# NOTES FOR TAS AND INSTRUCTORS, SNP LAB

## Week 1

### General information about the lab

This week in lab, we'll be starting procedures for the **SNP lab**: this will include isolation of cheek cell DNA and setting up PCR reaction for a particular region of the DNA.

Students will **isolate their cheek cell DNA** using a Qiagen kit. They will also set up a **PCR reaction**. The purpose of this lab is to determine the class' variation at a particular SNP (single nucleotide polymorphism) in the human genome. This SNP is in an intron of the cdk3 gene; some people have an "A" at the site, while others have a "G" at the site. We will be amplifying the region of DNA surrounding this site this week. Next week in lab, we will cut the amplified DNA with a restriction enzyme (Hpa II) which will recognize one version of the SNP but not the other.

*Cheek cell isolation*

Do not let students eat or drink for 30 minutes before they begin the **cheek cell isolation** procedure; there is a 15 minute air-drying period right after they scrape their cheek cells when it will be fine for them to eat. Tell the students to swab gently—we do not need blood cells in our samples! The students can air dry their swabs by sticking the handles of their swabs in empty 15-ml conical tubes which will be at each bench. Tell them not to throw these tubes away; we'll re-use them for all the lab sections.

There is only one tube of Proteinase K: TAs or instructors should dispense as students are ready, to be sure we have enough for all the students in the course. This tube can be kept on the side bench at room temperature.

*PCR*

The primers are in the freezer. The PCR master mix (includes dNTPs, TAQ, and buffer) is already in the PCR tubes, in the form of a dehydrated bead in each tube. These PCR tubes (with beads) are at room temperature; have students check to make sure there is a bead present before using the PCR tube.

Warn the students that the PCR tubes are specially designed with thin walls in order to prevent the tubes from insulating the reaction mixture. This makes the tubes pretty flimsy, so they should be quite careful.

You can have students can set up the PCR reactions on ice or using the racks at their bench—each lab table will have a rack with smallish holes for the PCR tubes (normal microfuge tube rack holes are too large). Alternatively, you could have them use normal sized-microfuge tubes as holders for the smaller tubes, and then a normal rack will work. Students should pipette the primers and their DNA into the PCR tubes using the special pipette tips which are stuffed with cotton; these tips are expensive, so there will be one box for P20 tips and one box for P200 tips at each lab bench. Groups across the bench will need to share, and they should NOT use these tips for the rest of the lab protocol.

TAs: please set up two additional PCR reactions using DNA which has already been isolated. Follow the set-up instructions in the lab manual. You can find the DNA in the same box which contains the primers. Students in your lab section will cut & run this DNA in a lane on their gel so they have at least one sample that we know should work and give them heterozygous results.

*Using the PCR Machine*

When you set up the PCR tubes in the PCR machine, ask your students to do two things:

1. Print (neatly) their names in the appropriate circle on the "PCR Machine Sign Up Sheet" for your lab section (see below).
2. After they have done that, they should print their name on the label with the row & column designation corresponding to where they signed the PCR Machine Sign Up Sheet. These labels should stay on the label sheet for now. (Labels are for printing out on Microtube Tough-Tags (from http://www.divbio.com) microfuge tube labels, catalog number TTLW-2016; they are in two files, called "PCR tube labels.doc" and "PCR tube labels part 2.doc".)

To run the PCR Machine: (This will need to be modified for your thermal cycler. The temperature profile we use is available in the student lab handout.)

1. Make sure all the tubes from both lab sections are in the machine. Close the lid of the machine all the way, including bringing the arm forward to lock the lid down.
2. Turn on the power switch on the back right side of the PCR machine.
3. Go to "USER" (push the F5 button).
4. Arrow down to "bio125."
5. Press "ACCEPT" (F1).
6. Press "RUN" (F1).
7. Arrow down to "bio125-beads1."
   *To show students what the cycles look like, you can press "VIEW" (F2) before going on.*
8. Press "START" (F1).
9. Enter 25 µl as the volume of the reaction, using the number pad keys.
10. Press "START" (F1).

Someone (TA or instructor) should return after the PCR reaction is complete and remove the tubes from the thermal cycler, label them, and move the tubes to the white cardboard box for your lab section:

After running the PCR machine, someone (TA or instructor) should come back and do the following:

1. Remove the PCR tubes from the machine, and as you do so, wrap the appropriate label around each microfuge tube (e.g. the tube coming out of the top left-hand corner should get the "A1" label). The labels will need to stick out like flags in order to be read later. (Share labels between the two simultaneous lab sections.)
2. Put the PCR tubes into the cardboard box labeled for your lab section. These boxes contain 96-well plates, which can be used as PCR tube racks; it helps if you can put the tubes in the box in the same arrangement as in the PCR machine.
3. Put the cardboard box into the freezer.

**TA Checklist for SNP Lab Week 1**

<u>After lab:</u>

- ❑ check the pipet tip boxes on the benches; if one is getting low, put a full box out at that bench; go to the stockroom for more tips if needed--try to keep a stash of 3 boxes of each size on the side bench. Make sure the stuffed pipet tips at each table are well-labeled; get an extra box if one looks low.

- check the glove stocks; there should be at least one full box of gloves in each size; if not, get back-up boxes from the stockroom
- check the stocks of microfuge tubes at each lab bench; if one of the glass jars is getting low, add an additional jar from the side bench or stockroom
- do a quick check of student benches, to make sure they're clean & all the extraneous tubes in the racks are thrown away (leave the 15 ml conical tube for drying cheek cells)

## Week 2

This week in lab we'll be doing these main activities:

- restriction enzyme digest of PCR products

  Students will take a subset of their DNA and digest this with Hpa II. Each lab group will also cut a sample of the heterozygote DNA the TAs PCRed last week. They may get close to using all the DNA in their PCR tube; having them spin their tubes down before opening might help. As long as they have a little of their uncut DNA left to run on the gel, they should be okay.

- gel electrophoresis of PCR products, cut and uncut

  Students will run a gel; each lab group will share a gel, with 8 lanes. One lane will contain a 100 bp marker. One lane will contain a sample of the TA-PCRed DNA, cut with Hpa II. The remaining six lanes will consist of cut and uncut DNA from each student in the lab group. The loading dye they're using this week will only contain the larger dye, so we don't have interference with the smaller dye band and the DNA bands we're interested in. ***At the end of lab, have students write their genotype (needs to be TWO letters, AG, AA, or GG) on a scrap of paper and put it anonymously into an envelope. Someone will compile the data for the lab sections so we can report back to the students.

**TA Checklist for SNP Lab Week 2**:

- During lab, you will need to dole out the Hpa II into each tube as the students get them ready. You might keep your own ice bucket handy for this.
- During lab, you will need to pipette the GelStar. Be sure the GelStar is thawed and ready when the students are.
- After lab, check stocks of tips, gloves, and microfuge tubes. Make sure students have thrown away all their used microfuge tubes (except the loading dye).

**DNA sequence surrounding the CDK3 SNP**

primer (or primer binding) sequences in blue
SNP in red, bold (SNP can be G or A)
HpaII cutting site in green dotted line; will only cut if SNP is a G

10 20 30 40 50

AAGGGCGTGT|AGCACAGCAT|AAAGACAGAG|CTAACTCAAT|GAGCGCCACT|TTC

ACAGGGA|AGATAAATAC|TGCACTTATC|CTGGGGGAGG|CTTC|C**G**GGTT|GAACA

ATCAG|TATACCCAAG|CCAGTTGTGT|ACAAAGGTCA|GGAAAGAGAC|CCTGGCCT

TG|GACTCAGAAA|GTGCCAGGGT|TATGTAAGAG|GCTGGCTGAT|GAGGGGAAAC

|TGTAGTCGGA|GCAGCAGCTG|GAGCCCACAT|GCACCTACCA|TGAGCAGGTC|CC

TGCCCTCT|GGCTCCAGAT|TGGGCACAAT|CTCTTCCAGT|CCCTTCCTGG|T

270 280 290 300 311

SNP @ # 96

Cut site between #94 & #95; if cut, leaves fragments of 94 bp and 217 bp

Forward primer: AAGGGCGTGTAGCACAGCATAAAG

Reverse primer: ACCAGGAAGGGACTGGAAGAGATT