Plasmid midiprep protocol (50 ml culture) – Spin Column Method

NOTES BEFORE STARTING

This protocol will work well for plasmids that have a medium to high copy number (say 20-200 copies per cell), and for plasmids that are small to medium in size (say 2-20 kb). For low copy plasmids (1-2 copies per cell) and/or very large plasmids (>20 kb) you need to use special procedures, and the below protocols will not work well, if at all.

The solutions needed for the plasmid prep are listed at the end of the protocol – make sure you have these ready before starting the procedure! Note that some of these are different from the “old school” solutions, and also some are different from plasmid kit buffers. These should work with any kind of silica column and could be adapted to free silica particles (e.g. diatomaceous earth).

1. Inoculate 50 ml of LB broth containing the appropriate antibiotic with a large loopful of your *E.coli* culture from a fresh plate of the same medium (‘fresh’ here means less than ~2 weeks old).
Grow overnight (16-24 h) at 37°C with shaking.

It is critical to remember to add the antibiotics to the LB broth. If you forget this, you won’t get any plasmid. Using the correct type and concentration of antibiotic correct is also essential. Some common antibiotics and their typical concentrations are shown below:

Ampicillin: 100 µg/ml — Chloramphenicol: 25 µg/ml (usually) or 12.5 µg/ml (for fosmids)

Kanamycin: 50 µg/ml — Tetracycline: 10 µg/ml (light-sensitive! wrap plates/broths in foil)

Streptomycin: 200 µg/ml — Gentamicin: 10 µg/ml

2. Aseptically transfer (pour) the cells into a 50 ml Falcon tube. Find or make a balance tube with the same volume of water. these, Estimating equal volumes by eye (+/- 1 ml) is OK for low speed centrifuges (e.g. Centaur /Centurion), but a balance should be used for more sensitive machines or when spinning at higher speeds (>3000g) (for these, need accuracy +/- 0.1 ml).

3. Centrifuge at 4000 rpm (~3000 g in Centaur/Centurion machine) for 10 minutes. This can be done cold or at room temp, it doesn’t matter. You can spin faster, up to say 7000 g, but above this point, faster is not better, and cell pellets will be hard to resuspend.

4. Pour off supernatants into culture waste, resuspend the cell pellet in 2.5 ml **buffer P1** by vortexing.

<<put on gloves and safety glasses (hazards: NaOH, SDS, guanidine)>>

5. Add 2.5 ml **buffer P2**. Mix by rapidly inverting the tube 10 times. Incubate 10 min at room temp. (No longer than 10 min!) The mixture should go clear and become viscous as the cells break open.

6. Add 3.5 ml **buffer N3**. Mix by rapidly inverting the tube 10 times. It is essential that the N3 buffer is thoroughly mixed in. Viscosity should disappear, and a white precipitate should appear. Incubate for 5 minutes at room temp. Centrifuge 3000 g for 15 min. *Carefully* pour off supernatant into a fresh 50 ml tube. Some of the white junk may carry over, but try to minimize this.

7. Place four spin columns (silica-based e.g. ‘Econospin’ brand) in their collection tubes and pipette 750µl of the clarified sample supernatant onto each column. Centrifuge for 30 seconds at ~10,000 x g and discard flow-through into culture waste. Repeat this procedure until all of the supernatant has been used up (ensure the supernatant is evenly distributed among the tubes at the last spin step).

8. Add 700 µl **buffer PB** to each column, centrifuge 30 sec at ~10,000 x g. Discard flow-through.

    <<can remove gloves and safety glasses>>

9. Add 700µl **buffer PE** to each column, centrifuge 30 sec at ~10,000 x g. Discard flow-through.

10. Repeat step 9.

11. Spin the ‘empty’ column one more time for 1 min at ~10,000 x g to remove the last traces of PE.

12. Discard collection tubes, and place the column part with the lid open on a clean ‘Kimwipe’. Place in 60 C° oven for ~10 min to evaporate residual ethanol. Avoid touching the ‘nipple’ at the bottom of the column with your fingers.

13. Place columns into Eppi tubes, and add 30 µl of **buffer EB** to each column. (ensure EB goes onto the membrane at the bottom, not the wall). Allow to sit for 2 min. Centrifuge 1 min at ~10,000 x g. Combine all the flow-through liquids into one tube. Note that you will only recover about 25 µl from each, giving a total of approx. 100 µl of plasmid DNA.

14. Digest 2 µl of the plasmid preparation with an appropriate restriction enzyme (eg. an enzyme that cuts once or twice) (see restriction digest protocol), and run on an agarose gel to evaluate yield. The Nanodrop is not accurate for plasmid preps.

PLASMID PREP SOLUTIONS (SPIN COLUMN METHOD) – based on recipes at openwetware.org

Buffer P1: 5 mM EDTA, pH 8. Sterilise by autoclaving. Then add RNAse to 300 µg/ml.

Usually this would be prepared by starting with a 0.5 M EDTA, pH 8 stock, and a 10 mg/ml aliquot of boiled RNAse.

Buffer P2: 1% SDS, 0.2 M NaOH. Not sterilised.

Prepare from stocks of 10% and 2 M NaOH (these don’t need to be sterilised, but its not a bad idea to prepare these and the final buffer in sterile bottles or tubes containing the correct amount of autoclaved RO water). <<safety glasses!>> Keep in a tube or bottle with minimal headspace and tightly closed. Will eventually develop precipitate of Na_2CO_3 – remake the solution if you see this. Recommend to make fresh approx. monthly.

Buffer N3: 4.2 M guanidine HCl, 0.9 M K-acetate, pH 4.8. Not sterilised.

Adjust the pH using glacial acetic acid <<safety glasses!>>

Buffer PB: 5 M guanidine HCl, 30% isopropanol. Not sterilised.

Prepare in a sterile bottle or tube containing the correct amount of autoclaved RO water.

Buffer PE: 10 mM Tris-HCl, pH 8, 80% ethanol. Not sterilised.

Prepare in a sterile bottle or tube containing the correct amount of autoclaved RO water.

Buffer EB: 10 mM Tris-HCl, pH 8. Autoclaved.

Usually this would be prepared by diluting a 1M Tris-HCL pH 8 stock.