Yeast DNA Prep

Introduction

Protocol to prep plasmid DNA from yeast cells. Preps both plasmid and genomic DNA. Protocol for prepping an entire plate; for selection of correctly assembled plasmids, the prepped DNA is transformed into E. coli, and transformation can be followed by colony pcr to select for the correct plasmids.

Materials

- H2O
- Plasmid release solution
- Tris/EDTA solution (EDTA concentration doesn't matter)
- DNA resuspension buffer of your choice (water is also an option)
- Phenol chloroform isoamyl alcohol
  - Phenol
  - Chloroform isoamyl alcohol

Procedure

DNA prep

1. Grow yeast colonies; the more the better, can preferably be a lawn. Can grow for many days, but for longer times keep plates in a plastic bag so that they don't dry.
2. Add 1 ml of H2O in an eppendorf tube.
3. Add cells to water. Use a z-shape rod if you have a lawn; if single colonies, pick them individually with a loop.
4. Centrifuge to pellet the cells.
5. Pour supernatant off; be gentle, don't shake the tube, some supernatant remains.
6. Add 200 uL plasmid release solution.
7. Resuspend pellet by vortexing or back/forth pipetting.
8. Add glass beads.
9. Add 200 uL phenol chloroform isoamyl alcohol

   Mix 1:1 phenol and chloroform isoamyl alcohol - make a mastermix
10. Close tubes, make sure they are properly closed and no glass beads are preventing the tube from closing. (that phenol chloroform won't leak - good to write labels on multiple places on tube in case some of it washes away)

    Note: careful with phenol chloroform isoamyl alcohol!
    Note: all phenol chloroform waste (e.g. tubes that had it in them) are put to special waste

11. Place in vortex in cold room for 5-6 minutes
12. Spin down briefly before opening so that phenol chloroform stuff isn't on the cap and doesn't get all over the place.
13. Add 200 uL Tris/EDTA in tube - then close and mix (shake)
14. Centrifuge for 10-15 minutes at 13,000-13,500 rcf
15. Collect supernatant into a new tube - careful that you don't get pellet interface with you!
16. Add double the supernatant volume of ethanol (e.g. 96 % ethanol works, e.g. 1 ml), then mix
17. Keep on ice for 20 minutes
18. (A fluffy precipitate should have formed; if not, mix and continue incubation on ice until you get it.)
19. Centrifuge for 15 minutes at 13,000-13,500 rcf to get a pellet. (This is the DNA, both genomic and plasmid, and the pellet contains also e.g. sugars)
20. Remove supernatant, let the pellet dry.
21. Suspend the pellet in buffer of your choice, in as small a volume as possible; depending on the size of the pellet, this could be 50 uL / 100 uL / 200 uL.
22. Transform 5 uL to chemically competent cells and screen transformants using colony PCR for correct plasmids.

    If using electroporation, 1-2 uL can be used.