QIAGEN Plasmid Maxiprep to Isolate Cholinesterase Expression Plasmids for \textit{In Vitro} Expression in COS-7 Cells

See p. 16 in Qiagen manual attached. In each case in the QIAGEN protocol, where you are given a choice of volumes, use the second, red, larger volume.

Notes to the steps in the procedure. I will do the first two steps for you, then each pair of students will process the 200 mls of final culture. We will isolate expression plasmids from the bacteria for AChE from \textit{D. viviparus} for \textit{in vitro} expression and biochemical characterization.

1. I will incubate a single colony from a fresh streak plate in 3 ml of LB-amp (3 ul of amp stock) in a snap-cap culture tube. It is very important that the streak plate is made the day before the broth culture is inoculated. Thus, if the Qiagen prep is to be done on Thursday, a streak plate should be made on Tuesday; I will do this. I will inoculate the broth culture at approximately 11 A.M. on Wednesday. Incubate the broth culture with vigorous shaking for 8 hours at 37°C.

2. Then I will add a 200 ul aliquot of this starter broth culture to an Erlenmeyer flasks containing 200 ml of LB amp (and 200 μl of amp stock), and grow at 37°C with vigorous shaking for 16 hours (from 7 P.M. until 1 P.M.).

This is where you start. Use RED volumes. Get ice. \textbf{This procedure will take the entire laboratory time.}

3. Use screw-top centrifuge bottles. Drain the tube on a KimWipe. Try to coordinate centrifugations.

4. Resuspend the pellet completely or you will not get a good yield. Use a 5 ml pipetman. Read the directions for using LyseBlue, attached at the end of the protocol. Change tips between reagents in the following steps.

5. No comments.

6. No comments.

7. No comments.

8. No comments.

9. Tape the QIAfilter to the QIAGEN-tip.

10. No comments.

11. No comments.
12. Elute into 35 ml plastic centrifuge tubes.

13. Precipitate DNA by adding 10.5 mls of isopropanol. Mix. Incubate the test tube at -70°C for 20 minutes. Centrifuge as indicated. **Mark the outside edge of the tube facing away from the rotor prior to adding the ethanol. Position the tube correctly in the centrifuge so the mark is in the appropriate position. You may not be able to see the pellet, but do not worry as this is normal.**

14. Delete this wash. Instead, carefully remove the tube from the centrifuge. Do not shake the tube. Pour off the isopropanol into a 50 ml conical tube. Drain the tube on a Kimwipe, tapping gently.

15. Air dry the pellet, which may be at the bottom or as a smear on the side of the tube for 5-10 minutes and dissolve in 100 ul of sterile distilled water. It is very important to wash the sides of the tube by rolling the water around the tube in all areas where the smear is in the tube.

16. Transfer the DNA to a microfuge tube. Label the tube Lungworm AChE, date, initials.

**QUANTIFICATION OF DNA CONCENTRATION**

The DNA is quantified and its purity is assessed spectrophotometrically at 260nm and 280nm. We will use the nanodrop uv-vis spectrophotometer in Dr. Repasky’s laboratory. The spectrophotometer will be configured for use.

1. Follow the instructions for the nanodrop spectrophotometer.
2. Add 1ul water and initialize machine.
3. Wipe the pedestal clean with a Kimwipe, add 1 ul water and blank the instrument.
4. Wipe the pedestal clean, and add 1 ul of sample and read.
5. Wipe the pedestal clean and rinse with some water on a Kimwipe.
6. Write the concentration of DNA on the side of the microfuge tube.
7. The 260/280 ratio determines the purity of the DNA. For pure DNA it is $>1.8$. 