Preparing Fragment Analysis Samples for 3730 Run

1.) Diluting the Tagged Products
Following the PCR with tagged primers the PCR will need to be diluted – the neat PCR would be off-scale on the 3730. Anytime you add a new primer set to a panel or build a new panel you will need to run dilution tests to see what the optimal dilution is for the primer set / panel. Keep in mind that dilution tests are most useful for samples where the genomic DNA template has been normalized so that you can anticipate the generation of PCR amplicons is the pretty much the same from one sample to the next.

A good way to begin the dilutions is to dilute the PCR 1:5 or 1:10 with water. For a 1:10 dilution for a 10ul PCR add 90ul water, seal, and vortex. Then you will dilute this further to the final optimal dilution which can be anywhere from 1:100 up to 1:600, generally running at 1:200 or 1:300, depending upon how robust the PCR is. For example, for a final 1:300 – take 3ul of your 1:10 diluted PCR and add this to 27ul of water. For a final 1:200 take 3ul of your 1:10 diluted PCR and add this to 57ul of water. For a final 1:300 take 3ul of your 1:10 diluted PCR and add this to 87ul of water. You get the idea.

Dilution tests are run on 8 samples for each new primer set or panel. So if you are working with a new primer set or panel select a column of samples, prepare dilutions of 1:100, 1:200, and 1:300. Then prepare the run plate with the exception of using formamide WITHOUT added Liz. Dilutions runs are free; however we will charge you for any samples which have the Liz standard in them. So pay close attention to the formamide you add to your samples. If you want to add Liz to one sample for each column of eight, you will be billed only for that one sample out of eight that appears with Liz. Thus, for each sample in the dilution run will consist of:

- 10ul formamide
- 7ul distilled water
- 1ul diluted PCR
- 18ul final volume per well

Once you have determined the correct dilution you are ready to dilute all samples run with that primer set/panel. If you are multiplexing individual PCR into a single 3730 run you can combine PCR at this point, diluting each to the appropriate final dilution.

2.) Preparing the Liz Standard / Formamide

Fragment analysis samples are run in formamide and water. In order to maintain the service contract on our 3730 here at Nevada Genomics Center we run all fragment analysis samples using Life Technologies Hi-Di™ Formamide, catalog #4311320. The formamide is available through NGC’s Life Tech cabinet. This is the only formamide you may use for samples brought into NGC for 3730 runs.

The size standard ladder used for the 36cm capillaries in the NGC 3730 is the Life Technologies GeneScan™ 500 LIZ™ Size Standard, catalog #4322682. The Liz Standard is available through NGC’s Life Tech cabinet.

Once you have both the formamide and the Liz you will dilute the Liz using 4.5ul of Liz per 1ml of formamide. We recommend you prepare the formamide/Liz mix as needed, 1ml at a time. Once mixed this should be stored in the refrigerator, wrapped or in a box as the Liz is fluorescent and should be kept from light exposure.

3.) Preparing the 3730 Run Plate

Each sample prepared for a 3730 run will consist of:

- 10ul Liz/formamide
- 7ul distilled water
- 1ul diluted PCR
- 18ul final volume per well
Our 3730 arrays run the odd columns first and then the even columns; **so if you have less than 48 samples please load them in the odd columns only** (1A-1H, 3A-3H, etc…). For example, if you have 32 samples you will have four columns with sample: 1, 3, 5, and 7. Please do not cut the 96 well plates or bring samples in strip tubes; the fragment analysis samples ready to run on the 3730 must be in 96 well plates. If you are unsure whether the 96 well plates you have on hand in your lab are appropriate for our 3730 please email us or stop-by with a plate and we can check it for you.