Electroporation of *Pichia*

2003-09-17 (Based on Invitrogen protocol)

Materials

- 5-100 ug pure plasmid DNA containing gene of interest, linearized by restriction digestion (p270 can be linearized with SacI, PmeI, and BstX)
- Extra YPD media
- YPDS plates (with proper antibiotic, ie, 100 and 500 ug/mL Zeocin)
- 500 mL YPD Media (in a 2L baffled flask)
- 1 L cold, sterile ddH_2O
- 25 mL cold, sterile 1M sorbitol
- Electroporation cuvettes (0.2 cm gap width)
- Incubator/shaker set at NO HIGHER THAN 30^0C. If the incubator doesn't keep consistent heat, set it at 28^0C.
- A Pichia strain, streaked out on a YPD plate. (We have X-33 strain)

NOTE: Because this protocol is time-consuming, I recommend doing multiple transformation simultaneously (ie, several different plasmids, or different amount of plasmid, etc). This will save you time in the end.

Protocol

1. Prepare DNA (ie, linearize, phenol-chloroform extraction, precipitation, resuspend in 5-10 µL water). I usually linearize an entire Qiagen Midi-prep of the plasmid I want to transform in to *Pichia*—the transformation efficiency is low, so more DNA is better. To do this, I follow the Midi-prep instruction and dissolve the resulting plasmid DNA in 180 µL ddH_2O, to which I add 20 µL of the appropriate restriction enzyme buffer, and then 5 µL of the enzyme. I let the digestion take place overnight, and the following morning I do the phenol-chloroform extraction and ethanol precipitation, and resuspend all the DNA in **10 µL ddH_2O**, (not TE or any other buffer—no salt for electroporation). I will start this digestion going the same day as I pick the colony of *Pichia* (Step 2).

2. Pick a single colony of your *Pichia* strain to grow in 10 ml YPD overnight at 30^0C in a 125 ml sterile flask.

3. Innoculate 500 mL YPD in a 2 L flask with 0.1-0.5 ml of the overnight culture. Grow overnight again to an O.D. of 1.2-1.5.

4. Centrifuge the cells at 1500 x g for 5 minutes at 4^oC. Resuspend the pellet with 500 mL of ice-cold, sterile water.

5. Centrifuge the cells as in step 3, and then resuspend the pellets in 250 mL of ice-cold, sterile water.

6. Centrifuge the cells again, then resuspend in 20 ml of ice-cold 1M sorbitol. Transfer to a 50 ml conical tube.

7. Centrifuge the cells again, the resuspend the pellet inn 1 mL of ice-cold 1M sorbitol for a final volume of about 1.5 mL. *Keep the cells on ice and use that day—do not store the cells!*

8. Mix 40 μL of the cells from step 6 with **linearized DNA** prepared as per step 1, and then transfer to an ice-cold 0.2 cm electroporation cuvette. Be sure to tap the cells down to the bottom of the cuvette

9. Incubate the cuvette with the cells and DNA on ice for 5 minutes.

10. Pulse the cells according to the manufacturer's instructions for yeast (ie, *Pichia pastoralis Saccharomyces cerevisiae).*

    a. The Bio-Rad Genepulser (Andrew Craig's Lab next door) has a built-in protocol for *Pichia* (25 uF, 200 ohm, 2000 V)

11. Immediately add 1 ml of ice-cold 1M sorbitol to the cuvette. Transfer the cuvette contents to a sterile 15 ml tube.

12. Let the tube incubate at **30^{o}C without** shaking for 1-2 hours.

13. Spread 10, 25, 50, 100, and 200 μL each on separate, labelled YPDS plates containing 100 ug/ml Zeocin (or whatever antibiotic is present on the plasmid).
    a. NOTE: To directly select for multi-copy recombinants, try plating 100-200 ul of the transformation mix on YPDS plates containing increasing concentrations of antibiotic (ie, 100, 500, and 1000 ug/mL Zeocin)

14. Incubate plates from 3-10 days at 30^{o}C until colonies form.