Yeast Transformation (long)

1) Set up 5 mL overnight culture in YPD or YP

2) Transfer 0.5 mL of overnight to fresh 25 or 50 mL culture. Let grow 3-5 hours until log phase → OD_660 between 0.3-0.8 (0.4-0.5 starting). 10 ml/100 ml culture is better.

3) Transfer 25/50 mL culture to sterile orange-capped tube.

4) Spin 5 min @ speed 7.

5) Decant supernatant, resuspend in 1 mL Li acetate (5 mL); Respin.

5a) Non-specific DNA boiling 5 min → ice 5 min

6) Decant liquid twice. Resuspend pellet w/ remaining liquid 10 mL. → 25 mL culture → 125 mL → 50 mL culture → 250 mL → 500 mL

7) Transfer 50 mL of cell suspension to 1.7 mL epi. 10 mL (per reaction) → ice 15 mins

8) For each reaction add: 5 mL of non-specific DNA (salmon sperm DNA) and 5 mL transforming DNA (not to mock though). 1 mL if it is a CEN plasmid!

9) Add 250 mL TE/lithium acetate/PEG to tube and resuspend gently (invert).

10) Incubate in 32°C shaker for 30 mins.

11) Transfer to 42-44°C water bath for 15 mins (Elaines bath).

12) Pellet cells in microfuge 1 min @ speed 10.

13) Aspirate media from pellet. Add 250 mL 1 M sorbitol, resuspend.

14) Transfer entire mixture to selective media plate. → Incubate in 32°C room.

* For 641R, etc. plates, after step 12, place pellet in 1 mL nonselective media (YPD, YP), plate test tube in rotating wheel in 25°C room for 18 hours. ρxate. Spin down 1 min @ speed 10, resuspend in 250 mL sorbitol, plate.