

DNA Amplification (Maxi prep)

(6-8 hours, need the plate with pre-grown bacteria as in miniprep)

0. Autoclave the bottles and tubes used in step 4 and step 5 before hand.

Day 1 (Morning) 1. Pick a single colony from a freshly streaked selective plate and incubate a starter culture of 2 ml LB medium containing the appropriate selective antibiotic (amp). Incubate for approx. 8 h at 37°C with vigorous shaking (approx. 300 rpm).

Day 1 (Afternoon) 2. Dilute the starter culture 1/250 into 500 ml of selective LB medium. Grow at 37°C for 12–16 h with vigorous shaking (approx. 300 rpm). Use a tube or flask with a volume of at least 4 times the volume of the culture.

Day 2 (Morning) 3. Prepare an ice tray and prechill P3 buffer in the Fridge.

4. Transfer the bacterial mix to the big tubes for centrifuge, be sure to weigh the tubes to ensure balance. Harvest the bacterial cells by centrifugation at 6000 x g (6000 RPM) for 15 min at 4°C. The Beckman Centrifuge (Model J2-21) is located in Bill Greeno's lab in BI room 2712. The key to Bill Greener's Lab is in the basement. We need to bring timer because the timer of the centrifuge is broken. At this step we are using motor JA10. Set temperature to 4°C (green) 20°C (red), and keep timer to hold position to keep the temperature.

5. Resuspend the bacterial pellet in 10 ml Buffer P1. Then transfer the mix to a smaller tube for centrifuging in JA20 motor at higher speed. Ensure that RNase A has been added to Buffer P1. The bacteria should be resuspended completely by vortexing or pipetting up and down until no cell clumps remain.

6. Add 10 ml Buffer P2, mix thoroughly by gently inverting the sealed tube 10 times, and incubate at room temperature (15–25°C) for 5 min. Do not vortex, as this will result in shearing of genomic DNA. The lysate should appear viscous and the color should turn blue. Do not allow the lysis reaction to

proceed for more than 5 min. After use, the bottle containing Buffer P2 should be closed immediately to avoid acidification from CO₂ in the air.

7. Add 10 ml of chilled Buffer P3 (pre-chilled in ice tray), mix immediately and thoroughly by gently inverting ~10 times, and incubate on ice for 20 min. Precipitation is enhanced by using chilled Buffer P3 and incubating on ice. After addition of Buffer P3, a fluffy white material forms and the lysate becomes less viscous, color turns blue. The precipitated material contains genomic DNA, proteins, cell debris, and KDS. The lysate should be mixed thoroughly to ensure even potassium dodecyl sulfate precipitation. If the mixture still appears viscous, more mixing is required to completely neutralize the solution.

8. Centrifuge at $\geq 20,000 \times g$ for 30 min at 4°C (at 12KRPM on JA20 motor). Remove supernatant containing plasmid DNA promptly. Before loading the centrifuge, the sample should be mixed again. After centrifugation the supernatant should be clear.

9. Centrifuge the supernatant again at $\geq 20,000 \times g$ (at 12 KRPM on JA20 motor) for 15 min at 4°C. Remove supernatant containing plasmid DNA promptly. This second centrifugation step should be carried out to avoid applying suspended or particulate material to the QIAGEN-tip.

10. Equilibrate a QIAGEN-tip 500 by applying 10 ml Buffer QBT, and allow the column to empty by gravity flow.

11. Apply the supernatant from step 8 to the QIAGEN-tip and allow it to enter the resin by gravity flow. The supernatant should be loaded onto the QIAGEN-tip promptly. If it is left too long and becomes cloudy due to further precipitation of protein, it must be centrifuged again or filtered before loading to prevent clogging of the QIAGEN-tip.

12. Wash the QIAGEN-tip with 2 x 30 ml Buffer QC. Allow Buffer QC to move through the QIAGEN-tip by gravity flow. The first wash is sufficient to remove all contaminants in the majority of plasmid DNA preparations. The second wash is especially necessary when large culture volumes or bacterial strains producing large amounts of carbohydrates are used.

13. Transfer the column tip to the top of a clean tube. Hold it with hand if necessary. Elute DNA with 15 ml Buffer QF. If you wish to stop the protocol

and continue later, store the elution at 4°C (Not good for longer than overnight).

14. Precipitate DNA by 10.5 ml (0.7 volumes) room-temperature isopropanol (molecular grade) to the eluted DNA. Mix and centrifuge immediately at $\geq 15,000 \times g$ (11 KRPM) for 30 min at 4°C. Carefully decant/discard the supernatant. All solutions should be at room temperature in order to minimize salt precipitation, although centrifugation is carried out at 4°C to prevent overheating of the sample. Isopropanol pellets have a glassy appearance and may be more difficult to see than the fluffy, salt-containing pellets that result from ethanol precipitation. Marking the outside of the tube before centrifugation allows the pellet to be more easily located. Isopropanol pellets are also more loosely attached to the side of the tube, and care should be taken when removing the supernatant.

15. Wash DNA pellet by pipet up and down with 1 ml of room-temperature 70% ethanol. Use pipet tip to break the pellet if necessary. Centrifuge at $\geq 15,000 \times g$ (top speed 13.2 RPM at 4°C in B500B) for 10 min. Here we use the centrifuge labelled "Eppendorf centrifuge 5415R" in B500B. Before centrifuge, we need to press the FastTemp button to cool down to 4°C. After centrifuge, carefully decant the supernatant without disturbing the pellet.

16. Air-dry the pellet for 5–10 min, and redissolve the DNA in a suitable volume of buffer (500ul) Redissolve the DNA pellet by rinsing the walls to recover all the DNA. Pipetting the DNA up and down to promote resuspension may cause shearing and should be avoided. Overdrying the pellet will make the DNA difficult to redissolve.