King Tut DNA Electrophoresis Lab

Protocol:

_____ 1. Place the gel casting tray into the electrophoresis unit so that the rubber gaskets are against the sides of the unit. (Wet the rubber gaskets to help the tray slide into place more easily.) This will create the casting chamber so the liquid agarose will not leak out. Place the six-well comb in the groove at the **end** of the casting tray. The comb makes the wells (holes) in the gel where the DNA will be loaded.

_____ 2. Each group of students needs to weigh 0.32 grams of agarose and add to 20 ml 0.5X TBE in a 125 ml flask. This makes a 0.8% agarose gel. (0.32 grams agarose in 40 ml 0.5x TBE).

_____ 3. Microwave for 30 -60 seconds on high or until the agarose is completely dissolved.

_____ 4. Add an additional 20 ml of 0.5X TBE and mix. (Total volume is 40 ml).

_____ 5. Add 2 drops of Carolina Blu concentrate and mix gently. Be careful not to incorporate lots of bubbles in the mixing process. Allow the agarose to cool slightly before pouring into the casting tray.

_____ 6. Pour the agarose into the gel casting tray in the electrophoresis apparatus. The solidification process will take about 10 minutes.

_____ 7. After the gel is cool and solidified, remove the comb. If the DNA is to be loaded immediately, lift the gel tray out and turn 90˚. Make sure the wells are placed at the end with the black electrode!!! *(If the DNA will be loaded at another time, gels can be stored overnight in a zip lock bag with a small amount of buffer: Slide the gel off the tray into the bag and seal. Remember the gels are fragile so be very gentle putting in and taking out of the bags.)*

_____ 8. Add 300 ml of 0.5X TBE buffer to cover the gel.

_____ 9. Load 20 µl samples from each tube of DNA into the wells. **Be careful not to cross contaminate!** Use a new pipette tip for each DNA sample. Quickly close the lids to the micro-centrifuge tubes to maintain the integrity of the DNA samples.

_____ 10. Place the lid on the electrophoresis unit with the black connector closest to the wells.

_____ 11. **Safety: Use caution here.** Make certain the lid is on the gel before connecting the unit to electricity. Run the gel at 125 Volts for 30-60 minutes, depending on class time.

_____ 12. When the loading dye has traveled at least half the distance of the gel, the DNA has traveled sufficient distance to show distinct separation of the bands. Turn the power supply off; unplug the wires from the power supply; remove the lid from the electrophoresis unit.

_____ 13. Remove the gel from the electrophoresis tank and place the gel in the staining tray.

_____ 14. Add 50 ml of the Final Carolina Blu stain to cover the gel. Periodically move the staining tray back and forth to ensure even staining of the gel.

_____ 15. Using a gloved hand, hold the gel securely while pouring the Final Carolina Blu stain back into the container (the stain can be used up to 8 times). **Do not waste it!**

_____ 16. Rinse the excess stain from the gel with distilled water. Carefully place the gel in the large plastic tray for destaining. Destain the gel by covering with distilled water (Chlorine ions in tap water may affect the dye). The gel can be destained overnight in distilled water. Multiple gels can be placed in the same destaining tray. To identify the gels, notch the corners to provide a unique identification for each student.

Gel Analysis 

1. Draw the gel and label lanes 1-6.
2. Once the gel has been stained and destained, draw the bands produced in each lane.
3. Compare the samples to find patterns. What similarities do you observe that indicate possible family relationships?