GenomeQuébec CES | CENTRE D'EXPERTISE ET DE SERVICES

Centre d'expertise et de services Génome Québec

Effective date: 2024-04-25

SEQUENCING SERVICES

User Guide

Preparation of Amplicons Librairies

Illumina Technology

Version 06

Table of Contents

TABLE OF CONTENTS **2**

GENERAL INFORMATION **3**

SAMPLE PREPARATION **3**

Starting Material 3
Sample Plating 3
Plates and Adhesive Films 3
Requirements 4

SERVICE REQUEST FORM AND SAMPLE SUBMISSION **4**

Service Request Form 5
Sample Submission 5

SAMPLE SHIPMENT PREPARATION **6**

Waybill 6
Package Preparation 6
Samples Shipment 6

FOR MORE INFORMATION **6**

Client Management Office 6

General Information

This document describes the procedure to follow when requesting a high-throughput sequencing service (Illumina technologies) requiring the preparation of an amplicon library (Juno™ multiplex PCR, 16S, ITS, 18S, etc.) or only indexing of amplicons (PCR products supplied by the customer).

To avoid any delay in the processing of the request, carefully follow the instructions in this guide.

Note that delays in the processing of samples will vary depending on the size of the project. It is recommended to contact the Client Management Office for information regarding processing time.

Sample Preparation

Starting Material

- The starting material for amplicon library preparation is genomic DNA (gDNA).
- The starting material for amplicon indexing is an unpurified PCR product.

Samples must be of the highest quality and purity. Resuspend the DNA in pure water or in 10 mM Tris-HCl pH 7.5-8.5 (without EDTA because its presence can inhibit certain enzymatic reactions). Do not resuspend DNA in buffers containing detergents (e.g.: SDS) or other additives that could inhibit enzymatic reactions during library preparation.

Sample Plating

- Samples must be sent in 96-well plates, regardless of the number.
- Each plate must contain only samples belonging to a single project.
- Arrange samples in the plate in the same order as identified on the submission form.

Plates and Adhesive Films

Recommended 96-well Plates

The procedures have been optimized to process a high throughput of samples. Any other plate format could damage the liquid handling robots used during procedures as well as increase delays. Therefore, samples submitted in plates not conforming to the specifications included in this procedure will be transferred to an acceptable plate. Fees will be billed to the user.

Skirted PCR plates must be used.

Five types of skirted plates are accepted:

- Eppendorf twin.tec, Cat# 951020401

- Corning Thermowell GOLD, Cat# 3752
- Axygen 96-well PCR Microplate, Cat# PCR96FSC
- BioRad Hard-Shell 96-Well PCR Plates, skirted, Cat# HSP9601
- 96 well 0.2mL Skirted PCR plate - Gray ultra rigid frame, Cat# IST-601-096GCT

Unaccepted 96-well Plates

- 96-well plate for cell culture
- 96-well PCR plate without skirt (or flanges)
- Half-skirted 96-well PCR plate

Recommended Adhesive Films

- Clear adhesive film:
  Adhesive PCR Films; Thermo Fisher Scientific, Cat#AB-0558
- Aluminum adhesive film:
  Aluminum Foils for PCR and Cold Storage; VWR, Cat# 60941-074

Requirements

Samples Volume and Concentration

For gDNA samples intended for the preparation of amplicon libraries (16S, ITS, 18S, etc.), it is important to provide a sufficient quantity of good quality gDNA in order to be able to carry out the entire project, i.e. 10 µL to be analyzed by fragment. No concentration value is necessary.

For unpurified PCR product samples intended for amplicon indexing, a volume between 20 and 25 µL should be sent for each sample. No concentration value is necessary. No purification is necessary. If the minimum sample volume is not met, diluent will be added to reach the required volume without any notice.

For samples requiring Juno™ multiplex PCRs, it is important to provide a minimum quantity of 6 µL of good quality genomic DNA in order to be able to complete the entire project. The concentration of genomic DNA samples must be at least 25 ng/µL.

Identification

All plates must be clearly identified, and the identification must match exactly what is indicated in the Sample Submission (field: “Plate Name”).

The identification must be made on one side of the plate and not on the sealant.

Service Request Form and Sample Submission

All service request forms and sample submissions must be done on the web through Nanuq by using a user account. To obtain a user account, contact the Client Management Office.

The laboratory work will only begin once all documentation has been submitted. Incomplete documentation will result in delays.

Service Request Form

1. Log into Nanuq.
2. Click "Add new request" under the section "Request" and follow the instructions.

   The "new request" option should not be used for an existing request.

   Do not use the "Back" button in your browser to go back to previous pages. Use the left-hand menu to navigate the form.

   Click "Next" to move to the subsequent page of the request.

   At any time during the process, save the work by clicking "Save and continue later". Drafts are accessible through "My request lists" under "Request". Requests will stay in drafts until they are submitted. To change a request under draft, click "Modify" in the left-hand menu.

   To request the return of samples once the project is complete, go to the "Sample Information" tab and provide the information requested.
3. Click on "Submit" for the request to be approved by the Client Management Office. Unsubmitted requests will not be processed.

Sample Submission

Once the service request is complete and submitted, submit the samples.

1. Log into Nanuq.
2. If applicable, find the request using "My request list" and open it.
3. Click on the "Sample submission" tab followed by "Add new samples".
4. Follow the instructions on the screen.
   - Choose "Next Generation Sequencing" as the service type, and then the required technology.
   - For PCR products (amplicon indexing), choose "Amplicon" as Sample Category.
   - For DNA requiring PCR types 16S, 18S, ITS (or others), choose "DNA metabarcoding" as Sample Category.
   - For DNA requiring multiplex PCRs (Juno – Fluidigm), choose "non-metabarcoding DNA" as Sample Category.
5. Make sure the status of the sample submission is "Submitted" by going to the "Sample submission" tab in the Service request.

Repeat these steps to add a new sample to the request or send replacement samples.

Sample Shipment Preparation

Waybill

Once samples are submitted, go back to the "Sample submission" tab, select the submission(s) associated with the package being prepared and click on "Print Waybill." By default, only one copy will print. However, two copies are required.

Package Preparation

The plates must be properly sealed and placed in a Ziploc bag.

The plates must be placed in a transport-resistant container.

If shipping from Canada for an overnight or same-day delivery and the samples are not frozen, include enough ice packs to keep the contents cold. However, if the samples are already frozen, send the contents on enough dry ice so that the samples remain frozen until arrival at the destination and thus minimize freeze-thaw cycles.

If shipping from outside Canada, the package must contain sufficient dry ice so that the samples remain frozen until arrival at the destination and thus minimize freeze-thaw cycles. Also, thawing during transport can cause loss of adhesion of the sealer on the plate which can lead to loss of samples or cross-contamination.

If the shipment contains heavy objects that may damage the contents during transport (e.g. dry ice blocks, ice packs), it is recommended to protect the samples against impacts.

One copy of the waybill must accompany the samples. Make sure that the waybill stays dry by placing it in a sealed plastic bag (type of Ziploc).

Samples crossing the Canadian border should be sent at the beginning of the week to avoid the risk of delays and of having the samples remain in the carrier's warehouse over the weekend. Messages such as "non-biohazardous biological samples", "Purified DNA from [species]", "For research use only", and "No commercial value" on the commercial invoice will help expedite customs clearance.

Samples Shipment

The delivery address and instructions for the shipping of samples are found on the waybill.

One copy of the waybill must be clearly visible on the outside of the package. It can be taped directly to the package or placed in a clear protective envelope taped to the package.

For More Information

Client Management Office

Telephone: 514-398-7211
Email: infoservices@genomequebec.com