Detailed Maxi-prep Overall Procedure (by Steven Chang)

Step 1: Transformation

(Two days before maxi-prep. Starting at late afternoon. Duration: ~2 hours)

1. Get the ice bucket and keep DH5a chemical competent cells (from the -80°C freezer) in ice for 5~10 minutes for them to thaw.
2. Turn on the heat shock machine to low setting at 42°C. Add water to the surface to make a water bath for even heat-up.
3. Put LB plate with the appropriate antibiotics at 37°C incubator to warm up.
4. Add 1μL DNA to DH5a, keep the vial in ice for 30mins.
5. Put 950μL LB media to round bottom tube (14mL) next to fire to ensure sterilization.
6. Heat shock the vial of DH5a for 45sec at 42°C. If the thermometer reads a higher temperature, add some water to cool it down first.
7. Cool down in ice for 2mins.
8. Add DH5a to LB media, incubate in shaker at 37°C for 1 hour. (In the warm room)
9. Add 50μL of the resulted LB media with DH5a to the center of the plate.
10. Soak the spreader in ethanol. Start gas fire and sterilize the spreader using fire 2 to 3 times.
11. Cool down the spreader by letting the spreader to touch the periphery of the LB plate. Let it cool before spreading the DH5a cells.
12. Spread the DH5a cells using the spreader
13. Put the plate upside down in the incubator for overnight (16~18 hours)

Step 2: Growing Bacteria

(One day before maxi-prep, starting around noon)

1. Next to a fire, put 5mL LB with the appropriate amount of antibiotics in a round bottom tube. (For ampicillin, use 1:1000 dilution i.e. 5μL. For kanamycin, use 1:500 dilution i.e. 10μL.)
2. Pick one medium sized colony by using a 10 or 200μL pipette tip to touch the bacteria colony and put the tip into the prepared LB media.
3. Incubate in shaker at 37°C for 8 hours.
4. Be sure to have a sterile 250mL LB medium prepared in a 1L or 2L Erlenmeyer flask.
5. Check if the LB medium becomes cloudy by swirling the tube and see if there are things floating inside. If not, wait longer.
6. If yes, put 0.5mL of the culture into 250mL LB with the appropriate amount of antibiotics (see dilution ratio above) next to a fire.
7. Incubate in shaker at 37°C for 12~16 hours.

Step 3: Maxi-prep using Hispeed plasmid maxi kit.

(Duration 2.5~3 hours. Procedure can also be found in the kit booklet.)

1. Turn on the centrifuge and put in the big rotor to cool it down. Put the P3 buffer in the fridge to pre-chill it.
2. Pour the incubated medium with bacteria cells into a 500mL centrifuge bottle. Prepare another bottle to balance the weight.
3. Harvest the bacterial cells by centrifugation at 6000 rpm for 15 minutes at 4°C.

4. Discard the liquid. Resuspend the pellet in 10mL P1 buffer. (P1 buffer is in the 4°C fridge.)
5. Add 10mL P2 buffer and vigorously invert the bottle in room temperature for 5 minutes. Mixture should turn blue.
6. Add 10mL chilled P3 buffer and mix immediately by vigorous inverting. Make sure all the blue color is gone.
7. Centrifuge at 6000rpm for ~2minutes. Turn off the centrifuge after done.
8. Assemble the cartridge without the plunger. Pour the supernatant to the cartridge and wait for 5 minutes. Don't pour in the solid.
9. During this time, assemble and equilibrate the column with 10mL QBT.
10. Remove the cap of the cartridge and using plunger to allow the liquid to flow through the column.
11. Wash the column with 60mL QC. (The liquid level should reach to the "A" of QUAGEN)
12. Elute DNA with 15mL QF to a new clean tube (the plastic 50mL centrifuge tube).
13. Add 10.5mL isopropanol to the eluted DNA. Incubate for 5 minutes.
14. During the incubation, remove the plunger from the 30mL syringe and attach QIA precipitator maxi module (white part to the syringe). Prepare some 70% ethanol.
15. Transfer the mixture to the syringe and push with plunger through the filter. Discard the liquid.
16. Detach the QIA precipitator first before pull out the plunger and re-attach afterwards. NEVER PULL OUT THE PLUNGER WITH THE QIA PRECIPITATOR ON THE SYRINGE.
17. Transfer 2mL 70% ethanol to the syringe and push with plunger through the filter.
18. Push air through several times to remove residual ethanol. Dry the tip of the QIA precipitator on a paper each time.
19. Detach the QIA precipitator from the 30mL syringe and attach it to the 5mL syringe (remove the plunger first!).
20. Set the QIA precipitator with the 5mL syringe on a 1.5mL collecting vial. Add 1mL of TE to the syringe. Push the plunger to elute the DNA.
21. Use a pipette to collect the eluted TE with DNA, re-add it to the syringe, and push the plunger to elute the DNA again.
22. Measure the DNA concentration. (Dilute it 100 times in molecular water and enter a dilution factor of 100 to the machine when measuring.)