Sanger Sequencing
User Guide

McGill University and Génome Québec Innovation Centre
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**WARNING!**

Sequencing Services reserves the right to refuse any and all samples that do not conform to the guidelines expressed herein without compensation.
Quote Request

Contact the Client Management Office for more information regarding prices or quote requests:

Phone: 514-398-7211
E-mail: infoservices@genomequebec.com

Service Request

To complete a service request:

1. Download the Sequencing Services request form.
2. Carefully complete the form following the given instructions.
3. Send the following to the Client Management Office where applicable:
   a. A copy of the ethics review committee’s approval form(s) for all submitted samples obtained from human subjects.
   b. A Purchase Order number, if it is the chosen method of payment.
4. Print, sign and send completed form by e-mail to the Client Management Office with a copy of the Excel file.

Note: Within 24 hours a password and user name to access Nanuq, Génome Québec’s web application will be forwarded, after which on-line sample submission is possible.

Billing Policy

Sanger Sequencing Services invoices projects at regular intervals:

- Every week for 60 samples or more.
- Once at the end of the month for less than 60 samples.

The information required for billing must be indicated in the Sequencing Services request form.
Method of Payment

Payment of invoices can be done by:

- Check (purchase order number mandatory to be indicated in the sample submission form)
- Credit card
- Wire transfer

See details in the Payment Instructions.

**Important:**
For security purposes do not enter credit card information in the Sequencing Services request form. A separate form will be sent at the time of invoicing.

Sample Preparation and Submission – General Guidelines

It is crucial that the guidelines mentioned in the User Guide be carefully followed so that unnecessary delays can be avoided.

Note that sequencing turnaround time is 2 to 4 working days following the date of reception. However, this may vary depending on demand.
Sample Submission Requirements

Acceptable Tube and Plate Formats

Specific material must be used for sample submission due to technical requirements.

**Only accepted formats:**

- PCR “strip” tubes
- PCR 96-well plates
- PCR 384-well plates

**PCR “strip” tubes with “strip” caps**

- ACCEPTED
  - PCR “strip” tubes

- 2 ml, 1.5 ml or 0.5 ml tubes are NOT ACCEPTED

- Single PCR tubes are NOT ACCEPTED
Unskirted or half-skirt PCR 96-well plates

IMPORTANT!
A 96-well plate is required for all submissions of 48 samples or more.
**PCR 384-well plates**
PCR 384-well plates may only be used for a submission of a full 384-well plate.

**Suggested products**
- **PCR “strip” tubes:**
  - VWR, 0.2 ml PCR strip tubes with 12 well, catalogue number: 53509-300
  - VWR, strip domed caps for 12 well strips, catalogue number: 53509-302
- **PCR 96-well plates:** Thermo Scientific, semi-skirted 96-well PCR plate, catalogue number: AB-1400-L
- **PCR 384-well plates:** Thermo Scientific, 384-well PCR plate, catalogue number: AB-2384
- **Plate sealers:** Thermo Scientific, Adhesive Sealing Sheets, catalogue number: AB-0558

**IMPORTANT!**
Avoid aluminum sealants and sealants that do not withstand freezing.

Some Taq polymerase mixtures contain additives that destroy the adhesive of plate sealants, resulting in leakage and cross-contamination. Consequently, it is strongly recommended that plates be sealed using 8- or 12-capping strips that form a tight seal on each well.
Sample and Primer Organization

**PCR “strip” tubes**

Group all samples one after the other. The tubes must be labelled with the corresponding well ID on the sample submission form: A01, A02, A03, etc.

The same method is applicable to primers. They must be aliquoted in as many PCR “strip” tubes as there are different samples and in the same order as their associated DNA samples. The tubes must be labelled with the corresponding well ID on the sample submission form.

**PCR 96-well plates**

Group all samples one after the other starting with well A01 and following the plate order, from A01 to A12, B01 to B12 and so on.

The primers must be aliquoted in as many wells as there are different samples and in the same order as their associated DNA samples.

They may be added to the sample plate however the primers must be aliquoted after the full set of samples, beginning in the first well of the row below the last samples. For submission of more than 48 samples, a new plate must be used. (See examples below)

Plate 1 of the sample submission form must be completed before beginning plate 2.

**Download examples**

[Excel] [Sample Submission Form - sample and primer organization in a 96-well plate](#)

[Excel] [Sample Submission Form - sample and primer organization in PCR “strip” tubes](#)

A sample to be sequenced with more than one primer must be aliquoted in as many tubes/wells as there are different primers. (See image below)

A primer to be used with more than one sample must be aliquoted in as many tubes/wells as there are different samples. (See image below)

A single submission can contain multiple types of DNA such as, plasmid DNA, purified and non-purified PCR product, phage and BAC DNA. The samples however must be grouped by DNA type. (See image below)

All samples to be sequenced with primers provided by the Sequencing Services (see Primer preparation) must be grouped together and per primer. (See image below)
# Samples and primers submission

Sample submission form for sequencing - 96 well plate or strip tubes - Sequencing Service

**Version 1.7e**

**Service Type:** Sequencing  
**Project Type:** Sequencing

**Sample submission details:**
- **Instructions:**)
  - If the Type of DNA is Primer, only the "Sample Name" and "Project Name" columns are required.
  - Name the file (e.g., example.ddn) and save the file in the Sequencing Service directory.
  - All samples must be submitted in 96 well PCR microplates, with no less than 48 samples.

**Concentration and quantity required for each sample to be sequenced:**

- **Primer/Probe:** Not specified
- ** Primer:** Not specified
- ** Input minimum:** 25 pmol
- ** 5% minimum:** 25 pmol
- ** 30% minimum:** 5 pmol
- ** Input maximum:** 100 pmol
- ** 5% maximum:** 100 pmol
- ** 30% maximum:** 100 pmol

**Oligo-primer:** The primer (50 pmol) products that higher DNA concentrations are required to achieve a good quality of PCR amplification. The primers must be sent to the sequencing service with the samples. For more details consult the Sequencing Service.

**Diluting submissions:** All samples must be submitted in a 96 well PCR microplate, with no less than 48 samples. Absolutely NO 1.5 ml or 8.0 ml Eppendorf tubes.

**Purchase Order Number:** P12345

## Plate or Tube Set Name

<table>
<thead>
<tr>
<th>Well</th>
<th>Sample name</th>
<th>Primer</th>
<th>Project name</th>
<th>Type of UHA</th>
<th>Use of template (base pairs)</th>
<th>Information on UHA</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>P1</td>
<td>P1</td>
<td>example</td>
<td>Not Purified PCR</td>
<td>500</td>
<td></td>
</tr>
<tr>
<td>A2</td>
<td>P2</td>
<td>P2</td>
<td>example</td>
<td>Not Purified PCR</td>
<td>500</td>
<td></td>
</tr>
<tr>
<td>A3</td>
<td>P3</td>
<td>P3</td>
<td>example</td>
<td>Purified PCR</td>
<td>500</td>
<td></td>
</tr>
<tr>
<td>A4</td>
<td>P4</td>
<td>example</td>
<td>Purified PCR</td>
<td>500</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A5</td>
<td>P5</td>
<td>P5</td>
<td>example</td>
<td>Purified PCR</td>
<td>500</td>
<td></td>
</tr>
<tr>
<td>A6</td>
<td>P6</td>
<td>example</td>
<td>Purified PCR</td>
<td>500</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A7</td>
<td>P7</td>
<td>example</td>
<td>Purified PCR</td>
<td>500</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A8</td>
<td>P8</td>
<td>example</td>
<td>Purified PCR</td>
<td>500</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A9</td>
<td>P9</td>
<td>example</td>
<td>Purified PCR</td>
<td>500</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A10</td>
<td>P10</td>
<td>example</td>
<td>Purified PCR</td>
<td>500</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B1</td>
<td>P11</td>
<td>example</td>
<td>Purified PCR</td>
<td>500</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B2</td>
<td>P2</td>
<td>P2</td>
<td>example</td>
<td>Purified PCR</td>
<td>500</td>
<td></td>
</tr>
<tr>
<td>B3</td>
<td>P3</td>
<td>P3</td>
<td>example</td>
<td>Purified PCR</td>
<td>500</td>
<td></td>
</tr>
<tr>
<td>B4</td>
<td>P4</td>
<td>example</td>
<td>Purified PCR</td>
<td>500</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B5</td>
<td>P5</td>
<td>P5</td>
<td>example</td>
<td>Purified PCR</td>
<td>500</td>
<td></td>
</tr>
<tr>
<td>B6</td>
<td>P6</td>
<td>example</td>
<td>Purified PCR</td>
<td>500</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B7</td>
<td>P7</td>
<td>example</td>
<td>Purified PCR</td>
<td>500</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B8</td>
<td>P8</td>
<td>example</td>
<td>Purified PCR</td>
<td>500</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B9</td>
<td>P9</td>
<td>example</td>
<td>Purified PCR</td>
<td>500</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B10</td>
<td>P10</td>
<td>example</td>
<td>Purified PCR</td>
<td>500</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Primers provided by the Sequencing Service:**
- TT, T2, UPA, K2K, 14K, Beinwaks, 17Normac, pGEM, pGEM R
Physical organization of samples and primers

Samples and primers must be organized in exactly the same positions as indicated in the sample submission form.

### Clearly label samples and correctly seal plates and/or cap tubes

Clearly label each tube with a well ID number: A01, A02, A03, etc.

#### How to identify and seal both plates and tubes?

Samples and primers tubes must be identified with the sample submission form reference well ID: A01, A02, A03, etc.

Proper capping of PCR « strip » tubes with their matching « strip » caps will help avoid evaporation which can negatively impact sequencing results.

It is mandatory to submit 48 or more samples in a 96-well plate.

Label the plate with the exact plate name as indicated in the sample submission form. Do not label the plate cover nor the plate seal.

Evaporation can negatively impact sequencing results. This can be avoided by proper sealing of plates with a good quality plate seal that can withstand freezing. Make sure that the perimeter of each individual well is adequately sealed.

**NB:** Some Taq polymerase mixtures contain additives that destroy the adhesive of plate sealants, resulting in leakage and cross-contamination. Consequently, it’s strongly recommended that plates be sealed using 8- or 12-capping strips that form a tight seal for each well.
WARNING!
Do not use parafilm or tape to seal the plate, nor to package plates and/or tubes together.

What NOT to do!

PCR 384-well plates
Only full 384-well plates can be submitted in the 384-well plate format.

The primers must be aliquoted in a 384-well plate and must be in the same order as their associated DNA samples.
Sample Submission Form
Please complete the sample submission form as recommended below.

| Sample Name: | Each sample name should be unique such that two sequences done with the same template but different primers can be easily differentiated from each other. |
| Primers: | Only one primer per well is permissible. |
| Purchase Order Number: | is generated by your institution's accounting department |
| Name given to the plate or collection of tubes: | Please give each plate or group of tubes a unique name. |
| Fragment size: | BAC DNA generally between 60,000 and 250,000 base pairs. Indicate the fragment size of submitted PCR products. |
| Type of DNA: | Choose the DNA type from the scroll down menu provided. Do not directly type it in. |
| Project Name: | Fill in the exact same project name chosen when the account was opened. |

<table>
<thead>
<tr>
<th>Veil</th>
<th>Sample name</th>
<th>Primer</th>
<th>Project name</th>
<th>Type of DNA</th>
<th>Size of template</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A</td>
<td>A1</td>
<td>P1</td>
<td>example</td>
<td>BAC</td>
<td>500</td>
</tr>
<tr>
<td>1B</td>
<td>A2</td>
<td>P2</td>
<td>example</td>
<td>BAC</td>
<td>500</td>
</tr>
<tr>
<td>1C</td>
<td>A3</td>
<td>P3</td>
<td>example</td>
<td>BAC</td>
<td>500</td>
</tr>
</tbody>
</table>

Note: The table contains example data and should be filled in according to the instructions provided.
DNA Sample Preparation

Volume and concentration required per sequencing sample:

<table>
<thead>
<tr>
<th>Volume</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unpurified PCR*</td>
<td>20 μl minimum</td>
</tr>
<tr>
<td>Purified PCR</td>
<td>7.5 μl minimum</td>
</tr>
</tbody>
</table>

**PCR amplifications must be verified on an agarose gel** and a copy of the gel picture must be sent to the sequencing service with the samples. **PCR products of less than 250 bp** result in lower quality sequences due to an oversaturation phenomenon whereby they appear much more intense than usual and to the compression of bases at the beginning of the sequencing read, which is an inherent limitation of the 3730xl technology.

**PCR products greater than 2000 bp** can be difficult to sequence because the concentration of DNA submitted is generally too low. The longer the PCR products the higher DNA concentrations are required to achieve good quality sequencing.

### Double bands

Even faint unspecific bands can negatively impact sequencing results. To eliminate the unspecific band, the PCR conditions must be optimized. As a last resort use a gel extraction kit to purify the band of interest. In that case send the gel agarose picture of the PCR products after the gel extraction to the sequencing service.

### Unspecific band

Verify that the band has the expected size.

### Required bands

Only one bright bands.

### Faint bands

Could result in low quality sequences. Reoptimize the PCR.

<table>
<thead>
<tr>
<th>Volume</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasmid DNA or phage</td>
<td>7.5 μl minimum 100–500 ng/μl</td>
</tr>
<tr>
<td>BAC/PAC end sequencing</td>
<td>20 μl minimum 500 ng/μl minimum</td>
</tr>
</tbody>
</table>

*Purification of PCR products (the removal of unincorporated dNTPs and unused PCR primers) is included with the sequencing service.

- **PCR products** requiring purification must have one amplified product (only one band on the gel) because an additional amplification product cannot be eliminated by the purification step.
- Be careful to remove all traces of Phenol/Chloroform or Ethanol from plasmid DNA samples. Resuspend DNA in 10 mM Tris-HCl (pH 8) or water. Do not use solutions containing EDTA.

- It is **very important to verify the quality and quantity of the sample** even though DNA Extraction Kits from established brand names give very good results when used in accordance to manufacturers’ instructions. The OD260/OD280 must be between 1.7 and 1.9.

**IMPORTANT!**
It is the responsibility of the client to provide an adequate amount of quality DNA. (see Troubleshooting).
Primer Preparation

Concentration and volume required per sequencing sample:

<table>
<thead>
<tr>
<th>Volume</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primer</td>
<td>10 µl</td>
</tr>
</tbody>
</table>

The sequencing service provides the following standard set of common primers free of charge:

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>T7</td>
<td>5’ - TAATACGACTCCTATAGGG - 3’</td>
</tr>
<tr>
<td>T3</td>
<td>5’ - AATTAACCTCACTAAAGGG - 3’</td>
</tr>
<tr>
<td>SP6</td>
<td>5’ - TATTTAGGTGACACTATAG - 3’</td>
</tr>
<tr>
<td>M13 forward</td>
<td>5’ - GTAAAACGACGGCCAGT - 3’</td>
</tr>
<tr>
<td>M13 reverse</td>
<td>5’ - GGAAACAGCTATGACCAGT - 3’</td>
</tr>
<tr>
<td>BGH reverse</td>
<td>5’ - TAGAAGGCACAGCGGAGG - 3’</td>
</tr>
<tr>
<td>T7 terminator</td>
<td>5’ - GCTAGTTATTGACTACACGG - 3’</td>
</tr>
<tr>
<td>pGEXF</td>
<td>5’ – GGGCTTGGAAGCCACGTTTGTGTCG - 3’</td>
</tr>
<tr>
<td>pGEXR</td>
<td>5’ – CCGGGAGCTGACTGTCAGAGG - 3’</td>
</tr>
<tr>
<td>CMV-F</td>
<td>5’ - CGC AAA TGG GCG GTA GGC GTG - 3’</td>
</tr>
</tbody>
</table>

Be aware that when selecting a sequencing primer:

- Resulting sequences are only clearly readable 30 to 60 bases from the 3’ end of the primer.
- Good quality template provided in sufficient amounts can produce up to 800 bases of good quality sequence.
- It is the responsibility of the customer to determine which primers are required to sequence the samples.

The correct designing of a sequencing primer is essential for good results:

- Primer length should be between 18 and 24 bases.
- G/C ratio should be between 40 to 60%.
- Primer annealing temperature must be greater than 50°C.
- Avoid designing primers upstream of homopolymeric or heteropolymeric regions (A, C, G or T repeats) because they are extremely difficult to sequence.

Note:
Sequencing Services does not offer a service of primer synthesis.
The following Web site for primer design: [http://frodo.wi.mit.edu/primer3/](http://frodo.wi.mit.edu/primer3/) is highly recommended.
Sample Submission

On-line Sample Submission

Access to the web application, Nanuq, is given once an account has been opened. After which sequencing samples must be submitted on-line via this application.

[PDF] “How to submit samples”

Where to Send your Samples

The samples can be sent by mail or delivered in person between 9:00 AM and 5:00 PM, Monday to Friday.

BaseXpress service samples must arrive at the laboratory before 11:00 AM, Monday to Friday.

Samples must be addressed to:

**Sanger Sequencing Services**
McGill University and Génome Québec Innovation Centre
740 Dr.-Penfield Avenue, Room 7300
Montréal, QC, H3A 1A4
Tel: 514-398-3311, ext. 00522

**IMPORTANT!**
The waybill from the online submission step must be printed and included in the package.

Sequencing Services is not responsible for samples that arrive in damaged plates or “strip” tubes, that have evaporated, arrived late due to shipping delays or delays resulting from mislabelled submissions.

All samples are kept at 4°C for a maximum of two weeks after having been sequenced. Unclaimed samples will be discarded.
Transmission of Results

All the results are directly available on the Web application Nanuq and may be viewed and downloaded as chromatograms, FASTA text and GenBank text.

Downloaded samples may be viewed using Chromas. A free software version for PCs is available at the following address: [http://www.technelysium.com.au/chromas.html](http://www.technelysium.com.au/chromas.html).

Nanuq sends an automatic message when the results are available to the individual who submitted the samples.

Sequencing turnaround time is 2 to 4 working days following the date of reception. However, this may vary depending on demand. It is crucial that the guidelines be carefully followed so that unnecessary delays can be avoided.

All sequences are available on Nanuq for a minimum of one year. After this time, they are archived but can be retrieved upon request. Customers may request the removal of their data from Nanuq at any given time.
Samples of good quality with recommended concentrations usually generate up to 800 bases of good quality sequence.

Short PCR fragments, less than 250 bp, result in lower quality sequences due to an oversaturation phenomenon whereby they appear much more intense than usual and to the compression of bases at the beginning of the sequencing read, which is an inherent limitation of the 3730xl technology.

For more on troubleshooting, consult the tutorial:

[PDF] Results Interpretation and Troubleshooting Tutorial

Note: If sequencing fails, a limited number of samples will be tested upon request free of charge.

The reactions will be repeated at no extra cost if it is determined that their failure was due to a problem with the equipment, the sequencing reaction kit or human error.

Failed reactions due to the following reasons are responsibility of the customer and will be invoiced as such:

- samples or primers not submitted according to specified requirements
- poor template quality
- poor primer quality
- presence of secondary structure
- presence of homopolymeric or heteropolymeric sequences
- treated DNA samples (for example, bisulphite treated)

For any questions about the results, contact Sequencing Services.
For More Information

Client Management Office

McGill University and Génome Québec Innovation Centre

Phone: 514-398-7211
E-mail: infoservices@genomequebec.com

Sanger Sequencing Services

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