

Protocol for Transformation *Candida albicans*.

For the transformation, inoculate 5ml YPD + uridine with BWP17 strain and grow overnight at 30oC.

In a sterile flask, add 30 ml of YPD + uridine and inoculate with 300 ul of BWP17 overnight culture and grow at 30oC for 4 hours. **DON'T GO OVER 4.5HOURS OR TRANSFORMATION EFFICIENCY DROPS.**

Pellet 30 ml of cells 1700 rpm X 5 min. in the Eppendorf table top centrifuge. **REMEMBER TO USE BALANCE TUBE.**

Resuspend cells in 5ml of TE (BIO101 kit) to wash and transfer to 15 ml conical tube.

Pellet in centrifuge 1700rpm X 5 min **REMEMBER THE BALANCE TUBE..**

Gently resuspend cells in 3 ml of lithium/cesium acetate solution (BIO101 kit) and incubate at 30oC for 30 min on the roller drum.

Pellet cells 1700rpm X 5min.

Gently resuspend in 300 ul TE. Cells now ready for transformation.

In a sterile 1.5 ml microcentrifuge tube add the following:

- 100 ul of cells
- 5 ul of carrier DNA (BIO101 kit, stored in cold room)
- 5 ul of histamine (BIO101 kit stored in cold room)
- 1 ug of plasmid.

Control: Set up parallel reaction but instead of plasmid, add equal volume of sterile TE from the BIO101 kit.

Gently mix all together and incubate at room temperature for 15 minutes.

In a separate 15 ml conical tube prepare the following (enough 2 transformations):

- 2.4 ml of PEG (BIO101 kit) very thick take your time
- 0.6 ml of TE/Cation Mix (BIO101 kit)
- vortex vigorously, takes a while for two solutions to mix.

Add 1 ml of the PEG/TE/Cation mix you made to each of the transformation reactions in the microfuge tubes. Mix cells into solution gently by shaking, not vortexing.

Incubate on the roller drum inside test tube for 3 hours.

Heat shock 42oC for 1 hour

Pellet cells in microcentrifuge 1 minute full speed.

Remove supernatant and resuspend pellets in 200 ul sterile PBS (by pipetting).

Plate 100 ul cells per plate of appropriate selective medium. Total 4 plates. (two for control and two for plasmid transformation)

Incubate plates at 30oC for 3 to 4 days.