UCDNA SEQUENCING FACILITY
Fragment Analysis Information

Services

- The UCDNA Sequencing Facility offers a run-only fragment analysis service on our ABI 3130XL Genetic Analyzer, meaning that you are responsible for analyzing your own data. If you are interested in starting a project please read through this information, then contact us at 530-754-9259 or dnadivas@ucdavis.edu.
- We offer three size standards for your convenience, ROX500, Gel Company ROX500, and LIZ500. ROX500 and Gel Company ROX500 are compatible with filter set D on the 3130XL and the following 3 dyes: 6-FAM, HEX, and NED. LIZ500 is compatible with filter set G5 on the 3130XL and the following 4 dyes: 6-FAM, VIC, NED, and PET.

Sample Submission

- Please be sure that your PCR reactions are optimized before beginning a fragment analysis project (see "Getting Started" below). Once you have optimized your PCR reactions, you will need to schedule your fragment analysis run with us **in advance** as we have multiple customers and limited space.
- On the day of your scheduled run, please provide us with **1µL** of your product in each well of your 96-well plate. **Label your plate and the cover with your name and the date**. We will then add the DI formamide and size standard to each well and provide you with gel running services only on our ABI 3130XL. We will e-mail you the unanalyzed files and then you will be responsible for the analysis and fragment size calling from the files (see "Data Analysis" below).
- Your first 96 samples are complementary "test" runs to get you started so you can see how you are doing on primer optimization. (Feel free to submit these test samples in multiples of 16 at a time if you wish--so not all 96 need to be submitted at once). **We highly recommend that you do a series of dilutions on these free samples to see which dilution/s will work the best.** Each set of 96 thereafter will cost you **$152.00** for UC customers. Non-UC pricing is **$203** per plate of 96 samples. Billing is done on a monthly recharge basis at the end of each month.

Helpful Hints...Getting Started

- Dye-labeled primers are expensive. Therefore, you don't want to order fluorescently labeled primers until you're sure that everything is working properly. You should start your PCR reactions off with unlabeled primers and run them on agarose or acrylamide gels to make sure that your primers behave well, whether developing multiplex reactions or optimizing lane multiplexing (see "multiplexing" below). You want to ensure that each primer behaves identically when run alone and when scored in whichever multiplex strategy you choose. Be forewarned that you will have to re-optimize the reactions for scoring on the 3130XL.
- The best way to design overlap between your primers to maximize the number that can be scored in a single capillary is to do a quick-and-dirty test of your loci by amplifying your unlabeled primers using a mixture of ~25 individuals spanning the diversity of samples likely to be included in the study. This mixed template PCR will provide a smear of amplification that approximates the range of allelic sizes you'll likely encounter in your research and gives you an idea of the approximate range of fragment sizes you'll amplify at that locus. Add 50% of that size range on either side of the observed smear (e.g., a smear encompassing 100-230bp should leave at least 65bp on either side for rare alleles outside the observed range) before you consider stacking another locus labeled in the same dye color above or below that one. **The minimum product size is 76bp and the maximum product size is 489bp** – anything outside this size range will not have sufficient ladder bands to be scored by the machine accurately and should not be used for data analysis.

Next Step...Labeled Primers

- Now that you have optimized your unlabeled primers on a tabletop gel, it is time to order fluorescently labeled primers for your fragment analysis project. Depending on which dye set and size standard you choose, you will have either 3 or 4 dyes available. NED is a proprietary dye which is only available through Applied Biosystems Life Technologies. The other dyes can be purchased through independent oligo vendors such as Operon. Amplification products from PCR with dye labeled primers will fluoresce differently with each dye label (see "multiplexing" below) and will require optimization to equalize the intensities on the gel image.
- We recommend that an initial PCR using final primer concentrations of 0.05, 0.1, 0.25 & 0.5µM be run on a variety of DNA templates across a temperature range of roughly 10°C around primer T_m. Temperature optimization is not necessary but recommended for best results. DNA concentration

and ratio of template to primer can greatly affect amplification and should be standardized across all samples for each locus. Primers used to amplify each locus should have approximately equal intensity prior to multiplexing attempts (see below).

Multiplexing

- Multiplexing is simply the use of a single capillary on a GeneScan run to run multiple independent loci for the same individual. This is made possible by the fact that the ABI collection software detects multiple different colored dyes, and although one dye is used for the internal size standard in each lane, the remaining dyes can be used to label primers amplifying independent loci that produce overlapping fragments. The different dyes have variable signal strengths, and this is something that you need to take into account when developing successful multiplexes. In general, 6-FAM (blue) has the highest intensity, followed by NED (yellow), and HEX (green). The 6-FAM can sometimes over-shadow the others, so as explained above, you will need to vary the amount of each label included in the multiplex reactions to equalize the intensity of each primer label on the 3130XL.
- We recommend that you use a post-PCR multiplex strategy which involves the individual amplification of each locus and the subsequent combining of the completed PCR reactions into one well to be run in a single capillary and scored simultaneously on the sequencer. This also allows post-amplification mixing of loci to overcome problems with particularly weak or strong signals.

Data

- There are three possible analysis software packages available to our customers. A GeneMapper software license is available from Life Technologies for a few thousand dollars. Alternatively, there are two free software packages available to download from our website--STRand (a UC Davis developed software) or Life Technologies Peak Scanner. If you analyze your gel using STRand software, you must apply a matrix to your samples. For the first few gels, until you have everything optimized, you will want to use the “identity matrix” that STRand provides. After you have optimized your samples on the 3130XL, you will want to run your own samples to generate a matrix that will be specific for your data. In order to do this, you must have the three dyes separated into individual wells to serve as a standard. You will need 3 samples with only NED, 3 samples with only HEX, and 3 samples with only 6-FAM (i.e. no multiplexed reactions in these 9 samples). We will add 3 samples with only ROX to this gel. With the remaining 84 spaces, you may run regular multiplexed reactions. When you get this gel back you will need to follow the instructions in STRand to create a new matrix. You can then apply your new customized matrix to your samples from that point on.
- **Note on using STRand with 3130XL files:** When using STRand with 3130XL data files, you don’t use the Add Gel or Find Lanes buttons. Instead, put your sample files from a run into a folder that contains only the sample files and no other folders. In the File menu, click Add Sample Files. This will allow you to select the folder that your sample files are in. There is a setting that cuts off the beginning of the sample to eliminate primer peaks. The default is 3000 but to be safe, you should set it to “0”. Then use the electropherogram to decide where to cut the sample off. Make any adjustments needed and click DNA Type to analyze.

**When submitting your samples for fragment analysis, please aliquot your samples as follows: numbers 1 through 8 will go into wells A1 through H1, samples 9 through 16 will go into wells A2 through H2, etc. Write your name and the date on the plate and then cover your plate with a foil cover and write your name and date on the cover as well.