



University of Southampton iGEM 2009 Protocol:
PureYield Plasmid Prep:

Maxi Plasmid Prep Protocol:

1. Grow a starter culture of cells
 - a. Add 25µl ampicillin to 5ml autoclaved LB medium to achieve a final concentration of 100µg/ml.
 - b. Transfer a single colony from the agar plate to the medium
 - c. Place in the shaking incubator and grow at 37 °C for 6-8 hours
2. Make two 250 ml LB medium by dissolving 5g powdered LB in 250ml sterilised water. Autoclave using a Media programme.
3. Overnight culture
 - a. Add 1.25ml ampicillin to each flask of the autoclaved medium to achieve a concentration of 100 µg/ml
 - b. Pipette 1 ml of the starter culture (from step 1) into each flask
 - c. Place in the shaking incubator at 37 °C overnight
4. Harvesting of cells
 - a. Split each flask of cells into 3 x 50 ml falcon tubes and centrifuge (5,000 x g) for 10 minutes, at 4 °C. Discard the supernatant. This should result in three pellets for each individual batch started
5. Lysing cells
 - a. To each pellet add 4 ml resuspension buffer and vortex until all cells are resuspended
 - b. Combine pellets from each original batch into a single falcon tube
 - c. Add 12 ml cell lysis buffer, invert gently three times to mix contents and incubate for 3 minutes at room temperature
 - d. Add 12 ml neutralisation solution and invert gently 15 times to mix
 - e. Centrifuge at 7,000 x g for 30 minutes
6. Purifying the plasmid DNA
 - a. Pour the lysate into columns on a vacuum manifold
 - b. Apply the vacuum and allow the lysate to be pulled through.
 - c. Remove the clearing column and wash the lower column with 5 ml endotoxin removal wash, followed by 20 ml column wash and allow to clear
7. Elution of DNA
 - a. Allow the columns to dry under vacuum for 10 minutes. Ensure that no ethanol residue remains
 - b. Elute the plasmid from the column into a microcentrifuge tube using 1ml nuclease free water



8. Check the yield and purity of DNA by using the Nanodrop and by Gel Electrophoresis (see Gel Electrophoresis Protocol)