GenomeQuébec CES | CENTRE D'EXPERTISE ET DE SERVICES

Centre d'expertise et de services Génome Québec

FEBRUARY 6, 2024

SEQUENCING SERVICES

Centre d'expertise et de services Génome Québec

User Guide : Sanger Sequencing

Version 4.3

Table of Contents

WARNING!

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

Service Request

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 Service request form.

2. Carefully complete the form following the given instructions.

3. Print, sign and send completed form by e-mail to the Client Management Office and include also:

   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.

Note: Within 24 hours a password and username 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 Service 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 Service 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

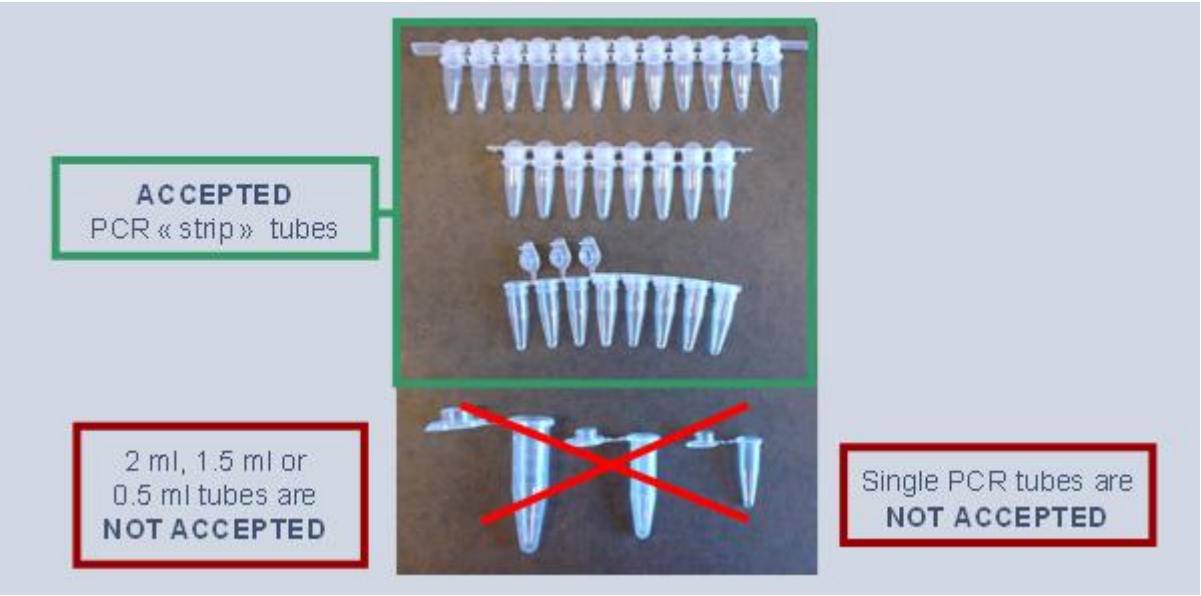
Acceptable Tube and Plate Formats

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

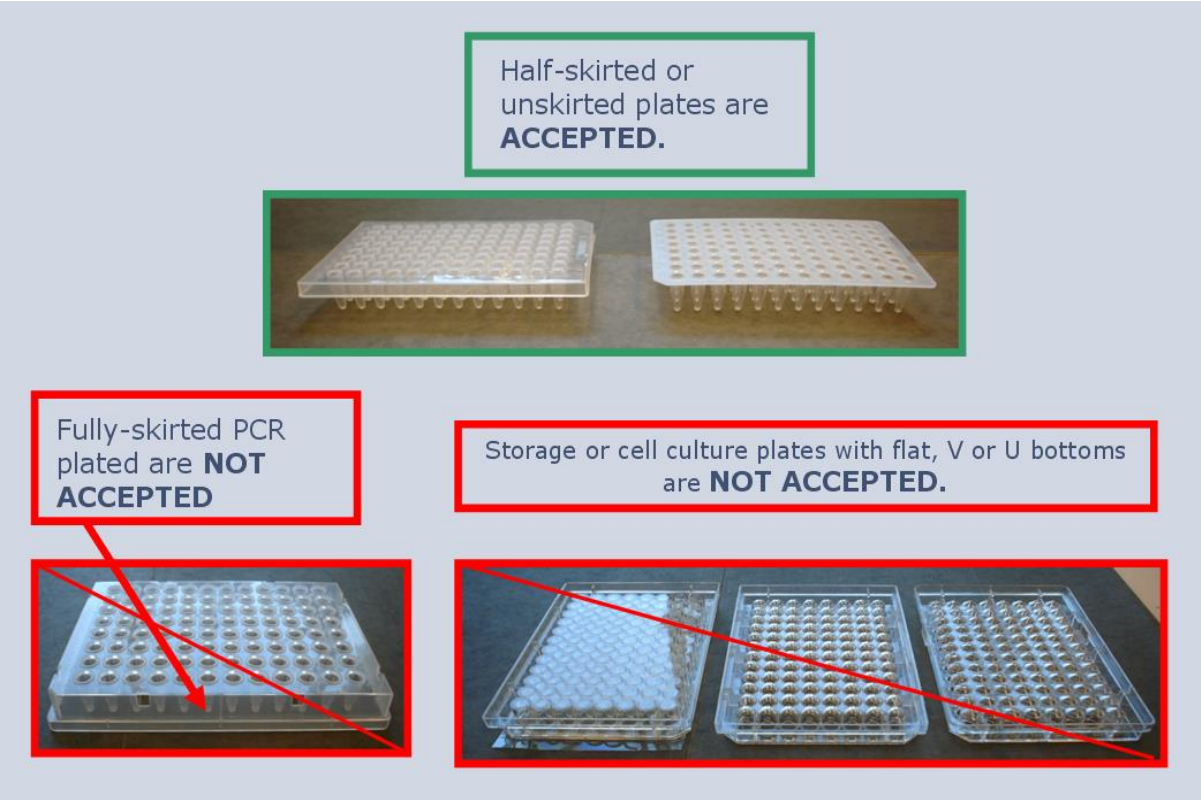
PCR "strip" tubes with "strip" caps



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.

PCR 384-well plate are **ACCEPTED**

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 96-well 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.

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.

Examples for sample and primer submission

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 Service (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

Instructions All fields are mandatory except for **'Information on DNA'**
If the Type of DNA is 'Primer', only the 'Sample Name' and 'Project Name' columns are required
Names must be 50 charaters or less, and can have only alphanumeric characters including the following 3 characters: - (hypen), _ (underscore) and .(dot)

Concentration and quantity required for each sample to be sequenced

Plasmid / Phage	Not purified PCR	Purified PCR	BAC	Primer
7.5 µl minimum	20 µl minimum	7.5 µl minimum	20 µl minimum	10 µl minimum
100 to 500 ng/µl	The longer the PCR products the higher DNA concentrations are required to achieve good quality sequencing. 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. For more details consult our User Guide.		500 ng/µl minimum	5 µM

Primers provided by the Sequencing Service
T7, T3, SP6, M13F, M13R, BGHreverse, T7 terminator, pGEXF, pGEXR

Obigatory submissions format: all samples must be submitted in a 96 well PCR microplate or if there are less than 48 samples, they may be submitted in strip tubes. **'Absolutely NO 1.5 ml or 0.6 ml Eppendorf like tubes'**

Purchase Order Number	P012345
1 - Plate or Tube Set Name	Example_1.2

Well	Sample name	Primer	Project name	Type of DNA	Size of template (base pairs)	Information on DNA
A01	PCR1_P1	P1	example	Not Purified PCR	500	
A02	PCR1_P2	P2	example	Not Purified PCR	500	
A03	PCR1_P3	P3	example	Not Purified PCR	500	
A04	PCR2_P1	P1	example	Purified PCR	650	
A05	PCR2_P2	P2	example	Purified PCR	650	
A06	PCR2_P3	P3	example	Purified PCR	650	
A07	plasmid1_T7	T7	example	Plasmid	1000	
A08	plasmid2_T7	T7	example	Plasmid	1000	
A09	plasmid1_T3	T3	example	Plasmid	1000	
A10	plasmid2_T3	T3	example	Plasmid	1000	
A11						
A12						
B01	P1		example	Primer		
B02	P2		example	Primer		
B03	P3		example	Primer		
B04	P1		example	Primer		
B05	P2		example	Primer		
B06	P3		example	Primer		
B07						
B08						
B09						
B10						

Physical organization of samples and primers

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

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

Version 1.7e
Service Type: Sequencing
Project Type: Sequencing

Instructions All fields are mandatory except for "**Information on DNA**"
If the Type of DNA is 'Primer', only the 'Sample Name' and 'Project Name' columns are required
Names must be 50 chararters or less, and can have only alphanumeric characters including the following 3 characters: - (hypen), _ (underscore) and .(dot)

Concentration and quantity required for each sample to be sequenced

Plasmid / Phage	Not purified PCR	Purified PCR	BAC	Primer
7.5 µl minimum	20 µl minimum	7.5 µl minimum	20 µl minimum	10 µl minimum
100 to 500 ng/µl	The longer the PCR products the higher DNA concentrations are required to achieve good quality sequencing. 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. For more details consult our User Guide.		500 ng/µl minimum	5 µM

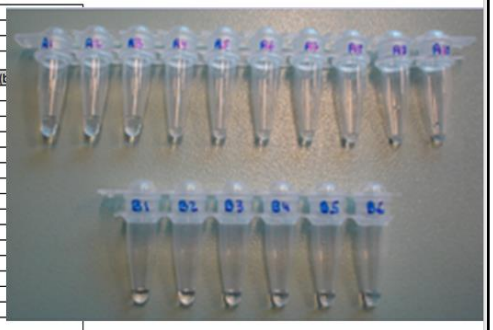
Primers provided by the Sequencing Service
T7, T3, SP6, M13F, M13R, BGHreverse, T7 terminator, pGEXF, pGEXR

Obligatory submissions format: all samples must be submitted in a 96 well PCR microplate or if there are less than 48 samples, they may be submitted in strip tubes. ***Absolutely NO 1.5 ml or 0.6 ml Eppendorf like tubes***

Purchase Order Number	P012345
1 - Plate or Tube Set Name	Example_1.2

Well	Sample name	Primer	Project name	Type of DNA	Size of template (b
A01	PCR1_P1	P1	example	Not Purified PCR	500
A02	PCR1_P2	P2	example	Not Purified PCR	500
A03	PCR1_P3	P3	example	Not Purified PCR	500
A04	PCR2_P1	P1	example	Purified PCR	650
A05	PCR2_P2	P2	example	Purified PCR	650
A06	PCR2_P3	P3	example	Purified PCR	650
A07	plasmid1_T7	T7	example	Plasmid	1000
A08	plasmid2_T7	T7	example	Plasmid	1000
A09	plasmid1_T3	T3	example	Plasmid	1000
A10	plasmid2_T3	T3	example	Plasmid	1000
A11					
A12					
B01	P1		example	Primer	
B02	P2		example	Primer	
B03	P3		example	Primer	
B04	P1		example	Primer	
B05	P2		example	Primer	
B06	P3		example	Primer	
B07					
B08					
B09					
B10					

The samples are aliquoted in tubes labelled A01 to A10.



The primers are aliquoted in tubes labelled B01 to B06.

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

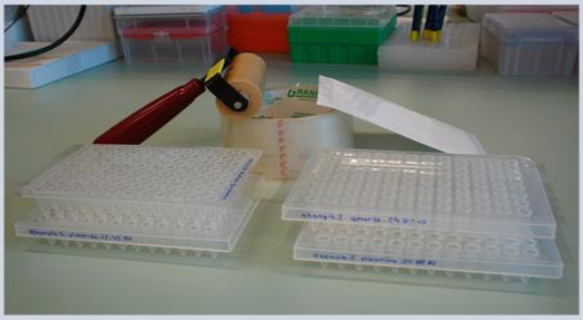
Clearly label each tube with a well ID number: A01, A02, A03, *etc*.

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.



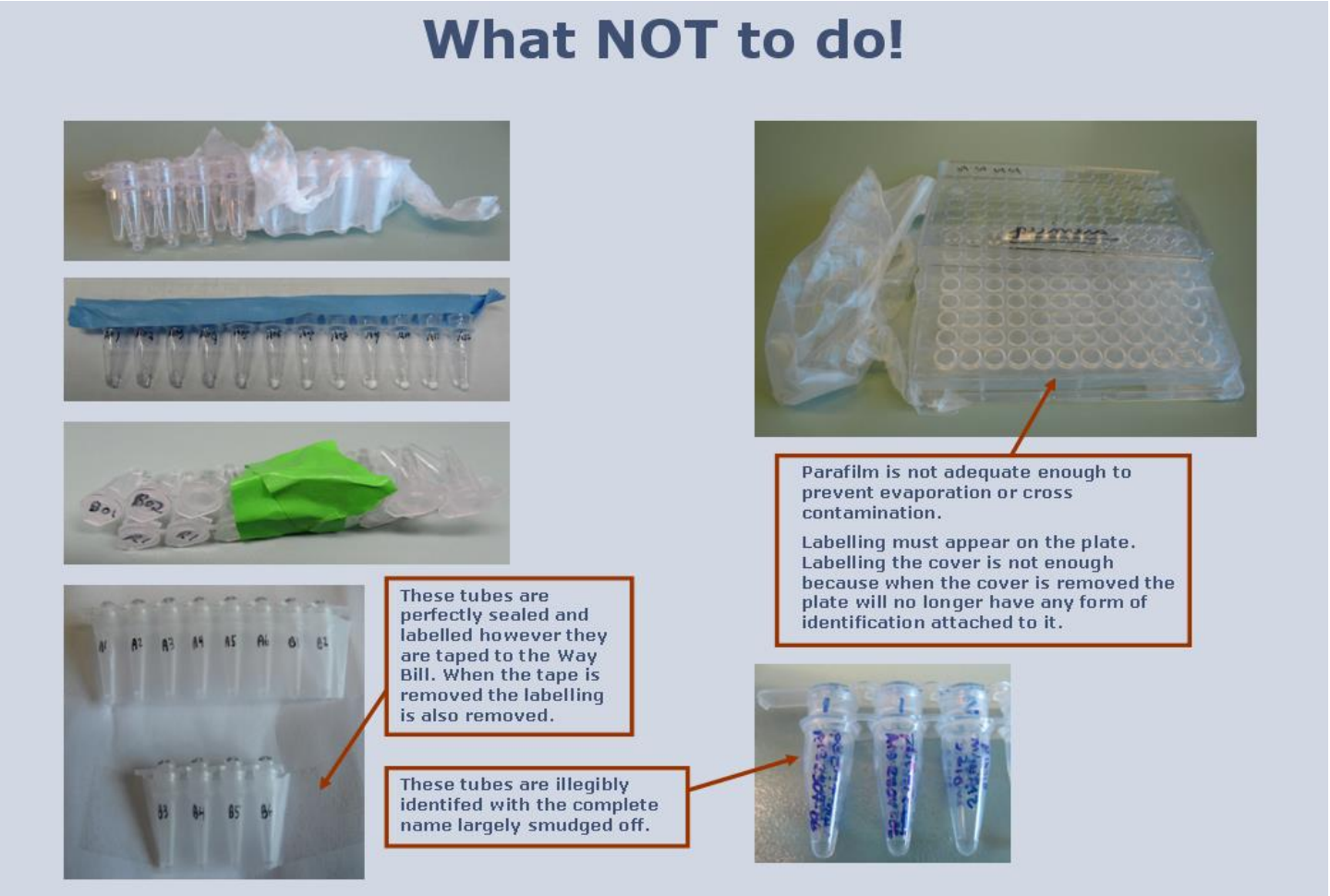
Label the plate with the exact plate name as indicated in the sample submission form. Do not label the plate cover nor the plate seal.

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 recommend that plates be sealed using 8- or 12-capping strips that form a tight seal for each well.

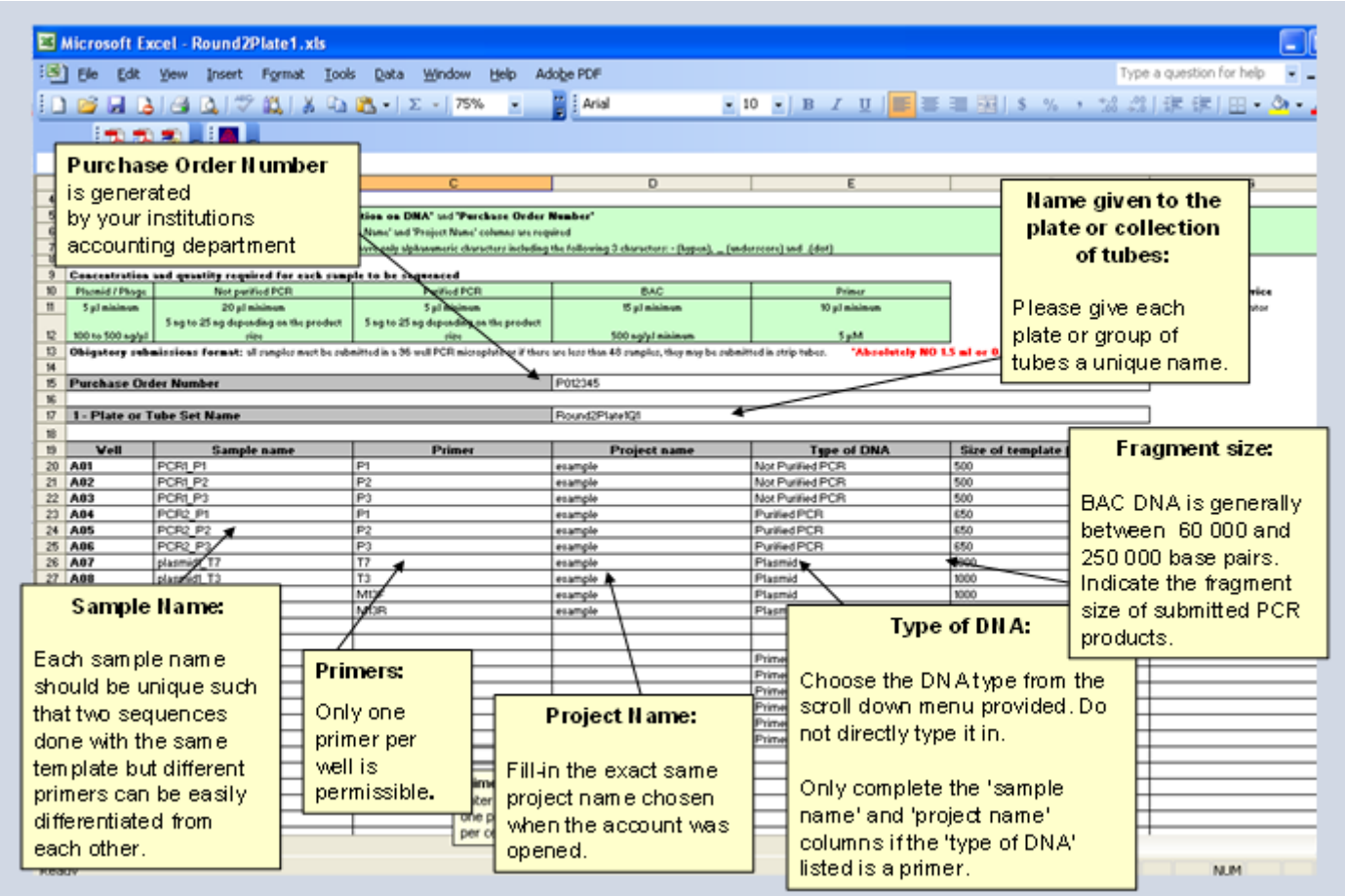
WARNING!

Do not use parafilm or tape to seal the plate, nor to package plates and/or tubes together.



Sample Submission Form

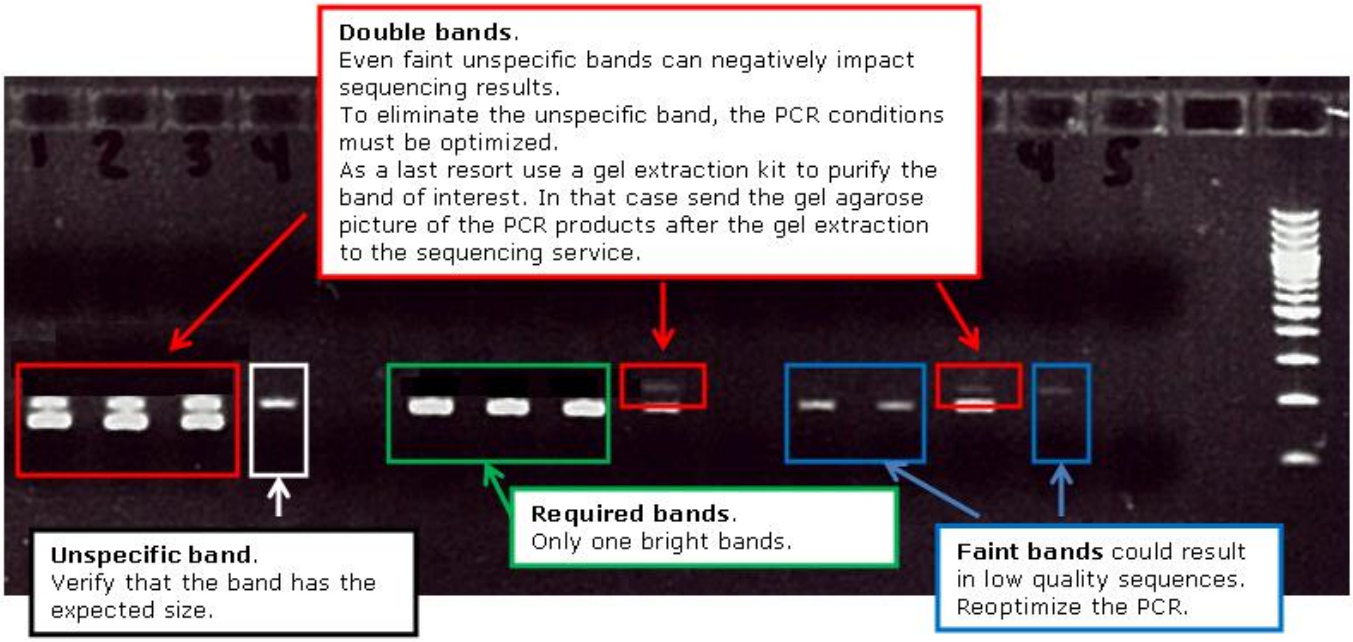
Please complete the sample submission form as recommended below.



DNA Sample Preparation

Volume and concentration required per sequencing sample:

	Volume	Concentration
Unpurified PCR*	20 µl minimum	**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.
Purified PCR	7.5 µl minimum	



	Volume	Concentration
Plasmid DNA or phage	7.5 µl minimum	100–500 ng/µl
BAC/PAC end sequencing	20 µl minimum	500 ng/µl minimum

*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:

	Volume	Concentration
Primer	10 µl	5 µM

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

T7	5′ - TAATACGACTCACTATAGGG - 3′
T3	5′ - AATTAACCCTCACTAAAGGG - 3′
SP6	5′ - TATTTAGGTGACACTATAG - 3′
M13 forward	5′ - GTAAAACGACGGCCAGT - 3′
M13 reverse	5′ - GGAAACAGCTATGACCATG - 3′
BGH reverse	5′ - TAGAAGGCACAGTCGAGG - 3′
T7 terminator	5′ - GCTAGTTATTGCTCAGCGG - 3′
pGEXF	5′ – GGGCTGGCAAGCCACGTTTGGTG - 3′
pGEXR	5′ – CCGGGAGCTGCATGTGTCAGAGG - 3′
CMV-F	5' - CGC AAA TGG GCG GTA GGC GTG - 3'

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 Service does not offer a service of primer synthesis.
The following Web site for primer design: 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

IMPORTANT!

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

Sequencing Service 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.

Address for sending samples

Please refer to the waybill for instructions on how to ship your samples.

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.

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.

Troubleshooting and Resequencing Policy

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 Service.

For More Information

Client Management Office

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

Sanger Sequencing Service

Phone: 514-345-4931 Ext.: 3088
E-mail: sangersequencing@genomequebec.com