

LAST WEEK (11/11):



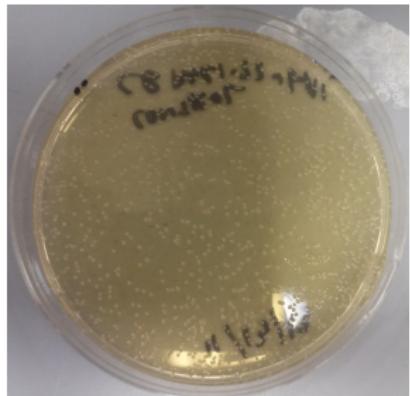
**Figure :** Colony PCR FAR-WT and CORE

- ▶ Did a PCR w/ Genomic DNA on 11/14.

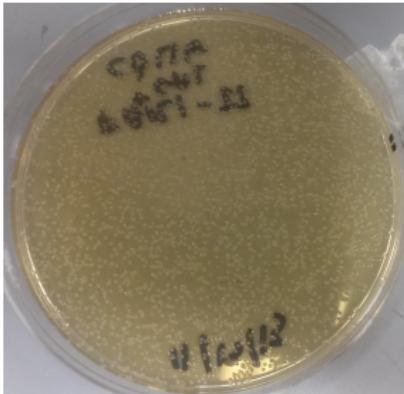
# Ligation for plasmid creation:

Lab Meeting II

Carles Boix



**Figure :** Ligation Control



**Figure :** pMM86 with FAR1-22  
ligation

## STEPS FOR PLASMID CONSTRUCTION

- Miniprep and digest pMM86
- Miniprep pGAL-FAR1-22 (pTCN113)
- PCR amplify FAR1-22 fragment
- Colony PCR amplify FAR1-WT fragment
- Digest both fragments (FAR1-WT)**
- Gel extraction of fragments
- Ligation
- Transformation into bacteria
- Miniprep and sending to sequencing (FAR1-22)**
- Transformation into yeast

# Transformation of ZEV with CORE

Lab Meeting II

Carles Boix

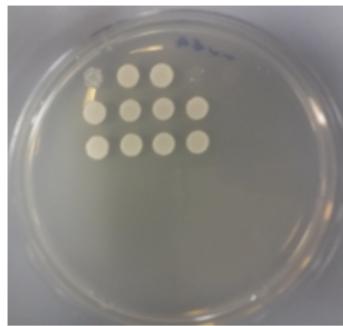


Figure : URA-

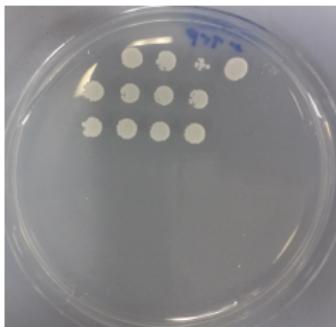


Figure : TRP-

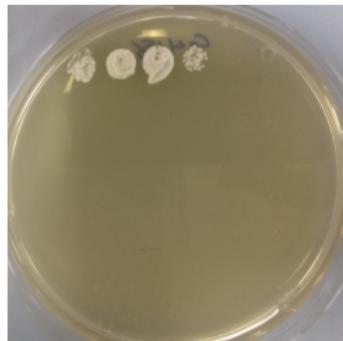


Figure : G418

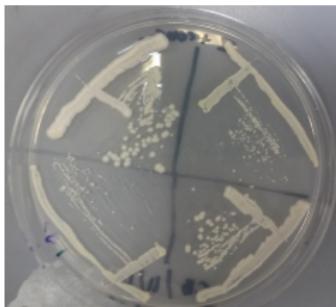


Figure : URA-

## STEPS FOR GENOMIC STRAIN CONSTRUCTION

- Miniprep pMM299 (ZEVpr)
- Miniprep pCORE
- Miniprep pGAL-FAR1-22 (pTCN113)
- PCR amplify CORE fragment
- PCR amplify FAR1-22 fragment
- Colony PCR amplify FAR1-WT fragment
- Colony PCR amplify ZEVpr fragment
- Transform ZEV yeast with CORE
- Transform ZEV+CORE with ZEVpr + FAR fragment**
- Send to sequencing.
- Transform ZEV + FAR with the blue light plasmids

## THIS WEEK (11/18):

### Plasmid Construction:

- ▶ Miniprep of pMM86 + FAR1-22. (pGAL + FAR1-22)
- ▶ Digestion, gel extraction, ligation of FAR1-WT
- ▶ Miniprep + sequencing of FAR1-WT + pMM86.

### Genomic Construction:

- ▶ Transform ZEV + CORE with FAR1-WT/ FAR1-22 and ZEVpr (homologous recombination).

### Controls:

- ▶ Build blue light rig.
- ▶ Transform ZEV strain with the two blue light plasmids.