GENOMICS SEQUENCING CENTER
Sample Submission Requirements

GSC Sanger Sequencing Services:

Since Watson first discovered the structure of DNA, many advances have been made to enable researchers to study and dissect this macromolecule. Of primary interest for gene discovery efforts is the ability to precisely unravel the sequence of nucleotides comprising genes of interest. This unraveling process, called DNA sequencing, is the main focus of the Fralin Life Sciences Institute (FLSI) Genomics Sequencing Center (GSC).

DNA templates are isolated from a variety of sources, including plasmids and PCR reactions. These templates are then used in conjunction with template-specific primers, fluorescently labeled nucleotides and DNA polymerase to generate labeled fragments of complimentary DNA. These fragments are then analyzed on an automated 48-capillary electrophoresis ABI 3730xl DNA Analyzer and detected by a laser to generate a string of nucleotides representing the DNA sequence of the starting template. Our DNA Sequencing includes our setting up the cycle sequencing reactions, performing clean-up, running the samples on the ABI 3730xl, and delivering the sequence data electronically.

DNA Preparation:

The quality of the starting template is the key factor in the quality of DNA sequence data. Sequencing by capillary electrophoresis is highly sensitive to sample contamination. Potential contaminants include: proteins, RNA, chromosomal DNA, non-specific PCR products, residual salts, organic chemicals (i.e. phenol, chloroform or ethanol), and residual detergents, as well as excess PCR primers, NTPs, enzyme and buffer components from PCR reactions.

PCR products should be purified by one of the following methods and submitted in water, not buffer:

- gel purification
- PCR purification columns (such as Qiagen kits)
- enzymatic treatment (such as ExoSAP-IT)

Please Note:

- Always include the optional PB wash step in the Qiagen kits as an added precaution to remove contaminants that might affect the sequence quality.
- Final elution of DNA must be done in ***ddH20*** rather than TE or the EB provided with the kit. This has been found to minimize potential problems in the sequencing reactions.
- It is very important to accurately quantitate your sample on a good spectrophotometer (50 ug/ml solution of dsDNA gives an A260 = 1) or Nanodrop instrument.

Primer Guidelines:

A critical component in successful sequencing is the design and selection of primers. We recommend the following parameters for custom primers:

- Length of 18-22 bases
- Tm of 55-60oC
- Minimize hairpins
- Minimize primer/dimer formation (self-complementary)
- Custom oligos should be resuspended in water at 3.2 pmol/ul

Sanger Sample Preparation:

- DNA Samples and primers must be combined by the researcher prior to submission according to our specifications in the chart below. Add 10ul of DNA (at concentration listed below) to 3 ul of primer (at 3.2 pmol/ul) for a total of 13ul.

Template Type	Size	Conc. (ng/ul)	Volume to add (ul)		Total Volume to submit (ul)
			DNA Template	Primer - 3.2 pm/ul	
Plasmids	100 (min) - 15,000 bp (max)	**100**	10	3	13
PCR products	100-200 bp	**1**	10	3	13
	200-500 bp	**2**	10	3	13
	500-1000 bp	**5**	10	3	13
	1000-2000 bp	**10**	10	3	13
	> 2000 bp	**25**	10	3	13

- Due to our using an automated liquid handling system for processing samples, we must have the volumes listed above and cannot accept anything less.
- Samples+primers must be submitted in ***0.2 mL PCR 8-tube strips with removable caps (no 12-tube strips)***. You must use strips with *removable caps* due to our using a liquid handling robot to process samples. If you have 48 or more samples, we will also accept PCR plates (see below).
- Each striptube in the submission must be numbered sequentially (as shown below) in the same order as the samples are submitted in the LIMS.
  Do not label the striptubes with the actual sample ID as the tubes are too small for the writing to be legible. Please label one end of the strip with the ***PI's initials***. Tubes must be clearly labeled using black or blue marker.

Example:

- <u>If submitting ***48 or more*** samples, you may submit them in a PCR plate:</u>

  1. Label the plate with the PI's name and the date.
  2. Your LIMS submission ***MUST*** be in the ***exact*** order as the order of the samples in the strip tubes/plate.

  For plates, arrange your samples vertically (A1 through H1, A2 through H2, etc.) as shown below:

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	9	17	25	33	41	49	57	65	73	81	89
B	2	10	18	26	34	42	50	58	66	74	82	90
C	3	11	19	27	35	43	51	59	67	75	83	91
D	4	12	20	28	36	44	52	60	68	76	84	92
E	5	13	21	29	37	45	53	61	69	77	85	93
F	6	14	22	30	38	46	54	62	70	78	86	94
G	7	15	23	31	39	47	55	63	71	79	87	control
H	8	16	24	32	40	48	56	64	72	80	88	control

- When you bring your samples to the collection box in the 2nd floor lobby of Steger Hall, please place all of your tubes (or your plate) in a plastic bag available at the desk. Write the PI's name on the slip of paper provided and place it in the bag. If there will be more than one submission per day from the same lab, please identify in some way which submission the samples go with so we can differentiate between them. Seal the bag securely. Place the bag containing your sample tubes into the collection box on the desk.

Clarity LIMS Registration Instructions:

Please note that in the Clarity LIMS, *anyone* working on a project may register and not just the primary investigator (PI). This will allow the person submitting the samples (Post Doc, graduate students, undergrads, technicians, etc.) to enter their email address and receive the data directly when the results are posted. Please be sure to enter the head of the lab's name and not the Post Doc, grad student, undergrad, or technician's name in the *PI entry space* on the registration form as the billing process is organized according to the PI's name.

The Clarity LIMS may be accessed at: *https://lims.flsi.vt.edu/lablink/Welcome.do*

Registration Instructions:

Log in to *LabLink* in the Clarity LIMS by accessing it at *https://lims.flsi.vt.edu/lablink/Welcome.do*

- The sign-in page will open. In the "*LabLink*" sidebar along the left-hand side of the page, click on the '***Request a User ID***' link.
- Enter all required information (those entry boxes outlined in red) and click '*submit*'.
- Please note that "*Accounts Admin*." Refers to the name of the fiscal person or bookkeeper in your department.
- If information is correct, click '*Agree*'; if information is not correct, click '*Disagree*' and re-enter correctly.
- Log out and wait for the registration approval via email.

The new sample submission sheets and sample submission instructions will be available in the left-hand sidebar on the screen where you submit a project. If you encounter any problems or have any questions, feel free to contact us by email at *flsi-sangerseq-g@vt.edu* or telephone us at (540) 231-1229.

Deliverables:

Upon completion of the sequencing run, you will be notified via email through the LIMS that your results are available for you to download. Log into your LIMS account and save these files to your computer (.ab1 files for Sanger sequencing). There are several software programs available online for viewing this type of sequencing file. We use *Sequence Scanner 2* which is free downloadable software from ABI available through Life Technologies.

Sample Drop-off:

Samples may be dropped off at Steger Hall in the Sanger sample collection box located in the 2nd floor lobby outside of the GSC between the hours of 8:00 am and 4:30 pm. Samples must be submitted in the LIMS and placed in the cooler by 9:00 am to be processed that same day.

When dropping off your samples in the GSC's Sanger sample collection box, please place your samples in a plastic bag (available at the desk). Write the PI's name on the slip of paper provided and place it in the bag. If there will be more than one submission per day from the same lab, please identify in some way which submission the samples go with so we can differentiate between them. Seal the bag securely. Place the bag containing your sample tubes into the collection box on the desk.

If sending samples via mail, pack the samples with ice packs and include an information sheet which has the researcher's name and contact information. Also indicate that the samples are intended for Sanger sequencing. Send the package to:

Genomics Sequencing Center
Fralin Life Sciences Institute of Virginia Tech
1015 Life Science Circle
Blacksburg, VA 24061
Phone: (540) 231-1229

Turnaround Times:

Our turnaround time is 48 hours (Monday through Friday) but we make every effort to complete the work within 24 hours. Our lab is closed on weekends so any Sanger samples submitted after 9:00 am on Friday will not be processed until Monday.

Thank you for using the Genomics Sequencing Center for your sequencing needs!