

LAST TWO WEEKS (11/25 AND 12/02):

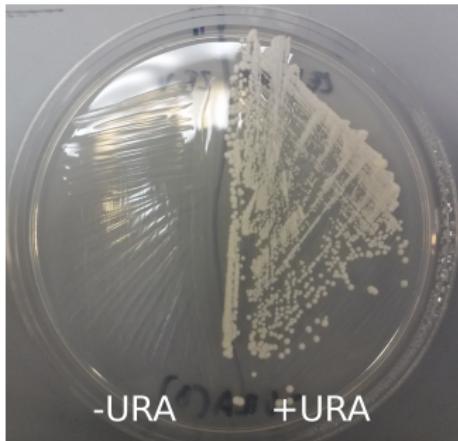


Figure : Test of SC -URA plate

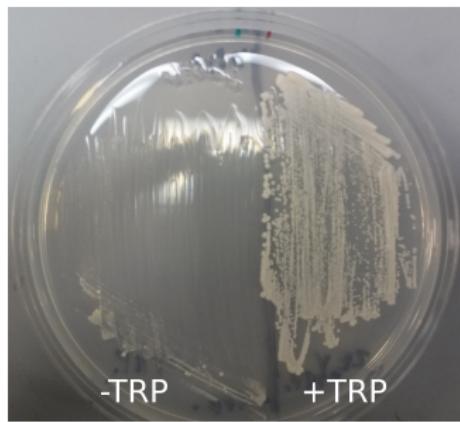
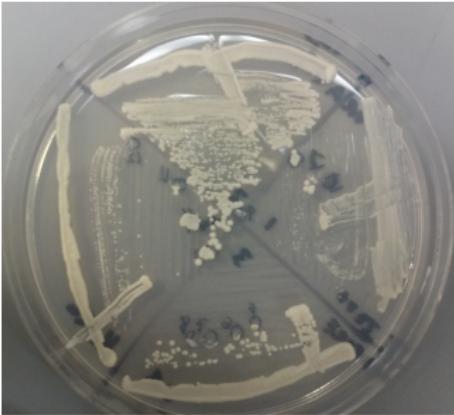
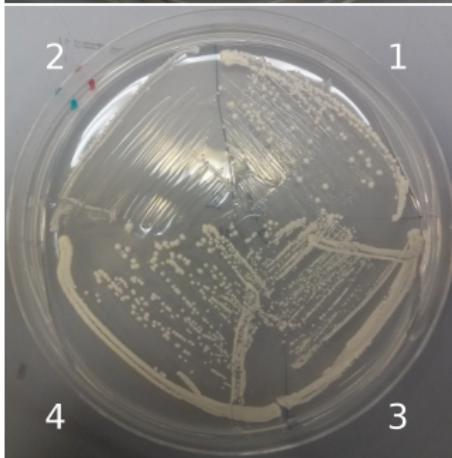
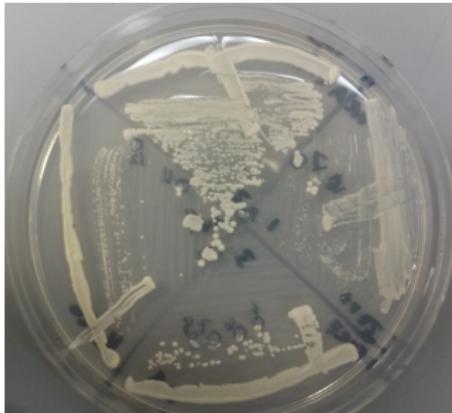


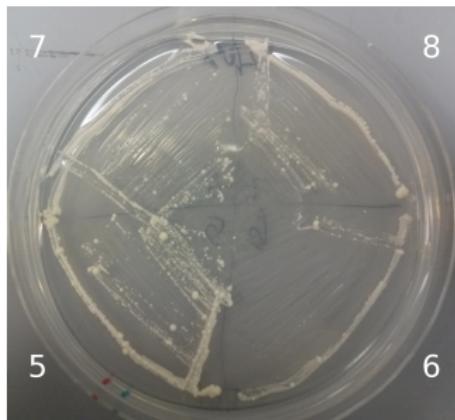
Figure : Test of SC -TRP plate



- ▶ Restreak transformants onto -URA from original G418.

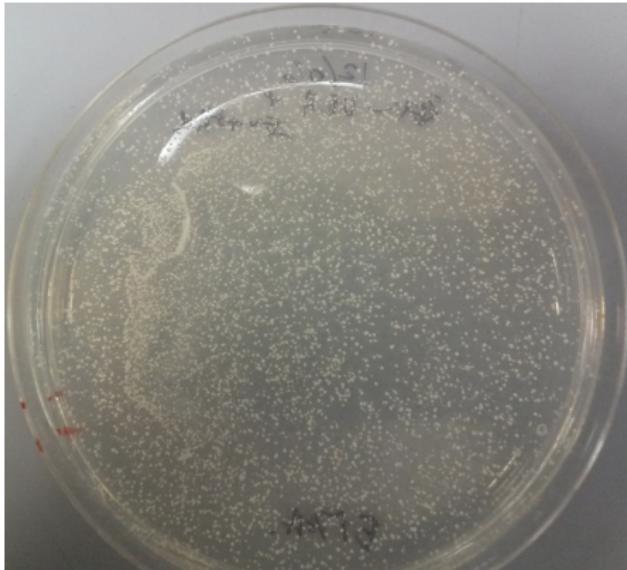


- ▶ Restreak transformants onto -URA from original G418.
- ▶ Pick colonies to streak onto -TRP



- ▶ Possibility: The CORE TRP KO fragment recombined with ClonNAT resistance.
- ▶ Replica plated onto G418+ClonNat; all colonies have G418+ClonNAT resistance.

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- ▶ Replica plated onto G418+ClonNat; all colonies have G418+ClonNAT resistance.
- ▶ Transformation of ZEV yeast w/ FAR + ZEVpr to knockout TRP directly.



PLASMID CONSTRUCTION:

Homologous recombination:

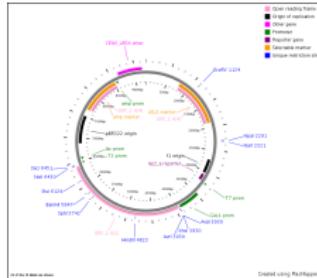


Figure : pGAL + FAR1-22

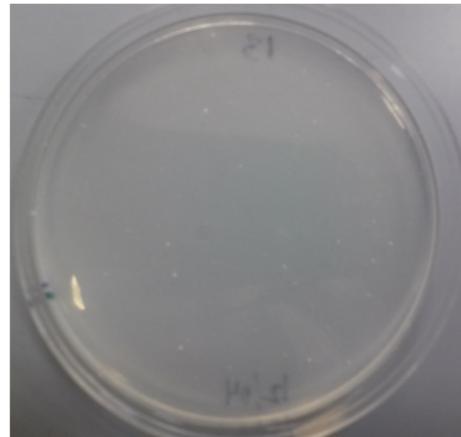
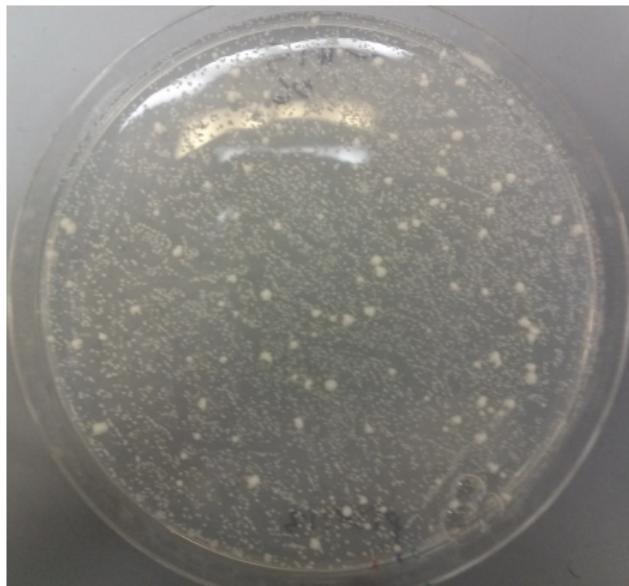


Figure : pGAL + FAR1-WT

- ▶ **Backup:** Transform the plasmid that we know to contain FAR1-22 under the GAL promoter into a *-HIS* strain



THIS WEEK (12/09) AND NEXT?

Test pGAL + FAR1-22:

- ▶ Raffinose starvation.
- ▶ Glucose/Galactose gradient.
- ▶ FACS experiments with DNA dye (against untransformed -HIS strain)

- ▶ Continue construction of pGAL + FAR1-22 and pGAL + FAR1-WT.

Today!

- ▶ Colony PCR colonies from 5-FAA plate to confirm TRP KO. (Will use primers for ZEVpr fragment, which is smaller).
- ▶ PCR of ZEVpr, FAR1-22, and **FAR1-WT** to do genomic construction by 5-FAA again.
- ▶ Set up raffinose starvation tomorrow.
- ▶ Set up galactose/glucose media. Dilutions + plates.