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TITLE:

A Deep-Sequencing-Assisted, Spontaneous Suppressor Screen in the Fission Yeast *Schizosaccharomyces pombe*

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KEYWORDS:
suppressor mutation, genetic interaction, spontaneous phenotypic recovery, whole-genome sequencing, bioinformatics analysis, fission yeast

SUMMARY:
We present a simple suppressor screen protocol in fission yeast. This method is efficient, mutagen-free, and selective for mutations that often occur at a single genomic locus. The protocol is suitable for isolating suppressors that alleviate growth defects in liquid culture that are caused by a mutation or a drug.

ABSTRACT:
A genetic screen for mutant alleles that suppress phenotypic defects caused by a mutation is a powerful approach to identify genes that belong to closely related biochemical pathways. Previous methods such as the Synthetic Genetic Array (SGA) analysis, and random mutagenesis techniques using ultraviolet (UV) or chemicals like ethyl methanesulfonate (EMS) or N-ethyl-N-nitrosourea (ENU), have been widely used but are often costly and laborious. Also, these mutagen-based screening methods are frequently associated with severe side effects on the organism, inducing multiple mutations that add to the complexity of isolating the suppressors. Here, we present a simple and effective protocol to identify suppressor mutations in mutants which confer a growth defect in *Schizosaccharomyces pombe*. The fitness of cells with a growth deficiency in standard rich liquid media or synthetic liquid media can be monitored for recovery using an automated 96-well plate reader over an extended period. Once a cell acquires a suppressor mutation in the culture, its descendants outcompete those of the parental cells. The recovered cells that have a competitive growth advantage over the parental cells can then be isolated and backcrossed with the parental cells. The suppressor mutations are then identified using whole-genome sequencing. Using this approach, we have successfully isolated multiple suppressors that alleviate the severe growth defects caused by loss of Elf1, an AAA+ family ATPase that is important in nuclear mRNA transport and maintenance of genomic stability. There

are currently over 400 genes in *S. pombe* with mutants conferring a growth defect. As many of these genes are uncharacterized, we propose that our method will hasten the identification of novel functional interactions with this user-friendly, high-throughput approach.

INTRODUCTION:

The basis of understanding functional links between genes relies on the ability to identify the molecular mechanism(s) by which complex genetic traits diverge to produce diverse phenotypes[1]. In the fission yeast, *Schizosaccharomyces pombe* (*S. pombe*), the majority of protein-coding genes are dispensable for viability[2]. This result does not speak to the unimportance of these genes, but rather to the intricate compensatory mechanisms underlying the biochemical pathways to which such genes belong. Dissecting these compensatory mechanisms has generated epistasis maps, which have uncovered comprehensive genetic interactions and broadened our understanding of functional biochemical pathways[3,4].

High-throughput methods (e.g., Synthetic Genetic Array analysis, or SGA) have been developed to identify genome-wide genetic interactions in budding yeast, and have been expanded for use in fission yeast[5,6]. Such approaches often rely on a library of strains containing all viable single protein-coding gene deletions (around 3,300 haploid deletion mutants covering over 92% of the fission yeast genome), and require a robotic arm to perform the genetic crosses between the strain of interest and all possible strains in the library[6]. Further, SGA techniques depend on the ability of library strains to have proper and efficient mating, a phenotype that is abnormal to 444 currently characterized genes in *S. pombe*[2].

Despite the complexity of genetic interactions, comparing the phenotype of a strain carrying mutations in two genes to the phenotype of two strains carrying individual mutations of each gene can have one of two notable outcomes: 1) The double mutant phenotype is worse than the expected multiplicative parental phenotypes in the form of sickness or, in the most extreme case, lethality. This is referred to as a negative genetic interaction, and is generally a sign that the two genes act in parallel biological pathways. 2) The double mutant phenotype is better than the expected combination of parental phenotypes, also known as a positive genetic interaction. A positive genetic interaction is particularly interesting because it indicates that these genes function in the same process. Two positively interacting genes have three potential relationships: a mutant gene may up-regulate the expression of the other gene in a parallel pathway, the two genes may work in concert within the same pathway downstream of one another, or the two genes encode proteins that interact directly with each other. Therefore, positive genetic interactions can be used to map gene regulatory nodes and classify uncharacterized genes in biochemical pathways[7,8].

A suppressor is a mutation that can alleviate the sickness phenotype of the mutation of another gene, typically representing a positive genetic interaction between the two genes[9,10]. Suppressor mutations on a different locus from that of the mutation they suppress are known as extragenic suppressors. They are especially valuable in studying non-viable genetic mutations by synthetically rescuing the lethal phenotype (also known as the Lazarus effect)[11]. They also have potential therapeutic applications in treating hereditary diseases[12,13].

For all of these reasons, the identification of suppressor mutations in various model organisms has been widely utilized to facilitate our understanding of various biochemical pathways[14–16]. Screening for suppressors is usually based on the phenotype of the mutation in question and requires conducting random mutagenesis to isolate the mutations that would alleviate the phenotype. Almost all model organisms have established random mutagenesis methods. For example, N-ethyl-N-nitrosourea (ENU) and ethylmethanesulfonate (EMS), two mutagens that are capable of inducing point mutations in DNA, are widely employed in various models from bacteria to mice[17–19]. In addition, manganese chloride has long been used in yeasts for the ability of the manganese cation to inhibit DNA repair pathways[20]. Another common approach is UV-induced mutagenesis, which generates genome-wide mutagenic pyrimidine dimers[21,22].

Although the utilization of chemical mutagenesis to identify suppressor mutations has been popular, the method has many drawbacks, including the use of dangerous chemicals, highly variable success rates, and the introduction of extra confounding variables presented by the negative side effects of the mutagen on multiple cellular processes[23,24]. Additionally, chemical mutagenesis often induces multiple mutations in the genome which adds to the complexity of using genetic and sequencing techniques to identify the exact mutation that conferred the suppressor phenotype in the organism[25].

To address the shortcomings of current mutagenesis approaches, we present a method to screen for spontaneous suppressor mutations in fission yeast that does not rely on any mutagens or a deletion library. The method isolates suppressors through a positive selection assay. The principle of this method is based on the growth advantage of the mutated suppressor subpopulation in the liquid culture, which can be monitored by an automated plate reader. Mating and meiosis are used only if one would like to clean up the genetic background or confirm the presence of monogenic alleles of suppressors before whole-genome sequencing. If the suppression phenotype is caused by a single mutation, the suppressor phenotype will segregate 2:2 after backcrossing with the parental strains. The suppressor mutations can then be identified using whole-genome sequencing. We propose that this method is applicable for screening suppressors in all microorganisms that can grow to a large population in liquid culture.

PROTOCOL:

1. Strain construction and preparation

1.1. Generate a mutation or a deletion of the gene (*yfm*, *your favorite mutation*) using standard site-directed mutagenesis (SDM) as described previously[26].

1.2. Before starting the screen, (optimally) backcross the mutant strains with a wild-type strain to clean the genetic background and generate fresh-born mutant cells as the parental strains. Streak the parental strain to individual colonies on standard rich media plates. Randomly pick eight to sixteen independent colonies (biological replicates) with the desired mutations for the plate reader assay (see 3.1).

NOTE: This protocol is effective only when the parental strains have a growth defect in liquid media (minimal or rich, with or without drug, or with temperature shifts that cause the growth defect). All parental strains should be haploid and thus able to be genetically crossed with other haploid strains with a complementary mating type.

2. Plate reader assay

2.1. With a sterile applicator, take a small amount of each of the colonies prepared in step 1.1 (no exact amount necessary to inoculate the starting culture) and place in a 96-well polystyrene microplate. Suspend each of the colonies in 200 µL of appropriate liquid media (rich or minimal, with or without drug). Include a blank well for every row on the plate containing 200 µL of the same media (no cells).

2.2. Run the following protocol on a plate reader detection software connected to an automated microplate-reader: Set a kinetic program for 24 h and temperature at 30 °C, with continuous fast orbital shaking (425 cpm, 3 mm amplitude). Set the optical reads to measure light scatter at a wavelength of 600nm for optical density, and set the light to read from below the plate at a reading frequency of 2 min (721 total reads over a 24-h period per well).

2.3. After 24 h, record the final blanked optical density readings (blanked OD600) and use the following formula to determine the volume needed to dilute each of the samples down to O.D. = 0.1:

$$\frac{desiredfinalO.D.\times desiredfinalvolume}{recordedblankedO.D.}=\frac{0.1\times 200}{recordedblankedO.D.}$$

NOTE: Export the data from the plate reader software and use a spreadsheet software to insert the above formula as a function to batch process the dilution volume to be used from each experimental well.

2.4. Every 24 h, dilute each of the samples using the same media as day 0 to O.D. = 0.1 (about 1.5 x 10^6 cells/mL) using the formula indicated in step 2.3. Save all growth curves generated daily and note any individual colony that shows an increased growth rate, judged by a final O.D. that is significantly higher than the rest of the cohort with the same genetic background or by a growth curve that is similar to that of wild-type colonies.

NOTE: This assay usually takes about 7-14 days. Perform all steps under sterile conditions.

3. Selection of suppressor colonies and confirmation of phenotype.

3.1. From the last day of the plate reader assay (step 2.4), save the liquid cultures that have a noticeably recovered growth rate, presumably by gaining a suppressor mutation that can alleviate the phenotype of the parental mutation. Transfer and mix 250 µL liquid culture to a

cryotube containing 250 µL of 50% glycerol. Flash freeze the cells in liquid nitrogen and save the strains in -80°C indefinitely.

3.2. To confirm that the suppressor mutation is a genetically heritable element, use standard genetic crossing methods to cross *yfm P* (for parental, the strain used at the beginning of the plate reader assay) with *yfm S* (for suppressor, the strain saved at the end of the plate reader assay). If the suppressor mutation is indeed a genetically heritable element, *yfm P* × *yfm S* should yield tetrads in which two colonies have the sickness phenotype of the parental strain and two colonies have the recovered growth rate of the suppressor strain.

3.3. From the cross of step 3.2, pick three colonies with the suppressor phenotype (S strain) and three colonies with the parental phenotype (P strain) from the same genetic cross (3 biological replicates for each), and proceed with the genomic DNA extraction and sequencing steps below.

NOTE: Steps 3.2 and 3.3 are highly recommended, but are not required. Alternatively, one can spread recovered liquid culture collected from 3.1 on rich medium into single colonies, then randomly pick three colonies as biological triplicates for whole-genome sequencing without further genetic confirmation. In this case, three biological triplicates of parental strain should be used for genomic sequencing comparison.

4. Genomic DNA extraction, library production and sequencing.

4.1. For DNA extraction, library preparation, and sequencing, randomly pick three biological replicates per *yfm P* strain, and three biological replicates of each individually arisen *yfm S* strains from the genetic crosses (step 3.2) or from the plates that have been spread to obtain single colonies of the S strain (step 3.3 note).

4.2. Grow strains in 10 mL cultures in rich media to mid-log phase (O.D. = 0.5–0.8, about 0.75–1.2 x 10^7 cells/mL), and use a shaking incubator to grow liquid cultures at 30 °C with continuously shaking at 250 rpm. Collect cells by centrifugation at 4 °C for 5 min at 1000 x *g*.

4.3. Suspend pelleted cells in 400 µL of DNA extraction buffer (2% Triton X-100, 1% SDS, 100 mM NaCl, 10 mM Tris-Cl (pH 8.0), 1mM Na_2-EDTA), then add 400 µL of glass beads and 400 µL of 25:24:1 phenol:chloroform:isoamyl alcohol. Vortex vigorously for 2 min at 4 °C.

4.4. Add an additional 200 µL of the DNA extraction buffer and mix by inverting several times. Centrifuge for 5 min at 4 °C at 20,000 x *g*.

4.5. Transfer the aqueous phase to a clean tube, add 20 µg of RNase A/T1 mix, and incubate at 37 °C for 15 min.

4.6. Add an equal volume of 25:24:1 phenol:chloroform:isoamyl alcohol, spin for 5 min at 4 °C at 20,000 x *g*, then transfer the aqueous phase to a clean tube.

4.7. Add an equal volume of chloroform, mix by inverting several times, then spin for 5 min at 4 °C at 20,000 x *g*, then transfer the aqueous phase to a clean tube.

4.8. Precipitate DNA with two volumes of 100% ethanol plus 10% volume of 3 M NaOAC (pH 4.3) at -20 °C for at least 2 h, then spin for 5 min at 4 °C at 20,000 x *g* and collect the pellet.

4.9. Wash pellet (precipitated DNA) twice with chilled 70% ethanol (Centrifuge at 20,000 x *g*, 5 min, 4 °C) and suspend the pellet in 50 µL of 10 mM Tris Buffer (pH 7.4).

4.10. Use a library prep kit (see **Table of Materials**) per manufacturer's recommendations to prepare the whole-genome sequencing library.

NOTE: We recommend the kit listed in the **Table of Materials** because it allows the construction of the genomic library without PCR amplification, which minimizes error mutations generated during PCR amplification. In addition, during the genomic library preparation, do not allow the beads to fully dry by shortening the bead drying time to 1–2 min.

4.11. For shearing parameters during the library preparation, use a focused sonicator (see **Table of Materials**) and set the duty factor to 20%, peak power to 175 W, with 200 cycles per burst, and frequency sweeping mode at 5.5°C to 6 °C for 45 s. Alternatively, use a DNA and chomatin shearing system (see **Table of Materials**) with the following settings: 50% amplitude at 4 °C with pulse mode, 15 s on and 15 s off for 10 min, with a total processing time of 20 min.

4.12. It is essential to handle the hazardous materials used in this step with care. Consult the appropriate Material Safety Data Sheets and institution's Environmental Health and Safety Office for handling NaOAC, ethanol, 25:24:1 phenol:chloroform:isoamyl alcohol and chloroform.

4.13. Sequence the resulting genomic libraries. The entire sequencing reads should cover at least three times of the entire genome with the resolution at a single nucleotide range. Paired-ended sequencing (or latest technology) is recommended.

5. Bioinformatics analysis for the identification of the suppressor mutations

5.1. Perform the bioinformatics analysis to focus on the genomic changes that are consistently identified between parental and suppressed *yfm* strains in all biological replicates.

NOTE: The complete pipeline process is described below, but, additionally, two plain-text BASH-script files, fastq_to_vcf.sh and vcfprocess.sh, are included as supplemental materials to show examples of the workflow from processing of reads to VCF variant files and processing and intersection of the VCF files, respectively.

5.2. Trim short-reads using SHEAR (https://github.com/jbpease/shear) following the command lines (all other options default):

```
shear.py --fq1 $FASTQ1 --fq2 $FASTQ2 --out1 $OUTFQ1 --out2 $OUTFQ2 \
```

```
--barcodes1 $BARCODE --platform TruSeq --trimqual 20:20 \
--trimpolyat 0 --trimambig --filterlength 50 --filterunpaired
```

5.3. Map reads to the *S. pombe* reference genome v2.30 obtained from PomBase (ftp://ftp.ebi.ac.uk/pub/databases/pombase/pombe/Chromosome_Dumps/fasta/) using BWA v0.7.15[27]. Use the following command line (all other options default):

```
bwa mem -t 8 $GENOME $OUTFQ1 $OUTFQ2 > $SAM1
```

5.4. Put alignment SAM files through GATK best practices pipeline[28] for variant calling using GATK v3.6[29], PicardTools v2.5.0 (http://broadinstitute.github.io/picard), and SAMtools v1.3.1[30]. Use the following command lines and parameters (all other options default):

```
java -Xmx30g -jar picard.jar AddOrReplaceReadGroups INPUT=$SAM1 \
OUTPUT=$BAMMARKED RGID=1 RGLB=lib01 RGPL=illumina \
RGPU=$BARCODE RGSM=$SAMPLENUMBER
samtools fixmate -O bam $BAMMARKED $BAMFIXED
samtools sort -O bam -o $BAMSORTED -T /home/peasejb/tmp $BAMFIXED
samtools index $BAMSORTED
java -Xmx30g -jar GenomeAnalysisTK.jar -T HaplotypeCaller \
-R $GENOME -I $BAMSORTED --genotyping_mode DISCOVERY \
-stand_emit_conf 10 -stand_call_conf 30 -o $VCFRAW
```

5.5. Compress and index VCF files using tabix:

```
bgzip $VCFRAW.vcf
tabix $VCFRAW.vcf.gz
```

5.6. Compare VCF files among parental and suppressor strains sequencing replicates using BCFtools v1.3.1[27]. Use the following command lines and parameters (all other options left default):

```
bcftools isec -n+1 $VCFPARENTAL1.gz $VCFPARENTAL2.gz $VCFPARENTAL3.gz \
$VCFMUTANT1.gz $VCFMUTANT2.gz $VCFMUTANT3.gz > common_variants.list
```

NOTE: This command yielded a file encoded with binary patterns where sequence variants appearing in the first mutant only would be binary-encoded as "000100", the second mutant only as "000010", all three mutants as "000111," etc. These files were generated for each set of parental and mutant replicate VCF files.

5.7. Compile the variant intersection list files together with the file name appended to each line using the UNIX grep command:

```
grep "." *.list > all.list
```

5.8. Cross-reference the complete variant list with the current GFF3 annotation file (ftp://ftp.ebi.ac.uk/pub/databases/pombase/pombe/Chromosome_Dumps/gff3/schizosaccharomyces_pombe.chr.gff3) using a custom Python script (variant_characterize.py) to identify

consistent SNP sites in protein-coding regions (synonymous and non-synonymous), 5' and 3' UTRs, and ncRNA.

```
python3 variant_characterize.py --list common_variants.list \
--gff schizosaccharomyces_pombe.chr.gff3 \
--fasta Schizosaccharomyces_pombe.ASM294v2.30.dna.genome.fa \
--pattern 000100 --out all.list.filter.000100
```

Repeat this script modifying the --pattern and the suffix of the output file (--out) using the binary patterns: 000010, 000001, 000110, 000011, 000101, and 000111

5.9. Combine the output from all these script runs in a tab-separated file, to be viewed as a spreadsheet. The annotated table of variants includes those that appear in either one or both mutant strain(s) relative to the background. The binary flag field denotes either appearance in a single mutant strain (000100, 000010, 000001), two mutant strains (000011, 000101, 000110), or all three mutant strains (000111).

5.10. Analyze the output annotated lists of variants not found in the parental samples, but found in one, two, or all three mutant samples. The annotation denotes both the genomic location and class of variant (synonymous/non-synonymous in a coding region, 3'/5' UTR, non-coding, etc.). From this list of candidate mutations, an example of a strongly relevant candidates might be a non-synonymous coding variant appearing consistently in all three strains. Another type of strong candidate would be an accumulation of various non-synonymous or putative regulatory mutations in the mutant strains appearing close together or within the same gene.

REPRESENTATIVE RESULTS:

Slow-growing mutants show phenotypic recovery in liquid culture

We selected three mutants involved in a variety of biological pathways with a sick, slow-growing, phenotype: AAA family ATPase Elf1, Histone Deacetylase Clr6, and Exon Junction Complex component Fal1. A wild-type strain and strains carrying mutations of these three genes that had been backcrossed with wild-type strains were streaked to individual colonies, and 16 single colonies were randomly selected to be cultured in rich liquid media using the 96-well plate as described above. Growth curves of individual colonies were recorded at the initial time point (day 0) and for 6 days with continuous monitoring using the plate reader. As expected, wild-type colonies show no noticeable changes in their growth curves throughout the experiment[31] (**Figure 1**). Notably, four colonies with the *elf1Δ* background and one *fal1Δ* colony show a dramatic shift in growth from slow-growing to some varying levels of growth similar or close to that of wild-type colonies. Dramatically, all *clr6-1* mutants show a consistent phenotypic recovery, growing at a faster rate by the end of the assay[31] (**Figure 1**). To characterize the different phenotypes, we refer to the original strains that are slow-growing as "P strains" (or parental strains) and to the strains showing phenotypic recovery as "S strains" (or suppressed strains). Please note that **Figure 1** is an example of one round of the screening experiment, and does not represent the total non-complementary suppressors identified and sequenced in the following representative results.

Phenotypic recovery is attributed to heritable traits

S. pombe can grow as a haploid in rich media, but two haploid strains with complementary mating types mate under nitrogen starvation. Meiosis in fission yeast entails a round of duplication followed by two rounds of cell division. The sexual cycle results in the formation of four haploid spores carrying the genetic material of the parental strain with 2:2 segregation of genetic traits following the rules of classical Mendelian genetics (**Figure 2A**). When grown on the same plate for the same amount of time, we confirmed the 2:2 segregation when back-crossing all suppressed strains (S strains) with their parental strains (P strains), which resulted in 2 small (growth defect) and 2 large (suppressor phenotype) colonies. Individual examples for suppressed *elf1Δ*, *clr6-1* and *fal1Δ* cells are shown in **Figure 2B**. We have confirmed that all isolated S strains carry a monogenic genetic element that suppresses the slow-growing phenotype of their P strains (data not shown).

Whole-genome sequencing successfully identifies suppressor mutations

As an example, we used paired-end whole genome sequencing to identify the genetic elements responsible for the phenotypic recovery in *elf1Δ S* strains. A more complete description of data analysis is available online[31]. Briefly, we employed biological triplicates of two independently generated *elf1Δ P* strains and biological duplicates of five non-complementary groups of *elf1Δ S* strains, each of which contains different suppressors. After we obtained a list of annotated variants from bioinformatics analysis (6.1-10), we prioritized certain classes of variants that were relevant to our analysis. We focused on identification of consistent genomic changes that were identical in all of the biological replicates of individual *elf1Δ S* strains compared with their parental *elf1Δ P* strains (**Figure 3** and **Supplemental Tables 1-4**). We identified five nonsynonymous changes at CDS regions in all five different *elf1Δ S* strains, including *rli1⁺, SPBPJ4664.02, cue2⁺* and *rpl2702⁺*. Both S-A1 and S-A2 contain mutated *SPBPJ4664.02*, although the mutations occur at different amino acids. Because *SPBPJ4664.02* is a long gene (11,916 nucleotides) with hundreds of repeats, the mutations were unable to be confirmed by performing PCR followed by sequencing. S-A3 contains a deletion mutant in *rli1* that is consistent in both biological duplicates. However, the mutant did not co-segregate with the S phenotype in *elf1Δ* background. We identified a *cue2* mutant (*cue2-1*) in S-B1, with the amino acids 396-400 missing. S-B2 contains an *rpl2702* mutant (*rpl2702-1*), which changes amino acid at position 45 from Glycine to Aspartate[31]. Both *cue2-1* and *rpl2702-1* were confirmed as *elf1Δ* suppressors as shown below.

Genetic confirmation of identified suppressor mutations verifies the heritability of the recovery phenotype

Two of the identified nonsynonymous changes, *cue2-1* and *rpl2702-1*, were reconstructed in the lab using standard protocols for site-directed mutagenesis. Double mutant strains *cue2-1 elf1Δ P* and *rpl2702-1 elf1Δ P* were crossed with the complimentary *elf1Δ P* strain[31] (**Figure 4**). If the nonsynonymous mutations, identified through this screen, were sufficient to suppress *elf1Δ P*, then the resulting tetrads would show a 2:2 small to large ratio in the colonies resulting from the 4 spores in each tetrad. Indeed, genetic crossing showed that the identified suppressor mutations are successful in suppressing the slow-growing phenotype of *elf1Δ P* and are heritable.

FIGURE LEGENDS:

Figure 1: Phenotypic recovery can be monitored by recording growth curves in a plate reader. Sixteen single colonies of wild-type (WT), *elf1Δ*, *clr6-1*, and *fal1Δ* were placed in a 96-well plate. Growth curves were recorded over a 24-h period and colonies were re-diluted daily in rich media. The growth defect is evident by the low absorbance (O.D.) at the end of the 24 h period on day 0. Phenotypically recovered strains are those that display a growth curve similar, or close to, that of wild-type over the 24-h period on day 6. Four colonies of *elf1Δ*, one colony of *fal1Δ*, and all colonies of *clr6-1* showed various levels of phenotypic recovery after 6 days.

Figure 2: Genetic crossing can confirm that the phenotypic recovery is attributed to a single heritable allele. (**A**) When fission yeast cells are subjected to nitrogen starvation, two haploid cells with a complementary mating type can generate a zygote which sporulates to generate a tetrad of 4 spores. The parental genetic materials will segregate during meiosis following the rules of Mendelian genetics. (**B**) Phenotypically recovered colonies (labeled S, for suppressed) with indicated parental genotypes were back-crossed with their complimentary parental colony (which shows no phenotypic recovery, labeled P, for parental). Genetic crosses showing 2:2 small (poor fitness) to large (recovered fitness) colonies demonstrate that the phenotypic recovery is heritable and can be attributed to a single genetic element. Red boxes are colonies carrying the suppressor allele, and blue boxes are colonies carrying the parental allele. This figure has been modified from Marayati et al., 2018[31].

Figure 3: Analysis of genome-wide sequencing data to identify genetic elements responsible of phenotypic recovery. Three biological replicates of two parental "P" strains (P-A and P-B), and two biological replicates of five phenotypically recovered switched "S" strains (S-A1, S-A2, and S-A3 recovered from P-A; S-B1 and S-B2 from P-B), were sequenced and the mutations were organized as a list of each mutation in the recovered strain as compared to the parental strain genome it was derived from (e.g., P-A vs. S-A1, etc.). The total number of detected mutations across the entire genome of all such pairwise comparisons was 660. A total of 44 mutations were identified when only mutations that occur in both biological replicates of the same "S" strain were selected. Out of 44 mutations, 12 mutations were insertion/deletion (INDEL) or non-synonymous mutations. Out of the 12 INDEL or non-synonymous mutations, five occurred in the protein coding sequence. The five mutations potentially correlate with the single genetic element responsible for the phenotypically recovered strains: a non-synonymous mutation in *SPBPJ4664.02* found in S-A1 and S-A2, INDEL in *rli1* found in S-A3, INDEL in *cue2* found in S-B1, and non-synonymous mutations in *rpl2702* found in S-B2. Detailed sequence information on the mutations and the filtered background is included in **Supplemental Tables 1-4**.

Figure 4: Confirmation of the suppressors identified through whole-genome sequencing. Results from whole-genome sequencing were confirmed by independently generating the mutations and performing genetic crosses to confirm the phenotypic recovery by crossing an *elf1Δ cue2-1* strain with an *elf1Δ P* strain, and *elf1Δ rpl2702-1* with *elf1Δ P* strain. Three representative vertical tetrads are shown. Red boxes are double-mutant colonies (*elf1 cue2-1,* or

elf1 rpl2702-1); blue boxes are *elf1Δ* colonies. This figure has been modified from Marayati et al., 2018 [31].

DISCUSSION:

The protocol described here represents a novel and simple screen for spontaneous suppressor mutations detectable through phenotypic recovery of mutations conferring slow growth in fission yeast, a phenotype characteristic of over 400 genes in *S. pombe*, the function of many of which remains unknown[2,32]. Previous methods have taken other approaches to screen for suppressor mutations in microorganisms, including the use of mutagens[21], or the application of a temperature shift in temperature-sensitive mutant backgrounds[33]. In contrast, this protocol shows that phenotypic recovery occurs without additional environmental/chemical interference and highlights the fitness advantage of the rise of suppression mutations eventually taking over the resources available in liquid culture. This screen allows the isolation of both bypass suppressors or interaction suppressors because it is effective for both loss-of-function mutations such as *elf1Δ* or *fal1Δ* and point mutations such as *clr6-1*, as long as the mutants demonstrate fitness defects in liquid culture.

So far, all recovered S strains that we have investigated have demonstrated varying degrees of phenotypic recovery. As detected through genetic crossing, the recovered phenotype is attributable to a single genetic element and is heritable (examples shown in **Figure 2**). This is one of the most significant advantages of this method compared to chemical-based or UV-based suppressor screens, which often target multiple genomic loci. It is common to observe one or two colonies recovered out of 16 colonies/strain (around 10%) within a week. However, we did notice that certain mutants, such as the loss-of-function of Rrp6, the nuclear-specific exosome subunit, never recovered to the almost wild-type growth rate observed in *elf1Δ* cells[31]. It is likely that the function of Rrp6 can only be partially compensated by suppressors, unlike the function of the other mutants tested, including *fal1Δ*, which was shown to cause a severe meiotic defect through its important function in regulatory splicing[34]. We believe that alternative suppressor screening methods would be subject to the same problem when *yfg* has unique, non-replaceable roles in cell growth.

Before performing genomic sequencing, it is optimal to back-cross the phenotypically recovered colonies, identified from the plate-reader, with the parental strains to clear the genetic background and obtain biological replicates. In addition, deep whole-genome sequencing identifies hundreds of single nucleotide changes, most of which are not identical between biological replicates that are of little interest for the screening. For example, we found a total of 660 genomic alterations across all three chromosomes between the two *elf1Δ* P and the five different S strains (**Figure 3**). We did not commonly observe identical mutations between sequenced biological replicates of each strain, suggesting that either new mutations may arise during the culturing of *elf1Δ* cells before genomic library construction or random errors may be introduced during the library construction and sequencing. Hence, isolating mutations that are consistent across biological replicates is an important aspect in the successful identification of suppressors using whole-genome sequencing.

We identified and confirmed two suppressors in CDS regions in five sequenced S strains. Although mutations in *SPBPJ4664.02* were detected in both S-A1 and S-A2 strains, it is unlikely that *SPBPJ4664.02* is a valid suppressor because S-A1 and S-A2 do not contain a suppressor on the same gene as they are not complementary with each other (data not shown). We also did not confirm *rli1* in S-A3, which did not co-segregate with the S phenotype when backcrossed with *elf1Δ.* Alternatively, we found specific mutations in non-coding regions in S-A1, S-A2 and S-A3. It is possible that these altered non-coding genomic regions alleviate the *elf1Δ* phenotype, which will be addressed in our future studies. Compared to traditional methods such as a linkage assay, which may take years to map a genetic mutation, we identified two suppressors within two months after confirming that a monogenic element caused the S phenotype. With the fast development of whole-genome sequencing technology, we are optimistic that this method will be more efficient to identify consistent genetic mutations in the foreseeable future.

In summary, this protocol provides step-by-step directions to successfully identify suppressor mutations for any gene of interest with a slow-growing defect in liquid culture. The simplicity of this assay allows for the large-scale screening of multiple genetic backgrounds of interest with little hands-on training. There is room to further automate the process by using a liquid handling robot to perform the daily dilutions. Since laboratory manipulation of microorganisms inevitably requires growth in liquid culture, a process that is inherently selective for fitness, we propose that this protocol can be broadly applied to other large-population model organisms such as bacteria and other yeast species.

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DISCLOSURES:

The authors declare no endorsements by the manufacturers of the instruments used in this method, and no competing financial interests.

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Figure 1

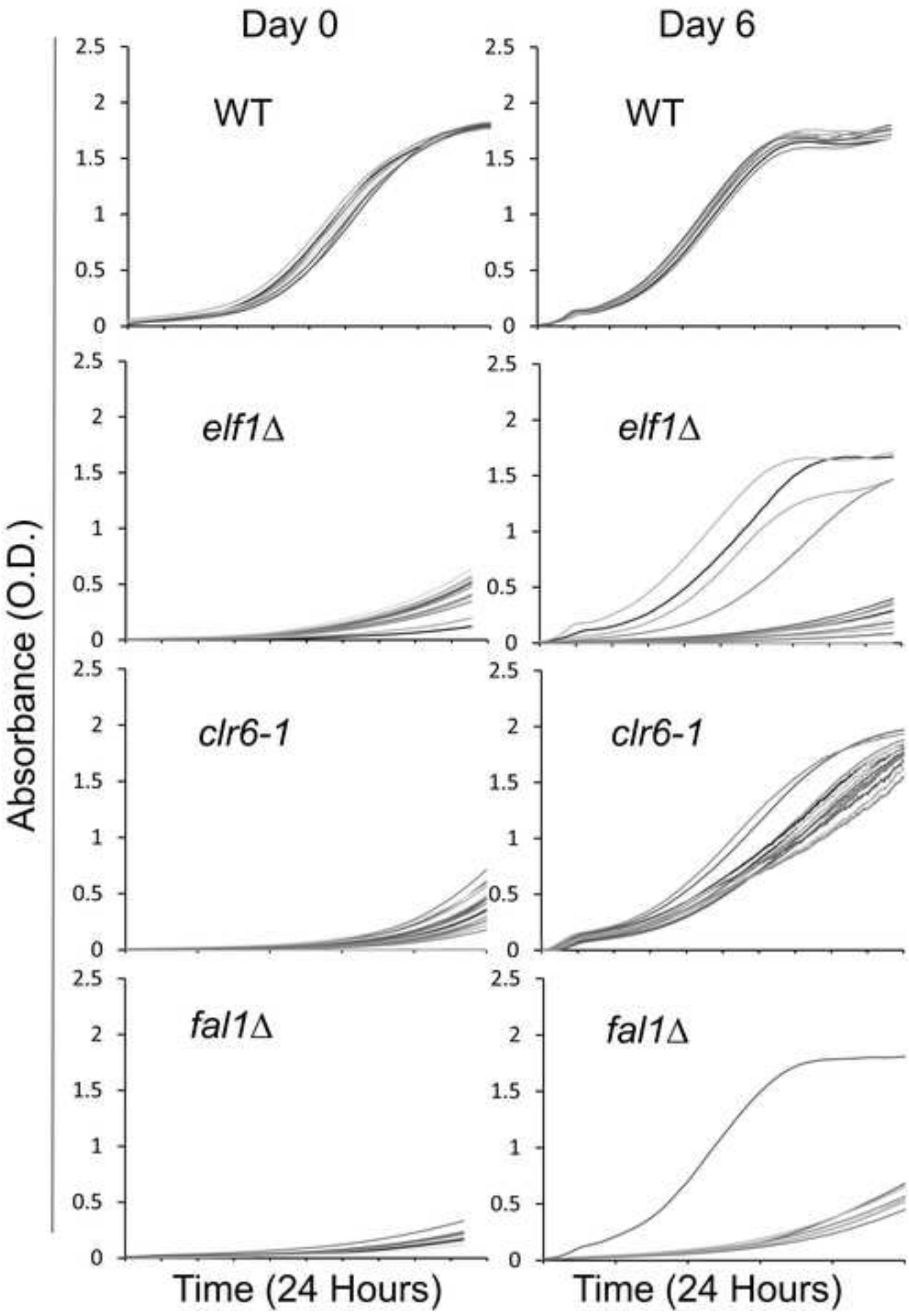


Figure 2

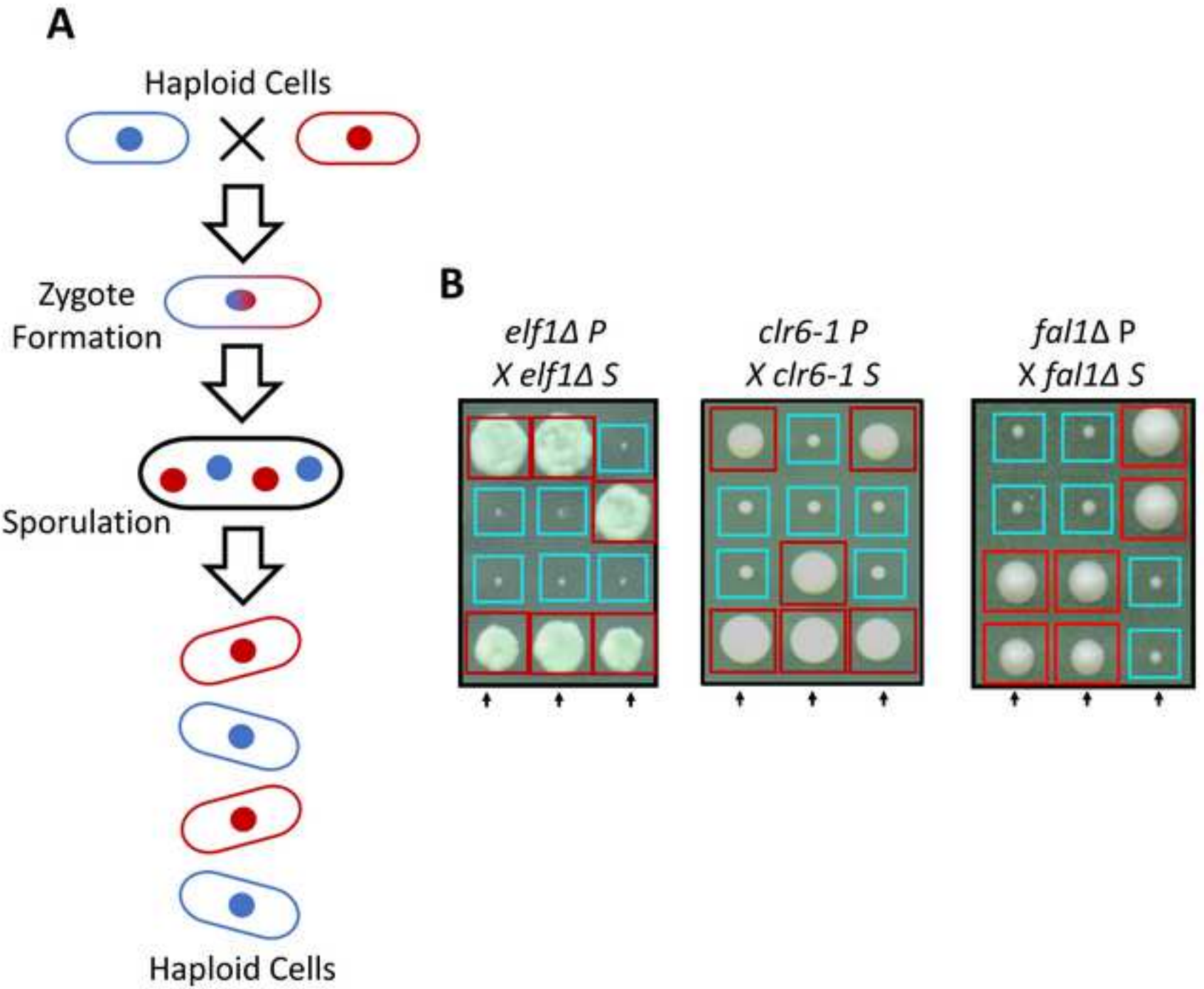
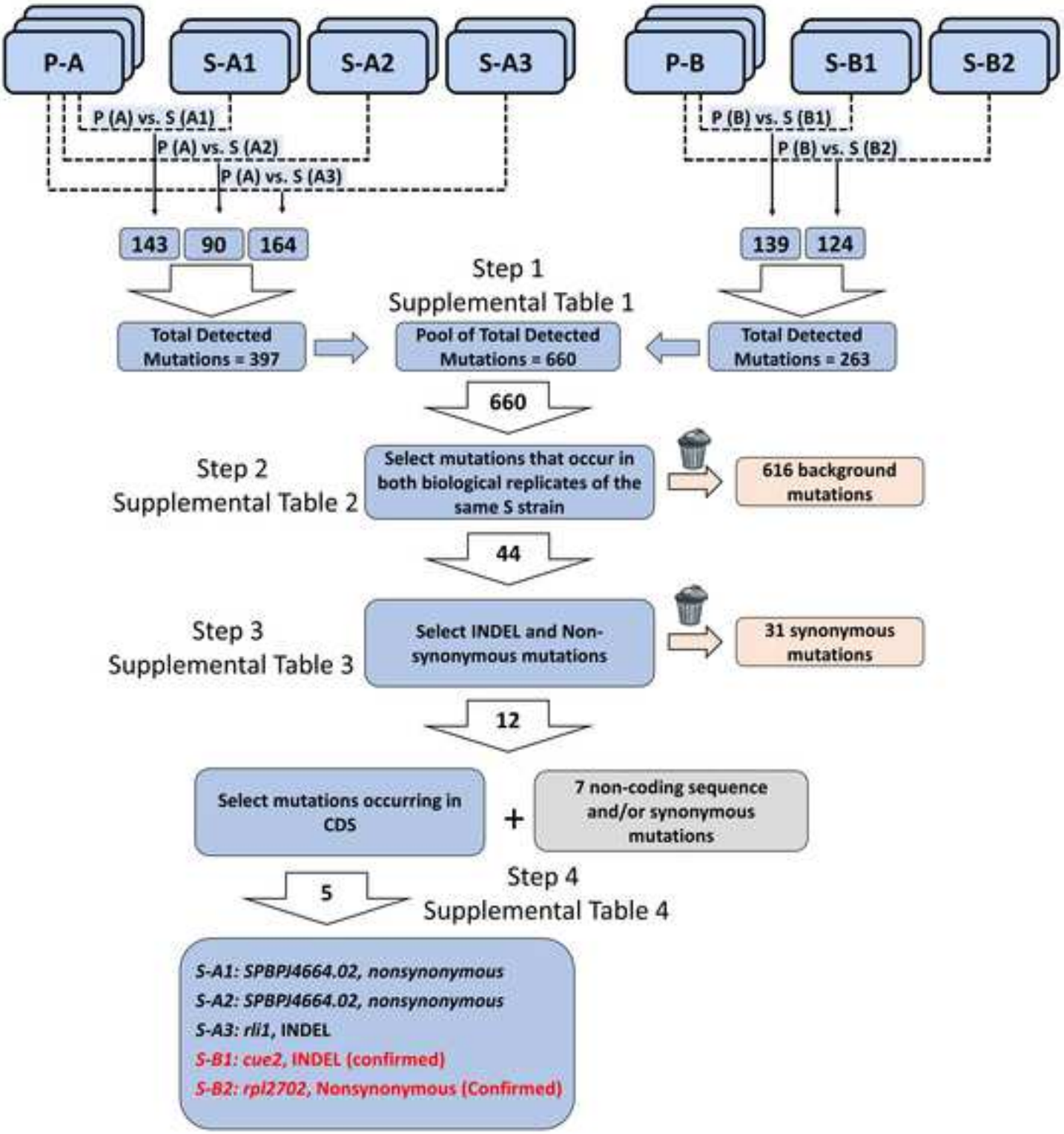
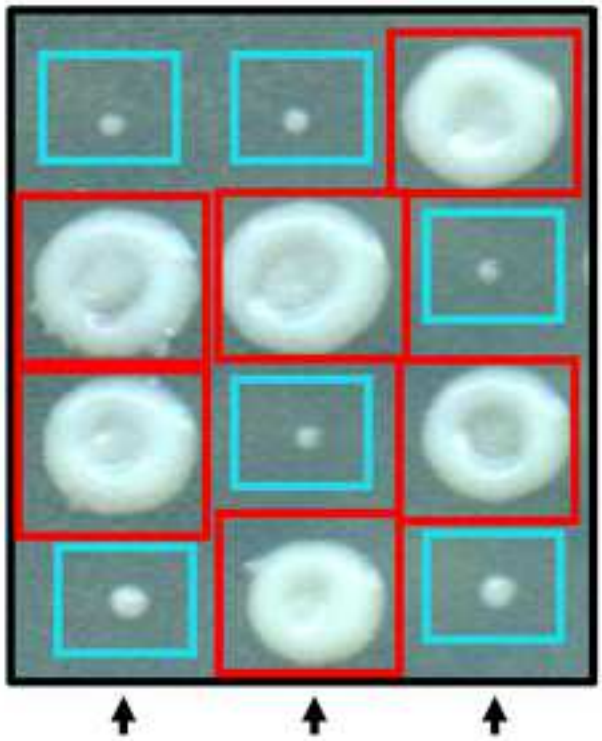


Figure 3



A *elf1Δ cue2-1* X *elf1ΔP*



B *elf1Δ rpl2702-1* X *elf1ΔP*

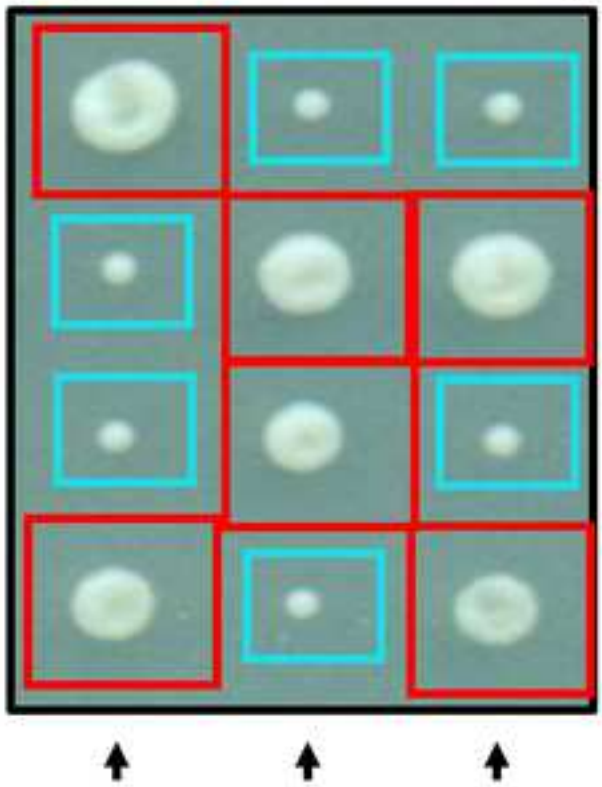


Table of Materials

Click here to access/download;Table of Materials;Materials and equipment_Marayati et al_final.xlsx

Name of Material/ Equipment	Company	Catalog Number
Adenine, Powder	Acros Organics	147441000
Bacteriology Petri Dish	Corning, Falcon	C351029
D-Glucose Anhydrous, Powder	Fisher Chemical	D16-1
Difco Agar, Granuated	Becton, Dickinson and Co.	214530
DNA extraction buffer		
Focused-ultrasonicator	Covaris Inc.	S220
Gen5 Data Collection and Analysis Software	Biotek, Inc.	GEN5SECURE
Hydrochloric Acid 1N, Liquid	Fisher Chemical	SA48-4
Liquid Rich Media (liquid YEA)		
Microplate Reader, Synergy H1 Hybrid Multi-Mode Reader	Biotek, Inc.	BTH1MG
Rich Media agar plates (YEA plates)		
RNase A/T1 mix	Thermo Fisher Scientific	EN0551
Sterile Polystyrene Inoculating Loop	Corning, Inc.	OS101
Sterile workspace and burners		
Tissue Culture Plate, 96-well Optical Flat Bottom with Low Evaporation Lid	Corning, Falcon	C353072
TruSeq DNA PCR-Free LT/HT Library Prep Kit	Illumina, Inc.	20015962
Yeast Extract, Powder	Fisher Chemical	BP1422-500

Comments/Description
Use at 75 mg/L to make liquid and solid rich media (YEA)
100×15mm, use to grow strains to single colonies on solid rich media
Use at 30 g/L to make liquid and solid rich media (YEA)
Use at 20 g/L to make solid rich media (YEA)
2% Triton X-100, 1% SDS, 100 mM NaCl, 10 mM Tris-Cl (pH 8.0), 1mM Na_2-EDTA
Alternatively, use QSonica Q800R sonicator/DNA and chromatin shearing system
Or equivalent, must be compatible with the micro-plate reader, use to export data readings from the micro-plate reader
Use to adjust pH to 5.5 in liquid and solid rich media
30 g/L D-Glucose, 5 g/L Yeast Extract, 75 mg/L Adenine, pH adjusted to 5.5 with 1 M HCl
Or equivalent, must read visible light at 600nm wavelength range
30 g/L D-Glucose, 5 g/L Yeast Extract, 75 mg/L Adenine, 20 g/L Agar, pH adjusted to 5.5 with 1 M HCl.
Use according to manufacturer recommendation
Or equivalent, use to transfer colonies from agar plates to 96-well plate
Or equivalent, must have optical flat bottom for micro-plate ready
Use to prepare the whole-genome sequencing library
Use at 5 g/L to make liquid and solid rich media (YEA)

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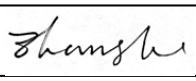
Signature:	
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Editorial comments:

1. Protocol to be filmed should have detailed, step-by-step directions highlighted, which is not necessarily the case; e.g.:
-Section 2: This section is fairly vague (and is perhaps not necessary to film; note that we can show the point that the strains should have a growth defect in another section of the video).

We agree. We don't need to film this section, but we need to be clear that having a growth defect in liquid culture (mutation or drug) is a pre-requirement for the screen.

-Section 6: You have indicated that bioinformatic analysis should be filmed, but not highlighted any of the steps; also the last step highlighted is fairly vague. Please

We won't film the bioinformatics analysis. We will only film the content in Figure 3, and the highlighted portion of step 6.10.

2. 6.6-6.8: It looks like this is geared to the analysis you did for the results (including in the provided scripts); how might it be altered for the protocol as presented, with all sequencing done in triplicate?

Thank you for this important point. We have revised the script in the protocol to better align with the protocol and to analyze triplicates.

3. Results: '...when backcrossing all yfm S with yfm P...' (first paragraph)-the use of the generic 'yfm' seems out of place in this section, which should be focused on specific results. Could this be '…suppressors with parentals' (or similar)? See also later in the paragraph.

We agree that it seems out of place. We have revised the text to "When grown on the same plate for the same amount of time, we confirmed the 2:2 segregation when back-crossing all suppressed strains (S strains) with their parental strains (P strains), which resulted in 2 small (growth defect) and 2 large (suppressor phenotype) colonies. Individual examples for suppressed *elf1*Δ, *clr6-1* and *fal1*Δ cells are shown in Figure 2B. We have confirmed that all isolated S strains carry a monogenic, genetic element that suppresses the slow-growing phenotype of their P strains (data not shown)".

4. Table 1: There should be one sheet per uploaded table; either split into 4 tables or upload as supplemental material. Note also that a 650+ row table will be tricky to include within the final manuscript.

We have split the table into 4 tables and uploaded the tables as supplementary material. We have modified the text to reflect the changes.

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Chromosome	Position	Gene ID	Coding/Exon ID	Original Sequence
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I	3160042	transcript:SPAC3G9.11c.1	-	C
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I	3160038	transcript:SPAC3G9.11c.1	-	C
I	3160037	transcript:SPAC3G9.11c.1	-	A
I	3160036	transcript:SPAC3G9.11c.1	-	G
I	3160028	transcript:SPAC3G9.11c.1	-	G
II	1952886	-	gene:SPN	A
II	693499	transcript:SPBPJ4664.02.1	CDS:SPB	AGC
II	692303	transcript:SPBPJ4664.02.1	CDS:SPB	CTG
II	692301	transcript:SPBPJ4664.02.1	CDS:SPB	GTT
II	670967	transcript:SPBC947.04.1	CDS:SPB	TAT
II	413474	transcript:SPBC106.18.1	-	C
II	413473	transcript:SPBC106.18.1	-	A
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III	1712983	transcript:SPCC191.06.1	-	G
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III	1529707	transcript:SPCC584.01c.1	-	C
III	1529703	transcript:SPCC584.01c.1	-	G
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II	1389161	transcript:SPBC8D2.16c.1	-	C
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II	1264820	-	gene:SPN	A
II	1264817	-	gene:SPN	G
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II	890630	-	gene:SPN	A
II	890630	transcript:SPBC713.14c.1	-	A
III	822725	transcript:SPCC1393.11.1	-	C
II	716295	transcript:SPBC119.02.1	-	G
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II	1312250	-	gene:SPN	C
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II	153538	transcript:SPBC1683.07.1	-	G
II	153535	transcript:SPBC1683.06c.1	-	G
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III	2309611	-	gene:SPN	TA
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II	520627	-	gene:SPN	C
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III	237048	transcript:SPCC1529.01.1	-	G
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II	113239	transcript:SPBC359.03c.1	-	G
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II	2579484	transcript:SPBC2G5.02c.1	-	C
I	2401096	-	-	C
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II	2203175	transcript:SPBC14C8.01c.1	-	T
II	2197587	transcript:SPBC17G9.13c.1	-	C
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III	2167597	transcript:SPCC1620.12c.1	-	T
III	2167592	transcript:SPCC1620.11.1	CDS:SPC	CAAA
III	2167592	transcript:SPCC1620.12c.1	-	CAAA
III	2167590	transcript:SPCC1620.11.1	CDS:SPC	C
III	2167590	transcript:SPCC1620.12c.1	-	C
III	2167588	transcript:SPCC1620.11.1	CDS:SPC	CTT
III	2167588	transcript:SPCC1620.12c.1	-	T
II	2132608	transcript:SPBC1711.01c.1	CDS:SPB	AAA
II	2132607	transcript:SPBC1711.01c.1	CDS:SPB	AAA
II	2132603	transcript:SPBC1711.01c.1	CDS:SPB	AAG

II	2132602	transcript:SPBC1711.01c.1	CDS:SPB	AAG
III	2074540	transcript:SPCPB1C11.02.1	-	C
I	1955853	-	gene:SPN	T
I	1955853	-	gene:SPN	T
III	961176	transcript:SPCC1742.01.1	CDS:SPC	ACA
II	890288	-	gene:SPN	GAAA
II	890288	transcript:SPBC713.13.1	CDS:SPB	GAAA
II	890288	transcript:SPBC713.14c.1	CDS:SPB	GAAA
II	693499	transcript:SPBPJ4664.02.1	CDS:SPB	AGC
II	692442	transcript:SPBPJ4664.02.1	CDS:SPB	AGT
II	670963	transcript:SPBC947.04.1	CDS:SPB	GCA
I	453369	transcript:SPAC23E2.03c.1	-	G
III	208132	transcript:SPCC1235.17.1	-	G
III	46959	transcript:SPCC1884.02.1	-	A
III	46959	transcript:SPCC757.02c.1	-	A
II	4165206	transcript:SPBC14F5.06.1	CDS:SPB	G
I	3477396	transcript:SPAC328.02.1	-	CA
I	3160042	transcript:SPAC3G9.11c.1	-	C
I	3160041	transcript:SPAC3G9.11c.1	-	G
I	3160038	transcript:SPAC3G9.11c.1	-	C
I	3160037	transcript:SPAC3G9.11c.1	-	A
I	3160036	transcript:SPAC3G9.11c.1	-	G
I	3160028	transcript:SPAC3G9.11c.1	-	G
III	1529703	transcript:SPCC584.01c.1	-	G
II	1389165	transcript:SPBC8D2.16c.1	-	A
II	465486	transcript:SPBC428.11.1	-	C
I	5426666	-	gene:SPN	G
I	5426664	-	gene:SPN	A
I	5271466	-	gene:SPN	A
I	5271432	-	gene:SPN	C
I	5173430	-	gene:SPN	G
I	4926094	transcript:SPAC1B3.01c.1	-	TAAA
I	4926093	transcript:SPAC1B3.01c.1	-	A
I	4926081	transcript:SPAC1B3.01c.1	-	TGAATGC
I	4926078	transcript:SPAC1B3.01c.1	-	A
I	3995046	transcript:SPAPB15E9.06.1	-	C
II	3980468	-	gene:SPN	C
II	3856940	-	gene:SPN	A
II	3856937	-	gene:SPN	C
II	3856935	-	gene:SPN	C
II	3856934	-	gene:SPN	A
II	3856931	-	gene:SPN	G
II	3856928	-	gene:SPN	A
II	3856922	-	gene:SPN	TGGAAGA

II	3799350	transcript:SPBC21C3.02c.1	-	G
I	3645103	transcript:SPAC8C9.03.1	-	C
I	3580034	-	gene:SPN	C
I	3580034	transcript:SPAC17A2.12.1	-	C
I	3576929	transcript:SPAC17A2.11.1	-	C
II	3512948	-	gene:SPN	A
II	3380128	-	gene:SPN	C
II	3329998	-	gene:SPN	G
I	3301568	-	gene:SPN	G
I	3301565	-	gene:SPN	C
II	3161493	transcript:SPBC609.01.1	-	A
I	3160024	transcript:SPAC3G9.11c.1	-	A
II	3050441	transcript:SPBC13E7.05.1	CDS:SPB	AAT
II	2591933	transcript:SPBC2G5.07c.1	-	G
II	2591911	transcript:SPBC2G5.07c.1	-	G
II	2579477	transcript:SPBC2G5.02c.1	-	G
II	2132606	transcript:SPBC1711.01c.1	CDS:SPB	AAA
II	2132604	transcript:SPBC1711.01c.1	CDS:SPB	T
II	2132601	transcript:SPBC1711.01c.1	CDS:SPB	C
III	2069621	transcript:SPCPB1C11.01.1	-	G
II	1964110	-	gene:SPN	C
III	1712983	transcript:SPCC191.05c.1	-	G
III	1712983	transcript:SPCC191.06.1	-	G
III	1674639	-	gene:SPN	A
II	1389161	transcript:SPBC8D2.16c.1	-	C
III	1302685	transcript:SPCC1322.08.1	-	C
III	1302674	transcript:SPCC1322.08.1	-	C
I	1148170	transcript:SPAC56F8.14c.1	-	A
I	1148167	transcript:SPAC56F8.14c.1	-	A
II	1104979	-	gene:SPN	G
II	1104979	transcript:SPBC1709.04c.1	-	G
II	1045366	transcript:SPBC337.08c.1	CDS:SPB	CTT
II	1045363	transcript:SPBC337.08c.1	CDS:SPB	CAC
II	1019534	transcript:SPBC146.10.1	-	T
III	961170	transcript:SPCC1742.01.1	CDS:SPC	GGT
III	958265	-	gene:SPN	A
III	958265	-	gene:SPN	A
III	958265	-	gene:SPN	A
III	958262	-	gene:SPN	TGAA
III	958262	-	gene:SPN	TGAA
III	958262	-	gene:SPN	TGAA
III	827274	transcript:SPCC1393.13.1	-	T
III	822725	transcript:SPCC1393.11.1	-	C
II	695689	transcript:SPBPJ4664.02.1	CDS:SPB	CTG

II	695630	transcript:SPBPJ4664.02.1	CDS:SPB	CCAATTACTAGTTC
II	693522	transcript:SPBPJ4664.02.1	CDS:SPB	AGT
II	693520	transcript:SPBPJ4664.02.1	CDS:SPB	TCT
II	693508	transcript:SPBPJ4664.02.1	CDS:SPB	CCA
II	693499	transcript:SPBPJ4664.02.1	CDS:SPB	AGC
II	693493	transcript:SPBPJ4664.02.1	CDS:SPB	CTG
II	670973	transcript:SPBC947.04.1	CDS:SPB	ACT
II	670965	transcript:SPBC947.04.1	CDS:SPB	CCC
III	568658	-	gene:SPN	C
III	568658	transcript:SPCC1672.04c.1	-	C
II	520668	-	gene:SPN	G
II	520666	-	gene:SPN	A
II	520664	-	gene:SPN	TGA
II	520661	-	gene:SPN	G
II	520659	-	gene:SPN	G
III	467918	transcript:SPCC4G3.03.1	-	C
I	453369	transcript:SPAC23E2.03c.1	-	G
III	237048	transcript:SPCC1529.01.1	-	G
III	69566	transcript:SPCC757.11c.1	-	T
III	69562	transcript:SPCC757.11c.1	-	C
III	69559	transcript:SPCC757.11c.1	-	C
III	69555	transcript:SPCC757.11c.1	-	T
I	5321726	-	gene:SPN	A
I	5321721	-	gene:SPN	A
I	4911430	-	gene:SPN	A
II	4209406	transcript:SPBC342.04.1	-	C
I	3993810	transcript:SPAPB15E9.02c.1	-	T
I	3993810	transcript:SPAPB15E9.06.1	-	T
I	3992214	-	gene:SPN	C
I	3992214	transcript:SPAPB15E9.02c.1	CDS:SPA	C
I	3992188	-	gene:SPN	G
I	3992188	transcript:SPAPB15E9.02c.1	CDS:SPA	G
II	3980468	-	gene:SPN	C
I	3645141	transcript:SPAC8C9.03.1	-	G
I	3645139	transcript:SPAC8C9.03.1	-	C
I	3645128	transcript:SPAC8C9.03.1	-	A
I	3610418	-	gene:SPN	G
I	3610418	-	gene:SPN	G
I	3610418	transcript:SPAC17G6.11c.1	-	G
I	3610417	-	gene:SPN	A
I	3610417	-	gene:SPN	A
I	3610417	transcript:SPAC17G6.11c.1	-	A
I	3580064	-	gene:SPN	TACAATTAA
I	3580064	transcript:SPAC17A2.12.1	-	TACAATTAA

I	3580061	-	gene:SPN	A
I	3580061	transcript:SPAC17A2.12.1	-	A
II	3380128	-	gene:SPN	C
I	3301538	-	gene:SPN	C
I	3300820	-	gene:SPN	G
I	3166796	-	-	T
I	3166796	-	gene:SPN	T
I	3160021	transcript:SPAC3G9.11c.1	-	C
I	3033347	-	gene:SPN	C
I	2416364	transcript:SPAC6B12.04c.1	-	A
I	2416364	transcript:SPAC6B12.18.1	-	A
I	2231762	transcript:SPAC18G6.09c.1	-	T
II	2204920	-	gene:SPN	G
II	2203175	transcript:SPBC14C8.01c.1	-	T
III	2074540	transcript:SPCPB1C11.02.1	-	C
II	1952889	-	gene:SPN	C
II	1952886	-	gene:SPN	A
III	1712983	transcript:SPCC191.05c.1	-	G
III	1712983	transcript:SPCC191.06.1	-	G
I	1665245	transcript:SPAC343.21.1	-	G
I	1665240	transcript:SPAC343.21.1	-	T
II	1554361	-	gene:SPN	C
II	1389160	transcript:SPBC8D2.16c.1	-	TC
I	1359503	-	gene:SPN	T
II	1104985	-	gene:SPN	C
II	1104985	transcript:SPBC1709.04c.1	-	C
II	1104984	-	gene:SPN	A
II	1104984	transcript:SPBC1709.04c.1	-	A
II	1104981	-	gene:SPN	AAC
II	1104981	transcript:SPBC1709.04c.1	-	AAC
III	961171	transcript:SPCC1742.01.1	CDS:SPC	ACT
II	890282	-	gene:SPN	T
II	890282	transcript:SPBC713.13.1	CDS:SPB	ACC
II	890282	transcript:SPBC713.14c.1	CDS:SPB	TAC
II	890279	-	gene:SPN	G
II	890279	transcript:SPBC713.13.1	CDS:SPB	CTA
II	890279	transcript:SPBC713.14c.1	CDS:SPB	CCT
II	890277	-	gene:SPN	T
II	890277	transcript:SPBC713.13.1	CDS:SPB	CTA
II	890277	transcript:SPBC713.14c.1	CDS:SPB	ATT
II	890273	-	gene:SPN	G
II	890273	transcript:SPBC713.13.1	CDS:SPB	CTT
II	890273	transcript:SPBC713.14c.1	CDS:SPB	TCT
II	693574	transcript:SPBPJ4664.02.1	CDS:SPB	TACACCAATTACTA

II	692442	transcript:SPBPJ4664.02.1	CDS:SPB	AGT
I	414099	transcript:SPAC31A2.12.1	-	C
I	414098	transcript:SPAC31A2.12.1	-	C
II	3883700	transcript:SPBC211.05.1	-	A
II	3883700	transcript:SPBC211.06.1	-	A
I	2327021	-	gene:SPN	C
I	2327021	transcript:SPAC6C3.03c.1	-	C
I	1905516	transcript:SPAC110.05.1	-	C
III	1302685	transcript:SPCC1322.08.1	-	C
II	1045183	transcript:SPBC337.08c.1	CDS:SPB	GAT
III	180262	transcript:SPCC1235.03.1	CDS:SPC	CCGATCTCTTGCTA
I	5322103	transcript:SPAP8A3.05.1	-	A
I	5078848	transcript:SPAC1006.05c.1	-	G
I	4235601	transcript:SPAC1F7.08.1	-	A
II	3856940	-	gene:SPN	A
II	3856937	-	gene:SPN	C
II	3856935	-	gene:SPN	C
II	3856934	-	gene:SPN	A
II	3856931	-	gene:SPN	G
II	3856930	-	gene:SPN	G
II	3856923	-	gene:SPN	GGAAGA
II	3856896	-	gene:SPN	A
I	3580034	-	gene:SPN	C
I	3580034	transcript:SPAC17A2.12.1	-	C
I	3578659	-	gene:SPN	G
I	3578659	transcript:SPAC17A2.11.1	-	G
II	3513908	-	gene:SPN	A
II	3513908	-	gene:SPN	A
II	3513904	-	gene:SPN	A
II	3513904	-	gene:SPN	A
II	3513900	-	gene:SPN	A
II	3513900	-	gene:SPN	A
II	3512948	-	gene:SPN	A
II	3512947	-	gene:SPN	A
II	3498954	transcript:SPBPB7E8.02.1	-	A
I	3477403	transcript:SPAC328.02.1	-	A
II	3410437	-	gene:SPN	G
II	3380128	-	gene:SPN	C
II	3161493	transcript:SPBC609.01.1	-	A
I	2932642	-	gene:SPN	C
I	2932642	transcript:SPAC2E1P3.04.1	-	C
II	2826100	transcript:SPBC19C7.04c.1	-	G
I	2401096	-	-	C
I	2231735	transcript:SPAC18G6.09c.1	-	C

II	2197587	transcript:SPBC17G9.13c.1	-	C
II	2132612	transcript:SPBC1711.01c.1	CDS:SPB	CGT
II	2132601	transcript:SPBC1711.01c.1	CDS:SPB	CTTTTTTTTA
III	2113232	-	gene:SPN	G
III	2074510	transcript:SPCPB1C11.02.1	-	C
II	1952900	-	gene:SPN	G
II	1952897	-	gene:SPN	C
II	1941589	-	gene:SPN	G
III	1925626	transcript:SPCC74.02c.1	-	C
II	1750208	-	gene:SPN	A
II	1554383	-	gene:SPN	G
I	1359552	-	gene:SPN	T
I	1359548	-	gene:SPN	T
III	1302674	transcript:SPCC1322.08.1	-	C
III	1239230	transcript:SPCC645.06c.1	-	G
I	1152925	transcript:SPAC56F8.15.1	-	C
II	1104941	-	gene:SPN	G
II	1104941	-	gene:SPN	G
II	1104941	transcript:SPBC1709.04c.1	-	G
III	961170	transcript:SPCC1742.01.1	CDS:SPC	GGT
II	716295	transcript:SPBC119.02.1	-	G
II	694720	transcript:SPBPJ4664.02.1	CDS:SPB	AAC
II	693574	transcript:SPBPJ4664.02.1	CDS:SPB	TACACCAATTACTA
III	568680	-	gene:SPN	TTA
III	568680	transcript:SPCC1672.04c.1	-	TTA
II	561064	-	gene:SPN	T
I	232681	transcript:SPAC24B11.13.1	-	A
III	231447	-	gene:SPN	C
III	208136	transcript:SPCC1235.17.1	-	T
II	153578	transcript:SPBC1683.06c.1	-	G
II	153578	transcript:SPBC1683.07.1	CDS:SPB	AGC
I	5173430	-	gene:SPN	G
I	5123329	-	gene:SPN	G
I	5123329	transcript:SPAC29A4.11.1	-	G
I	5078848	transcript:SPAC1006.05c.1	-	G
I	3993810	transcript:SPAPB15E9.02c.1	-	T
I	3993810	transcript:SPAPB15E9.06.1	-	T
II	3799433	transcript:SPBC21C3.02c.1	-	C
I	3610433	-	gene:SPN	G
I	3610433	-	gene:SPN	G
I	3610433	transcript:SPAC17G6.11c.1	-	G
I	3610425	-	gene:SPN	G
I	3610425	-	gene:SPN	G
I	3610425	transcript:SPAC17G6.11c.1	-	G

I	3610417	-	gene:SPN	A
I	3610417	-	gene:SPN	A
I	3610417	transcript:SPAC17G6.11c.1	-	A
I	3576439	-	gene:SPN	G
II	3540075	transcript:SPBC1105.17.1	-	A
II	3512948	-	gene:SPN	A
II	3380176	-	gene:SPN	G
II	3380174	-	gene:SPN	TCG
II	3380169	-	gene:SPN	TAAC
II	3380164	-	gene:SPN	TCCA
II	3380151	-	gene:SPN	A
II	3161493	transcript:SPBC609.01.1	-	A
I	3033347	-	gene:SPN	C
II	2579458	transcript:SPBC2G5.02c.1	-	C
I	2525182	-	gene:SPN	G
I	2231762	transcript:SPAC18G6.09c.1	-	T
I	2231735	transcript:SPAC18G6.09c.1	-	C
II	2203098	transcript:SPBC14C8.01c.1	-	G
II	2203097	transcript:SPBC14C8.01c.1	-	G
II	2203092	transcript:SPBC14C8.01c.1	-	G
II	2203090	transcript:SPBC14C8.01c.1	-	T
II	2203088	transcript:SPBC14C8.01c.1	-	T
II	2203086	transcript:SPBC14C8.01c.1	-	C
III	2069621	transcript:SPCPB1C11.01.1	-	G
II	1955514	-	gene:SPN	G
II	1818108	transcript:SPBC9B6.03.1	-	C
II	1750216	-	gene:SPN	C
II	1678218	transcript:SPBC19C2.04c.1	-	AT
I	1494950	transcript:SPAC5D6.10c.1	CDS:SPA	AGA
I	1494950	transcript:SPAC5D6.12.1	-	G
I	1494938	transcript:SPAC5D6.10c.1	CDS:SPA	C
I	1494938	transcript:SPAC5D6.12.1	-	C
III	1302674	transcript:SPCC1322.08.1	-	C
II	1171363	transcript:SPBC409.19c.1	-	T
II	1171362	transcript:SPBC409.19c.1	-	T
II	1078283	transcript:SPBC1734.09.1	-	G
II	837481	transcript:SPBC36.02c.1	-	G
II	837476	transcript:SPBC36.02c.1	-	G
II	837475	transcript:SPBC36.02c.1	-	T
III	761531	transcript:SPCC1020.10.1	-	C
II	693100	transcript:SPBPJ4664.02.1	CDS:SPB	CAGTTCTACACCA
II	670978	transcript:SPBC947.04.1	CDS:SPB	ACT
II	670968	transcript:SPBC947.04.1	CDS:SPB	TAT
II	561064	-	gene:SPN	T

II	521748	-	gene:SPN	A
III	431910	transcript:SPCC306.11.1	-	C
III	431906	transcript:SPCC306.11.1	-	G
III	365747	transcript:SPCC594.06c.1	-	CA
III	207507	transcript:SPCC1235.17.1	-	C
II	154365	-	gene:SPN	G
II	154365	transcript:SPBC1683.07.1	CDS:SPB	GGG
II	113240	transcript:SPBC359.03c.1	-	T
III	69609	transcript:SPCC757.11c.1	-	G
I	43286	transcript:SPAC977.07c.1	CDS:SPA	CGT
I	3645128	transcript:SPAC8C9.03.1	-	A
I	3645117	transcript:SPAC8C9.03.1	-	C
III	1939467	-	gene:SPN	G
III	1939467	transcript:SPCC74.05.1	CDS:SPC	GGT
II	694252	transcript:SPBPJ4664.02.1	CDS:SPB	AAC
I	5321699	-	gene:SPN	C
I	4235601	transcript:SPAC1F7.08.1	-	A
I	4140105	-	gene:SPN	A
I	3993810	transcript:SPAPB15E9.02c.1	-	T
I	3993810	transcript:SPAPB15E9.06.1	-	T
II	3856940	-	gene:SPN	A
II	3856937	-	gene:SPN	C
II	3856935	-	gene:SPN	C
II	3856934	-	gene:SPN	A
II	3856931	-	gene:SPN	G
II	3856930	-	gene:SPN	G
II	3856923	-	gene:SPN	GGAAGA
II	3856896	-	gene:SPN	A
I	3610417	-	gene:SPN	A
I	3610417	-	gene:SPN	A
I	3610417	transcript:SPAC17G6.11c.1	-	A
II	3410437	-	gene:SPN	G
II	3380128	-	gene:SPN	C
II	3161493	transcript:SPBC609.01.1	-	A
I	3033347	-	gene:SPN	C
I	2327021	-	gene:SPN	C
I	2327021	transcript:SPAC6C3.03c.1	-	C
II	2204920	-	gene:SPN	G
II	2197587	transcript:SPBC17G9.13c.1	-	C
II	2132606	transcript:SPBC1711.01c.1	CDS:SPB	AAA
II	2132604	transcript:SPBC1711.01c.1	CDS:SPB	T
II	2132601	transcript:SPBC1711.01c.1	CDS:SPB	C
III	2113205	-	gene:SPN	C
II	1964110	-	gene:SPN	C

I	1954162	-	gene:SPN	C
I	1954162	-	gene:SPN	C
I	1905516	transcript:SPAC110.05.1	-	C
III	1674668	-	gene:SPN	A
III	1674667	-	gene:SPN	G
III	1674665	-	gene:SPN	C
III	1674664	-	gene:SPN	A
III	1674642	-	gene:SPN	C
I	1494950	transcript:SPAC5D6.10c.1	CDS:SPA	AGA
I	1494950	transcript:SPAC5D6.12.1	-	G
III	1239230	transcript:SPCC645.06c.1	-	G
I	1149920	transcript:SPAC56F8.13.1	CDS:SPA	A
I	1149920	transcript:SPAC56F8.14c.1	-	A
III	970548	-	gene:SPN	C
III	717698	-	gene:SPN	TA
II	695620	transcript:SPBPJ4664.02.1	CDS:SPB	C
II	695615	transcript:SPBPJ4664.02.1	CDS:SPB	C
II	695611	transcript:SPBPJ4664.02.1	CDS:SPB	T
II	695473	transcript:SPBPJ4664.02.1	CDS:SPB	CTC
II	695466	transcript:SPBPJ4664.02.1	CDS:SPB	AGT
II	695464	transcript:SPBPJ4664.02.1	CDS:SPB	TCT
II	695443	transcript:SPBPJ4664.02.1	CDS:SPB	AGT
II	695442	transcript:SPBPJ4664.02.1	CDS:SPB	AGT
II	694982	transcript:SPBPJ4664.02.1	CDS:SPB	TCA
II	693169	transcript:SPBPJ4664.02.1	CDS:SPB	CTG
II	670970	transcript:SPBC947.04.1	CDS:SPB	ACA
III	568658	-	gene:SPN	C
III	568658	transcript:SPCC1672.04c.1	-	C
II	561064	-	gene:SPN	T
I	442783	transcript:SPAC23E2.01.1	-	A
III	237023	transcript:SPCC1529.01.1	-	A
III	208136	transcript:SPCC1235.17.1	-	T
II	153578	transcript:SPBC1683.06c.1	-	G
II	153578	transcript:SPBC1683.07.1	CDS:SPB	AGC
I	4514505	transcript:SPAC27D7.03c.1	-	C
I	3993829	transcript:SPAPB15E9.02c.1	-	G
I	3993829	transcript:SPAPB15E9.06.1	-	G
I	3993828	transcript:SPAPB15E9.02c.1	-	G
I	3993828	transcript:SPAPB15E9.06.1	-	G
II	3856896	-	gene:SPN	A
I	3580034	-	gene:SPN	C
I	3580034	transcript:SPAC17A2.12.1	-	C
I	3578659	-	gene:SPN	G
I	3578659	transcript:SPAC17A2.11.1	-	G

II	3380153	-	gene:SPN	A
II	3161493	transcript:SPBC609.01.1	-	A
I	3160024	transcript:SPAC3G9.11c.1	-	A
I	3033365	-	gene:SPN	C
I	3033347	-	gene:SPN	C
I	2906433	transcript:SPAPYUK71.03c.1	CDS:SPA	TA
I	2525182	-	gene:SPN	G
I	2416348	transcript:SPAC6B12.04c.1	-	C
I	2416348	transcript:SPAC6B12.18.1	-	C
II	2197630	transcript:SPBC17G9.13c.1	-	T
II	2197629	transcript:SPBC17G9.13c.1	-	T
II	2197623	transcript:SPBC17G9.13c.1	-	T
II	2197621	transcript:SPBC17G9.13c.1	-	T
II	2197617	transcript:SPBC17G9.13c.1	-	C
II	2197616	transcript:SPBC17G9.13c.1	-	C
II	2197610	transcript:SPBC17G9.13c.1	-	G
I	1955758	-	gene:SPN	T
I	1954162	-	gene:SPN	C
I	1954162	-	gene:SPN	C
I	1719470	transcript:SPAC664.09.1	-	A
I	1719469	transcript:SPAC664.09.1	-	G
III	1712983	transcript:SPCC191.05c.1	-	G
III	1712983	transcript:SPCC191.06.1	-	G
III	1702691	-	gene:SPN	C
III	1702691	transcript:SPCC417.15.1	-	C
II	1684533	transcript:SPBC19C2.05.1	-	C
III	1674618	-	gene:SPN	C
II	1389139	transcript:SPBC8D2.16c.1	-	C
II	1171363	transcript:SPBC409.19c.1	-	T
II	1171355	transcript:SPBC409.19c.1	-	T
II	1045366	transcript:SPBC337.08c.1	CDS:SPB	CTT
II	1045363	transcript:SPBC337.08c.1	CDS:SPB	CAC
III	970548	-	gene:SPN	C
II	694291	transcript:SPBPJ4664.02.1	CDS:SPB	AGT
II	694290	transcript:SPBPJ4664.02.1	CDS:SPB	AGT
II	693100	transcript:SPBPJ4664.02.1	CDS:SPB	CAGTTCTACACCA
II	692442	transcript:SPBPJ4664.02.1	CDS:SPB	AGT
II	670962	transcript:SPBC947.04.1	CDS:SPB	GCA
II	561064	-	gene:SPN	T
II	465514	transcript:SPBC428.11.1	-	A
II	465513	transcript:SPBC428.11.1	-	C
III	431932	transcript:SPCC306.11.1	-	A
III	365747	transcript:SPCC594.06c.1	-	CA
I	239258	-	gene:SPN	A

I	239258	-	gene:SPN(	A
III	237048	transcript:SPCC1529.01.1	-	G
III	69609	transcript:SPCC757.11c.1	-	G

Table _seqchanges2_: Changes identified between all triplicates of P elf1Δ and one or both du

Altered Sequence	Original Amino Acid	New Amino Acid	Type of Change
C	-	-	INDEL
T	-	-	five_prime_UTR
T	-	-	five_prime_UTR
T	-	-	five_prime_UTR
T	-	-	five_prime_UTR
T	-	-	five_prime_UTR
T	-	-	five_prime_UTR
T	-	-	ncRNA
AGT	S	S	SYN
TTG	L	L	SYN
GCT	V	A	NONSYN
TAC	Y	Y	SYN
T	-	-	five_prime_UTR
T	-	-	five_prime_UTR
CTT	-	-	INDEL
CTT	-	-	INDEL
CAAAAAAA	-	-	INDEL
A	-	-	INDEL
CT	-	-	INDEL
CT	-	-	INDEL
A	-	-	ncRNA
A	-	-	five_prime_UTR
ATTTTTTTTTTTTTT	-	-	INDEL
A	-	-	three_prime_UTR
A	-	-	three_prime_UTR
GAAAAAAAAAAAAAAAAAAAAAAAAA	-	-	INDEL
T	-	-	three_prime_UTR
T	-	-	three_prime_UTR
T	-	-	ncRNA
C	-	-	INDEL
TAAAAAAAAAAAAAAAAAAAAAAAAG	-	-	INDEL
T	-	-	ncRNA
T	-	-	ncRNA
A	-	-	three_prime_UTR
CAAAAAAAAAAAAAAAAAAAAAAAAA	-	-	INDEL
ATA	K	I	NONSYN
AAT	K	N	NONSYN
TAG	K	*	NONSYN
AGG	K	R	NONSYN
T	-	-	five_prime_UTR
CAAAAAAAAAAAAAAAAAAAAAAAAA	-	-	INDEL

CAAAAAAAAAAAAAAAAAAAAAAAAA	-	-	INDEL
T	-	-	ncRNA
T	-	-	five_prime_UTR
GAAAAAAAAAAAAAAAAAAAAAAAAA	-	-	INDEL
GAAAAAAAAAAAAAAAAAAAAAAAAA	-	-	INDEL
A	-	-	INDEL
A	-	-	five_prime_UTR
A	-	-	five_prime_UTR
CAAAAAAAAAAAAAAAAAAAAAAAAA	-	-	INDEL
CAAAAAAAAAAAAAAAAAAAAAAAAA	-	-	INDEL
T	-	-	five_prime_UTR
T	-	-	five_prime_UTR
A	-	-	ncRNA
A	-	-	ncRNA
T	-	-	ncRNA
T	-	-	ncRNA
A	-	-	ncRNA
T	-	-	ncRNA
T	-	-	five_prime_UTR
T	-	-	three_prime_UTR
GTTTTTTT	-	-	INDEL
AAT	N	N	SYN
CTG	L	L	SYN
GTT	L	V	NONSYN
ACT	S	T	NONSYN
TCA	S	S	SYN
CCG	P	P	SYN
CTT	L	L	SYN
CTTTTTTTTTTTCCTTTTTTTTTTTTTTTTI	-	-	INDEL
GAAAAAAAAAA	-	-	INDEL
T	-	-	three_prime_UTR
A	-	-	three_prime_UTR
T	-	-	ncRNA
T	-	-	ncRNA
A	-	-	three_prime_UTR
A	-	-	three_prime_UTR
A	-	-	ncRNA
A	-	-	three_prime_UTR
T	-	-	five_prime_UTR
T	-	-	five_prime_UTR
T	-	-	three_prime_UTR
A	-	-	five_prime_UTR
A	-	-	five_prime_UTR
A	-	-	five_prime_UTR

A	-	-	five_prime_UTR
T	-	-	three_prime_UTR
GAAAAAAAAAAAAAAAAAAAAAAAAA	-	-	INDEL
GAAAAAAAAAAAAAAAAAAAAAAAAA	-	-	INDEL
CTTTTTTTTTTTTTTTTTTTCAATTTTTTT	-	-	INDEL
GA	-	-	INDEL
ATTTTTTTTTTTTTTTTTTTTTTTTTTTTT	-	-	INDEL
ATTTTTTTTTTTTTTTTTTT	-	-	INDEL
GTTTTTTTCTTTTTTCTTTTTTTTTTTTTT	-	-	INDEL
CTTTTTTTTTTTTTTTTTT	-	-	INDEL
A	-	-	five_prime_UTR
T	-	-	ncRNA
T	-	-	ncRNA
CAAAAAAAAAAAAAAAAAAAAAAAAA	-	-	INDEL
T	-	-	three_prime_UTR
T	-	-	ncRNA
T	-	-	ncRNA
A	-	-	three_prime_UTR
A	-	-	three_prime_UTR
GTC	D	V	NONSYN
A	-	-	three_prime_UTR
GAAAAAAAAAAAAAAA	-	-	INDEL
GAAAAAAAAAAAAAAA	-	-	INDEL
CTTTT	-	-	INDEL
T	-	-	ncRNA
T	-	-	ncRNA
ATTTTTTTTTTTT	-	-	INDEL
CTTTTTTTTTTT	-	-	INDEL
A	-	-	three_prime_UTR
GAAAAAAAAAAAAAAAAAAAAAAAAA	-	-	INDEL
T	-	-	three_prime_UTR
A	-	-	three_prime_UTR
A	-	-	three_prime_UTR
T	-	-	five_prime_UTR
AGT	S	S	SYN
A	-	-	five_prime_UTR
AAC	S	N	NONSYN
T	-	-	five_prime_UTR
T	-	-	five_prime_UTR
T	-	-	five_prime_UTR
T	-	-	five_prime_UTR
A	-	-	INDEL
A	-	-	INDEL
AGTTAGTC	-	-	INDEL

AGTTAGTC	-	-	INDEL
A	-	-	five_prime_UTR
A	-	-	five_prime_UTR
ACATACCAT	-	-	INDEL
ACATACCAT	-	-	INDEL
GAA	-	-	INDEL
GAA	-	-	INDEL
GGTC	-	-	INDEL
GGTC	-	-	INDEL
C	-	-	five_prime_UTR
C	-	-	five_prime_UTR
T	-	-	five_prime_UTR
T	-	-	five_prime_UTR
TAAAAAAAAAAAAAAAAGAAAAAAAA/	-	-	INDEL
TAAAAAAAAAAAAAAAAGAAAAAAAA/	-	-	INDEL
T	-	-	five_prime_UTR
TTG	L	L	SYN
GCT	V	A	NONSYN
GCT	A	A	SYN
T	-	-	ncRNA
T	-	-	five_prime_UTR
T	-	-	five_prime_UTR
A	-	-	INDEL
GTTCTTTTTTTTTTTTTTTTTT	-	-	INDEL
A	-	-	five_prime_UTR
A	-	-	five_prime_UTR
T	-	-	five_prime_UTR
T	-	-	five_prime_UTR
T	-	-	five_prime_UTR
ATTTTTTTTTTT	-	-	INDEL
CTTTTTTTTTTTTTTTTTTTT	-	-	INDEL
C	-	-	INDEL
ATTTTTTTTTTTTTTTTTCTTTTTTTTTTTTT	-	-	INDEL
T	-	-	five_prime_UTR
T	-	-	five_prime_UTR
T	-	-	five_prime_UTR
T	-	-	five_prime_UTR
T	-	-	five_prime_UTR
CTTTTTTT	-	-	INDEL
T	-	-	ncRNA
T	-	-	ncRNA
T	-	-	INDEL
A	-	-	three_prime_UTR
CAAAAAAAAAAAAA	-	-	INDEL

T	-	-	INDEL
T	-	-	ncRNA
T	-	-	ncRNA
T	-	-	ncRNA
A	-	-	five_prime_UTR
T	-	-	five_prime_UTR
T	-	-	five_prime_UTR
AAA	T	K	NONSYN
A	-	-	three_prime_UTR
CTTTTTTTTTTTT	-	-	INDEL
TAAAAAAAAAAAAAAAAAAAAAAAAAA	-	-	INDEL
T	-	-	ncRNA
T	-	-	ncRNA
TAAAAAAAAAAAAAAATAAAAAAA	-	-	INDEL
GAAAAAAAAAAAAAAAAAAAAAAAA	-	-	INDEL
CTTTTTTTTTTTTTTTGT	-	-	INDEL
GTAAAAAAAAAAAAAAAAAAACTTGAA	-	-	INDEL
GAAAAAAAAAAAAAAAAAAAAAAAAA	-	-	INDEL
GAAAAAAAAAAAAAAAAAAAAAAAAA	-	-	INDEL
T	-	-	five_prime_UTR
T	-	-	five_prime_UTR
T	-	-	three_prime_UTR
CTTTTTTTTTTTTTTTTTTTTTTATTTTTTTT	-	-	INDEL
CTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT	-	-	INDEL
CTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT	-	-	INDEL
T	-	-	INDEL
ATTTTTTTTTTTTT	-	-	INDEL
TTT	Y	F	NONSYN
A	-	-	five_prime_UTR
A	-	-	intron
A	-	-	three_prime_UTR
A	-	-	three_prime_UTR
CAAAAAAAAAAAAAAAAAAAAAAAA	-	-	INDEL
TGC	F	C	NONSYN
G	-	-	three_prime_UTR
C	-	-	INDEL
C	-	-	INDEL
CT	-	-	INDEL
CT	-	-	INDEL
CGT	L	R	NONSYN
G	-	-	three_prime_UTR
ATA	K	I	NONSYN
AAT	K	N	NONSYN
TAG	K	*	NONSYN

AGG	K	R	NONSYN
T	-	-	three_prime_UTR
TA	-	-	INDEL
TA	-	-	INDEL
TCA	T	S	NONSYN
G	-	-	INDEL
G	-	-	INDEL
G	-	-	INDEL
AGT	S	S	SYN
ACT	S	T	NONSYN
TCA	A	S	NONSYN
GAAAAAAAAAAAAAAAAA	-	-	INDEL
A	-	-	three_prime_UTR
AATAT,AATATAT	-	-	INDEL
AATAT,AATATAT	-	-	INDEL
GTGTATTGAAGTTAATCCTACCGATCGCA	-	-	INDEL
C	-	-	INDEL
T	-	-	five_prime_UTR
T	-	-	five_prime_UTR
T	-	-	five_prime_UTR
T	-	-	five_prime_UTR
T	-	-	five_prime_UTR
T	-	-	five_prime_UTR
A	-	-	five_prime_UTR
T	-	-	five_prime_UTR
T	-	-	five_prime_UTR
T	-	-	ncRNA
T	-	-	ncRNA
T	-	-	ncRNA
CTTTTTTTTTTTTTTTTTTTTTTATTTTTTT	-	-	INDEL
GT,GTTTTTTTTTTTTTTTTTTTTATTTTTTT	-	-	INDEL
T	-	-	INDEL
T	-	-	three_prime_UTR
T	-	-	INDEL
G	-	-	three_prime_UTR
T	-	-	three_prime_UTR
CTTTTTTTTTTTTTTTTTTTTTTATTTTTTTT	-	-	INDEL
T	-	-	ncRNA
T	-	-	ncRNA
T	-	-	ncRNA
T	-	-	ncRNA
T	-	-	ncRNA
ATTTTTTT	-	-	INDEL
T	-	-	INDEL

GA	-	-	INDEL
CTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT	-	-	INDEL
CTTTTTTTTTTT	-	-	INDEL
CTTTTTTTTTTT	-	-	INDEL
CT,CTTTTTTTTTTTTTTTTTTTTTTTTTTT	-	-	INDEL
ATTTTTTTTTTTTTTTT	-	-	INDEL
CTTTTTTTTTTTTTTTTTTTTTTTTTTTTT	-	-	INDEL
T	-	-	ncRNA
T	-	-	ncRNA
T	-	-	ncRNA
ATTTTTTTTTTTTTTTTTTTTTTTTTTTTT	-	-	INDEL
T	-	-	five_prime_UTR
AAC	N	N	SYN
A	-	-	three_prime_UTR
A	-	-	three_prime_UTR
A	-	-	five_prime_UTR
GAA	K	E	NONSYN
TAA	-	-	INDEL
CCA	-	-	INDEL
T	-	-	five_prime_UTR
CTTTTTTT	-	-	INDEL
GAAAAAAAAAAAAAA	-	-	INDEL
GAAAAAAAAAAAAAA	-	-	INDEL
T	-	-	ncRNA
T	-	-	five_prime_UTR
T	-	-	five_prime_UTR
CTTTTTTTTTTTTTTTTTTTTTTTTTTTTT	-	-	INDEL
T	-	-	three_prime_UTR
T	-	-	three_prime_UTR
A	-	-	ncRNA
A	-	-	five_prime_UTR
CTA	L	L	SYN
CAT	H	H	SYN
TTTATGTC	-	-	INDEL
CGT	G	R	NONSYN
ATTTTT	-	-	INDEL
ATTTTT	-	-	INDEL
ATTTTT	-	-	INDEL
T	-	-	INDEL
T	-	-	INDEL
T	-	-	INDEL
C	-	-	three_prime_UTR
T	-	-	three_prime_UTR
CTC	L	L	SYN

C	-	-	INDEL
ACT	S	T	NONSYN
TCA	S	S	SYN
CCG	P	P	SYN
AGT	S	S	SYN
CTT	L	L	SYN
ACG	T	T	SYN
CGC	P	R	NONSYN
CTTTTTTTTTTTTTTTTTTTTTTTATTTTTT	-	-	INDEL
CTTTTTTTTTTTTTTTTTTTTTTTATTTTTT	-	-	INDEL
T	-	-	ncRNA
ATTTTTTTTTTTT	-	-	INDEL
T	-	-	INDEL
T	-	-	ncRNA
T	-	-	ncRNA
A	-	-	five_prime_UTR
GAAAAAAAAAAAAAAAAA	-	-	INDEL
GAAAAAAAAAAAAAAAAAAAAAAA	-	-	INDEL
A	-	-	three_prime_UTR
A	-	-	three_prime_UTR
A	-	-	three_prime_UTR
A	-	-	three_prime_UTR
T	-	-	ncRNA
T	-	-	ncRNA
AGTTTTTTTTTTTTTTTTTTTTTTTTTTTT	-	-	INDEL
T	-	-	three_prime_UTR
TAAAAAAAAAAAAAAAAGAAAAAAAA	-	-	INDEL
TAAAAAAAAAAAAAAAAGAAAAAAAA	-	-	INDEL
CAAAAAAAAAAAAAAAAAAAAAAAAAA	-	-	INDEL
CAAAAAAAAAAAAAAAAAAAAAAAAAA	-	-	INDEL
GAAAAAAAAAAAAA	-	-	INDEL
GAAAAAAAAAAAAA	-	-	INDEL
CTTTTTTTTTTTTTTTTTTTTATTTTTTTTT	-	-	INDEL
T	-	-	three_prime_UTR
T	-	-	three_prime_UTR
T	-	-	three_prime_UTR
A	-	-	ncRNA
A	-	-	ncRNA
A	-	-	five_prime_UTR
T	-	-	ncRNA
T	-	-	ncRNA
T	-	-	five_prime_UTR
T	-	-	INDEL
T	-	-	INDEL

T	-	-	ncRNA
T	-	-	five_prime_UTR
CTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT	-	-	INDEL
T	-	-	ncRNA
GGGGGGCTTTTTTTTCTTTTTTTTTTTTTTT	-	-	INDEL
TTTTA	-	-	INDEL
TTTTA	-	-	INDEL
A	-	-	five_prime_UTR
CTTTTTTTTTTTTTTTTTT	-	-	INDEL
T	-	-	three_prime_UTR
T	-	-	five_prime_UTR
A	-	-	three_prime_UTR
A	-	-	ncRNA
A	-	-	three_prime_UTR
T	-	-	three_prime_UTR
T	-	-	ncRNA
T	-	-	ncRNA
GAAAAAAAAAAAAAAAAAAAAAAAAA	-	-	INDEL
GAAAAAAAAAAAAAAAAAAAAAAAAA	-	-	INDEL
A	-	-	five_prime_UTR
A	-	-	five_prime_UTR
CTTTTTTTTTTTTTTTTTT	-	-	INDEL
T	-	-	INDEL
A	-	-	ncRNA
CTTTTTTTTTT	-	-	INDEL
CTTTTTTTTTT	-	-	INDEL
T	-	-	ncRNA
T	-	-	five_prime_UTR
A	-	-	INDEL
A	-	-	INDEL
ACG	T	T	SYN
A	-	-	ncRNA
TCC	T	S	NONSYN
TTC	Y	F	NONSYN
A	-	-	ncRNA
TTA	L	L	SYN
CTT	P	L	NONSYN
A	-	-	ncRNA
CTT	L	L	SYN
TTT	I	F	NONSYN
A	-	-	ncRNA
TTT	L	F	NONSYN
TTT	S	F	NONSYN
T	-	-	INDEL

ACT	S	T	NONSYN
T	-	-	three_prime_UTR
T	-	-	three_prime_UTR
AAT	-	-	INDEL
AAT	-	-	INDEL
A	-	-	ncRNA
A	-	-	five_prime_UTR
T	-	-	three_prime_UTR
T	-	-	five_prime_UTR
GAC	D	D	SYN
C	-	-	INDEL
ATTTTTTTTTTTTTTTTATTTTTTTTTTTTTT	-	-	INDEL
GAAAAAAAA	-	-	INDEL
T	-	-	five_prime_UTR
T	-	-	ncRNA
T	-	-	ncRNA
T	-	-	ncRNA
T	-	-	ncRNA
T	-	-	ncRNA
T	-	-	ncRNA
G	-	-	INDEL
ATTTTTTTT	-	-	INDEL
CTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT	-	-	INDEL
CTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT	-	-	INDEL
T	-	-	ncRNA
T	-	-	three_prime_UTR
T	-	-	ncRNA
T	-	-	ncRNA
T	-	-	ncRNA
T	-	-	ncRNA
T	-	-	ncRNA
T	-	-	ncRNA
T	-	-	ncRNA
ATTT	-	-	INDEL
T	-	-	five_prime_UTR
C	-	-	three_prime_UTR
T	-	-	ncRNA
CTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT	-	-	INDEL
ATTTTTTTTTTTTTTTTTTTTTTTTTTTTTT	-	-	INDEL
CTTTTTTTTTTTTAATTTTT	-	-	INDEL
CTTTTTTTTTTTTAATTTTT	-	-	INDEL
GAAAAAAAAAAAAAAAAAAAAAAAAA	-	-	INDEL
CAAAAAAAAAAAAAAAAAAAGAAAAA	-	-	INDEL
CAAAAAAAAAAAAAAAAAAAAAAAAA	-	-	INDEL

CAAAAAAAAAAAAAAAAAAAAAAAAA	-	-	INDEL
TGT	R	C	NONSYN
C	-	-	INDEL
A	-	-	ncRNA
CTTTTTTTTTTTTTTTGTTT	-	-	INDEL
T	-	-	ncRNA
T	-	-	ncRNA
T	-	-	ncRNA
CAAAAAAAAAAAAAA	-	-	INDEL
T	-	-	ncRNA
T	-	-	ncRNA
A	-	-	ncRNA
A	-	-	ncRNA
CTTTTTTTTTTTTTTTTTTTTTTTTTTTTT	-	-	INDEL
A	-	-	three_prime_UTR
T	-	-	three_prime_UTR
GTTTTTTTTTTTTTTTTTTTTTTTTTTTTT	-	-	INDEL
GTTTTTTTTTTTTTTTTTTTTTTTTTTTTT	-	-	INDEL
GTTTTTTTTTTTTTTTTTTTTTTTTTTTTT	-	-	INDEL
TGT	G	C	NONSYN
GTTTTTTT	-	-	INDEL
AAT	N	N	SYN
T	-	-	INDEL
T	-	-	INDEL
T	-	-	INDEL
TAAAAAAAAAAAAAAAAAAAAAAAAA	-	-	INDEL
T	-	-	three_prime_UTR
T	-	-	ncRNA
A	-	-	three_prime_UTR
A	-	-	five_prime_UTR
AAC	S	N	NONSYN
GT,GTTTTTTT	-	-	INDEL
A,GAAAAAAAAAAAAAAAAAAAAAAAA	-	-	INDEL
A,GAAAAAAAAAAAAAAAAAAAAAAAA	-	-	INDEL
GAAAAAAAAAAAAAAAAAAAAAAAAA	-	-	INDEL
TAAAAAAAAAAAAAAAAAAAAAAAAA	-	-	INDEL
TAAAAAAAAAAAAAAAAAAAAAAAAA	-	-	INDEL
A	-	-	three_prime_UTR
T	-	-	ncRNA
T	-	-	ncRNA
T	-	-	five_prime_UTR
A	-	-	ncRNA
A	-	-	ncRNA
A	-	-	five_prime_UTR

T	-	-	ncRNA
T	-	-	ncRNA
T	-	-	five_prime_UTR
GAAAAAAAAAAAAAAAAAAAAAAAA	-	-	INDEL
T	-	-	three_prime_UTR
ATTTTTTTTTTTTTTTTTTTTTTTTTTTTTT	-	-	INDEL
GTTTTTTTTTTTTTT	-	-	INDEL
T	-	-	INDEL
T	-	-	INDEL
T	-	-	INDEL
T	-	-	ncRNA
ATTTTTTTTTTTTTTTTTTTTTTTTTTTTTT	-	-	INDEL
CTTTTTTTTTTTTTTTTTTT	-	-	INDEL
CAAAAAAAAAAAAAAAAAAAAAAAAA	-	-	INDEL
T	-	-	ncRNA
A	-	-	three_prime_UTR
CAAAAAAAAAAAAAAAAAAAAAAAAA	-	-	INDEL
A	-	-	three_prime_UTR
A	-	-	three_prime_UTR
A	-	-	three_prime_UTR
A	-	-	three_prime_UTR
A	-	-	three_prime_UTR
A	-	-	three_prime_UTR
T	-	-	five_prime_UTR
A	-	-	ncRNA
CTTTTTTTTTTTTTTTTTTTTTATCGTTTT	-	-	INDEL
T	-	-	ncRNA
A	-	-	INDEL
AAA	R	K	NONSYN
A	-	-	five_prime_UTR
CAAAAAAAAAAAAAAAAAAAAAAAAA	-	-	INDEL
CAAAAAAAAAAAAAAAAAAAAAAAAA	-	-	INDEL
CTTTTTTTTTTTTTTTTTTTTTTTTTTTTT	-	-	INDEL
TAAAAAAAAA	-	-	INDEL
A	-	-	three_prime_UTR
A	-	-	three_prime_UTR
A	-	-	three_prime_UTR
A	-	-	three_prime_UTR
A	-	-	three_prime_UTR
A	-	-	five_prime_UTR
C	-	-	INDEL
GCT	T	A	NONSYN
TGT	Y	C	NONSYN
TAAAAAAAAAAAAAAAAAAAAAAAAA	-	-	INDEL

T	-	-	ncRNA
T	-	-	three_prime_UTR
T	-	-	three_prime_UTR
C,AA	-	-	INDEL
A	-	-	three_prime_UTR
C	-	-	ncRNA
GGC	G	G	SYN
TAAAAAAAAAAAAAAAAAAAATTAAAAA	-	-	INDEL
A	-	-	three_prime_UTR
TGT	R	C	NONSYN
T	-	-	three_prime_UTR
CTTTTTTTT	-	-	INDEL
A	-	-	ncRNA
GAT	G	D	NONSYN
AAT	N	N	SYN
T	-	-	ncRNA
T	-	-	five_prime_UTR
T	-	-	ncRNA
TAAAAAAAAAAAAAAAAAAAAAAAAAA	-	-	INDEL
TAAAAAAAAAAAAAAAAAAAAAAAAAA	-	-	INDEL
T	-	-	ncRNA
T	-	-	ncRNA
T	-	-	ncRNA
T	-	-	ncRNA
T	-	-	ncRNA
T	-	-	ncRNA
G	-	-	INDEL
ATTTTTTTT	-	-	INDEL
T	-	-	ncRNA
T	-	-	ncRNA
T	-	-	five_prime_UTR
GTTTTTTTTTTTTTTTTTCCTTTCTTAAAAT	-	-	INDEL
CTTTTTTTTTTTTTTTTTTT	-	-	INDEL
ATTTTTTTTTTTTTTTTTTTTTTTTTTTTTT	-	-	INDEL
CTTTTTTT	-	-	INDEL
A	-	-	ncRNA
A	-	-	five_prime_UTR
A	-	-	ncRNA
CAAAAAAAAAAAAAAAAAAAAAAAAA	-	-	INDEL
GAA	K	E	NONSYN
TAA	-	-	INDEL
CCA	-	-	INDEL
CT	-	-	INDEL
CTTTTTTT	-	-	INDEL

CAAAAAAAAAAAAA	-	-	INDEL
CAAAAAAAAAAAAA	-	-	INDEL
T	-	-	three_prime_UTR
T	-	-	ncRNA
T	-	-	ncRNA
T	-	-	ncRNA
T	-	-	ncRNA
A	-	-	ncRNA
AAA	R	K	NONSYN
A	-	-	five_prime_UTR
A	-	-	three_prime_UTR
ACCCAAAAAAAAAAAAACAAAAAAAAA/	-	-	INDEL
ACCCAAAAAAAAAAAAACAAAAAAAAA/	-	-	INDEL
CTTTTTTTTTTTTTTTTTTTTTTTTTTTT	-	-	INDEL
T	-	-	INDEL
CACATCCACACCAATTACTAGTTCAACTG	-	-	INDEL
CAACTGCTT	-	-	INDEL
TTCTACACCAATTACTA	-	-	INDEL
CTG	L	L	SYN
ACT	S	T	NONSYN
TCA	S	S	SYN
AGA	S	R	NONSYN
ACT	S	T	NONSYN
CCA	S	P	NONSYN
CTT	L	L	SYN
ACG	T	T	SYN
CTTTTTTTTTTTTTTTTTTTTTTTAATTTT	-	-	INDEL
CTTTTTTTTTTTTTTTTTTTTTTTAATTTT	-	-	INDEL
TAAAAAAAAAAAAAAAAAAAAAAAAA/	-	-	INDEL
T	-	-	five_prime_UTR
T	-	-	five_prime_UTR
G	-	-	three_prime_UTR
A	-	-	five_prime_UTR
AAC	S	N	NONSYN
CAAA	-	-	INDEL
A	-	-	five_prime_UTR
A	-	-	five_prime_UTR
A	-	-	five_prime_UTR
A	-	-	five_prime_UTR
ATTTTTTTTTTTTTTTTTTTTTTTTTTTT	-	-	INDEL
CTTTTTTTTTTTTTTTTTTTTTTTTATTTT	-	-	INDEL
CTTTTTTTTTTTTTTTTTTTTTTTTATTTT	-	-	INDEL
T	-	-	ncRNA
T	-	-	three_prime_UTR

ATTTTTTTTTTTTTTTTTTTTTTT	-	-	INDEL
ATTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT	-	-	INDEL
ATTTTTTTTTTTTT	-	-	INDEL
CCTTTTTTTTTTTTTT	-	-	INDEL
CTTTTTTTTTTTTTTTTT	-	-	INDEL
T	-	-	INDEL
T	-	-	ncRNA
CTTTTTTTTTTTTTTTTTATTTATTTTTTTT	-	-	INDEL
CTTTTTTTTTTTTTTTTTATTTATTTTTTTT	-	-	INDEL
A	-	-	five_prime_UTR
A	-	-	five_prime_UTR
A	-	-	five_prime_UTR
A	-	-	five_prime_UTR
A	-	-	five_prime_UTR
A	-	-	five_prime_UTR
A	-	-	five_prime_UTR
G	-	-	ncRNA
CAAAAAAAAAAA	-	-	INDEL
CAAAAAAAAAAA	-	-	INDEL
T	-	-	five_prime_UTR
T	-	-	five_prime_UTR
GAAAAAAAAAAAAAAAAAAAAAAAAAA	-	-	INDEL
GAAAAAAAAAAAAAAAAAAAAAAAAAA	-	-	INDEL
A	-	-	ncRNA
A	-	-	three_prime_UTR
A	-	-	three_prime_UTR
CTTTTTT	-	-	INDEL
CT	-	-	INDEL
TAAAAAAAAA	-	-	INDEL
A	-	-	three_prime_UTR
CTA	L	L	SYN
CAT	H	H	SYN
CTTTTTTTTTTTTTTTTTTTTTTT	-	-	INDEL
AGA	S	R	NONSYN
ACT	S	T	NONSYN
C	-	-	INDEL
ACT	S	T	NONSYN
GTA	A	V	NONSYN
TAAAAAAAAAAAAAAAAAAAAAAAA	-	-	INDEL
T	-	-	five_prime_UTR
T	-	-	five_prime_UTR
T	-	-	three_prime_UTR
C,AA	-	-	INDEL
T	-	-	ncRNA

T	-	-	ncRNA
GAAAAAAAAAAAAAAAAAAAA	-	-	INDEL
A	-	-	three_prime_UTR

plicates of S elf1Δ strains.

Compared Strains
P (A) vs. S (A1 a and b)
P (A) vs. S (A1 a and b)
P (A) vs. S (A1 a and b)
P (A) vs. S (A1 a and b)
P (A) vs. S (A1 a and b)
P (A) vs. S (A1 a and b)
P (A) vs. S (A1 a and b)
P (A) vs. S (A1 a and b)
P (A) vs. S (A1 a and b)
P (A) vs. S (A1 a and b)
P (A) vs. S (A1 a and b)
P (A) vs. S (A1 a and b)
P (A) vs. S (A1 a and b)
P (A) vs. S (A1 a and b)
P (A) vs. S (A1a)
P (A) vs. S (A1a)
P (A) vs. S (A1a)
P (A) vs. S (A1a)
P (A) vs. S (A1a)
P (A) vs. S (A1a)
P (A) vs. S (A1a)
P (A) vs. S (A1a)
P (A) vs. S (A1a)
P (A) vs. S (A1a)
P (A) vs. S (A1a)
P (A) vs. S (A1a)
P (A) vs. S (A1a)
P (A) vs. S (A1a)
P (A) vs. S (A1a)
P (A) vs. S (A1a)
P (A) vs. S (A1a)
P (A) vs. S (A1a)
P (A) vs. S (A1a)
P (A) vs. S (A1a)
P (A) vs. S (A1a)
P (A) vs. S (A1a)
P (A) vs. S (A1a)
P (A) vs. S (A1a)
P (A) vs. S (A1a)
P (A) vs. S (A1a)
P (A) vs. S (A1a)

P (A) vs. S (A1a)
P (A) vs. S (A1a)
P (A) vs. S (A1a)
P (A) vs. S (A1a)
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P (A) vs. S (A1b)
P (A) vs. S (A2 a and b)
P (A) vs. S (A2 a and b)
P (A) vs. S (A2 a and b)
P (A) vs. S (A2 a and b)
P (A) vs. S (A2 a and b)
P (A) vs. S (A2 a and b)
P (A) vs. S (A2a)
P (A) vs. S (A2a)
P (A) vs. S (A2a)
P (A) vs. S (A2a)
P (A) vs. S (A2a)
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P (A) vs. S (A3 a and b)
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P (A) vs. S (A3 a and b)
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P (A) vs. S (A3b)
P (A) vs. S (A3b)
P (A) vs. S (A3b)
P (B) vs. S (B1 a and b)
P (B) vs. S (B1 a and b)
P (B) vs. S (B1 a and b)
P (B) vs. S (B1 a and b)
P (B) vs. S (B1 a and b)
P (B) vs. S (B1 a and b)
P (B) vs. S (B1 a and b)
P (B) vs. S (B1 a and b)
P (B) vs. S (B1a)
P (B) vs. S (B1a)
P (B) vs. S (B1a)
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P (B) vs. S (B2b)
P (B) vs. S (B2b)
P (B) vs. S (B2b)

Chromosome	Position	Gene ID	Coding/Exon ID	Original Sequence
II	413473	transcript:SPBC106.18.1	-	A
II	413474	transcript:SPBC106.18.1	-	C
II	465486	transcript:SPBC428.11.1	-	C
III	1302685	transcript:SPCC1322.08.1	-	C
II	1389165	transcript:SPBC8D2.16c.1	-	A
III	1529703	transcript:SPCC584.01c.1	-	G
I	2327021	transcript:SPAC6C3.03c.1	-	C
I	3160028	transcript:SPAC3G9.11c.1	-	G
I	3160028	transcript:SPAC3G9.11c.1	-	G
I	3160028	transcript:SPAC3G9.11c.1	-	G
I	3160036	transcript:SPAC3G9.11c.1	-	G
I	3160036	transcript:SPAC3G9.11c.1	-	G
I	3160037	transcript:SPAC3G9.11c.1	-	A
I	3160037	transcript:SPAC3G9.11c.1	-	A
I	3160038	transcript:SPAC3G9.11c.1	-	C
I	3160038	transcript:SPAC3G9.11c.1	-	C
I	3160041	transcript:SPAC3G9.11c.1	-	G
I	3160041	transcript:SPAC3G9.11c.1	-	G
I	3160042	transcript:SPAC3G9.11c.1	-	C
I	3160042	transcript:SPAC3G9.11c.1	-	C
I	3477396	transcript:SPAC328.02.1	-	CA
III	180262	transcript:SPCC1235.03.1	CDS:SPCC1235.03.1:pep	CCGATC
I	3477396	transcript:SPAC328.02.1	-	CA
I	3645117	transcript:SPAC8C9.03.1	-	C
II	3883700	transcript:SPBC211.05.1	-	A
II	3883700	transcript:SPBC211.06.1	-	A
I	3993810	transcript:SPAPB15E9.02c.1	-	T
I	3993810	transcript:SPAPB15E9.06.1	-	T
II	4165206	transcript:SPBC14F5.06.1	CDS:SPBC14F5.06.1:pep	G
III	1939467	-	gene:SPNCRNA.1245	G
II	1952886	-	gene:SPNCRNA.1481	A
I	2327021	-	gene:SPNCRNA.813	C
III	1939467	transcript:SPCC74.05.1	CDS:SPCC74.05.1:pep	GGT
II	692301	transcript:SPBPJ4664.02.1	CDS:SPBPJ4664.02.1:pep	GTT
II	692301	transcript:SPBPJ4664.02.1	CDS:SPBPJ4664.02.1:pep	GTT
II	670961	transcript:SPBC947.04.1	CDS:SPBC947.04.1:pep	GCA
II	1045183	transcript:SPBC337.08c.1	CDS:SPBC337.08c.1:pep	GAT
II	692303	transcript:SPBPJ4664.02.1	CDS:SPBPJ4664.02.1:pep	CTG
II	692303	transcript:SPBPJ4664.02.1	CDS:SPBPJ4664.02.1:pep	CTG
II	694252	transcript:SPBPJ4664.02.1	CDS:SPBPJ4664.02.1:pep	AAC
II	693499	transcript:SPBPJ4664.02.1	CDS:SPBPJ4664.02.1:pep	AGC

II	670967	transcript:SPBC947.04.1	CDS:SPBC947.04.1:pep	TAT
I	1905516	transcript:SPAC110.05.1	-	C
I	3645128	transcript:SPAC8C9.03.1	-	A

Altered Sequence	Original Amino Acid	New Amino Acid	Type of Change	Compared Strains
T	-	-	five_prime_UTR	P (A) vs. S (A1 a and b)
T	-	-	five_prime_UTR	P (A) vs. S (A1 a and b)
T	-	-	five_prime_UTR	P (A) vs. S (A3 a and b)
T	-	-	five_prime_UTR	P (B) vs. S (B1 a and b)
T	-	-	five_prime_UTR	P (A) vs. S (A3 a and b)
A	-	-	five_prime_UTR	P (A) vs. S (A3 a and b)
A	-	-	five_prime_UTR	P (B) vs. S (B1 a and b)
T	-	-	five_prime_UTR	P (A) vs. S (A1 a and b)
T	-	-	five_prime_UTR	P (A) vs. S (A2 a and b)
T	-	-	five_prime_UTR	P (A) vs. S (A3 a and b)
T	-	-	five_prime_UTR	P (A) vs. S (A1 a and b)
T	-	-	five_prime_UTR	P (A) vs. S (A3 a and b)
T	-	-	five_prime_UTR	P (A) vs. S (A1 a and b)
T	-	-	five_prime_UTR	P (A) vs. S (A3 a and b)
T	-	-	five_prime_UTR	P (A) vs. S (A1 a and b)
T	-	-	five_prime_UTR	P (A) vs. S (A3 a and b)
T	-	-	five_prime_UTR	P (A) vs. S (A1 a and b)
T	-	-	five_prime_UTR	P (A) vs. S (A3 a and b)
C	-	-	INDEL	P (A) vs. S (A1 a and b)
C	-	-	INDEL	P (B) vs. S (B1 a and b)
C	-	-	INDEL	P (A) vs. S (A3 a and b)
CTTTTTT	-	-	INDEL	P (B) vs. S (B2 a and b)
AAT	-	-	INDEL	P (B) vs. S (B1 a and b)
AAT	-	-	INDEL	P (B) vs. S (B1 a and b)
TAAAAA	-	-	INDEL	P (A) vs. S (A2 a and b)
TAAAAA	-	-	INDEL	P (A) vs. S (A2 a and b)
GTGTAT	-	-	INDEL	P (A) vs. S (A3 a and b)
A	-	-	ncRNA	P (B) vs. S (B2 a and b)
T	-	-	ncRNA	P (A) vs. S (A1 a and b)
A	-	-	ncRNA	P (B) vs. S (B1 a and b)
GAT	G	D	NONSYN	P (B) vs. S (B2 a and b)
GCT	V	A	NONSYN	P (A) vs. S (A1 a and b)
GCT	V	A	NONSYN	P (A) vs. S (A2 a and b)
GCT	A	A	SYN	P (A) vs. S (A2 a and b)
GAC	D	D	SYN	P (B) vs. S (B1 a and b)
TTG	L	L	SYN	P (A) vs. S (A1 a and b)
TTG	L	L	SYN	P (A) vs. S (A2 a and b)
AAT	N	N	SYN	P (B) vs. S (B2 a and b)
AGT	S	S	SYN	P (A) vs. S (A1 a and b)

TAC	Y	Y	SYN	P (A) vs. S (A1 a and b)
T	-	-	three_prime_UTR	P (B) vs. S (B1 a and b)
T	-	-	three_prime_UTR	P (B) vs. S (B2 a and b)

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Chromosome	Position	Gene ID	gene name
I	3477396	transcript:SPAC328.02.1	dbl4
III	180262	transcript:SPCC1235.03.1	cue2
I	3477396	transcript:SPAC328.02.1	dbl4
I	3645117	transcript:SPAC8C9.03.1	cgs1
II	3883700	transcript:SPBC211.05.1	sap10
II	3883700	transcript:SPBC211.06.1	sap10
I	3993810	transcript:SPAPB15E9.02c.1	dubious
I	3993810	transcript:SPAPB15E9.06.1	dubious
II	4165206	transcript:SPBC14F5.06.1	rli1
III	1939467	transcript:SPCC74.05.1	rpl2702
II	692301	transcript:SPBPJ4664.02.1	unkown glycoprotein
II	692301	transcript:SPBPJ4664.02.1	unkown glycoprotein

Coding/Exon ID	Original Sequence	Altered Sequence	Original Amino Acid	New Amino Acid	Type of Change
-	CA	C	-	-	INDEL
CDS:SPCC1235.03.1:pep	CCGATC	C	-	-	INDEL
-	CA	C	-	-	INDEL
-	C	CTTTTTT	-	-	INDEL
-	A	AAT	-	-	INDEL
-	A	AAT	-	-	INDEL
-	T	TAAAAA	-	-	INDEL
-	T	TAAAAA	-	-	INDEL
CDS:SPBC14F5.06.1:pep	G	GTGTAT	-	-	INDEL
CDS:SPCC74.05.1:pep	GGT	GAT	G	D	NONSYN
CDS:SPBPJ4664.02.1:pep	GTT	GCT	V	A	NONSYN
CDS:SPBPJ4664.02.1:pep	GTT	GCT	V	A	NONSYN

Compared Strains

P (A) vs. S (A1 a and b)
P (B) vs. S (B1 a and b)
P (A) vs. S (A3 a and b)
P (B) vs. S (B2 a and b)
P (B) vs. S (B1 a and b)
P (B) vs. S (B1 a and b)
P (A) vs. S (A2 a and b)
P (A) vs. S (A2 a and b)
P (A) vs. S (A3 a and b)
P (B) vs. S (B2 a and b)
P (A) vs. S (A1 a and b)
P (A) vs. S (A2 a and b)

Chromosome	Position	Gene ID	
II	4165206	transcript:SPBC14F5.06.1	rli1
III	180262	transcript:SPCC1235.03.1	cue2
III	1939467	transcript:SPCC74.05.1	rpl2702
II	692301	transcript:SPBPJ4664.02.1	unknown glycoprotein
II	692301	transcript:SPBPJ4664.02.1	unknown glycoprotein

Coding/Exon ID	Original Sequence	Altered Sequence	Original Amino Acid	New Amino Acid
CDS:SPBC14F5.06.1:pep	G	GTGTATTGAA	-	-
CDS:SPCC1235.03.1:pep	CCGATCTCTTGCTATG	C	-	-
CDS:SPCC74.05.1:pep	GGT	GAT	G	D
CDS:SPBPJ4664.02.1:pep	GTT	GCT	V	A
CDS:SPBPJ4664.02.1:pep	GTT	GCT	V	A

Type of Change	Compared Strains
INDEL	P (A) vs. S (A3 a and b)
INDEL	P (B) vs. S (B1 a and b)
NONSYN	P (B) vs. S (B2 a and b)
NONSYN	P (A) vs. S (A1 a and b)
NONSYN	P (A) vs. S (A2 a and b)

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