*Note – Requires 2 incubators are set to 28°C and 22°C, respectively.*

***Prepare strains***

Transform *Agro* strain with pHKJF03 binary vector (see e- poration of Agro protocol).

S/O transformed *Agro* strain on a kanamycin & streptomycin LB plate. Incubate at RT (or 28*°C* for faster growth) and allow ~3 days for singles to form. (binary vector selection – kan; Ti plasmid selection - strep)

Streak yeast strain to be transformed to singles on YPD.

***O/N transformation cultures***

When both *Agro* and yeast singles are ready, inoculate *Agro* into 5ml LB +kan +strep and inoculate yeast into 10 ml YPD. Incubate *Agro* O/N on a 200 rpm shaker @ 28*°C*. Incubate yeast O/N in species optimal conditions (*Hanseniaspora occidentalis* on a RT 250 rpm shaker).

***Transformation AM***

Dilute yeast O/N 1:10 by inoculating into 50 ml YPD. Incubate on shaker until mid-log. (5-6 hours for *S. cerevisiae*  - 10 -12 hours for *H. occidentalis*)

When ~6 hours are remaining in yeast growth, pellet 2ml of *Agro* culture for 3 min @ 15,000g.

Remove supernatant. Wash the pellet with 1 ml of freshly prepared IM media. Resuspend in 200 ul IM media.

Add 5 ul AS solution to 5ml IM media.

Transfer all 200 ul *Agro* cells to 5 ml IM +AS media. Incubate for 5-6 hours on a shaker @ 28*°C*.

***Transformation PM***

Harvest yeast culture(s) in 50ml Falcon tubes. Spin @ 3,000g for 5 min.

Pour off supernatant and wash in 25 ml IM media. Resuspend in 500 ul IM media.

Prep an IM +AS plate by laying 4-5 rectangular pieces of sterile filter paper on top.

Transfer 60 ul of *Agro* culture into a 1.5 ml tube. Add 60 ul of yeast slurry. Pipette up and down to mix. Repeat for each piece of filter paper to be inoculated.

Spot 100ul of *Agro-*yeast cocktail to each piece of filter paper.

Incubate plate on bench top for 3 to 9 days.

***Recovering transformants***

Use forceps to transfer each piece of filter paper to the bottom of a sterile 50 ml Falcon tube.

Add 2ml .9% NaCl sol’n to each tube. Set tube on an angle to soak the filter paper the sol’n.

Vortex vigorously to resuspend cells.

Prepare a 1:50 and 10-5 dilution for each sample. Plate the following and incubate 3-5 days.

|  |  |  |  |
| --- | --- | --- | --- |
| **Media** | **Volume** | **Dilution** | **Selects for** |
| YPD +cefo | 100ul | 10-5 | Yeast cfus |
| YPD +cefo +G418 | 200ul | 1:50 | Transformants |
| LB +rif | 100ul | 10-5 |  |

***Media & Reagents***

*Sol’n “K” Sol’n “MN” Ca+ Sol’n Microspores*

17 g KH2PO4 6 g MgSO4 500 mgCaCl2 10 mg Na2MoO4

21.75 g K2HPO4 3 g NaCl 50 ml H2O 10 mg MnSO4

100 ml H2O 200 ml H2O 10 mg ZnSO4

10 mg CuSO4

*Fe+ Sol’n NH4 Sol’n MES* 10 mg H3BO3

10 mg FeSO4 20 g NH4NO3 97.6 g MES 100 ml H2O

100 ml H2O 100 ml H2O HCl to pH 5.5

500 ml H2O

*AS sol’n*

392.38 mg Acetosyringone

10 ml DMSO

*Induction Media*

*IM broth*  *IM agar*

0.4 ml sol’n “K” 0.4 ml sol’n “K”

10 ml sol’n “MN” 10 ml sol’n “MN”

2.5 ml Microspores 2.5 ml Microspores

5 ml Fe+ sol’n 5 ml Fe+ sol’n

0.5 ml Ca+ sol’n 0.5 ml Ca+ sol’n

1.25 ml NH4 sol’n 1.25 ml NH4 sol’n

20 ml MES 20 ml MES

2.85 ml 87% glycerol 2.85 ml 87% glycerol

5 ml glucose 2.5 ml glucose

452.5 ml H2O 9 g agar

452.5 ml H2O

500 ul AS after autoclave