# Sanger Sequencing Service Options & Fragment Analysis

Sanger Sequencing is based on sequencing specific genomic regions of interested of no more than 800bp in a single sequence. These sequences will be viewed with chromatograms.

### Requisites for Sanger Sequencing & Fragment Analysis using the SeqStudio:

- User is responsible to have read and agree to our Terms of Agreement.
- If sending more than 16 reactions, use PCR strip tubes or PCR plates.
- If sending less than 16 reactions, use PCR tubes. Do not send 1.5mL tubes.
- If sequencing or genotype PCR products, send copy of agarose picture of product & product quantification using NanoDrop or Qubit.
- If you are performing fragment analysis it is important to perform a dilution test of all samples and depending of the total number of samples this test should be include in the Quote, contact sgf for details.
- Please fill out our **Sample Submission Form** and send it to us via e-mail.

#### This form is mandatory and is located in this page.

- Naming Samples:
  - Name *tubes* using consecutive numbers.
  - In the Sample Submission Form, name samples with consecutive numbers, and keep a file with your sample names for reference.
    - For example:
    - If you have 3 samples, each sample sequenced Forward and Reverse, naming would be:
    - **1**, 2, 3, 4, 5, 6,
    - where 1-3 will be samples 1 to 3, Forward and 4-6 will be the same samples but sequenced with the Reverse primer.
- Ask for a Quote for Sequencing or Fragment Analysis Services at sgf.upr@gmail.com.

# **Product Quantity and Quantification:**

Template Length	Quantity/reaction
100-200 bp	1-3  ng
200-500 bp	3 - 10  ng
500-1000 bp	5 – 20 ng
1000-2000 bp	10 – 40 ng
<2000 bp	40 – 100 ng
Plasmids	200 – 500 ng

# **Sequencing Services:**

## **Full Service Sequencing (FSS)**

For Full Service Sequencing, the user provides their quantified, pure PCR product and primer, each separately. SGF will be charged of purifying the PCR product and perform the sequencing reaction. Currently, the PCR purification method is Exo-SAP (see the "Protocols" tab for details) and the Sequencing Chemistry being used is the Big Dye Terminator v3 from BioAnalytical Instruments.

#### **Ready to Sequence Service (RSS)**

For Ready to Sequence Services, the user provides their quantified and purified PCR product (or miniprep product for plasmids), and primer. SGF will perform Cycle Sequencing accordingly.

## Ready to Load (RTL)

For Ready to Load Services, the user must perform their sequencing reaction and post purification. Samples must be either resuspended in Formamide or dry. These samples must be on a semi-skirted 96 well plate. See Protocols Section for details regarding specific sequencing reagents required).

### We currently provide the following primers and are included in the price.

M13F (-20, -21)	5' TGT AAA ACG ACG GCC AGT 3'
M13F (-41)	5' CGC CAG GGT TTT CCC AGT CAC GAC 3'
MI3R (-27)	5' CAG GAA ACA GCT ATG AC 3'
MI3R (-48)	5' AGC GGA TAA CAA TTT CAC ACA GG 3'
T3 Prom	5' AAT TAA CCC TCA CTA AAG GG 3'
T7 Prom	5' TAA TAC GAC TCA CTA TAG GG 3'
SP6 Prom	5' TAC GAT TTA GGT GAC ACT ATA G 3'
16S RNA F	5' AGA GTT TGA TCC TGG CTC AG 3'
16S RNA R	5' ACG GCT ACC TTG TTA CGA CTT 3'
ITS 4	5' GGA AGT AAA AGT CGT AAC AAG G 3'
ITS 5	5' TCC TCC GCT TAT TGA TAT GC 3'
MatK F	5' TAA TTT ACG ATC AAT TCA TTC 3'
MatK R	5' ACA AGA AAG TCG AAG TAT 3'
rbel F	5' ATG TCA CCA CAA ACA GAG ACT AAA GC 3'
rbel R	5' GTA AAA TCA AGT CCA CCR CG 3'
FishF1	5' CAA CCA ACC ACA AAG ACA TTG GCA C 3'
FishF2	5' CGA CTA ATC ATA AAG ATA TCG GCA C 3'
FishR1	5' TAG ACT TCT GGG TGG CCA AAG AAT CA 3'
FishR2	5' ACT TCA GGG TGA CCG AAG AAT CAG AA 3'

## **Two Options to Submit Samples:**

## Ship samples to (Fedex or UPS):

Att. Silvia Planas Molecular Sciences Research Center Second Floor Carretera #1, Esquina Carr. #8838 Sector El Cinco San Juan, Puerto Rico 00926

Or Personally Drop Off samples using the same address