SAMPLE SUBMISSION GUIDE FOR SANGER SEQUENCING

Registration is mandatory

Registration to our integrated and secured management system is free and can be done at the following address: https://pag.ibis.ulaval.ca/seq/en/enregistrer.php

Once you have registered, you will be provided with a user name and password required to submit samples and access your results. The data in our system is organised by principal investigator and everyone registered under the same PI has access to the data from the entire group.

A payment method is required for sample submission

Customers from Université Laval must supply a valid account number to be invoiced internally. For customers outside Université Laval, we accept most usual payment methods. Below you will find the information that must be provided for each method.

Purchase order: We need the PO number and attach a copy of the PO (in PDF format), particularly if there are special instructions for payment.

Credit Card: We accept Visa and Mastercard. You must supply the contact information (name, address and email) of the person responsible for the payment. A link to a secured payment site will be sent to that person. Please note that we will invoice at the moment we feel confident that we can deliver the complete service under our high quality standards. DO NOT ENTER CREDIT CARD INFORMATION ON OUR SAMPLE SUBMISSION SITE.

Prepayment: We can generate an invoice readily for bank transfer. You must supply the contact information (name, address and email) of the person responsible for the payment. Please note that we will invoice at the moment we feel confident that we can deliver the complete service under our high quality standards.

How to request Sequencing?

To place an order, you must download this Excel file and complete with all the information we need for sequencing your samples.

Send your file by email to seq-request@ibis.ulaval.ca. Shortly after, you will receive a confirmation.

Please write the ticket number provided in the subject line of the confirmation email in the box dedicated to this in the Excel File and print a copy that you will send along with the samples.

Sample preparation

General instructions for preparing DNA

- Sanger sequencing requires particular attention on how the DNA and primers are prepared. It is primordial that you follow attentively the following instructions.
- The DNA and primers must respect the highest quality standards and must be provided in amounts described below.
- **DNA quality** is the most important factor driving the success of Sanger sequencing. Preparations of bad quality containing salts, RNA or other inhibitors will inevitably result in bad reactions.
- DNA can be supplied in nanopure water or in a Tris buffered solution (10 mM Tris pH 8.0). Please avoid using TE as EDTA tends to inhibit polymerases used in sequencing.
- For preparing plasmids, we recommend strains DH5 alpha, DH10B, HB101 or XL1-Blue. Please avoid using strain JM101 and strains from the same lineage.
- We can perform sequencing with general and specific primers.

Suggested procedures

- **Purification**
  - Plasmids, lambda clones (phages), M13 clones (single stranded DNA): Any commercial kits should give high quality material, cesium chloride is also acceptable.
  - PCR products: Commercial kits will perform well. If multiple bands are observed, agarose gel purification is strongly recommended.

- **Quantification**
  - For plasmids, it can be performed using a spectrophotometer at 260 nm **(Important notice: it is impossible to quantify unpurified PCR products this way as primers and nucleotides also contributes to absorbance at 260 nm)**

Solution must be free of RNA, proteins or other contaminants that absorb light at 260 nm. Please verify that the ratios 260/280 and 260/230 are above 1.7 to ensure good quality.

- Fluorometry
- Agarose gel **(recommended for unpurified PCR products)** Done by comparing the intensity of the PCR product to known standards (low or high DNA mass ladder).

- **DNA quality**
  - It is strongly recommended to verify the quality of the DNA on an agarose gel.

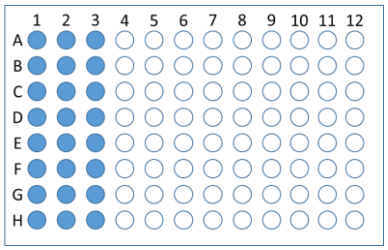
Preparing for shipping

- **Responsibility**
  - Customer have to ensure that their sample meet the platform requirements;
  - We are not responsible for any damage that might occur during transport;
  - We could refuse to perform analysis if samples do not meet requirements;
  - Additional fees could apply if we have to perform adjustments on your samples (changing tubes or concentrations)

- **Vessel specifications**
  - **Purified DNA samples** must be submitted in PCR plates or 1.5 ml tubes (for primers and orders less than 8 samples).
  - In the case of **unpurified PCR products**, semi-skirted PCR plates must be used that are compatible with ABI thermocyclers. They will be used directly to perform ExoProStar™ treatment. An example is given below.

  - Ensure that tubes and plates are labelled with ticket number.

- For incomplete plates, please use a column layout as in the following example:



- Ensure that the plates are sealed correctly to avoid cross contamination during transport. We recommend the use of Abgene 0558 sealing films (AB-0558) or equivalent. We must be able to remove the sealing film to work on your samples.
- Please, do not leave empty wells between samples. However, if you have a complete plate, please leave an empty well. We will use this for our control.

- **Concentrations and volumes**
  - The following table provides requirements depending on the type of material shipped for sequencing. If you want multiple reactions per sample, please adjust accordingly. There is one exception, for unpurified PCR fragments, we can perform 4 sequencing reactions with 5 ul.

DNA type	Concentration	Volume
Phage	100 ng/µl	10 µl
Plasmids	100 ng/µl	10 µl
Purified PCR product		
- Lenght <1 kbp	2 ng/µl	10 µl
- Lenght 1-2kbp	5 ng/µl	10 µl
- Lenght >2kbp	10 ng/µl	10 µl
Unpurified PCR product[1]	>10 ng/µl	5 µl Exactly
DNA single strand	25 ng/µl	10 µl

Note 1 : 4 reactions can be done with 5 µl

Primers

- **General primers**

Below is the list of primers frequently used and provided for free:

Nom	Sequence 5'-3'	Tm
M13 Reverse (-20)[1]	CAG GAA ACA GCT ATG AC	48.5°C
M13 Reverse (-48)[1]	AGC GGA TAA CAA TTT CAC ACA GGA	69.4°C
M13 Forward (-21)[1]	TGT AAA ACG ACG GCC AGT	59.8°C
M13 Forward (-47)[1]	CGC CAG GGT TTT CCC AGT CAC GAC	79.0°C
T3 promoter	CGA AAT TAA CCC TCA CTA AAG G	62.5°C
T7 promoter[2]	TAA TAC GAC TCA CTA TAG GG	51.4°C
T7 terminator	GCT AGT TAT TGC TCA GCG G	60.5°C
SP6 promoter	TAT TTA GGT GAC ACT ATA G	43.5°C
(T)24(A/C/G)[3]	TTT TTT TTT TTT TTT TTT TTT TTT (A/C/G)	53.5°C

[1]For M13 forward and M13 reverse, please provide position. If not specified, we will use M13 Reverse (-48) and M13 Forward (-47) by default

[2] Vectors pCI et pSI from Promega have a T7 promoter primer that is different from the one we supply. The 3' base is different.

[3]For sequencing cDNA clones using the polyA tail (>18As)

- **Specific primers**

They must be supplied by the customer. Please follow these guidelines :

  - The concentration must be at 1.5 µM and we require 5 µl per reaction.

- **Tricks for the design of specific primers**

  Multiple software can help you in this task. Here are the basics for good sequencing primers:

  - High purity (should no longer be an issue with commercial providers)
  - Length: 18-24 bases with a GC content above 50%
  - Melting temperature between 50°C and 60°C
  - Avoid secondary structures
  - Verify that there are no alternative binding sites
  - Avoid mismatches
  - Avoid repeats

Instructions for shipping

Protect the plates to ensure that they will not be damaged during transport. We recommend the use of a styrofoam container and ice packs as a refrigerant. Don't use dry ice as it tends to make seals peel-off. We strongly recommend shipping only on Mondays and Tuesdays.

Please ship at the following address:

Plate-forme d'analyses genomiques

Pavillon CE-Marchand

1030, rue de la Medecine

Local 0147 (Reception des marchandises)

Universite Laval

Quebec (Quebec) G1V 0A6

CANADA

Within Canada

Use an overnight carrier service with next morning delivery.

International

Use the fastest carrier possible. It is important to advise us upon shipping so we can speed up customs clearance. A pro-forma invoice must accompany the package. We can provide a template upon request. Please send carrier information and tracking number to sequencage@ibis.ulaval.ca .