# Natural variation in an essential host gene underlies resistance to a parasitic plasmid in budding yeast

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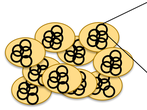
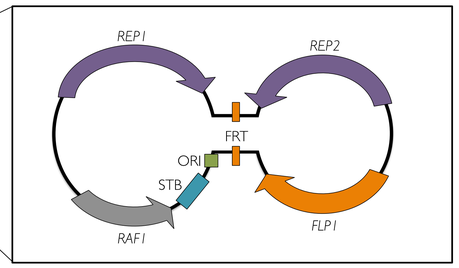
## The 2-micron plasmid is a selfish element

The 2-micron plasmid is a genetic parasite found specifically in budding yeasts. The plasmid must hijack host cellular machinery to accomplish its own replication and segregation during the host cell cycle.

Yeast without the 2-micron plasmid grow 1-3% faster than yeast with the plasmid



~50 copies plasmid per haploid cell




Budding yeast and 2-micron plasmids are a powerful, molecularly tractable system for exploring how a naturally occurring parasite and host interact.

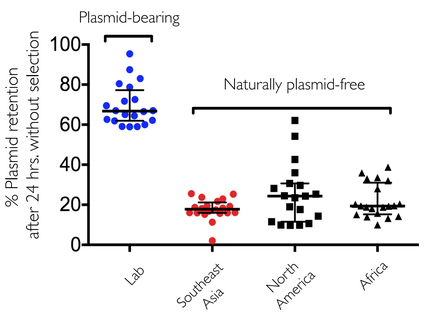
## Heritable restriction of 2-micron in natural yeast strains.

Most *Saccharomyces cerevisiae* strains have the 2-micron, but not all.

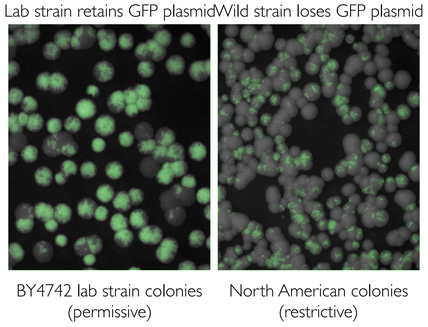
We screened ~60 natural *S. cerevisiae* isolates:
3 strains naturally do not carry the 2-micron plasmid.

When a reporter 2-micron is reintroduced, these strains rapidly, and reproducibly, lose it again.

### Plasmid Loss




### Colony sectoring

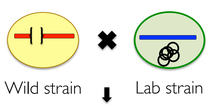



BY4742 lab strain colonies (permissive)

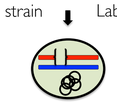
North American colonies (restrictive)

## How are these naturally evolved yeast cells fighting the 2-micron?

It could be due to loss of a host susceptibility factor

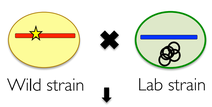



Complementation of missing host factor that plasmid requires



OR

The host could have evolved a restriction factor




Dominant acting restriction factor fights plasmid

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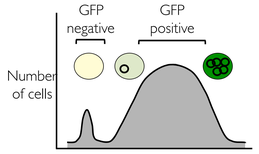
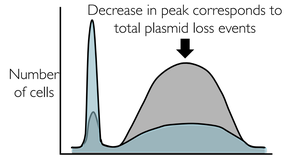
We are using a genetic mapping strategy to determine which genes underlie this plasmid loss trait.

## Developing a high throughput phenotyping assay to facilitate genetic mapping

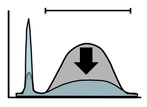


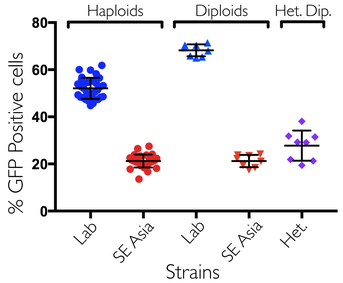




$$\% \text{ Plasmid retention} = \frac{\% \text{ GFP positive cells in non-selection}}{\% \text{ GFP positive cells in selection}}$$

We developed GFP-2-micron reporter assay that allows for single-cell plasmid phenotyping by flow cytometry.

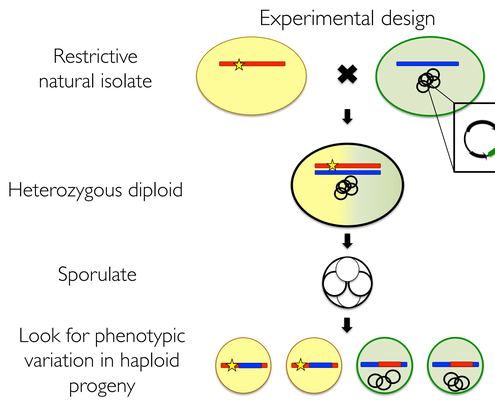
## Rapid plasmid loss is dominant in the heterozygous diploid.






Rapid plasmid loss in the heterozygote is consistent with a dominant acting restriction factor

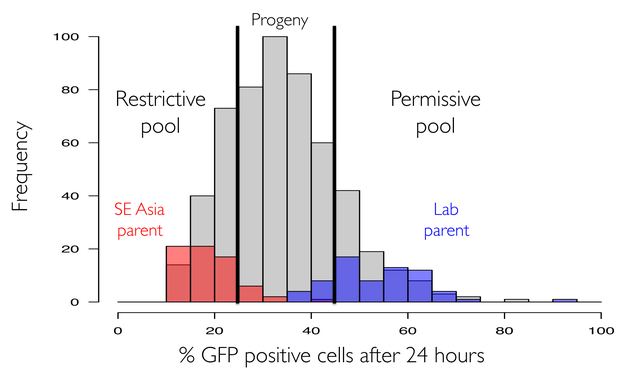
## Identifying plasmid restriction factors by QTL mapping.



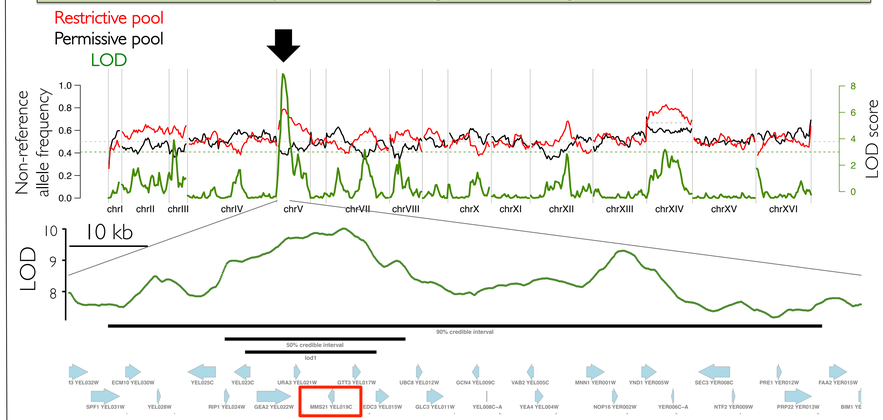

2-micron reporter plasmid: native plasmid sequence plus drug marker and GFP

Use bulk segregant analysis and quantitative trait loci (QTL) mapping to identify genomic regions contributing to plasmid restriction

## Progeny pooled based on parent phenotype distribution.




## QTL analysis identifies genomic region of interest.




| | Whole region | Filtered for SNPs correlated with plasmid presence/ absence in across strains |
|---|---|---|
| 90% Confidence interval | 91kb, 54 genes | 15 SNPs, 11 genes |
| 50% Confidence interval | 23kb, 16 genes | 6 SNPs, 4 genes |

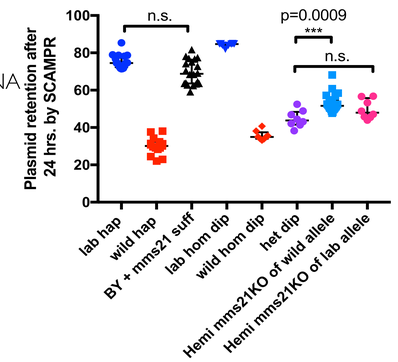
## Candidate genes of interest

MMS21

- Essential SUMO E3 ligase (one of 3 mitotic in yeast)
- SMC5-SMC6 complex member
- Double knockout of 2 other SUMO E3 ligases (siz1/siz2) shows "nibbled" colony phenotype
- Anchors dsDNA breaks to nuclear periphery
- Removal of X-shaped DNA structures during DNA replication and repair
- Facilitates proper repair of repetitive/high copy regions

Ragi *MMS21* underlies a small, but significant, portion of rapid plasmid loss phenotype

**Ragi *MMS21* allele contributes to plasmid loss phenotype**




The Ragi *MMS21* allele alone is not sufficient to confer plasmid resistance, but a hemizygous knockout in the heterozygous diploid background shows ragi *MMS21* is necessary for the full Ragi plasmid-loss phenotype


NTD has helices that are essential for binding SMC5/6, DNA repair and host viability. The RING domain is important for sumoylation, but not essential for the host viability!

## Future directions

- Validation of other variants in the ChrV locus, other genomic loci of interest
- What is the mechanism of plasmid restriction?
- How specifically does the MMS21 variant decrease plasmid stability?
- Do restrictive gene(s) show signs of rapid evolution across yeast?

The 2-micron is a selfish element naturally found in yeast.
Some strains restrict the 2-micron.
We have mapped this trait to a region of Chromosome V.
We are currently validating this region and testing candidate genes.
We hope to understand the mechanism of parasite resistance of these natural yeast isolates.