Plasmid miniprep protocol (5 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 standard plasmid kit buffers. These should work with any kind of silica-based purification column and could also be adapted to purification using free silica particles (e.g. diatomaceous earth).

1. Inoculate 5 ml of LB broth in a narrow-neck McCartney bottle containing the appropriate antibiotic with a 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. Centrifuge the culture at 4000 rpm (~3000 g in Centaur/Centurion machine) for 10 minutes. You can spin it in the McCartney bottle, there is no need to decant into a centrifuge tube. Make sure you find appropriate balance(s) if you have an odd number of bottles – these should be weighed since it's hard to estimate volumes in McCartneys by eye, and the bottles may have different weights.

3. Pour off supernatant into culture waste, resuspend the cells in 1 ml of **buffer EB** by vortexing. Transfer to 1.5 ml Eppendorf tube. Centrifuge 1 min at ~10,000 g. Pour off supernatant, keep pellet.

(this step efficiently transfers all the cells into a smaller tube…it is possible to skip this step and directly resuspend in 250 µl buff. P1 in the McCartney bottle, but this risks losing a lot of cells)

4. Resuspend the cell pellet in 250 µl **buffer P1** by vortexing.

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

5. Add 250 µl **buffer P2**. Mix by rapidly inverting the tube ~5 times. Incubate **5 min** at room temp. The mixture should go clear and become viscous as the cells break open.

6. Add 350 µl **buffer N3**. Mix by rapidly inverting the tube ~5 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 for 5 min at ~15,000 g.

7. Place a spin column (silica-based e.g. 'Econospin' brand) into its collection tube (if it isn't already set up that way), and carefully pipette 750µl of the plasmid extract supernatant onto the column.

Avoid the white pellet and any white junk that may be floating on top. Centrifuge 30 seconds at ~15,000 g. Discard flow-through into culture waste.

8. Add 750 µl **buffer PB** to the column, centrifuge 30 sec at ~15,000 x g. Discard flow-through.

9. Add 750µl **buffer PE** to the column, centrifuge 30 sec at ~15,000 x g. Discard flow-through.

10. Repeat step 9.

11. Spin the column 1 min at ~15,000 x g to remove the last traces of PE, discard collection tube, 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.

12. Place column into an Eppi tube, 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 ~15,000 g.

13. 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. Note that the Nanodrop may not be 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. Fresh RNase should be added at least once a month, otherwise not all RNA will degrade and it will contaminate your plasmid prep (A_{260}/A_{280} ratio will be ~2 rather than 1.8 for pure DNA)

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.