# MINI PREPS

In this lab, you are going to purify plasmid DNA from bacterial. Each group will pick four colonies: one for each plasmid from your new plate (pDONR/221 containing excisionase or containing integrase) and one for each plasmid from the TAs plate. Please use the following protocol:

**Lab day I (second day of lab 2)**

1. Get four test tubes and put 5ml of LB (containing 50 μg/ml kanamycin) to each tube.

2. **INOCULATE** each of the LB medium with a single colony and incubate the culture overnight at 37 °C

   Note: This process will take less than an hour for the class. I am hoping that there will be volunteers from each group to **HARVEST** the culture in the next morning (steps 3 and 4 below) which will also take less than half an hour.

3. Spin 1.5 ml of culture at 5,000 for 1 min at RT,

4. Decant supernatant by hand and remove excess medium by aspiration of pipetting. Store the pellet at -20 for use in the next lab session.

**Lab day II (first day of lab 3)**

5. Add 100 μl solution I (4°C) to each of the tube with the pellet from step 4 and vortex vigorously to **RESUSPEND THE CELL**. Keep on ice.

6. Add 200 μl solution II (RT) to **LYSE THE BACTERIA**. Mix by inverting the tubes 2 or 3 times. DO NOT VORTEX. Keep on ice for 3 min.

7. Add 150 μl solution III (4°C) and immediately mix by inverting 4 to 6 times to **NEUTRALIZE THE SAMPLE AND PRECIPITATE PROTEINS**, etc. Keep on ice for 20 min.

8. Spin at 13,000 for 5 min at RT.

9. Transfer the supernatant into new microcentrifuge tubes (remember to label your tubes). Avoid transferring white junk.

10. Add 1 ml isopropanol (RT) to **PRECIPITATE THE PLASMID DNA**. Invert gently 1 or 2 times. Spin at 13,000 for 5 min.

11. Decant supernatant by hand, then rinse pellet with 70% EtOH (4°C), do not shake.

12. Decant EtOH and spin in speed vacuum on medium heat until pellet dries out (about 5-10 min). For this step, you can let the sample air dry.

13. Re-dissolve the Nucleic Acid in 20 μl 1X TE pH = 8.0. Store at -20°C.

*Solution I (Store at 4C)*

50 mM Glucose
25 mM Tris (pH 8.0)
10 mM EDTA (pH 8.0)

*Solution II (Prepare freshly)*

0.2 M NaOH
1% SDS

To 8.8 ml of water add 1 ml of 10% SDS and 0.2 ml of 10 N NaOH.

*Solution III (Store at 4C) (Alkaline lysis)*

To 60 ml of 5 M KAc (29.44 g) add 11.5 ml of glacial acetic acid and 28.5 ml of distilled water.