Plasmid Extraction – QIAGEN Plasmid **Midi Kit**

*The **Midi kit** recommendation:*

- *High-copy plasmids: using 25 ml bacterial culture; yield 75-100 ug*
- *Low-copy plasmids: using 100 ml bacterial culture; yield 20-100 ug*

Bacterial culture, harvest, and lysis

1. Pellet overnight LB culture at 6000 X g for 15 min at 4°C
   - Prepare **eight** 1.5 ml tubes for each plasmid
   - Add equal amount of LB culture to each tube:
     - If there are **25 ml** LB culture: Add 1.5 ml to each 1.5 ml tube ➔ spin ➔ remove the supernatant ➔ add another 1.5 ml to each tube ➔ spin ➔ remove the supernatant
   - If you wish to stop the protocol and continue later, freeze the cell pellets at **-20 °C**

2. Homogeneously resuspend the bacterial pellet in **0.5 ml** Buffer P1
   - Buffer P1 = cell resuspension solution (with RNase A; store at 4 C freezer)
   - The bacterial should be resuspended completely by vortexing until no cell clumps remain

3. Add **0.5 ml** Buffer P2, mix thoroughly by inverting 4-6 times, and incubate at RT for 5 min
   - Buffer P2 = cell lysis buffer (contains detergent SDS, very alkaline)
   - **Mix gently; do NOT vertex, which will shear the chromosomal DNA**
   - All DNA becomes denatured to its single stranded form
     - Chromosomal DNA is long and is attached to broken pieces of the cell membrane
     - Plasmid DNA is linked, so it forms two attached circles

4. Add **0.5 ml** chilled Buffer P3, mix thoroughly by vigorously inverting 4-6 times, and incubate on ice for 5 min (precipitation is enhanced)
   - P3 = neutralization buffer/DNA binding buffer
   - Contains potassium acetate; the KAc forms a precipitate when it interacts with SDS. At the same time, denatured proteins co-precipitate with SDS.
   - At the neutral pH, all DNA tried to re-nature; plasmid can easily re-form to its double stranded form. Chromosomal DNA finds it difficult to re-nature b/c it has no reference point and homologous pieces of DNA may be blocked from finding each other by the cell debris present

5. Centrifuge at maximum speed for 10 min at 4°C
   - Dense cell debris is pelleted to the bottom of the tube. Chromosomal DNA is also pelleted along with the cell debris

6. Carefully transfer the supernatant to new tubes, and spin again at maximum speed for 5 min at 4°C

Bind, wash, and elute plasmid DNA on QIAGEN-tip

7. Equilibrate a **QIAGEN-tip 100** by applying **4 ml** Buffer QBT and allow column to empty by gravity flow

8. Apply the supernatant (from step 6) to the QIAGEN-tip 100 and allow it to enter the resin by gravity flow
- **1.5 ml supernatant from each 1.5 ml tube**; as there are **eight** tubes, we have **12 ml supernatant** for each plasmid
- Plasmid DNA binds to the filter membrane

9. Wash the QIAGEN-tip 100 with **2 X 10 ml** Buffer QC. Allow Buffer QC to move through the QIAGEN-tip 100 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 are used

10. Elute DNA with **5 ml** Buffer QF into clean 50 ml tube

Precipitate, wash, and redissolve plasmid DNA

11. Aliquot 5 ml DNA elution to **four 2 ml tubes**; each tube has **1.25 ml elution**

12. Precipitate DNA by adding **0.7X (0.875 ml)** RT isopropanol to the eluted DNA and mix. Centrifuge at maximum speed for 30 min at 4°C. Carefully decant supernatant
- Invert the tube several times to homogenize the solution

13. Wash DNA pellet with **1 ml** RT 70% ethanol and centrifuge at maximum speed for 10 min. Carefully decant supernatant.
- To wash the DNA, invert the tube several times to resuspend the pellet

#14. **Pool the pellet from five tubes into one**
- For each tube, remove 0.8 ml supernatant; using a cutted tip resuspend the pellet, and **pool all pellets** into a single tube; spin again for 5 min; remove the supernatant carefully with pipette tips
- If there are ethanol stay on the wall of the tube, quick spin the tube, and use a pipette to remove the remaining ethanol at the bottom of the tube

15. Air-dry pellet for 5-10 min and re-dissolve DNA in 20 μl autoclaved de-ionized water.
- Avoid pipette up and down which may cause plasmid shearing