4/11/03

Yeast Transformation (long)

1) Set up 5mL overnight culture in YPD/YPG

2) Transfer 0.5mL of overnight to fresh 25 or 50mL culture. (0.1–0.2 starting)
   Let grow 3–5 hours → till log phase → OD_{660} between 0.3 + 0.8
   2–3 cell div. ↳ higher is better.

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

4) Spin 5 min @ speed 7.

5) Decant supernatant, resuspend in TeLi acetate (5mL). Respin
   ↳ 5 min @ speed

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

6) Decant liquid twice. Resuspend pellet w/ remaining liquid.
   ↳ 25mL culture → 125 µL ± ~~50~~ µL
   ↳ 50mL culture → 250 µL ± 50 µL

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

8) For each reaction add 5 µL of nonspecific DNA (salmon sperm DNA) and 5 µL transforming DNA (not to mock though) <u>1 µl</u> if it is a <u>CEN plasmid</u>!

9) Add 250 µL 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 (Elaine's bench).

12) Pellet cells in microfuge 1 min @ <u>speed 10</u>.

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

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

* For G418, etc. plates, after step 12, place pellet in 1ml nonselective media (YPD, YPG), place test tube in rotating wheel in 25°C room for 18 hours. ~~Then plate.~~ Spin down 1 min @ speed 10, (↳ aspirate supernatant) resuspend in 250 µL sorbitol, plate.