

# Fragment Analysis FAQ's

## Fragment Analysis: Placing an order

### What fragment analysis services does the facility provide?

The facility uses an ABI 3130-*Avant* Genetic Analyzer to resolve and analyze fluorescently labeled DNA fragments for microsatellite analysis, AFLP and T-RFLP analysis and SNP detection. The following filter sets and dyes are available:

- Filter D: 6-Fam, Hex, Ned, Rox-size std only
- Filter G: 6-Fam, Vic, Ned, Pet, Liz-size standard only
- Filter F: 5-Fam, Joe, Ned, Rox-size std only
- Filter E: for use with Applied Biosystem's SNaPshot kit
- PowerPlex: Fluorescein, JoeA, TMR, CXR

The facility provides the following size standards: GS120 Liz, GS500Rox, GS500Liz, GS1200Liz, ILS-600, and Bioventure 1000 (a 1000 bp Rox labeled ladder). Samples will be analyzed with GeneMapper 5.0.

## How do I place an order?

1. Login to iLab.
2. Find the 'Request for Services' tab, and then locate 'Sample Submission and Laboratory Services' heading, open the VCC DNA Analysis Facility folder.
3. Select the [Fragment Analysis] - Full 96 well plate (per plate) or the [Fragment Analysis] Genescan Run(per sample) and fill out necessary information on the order form. \*\*Note: Please adjust the quantity field in both locations on the order form.

1) Forms and Request Details

May 13 04:26 PM [Fragment Analysis] - Genescan Run (per sample) VCC DNA Analysis Facility

Quantity: 1.0 \$5.74 Billing Status: Not Ready To Work Status: Proposed

View Form Genescan Visible Not Started

**ATTENTION: Please adjust the Quantity above to reflect the number of samples you are submitting for this order. Fill out all information below, SAVE the completed form and then SUBMIT the form.**

Number of Samples (Be sure to change the Quantity field above as well!)

Type of Analysis

- Microsatellite Markers
- AFLP/RFLP
- SNP(SNaPshot)
- Spectratyping

Filter Set

- Filter D:6-Fam-Hex-Ned-Rox-(size std only)
- Filter G:6-Fam-Vic-Ned-Pet-Liz-(size std only)
- Filter F:-5-Fam-Joe-Ned-Rox-(size std only)
- Filter E for use with Applied Biosystem's SNaPshot kit
- PowerPlex:Fluorescein-JoeA-TMR-CXR

Size Standard

- 120bp
- 500bp
- 600bp (CXR only)
- 1000bp (Rox only)
- 1200bp (Liz only)

Download excel Sample Sheet Genescan\_upload.xls

Upload Excel Sheet please upload

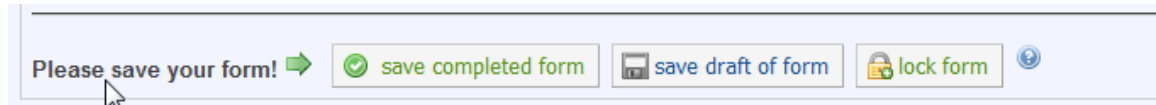
Save Progress

4. All Genescan order forms (not just those in 96 well plates) need to have an excel file attached listing the sample names.

(The first time you place an order you will need to download an excel file template. To do this, find 'Download Excel Sample Sheet' click on the link "Genescan\_upload.xls". You can now keep a copy of this template on your computer. When you want to submit an order use this template to fill in the sample names, save it with your name and the date and then this file can be attached to your online order form. If your samples are in a 96 well plate be sure the sample name matches with the plate location. If your samples are in tubes, disregard the well location and just list the sample names starting in the A1 location.)

5. Fill out all information, browse to locate the excel file with your sample names and then click 'please upload' at the bottom of the page.

When you have completed filling out the service request, please press 'save completed form'



6. Remember to press 'submit request to core'



### **How do I print a copy of my service request?**

After your service request has been submitted, locate the pencil icon on the right hand side of the screen. We suggest that you print to the web format.



### **Where do I put my samples?**

Samples should be put on the top shelf in the refrigerator marked "Fragment Analysis". Please clearly label the rack or 96 well plate with your name and the date. After the samples have been processed they will be moved to the bottom shelf. Please remove old samples!

## When will my samples be run and how will I be notified?

Turn-around is usually 1-2 days. We occasionally experience periods of high demand and it may take 3-4 days. Cancer-qualifying projects will receive priority during these times.

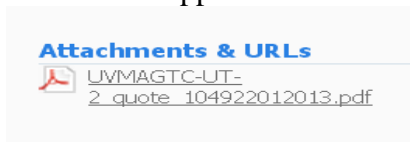
You will receive an email from iLab when your data is ready. We sometimes edit this message to have specific remarks about how your samples ran, so please look at this message!

## How do I download my data from iLab?

You will receive an email from iLab letting you know you have data available. Please login to iLab and navigate to your service request. To find your data, open your service request by clicking the blue arrow.



Your data will be attached to your service request under the heading “Attachments and URL’s in a zipped file.



Service requests are only stored on the iLab system for 18 months. We strongly urge you to download your data to a safe place.

## Fragment Analysis: Sample information

### What dyes can I label my primer with?

Primers can be labeled with 6-Fam, Hex, Ned, Vic, or Pet. If you are multiplexing your PCR, or want to pool PCR products to determine more than one marker per injection, be sure the combination of dyes falls within one of the filter sets below.

Filter D:	Filter G:	FilterF:	PowerPlex:
6-Fam	6-Fam	5-Fam	Fluorescein
Hex	Vic	Joe	JoeA
Ned	Ned	Ned	TMR
Rox (size std only)	Pet	Rox (size std only)	CXR (size std only)
	Liz (size std only)		

### **Can I have more than one marker labeled with the same dye?**

Yes, as long as the expected allele size ranges do not overlap.

### **Can I multiplex my PCR reaction?**

Yes, you can simultaneously amplify multiple DNA targets using multiple primer pairs. You can use the same dye to label more than one target if the expected allele sizes don't overlap. For targets with a similar allele size range, be sure to use different dyes (but from the same filter set). Optimizing a multiplex PCR can be tricky, please see Tim for suggestions if you are having trouble.

### **What size range of fragments can be resolved?**

We can resolve fragments from 35bp to 1kb. The resolution for sizing fragments is best under 600bp.

### **What is the plus A artifact, and how can I get rid of it?**

Plus A peaks are created when the Taq polymerase adds a non-templated nucleotide (usually an A) to the 3' end of the double-stranded DNA. This addition is generally not absolute, a fraction of the amplicons generated receive the 'A' and a fraction do not. This can result in an ambiguity of allele calls.

There are two approaches to addressing this plus A artifact: modification of the thermal cycling conditions or a modification (tailing) of the reverse primer. You can promote the addition of the 'A' peak by increasing the duration of a 72°C final extension from 10 to 30 minutes. This will encourage all fragments to end up in the plus A state. You may need to optimize this for each microsatellite marker. Modifying the reverse primer by adding a 7-base tail can also be done to encourage the addition of the plus A. For more information read the Applied Biosystems User Bulletin: Plus A Artifacts which is available from their website.

### **Does the PCR product need to be cleaned before bringing it to the facility?**

No, the PCR product does not need to be cleaned before bringing to the facility. If you have done your reactions under oil, we do ask that you transfer the sample from under the oil to a new tube.

### **Do I need to dilute my PCR product before bringing it to the facility?**

Yes, you will most likely need to make a dilution. The facility would like to load 1uL of PCR product. If your samples are very strong, with rfu's (relative fluorescent units) greater than 6000, a dilution should be made. The facility is happy to run a few samples for you for free to help you determine what dilution would be best. Generally, a 1/10 or 1/20 dilution is a good place to start.

**I have multiple PCR products that I want to run in a single injection, should I pool the products before bringing to the facility?**

If you are starting a new project, it would be best for you to bring your samples to the facility in separate tubes first. The intensity of emitted fluorescence varies for each dye. 6Fam, Vic and Tet generate higher signals than Ned or Hex. We will want to run them separately first to determine the proper pooling ratio so that all markers will be in a good range (500-4000rfu's). Once we have worked out the conditions, we will ask that you pool the products so that we can take 1uL of pooled product to run on the instrument.

**Fragment Analysis: Format of data**

**What format will my fragment analysis data be in?**

The data files generated have a .fsa extension.

**What software is available to view my fragment analysis data?**

The facility has a copy of GeneMapper® 5.0 that you can use to analyze your microsatellite markers, AFLP/T-RFLP, or SNP data generated using the Applied Biosystems SNaPshot System.

Data can also be analyzed in a new freeware from Applied Biosystems called Peak Scanner™.

To download Peak Scanner™ go to:

[http://www.appliedbiosystems.com/support/software\\_community/free\\_ab\\_software.cfm](http://www.appliedbiosystems.com/support/software_community/free_ab_software.cfm)

Please contact the facility for a tutorial on using these software packages.

5/13/13

