DNA Fingerprinting - Week 2

Due to time constraints, samples of the PCR products made last week have already been run on a 1.5% agarose gel. Images of these gels are available in the class folder on the P: drive. You will be able to use these images to determine the D1S80 genotype of all the individuals in the class.

During this lab you will prepare, run and photograph gels for the next class. While the gels are running, you will analyze the data for your class. Consider yourself the technician in a crime lab. The quality of your work will potentially affect the outcome of the trial. Work carefully!!!!!!!!!!!!!!!!!

Separation of D1S80 alleles using agarose gels. You will work in groups of four; each group should have DNA from all class members (14-16 depending on class size), a control with NO DNA, and one unknown. Later, you will analyze a gel that was prepared by the previous class.

1. Add 6µl of 6X gel loading buffer to each sample. This contains a dye that will make it possible to visually follow the progress of the migrating DNA fragments.
   - In the left most lane of the gel, load 10µl of the DNA ladder,
   - Load 20 µl of the control (0),
   - Load 20 µl of the class samples in consecutive lanes of the gel (sample 1-16),
   - Load 20 µl of the unknown sample,
   - Carefully record the contents of each lane.

2. **Gel Prep** - You will be using a 1.5% agarose gel. Each table will prepare one gel using instructions provided.

3. Run the gel at ~ 125v until the bromophenol blue dye (dark blue) is near the end of the gel.

4. Examine the electronic image of your gel on the Chemimager device in the darkroom (3rd floor). Align the front of the wells with the zero mark. Save the image in the appropriate class folder at p: \class\biology\bio110

Why have we gone to all this work? Rumor has it that a member of the class left a partially empty KC Biology Nalgene bottle on the side bench in the lab. We would like to find the owner so we swabbed the lip of the bottle, isolated the DNA and amplified it using PCR. We hope that it will match one of the class members so that we can determine the rightful owner. After all, these really are nice bottles.....

Data Analysis

You are now ready to analyze the gels that were made using your DNA. The first step is to determine the sizes of the fragments. Once this is done you can determine the number of VTNRs for each individual and determine the owner of the Nalgene bottle that was left in the lab.
If you are fuzzy about how to use a standard curve, refer to the information provided at the web site or review the information provided in last week’s lab.

Begin by measuring the distance migrated by each of the fragments in the ladder. Measure from the front of the well to the leading edge of the DNA band. Do the same for each individual’s band(s). Measurements can be done the old fashioned way with a standard ruler or in Scion Image.

Using Scion Image for gel analysis.

To use Scion Image to analyze gels they must be saved in .tif format. Gels should be located in the class folder labeled DNA gels. If not there use the old fashioned way to measure your gels.

- **Open** Scion Image
- **Retrieve** your image from the class folder
- **Optional** - Use this technique if precise measurements are required. **Analyze ➤ Set Scale ➤** select mm ➤ measure a known distance using dashed line ➤ set scale to measured distance ➤ ok
- **Analyze ➤ Options ➤** check only **Perimeter/length**
- If the gel is dark **Edit ➤ Invert** to lighten the image. If you do not invert the image you may want to select “white” in the LUT tool bar.
- Select the diagonal line tool in the tool bar

  - **Draw** a line from the lower edge of the well to the leading edge of the first band in the ladder. A 1Kb plus ladder is shown on the right. Note the relative positions of the bands.
  - Ctrl 1 (makes the measurement) Ctrl 2 opens the box so you can view the data.
  - To make the next measurement, left click on the bottom of the line and move up to the leading edge of the next band. Ctrl 1, Ctrl 2. Repeat to measure the bands in the ladder.
  - Now measure all of the unknown bands proceeding from left to right.

Using Minitab to create a standard curve.

A standard curve to relate the distance DNA migrates to the fragment size can be created using a modification of the regression analysis with a fitted line plot.

\[
C1 = \# \text{ base pairs for each fragment in the 1KB ladder}
\]
\[
C2 = \text{distance migrated by fragments of the 1KB ladder}
\]

```
Stat ➤ Regression ➤ Fitted line plot ➤ Y (C1) ➤ X (C2) ➤ Options
(✓ log10 Y, ✓ display logscale for Y)
```

Provide a caption that is clear. Include the equation for the regression. Edit the axis labels if necessary.

**Note:** the log_{10} base pairs for the unknown fragments can be calculated from the regression equation.
Continue Data Analysis.

C₃ = distance migrated for each class members fragments
C₄ = Code for fragment source
C₅ - Use the \textit{Calc menu} and the regression equation calculated above to determine the \( \log_{10} \) base pairs for the unknown fragments in C₃.
C₆ - Use the \textit{Calc menu} to convert from \( \log_{10} \) base pairs to base pairs by taking the antilog of the data in C₅. You now have the fragment size for each individual calculated in base pairs.
C₇ - Use the \textit{Calc menu} to calculate the number of repeats the D1S80 locus for each individual.

Assume that a fragment with no repeats has 142 bp. Subtract 142 from the number of base pairs in the fragments. Then divide the result by 16 to get the approximate number of repeats for each individual in the class.

How variable were the results? Compare the number of VTNRs you got for each individual to the number other groups got. Can you identify the owner of the Nalgene bottle based on your group data? The class data?

Assignment:

At the end of the class you are expected to submit the following:

- A graph containing the standard curve for a 1KB plus ladder,
- A table showing the fragment sizes and number of VTNR for each class member,
- A captioned/labeled copy of the gel for your table,
- Indicate who contributed the unknown DNA, and defend your answer taking into consideration the variability of the data from the other groups.

The above assignment may be modified by your instructor.