# Evolutionary Rescue of Human Disease Mutations

Brooke Dubyna[1], Ryan Vignogna1, Ethan Perlstein2, Gregory Lang1

1Department of Biological Sciences, Lehigh University, Bethlehem PA
2 Perlara PBC, Berkeley, CA

## Abstract

Several thousand human disease-associated alleles have clear yeast orthologs, making yeast an ideal model system for advancing a basic understanding of human disease.

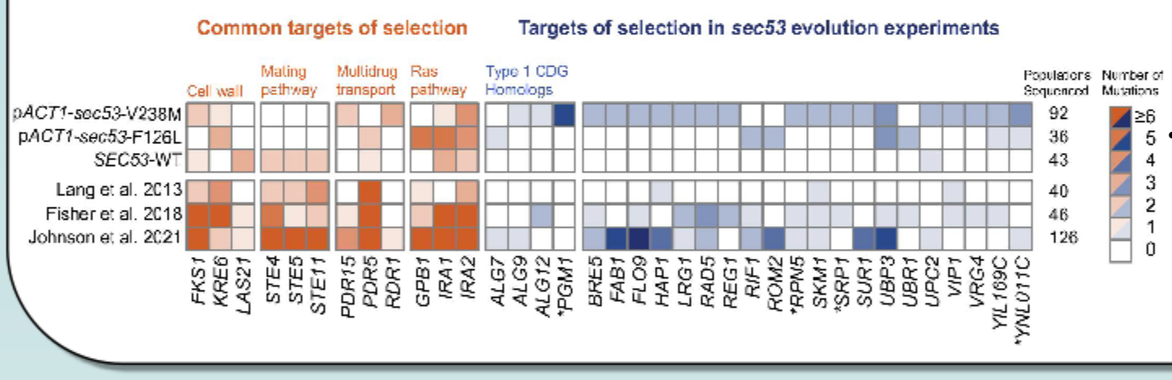
We used CRISPR/Cas9 editing to generate 51 yeast strains with 11 human disease-associated variants, focusing on human disease genes involved in protein glycosylation, ribosome maturation, mitochondrial function, and RNA regulation. We find that most of these alleles recapitulate as fitness defects in yeast. To enrich for suppressors, we evolved these populations for 1,000 generations and performed whole genome sequencing. We identified both known and previously unknown suppressor mutations. For example, we identify mutations in *DPM1* and *RPS9B* that compensate for disease phenotypes of *ALG7* and *SDO1*, respectively. Reconstructed strains harboring evolved suppressor mutations and the original disease alleles demonstrate that these suppressors rescue fitness and may compensate for defects in molecular function. By exerting continuous selective pressure over thousands of generations, experimental evolution allows us to select for suppressor mutations, including those with modest fitness effects or occurring at lower frequencies, which are underrepresented in traditional genetic screens.

Our results will map local genetic interaction networks centered around disease-associated alleles, providing greater insights into disease phenotypes and informing potential targeted therapies for disease management in patients with rare disorders.

## Background

We use experimental evolution to identify mutations that compensate for human disease-associated alleles.

| Human Variant | Yeast Equivalent | Effect | Relative Enzyme Activity | Relative Growth Rate p*SEC53* (1x) | Relative Growth Rate p*ACT1* (2x) |
|---|---|---|---|---|---|
| *PMM2* | *SEC53* | Wild-type | 1.00 | 1.00 | 1.00 |
| *pmm2*-V231M | *sec53*-V238M | Decreased Stability | 0.39 | 0.32 | 0.67 |
| *pmm2*-F119L | *sec53*-F126L | Reduced Dimerization | 0.25 | 0.27 | 0.57 |



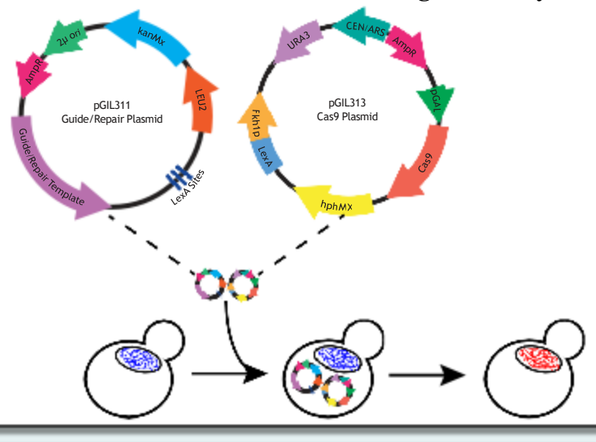

- Previous work in our lab focused on two disease-associated alleles of *PMM2*.
- We evolved the mutant strains for 1,000 generations and performed whole genome sequencing.
- We found that mutations in *PGM1* compensate for slow growth and partially restore glycosylation defects.

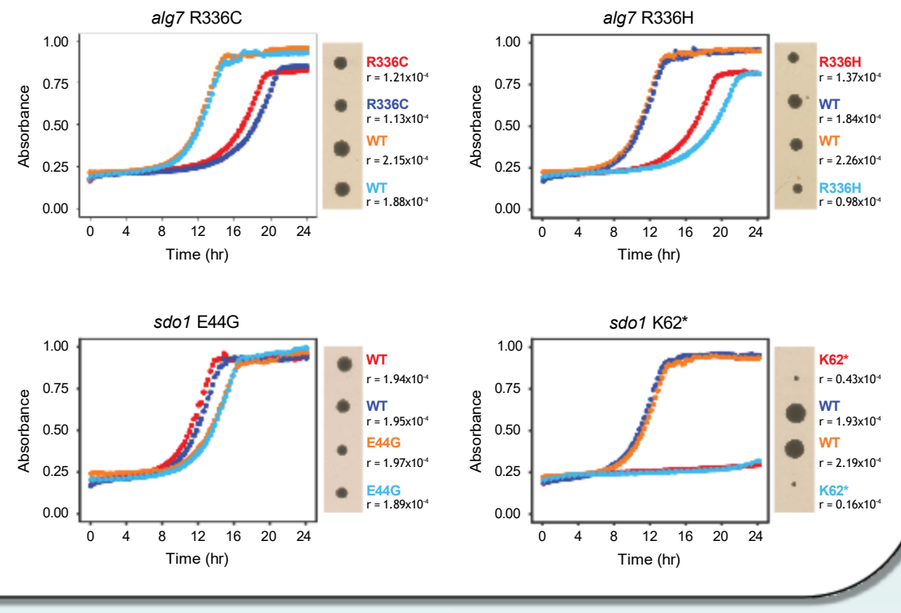
## Construction of yeast strains containing human disease-associated alleles

| Human Disease | Human Gene | Yeast Ortholog | Human Variant | Yeast Equivalent | Biological Process |
|---|---|---|---|---|---|
| Congenital disorder of glycosylation, type Ij | *DPAGT1* | *ALG7* | Y140C<br>R301C<br>R301H | Y171C<br>R336C<br>R336H | Protein N-linked glycosylation |
| Complex V deficiency, nuclear type 1 | *ATPAF2* | *ATP12* | W94R | W103R | Mito. ATP synthase complex assembly |
| Pseudouridine synthase deficiency | *PUS3*<br>*PUS7* | *DEG1*<br>*PUS7* | S394Cfs<br>D503Y | *deg1*Δ<br>D478Y | Pseudouridine synthesis |
| Shwachman-Diamond syndrome 1 | *SBDS* | *SDO1* | E44G<br>K62* | E44G<br>K62* | Cytosolic ribosome assembly |
| Leigh syndrome (Complex IV), nuclear type 1 | *SURF1* | *SHY1* | G124E<br>I246T<br>Y274D | G137E<br>F249T<br>Y344D | Mito. respiratory chain complex IV assembly |

- We selected multiple alleles across six genes associated with rare monogenic disorders.
- We transformed yeast with guide/repair and Cas9 plasmids.

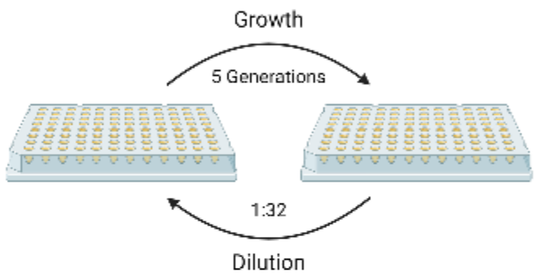
- We constructed 51 yeast strains with human disease-allele replacements.
- Strains included haploids and homozygous, heterozygous, and compound heterozygous diploids.
- Disease-associated alleles cause slow growth in yeast.

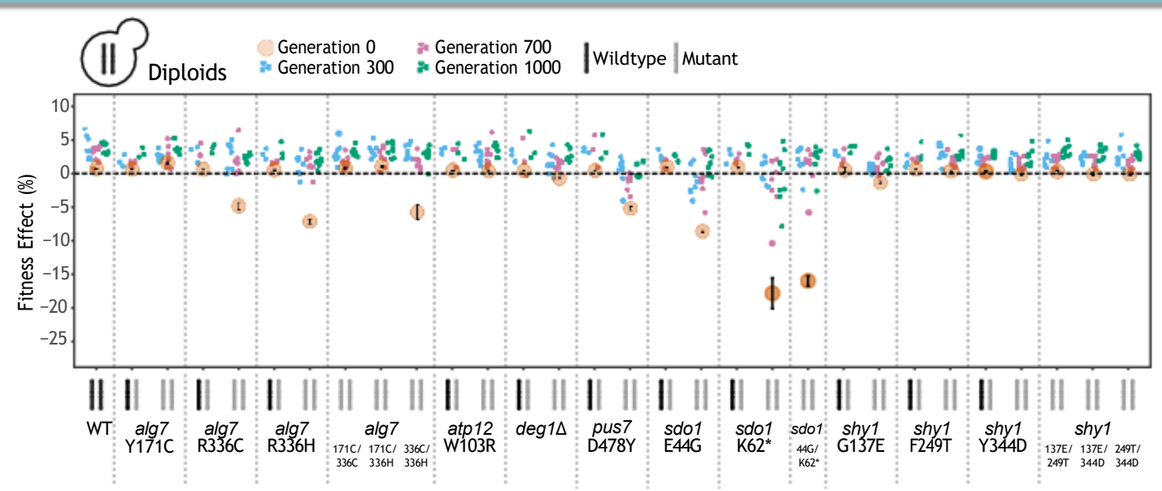



## Compensatory mutations arise over the course of experimental evolution

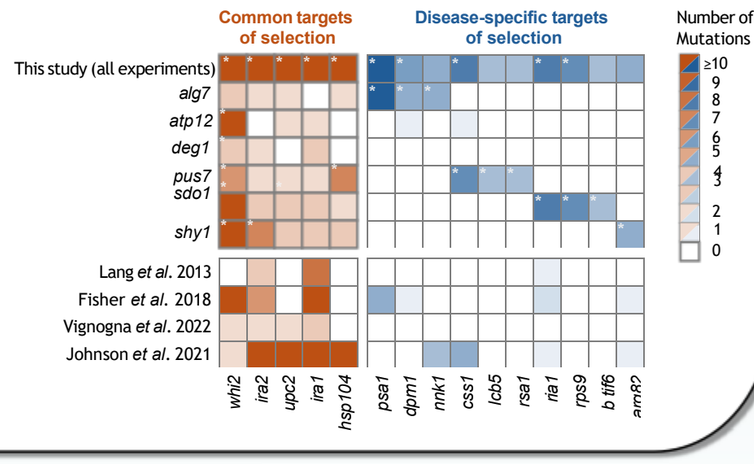
- Experimental evolution allows us to evolve multiple populations in parallel to study adaptation in real time.
- We evolved replicates of each population for 1,000 generations.



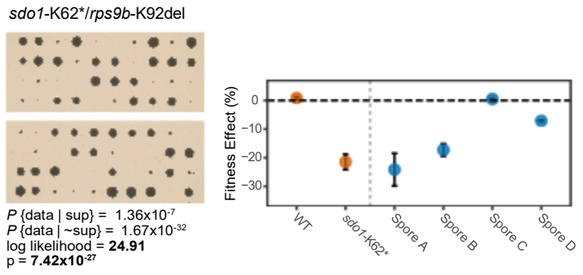

- We measured fitness effects over the course of the evolution experiment using a competitive fitness assay.
- Heterozygotes performed similar to wildtype, consistent with autosomal recessive inheritance.
- Haploids and homozygotes have fitness defects relative to wildtype at Generation 0.
- All strains increased in fitness over 1,000 generations, suggesting that compensatory mutations arose which could overcome deficiencies caused by the disease alleles.

- We isolated single clones from each population and performed whole genome sequencing to identify mutations that arose more often than expected by chance.
- Genes that were commonly mutated in one disease background are putative adaptive targets of selection.
- We tend to observe compensatory mutations occuring in the same or related pathways to their respective ancestral disease-allele.

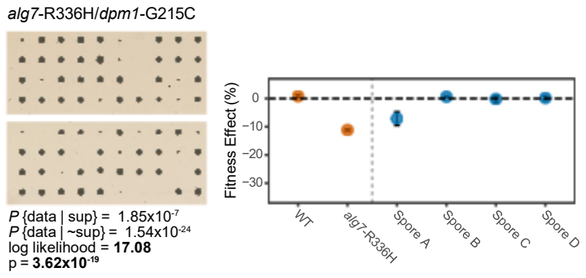



## Compensatory mutations rescue fitness defects

*sdo1*-K62**rps9b*-K92del




*alg7*-R336H/*dpm1*-G215C




| Mating Type | Genotype | Genotype |
|---|---|---|
| *MAT*α | WT/*dpm1*-G215C | WT/*dpm1*-G215C |
| *MAT*α | *alg7*-R336H/WT | *alg7*-R336H/*dpm1*-G215C |
| *MAT***a** | WT/*dpm1*-G215C | WT/WT |
| *MAT***a** | *alg7*-R336H/WT | *alg7*-R336H/WT |



- We reconstructed double mutants harboring both the original mutation and evolved suppressor.
- Tetrad dissections followed a 1:4:1 ratio of segregation patterns, consistent with the evolved mutations acting as suppressors.
- Spores inheriting both alleles displayed increased fitnes.
- Double mutants with *alg7*-R336 and a *DPM1* or *PSA1* suppressor had halos restored to intermediate or wildtype sizes.
- Suppressors could be acting to restore or compensate for molecular defects in glycosylation.

## References and Support

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