

Hello and welcome to the University of Illinois DNA Services Facility! Here are some highlights of what we offer:

- Analysis of fluorescently-labeled fragments up to 1000bp in length on the ABI 3730x1 genetic analyzers
- High run-to-run size-calling consistency with low background noise
- Fast analysis and automated allele-call editing with GeneMapper® software V4.0
- Quick turnaround (24-36 hours) and low prices

Frequently Asked Questions

1. Which size standard should I use?

There are several size standards available that are labeled with either **ROX** (for 3-dye multiplexing) or **LIZ** (for 4-dye multiplexing) fluorophores. Your choice will be based on both the expected sizes of your fragments and how many dyes you want to multiplex. We stock all size standards here at the facility and they are included in the analysis price. Here is a complete list of our size standards:

- **LIZ120** – analyzes fragments that are 50-120bp in length with at least 4-dye multiplexing
- **ROX400** – analyzes fragments that are 50-400bp in length with at least 3-dye multiplexing
- **ROX500** – analyzes fragments that are 50-500bp in length with at least 3-dye multiplexing
- **LIZ500** – analyzes fragments that are 50-500bp in length with at least 4-dye multiplexing
- **LIZ600** – analyzes fragments that are 50-600bp in length with at least 4-dye multiplexing
- **ROX1000** – analyzes fragments that are 50-1000bp in length with at least 3-dye multiplexing

2. Which fluorescent dyes can I use to label my primer(s)?

Once you have selected a size standard, use this chart to determine which dyes are acceptable for labeling your primers. These are the only dye combinations that are approved by Applied Biosystems for the 3730. For questions about alternative fluorophores, please contact Laura Guest at lguest@illinois.edu or 217-333-9520.

Size Standard Label	Compatible Primer Labels
LIZ120	dR110 dR6G dTAMRA dROX
ROX400, ROX500, ROX1000	6-FAM HEX NED
LIZ500, LIZ600	6-FAM VIC NED PET

3. What DNA concentration should I use?

Unfortunately there is no single answer for every project. Fluorescence on the 3730x1 is **relative**; that is, the intensity (height) of an individual peak is affected by its own concentration *and* the concentrations of other peaks in the sample. To help determine optimal DNA concentrations, we offer one free test plate to each new user. We recommend that you select up to 12 samples that represent a variety of marker sets for your project and perform serial dilutions from 1/2 to 1/64. Load **5uL** of each undiluted sample in the top row, followed by **5uL** of each dilution in successive rows. Please leave row “H” open on the test plate for the control. Use the following chart as a guide:

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	Sample 10	Sample 11	Sample 12
A	undiluted	undiluted	undiluted	undiluted	undiluted	undiluted	undiluted	undiluted	undiluted	undiluted	undiluted	undiluted
B	1/2	1/2	1/2	1/2	1/2	1/2	1/2	1/2	1/2	1/2	1/2	1/2
C	1/4	1/4	1/4	1/4	1/4	1/4	1/4	1/4	1/4	1/4	1/4	1/4
D	1/8	1/8	1/8	1/8	1/8	1/8	1/8	1/8	1/8	1/8	1/8	1/8
E	1/16	1/16	1/16	1/16	1/16	1/16	1/16	1/16	1/16	1/16	1/16	1/16
F	1/32	1/32	1/32	1/32	1/32	1/32	1/32	1/32	1/32	1/32	1/32	1/32
G	1/64	1/64	1/64	1/64	1/64	1/64	1/64	1/64	1/64	1/64	1/64	1/64
H												

4. Do I have to clean-up the samples before I submit them?

No. Purification is rarely needed. A clean-up step is only necessary if the samples have high salt concentrations (usually from RE buffers used in TRFLP analysis) that will interfere with the electrokinetic injection on the 3730x1. The results of the dilution test plate will help us determine if a purification step is required.

5. **What's the required sample volume?**

For low or medium throughput projects, please submit **5-10uL** of each sample in 1.5mL tubes (for low-throughput orders of <96 samples) or in 96-well plates (for medium- or high-throughput orders of 96+ samples). If using tubes, please label the lid of the tube with the sample name and the side of the tube with your last name and the date. Sample names on the tubes must **exactly** match the names entered in the order form.

If using 96-well plates, please label the plate with the order number, your last name and the date. Seal the plate with high-quality adhesive tape such as Qiagen Tape Pads (Cat. #19570) or BioRad Microseal 'B' Adhesive Seals (Cat. #MSB-1001) to prevent evaporation and/or cross-contamination.

6. **How do I submit a low-throughput (LT) or medium-throughput (MT) order?**

At this time, LT and MT orders are processed through the Core Facility. Please use the CoreLIMS website for placing orders and retrieving data. To do so:

- a. Create a login account at <https://unicorn.biotec.uiuc.edu> and login to the site.
- b. Go to the [Payment Manager](#) link (near the bottom) and enter a payment option (credit card or PO#). You only need to do this when you first submit samples or anytime you want to change the payment information.
- c. Back on the main menu, select the [Fragment Analysis Order Form](#) link.
- d. Enter the # of samples to be analyzed (96 is the max per order) and hit SUBMIT.
- e. At the top of the next page, choose a size standard from the drop-down box.
- f. Fill in the table below with sample names, the type of reaction and fluorescent labels of the primer(s). If you have many samples, a FILL button is available for each column of the table to fill down. If you are submitting a 96-well plate, you may find it easier to just label the samples 1-96 by using the "Index" button (in the Sample Name column).
- g. When submitting a 96-well plate, please note in the Comments field whether your samples are organized **horizontally** (A1, A2, A3, etc.) or **vertically** (A1, B1, C1, etc.) in the plate.
- h. When finished, hit SUBMIT at the bottom.
- i. Print out 2 copies of the completed form and send 1 with your samples to:
DNA Core Sequencing Facility
1201 W. Gregory Dr.
334 ERML
Urbana, IL 61801

7. **How do I submit a high-throughput (HT) order?**

You will need to come in, set up a HT project and sign a contract before submitting samples. Please contact Laura at lguest@illinois.edu or 217-333-9520 for more information.

8. **What is the price?**

The price depends on the size of your order and are listed below. Prices include addition of your choice of size standard, electrophoresis on the ABI 3730xl and access to GeneMapper analysis software.

Project Size	UIUC	Other IL universities	Out-of-state universities, government facilities, private companies or international laboratories
Low-Throughput <i>1-95 samples</i>	\$2.94 ea	\$3.23 ea	\$3.68 ea
Medium- and High-Throughput <i>at least 1 96-well plate</i>	\$100.00/plate	\$110.00/plate	\$125.30/plate

9. **How will I know when my data is ready?**

You will receive an automated e-mail from the CoreLIMS site (for LT or MT orders) or the HT pickup site (for HT orders) when your data is ready.

10. **What software can I use for analysis?**

The .fsa files generated by the 3730xl can be analyzed with GeneMapper (Applied Biosystems), PeakScanner (Applied Biosystems), or GeneMarker (SoftGenetics). We provide our customers with free access to the GeneMapper software via a remote desktop connection. To set-up a GeneMapper account, please complete the GeneMapper® Access Form and send it to Laura at lguest@illinois.edu. You will then receive complete instructions on how to set up a connection to the remote desktop.

11. How do I schedule a GeneMapper session?

Go to <http://titan.biotech.uiuc.edu/calendar> and register. Once a web administrator confirms that you are a UIUC customer, you'll be granted access to the scheduler. To reserve a GeneMapper session on the remote desktop, log on to the calendar. Select [Go to the Online Scheduler](#) under 'My Quick Links'. Choose [GeneMapper Remote](#) in the 'View schedule' drop-down box. On the calendar, click on the time slot you want to reserve. In the 'New Reservation' window that appears, choose the starting and ending times of your session (please remember the time limit of 4 hours/day) and click **Save** to make your reservation. The scheduler will send you an e-mail confirming your reservation. Use the 'My Reservations' section on the control panel to view, edit or delete reservations.

12. How do I use GeneMapper on the remote desktop?

Here are basic instructions that will get you started. You may also receive free hands-on training – please contact Laura to set up an appointment.

- a. Log on to the remote desktop using the instructions that were emailed to you.
- b. Retrieve your sample files from the CoreLIMS or HT pickup site. Download the files onto the **remote desktop** (not your computer's desktop).
- c. Login to the GeneMapper software. A blank project window will open up.
- d. Go to [File > Add Samples to Project](#). In the window that appears, select the folder containing your sample files and click [Add To List](#), then [Add & Analyze](#). Enter a unique name for the project and hit OK.
- e. After the software analyzes your samples it will assign PQV (Processed Quality Value) scores to each one. There are 3 different PQVs: **green squares** (Pass), **yellow triangles** (Check) and **red octagons** (Fail). The PQVs provide a visual 'snapshot' of the success of each sample. The PQV columns will be to the right of your sample names. You can proceed with sizing analysis only if all of your PQVs are passing (**green squares**).
 - The first 3 columns – **SFNF** (*Sample File Not Found*), **MNF** (*Matrix Not Found*), and **SNF** (*Size Standard Not Found*) – should **always** have **green squares**. If they do not, please contact the facility.
 - The next column, **OS** (*Off-Scale*), judges the signal intensity of all the peaks in a particular profile. If all of the peaks are within the acceptable RFU (relative fluorescent units) range, the column will have a **green square**. A **yellow triangle** indicates that one or more of the peaks is fluorescing too brightly and need to be checked out. These samples will usually need to be diluted before proceeding. Two main problems result from off-scale samples:
 - i. An off-scale peak can appear split and be sized incorrectly by the software.
 - ii. The excessive signal from an off-scale peak can bleed-over into adjacent capillaries and produce artifacts in other samples ('ghost' peaks that aren't really present).
 - The last PQV column is **SQ** (*Sizing Quality*). With every size standard (except for ROX1000), all samples should have **green squares** in this column. If they have either a **yellow triangle** or a **red octagon**, it means that there is a problem with the size standard for that sample. Please contact Laura on how to troubleshoot this problem.

Note: ROX1000 standard is not manufactured by ABI and therefore does not perfectly fit the best-fit sizing curve built in to GeneMapper. As a result, samples analyzed with the ROX1000 standard will always have red octagons in the SQ column. Customers will need to override this PQV; please see "SQ Override Instructions.doc" for information on how to do this.

We make every effort to help our clients set-up projects and obtain data as quickly as possible. Please don't hesitate to contact me if you have any additional questions.

Sincerely,
Laura Guest
UIUC Core DNA Facility
lguest@illinois.edu
217-333-9520