GENOMICS SEQUENCING CENTER

Sample Submission Requirements

Sanger Sequencing

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. We highly recommend the use of the Qiagen series of DNA prep kits as we have found these kits to give consistently high quality sequencing results*.

> * *This does not represent an endorsement of Qiagen products. There are other products on the market which can give good quality template.*

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.
- ***Please notify us at bi-gscinfo-g@vt.edu if your template is GC or AT rich (>50%) so that we can adjust the chemistry accordingly.***

Sample Preparation

- DNA Samples and primers must be combined by the researcher prior to submission according to our specifications in the chart below:

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

- Primers should have a concentration of ***3.2 pmol/uL*** (see below for more information on primers).
- 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*** (no 12-tube strips) with *removable caps.* If you have 48 or more samples, we will also accept PCR plates (see below).
- Each striptube in the submission must be numbered consecutively (in the same order 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***.

  Example:

- Tubes must be clearly labeled in black or blue marker. Do not use tape!

- If submitting ***48 or more*** samples, you may submit them in a PCR plate:
  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 at the main desk of VBI, 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 write the name of the first sample of the project on the paper as well so we can differentiate between the submissions*]. Seal the bag securely. Place the bag containing your sample tubes into the cooler on the desk.

Primer Guidelines

All primers must now be combined with the sample by the researcher prior to submission. Please submit all primers under the "Custom Primer" heading in the LIMS.

Design 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)
- Provide custom oligos resuspended in water at 3.2 pmol/ul

Design suitable custom sequencing primers using:

PrimerQuest
http://scitools.idtdna.com/Primerquest/

Check your existing primer's Tm, hairpin formation, and more using:

OligoAnalyzer
http://www.idtdna.com/analyzer/Applications/OligoAnalyzer/