Miniprep DNA extraction (Qiagen)

Materials:

- Liquid broth cell culture
- Resuspension solution (containing RNase A)
- Lysis solution
- Neutralisation solution
- Wash solution (treated with ethanol)
- Elution buffer
- GeneJET spin column

Protocol:

1. Spin liquid broth cell culture for 10 minutes at 4000g to pellet cells, decant and discard all supernatant.
2. Resuspend the pelleted cells in 250 µl of the Resuspension Solution.
3. Transfer the cell suspension to a microcentrifuge tube, the bacteria should be resuspended completely by vortexing or pipetting up and down until no cell clumps remain.
4. Add 250 µl of the Lysis Solution and mix thoroughly by inverting the tube 5 times until the solution becomes viscous and slightly clear. Do not vortex to avoid shearing of chromosomal DNA. Do not incubate for more than 5 min to avoid denaturation of supercoiled plasmid DNA.
5. Add 350 µl of the Neutralization Solution and mix immediately and thoroughly by inverting the tube 5 times.
6. Centrifuge for 5 min to pellet cell debris and chromosomal DNA.
7. Transfer the supernatant to the supplied GeneJET spin column by decanting or pipetting. Avoid disturbing or transferring the white precipitate.
8. Centrifuge for 1 min. Discard the flow-through and place the column back into the same collection tube.
9. Add 500 µl of the Wash Solution to the GeneJET spin column. Centrifuge for 60 seconds and discard the flow-through.
10. Repeat step 9.
11. Centrifuge for an additional 1 min to remove residual Wash Solution. This step is essential to avoid residual ethanol in plasmid preps.
12. Transfer the GeneJET spin column into a fresh 1.5 mL microcentrifuge tube. Add 50 µl of the Elution Buffer to the centre of GeneJET spin column membrane to elute the plasmid DNA. Take care not to contact the membrane with the pipette

tip. Incubate for 2 min at room temperature and centrifuge for 2 min. To increase DNA concentration, try eluting first with 30 µl elution buffer, followed by 20 µl in a separate step.