Multicomponent Analysis

Necessary reagents:

- GenomeLAB Human STR Primer Set (PN A20100)
- Taq DNA Polymerase (Amplitaq GOLD or equivalent)
- PCR machine
- 0.2 ml tubes for PCR set up
- 0.2 ml PCR tube racks
- lab marker
- pipettemen
- pipette tips
- beakers for disposal of tips
- 1.5 ml tubes for dilution
- sterile water

Part I: PCR set up using D5S818, D13S317 and Amelogenin loci

Nomenclature for STR loci has to do with chromosomal location. Therefore D5S818 is located on Chromosome 5; D13S317 is located on Chromosome 13. Amelogenin is located on the X and Y chromosomes, although product sizes differ, making the chromosome distinguishable.

D5S818 and D13S317 are simple STRs. For D5S818, there is a repeat unit of $[AGAT]_n$, where $n$ is the number of repeats. From this, then the number of repeat units will determine basepair size of the fragment amplified.

To use an example, if you have 5 repeats of a D5 STR, then you have

$$AGAT \ AGAT \ AGAT \ AGAT \ AGAT$$

which equals 20 basepairs

Then if you have 10 repeats,

$$AGAT \ AGAT \ AGAT \ AGAT \ AGAT \ AGAT \ AGAT \ AGAT \ AGAT$$

which equals 40 basepairs.

Note: Actual product size is determined by PCR Primer locations as discussed in class. For the example above then, if you were comparing 5 repeat unit sample to the 10 repeat unit sample, they would be 20 basepairs in size different from each other (10 repeats being 20 basepairs larger).

We will be amplifying each of these regions in class tonight with our isolated and quantitated human genomic DNA samples. If you failed to isolate suitable amounts of DNA, please see instructor.
1. **EACH STUDENT** will perform **TWO PCR** reactions (or how ever many). Label one tube for each reaction, and remember one for your manipulation blank. Please include your initials on each tube. Use a 0.2 ml tube.

2. For each tube appropriately, each person will need a total of 10 ng of DNA per reaction. If possible this should be done in a volume of 1 ul. Dilution of your DNA samples to 10 ng/uL may be necessary and should be done in sterile water (why?).

For Manipulation blanks (or samples) with no detectable DNA, 1 ul will be taken for amplification.

3. Assemble reactions as described according to the table below. If you have only two samples, individual reactions may be prepared, but if more than 2, making a mastermix with all components except the template DNA (or Manipulation blank) is recommended.

<table>
<thead>
<tr>
<th>Component</th>
<th>1 rxn amount (ul)</th>
<th>Mastermix for X samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>GenomeLab Human STR Primer Set</td>
<td>10 uL</td>
<td></td>
</tr>
<tr>
<td>Taq DNA Polymerase (5 U / uL)</td>
<td>0.6 uL</td>
<td></td>
</tr>
<tr>
<td>DNA template (10 ng/uL)</td>
<td>1 uL</td>
<td>--</td>
</tr>
<tr>
<td>Water (PCR grade)</td>
<td>8.4 uL</td>
<td></td>
</tr>
<tr>
<td><strong>Total reaction volume</strong></td>
<td><strong>20 ul</strong></td>
<td><strong>19 ul/reaction</strong></td>
</tr>
</tbody>
</table>

**NOTE:** Each primer set is tagged with a different fluorescent tag for multicomponent analysis.

4. Perform PCR. PCR parameters are listed below. This will be done in one PCR machine, so all tubes must be prepared and ready before starting. If your preparation is completed before the other students, please maintain your PCR tubes on ice until we are all ready to load the machine.

- 94 C for 5 minutes
- 30 cycles of
  - 94 C for 1 minute
  - 60 C for 1 minute
  - 70 C for 1 minute
- 60 C for 30 minutes
- 4 C HOLD
Part II: Analysis

1. Remove 2 ul of your samples, add 0.5 ul size standard and 37.5 ul sample load solution. If load solution Mastermix is available (likely), just take 38 ul of the mastermix plus the 2 ul of your samples (remember, you will have TWO, your DNA and your MB).

2. Place the appropriate 40 ul into a single well in a multiwell plate (provided). **BE SURE and LABEL** which wells you are using for each sample (so if you add your pooled buccal samples into well A2, label buccal samples or B and your initials. Your manipulation blank samples would go into the next well, A3, labeled MB and your initials).

3. Follow instructions for multicomponent run on Beckman and subsequent analysis.
QUICK Protocol

Part I: PCR
A. Label tubes
   0.2 ml PCR tubes
   Buccal and Manipulation blank
B. Prepare DNA
   Dilute if needed
   10 ng in 1 ul total volume buccal
   Use 1 ul directly from Manipulation blank sample
C. PCR

PART II: Analysis

Take 2 ul from buccal PCR, add to 38 ul Master sample load solution.
Take 2 ul from manipulation blank, add to 38 ul Master sample load solution.

2. Run on Beckman
   Add samples to plate
   Mark plate diagram with your two samples’ locations.

3. Analyzed as instructed.