

Hello and welcome to our facility!

Here are some highlights of what our service offers:

- 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 labeled with either **ROX** (for 3-dye multiplexing) or **LIZ** (for 4-dye multiplexing). Your choice of size standard 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 price. Here is a complete list of our size standards:

- **ROX400** – analyzes 50-400bp fragments; 3-dye multiplexing
- **ROX500** – analyzes 50-500bp fragments; 3-dye multiplexing
- **LIZ500** – analyzes 50-500bp fragments; 4-dye multiplexing
- **LIZ600** – analyzes 50-600bp fragments; 4-dye multiplexing
- **ROX1000** – analyzes 50-1000bp fragments; 3-dye multiplexing

2. What primer dye labels can I use?

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 will work on the 3730.

Size Standard Label	Size range	Primer Dye Labels			
ROX	up to 1000bp	6-FAM	HEX	NED	N/A
LIZ	up to 600bp	6-FAM	VIC	NED	PET

3. At what concentration should my samples be?

Unfortunately, there is no exact answer, so we offer one free test plate to each new user to help determine optimal DNA concentrations, instrument run parameters, etc. 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 dilution into a 96-well plate along with **5uL** of the undiluted sample. 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	full strength	full strength	full strength	full strength	full strength	full strength	full strength	full strength	full strength	full strength	full strength	full strength
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												

Please leave row “H” open on the test plate for the control. Please contact me at lguest@uiuc.edu or 217-333-9520 with additional questions.

4. What’s the required sample volume?

We ask for 5uL of each sample. We only load 1uL onto the machine, but we like to have extra in case troubleshooting is needed. We will store samples for up to 2 weeks after they are run so you may pick up the remainder at any time.

5. **How do I submit an order to the UIUC facility?**

- 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 Request Form" link.
- d. Enter the # of samples to submit (96 is the max per order).
- e. At the top of the next page, choose a size standard from the drop-down box.
- f. Fill out the table with the sample names, type of reaction and fluorescent labels. If you have many samples, a FILL button is available for each column of the table to fill down. Samples name in the site must EXACTLY match those on the tops of the tubes! If you are submitting samples in a 96-well plate, you may find it helpful to use the "Index" button to label them 1-96. Please also don't forget to write in the "Comments" field whether your samples are organized horizontally (A1, A2, A3, etc.) or vertically (A1, B1, C1, etc.) in the plate. When finished, hit SUBMIT at the bottom.
- g. 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

6. **What tubes/plates should I use for submitting my samples?**

- a. If your order is less than 96 samples (low-throughput), we ask that you use 1.5mL tubes. Please label the lid with the sample name and the side of the tube with your last name and the date.
- b. If your order is 96+ samples (medium-throughput), we ask that you use 96-well plates (any kind). Label the side of the plate with your order number, your last name and the date. Seal the plate with high-quality adhesive tape (such as Qiagen cat #19570) to prevent evaporation and/or contamination.

7. **What is the price?**

Prices include addition of size standard, electrophoresis on the ABI 3730xI and access to GeneMapper analysis software.

Project Size	UIUC	Other IL universities	Out-of-state univ or government	Industry
Low-Throughput <i>1-95 samples</i>	\$1.50 ea	\$1.65 ea	\$1.88 ea	\$1.95 ea
Medium-Throughput <i>1-11 96-well plates</i>	\$100.00/plate	\$110.00/plate	\$125.30/plate	\$130.00/plate

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

You will receive an automated e-mail from the server when your data is posted.

9. **How do I access the GeneMapper[®] software on the remote desktop in order to analyze my data?**

Please complete the GeneMapper[®] Access Form and send it to me at lguest@uiuc.edu. I will activate the account and send you complete instructions on how to set up a connection to the remote desktop.

10. **How do I schedule a GeneMapper session?**

Go to <http://titan.biotec.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.

11. **When my scheduled time arrives, how do I begin using GeneMapper on the remote desktop?**

- a. Log on to the remote desktop.
- b. Retrieve your sample files from the CoreLIMS website at <https://unicorn.biotec.uiuc.edu>.
 - i. Click on the "Data Retrieval" link in the Fragment Analysis section. Select your order number from the list and click SUBMIT.

ii. The next page will contain a table of data files for your order. To download results for an individual sample, right-click on the "seq" link next to each sample. To download the whole order at once, click on the "3730 sequence" button at the top of the page, then right-click on the "Download File" link that appears on the next page. The site will then download a .zip file of your data, so you will need to extract the files before proceeding to the next step (the WinZip software is available on the remote desktop).

12. GeneMapper is a completely new to me. How do I get started?

- a. Launch the GeneMapper software and enter your login name and password. A blank project window will open up.
- b. 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 name for the project (make it unique!) and hit OK.
- c. After the software analyzes your samples it will assign PQV (Processed Quality Value) scores to each. The PQVs come in 3 shapes: **green squares** (Pass), **yellow triangles** (Check) and **red octagons** (Fail) and provide a visual 'snapshot' of the success of each sample. You will notice the shapes in 5 columns to the left 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 in them. If they do not, please let me know.
 - The next column, **OS** (*Off-Scale*), judges the quality of the fluorescent signal obtained from all the peaks in a particular profile. If the sample passes and 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 flooding the detector. As a result, it will need to be diluted and rerun before proceeding. Two main problems result from off-scale samples:
 - * An off-scale peak can appear split and be sized incorrectly by the software.
 - * 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 the samples should have **green squares**. 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 us on how to proceed.

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 learn and navigate the GeneMapper software as quickly as possible. We can answer many questions on how to set up projects, obtain basic sizing information, adjust analysis parameters, etc. For any questions that we cannot answer, we will likely refer you to staff at Applied Biosystems, the developers of GeneMapper.

If you have any additional questions, please contact me at lguest@uiuc.edu or 217-333-9520.

Sincerely,
Laura Guest
UIUC Core DNA Facility