

DNA Maxi-prep (revised 9/06)

DAY 1:

1. Inoculate 500 mL TB + ampicillin (or appropriate antibiotic) + 10 mL 50X phosphate buffer with either 1 mL stock bacterial culture or transformation, allow to grow overnight (16-20 hrs) at 37°C while shaking at 250 rpm. (If using single colony directly from a plate, allow to grow longer)

DAY 2: (3.5 hours + overnight spin)

2. Pellet bacterial cells by spinning at 3000 rpm x 7 min (or 5000 rpm for 5 min) at 4°C. Discard supernatant and place pellets on ice. Can also store pellets at -20°C.
3. Add 25 mL Solution I and completely resuspend cells by placing tube in 37°C shaking incubator for about 5 min (or longer if needed). Place on ice x 10 min.
4. Add 50 mL Solution II and mix by shaking. Set on ice x 10 min.
5. Add 37.5 mL Solution III and mix by shaking. Set on ice x 10 min (OK to leave longer if needed).
6. Spin lysed cells at 7000 rpm for 15 min at 4°C.
7. Filter supernatant through 3 Kim-wipes or 2 coffee filters into fresh centrifuge tube, repeat if needed until no precipitate in tube. Add 60 mL isopropanol and mix by inverting tube several times, allow to stand at room temp x 20 min (OK to leave longer if needed).
8. Pellet DNA by spinning at 7000 rpm x 20 min at room temp. Discard supernatant and allow pellet to dry slightly (5min on bench, 5 min in 37°C incubator) but do not dry completely or DNA won't dissolve!
9. Add 3 mL 1X TE to pellet and resuspend in 37°C shaking incubator to dissolve completely.
10. Prepare 2 ultracentrifuge tubes for each DNA pellet – add ~2.5 mL saturated CsCl (upper phase) to tubes followed by 100 µL EtBr (10 mg/ml stock) and mix.
11. Divide suspended DNA into the two prepared tubes, mix gently and top off tubes with CsCl (goal 3:1 CsCl to TE/DNA).
12. Cap tubes with crimper and spin overnight at 65K at 22°C (or for 6 hours minimum).

DAY 3: (8.5 hours + overnight at -20°C)

13. Remove tubes from rotor, DNA should form pink band in the middle of centrifuge tube. Prick top of tube (gently – watch eyes!) with 18G needle attached to 5 mL syringe to create air vent, then extract band by piercing just below band.
14. Place band in a fresh UC tube (can combine into one tube if desired) and add 3:1 CsCl:1X TE. Respin for 5-7 hours at 65K at 22°C.
15. Extract bands from tubes (and combine bands if kept separated for second spin) into 15 mL conical tube.
16. Wash DNA 5 times with an equal volume of water-saturated butanol (top layer of 1:1 stock), aspirating top layer between washes (until pink color disappears). Consider doing this in hood.
17. Transfer bottom aqueous layer containing DNA into dialysis tubing and clip both ends, attach empty eppendorfs to float if needed. Dialyze in 4L 1X TE (can use 40 mL 1M Tris + 8 mL 0.5 M EDTA in 4L ddH₂O) for 1 hour. Dialyze 2 more hours or overnight in fresh 4L 1X TE.
18. Transfer DNA solution to 30 mL glass tubes. Measure total volume of sample and add 1/10 total volume of 3M Na acetate and 2.5X total volume cold 100% ethanol (in -20°C). Parafilm the openings, invert several times to mix and place at -20°C for 1 hour or overnight.

DAY 4: (2 hours)

19. Pellet precipitated DNA by spinning at 6000 rpm x 20 min at 4°C. Discard supernatant, resuspend pellet in 3-4 mL 70% ethanol/water (do not vortex) and respin. Discard supernatant and allow pellet to dry slightly (5 min on bench, 5 min in 37°C incubator – do not dry completely!).
20. Resuspend pellet in 500-1000 µL 1X TE/water depending on size of pellet, place in 55°C water bath x 5 min to dissolve DNA.
21. Check concentration in spectrophotometer:
 - 10 µL DNA + 990 µL H₂O in quartz cuvette (for 1:100 dilution)
 - 1 mL water + 10 µL TE for blank
 - Turn power on, press 4 (Abs ratio 260/280nm), set number of samples, autoprnt on
 - Load blank into slot 1, other samples behind, press measure
 - Clean cuvettes on vacuum flask → water then ethanol then acetone

Concentration ($\mu\text{g} / \mu\text{L}$): $(\text{OD}_{260} \times 50 \times \text{dilution factor})/1000$

22. Run analytical gel/digest.

Recipes:

Solution I

9g Glucose (Dextrose)
25ml 1M Tris, pH
8.0
20ml 0.5M EDTA, pH 8.0

ddH₂O to 1 L

Solution II

40ml 5N NaOH

50ml 20% SDS
ddH₂O to 1 L

Solution III

294g K acetate
115ml Glacial acetic
acid
ddH₂O to 1 L
(Dissolve K Acetate
before
adding GAA)