Cycle Sequencing Reaction Clean Up Lab

Purpose: The ethanol precipitation process described below is designed to remove any excess/unincorporated ddNTPs left over from cycle sequencing reaction, because they would migrate in the gel along with the DNA chains ending in a ddNTP, and cause blobs which inhibit the laser's ability to return the signal (= bad sequences in those areas)

1. Label the lid of a sterile 1.5 ml tube (in autoclaved beaker) with number and "cyc seq". Label the side of this tube with number lab instructor's initials. The clean cycle sequencing product will be stored in this tube.
2. Add **5.0 uL Prep solution** to tube.
   Ingredients: 2.0 uL 100mM EDTA
         2.0 uL 3M NaOAc
         1.0 uL Glycogen
3. Transfer the cycle sequencing product to a 1.5 mL tube.
4. Add 60 uL cold 95% EtOH.
5. Centrifuge at 13200 rpm for 15 minutes, then remove supernatant with pipette (avoiding the pellet).
6. Add 200 uL cold 70% EtOH to the *edge* of the tube to avoid dislodging the pellet.
7. Centrifuge at 13200 rpm for 5 minutes, then remove supernatant with pipette.
8. Add 200 uL 70% EtOH to the *edge* of the tube to avoid dislodging the pellet.
9. Centrifuge at 13200 rpm for 5 minutes, then remove supernatant with pipette.
10. Vacuum dry the samples until all of the EtOH has evaporated (15-30 minutes). (Lab Instructor will do this for you.)

Success of both the cycle sequencing reaction and its clean up is assayed by gel electrophoresis using a 6% polyacrylamide gel in the BECKMAN COULTER CEQ 8800 DNA Sequencer which contains capillary electrophoresis tubes and the laser which reads the signals from the fluorescently labeled ddNTPs. Students are not able to operate this instrument due to its delicacy and expense. The Sequencer relays the sequence to a computer and produces a hard copy of the sequence called an electropherogram.

Next lab is Electropherograms, Sequence Analysis/Interpretation, and GenBank. Answer these questions for next quiz, using the Internet site *http://www.ncbi.nlm.nih.gov/*
- For what is NCBI an acronym?
- What is GenBank?
- GenBank contains how many bases from how many species?
- What is BLAST?
- What is an Expect (E) value?
- What does NCBI do?