Yeast DNA precipitation

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Abstract

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.

Citation: Snehadri Sinha Yeast DNA precipitation. **protocols.io**
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Protocol

Step 1.
Grow yeast colonies; the more the better. Can grow for many days, but for longer times keep plates in a plastic bag so that they don't dry.

Step 2.
Add 1 ml of H2O in an eppendorf tube.

Step 3.
Add cells to water. Use a z-shape rod if you have colonies very densely; if more sparse colonies, pick them individually with a loop.

Step 4.
Centrifuge to pellet the cells.

Step 5.
Pour supernatant off; be gentle, don't shake the tube, some supernatant remains.

Step 6.
Add 200 uL plasmid release solution.

Step 7.
Resuspend pellet by vortexing or back/forth pipetting.

Step 8.
Add glass beads.

Step 9.

Add 200 uL phenol chloroform isoamyl alcohol.

Mix 1:1 phenol and chloroform isoamyl alcohol - make a mastermix

Step 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.

Step 11.
Place in vortex in +4 C for 5-6 minutes.

Step 12.
Spin down briefly before opening so that phenol chloroform isn't on the cap.

Step 13.
Add 200 uL Tris/EDTA in tube - then close and mix (shake)

Step 14.
Centrifuge for 10-15 minutes at 13,000-13,500 rcf

Step 15.
Collect supernatant into a new tube - careful that you don't get pellet interface with you!

Step 16.
Add double the supernatant volume of ethanol (e.g. 96 % ethanol works, e.g. 1 ml), then mix

Step 17.
Keep on ice for 20 minutes

Step 18.
(A fluffy precipitate should have formed; if not, try to mix and continue incubation on ice.)

Step 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)

Step 20.
Remove supernatant, let the pellet dry.

Step 21.
Suspend the pellet in water or 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.

Step 22.
Electroporate 1-2 uL and screen transformants using colony PCR for correct plasmids. Transformation with chemically competent cells might not be as efficient.