Yeast transformation

Materials:

- 1x LiOAc buffer
- PEG solution
- ss carrier DNA

Procedure:

- Start an overnight culture in YPD or selective media and shake overnight at an appropriate temperature for the strain. The goal is to obtain 50 ml of mid-log culture the following day. To this end, inoculate a 5 ml culture heavily and plan to back-dilute in the morning or inoculate a 50 ml culture lightly and hope for the best.
- Measure OD600 in the morning. You will need 5 OD units of cells for each transformation.
    - if OD600 is >1.0, dilute cells back to 0.1 and grow 4-6 hours.
    - if OD600 is 0.2 – 1.0, use immediately or dilute for use later in the day.
    - if OD600 is less than 0.2, continue growing cells.
      Note: Due to light scattering, accurate OD measurements can only be made between the range of 0.1 to 0.3 (note that the linear range may vary from one spectrophotometer to the next). Dilute accordingly before making measurements.
- Centrifuge the culture in 50 ml conical tube for 3-5 minutes at 3000g (2500 rpm) in a table-top centrifuge.
- Thaw single-stranded carrier DNA. Denature at 95°C for 5 minutes and cool on ice.
  Note: It is not necessary or desirable to boil the carrier DNA every time. Keep a small aliquot in -20°C freezer and boil after three or four freeze/thaw cycles.
- Pour off media, pipet off residual liquid and resuspend the cell pellet in 10 ml sterile milliQ H2O by vortexing.
- Spin down cells 3 minutes at 3000g (2500 rpm).
- Pour off water, pipet off residual liquid and resuspend pellet in 0.5ml 1xLiOAc buffer per 5 OD units of cells. Transfer 0.5 ml of cell suspension to a 1.5 ml eppendorf tube for each transformation. Spin down cells and discard the supernatant.
- Add 360ul of TRAFO mix to each cell pellet in the following order (if setting up multiple transformations, you can make a master mix):
    - 240 µl PEG (50%)
    - 90 µl 1X LiOAc
    - 10 µl denatured ssDNA
    - 1-5 µg of plasmid DNA or 25% of an efficient PCR reaction in no more than 20 µl of total volume. Concentrate DNA by EtOH precipitation if necessary.
      Note: include a no DNA control
- Vortex for 1 minute to mix the PEG with other components. Incubate in the 30°C shaker for 30 minutes at 100 rpm.
- Heat shock at 42°C for 15 min. TIMING IS IMPORTANT. You can use a metal heat block for this. Chill on ice immediately afterwards.

- Spin down at 3000rpm for 2 min. Remove the supernatant and add 100ul sterile miiliQ water to the cell pellet, resuspend by gentle pipetting.
- Resuspend in 200 µl YPD and spread cells on appropriate selective plates (see Notes)
- Incubate 2-3 days at appropriate temperature for the strain.

Notes

1. This protocol yields approximately 500-5000 colonies/µg of plasmid DNA. It is often wise to plate high and low volumes of cell suspension (e.g. 160 µl and 40 µl) to obtain plates with well-spaced transformants.
2. Integrating plasmids yield about 10-100 fold fewer transformants. A list of enzymes that digest the marker loci of pRS plasmids uniquely is provided below. Be sure that the enzymes do not digest your plasmid inserts.

   URA3: StuI, NcoI, NdeI (EcoRV if it's been deleted from poly-linker)
   LEU2: XcmI, AflII, BstEII @ 60°, AgeI
   TRP1: Bsu36I, BstZ17I, SnaBI, MfeI
   HIS3: NheI, MscI, NdeI
   ADE2: StuI, HpaI, AflII, AatII
3. To select for drug resistance markers (KANMX, NATMX or HYGMX), first plate on YPD and then replica plate to YPD+drug the following day.

Screening of Yeast colonies

Colony PCR (only short PCR products <1.5 kb)

Materials:

- 20 mM NaOH

Procedure:

- Pick several colonies and patch on a selective plate. If there are no colorimetric screening methods, vary size and place on the plate to cover a representative sample. Incubate 1-2 days
- Take a small amount of cells and resuspend in 15µL 20mM NaOH
- Incubate at 96°C for 15 min
- Vortex briefly and spin down the cell debies
- Use 2-5 µL in a PCR mix.

Crude genome prep for PCR (long, short and tricky fragments)

Materials:

- 0.2M LiAc containing 1% SDS.
- 100% EtOH
- 70% EtOH

Procedure:

- Pick and resuspend a swab of yeast cells into 100 uL LiAC/SDS Mix. (Can also use cell pellet from 100 uL O/N liquid culture)
- Heat to 75°C for 10 min.
- Add 300 uL 100% EtOH and vortex.
- Spin at max speed for 3 min. Remove supernatant.
- Resuspend pellet in 150 uL 70% EtOH.
- Spin at max speed for 3 min. Remove supernatant.
- Dry pellet briefly on heat block. Don't overdry.
- Add 50 uL GeneJet Elution Buffer and vortex thoroughly.
- Spin down at max speed for 1 min.
- Transfer 30 uL supernatant to clean tube.
- Nanodrop for [DNA].
- Use 1-2 uL in 50 uL PCR reaction.

Losing plasmids

Materials:

- Make sure to wash and autoclave replica plating velvets.

Procedure:

- Plate a confirmed strain on non-selective media. Make dilutions to make sure that one plate gives a proper number of colonies. Incubate O/N in 30°C. Don't forget to mark the direction of your plates!
- Next day, replica plate on selective media for the plasmid you want to get rid of. Incubate O/N in 30°C. Don't forget to mark the direction of your plates!
- Colonies that grow on non-selective media but not on selective media have lost the plasmid and can be continuously used.