# Bioinformatics Practicals In Sillico

### BC-7107

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## Introduction

Bioinformatics is the application of computational technology to handle the rapidly growing repository of information related to molecular biology. Bioinformatics combines different fields of study, including computer sciences, molecular biology, biotechnology, statistics and engineering. It is particularly useful for managing and analysing large sets of data, such as those generated by the fields of genomics and proteomics.

In this report we focus on the bioinformatics tools for mutant analysis through three different projects; mutations in gai and spy in Arabidopsis Thaliana, mutations in Saccharomyces cerevisiae and mutations as well as Denovo assembly in Lactobacillus Helveticus. We want to sort out new mutation with these tools and learn how to design a bioinformatics test. It includes the quality test, the annotation of our sequenced genomes and various analysis of these results. Thus, everything upstream of the analysis must be properly done, using several software described thereafter. Our machines are too week in order to analyse the data and performed the bioinformatics steps, thus, we will use a cluster dedicated for this lecture, in Bern Switzerland.

## Yeast Genome Analysis

### Introduction

The budding yeast Saccharomyces cerevisiae (S.cerevisiae) is a single-celled lower eukaryote belonging to the kingdom of fungi. Ever since its discovery, S.cerevisae has nourished human advancements in the field of fermented food products, alcoholic beverages (e.g. beer, which is the epony of S.cerevisae) and the production of biofuel.1 In addition to the contribution industrial fermentation, S.cerevisiae has become one of the most popular model organism for eukaryotic biology, due to its simple cellular architecture, cheap maintenance cost, fast growth, non-pathogenic nature (discussed in [1]), and homologies to human cells (e.g. ribosomes), which cannot be studied in prokaryotic model organism, such as E.coli [2]. In particular, the genetic analysis of S.cerevisiae has gained popularity in the scientific community since it was the first eukaryotic organism whose genome was fully sequenced. The haploid genome of S.cerevisiae consists of 16 linear chromosomes containing 6604 genes encoded within approximately 12 megabase-pairs (Mbp) [3]. The fact that genome of S.cerevisiae is quite small and almost completely void of intronic DNA, thus making it an ideal microorganism for the identification of mutations and single nucleotide polymorphisms.

Mating of two haploid yeast of opposite mating type (i.e. Mat a or  $\alpha$ ) gives rise to diploid cells that possess 32 chromosomes. This is a single eukaryotic organism with a division cycle of 90 minutes. Through the process of budding in which smaller daughter cells pinch, or bud, off the mother cell. S.cerevisiae forms colonies on agar plates in the laboratory in a few days with no special incubators required (best grows at  $30^{\circ}$ C).

#### TODO Thibault INTRO SERIEUSE SUR TOM1 - intro gene rapport yeast

Target of Myb protein1 (Tom1), is a gene involved in the ribosomal biogenesis in yeast and human respectively. In human, this gene is involved in several pathways including endocytosis, endosomal transport, intracellular protein transport, neutrophil degranulation and protein transport. [4] [5] It is located on the ch.22 (component UP000005640) and ch.4 in human and yeast respectively. As it is well conserved among the eukaryote, we can study the gene with yeast foe the raisons explained above. In yeast specifically, TOM1 was first described as gene involved in temperature sensitivity and could be supress by STM1. [6] TOM1 is a hect-domain, wherein has been identified as a conserved feature of E3 ubiquitin ligases group. It regulates transcriptional activation Through effectors ADA on coactivator proteins on the DNA. The action of TOM1 is to regulate through ubiquitination the temperature sensitivity. [7] A tom1-1 mutant has been isolated, and under electron microscopy and indirect immunofluorescence microscopy, it has been shown that the large nucleus contains duplicated DNA and short spindle and structures fragmentations.5. This show that the disruption of the system that impact the nuclear transport and the cell division in the G1 phase. [5] [8] TOM1 encode for a large 380KDa proteins with a hect-domain at its C terminus (homologous to E6-AO C terminus). Site-directed mutagenesis of the conserved cysteine residue (tom1C3235A) in the hect-domain, supposed to be necessary for thioester-bond formation with ubiquitin, abolished the gene function. After a test with the over production of a myc-tagged ubiquitinRA, it shows that TOM1 is a ubiquitin ligase. [8] More recently, TOM1 has been described as a fundamental macromolecular machine. The ribosomes biogenesis is much more complex in eukaryote cells as in bacteria, and it is involved in several fundamental cellular processes, including growth and cell division. [9] Ribosomes are subunits assemblage allowing the production of proteins. Subunits are made of RNA (rRNA) and specifies proteins (r-proteins) (Saccharomyces cerevisiae: 40S [18S rRNA, 33 RPs]; 60S [25S, 5.8S, 5S rRNA, 46 RPs]-Escherichia coli: 30S [16S rRNA, 21 RPs]; 50S [23S, 5S rRNA, 34 RPs]). [10] Recent studies have shown that defect in the biogenesis linked to a wide range of hereditary diseases like Alzheimer's and anemia. [11] Ribosomes are a mixture of almost 80 different protein and stick together through a scaffold made by the RNA as explain above. Each protein is express in one copy and each of these proteins are needed to assemble the ribosomes. However, the number of steps needed for the biogenesis is large and not totally known. Moreover, it is impossible for a cell to produce the exact number of the needed proteins, furthermore the same number of copies of all the proteins in a ribosome [12] It will build up the number needed and then degrade the leftover, wherein are ubiquinined by TOM1 and degraded in lysosomes. We can say that TOM1 act as a quality control on this mechanism during the anabolism and division phases of the cells, leading to a week and crucial homeostasis [12] Ribosome biogenesis is an intricate process involving many chaperons and assembly factors (>200 factors) and snoRNAs (75) [10] Two subunits are part of the final ribosomes, the 40S has one rRNA (18S) and 33 r-proteins. The 60S (comprises three rRNAs (25S, 5.8S, 5S) and 47 r-proteins subunit [10]

#### todo ci-dessous ça veut rien dire, la 2ème phrase est pas finie

The assembly and maturation of the ribosomes passes from the nucleus to the cytosol. ATP-dependent RNA helicases and three AAA-type ATPases (ATPases associated with various cellular activities). This suggests that the energy derived by these enzymes is required for ribosomes assembly. The absence of one of these proteins might stall ribosome biogenesis and terminate cell growth even under optimal growth conditions [9] [10].

To summarize, TOM1 is necessary to the ribosome's biogenesis and the elimination of the leftover building blocks by ubiquitination. Thus, the aim of this project is to identify the suppressor of this gene by high sequencing throughput with bioinformatics tools.

#### TODO on en fait référence nule part à cette figure -; nécessaire ?

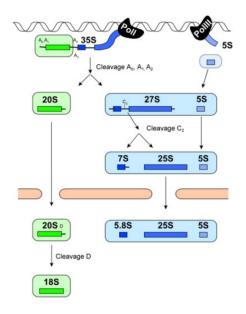


Figure 1: Simplified overview of the major steps in pre-rRNA processing.

### Methods

Quality control of sequences The high throughput sequencing method isn't infaillble, so the data will have contaminants, badly read sequences due to the intensity of the fluorescent signal, or the quality of the reagents that have a decaying quality with the number of sequencing cycles over time [13]. These errors might add a lot of false signals, useless extrawork, and complicate the

data analysis, therefore we need to get rid of them. In order to check for the quality of the data, we use the tool: fastqc [14] which will help us visualize our fasta file given by the sequencer.

**Trimming and bad quality removal** After the quality control check, we used the tool *trimmomatic* [15] with the given parameters of MINLEN: 130 to trim down the bad quality ends of the reads, keeping at least 130bp of the trimmed read, and the parameter SLIDINGWINDOW:4:15, thus removing the reads that have an average base pair quality score lower than 15. The next step was checking if the quality of the data has improved after the trimming process, by using again fastqc, on the trimmomatic fastq file output.

Sequence alignment against reference Since the fasta files gives no information about the sequences position in the yeast genome, we had to align all of the reads against the fasta file of a known yeast genome, or most likely a consensus of a yeast genome, containing the positional information.

However, in order to do that, we first had to index the reference fasta using the *bwa index* tool [16], which is a way of giving a sort of table of contents of our reference fasta file (in our case the R64-1-1.92.fa), that is used by the burrows-wheeler aligner algorithm. Subsequently, we used the *bwa mem* tool in order to align our sequenced data against the reference. Then, we have converted all the SAM [17] files containing our aligned sequences, into BAM files, a compressed binary format easier to work with.

Variant calling and annotation This has been done using only samtools mpileup, that took our reference file, and the aligned BAM files as an input, and gave us the binary format of the Variant Calling Format files (.vcf). Then we used beftools to convert them into vcf.gz files. This method was prefered considering the fact that our genome is a haploid yeast genome, and it doesn't need a complicated algorithm as used by the GATK pipeline. The tabix [18] tool was used on the vcf files, in order to index them properly.

In order to annotate the variants given by the vcf files, we had to use SNPeff tool on the vcf files that were merged together with all their indexes, and we also kept only the variants that were found in less than all 4 strains that we had to analyse, since we had to filter through all the variants that were different from the reference. The variants interesting to us, are indeed the ones that are specific to one of the mutants, and that's why we had to filter this way.

The results were then visualized by either reading the vcf files in xcel or in IGV.

 $\operatorname{TODO}$  Lionel WIP Gene  $\operatorname{TOM}$  ou autre polymorphisme trouvé à décrire dans le fichier vcf.

## Arabidopsis Thaliana Genome Analysis

TODO ALAIN: REVOIR INTRO, MERGE SPY

TODO LIONEL: CHANGER IMAGE

### Introduction

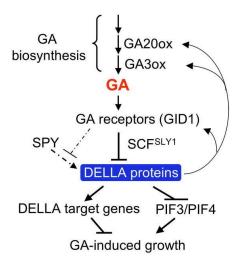
The plant Arabidopsis thaliana is a genetic model worldwide used in plant biology since 1995, when it has been promoted as model for molecular genetics. The genome is entirely sequenced in 200. ATH is a diploid organism of 114,5 to 125 million base pairs within 5 chromosomes (haploid). The germination to mature seed is done about 6 weeks and easy to cultivate in restricted space and produce a lot of seed. A wide range of mutants are already available and it growth from year to another through multinational research community of academic, government and industry laboratories. The importance of ATH is crucial and invaluable resources to fight the loss of crops due to plants diseases.

GAI Gibberellic-Acid Insensitive (GAI) is a gene in Arabidopsis thaliana in chromosome 1 which is involved in the regulation of plant growth. Precisely, it mediated the input signals and module the growth by decreasing the responsiveness to gibberellin [19]. Gibberellin is a tetracyclic diterpenoid growth factor and influence essentially the stem elongation and other plant developmental processes [20]. The main mutation involved a deletion of a 17 amino acid segment. The gai allele contains a deletion of 51-bp from within the GAI ORF, from close to the N terminus and confers a dominant dwarf phenotype. The mutation take place at the DNA-binding transcription factor activity and causes a dwarf phenotype. It acts as a repressor and a coactivator of the zinc finger transcription factors GAF1/IDD2 and ENY/IDD1 in regulation of gibberellin homeostasis and signalling of the gibberellin (GA) signalling pathway. The GAI (gai1-1 and gai 1-2, two mutations on the same gene) protein as normally a length of 533 AA and is normally located in the nucleus. The deleted segment is shown in yellow for DELLA, the common one [19] [21]. If it is mutated (gai) and the plant growth better, it is a gain of function gene, in contrary it is a loss of function. The cellular gai's component is in the nucleus and is described as a transcription region of DNA and bind it directly. The mutation in SPY (spy) is a suppressor of gai, conferring to the plant a normal phenotype. GA-deficient Arabidopsis mutants display characteristic phenotypes, including dark green leaves and a dwarf growth habit attributable to reduced stem elongation [19]. The gai mutation affects GA reception or subsequent signal transduction and does not result in GA deficiency [20].

10	20	30	40	50
MKRDHHHHHH	QDKKTMMMNE	EDDGNGM <mark>DEL</mark>	LAVLGYKVRS	<b>SEMA</b> DVAQKL
60	70	80	90	100
EQLEVMMSNV	QEDDLSQLAT	ETVHYNPAEL	YTWLDSMLTD	LNPPSSNAEY
110	120	130	140	150
DLKAIPGDAI	LNQFAIDSAS	SSNQGGGGDT	YTTNKRLKCS	NGVVETTTAT
160	170	180	190	200
AESTRHVVLV	DSQENGVRLV	HALLACAEAV	QKENLTVAEA	LVKQIGFLAV
210	220	230	240	250
SQIGAMRKVA	TYFAEALARR	IYRLSPSQSP	IDHSLSDTLQ	MHFYETCPYL
260	270	280	290	300
KFAHFTANQA	ILEAFQGKKR	VHVIDFSMSQ	GLQWPALMQA	LALRPGGPPV
310	320	330	340	350
FRLTGIGPPA	PDNFDYLHEV	GCKLAHLAEA	IHVEFEYRGF	VANTLADLDA
360	370	380	390	400
SMLELRPSEI	ESVAVNSVFE	LHKLLGRPGA	IDKVLGVVNQ	IKPEIFTVVE
410	420	430	440	450
QESNHNSPIF	LDRFTESLHY	YSTLFDSLEG	VPSGQDKVMS	EVYLGKQICN
460	470	480	490	500
VVACDGPDRV	ERHETLSQWR	NRFGSAGFAA	AHIGSNAFKQ	ASMLLALFNG
510	520	530		
GEGYRVEESD	GCLMLGWHTR	PLIATSAWKL	STN	

Figure 2: The DELLA protein GAI comes from the GRAS family protein 3 and located on the chromosome 1, locus:2006747 AT1G14920. Call DELLA because of the 17 AA deleted.

SPY For spy, three independent recessive mutations at the SPINDLY (SPY) locus of Arabidopsis confer resistance to the gibberellin (GA) biosynthesis inhibitor paclobutrazol. Paclobutrazol or  $\alpha$ -tert-Butyl- $\beta$ -(4-chlorobenzyl)-1H-1,2,4-triazole-1-ethanol, is a plant growth retardant. It is an antagonist of the plant hormone gibberellin. It works by inhibiting gibberellin biosynthesis by inhibiting endoplasmic reticulum monooxygenases. Relative to wild type, spy mutants exhibit longer hypocotyls, leaves that are a lighter green colour, increased stem elongation, early flowering, parthenocarpy, and partial male sterility. All of these phenotypes are also observed when wild-type Arabidopsis plants are repeatedly treated with gibberellin A3 (GA3). The spy-1 allele is partially epistatic to the ga1-2 mutation, which causes GA deficiency. In addition, the spy-1 mutation can simultaneously suppress the effects of the ga1-2 mutation and paclobutrazol treatment, which inhibit different steps in the GA biosynthesis pathway. This observation suggests that spy-1 activates a basal level of GA signal transduction that is independent of GA [21].



 $\label{eq:figure} Figure 3: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3243332/figure/i1543-8120-64-1-1-f21/$ 

### Methods

**TODO** Rares

### Results

**TODO** Rares

Discussion

# Lactobacillus Heleveticus Genome Assembly

### Introduction

The diverse bacteria involved in cheese production are essential for the texture and taste development but also, during the ripening process, the microbial changes helps to kill pathogens and reduce spoilage micro-organisms. *Lactobacillus helveticus* is a thermophilic lactic acid bacterium (LAB) used in the dairy industry as a starter or an adjunct culture for cheese manufacture [22]. By releasing **peptidoglycan hydrolases**(PGHs), it has the ability to digest the bacterial cell wall (gram+) inducing death of surrounding bacteria but also its autolysis.

The genomic plasticity of *Lactobacillus helveticus* leads to a high variation in PGHs activity from one strain to another. In a previous study, the activity of a PGH with an estimated size of 30kDa was tested by zymography in nine strains of *Lactobacillus helveticus* of which six were sequenced (see figure 4). Two phenotypes were shown: phenotype A exhibits PGH activity (strains **FAM8102c1c1**, **FAM23285** and **FAM19191**) and phenotype B does not (strains **FAM22016**, **FAM1450** and **FAM1213**).

The aim of this work was to detect potential genomic differences involved in the two different phenotypes by sequencing, assembling and compare the genome of the six strains using a previously annotated reference genome of *Lactobacillus helveticus* (NC\_010080). A potential candidate present only in the strains expressing a PGHs activity suggests that it might have been acquired by a viral insertion.

### Methods

Sequencing and genome assembly The six Lactobacillus helveticus strains FAM8102c1c1, FAM23285, FAM19191, FAM22076, FAM1450, FAM1213 were sequenced by Illumina sequencing. The following tasks were performed using the cluster provided by the University of Bern. FastQC [14] was used to check the quality of the reads and Trimmomatic [15] to filter out bad quality reads. SOAPdenovo as well as Spades were used to perform the genome assembly with the reads of each strains. For SOAPdenovo the k-mer sizes were set to 95, 85, 75 and 65. For Spades k-mere sizes were set to 21, 33, 55, 77 and 99 (default values). The four assemblies of SOAPdenovo and the assembly of Spades were compared using Abyss with a maximum number of contigs set to 1000. The best genome assemblies with the bigger N50 and a approximate genome size of 20Mbp (Genome size of Lactobacillus helveticus) were then chosen<sup>1</sup>.

Genome annotation and pan-genome analysis We used the *PROKKA* pipeline [23] to annotate the genome of the six best assemblies and the reference genome for *Lactobacillus helveticus* NC\_010080. *PROKKA* is an automated pipeline that annotates prokaryotic genomes. It locates open reading frames ans RNA regions on contigs and translates it to protein sequences, searching for protein homologues in public databases. The resulting standards .gff files containing the annotated genome for each strain are then used by *Roary* [24] to generate a pan-genome of the six strains. The result was then visualized with *Phandango* [25] allowing visualisation of phylogenetic tree, associated metadata and genomic information.

**Extraction of the genes for each phenotypes** Grep was applied to the files generated by *Roary* to extract the nine PHG's [22] labelled "Lhv\_" with *PROKKA* (table 2). The set of genes

 $<sup>^{1}</sup>$ Due to the temporary unavailability of the cluster, this operation has been performed by L. Falquet and the results were provided to the students afterwards.

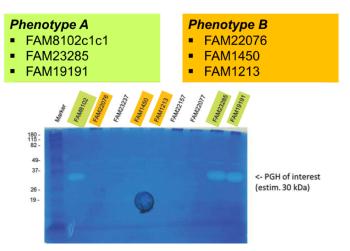


Figure 4: Phenotype A is expressing an active peptidoglycan hydrolase and phenotype B is not.

found in strains expressing phenotype A was then compared to the set of gene showing phenotype B. In table 1 we have the two PGHs present only in the three strains expressing the PGHs activity. The nucleotide sequences were then converted to amino acid sequences for further comparison.

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CLUSTAL format alignment by MAFFT L-INS-i (v7.310)

FAM19191 1K 003 MTSRQLGVDVAVYQGTSMTAYHNAGAKFGIAKLTEGTNYVNPKAHYQIKSLHANHMYVHA FAM23285 1K 004 MTSRQLGVDVAVYQGTSMTAYHNAGAKFGIAKLTEGTNYVNPKAHYQIKSLHANHMYVHA FAM8102 1K 0056 MTSRQLGVDVAVYQGTSMTAYHNAGAKFGIAKLTEGTNYVNPKAHYQIKSLHANHMYVHA FAM8102 1K 0056 MTSRQLGVDVAVYQGTSMTAYHNAGAKFGIAKLTEGTNYVNPKAHYQIKSLHANHMYVHA FAM8102 1K 0056 MTSRQLGVDVAVYQGTSMTAYHNAGAKFGIAKLTEGTNYVNPKAHYQIKSLHANHMYVHA FAM8102 1K 0056 YHFATFGYSVSRAKLEGKAFVKRAKAENISKKRFLWLDWESGSGNCVTGGKAASTKAILA FAM8102 1K 0056 YHFATFGYSVSRAKLEGKAFVKRAKAENISKKRFLWLDWESGSGNCVTGGKAASTKAILA FAM19191 1K 003 FMKVCHDAGYKVGLYSGASLLRNNIDTKQIVKKYGTCIWVASYPTDLAYTPNFNYFPSMD FAM8102 1K 0056 FMKVCHDAGYKVGLYSGASLLRNNIDTKQIVKKYGTCIWVASYPTDLAYTPNFNYFPSMD FAM8102 1K 0056 GVAIWQFCDNWKGLGVDGNISLIDLHKDSAGKKVTKPAEKPKPKPEKKTGVVYAPVINRN FAM812285 1K 004 GVAIWQFCDNWKGLGVDGNISLIDLHKDSAGKKVTKPAEKPKPKPEKKTGVVYAPVINRN FAM8102 1K 0056 GVAIWQFCDNWKGLGVDGNISLIDLHKDSAGKKVTKPAEKPKPKPEKKTGVVYAPVINRN FAM812285 1K 004 GVAIWQFCDNWKGLGVDGNISLIDLHKDSAGKKVTKPAEKPKPKPEKKTGVVYAPVINRN FAM812285 1K 004 GVAIWQFCDNWKGLGVDGNISLIDLHKDSAGKKVTKPAEKPKPKPEKKTGVVYAPVINRN FAM8102 1K 0056 FMWMIQLMDGNGHYTGKYIKTNTRWKYFDVKTIKGMKCYKLGTDKQWVPAKFLKVIE FAM8102 1K 0056 PNWMIQLMDGNGHYTGKYIKTNTRWKYFDVKTIKGMKCYKLGTDKQWVPAKFLKVIE FAM8102 1K 0056 PNWMIQLMDGNGHYTGKYIKTNTRWKYFDVKTIKGMKCYKLGTDKQWVPAKFLKVIE FAM8102 1K 0056 PNWMIQLMDGNGHYTGKYIKTNTRWKYFDVKTIKGMKCYKLGTDKQWVPAKFLKVIE
```

Figure 5: Alignment of amino acid sequences of group 2372 for the three strains.

### Results

Gene	Annotation	Avg group	FAM19191_	FAM23285_	FAM8102_
		size nuc	1K	1K	1K
group_2348	Lhv_2053	1121/41 kDa	FAM19191_	FAM23285_	FAM8102_
	Lysin		1K_00069	1K_00060	1K_00069
	(L.crispatus)				
	pseudo-				
	gene in				
	L.helveticus				
group_2372	Lhv_2053	893/ 33 kDa	FAM19191_	FAM23285_	FAM8102_
	Lysin		1K_00397	1K_00499	1K_00565
	(L.crispatus)				
	pseudo-				
	gene in				
	L.helveticus				

Table 1: Genes present only in the three strains with a PGH activity.

According to figure 4, the PGH involved is approximately 30kDa thus matches with group 2372. Looking at the alignment of the amino acid sequences (Figure 5) we see that the sequences are identical thus showing a great conservation between the three strains.

**Discussion** We can see that PGHs are present in all strains (table 2), therefore the phenotype observed in the figure 4 is not due to an absence of PGH.

Using BLASTp [26] with default parameters, the protein was searched to be a particular lysin (WP\_101853908.1) encoded by the pneumococcal bacteriophage Cp-1 [27]. To look further into this sequence, we could use PHASTER [28], the PHAge Search Tool - Enhanced Release, which helps identifying and annotate prophage sequences within bacterial genomes and plasmids.

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- A Supplementary figures Yeast Genome Analysis
- B Supplementary figures Arabidopsis Thaliana Genome Analysis
- C Supplementary figures Lactobacillus Helveticus Genome Assembly

Gene	Annotation	FAM1213 1K	FAM1450 1K	FAM19191 1K	FAM22076 1K	FAM23285 1K	FAM8102 1K
group_1103	Lhv_0549 N-acetylmuramidase	FAM1213_ 1K_01187	${ m FAM1450}_{-1} { m K}_{-00785}$	FAM19191_ 1K_01147	FAM22076_ 1K_00934	FAM23285_ 1K_01072	FAM8102_ 1K_01185
group_1218	Lhv_1433 Lysin	FAM1213_ 1K_01833	FAM1450_ 1K_00044	FAM19191_ 1K_01884	FAM22076_ 1K_01582	FAM23285_ 1K_01903	FAM8102_ 1K_01986
group_3457	Lhv_0649 Lysozyme	FAM1213_ 1K_00895	FAM1450_ 1K_00838	FAM19191_ 1K_01232	FAM22076_ 1K_00917	FAM23285_ 1K_01191	FAM8102_ 1K_01268
group_852	Lhv_1295 Enterolysin M23 family peptidase	FAM1213_ 1K_00043	FAM1450_ 1K_01113	FAM19191_ 1K_00150	FAM22076_ 1K_00164	FAM23285_ 1K_00217	FAM8102_ 1K_00225
group.862	Lhv_1059 LysM peptidoglycan-binding domain-containing protein	FAM1213_ 1K_00147	FAM1450_ 1K_00238	FAM19191_ 1K_00248	FAM22076_ 1K_00274	FAM23285_ 1K_00308	FAM8102_ 1K_00381
group_993	Lhv-1433 Lysin	${ m FAM1213}_{-}$ $1{ m K}_{-}00691$	${ m FAM1450}_{-1} \ 1{ m K}_{-01203}$	FAM19191_ 1K_01800	FAM22076_ 1K_00088	FAM23285_ 1K_01748	FAM8102_ 1K_01891
group_995	Lhv_0191 Amidase	$\begin{array}{c} {\rm FAM1213.} \\ {\rm 1K\_00700} \end{array}$	$\begin{array}{c} {\rm FAM1450}_{-} \\ {\rm 1K}_{-}00303 \end{array}$	FAM19191_ 1K_00506	FAM22076_ 1K_00064	FAM23285_ 1K_00566	FAM8102_ 1K_00638
group_1862	Lhv.2053 Lysin (L.crispatus) pseudogene in L.helveticus		FAM1450_ 1K_00045	FAM19191_ 1K_01885	FAM22076_ 1K_01583	FAM23285_ 1K_01904	FAM8102_ 1K_01987
group_1899	Lhv-2053 Lysin (L.crispatus) pseudogene in L.helveticus		FAM1450_ 1K_00267	FAM19191_ 1K_00615	FAM22076_ 1K_00716	FAM23285_ 1K_00607	FAM8102_ 1K_00746
group_1344	Lhv_1307 Enterolysin M23 family peptidase			FAM19191_ 1K_00162	FAM22076_ 1K_00152	FAM23285_ 1K_00229	FAM8102_ 1K_00237
group_1345	Lhv_0190 N-acetylmuramidase			FAM19191_ 1K_00507	FAM22076_ 1K_00063	FAM23285_ 1K_00565	FAM8102_ 1K_00639

Table 2: PGHs in common between all strains. Extracted from the files generated by Roary and labeled "Lhv\_" by PROKKA.