), which makes *L. plantarum* an advantageous microbe in e.g. wine making, was found to be upregulated in logarithmic phase of growth. The current study demonstrated that macroarrays printed on nitrocellulose filters with simple robotic systems can be analyzed by standard laboratory equipment and methods usually available in molecular laboratories. Using this technology, rapid and cost-efficient analysis of genome function of *L. plantarum* can be carried out e.g. in developing regions, where lactic acid fermentation of food and feed matrices is a common practice.

Keywords

Macroarray, Gene Expression, *L. plantarum*

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1. Introduction

Lactic acid bacteria (LAB) are widely used for the preservation of food and feed raw materials and to intensify the flavour and texture of fermented products. Of the lactobacilli commonly used in food processes, *Lactobacillus plantarum* is important in the production of many fermented foods of both plant (pickled vegetables, silage, sourdough) and animal (dry ferment sausages, fermented fish, cheese) origin [1]. This versatility and ecological flexibility is most likely associated with the genome size of *L. plantarum*, which is one of the largest known among LAB [2]. Based on complete genome sequencing, *L. plantarum* has a capacity to use a large variety of carbon sources and encompasses a relatively high number of regulatory functions concentrated within a defined genomic region, which was designated the lifestyle adaptation region [3]. These genomic features exhibit an efficient adaptation capacity of *L. plantarum* to versatile environmental conditions.

Due to the ability to maintain pH homeostasis at low external pH, *L. plantarum* is tolerant to acidic environment and often becomes the dominant LAB at the end of spontaneous vegetable fermentation [4]. Therefore, this species is common in vegetable and silage fermentations. Yet, spontaneous fermentation is generally poorly controlled and unstable, and the quality of products varies depending on fermented material and inherent microbiota. Spontaneously fermented vegetables may also contain among other things biogenic amines, which have been associated with certain toxicological characteristics and outbreaks of food poisoning. The formation of biogenic amines has been repressed by the use of a pure *L. plantarum* starter instead of spontaneous fermentation [5]. For the above reasons, well-characterized starter cultures with desirable properties would be of particular importance.

Previously, the technological properties of potential starter LAB could be determined almost exclusively in pilot- and full-scale food and feed production experiments. Today, the development of molecular techniques has made possible the exploitation of genomic and proteomic data for the observation of potential genotypic and phenotypic differences between individual strains in specific growth conditions. Studies based on *L. plantarum* DNA-microarrays [6], proteomic patterns [7]-[10] and sequencing technologies, like metagenomic sequencing and RNAseq [11]-[13] and transcriptional profiling [14]-[17] have been carried out to elucidate strain-specific differences in genome composition and adaptation to various growth conditions.

Both microarray and proteomic studies require specific laboratory facilities and expertise, which may not be available in all research laboratories. Yet, *L. plantarum* is used worldwide for food and feed fermentations and there is a growing demand for the design of starter cultures with well-characterized technological properties. For easy and cost-efficient monitoring of gene expression in *L. plantarum*, a macroarray based on a group of selected *L. plantarum* genes printed onto a nitrocellulose filter was designed in this work. Using the macrofilters designed, the expression of a selected set of *L. plantarum* genes was assayed in synthetic MRS medium and in extracted carrot juice. To compare the potential differences of starter gene expression in hygienic and contaminated cultivation media, the studied *L. plantarum* strain was cultivated in both sterile and contaminated (yeast and *Escherichia coli*) MRS and carrot juice.

2. Materials and Methods

2.1. Bacterial Strains and Growth Conditions

L. plantarum strain MLBPL1 has been isolated from sauerkraut [18] [19]. The strain was routinely grown in microaerophilic conditions at 32˚C and maintained in MRS broth (Difco, BD, Franklin Lakes, NJ, USA). For plating, MRS was solidified with 1.5% agar. *E. coli* DH5α, carrying the plasmid vector pBluescript, was grown in Luria Bertani broth supplemented with ampicillin (50 µg/ml) as a selective agent at 37˚C 200 rpm. For contamination cultivations, a yeast and an *E. coli* strain originating from spoiled vegetables were propagated in YGC broth at 30˚C and in Luria Bertani broth at 37˚C, respectively.

For macroarray analyses, *L. plantarum* strain MLBPL1 was grown in synthetic medium MRS and carrot juice. To simulate contaminated growth conditions, the spoiling *E. coli* and yeast strains were inoculated into MRS and carrot juice. Cultivations were performed using Spectra/Por Float-A-Lyzer dialysis tube (MWCO 100 kDa, Spectrum Laboratories, Rancho Dominguez, CA, USA) in order to make it easier to separate the *L. plantarum* cells, the vegetable matrix and the spoiling strains of yeast and *E. coli*. By using the dialysis tube, no filtering of plant material was needed and in addition, the cells of contaminating strains didn't interfere with the extraction of RNA. Carrot juice was prepared from fresh vegetables with a juice extractor. The extracted juice was centrifuged at 18,500 g for 40 min and pasteurized in a water bath at 95˚C for 30 min.

An overnight culture of MLBPL1, grown in MRS-medium at 32˚C, was used to inoculate MRS broth and

carrot juice. For MRS cultivation, a 1% inoculumn was used. For carrot juice cultivation and contamination cultivations, the inoculumn was centrifuged at 13,000 g for 3 min and the pellet was suspended into centrifuged (18,500 g for 40 min) and filter-sterilized (0.8/0.2 µm pore sizes) carrot juice (carrot cultivations) or MRS broth (MRS contamination cultivation), after which the suspension was transferred to a Spectra/Por Float-A-Lyzer dialysis tube. The tube was then transferred to a bottle containing carrot juice, contaminated MRS broth or contaminated carrot juice. In contamination cultivations, MRS broth and carrot juice were contaminated by inoculating them with 1% *E. coli* and yeast. The cultivations were performed at 32˚C. The growth was determined by plating onto MRS agar plates appropriate dilutions from the samples taken during the growth. The plates were incubated at 32˚C for 48 h, until single bacterial colonies appeared.

2.2. Extraction and Labeling of RNA

Bacterial cells of the *L. plantarum* strain MLBPL1 grown in MRS broth and carrot juice were harvested at exponential (6 h) and stationary phase (14 h) of growth by centrifugation for 3 min at 4˚C at 11,000 g. The collected cells were frozen immediately in liquid nitrogen and stored at −70˚C. Extraction of total RNA was carried out with SV total RNA isolation system (Promega, Madison, WI, USA) with some modifications to the protocol on the disruption of the cells. Briefly, bacterial cells thawed slowly on ice were first washed with sterile water treated with diethyl pyrocarbonate (DEPC) and collected by centrifugation. Next, the pellet was resuspended into 225 µl of SV RNA lysis buffer of the Promega kit and transferred to an eppendorf tube containing 100 µl of nitric acid-washed glass beads. The cells were disrupted with glass beads in a cell homogenizer as described before (Kahala, *et al.*, 2008). After that, the lysate was transferred to a new tube and 350 µl of SV RNA dilution buffer of the Promega kit was added per 175 µl of lysate. From this step on, the extraction was carried on as recommended by the manufacturer. Two technical duplicates from the RNA extraction on each culture medium and harvesting point were made. mRNA was enriched from total RNA samples by removing the 16S and 23S rRNAs with MICROB Express Bacterial mRNA Purification kit (Ambion, Austin, TX, USA) according to the instructions of the manufacturer. The RNA concentration was determined spectrophotometrically at 260 nm.

The integrity of the isolated prokaryotic RNA was determined by total RNA gel electrophoresis and Northern blot carried out as described by [20]. Total RNA samples, denatured with glyoxal and dimethylsulphoxide, were separated by size in a 1.0% (w/v) agarose gel in 10 mM sodium phosphate buffer, pH 6.5 followed by a transfer to a positively charged nylon membrane (Roche) and hybridization with a *ldhD*-specific 736 bp probe, amplified with primer pair 5'-AAGTTAGCCGACGAAGGG-3' and 5'-CCATGTTGTGAACGGCAG-3' targeted to *L. plantarum* strain D90339.1. The probe was labelled with digoxigenin-dUTP according to the instructions of the manufacturer (Roche). Luminescent DIG detection kit (Roche) was used for hybrid detection. To detect the potential residual chromosomal DNA in the isolated mRNA sample, primers 5'-AAGTTAGCCGACGAAGGG-3' and 5'-GGGCGTATAATTCGTCCAAA-3' designed to produce a 403 bp fragment from the target *ldhD* gene of *L. plantarum* strain D90339.1 were used. PCR-procedure using Dynazyme II DNA polymerase (Finnzymes, Espoo, Finland) was carried out in the reaction conditions recommended by the enzyme manufacturer.

cDNA was synthesized by RT from DNA-free mRNA and cDNA labelling was performed with an alkalilabile digoxigenin-11-dUTP (DIG) (Roche, Basel, Switzerland) in a reverse transcription reaction with Im-Prom-II Reverse Transcription System kit (Promega) as follows: 1 µg of mRNA was mixed with 0.5 µg of random hexamer primers provided by the kit manufacturer. The mixture was heated at 70˚C for 5 min and chilled on ice for 5 min. cDNA synthesis was carried out by combining RNA-primer mixture with 1 × ImProm-II reaction buffer, 5 mM $MgCl_2$, 0.5 mM dATP, 0.5 mM dGTP, 0.5 mM dCTP, 0.325 mM dTTP, 0.175 mM DIG-11-dUTP, 1 U/µl RNasin Ribonuclease Inhibitor, and 1 µl ImProm-II Reverse Transcriptase. Annealing was performed at 25˚C for 5 min, followed by extension at 43˚C for 1h and enzyme inactivation at 70˚C for 15 min. The labeled cDNA was purified with Microarray Target Purification kit (Roche) according to the instructions of the manufacturer.

2.3. PCR and Labeling of the Positive Control for Macroarray

Human-based HbGAM (heparin-binding growth-associated molecule) gene [21] inserted into the plasmid pBluescript (Agilent Technologies, Santa Clara, CA) was amplified with polymerase chain reaction (PCR) to be used as a positive control in macroarray analyses. PCR reactions were carried out with Dynazyme II DNA polymerase (Finnzymes, Espoo, Finland) using the reaction conditions recommended by the manufacturer. Bacte-

rial lysate of the *E. coli* strain DH5*α*, harbouring the recombinant pBluescript-HbGAM plasmid to be used as a template for PCR, was obtained by disrupting the cells with glass beads. To amplify the HbGAM gene, the primer pair 5'-GTAAAACGACGGCCAG-3' and 5'-CAGGAAACAGCTATGAC-3' targeting the plasmid was used. The amplified PCR product was purified with Wizard® SV Gel and PCR Clean-Up System (Promega) and labelled with DIG-11-dUTP using DIG-High Prime labeling kit (Roche).

2.4. Amplification of *L. plantarum* MLBPL1 Genes and Macroarray Printing

Primers were designed for the amplification of selected genes from the fully sequenced genome of *L. plantarum* WCFS1 [3]. The gene list and designed primers are listed in **Supplement 1**. To enable an easy re-amplification of the PCR-products, universal nucleotide sequences were added to the 5' termini of the specific forward and reverse primers. A nucleotide sequence 5' CCGCTGCTAGGCGCGCCGTG was added to the forward primers and, respectively, a nucleotide sequence 5' GCAGGGATGCGGCCGCTGAC was added to the reverse primers. Amplification of the selected genes was done as described for the positive control for macroarray. For the PCR reaction, a 10 pmol primer concentration and 20 ng of corresponding genomic template DNA were used in a 100 µl reaction volume in a 96 well PCR plate. The success of the PCR amplification was checked by analyzing 5 µl from the reactions on a 1% agarose gel. The obtained PCR products were purified using Montage PCR Purification 96 Well Plates (Millipore).

PCR fragments in the 96 well plates were transferred to 384 plates for printing on the nitrocellulose macroarray (**Supplement 2**). Purified PCR fragments were gridded in duplicate on nitrocellulose membranes with a QPix automated colony picker (Genetix Ltd., UK) using a 384-pin gridding head as described in [22].

2.5. Hybridization and Detection

Macroarrays were prehybridized for 2 h at 60˚C with 20 ml of DIG Easy Hyb buffer (Roche). Hybridizations were performed overnight at 60˚C with 6 ml DIG Easy Hyb buffer (Roche) containing 5 µl of labeled cDNA probe and HbGAM which was used as a positive control in hybridization reactions. After hybridization, macroarrays were washed twice at room temperature for 5 min with washing solution containing 2 × SSC (1 × SSC is 0.15 M NaCl and 15 mM sodium citrate) and 0.1% sodium dodecyl sulphate (SDS) and twice at 68˚C for 15 min with washing solution (0.1 × SSC, 0.1% SDS). Hybridized spots were detected with chemiluminesence-based DIG detection kit (Roche) using CDP-Star (Roche) as a substrate and chemiluminescence produced was detected by FluorChem (Alpha Innotech Corp., San Leandro, CA) gel image system. For reprobing, the DIG-labelled probe was removed with a following procedure. The membrane was rinsed thoroughly in sterile water, washed twice with 0.2 M NaOH containing 0.1% SDS at 37˚C for 20 min and rinsed with 2 × SSC for 5 min.

2.6. Statistical Methods

The DNA probes spotted on the macroarray were selected using results from the previous proteomics results using 2-DE and HPLC-ESI-MS/MS [8]. Additionally, computationally predicted expression values were used for choosing the remaining probes. Codon usage differences (codon bias) were used for predicting gene expression levels for all 3009 genes of *L. plantarum* (C) [3], and the set of 63 genes encoding ribosomal proteins (RB). Codon bias for a gene g with respect to gene set G was calculated by the formula

$$B(g|G) = \sum_a pa(g)\left[\sum_{(x,y,z)=a} \left| f(x,y,z) - g(x,y,z) \right|\right] \quad (1)$$

where $f(x,y,z)$ denotes a normalized frequency of the codon triplet (x,y,z) coding for an amino acid a in a gene g, $g(x,y,z)$ denotes the frequency of the codon triplet (x,y,z) in the gene set G, and $p_a(g)$ is the fraction of the amino acid a in the gene g [23].

The gene g was predicted as highly expressed if the relative codon bias

$$RCB = \frac{B(g|C)}{B(g|RB)} \quad (2)$$

exceeded 1.05. The genes obtaining the greatest *RCB* values were chosen for the DNA macroarray filter in addi-

tion to those identified by the HPLC-ESI-MS/MS.

After scanning of the macroarray images, the quantification of the hybridized signals and background subtraction were done by the TIGR Spotfinder image processing software [24]. After quantification and background correction, the signals were normalized using median array intensities. Finally, the expression levels for each gene and sample was obtained by taking median of the normalized intensity values across the two replicates of each sample. Gene expression levels were compared between MRS, carrot juice and contaminated versions of the growth media in exponential (6 h) and stationary (14 h) growth phases. The pair-wise comparisons were made using fold changes, ratios of the mean expression levels. Fold changes greater than two are reported in the results. All genes in the array were grouped into functional groups according to their main roles. Gene set enrichment analysis was made for the gene sets with fold change greater than two in order to test whether any functional group is overrepresented among the differentially expressed genes between two growth media. Analyses were made using SAS® (SAS for Windows 9.1).

3. Results

Growth rate of *L. plantarum* MLBPL1 cells was similar in MRS and carrot juice (**Figure 1**). Integrity and purity of the isolated RNA were demonstrated by Northern blot and PCR of the *ldhD* gene (data not shown).

The macroarray included 178 genes belonging to 18 main groups. The largest groups were energy metabolism (59 genes), protein synthesis (30 genes), protein fate (10 genes), regulatory functions (10 genes), cell envelope (9 genes) and DNA metabolism (9 genes). Of the 178 genes tested, 18 (10%) showed a mean fold change greater than 2.0 in at least one of the ten comparisons between growth media or growth phases (**Table 1**). The most frequent functions included were energy metabolism, cell envelope, protein fate and nucleotide metabolism.

3.1. Expression Levels of the Genes as a Function of Growth

The majority of the genes studied on the membranes showed no significant change in levels of expression during the growth or between the growth media. The number of the genes found to be regulated as a function of growth was clearly higher in MRS-based growth medium than in carrot juice, in which only genes involved in fatty acid and phospholipid metabolism showed differential expression in different growth phases. The function of the genes showing upregulation in logarithmic phase in MRS medium was mostly related to energy metabolism, but also to cell division, cell envelope biosynthesis and pyrimidine ribonucleotide biosynthesis. Generation of sufficient energy for growth in logarithmic phase is important and was evidenced in the MRS based medium.

In MRS cultivation, when entering in the stationary phase of growth, transcription of genes involved in energy metabolic pathways decreased and higher expression levels were found for genes involved in protein fate, protein folding and stabilization, like “folding” chaperones DnaK and GroEL. In contaminated MRS medium, especially the expression levels of genes involved in sugar metabolism pathways (*galK*, *lacM*) were found to be higher in logarithmic phase. This reflects higher demand for energy in the logarithmic phase and probably competition between the *Lactobacillus* and contaminating strains in the utilization of sugars that are needed for growth.

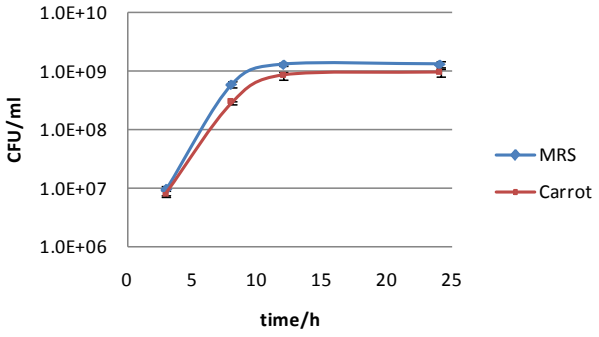

Figure 1. Growth of *L. plantarum* MLBPL1 in two growth media.

Table 1. Genes and their main roles showing mean fold change > 2.0 in ten comparisons among growth media and two growth phases. Minus and plus signs show which of the compared groups has higher expression: +: shows higher expression in the first; −: in the second group.

Main role	gene ORF	gene product	MRS vs MRS cont. 6 h	MRS vs MRS cont. 14 h	Carrot juice vs Carrot juice cont. 6 h	Carrot juice vs Carrot juice cont. 14 h	MRS vs Carrot juice 6 h	MRS vs Carrot juice 14 h	MRS 6h vs 14 h	MRS cont. 6 h vs 14 h	Carrot juice 6 h vs 14 h	Carrot juice cont 6 h vs 14 h
Energy metabolism—Pyruvate dehydrogenase	pdhB lp_2153	pyruvate dehydrogenase complex, E1 component, beta subunit	−2.20	-	-	-	-	-	-	-	-	-
Energy metabolism—Sugars	galK lp_3482	galactokinase	−3.01	-	-	-	-	-	-	+2.13	-	-
	lacM lp_3484	beta-galactosidase, small subunit	−2.30	-	-	-	-	-	-	+2.43	-	-
Energy metabolism—Glycolysis/ gluconeogenesis	pyk lp_1897	pyruvate kinase	-	-	-	-	-	−2.32	-	-	-	-
Energy metabolism—Pentose phosphate pathway	rpiA1 lp_0602	ribose 5-phosphate epimerase	-	-	-	-	-	-	+2.02	-	-	-
DNA metabolism—DNA replication, recombination	dnaN lp_0002	DNA-directed DNA polymerase III, beta chain	-	-	-	-	-	-	-	-	-	+2.15
Cellular processes—Cell division	ftsH lp_0547	cell division protein FtsH, ATP-dependent zinc metallopeptidase	-	−2.06	-	-	-	-	+2.28	-	-	-
Cell envelope—Biosynthesis and degradation	lp_0304	extracellular protein	-	-	-	-	-	-	+2.16	-	-	-
Cell envelope—Other	lp_2290	integral membrane protein	-	-	-	-	-	-	+2.41	-	-	-
Signal transduction—PTS	pts16ABC lp_2097	fructose PTS, EIIABC	-	−2.50	-	-	-	−2.86	-	-	-	-
Enzymes of unknown specificity	mleS lp_1118	malolactic enzyme	-	-	+2.32	+2.17	−2.56	−2.28	-	-	-	-
Fatty acid and phospholipid metabolism—Biosynthesis	fabF lp_1675	3-oxoacyl-[acyl-carrier protein] synthase II	-	-	-	-	-	-	-	-	+2.18	
Purines, pyrimidines, nucleosides, and nucleotides—Pyrimidine ribonucleotide biosynthesis	pyrD, lp_2697	dihydroorotate oxidase,	-	-	-	-	+3.74	-	-	+2.02	-	-
	pyrC, lp_2699	dihydroorotase	-	-	-	-	+3.36	-	+2.54	-	-	-
Transport and binding proteins—Amino acids, peptides	oppA lp_1261	oligopeptide ABC transporter, substrate binding protein	-	-	-	-	−2.41	-	-	-	-	-
Protein fate—Protein folding and stabilization	groEL lp_0728	GroEL chaperonin	-	-	-	-	-	-	−2.25	-	-	-
	dnaK lp_2027	heat shock protein DnaK	-	-	-	-	-	-	−2.17	-	-	-
Unknown function	typA lp_2146	GTP-binding protein TypA	-	-	-	-	-	-	+2.45	-	-	-
	lp_3092	fumarate reductase, flavoprotein subunit precursor, N-terminally truncated	-	-	-	-	-	-	−2.13	-	-	-

3.2. Expression Levels of the Genes between Different Growth Media

The mRNA level of several genes was shown to be regulated in response to different growth media. At the exponential (6 h) phase of growth, the genes encoding dihydroorotate oxidase and dihydroorotase enzymes were differentially expressed in MRS and carrot juice. They showed 3.7- and 3.4-fold higher expression in the MRS compared to carrot juice growth medium, respectively (**Table 1**). Differential expression ($p = 0.015$) of these genes encoding proteins involved in pyrimidine ribonucleotide biosynthesis is an indication of distinct gene regulation and, consequently, potentially different rate of pyrimidine biosynthesis in synthetic MRS compared to vegetable-based carrot juice cultivation medium.

Expression of malolactic enzyme (mle) gene was clearly higher in logarithmic phase when grown in plant based medium. Upregulation of cell division protein FtsH was observed in contaminated MRS 14 h compared to MRS 14 h, probably indicating higher stress response in contaminated MRS.

4. Discussion

This study focused on defining the differences in *L. plantarum* gene expression levels in different media and in different growth phases by the use of a simple and low-cost macroarray technique. Previously described DNA macroarray technique [22] has been further developed for studying gene expression profile of the industrially important lactic acid bacterium.

Fermentation conditions may dramatically affect functional characteristics of LAB [13]. Marked changes in expression levels upon entry in the stationary phase have been found out [25] [26]. Highly expressed genes are turned off or markedly repressed and genes, mostly inactive in the growing cells, begin to be expressed in the stationary phase [25] [26]. In this study, transcription of genes involved in energy metabolic pathways decreased in stationary phase and higher expression levels were found for genes like "folding" chaperones *DnaK* and *GroEL*. *GroEL* basal expression is enhanced by environmental stress, including elevated temperature, oxygen limitation, and nutrient deprivation [27] [28]. DnaK plays a central role in protein folding, refolding, translocation and in the stress conditions. The elevated expression of these genes is probably a response to the diminishing nutrients and high concentration of lactic acid in the medium which is known to cause stress especially in the late-stationary phase [29].

Proteomic studies by [10] has revealed significant changes on fermentation profiles of *L. plantarum* strains previously grown under food-like conditions compared to cultivation in MRS broth. In our study, expression of a malolactic enzyme (mle) gene in plant-based medium was found to be upregulated in logarithmic phase of growth. Mle enzymes, involved in decarboxylation of L-malic acid to L-lactic acid and CO_2 [30], have been purified from several lactic acid bacteria, including *Leuconostoc mesenteroides*, *L. plantarum*, and *Leuconostoc oenos* [31]. In several studies, *L. plantarum* has been shown to have malolactic activity and therefore is of interest in wine production [32]. The significance of malolactic activity of LAB in sauerkraut fermentation has also been reported. Conversion of malic acid into lactic acid before significant sugar metabolism may play some role in early fermentation [30] [33].

Higher expression of cell division protein FtsH in contaminated MRS compared to MRS probably indicated higher stress response in contaminated MRS. Functional studies have revealed an important role for FtsH in the bacterial stress response. In several bacteria, including *E. coli*, *B. subtilis*, *Lactococcus lactis*, *O. oeni*, *Helicobacter pylori*, and *L. plantarum*, *ftsH* expression is induced in response to heat and other stress factors controlled by additional regulators [34].

Macroarray was found to be an applicable method for studying expression of defined genes of *L. plantarum* during fermentation. Macroarray technology has been successfully applied also e.g. for the detection of pathogens in chicken samples [35] and studies on environmental samples for the presence of specific antibiotic resistance genes [36] communities of diazotrophs [37], and expression of 375 genes in *L. lactis* subsp. *lactis* IL1403 during stress conditions [38].

L. plantarum is encountered in a variety of environmental niches, which include dairy, meat and many vegetable or plant fermentations as well as the human gastrointestinal tract. Because of this flexibility and versatility, strains of this species have been traditionally used for food and feed preservation and as starters in the manufacture of fermented products. Formerly, the technological properties and suitability of certain strains to selected applications could be ensured almost exclusively by laborious and time-consuming food processing and preservation experiments. Today, the long history of use and on the other hand the development of molecular and ge-

nomic techniques have made *L. plantarum* one of the most studies food microbes. Modern DNA microarray [6], next-generation sequencing technologies [39] and especially transcriptomic studies are accurate and sensitive and have enabled the detailed examination of *L. plantarum* genome structure and function. The most advanced technologies, however, require specific instrumentation and have often high running costs, which may rule out their use in many cases. The current study demonstrated that macroarrays printed on nitrocellulose filters with simple robotic systems can be analyzed by standard laboratory equipment and methods usually available in molecular laboratories. Using this technology, rapid and cost-efficient analysis of genome function of *L. plantarum* can be carried out e.g. in developing regions, where lactic acid fermentation of food an feed matrices is a common practice, but research and analysis laboratories often lack the most expensive specific laboratory instrumentation.

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Supplement 1. Primers designed for PCR amplification of the selected genes.

ORF	Start	End	ORF Length	Strand	Gene	Product	FP 5'	FP Sequence	RP 5'	RP Sequence	PCR Fragment Length
lp_0175	151222	152475	1254	+	malE	maltose/maltodextrin ABC transporter, substrate binding protein	35	TAGCCACTGGTT TAGCACTC	428	TCAGATTCGATCG TTACAGG	393
lp_0230	208913	210751	1839	+	pts2CB	mannitol PTS, EIICB	51	AAAGAAACTGGG CGTTAAGG	456	AATCTTATCGGAA ATGGCGT	366
lp_0233	213329	214486	1158	+	mtlD	mannitol-1-phosphate 5-dehydrogenase	324	GCAAGCTAACAA CAATACGA	698	TTGTAGCCTTTCA TGTTACC	374
lp_0257	232292	233083	792	-	pepM	methionyl aminopeptidase	333	TGTTGCTGGTAC ACCATCAC	729	GTCATCGGTAACG ACAAGGG	396
lp_0302	278235	279038	804	-		extracellular protein	123	CCAAGCTCACAA CACTTCAG	507	AGCACTTGATGA AGCTTGAC	384
lp_0304	280089	280727	639	-		extracellular protein	44	CTAGTCTCTTTGC CATTGGT	586	AACGTGAAGCAA CATAACTG	542
lp_0330	301990	302853	864	+	fba	fructose-bisphosphate aldolase	30	ACAAGACGCATA CAAGAACC	514	CGGCTAAGATGTC GATACCA	484
lp_0447	401588	402862	1275	+	mvaA	hydroxymethylglutaryl-CoA reductase	212	TTGTTCCAATGG TTACTGAG	612	AATACTCAGTAA AGCGTCCT	400
lp_0480	428432	429031	600	+	rpoE	DNA-directed RNA polymerase, delta subunit	196	GGTAGTTTCATTT CGCTCGG	491	TCGTCTGTGTCAT CAACACC	295
lp_0537	485467	486429	963	-	ldhL1	L-lactate dehydrogenase	143	ACGCCCTTGATC TTGAAGAC	523	TGTAAGCATCAAC GGAACGA	380
lp_0539	487338	490865	3528	+	mfd	transcription-repair coupling factor	314	TAAACGCCTTAT TGCGTGAC	798	TTGTGCTAAATTA TCGGCCA	484
lp_0547	496382	498619	2238	+	ftsH	cell division protein FtsH, ATP-dependent zinc metallopeptidase	327	CCAAGTTTCTAA GTTGGCGG	750	TGAGAAGAATGG GACACCAG	423
lp_0576	534155	534961	807	+	pts9C	mannose PTS, EIIC	112	CTGATCGGATTA GTTACTGG	561	ACCGATTGATAA ACCATCAG	449
lp_0577	534979	535896	918	+	pts9D	mannose PTS, EIID	49	TGGATGCGTTCT AACTTCCT	506	TTAGAACCAGCTT TGTACCC	457
lp_0601	572690	574021	1332	-	pepC1	cysteine aminopeptidase	71	AAGTCATCGAAC GTAGTGTC	394	TCAACCAAGCAA CTTTACGG	323
lp_0609	579545	581035	1491	+	gltX	glutamate--tRNA ligase	185	ACAATTTGACGT GGTTAGGG	612	AAAGTTGTACGTT GGCATCC	427
lp_0619	587629	588054	426	+	rplK	ribosomal protein L11	161	TCCCTGTTGTGAT TACGGTG	418	CGACGGTGAATC CCATACTC	257
lp_0620	588155	588844	690	+	rplA	ribosomal protein L1	365	CCCAAGTTGGTC GTTTAGGT	678	TAAATCAACACGT ACCGCTG	313
lp_0621	589043	589546	504	+	rplJ	ribosomal protein L10	197	ATAAGGCCGGAT TTGAAGAC	441	AGGTGCTTGTAAC AAGTTGG	244
lp_0690	634153	636711	2559	+		integral membrane protein (putative)	123	GTGGCATCTGTT AAGCATGG	581	CCACTCAATAACC GAACGAC	458
lp_0692	637543	638553	1011	-	nrdF	ribonucleoside-diphosphate reductase, beta chain	54	GGCAATTAACTG GGATCGAG	489	TTGCTTCAATGGA TCTTCGT	435
lp_0715	656135	657187	1053	-	phnD	phosphonates ABC transporter, substrate binding protein (putative)	27	AGGTCTTTCATTC ATTTCGG	399	ATAAGTAGCGTCT TTCAAGG	372
lp_0728	665344	666969	1626	+	groEL	GroEL chaperonin	15	AAAGTTCTCTGA AGATGCAC	397	TAGCGTGTAATGA GTCAACC	382
lp_0757	691787	692707	921	+	galU	UTP--glucose-1-phosphate uridylyltransferase	153	AGACATTGTTAT CGTCACGG	615	ACCAATGATAGC AAGATCAC	462
lp_0786	720764	721354	591	-	clpP	endopeptidase Clp, proteolytic subunit	22	ATTGAACAGTCA TCACGTGG	338	AAACGCTTGCCCT TAGTACC	316
lp_0789	724979	726001	1023	+	gapB	glyceraldehyde 3-phosphate dehydrogenase	161	CTCATGGTACTTT GAACGCT	603	GAAGTTACCACC ACGTACAG	442
lp_0790	726122	727324	1203	+	pgk	phosphoglycerate kinase	439	GGTGATGTCTAC GTCAACGA	872	GAACCTTCAACA ACCTTGCT	433

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lp_0791	727351	728109	759	+	tpiA	triosephosphate isomerase	389	CATTAGAACAAC GTGAAGCC	736	GTTCCAAGAATG AATCAGGT	347
lp_0792	728191	729519	1329	+	enoA1	phosphopyruvate hydratase	239	TTGGTTACGATG TAACTGAC	724	CAAAGGCAATGG CAATATCC	485
lp_0800	736645	743064	6420	+		cell surface protein precursor	203	CTACGACTAGTA CAACAGGG	660	ATCTTTCGAGTCT GAGTCAC	457
lp_0923	854229	856649	2421	+		cell surface protein precursor	261	AACACAAACTAA AGTGGCGA	706	TAGCTTGATTTGC TTGGGTG	445
lp_0938	868001	870874	2874	+	hsdR	type I site-specific deoxyribonuclease, HsdR subunit	670	TTTACGACCACT TTCTTGAG	1109	ACCGCATTATTCA TCTTCTG	439
lp_0959	890255	891661	1407	-	pepD3	dipeptidase	15	TAAAGGTTCATG TACCACGA	388	GTAATACTAGGGT GCAAAGG	373
lp_1012	933125	934402	1278	+	serS2	serine-tRNA ligase	251	AGACGTTGGATG AAGAACGG	736	CGGGAATAACTTC ATCACGA	485
lp_1021	939961	943575	3615	+	rpoB	DNA-directed RNA polymerase, beta subunit	223	TTAGAACCAAAG TACACCGT	673	CCAACGTCAACAT CAAACTG	450
lp_1022	943593	947234	3642	+	rpoC	DNA-directed RNA polymerase, beta' subunit	168	GGATTGGGAATG TGCTTGTG	602	TGACCAGAAGCA TCCTTCAG	434
lp_1025	948830	949243	414	+	rpsL	ribosomal protein S12	16	CAATTAATTCGC AAAGGCCG	364	CTTCGACACCAGC AGTATCC	348
lp_1026	949260	949730	471	+	rpsG	ribosomal protein S7	91	ATGTTAGATGGT AAGCGCGG	432	CATCCGGTGAGTA TCTTCAC	341
lp_1027	949872	951968	2097	+	fusA2	elongation factor G	318	CGTATTGGATGC CCAATCAG	678	GTCATCGATGTCA GCTAAGG	360
lp_1033	953265	953894	630	+	rplC	ribosomal protein L3	34	GGAATGACGCAA GTCTTCAC	423	ATGGTAACGAGA ACCGTGAG	389
lp_1034	953913	954536	624	+	rplD	ribosomal protein L4	43	GGTGACGTTACT TTGAACGA	377	ACTGCAACTAAG CTGTTGTC	334
lp_1036	954853	955692	840	+	rplB	ribosomal protein L2	125	GTCGTAACAGTT ATGGTCAC	605	AATTCGTGTTGTT CATTGCC	480
lp_1040	956391	957044	654	+	rpsC	ribosomal protein S3	220	TGGTTCTGAAGT TGAAGCTC	296	CCATGAATAGTCG ATGTCAG	318
lp_1041	957047	957481	435	+	rplP	ribosomal protein L16	15	ACGGGTTAAGTA TCGTCGTG	422	GATTCGCCACCTA CTTCCTC	407
lp_1047	958742	959284	543	+	rplE	ribosomal protein L5	38	TCGTTCCAGCAT TAGTTGAC	469	TTACAATGTCTAA ACCGCGA	431
lp_1051	959949	960485	537	+	rplF	ribosomal protein L6	150	TAAATTCACTCG TCCATCTG	501	TTCATCGACGTAA CGAATCC	351
lp_1053	960906	961406	501	+	rpsE	ribosomal protein S5	101	CTGCATTAGTAA TCGTTGGG	442	TTAAGCTCTTTAA GCCTTCG	341
lp_1055	961635	962066	432	+	rplO	ribosomal protein L15	70	TCAAGTGGCCAA GGTAAGAC	375	GAACTTGCTTGCC TTAACTG	305
lp_1058	963427	964077	651	+	adk	adenylate kinase	90	GACCGGAGATAT CTTCCGTG	418	ACTTATGGTAAGT TGCACCG	328
lp_1062	965520	966464	945	+	rpoA	DNA-directed RNA polymerase, alpha subunit	99	TTTAGGGAATTC ACTTCGTC	533	TAGTTGACACGTT CGATTGG	434
lp_1070	973064	973990	927	+		lipoprotein precursor	52	CTTTGATGATTC AGTCGCAC	468	CTTTGATGATTCA GTCGCAC	416
lp_1077	978985	979428	444	+	rplM	ribosomal protein L13	47	GGTATGTAGTTG ATGCAACC	349	GGCCTAAAGAGT TGTGAGGA	302
lp_1118	1015132	1016775	1644	+	mleS	malolactic enzyme	117	AACAATTGATGA ACAGGCGA	547	CTGGCAGTACTTG TGATGGG	430
lp_1261	1146477	1148120	1644	+	oppA	oligopeptide ABC transporter, substrate binding protein	1305	AAACGGGAACTT CGATACGG	1624	TGAAGTTGTAGTT TGAGCCC	319
lp_1274	1157416	1159146	1731	+	ptsI	phosphoenolpyruvate-protein phosphatase	102	AGTTTCCGATAC GGATGCTG	411	CGTTACGTCGCGA ATATCAC	309
lp_1316	1210182	1212608	2427	+	leuS	leucine-tRNA ligase	33	ATGGCAACATTA TTGGAAGG	495	CGTTCCACCCATC ATATCAG	462
lp_1329	1224048	1224695	648	-	dgk2	deoxyguanosine kinase	213	CGATAACAACGT CCTCGACC	509	CATTTCCGATAAG CGGTCCA	296
lp_1468	1345128	1345925	798	+		ABC transporter, ATP-binding protein	251	CACGTAAGGGAC TCTTCCTC	728	TCAAGGGTCTTTG CTAAGTC	477
lp_1508	1378501	1381149	2649	+	polA	DNA-directed DNA polymerase I	289	CAGCTATTAGAC GCGTATGG	719	TGTTCGCGATCCT CAATCAG	430

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lp_1514	1385839	1387803	1965	+	thrS	threonine-tRNA ligase 1	1060	CGCTCATACCGT GAATTACC	1516	CGTTCTGGTAACA AGAAGTC	451
lp_1615	1467824	1470241	2418	+	priA	primosomal protein N'	62	GTTATCGAATTC CAACCGCC	506	ACTTGGTAGTACA CATCGAC	444
lp_1632	1485226	1488783	3558	+	smc	cell division protein Smc	166	TTACGCGGCACT AAGATGAC	586	CAATGATATCGTT CACGCGG	420
lp_1643	1496310	1502969	6660	+		cell surface protein precursor	122	TTAGCCAAGTTG AACAAGTG	491	AATTTACTGGTGA CACTTGG	369
lp_1767	1601959	1603302	1344	+		lysin	643	ACATACCACCCG TTAAGCTG	1096	CCGTGATATAACC ACCACTC	453
lp_1882	1699597	1700886	1290	-	rpsA	ribosomal protein S1	241	GTTGTCATTTCTC GAATTGG	692	TGAACCAAACCA TCAATTCC	451
lp_1897	1711809	1713569	1761	-	pyk	pyruvate kinase	243	ATCTGAATACAA GATCGGTG	676	CTTGTGATTCGAT CTTAGGG	433
lp_1899	1714731	1718081	3351	-	dnaE	DNA-directed DNA polymerase III, alpha chain	321	TGCAGGATTGTC GCTAACAG	736	CCAGTTGCTCATA GTCAGCC	415
lp_1941	1759378	1760784	1407	-	nox4	NADH oxidase	9	AATGATCATCGG TAGTACCC	382	CTTTCTCATGATC GATACCC	373
lp_2027	1826580	1828448	1869	-	dnaK	heat shock protein DnaK	14	AAATTATCGGGA TTGACCTC	474	CAAACCATAGGC TAAAGCTG	460
lp_2054	1857090	1857968	879	-	tsf	elongation factor TS	405	GATCAGTCTTCG TCGTTTCC	805	AACGAATGAATG AGCTAACC	400
lp_2055	1858071	1858874	804	-	rpsB	ribosomal protein S2	298	ACTTTGACTAAC TGGAACAC	597	AATAACGTCGAT GTCATCTG	299
lp_2057	1860110	1861108	999	-	ldhD	D-lactate dehydrogenase	181	GTATTGAACAAG TTAGCCGA	610	ACGTGATAACATC AGCTTGG	429
lp_2097	1889787	1891754	1968	+	pts16ABC	fructose PTS, EIIABC	229	ACCGTGATGTTT GCTAAGAG	676	GCTTAACACCTTC AGAACCA	447
lp_2118	1911390	1912712	1323	-	tig	trigger factor	851	CGCATGATGCAA TTGAAGAC	1261	CAATCAAGTCAA CGGCTTGG	410
lp_2119	1912916	1914103	1188	-	tuf	elongation factor Tu	497	ACGATATTCCTG TTATCCGT	919	TGAACTTCTTGTG GGTTTGG	422
lp_2146	1938778	1940616	1839	-	typA	GTP-binding protein TypA	356	TGGAACAACACC TAACACCA	798	ACGTTGAAGACC AAAGAACC	442
lp_2193	1979094	1980377	1284	-	ftsZ	cell division protein FtsZ	218	GTTCTAATCCTG ATGTTGGT	635	AAGTCCAAGTTA ACGTACCC	417
lp_2290	2066643	2069711	3069	-		integral membrane protein	99	GACATTGATTTG GGAAGTGG	572	ATTACAACGGCG GTAATCAG	473
lp_2324	2101355	2102593	1239	-	gshA	glutamate-cysteine ligase (putative)	175	ACACAGTTGGAG TTAGTCAC	577	AGCAGCCATAAA GATACGTC	402
lp_2331	2108055	2108663	609	+	rpsD	ribosomal protein S4	42	TTTGGGAATGTC CCTTTCAG	430	CACGAACTGAGA CAACTTGG	388
lp_2486	2222028	2224781	2754	-		cell surface protein precursor, GY family	581	CATTGACGAGTG CTACTCAG	984	GGTAGTCCCTGTA AATTCCA	403
lp_2502	2233585	2234937	1353	-	pgi	glucose-6-phosphate isomerase	62	GCGAAATGCAAG CTATGGTC	496	ACTTCGCGATTAA ACGTTCC	434
lp_2659	2362936	2365302	2367	-	xpk1	phosphoketolase	1069	ATGGCTGCTAAC CCAATCAC	1445	GCAAATAACCCA TGACGTCC	376
lp_2694	2393220	2396825	3606	-	rexB	ATP-dependent nuclease, subunit B	497	TGGCGATCGTTT ACAAAGAC	941	ACATTCGTATTCG GCTCCTC	444
lp_3001	2669470	2672694	3225	-		cell surface protein precursor (putative)	552	GACGGCTAGCTT GTATTTGG	869	TTAAGGCCATAA GTGTGCCC	317
lp_3075	2735786	2738533	2748	-		cell surface protein (putative)	66	ATATCGTGGATC ACGGACAG	457	CTAGCGATGCACC AAATGAC	391
lp_3114	2772613	2778711	6099	-		cell surface protein precursor	205	ACATTGGCTAGT AAGTCGGT	587	TTCGTAGCTACTG TAGCCGT	382
lp_3170	2827652	2828344	693	-	pmg9	phosphoglycerate mutase	208	GAAGAATCCGAC CAACTCTG	582	TTCGATGTACTTG CTTAAGG	374
lp_3174	2832356	2833549	1194	+	cfa2	cyclopropane-fatty-acyl-phospholipid synthase	137	TTCCAATGCGTG AAATTACC	595	GTTCTTGTGATAA CGTGACC	458
lp_3204	2854090	2855373	1284	-	nupC	nucleoside transport protein	49	GCCATTGCCTAC TTATTCTC	482	TGCTTCAATTGTA ACTGCGA	433
lp_3313	2949059	2951317	2259	+	pflB2	formate C-acetyltransferase	1310	CCAAAGCGATTC TTTATGCC	1790	AGTTTGGCATTCC GATAGAG	480

Continued

lp_3421	3037010	3038122	1113	-		extracellular protein, gamma-D-glutamate-meso-diaminopimelate muropeptidase (putative)	442	GCTGCTCAATCA AGTAGCAC	855	AGAGCAGTCCATT TGTTCGT	413
lp_3485	3094698	3096914	2217	-	melA	alpha-galactosidase	42	AGAACAACTTGT CTTTCACC	401	TCTTCGACATAAG TTTGTGG	359
lp_3551	3169067	3171478	2412	+	xpk2	phosphoketolase	1172	TCGATGATTATG CTTTGGAC	1467	ATTGTAAGCTTCC AACATCC	295
lp_3662	3282611	3285214	2604	-	adhE	bifunctional protein: alcohol dehydrogenase, acetaldehyde dehydrogenase	483	GGAAGCGATTAA AGCTGCTG	924	TTTACCAGCAACT GGACCAC	441
lp_3665	3286816	3287352	537	+	pdc	p-coumaric acid decarboxylase	53	ACACTTATGATA ACGGCTGG	472	CTTTGTAAGGTGC TTCACTG	419
lp_0002	1546	2685	380	+	dnaN	DNA-directed DNA polymerase III, beta chain	712	CCTTCTATTCTCG CTTACTC	1129	CGAACTGGTGTA ATCAACTG	417
lp_0006	4565	6511	649	+	gyrB	DNA gyrase, B subunit	1451	GTTTACGGCAAT GGGAACTG	1875	CCATCAACATCGA GAAGACC	424
lp_0061	60505	61350	282	+		acetoacetate decarboxylase (putative)	368	GAATGACAACAG TGCGACAG	802	TCATACCGACCCG TTAAGAG	434
lp_0129	114984	115406	141	+	hsp1	small heat shock protein	36	ATGTTGGATCCG TTTGAACG	368	AATCGTTAAGAC ACCGTCAG	332
lp_0184	164479	165345	289	-	sacK1	fructokinase	196	CCGATGATCCGA AATATGGT	587	AATATCTTTCCCA GCTTTCC	391
lp_0210	193763	194950	396	+	ack1	acetate kinase	693	TTAACGGGTGTC ACAATGGG	1099	CTGATGAGTCCTT CTTGACC	406
lp_0233	213329	214486	386	+	mtlD	mannitol-1-phosphate 5-dehydrogenase	636	CTCTTCTCCGTTA ATACTGG	1067	TTGTTCGATTGGT TCATCCT	431
lp_0244	220124	220672	183	+		oxidoreductase (putative)	134	TAACCAAGACTA CGATGCGG	498	CTAAGAATGATTG GGTGCCT	364
lp_0301	277394	278113	240	-		membrane-bound protease, CAAX family	160	TAAATCGTGACT GGCTACGG	561	GCCATAGTAGCG ATGTTGTC	401
lp_0313	288937	290139	401	+	ndh1	NADH dehydrogenase	778	CACACGTCATTG CTGATTCC	1179	CTTTACTGAAGAC CGTTCCT	401
lp_0329	300197	301564	456	-	acdH	acetaldehyde dehydrogenase	861	GAAGCAACTGTC ATGAACCT	1265	GAGTGTTAAGGA TGGTGCGA	404
lp_0466	417214	418050	279	+	purR	purine biosynthesis operon repressor	388	AAATCGACGCTG TTATGACC	785	AATCGCCTTGTCA CTATCAC	397
lp_0481	429204	430817	538	+	pyrG	CTP synthase	771	GACCAAATCGTC CTTGACCA	1214	TTCAGCAGAGTTA GCATCCT	443
lp_0566	522053	522880	276	+	nadE	NAD synthase	368	GGAAGCTAACCA ATTGACGA	777	CAGTCTTTAAGTA CCACGCC	409
lp_0575	533143	534117	325	+	pts9AB	mannose PTS, EIIAB	456	CCAGGGAAGTTG GAATATGG	811	TGAACTTTACCAA CTGAGTG	355
lp_0585	558254	561127	958	+		transcription regulator	2308	TGGTCGAAATGA AGACACGG	2732	AATGTCTGGCATC TCTTCGG	424
lp_0597	570711	571388	226	+	pgm2	phosphoglycerate mutase	228	CTGCCTGAGTAT AAGACGTG	635	GTGATAACAAATT GGCACTC	407
lp_0602	574097	574780	228	-	rpiA1	ribose 5-phosphate epimerase	198	GGTATCCCGATG AAATCCGT	623	CATGTCTAAGAAC AGGCCGT	425
lp_0725	663325	664002	226	+		hypothetical protein	198	GGTATCCCGATG AAATCCGT	623	CATGTCTAAGAAC AGGCCGT	425
lp_0737	674619	675185	189	+		ribosomal protein S30EA	163	AAGTCACCATTC CACTTCCC	547	TCAATCAAACCGT AACGACC	384
lp_0754	688895	689842	316	+	hprK	bifunctional protein: HPr kinase, P-ser-HPr phosphatase	373	TGACGAATTACT TGGAAGGT	762	ATTTCCGGTTCTG TTCTCCA	389
lp_0807	748192	749169	326	+	pta	phosphate acetyltransferase	449	CTCGGGTGCATT TATCATGC	930	CTTCTTCACTGCA TCCACGT	481
lp_0852	790676	792439	588	+	pox2	pyruvate oxidase	1360	GCTTGCCCGTTA TTAACGTG	1738	AGTAAGGCGCCT AATGATGG	378
lp_0853	792561	793469	303	-	pepR1	prolyl aminopeptidase	425	TGTCAACGTTAA CAAGTGTC	860	CAAATGGTCAAA GTAGACGG	435
lp_1005	927175	928857	561	+	als	acetolactate synthase	1155	AGTGATGACATG ACAGTGAC	1562	ATCAGCAGGTTG GTTTACCC	407
lp_1090	992601	993524	308	-	ttdA	L(+)-tartrate dehydratase, subunit A	537	TTCGTGATGGAT CGCATGAC	914	TTCGACTCCTTTG TTCAGTG	377

Continued

lp_1101	999592	1000521	310	+	ldhL2	L-lactate dehydrogenase	283	ACCGCAATACGA AGATTCTG	657	TTTGACCACCTTT ACCACGA	374
lp_1108	1005719	1006633	305	+	citE	citrate lyase, beta chain	483	TATACGACGGAC ATGAAGAC	869	TTTGGCTAAGCTC ATCACAC	386
lp_1148	1044413	1045876	488	+	gatA	glutamyl-tRNA amidotransferase, subunit A	1015	ATGTCTATGTCC GTAGTCGT	1424	TTGCGTTGCTTGT TCAAAGG	409
lp_1149	1045876	1047300	475	+	gatB	glutamyl-tRNA amidotransferase, subunit B	945	GTGTTGACCCAA ACTAAGGA	1352	CATGATTTGACCC ACGAGGA	407
lp_1200	1093199	1094146	316	+	galE2	UDP-glucose 4-epimerase	347	CCTATTTACGTCT TCGGCAG	788	CAATGACGTTTCT GCAACCT	441
lp_1250	1134493	1136007	505	-	gntK	gluconokinase	1015	CCCACGGACTCT TATTTCAC	1433	ATATAACTGGTGG TTGGCGG	418
lp_1273	1157150	1157416	89	+	hpr	phosphocarrier protein Hpr	11	AGAATTTCACGT TATCGCAG	242	CATCGTTTCAGTT AAAGCTG	231
lp_1301	1189935	1191122	396	+	metK	methionine adenosyltransferase	726	GTTATTGGTGGT CCTCAAGG	1140	ATGGCAAATCAA TATCGGTC	414
lp_1500	1373935	1374624	230	+	narI	nitrate reductase, gamma chain	260	GTACCATATGTT CGGTTCCT	671	CCGATAAACGAT GTAACGCC	411
lp_1521	1391554	1392585	344	-		oxidoreductase	633	AGTGAAGTACAT GCTTTAGG	1026	CCCATCCTTCAAT AACTACC	393
lp_1541	1407210	1408646	479	+	gnd2	phosphogluconate dehydrogenase (decarboxylating)	987	CGTCAAGCCCTC TACTTCAG	1407	ATAACCCTTCACG GTCAGTC	420
lp_1563	1428800	1429282	161	+	greA2	transcription elongation factor GreA	54	AAAGAGCTTGAA GACCTTCG	458	TTTAACCGTCATG GTACCGG	404
lp_1665	1518459	1519502	348	+	adh1	alcohol dehydrogenase	590	AATCGTCTTGAT GAGTCGCC	1003	TGCATGTCTTGAT AAGCCTG	413
lp_1675	1526214	1527446	411	+	fabF	3-oxoacyl-[acyl-carrier protein] synthase II	750	CAGATTCTTGGT GAAGTCGT	1170	TACTCAAAGCATA GGTTGCG	420
lp_1779	1610466	1612121	552	+	fhs	formate-tetrahydrofolate ligase	1159	CCGTCAAAGACT ATTGTGCC	1561	GTCAAAGCGACA ATGAAACC	402
lp_1783	1614564	1615646	361	+	py-rAA2	carbamoyl-phosphate synthase (glutamine-hydrolysing), small chain	469	CAACACCGAAAC CATTTCCT	843	TTTGGTTCGTAAT CACTTCG	374
lp_1817	1643232	1644257	342	+		ribitol-5-phosphate 2-dehydrogenase (putative)	421	CATTCATTGAGA TGGTCTCG	830	TCGTAACGTCAAT CCTTCTG	409
lp_1898	1713655	1714617	321	-	pfk	6-phosphofructokinase	325	CATTGCGCTTAA CGGAACAC	746	ATGAGCAATGGT ATTCCCAC	421
lp_1981	1793507	1794787	427	-	hisS	histidine-tRNA ligase	842	CTACACGACGAT CTGTGCTG	1256	ATCTTGATAGATG TCCGCCA	414
lp_2030	1830545	1831255	237	-	aldB	alpha-acetolactate decarboxylase	173	TGAGGATGGTCA AATTCGTG	587	CGCTTGTTCGACT TCAAACC	414
lp_2052	1855602	1856165	188	-	frr	ribosome recycling factor	125	TTCTGCAGATTA TTACGGTG	473	TAACTGATGTAAC TGGTCGT	348
lp_2086	1880695	1881213	173	-	apt	adenine phosphoribosyltransferase	71	TATTTCACCACT GATGGCTG	466	TGCAACTCTTTGA GTTCGAC	395
lp_2094	1886553	1887848	432	-		GTP-binding protein	865	CCGAAGCTAATC TGGAAGAC	1256	CGTATCATCATTC TTGGCAC	391
lp_2096	1888848	1889765	306	+	fruK	1-phosphofructokinase	457	CTGCTGGTGCTA ACTTTGTC	857	CGCAATGTCTTCT GAGAAGG	400
lp_2123	1917039	1917911	291	-	dapA1	dihydrodipicolinate synthase	321	GTTCCCTACTAC AACAAGCC	730	CTCATCTTGGGTG TTAAGTC	409
lp_2153	1945980	1946957	326	-	pdhB	pyruvate dehydrogenase complex, E1 component, beta subunit	568	CCGTTCCGTTAG ATAAAGCA	950	TGCCTCAATTTCA TCTTCAG	382
lp_2154	1946959	1948071	371	-	pdhA	pyruvate dehydrogenase complex, E1 component, alpha subunit	511	CTTATACTGGTG ATGGTGGG	906	TACGGTAACGAA TCAATGGA	395
lp_2189	1976850	1977548	233	-	divIVA	cell division initiation protein DivIVA	201	AATCAATCTATC TTGGTGGC	585	TTGCTTCTGAGTT TACGCTC	384
lp_2231c	2022077	2022661	195	-	ppiB	peptidylprolyl isomerase	290	GGCATTATCAAT GGCCAACG	566	TGTTTCGATCACA ACATCCT	276

Continued

lp_2256	2039314	2040324	337	-	ccpA	catabolite control protein A	625	AGGCTAAGATT CCGTTTGAC	1008	AATCAGCAGACTT GGTTGAG	383
lp_2301	2078957	2080099	381	-	recA	recombinase A	678	GCAGAACAGAT CAAGGAAGG	1077	TACTTTGACCTTT ACTGCCA	399
lp_2323	2100621	2101115	165	-	tpx	thiol peroxidase	150	ATGCCAGATAT TGATACGCG	463	GCAACGTAATTTG GCTCGTG	313
lp_2345	2120498	2121610	371	-	ddl	D-alanine-D-alanine ligase	616	CCGATGCGTTC AAATATGAC	1021	GCCGTATAACTAA TGCCCGA	405
lp_2349	2123935	2124852	306	-	hicD3	L-2-hydroxyisocaproate dehydrogenase	467	AAAGTATGTCG GACAAGCAG	863	TAACGACGCTCGT TCATCAG	396
lp_2359	2130199	2131200	334	-	mreB2	cell shape determining protein MreB	467	GACTAGTGATA TCGCTGTCC	853	CCACCAGTCAACG TAATTCC	386
lp_2366	2137118	2138632	505	-	atpA	H(+)-transporting two-sector ATPase, alpha subunit	950	AATTATCGAAA CGCAAGCTG	1364	ACGGGCAATATCA TCAACTG	414
lp_2544	2269594	2270949	452	+	npr2	NADH peroxidase	848	GACCTTAGTCC CATTTGCCC	1264	GCTAAGTCAGCAA CAGTCAG	416
lp_2596	2313948	2314751	268	-	pflA1	formate acetyltransferase activating enzyme	374	TGAGACAACTG GTTACGCAC	793	TTCACCCGTACTT TAACACC	419
lp_2598	2315865	2318309	815	+	pflB1	formate C-acetyltransferase	1991	AACCTCTTCTAT TTCGGCCA	2404	GTTTCTTTGGATA GGCCCAC	413
lp_2681	2380421	2381905	495	+	gpd	glucose-6-phosphate 1-dehydrogenase	937	CTTTCGTCGCTG GTAAAGTC	1355	AACGAATTTCCAC GAATCGG	418
lp_2690	2398345	2398983	213	-	pyrE	orotate phosphoribosyltransferase	97	GTATTCGCCAA CCAGAACAG	490	GTACCGGCATCAT TGATCAG	393
lp_2697	2398345	2398983	213	-	pyrE	orotate phosphoribosyltransferase	97	GTATTCGCCAA CCAGAACAG	490	GTACCGGCATCAT TGATCAG	393
lp_2699	2399695	2400612	306	-	pyrD	dihydroorotate oxidase	124	TGACGATTTCTT ATCCGGCG	527	GTTGGACAAACTG AACAGCG	403
lp_2702	2404915	2406207	431	-	pyrC	dihydroorotase	422	ATTTGGAACTG ATCCAGCGA	838	ATGTCTGTACAAG CAGTCGG	416
lp_2703	2406211	2407146	312	-	pyrB	aspartate carbamoyltransferase	869	GTTAGTTGCGG GATTGTTGG	1284	GCGTTTCCTTTCC ATATGCC	415
lp_2704	2407293	2407835	181	-	pyrR1	pyrimidine operon regulator	554	CAAAGAATGGT ATGGCCGTG	932	AACCTCCACTTGA GTTGCTG	378
lp_2728	2428365	2429495	377	-	purK1	phosphoribosylaminoimidazole carboxylase, ATPase subunit	11	AGTCGTTGATG CAATGACCA	411	GTCCACGATCGAC TAAGACC	400
lp_2766	2457282	2458373	364	+		hypothetical protein	680	AGCTAATGTTC AGCCCAGTG	1073	TAATACGGTGACA TGACCCA	393
lp_2807	2504803	2506059	419	-	tyrS	tyrosine-tRNA ligase	540	TCAAATGATGC GACTTATGG	950	GTATTGCCCAAAC TCATCGT	410
lp_2873	2558269	2559309	347	-	adh2	alcohol dehydrogenase	760	TCAACCAGGAT GATCGAGAC	1144	CCATTGATTCGAA TCGCACC	384
lp_3051	2712741	2713913	391	+	dhaT	1,3-propanediol dehydrogenase	440	AGCCTTAGCTG ACGTAATGG	856	CGTAAGCCAATGT TCTTCCA	416
lp_3092	2751334	2752746	471	+	gabD	succinate-semialdehyde dehydrogenase (NAD(P)+)	682	AAACATCATTA CGCGAAGCC	1092	CGTCTTTAAGCGC ATTAGTC	410
lp_3125	2791021	2792502	494	+		fumarate reductase, flavoprotein subunit precursor, N-terminally truncated	864	AAATTAGTCCC TGGCGATCC	1270	AGTTCTGGTAAGG AGCTGAG	406
lp_3265	2904444	2905385	314	+		cell surface hydrolase, membrane-bound (putative)	960	GGGAACTTCAT GGGCTTAGG	1371	ACGTATCACCAGT TAGTCCA	411
lp_3270	2910392	2911681	430	+	purA	adenylosuccinate synthase	474	CGTCACACGGA TATCATCCT	896	TTGATACTCGGCA GGATCGA	422
lp_3314	2951354	2952175	274	+	pflA2	formate acetyltransferase activating enzyme	850	AAGTCGGTGAT TTCATTCGT	1266	TAACGTTAGTTTG TTGGCGA	416
lp_3352	2981556	2981999	148	-	hsp3	small heat shock protein	398	GTTCGAACGTC TAATGAAGG	794	CTTGTACCCGTTG TAATCAG	396
lp_3403	3017894	3018754	287	-		oxidoreductase	64	TGGACGATTTG GTTAATGGA	431	AATATGATGGGTA TCCGCAG	367
lp_3480	3089259	3090263	335	-		UTP-galactose-1-P uridylyltransferase	321	GTTGACCTCTAC TTGATCCA	711	AGAAACCGTGTTG TAATGAC	390
lp_3484	3093639	3094598	320	+	lacM	beta-galactosidase, small subunit	486	GGTCTGCGGTTT ATCATACC	882	AACTATCAATGCC ACCGACC	396

Continued

lp_3534	3151784	3154084	767	+	agl5	alpha-glucosidase	1834	GTGACGACATAC TAGTTGCC	2242	AATTCAACTGTGA TCTGCTG	408
lp_3544	3163853	3164476	208	-	gph3	phosphoglycolate phosphatase (putative)	143	CGGTGAGATGAT CCTGAGAG	517	CCTGCATTCTTTG AAGCCTG	374
lp_3545	3164582	3165640	353	-	gutB	L-iditol 2-dehydrogenase	575	TGTTTCTGGGAT CACTAAGG	969	AGTGTTCAAGATC AAAGACC	394
lp_3549	3168152	3168919	256	+		transcription regulator	285	TTCCTAGATTAT GGCACCAC	685	GCGTCCACGTTAC TAATGTC	400
lp_3555	3174155	3174883	243	-	araD	L-ribulose 5-phosphate 4-epimerase	305	CTATGCAGCTGC TCAAATGG	716	TGCATGATCCTTA GAATGCG	411
lp_3583	3199392	3201506	705	-	clpL	ATP-dependent Clp protease, ATP-binding subunit ClpL	1698	ATCGCTACTTCT AATGCTGG	2110	GCTGCCGATATCA CAATCTC	412
lp_3586	3202767	3203867	367	-	lox	lactate oxidase	628	TCATGGAAATCT ATGCTGCT	1029	GCTCATCATTAAG GTGACTC	401
lp_3589	3206328	3208139	604	-	pox5	pyruvate oxidase	1319	GGTGTTTAATCT GGCTGGTG	1734	AATCTTGAGCTTC ATACCGT	415
lp_3592	3209669	3210514	282	-	rhaD	rhamnulose-1-phosphate aldolase	376	CTCGGTTGAAGC AAGATCCT	783	AACGCTTGATTAA GTCACGG	407
lp_3603	3219914	3220636	241	+		sugar-phosphate aldolase	258	CAACAAATTGAC GGTGTAGG	685	TGGTACTGCTTAA TTAGCCC	427

Supplement 2. Transfer of the amplified PCR products to 348 well plates for 384-pin gridding onto nitrocellulose membranes.

ORF	Gene	Product	Plate_96	96 Well	Target Plate	384 Well	384 Well
lp_0175	malE	maltose/maltodextrin ABC transporter, substrate binding protein	M2_1_96	A01	Plate_1	A01	A16
lp_0230	pts2CB	mannitol PTS, EIICB	M2_1_96	B01	Plate_1	C01	C16
lp_0233	mtlD	mannitol-1-phosphate 5-dehydrogenase	M2_1_96	C01	Plate_1	E01	E16
lp_0257	pepM	methionyl aminopeptidase	M2_1_96	D01	Plate_1	G01	G16
lp_0302		extracellular protein	M2_1_96	E01	Plate_1	I01	I16
lp_0304		extracellular protein	M2_1_96	F01	Plate_1	K01	K16
lp_0330	fba	fructose-bisphosphate aldolase	M2_1_96	G01	Plate_1	M01	M16
lp_0447	mvaA	hydroxymethylglutaryl-CoA reductase	M2_1_96	H01	Plate_1	O01	O16
lp_0480	rpoE	DNA-directed RNA polymerase, delta subunit	M2_1_96	A02	Plate_1	A02	A17
lp_0537	ldhL1	L-lactate dehydrogenase	M2_1_96	B02	Plate_1	C02	C17
lp_0539	mfd	transcription-repair coupling factor	M2_1_96	C02	Plate_1	E02	E17
lp_0547	ftsH	cell division protein FtsH, ATP-dependent zinc metallopeptidase	M2_1_96	D02	Plate_1	G02	G17
lp_0576	pts9C	mannose PTS, EIIC	M2_1_96	E02	Plate_1	I02	I17
lp_0577	pts9D	mannose PTS, EIID	M2_1_96	F02	Plate_1	K02	K17
lp_0601	pepC1	cysteine aminopeptidase	M2_1_96	G02	Plate_1	M02	M17
lp_0609	gltX	glutamate-tRNA ligase	M2_1_96	H02	Plate_1	O02	O17
lp_0619	rplK	ribosomal protein L11	M2_1_96	A03	Plate_1	A03	A18
lp_0620	rplA	ribosomal protein L1	M2_1_96	B03	Plate_1	C03	C18
lp_0621	rplJ	ribosomal protein L10	M2_1_96	C03	Plate_1	E03	E18
lp_0690		integral membrane protein (putative)	M2_1_96	D03	Plate_1	G03	G18
lp_0692	nrdF	ribonucleoside-diphosphate reductase, beta chain	M2_1_96	E03	Plate_1	I03	I18
lp_0715	phnD	phosphonates ABC transporter, substrate binding protein (putative)	M2_1_96	F03	Plate_1	K03	K18
lp_0728	groEL	GroEL chaperonin	M2_1_96	G03	Plate_1	M03	M18
lp_0757	galU	UTP-glucose-1-phosphate uridylyltransferase	M2_1_96	H03	Plate_1	O03	O18
lp_0786	clpP	endopeptidase Clp, proteolytic subunit	M2_1_96	A04	Plate_1	A04	A19

Continued

lp_0789	gapB	glyceraldehyde 3-phosphate dehydrogenase	M2_1_96	B04	Plate_1	C04	C19
lp_0790	pgk	phosphoglycerate kinase	M2_1_96	C04	Plate_1	E04	E19
lp_0791	tpiA	triosephosphate isomerase	M2_1_96	D04	Plate_1	G04	G19
lp_0792	enoA1	phosphopyruvate hydratase	M2_1_96	E04	Plate_1	I04	I19
lp_0800		cell surface protein precursor	M2_1_96	F04	Plate_1	K04	K19
lp_0923		cell surface protein precursor	M2_1_96	G04	Plate_1	M04	M19
lp_0938	hsdR	type I site-specific deoxyribonuclease, HsdR subunit	M2_1_96	H04	Plate_1	O04	O19
lp_0959	pepD3	dipeptidase	M2_1_96	A05	Plate_1	A05	A20
lp_1012	serS2	serine-tRNA ligase	M2_1_96	B05	Plate_1	C05	C20
lp_1021	rpoB	DNA-directed RNA polymerase, beta subunit	M2_1_96	C05	Plate_1	E05	E20
lp_1022	rpoC	DNA-directed RNA polymerase, beta' subunit	M2_1_96	D05	Plate_1	G05	G20
lp_1025	rpsL	ribosomal protein S12	M2_1_96	E05	Plate_1	I05	I20
lp_1026	rpsG	ribosomal protein S7	M2_1_96	F05	Plate_1	K05	K20
lp_1027	fusA2	elongation factor G	M2_1_96	G05	Plate_1	M05	M20
lp_1033	rplC	ribosomal protein L3	M2_1_96	H05	Plate_1	O05	O20
lp_1034	rplD	ribosomal protein L4	M2_1_96	A06	Plate_1	A06	A21
lp_1036	rplB	ribosomal protein L2	M2_1_96	B06	Plate_1	C06	C21
lp_1040	rpsC	ribosomal protein S3	M2_1_96	C06	Plate_1	E06	E21
lp_1041	rplP	ribosomal protein L16	M2_1_96	D06	Plate_1	G06	G21
lp_1047	rplE	ribosomal protein L5	M2_1_96	E06	Plate_1	I06	I21
lp_1051	rplF	ribosomal protein L6	M2_1_96	F06	Plate_1	K06	K21
lp_1053	rpsE	ribosomal protein S5	M2_1_96	G06	Plate_1	M06	M21
lp_1055	rplO	ribosomal protein L15	M2_1_96	H06	Plate_1	O06	O21
lp_1058	adk	adenylate kinase	M2_1_96	A07	Plate_1	A07	A22
lp_1062	rpoA	DNA-directed RNA polymerase, alpha subunit	M2_1_96	B07	Plate_1	C07	C22
lp_1070		lipoprotein precursor	M2_1_96	C07	Plate_1	E07	E22
lp_1077	rplM	ribosomal protein L13	M2_1_96	D07	Plate_1	G07	G22
lp_1118	mleS	malolactic enzyme	M2_1_96	E07	Plate_1	I07	I22
lp_1261	oppA	oligopeptide ABC transporter, substrate binding protein	M2_1_96	F07	Plate_1	K07	K22
lp_1274	ptsI	phosphoenolpyruvate-protein phosphatase	M2_1_96	G07	Plate_1	M07	M22
lp_1316	leuS	leucine-tRNA ligase	M2_1_96	H07	Plate_1	O07	O22
lp_1329	dgk2	deoxyguanosine kinase	M2_1_96	A08	Plate_1	A08	A23
lp_1468		ABC transporter, ATP-binding protein	M2_1_96	B08	Plate_1	C08	C23
lp_1508	polA	DNA-directed DNA polymerase I	M2_1_96	C08	Plate_1	E08	E23
lp_1514	thrS	threonine-tRNA ligase 1	M2_1_96	D08	Plate_1	G08	G23
lp_1615	priA	primosomal protein N'	M2_1_96	E08	Plate_1	I08	I23
lp_1632	smc	cell division protein Smc	M2_1_96	F08	Plate_1	K08	K23
lp_1643		cell surface protein precursor	M2_1_96	G08	Plate_1	M08	M23
lp_1767		lysin	M2_1_96	H08	Plate_1	O08	O23
lp_1882	rpsA	ribosomal protein S1	M2_1_96	A09	Plate_1	A09	A24
lp_1897	pyk	pyruvate kinase	M2_1_96	B09	Plate_1	C09	C24
lp_1899	dnaE	DNA-directed DNA polymerase III, alpha chain	M2_1_96	C09	Plate_1	E09	E24
lp_1941	nox4	NADH oxidase	M2_1_96	D09	Plate_1	G09	G24

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lp_2027	dnaK	heat shock protein DnaK	M2_1_96	E09	Plate_1	I09	I24
lp_2054	tsf	elongation factor TS	M2_1_96	F09	Plate_1	K09	K24
lp_2055	rpsB	ribosomal protein S2	M2_1_96	G09	Plate_1	M09	M24
lp_2057	ldhD	D-lactate dehydrogenase	M2_1_96	H09	Plate_1	O09	O24
lp_0002	dnaN	DNA-directed DNA polymerase III, beta chain	M2_2_96	A01	Plate_1	B01	B16
lp_0006	gyrB	DNA gyrase, B subunit	M2_2_96	B01	Plate_1	D01	D16
lp_0061		acetoacetate decarboxylase (putative)	M2_2_96	C01	Plate_1	F01	F16
lp_0129	hsp1	small heat shock protein	M2_2_96	D01	Plate_1	H01	H16
lp_0184	sacK1	fructokinase	M2_2_96	E01	Plate_1	J01	J16
lp_0210	ack1	acetate kinase	M2_2_96	F01	Plate_1	L01	L16
lp_0233	mtlD	mannitol-1-phosphate 5-dehydrogenase	M2_2_96	G01	Plate_1	N01	N16
lp_0244		oxidoreductase (putative)	M2_2_96	H01	Plate_1	P01	P16
lp_0301		membrane-bound protease, CAAX family	M2_2_96	A02	Plate_1	B02	B17
lp_0313	ndh1	NADH dehydrogenase	M2_2_96	B02	Plate_1	D02	D17
lp_0329	acdH	acetaldehyde dehydrogenase	M2_2_96	C02	Plate_1	F02	F17
lp_0466	purR	purine biosynthesis operon repressor	M2_2_96	D02	Plate_1	H02	H17
lp_0481	pyrG	CTP synthase	M2_2_96	E02	Plate_1	J02	J17
lp_0566	nadE	NAD synthase	M2_2_96	F02	Plate_1	L02	L17
lp_0575	pts9AB	mannose PTS, EIIAB	M2_2_96	G02	Plate_1	N02	N17
lp_0585		transcription regulator	M2_2_96	H02	Plate_1	P02	P17
lp_0597	pgm2	phosphoglycerate mutase	M2_2_96	A03	Plate_1	B03	B18
lp_0602	rpiA1	ribose 5-phosphate epimerase	M2_2_96	B03	Plate_1	D03	D18
lp_0725		hypothetical protein	M2_2_96	C03	Plate_1	F03	F18
lp_0737		ribosomal protein S30EA	M2_2_96	D03	Plate_1	H03	H18
lp_0754	hprK	bifunctional protein: HPr kinase, P-ser-HPr phosphatase	M2_2_96	E03	Plate_1	J03	J18
lp_0807	pta	phosphate acetyltransferase	M2_2_96	F03	Plate_1	L03	L18
lp_0852	pox2	pyruvate oxidase	M2_2_96	G03	Plate_1	N03	N18
lp_0853	pepR1	prolyl aminopeptidase	M2_2_96	H03	Plate_1	P03	P18
lp_1005	als	acetolactate synthase	M2_2_96	A04	Plate_1	B04	B19
lp_1090	ttdA	L(+)-tartrate dehydratase, subunit A	M2_2_96	B04	Plate_1	D04	D19
lp_1101	ldhL2	L-lactate dehydrogenase	M2_2_96	C04	Plate_1	F04	F19
lp_1108	citE	citrate lyase, beta chain	M2_2_96	D04	Plate_1	H04	H19
lp_1148	gatA	glutamyl-tRNA amidotransferase, subunit A	M2_2_96	E04	Plate_1	J04	J19
lp_1149	gatB	glutamyl-tRNA amidotransferase, subunit B	M2_2_96	F04	Plate_1	L04	L19
lp_1200	galE2	UDP-glucose 4-epimerase	M2_2_96	G04	Plate_1	N04	N19
lp_1250	gntK	gluconokinase	M2_2_96	H04	Plate_1	P04	P19
lp_1273	hpr	phosphocarrier protein Hpr	M2_2_96	A05	Plate_1	B05	B20
lp_1301	metK	methionine adenosyltransferase	M2_2_96	B05	Plate_1	D05	D20
lp_1500	narI	nitrate reductase, gamma chain	M2_2_96	C05	Plate_1	F05	F20
lp_1521		oxidoreductase	M2_2_96	D05	Plate_1	H05	H20
lp_1541	gnd2	phosphogluconate dehydrogenase (decarboxylating)	M2_2_96	E05	Plate_1	J05	J20
lp_1563	greA2	transcription elongation factor GreA	M2_2_96	F05	Plate_1	L05	L20
lp_1665	adh1	alcohol dehydrogenase	M2_2_96	G05	Plate_1	N05	N20

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lp_1675	fabF	3-oxoacyl-[acyl-carrier protein] synthase II	M2_2_96	H05	Plate_1	P05	P20
lp_1779	fhs	formate-tetrahydrofolate ligase	M2_2_96	A06	Plate_1	B06	B21
lp_1783	pyrAA2	carbamoyl-phosphate synthase (glutamine-hydrolysing), small chain	M2_2_96	B06	Plate_1	D06	D21
lp_1817		ribitol-5-phosphate 2-dehydrogenase (putative)	M2_2_96	C06	Plate_1	F06	F21
lp_1898	pfk	6-phosphofructokinase	M2_2_96	D06	Plate_1	H06	H21
lp_1981	hisS	histidine-tRNA ligase	M2_2_96	E06	Plate_1	J06	J21
lp_2030	aldB	alpha-acetolactate decarboxylase	M2_2_96	F06	Plate_1	L06	L21
lp_2052	frr	ribosome recycling factor	M2_2_96	G06	Plate_1	N06	N21
lp_2086	apt	adenine phosphoribosyltransferase	M2_2_96	H06	Plate_1	P06	P21
lp_2094		GTP-binding protein	M2_2_96	A07	Plate_1	B07	B22
lp_2096	fruK	1-phosphofructokinase	M2_2_96	B07	Plate_1	D07	D22
lp_2123	dapA1	dihydrodipicolinate synthase	M2_2_96	C07	Plate_1	F07	F22
lp_2153	pdhB	pyruvate dehydrogenase complex, E1 component, beta subunit	M2_2_96	D07	Plate_1	H07	H22
lp_2154	pdhA	pyruvate dehydrogenase complex, E1 component, alpha subunit	M2_2_96	E07	Plate_1	J07	J22
lp_2189	divIVA	cell division initiation protein DivIVA	M2_2_96	F07	Plate_1	L07	L22
lp_2231c	ppiB	peptidylprolyl isomerase	M2_2_96	G07	Plate_1	N07	N22
lp_2256	ccpA	catabolite control protein A	M2_2_96	H07	Plate_1	P07	P22
lp_2301	recA	recombinase A	M2_2_96	A08	Plate_1	B08	B23
lp_2323	tpx	thiol peroxidase	M2_2_96	B08	Plate_1	D08	D23
lp_2345	ddl	D-alanine-D-alanine ligase	M2_2_96	C08	Plate_1	F08	F23
lp_2349	hicD3	L-2-hydroxyisocaproate dehydrogenase	M2_2_96	D08	Plate_1	H08	H23
lp_2359	mreB2	cell shape determining protein MreB	M2_2_96	E08	Plate_1	J08	J23
lp_2366	atpA	H(+)-transporting two-sector ATPase, alpha subunit	M2_2_96	F08	Plate_1	L08	L23
lp_2544	npr2	NADH peroxidase	M2_2_96	G08	Plate_1	N08	N23
lp_2596	pflA1	formate acetyltransferase activating enzyme	M2_2_96	H08	Plate_1	P08	P23
lp_2598	pflB1	formate C-acetyltransferase	M2_2_96	A09	Plate_1	B09	B24
lp_2681	gpd	glucose-6-phosphate 1-dehydrogenase	M2_2_96	B09	Plate_1	D09	D24
lp_2690	pyrE	orotate phosphoribosyltransferase	M2_2_96	C09	Plate_1	F09	F24
lp_2697	pyrD	dihydroorotate oxidase	M2_2_96	D09	Plate_1	H09	H24
lp_2699	pyrC	dihydroorotase	M2_2_96	E09	Plate_1	J09	J24
lp_2702	pyrB	aspartate carbamoyltransferase	M2_2_96	F09	Plate_1	L09	L24
lp_2703	pyrR1	pyrimidine operon regulator	M2_2_96	G09	Plate_1	N09	N24
lp_2704	purK1	phosphoribosylaminoimidazole carboxylase, ATPase subunit	M2_2_96	H09	Plate_1	P09	P24
lp_2097	pts16ABC	fructose PTS, EIIABC	M2_1_96	A10	Plate_2	A01	A22
lp_2118	tig	trigger factor	M2_1_96	B10	Plate_2	C01	C22
lp_2119	tuf	elongation factor Tu	M2_1_96	C10	Plate_2	E01	E22
lp_2146	typA	GTP-binding protein TypA	M2_1_96	D10	Plate_2	G01	G22
lp_2193	ftsZ	cell division protein FtsZ	M2_1_96	E10	Plate_2	I01	I22
lp_2290		integral membrane protein	M2_1_96	F10	Plate_2	K01	K22
lp_2324	gshA	glutamate-cysteine ligase (putative)	M2_1_96	G10	Plate_2	M01	M22
lp_2331	rpsD	ribosomal protein S4	M2_1_96	H10	Plate_2	O01	O22
lp_2486		cell surface protein precursor, GY family	M2_1_96	A11	Plate_2	A02	A23

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lp_2502	pgi	glucose-6-phosphate isomerase	M2_1_96	B11	Plate_2	C02	C23
lp_2659	xpk1	phosphoketolase	M2_1_96	C11	Plate_2	E02	E23
lp_2694	rexB	ATP-dependent nuclease, subunit B	M2_1_96	D11	Plate_2	G02	G23
lp_3001		cell surface protein precursor (putative)	M2_1_96	E11	Plate_2	I02	I23
lp_3075		cell surface protein (putative)	M2_1_96	F11	Plate_2	K02	K23
lp_3114		cell surface protein precursor	M2_1_96	G11	Plate_2	M02	M23
lp_3170	pmg9	phosphoglycerate mutase	M2_1_96	H11	Plate_2	O02	O23
lp_3174	cfa2	cyclopropane-fatty-acyl-phospholipid synthase	M2_1_96	A12	Plate_2	A03	A24
lp_3204	nupC	nucleoside transport protein	M2_1_96	B12	Plate_2	C03	C24
lp_3313	pflB2	formate C-acetyltransferase	M2_1_96	C12	Plate_2	E03	E24
lp_3421		extracellular protein, gamma-D-glutamate-meso-diaminopimelate muropeptidase (putative)	M2_1_96	D12	Plate_2	G03	G24
lp_3485	melA	alpha-galactosidase	M2_1_96	E12	Plate_2	I03	I24
lp_3551	xpk2	phosphoketolase	M2_1_96	F12	Plate_2	K03	K24
lp_3662	adhE	bifunctional protein: alcohol dehydrogenase, acetaldehyde dehydrogenase	M2_1_96	G12	Plate_2	M03	M24
lp_3665	pdc	p-coumaric acid decarboxylase	M2_1_96	H12	Plate_2	O03	O24
lp_2728		hypothetical protein	M2_2_96	A10	Plate_2	B01	B22
lp_2766	tyrS	tyrosine-tRNA ligase	M2_2_96	B10	Plate_2	D01	D22
lp_2807	adh2	alcohol dehydrogenase	M2_2_96	C10	Plate_2	F01	F22
lp_2873	dhaT	1,3-propanediol dehydrogenase	M2_2_96	D10	Plate_2	H01	H22
lp_3051	gabD	succinate-semialdehyde dehydrogenase (NAD(P)+)	M2_2_96	E10	Plate_2	J01	J22
lp_3092		fumarate reductase, flavoprotein subunit precursor, N-terminally truncated	M2_2_96	F10	Plate_2	L01	L22
lp_3125		cell surface hydrolase, membrane-bound (putative)	M2_2_96	G10	Plate_2	N01	N22
lp_3265	purA	adenylosuccinate synthase	M2_2_96	H10	Plate_2	P01	P22
lp_3270	pflA2	formate acetyltransferase activating enzyme	M2_2_96	A11	Plate_2	B02	B23
lp_3314	hsp3	small heat shock protein	M2_2_96	B11	Plate_2	D02	D23
lp_3352		oxidoreductase	M2_2_96	C11	Plate_2	F02	F23
lp_3403	galE4	UDP-glucose 4-epimerase	M2_2_96	D11	Plate_2	H02	H23
lp_3482	galK	galactokinase	M2_2_96	E11	Plate_2	J02	J23
lp_3484	lacM	beta-galactosidase, small subunit	M2_2_96	F11	Plate_2	L02	L23
lp_3534	agl5	alpha-glucosidase	M2_2_96	G11	Plate_2	N02	N23
lp_3544	gph3	phosphoglycolate phosphatase (putative)	M2_2_96	H11	Plate_2	P02	P23
lp_3545	gutB	L-iditol 2-dehydrogenase	M2_2_96	A12	Plate_2	B03	B24
lp_3549		transcription regulator	M2_2_96	B12	Plate_2	D03	D24
lp_3555	araD	L-ribulose 5-phosphate 4-epimerase	M2_2_96	C12	Plate_2	F03	F24
lp_3583	clpL	ATP-dependent Clp protease, ATP-binding subunit ClpL	M2_2_96	D12	Plate_2	H03	H24
lp_3586	lox	lactate oxidase	M2_2_96	E12	Plate_2	J03	J24
lp_3589	pox5	pyruvate oxidase	M2_2_96	F12	Plate_2	L03	L24
lp_3592	rhaD	rhamnulose-1-phosphate aldolase	M2_2_96	G12	Plate_2	N03	N24
lp_3603		sugar-phosphate aldolase	M2_2_96	H12	Plate_2	P03	P24

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