**Plasmid Midiprep (using promega)**

Preculture(~10ml) LB

Inoculation from preculture into 100ml LB

Morning--------------------------------Evening------------------------------------Next day morning

* Inoculate a colony from the agar plate in a 5-10 ml LB media (with antibiotic) in a 50ml falcon as a preculture in the morning using a pipette tip. Incubate at 37°C in a shaker
* Inoculate 1 ml of this culture into a 100ml LB + antibiotic (in a conical flask of 1000ml capacity-this large flask will ensure proper aeration for the culture). Incubate the flask at 37°C in a shaker.
* After 12-16h (next day), pellet down the cells by pouring the culture in 50ml falcons and centrifuging at 5000rpm for 10 min, RT.
* Discard the supernatant
* Add 6ml of Solution I (Cell resuspension solution) to the pellet and resuspend the pellet using a 10ml pipette. Combine the pellet from the other falcon-in case you distributed your culture volume in 2 falcons. Pool up the total resuspended culture now into oakridge tubes.



Oakridge tube

* Check whether the pellet is completely resuspended and no smaller pellets are left.
* Add 6ml of Solution II (Cell lysis solution) from the side. Mix it well by inverting slowly 5-6 times.
* Add 10ml of Solution III (Neutralization Solution) to the tube and again mix slowly by inverting the tube till you see the precipitate and the solution becomes homogenous.
* Spin @ 15,000-15,500 rpm for 20-30 min RT, in a sorvall centrifuge (upstairs, ask someone for help if you are not familiar with it-**use rotor SS34**).

 

Sorvall Centrifuge; SS34 rotor

* While the tubes are being centrifuged, prepare the vacuum chamber (PROMEGA) and place the filter columns-first white and a blue filter over to that.
* Pour the supernatant over the blue filter column and allow it to pass into the white column by gravity (note: blue column is a filter column while the white one is a DNA purification column). Discard the blue column (the blue column is simply to ensure that no precipitate is left out in the white column which may create a problem during further steps)
* Use the vacuum pump upto 30 Pascals (check the indicator) to elute out the supernatant from the white column.
* Wash with 5 ml of Endotoxin removal solution.
* Once it is completely eluted out, add 20 ml of Wash buffer (check if it is supplemented with Ethanol). Drain it with vacuum.
* Run the vacuum for 1 min after draining the Ethanol; this is to completely dry the membrane column, so as to not contaminate the DNA with extra alcohol.
* Remove the white column from the Vacuum chamber and wipe the column nozzle on a kimwipe, if you happen to see some wash buffer droplets.
* Place the column on the DNA eluting chamber (blue case that holds a µfuge tube with a transparent open cap) that contains an open µfuge tube so as to elute the DNA directly into it.
* Add from 350-500µl of warm (40-45°C) nuclease free H2O (provided in the kit) onto the white column and wait for about a min.
* Switch on the vacuum pump and allow the DNA to be eluted into the µfuge tube. Try to increase the vaccum pressure to upto 50-60 pascals to ensure complete remove of the water containing DNA. Close the stopper once done, and only then switch off the pump (**It is important to close the stopper before switching off the pump so as to not damage the DNA membrane in the column**).
* Carefully take out the µfuge tube, close the lid and allow it to stand for 10 min.
* Spin down the tube in a centrifuge at 13,000 rpm for a min at RT to pellet down some debris or column fibres.
* Take the supernatant carefully into a new µfuge tube and measure the DNA concentration using a nanodrop.