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GENOTYPING SERVICES

User Guide

Microsatellites

Version 05

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General Information

This document describes the procedure to follow when requesting microsatellite marker genotyping and human cell line identification services. The detailed instructions for the preparation, the sample submission, the shipping requirements, as well as any additional information are all provided in this guide.

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 the microsatellite marker genotyping service may vary depending on the options chosen.

Option	Description	Starting Material
1 Read only	Reading of PCR products previously resuspended in formamide with a standard molecular weight scale	PCR product* previously resuspended in formamide + standard
2 Addition of formamide with standard + reading	Reading PCR products to be resuspended in formamide with the standard 500Liz molecular weight scale	PCR product*
3 Addition of formamide with standard + reading + analysis	Reading of PCR products to be resuspended in formamide with the standard 500Liz molecular weight scale and analysis of chromatograms	PCR product*
4 Reading + analysis	Reading of PCR products previously resuspended in formamide with a standard molecular weight scale and analysis of chromatograms	PCR product* previously resuspended in formamide + standard
5 PCR + reading + analysis	Carrying out PCR amplification from genomic DNA (gDNA), reading and analysis of the 10 markers**	Genomic DNA (human only)
6 PCR + reading + analysis	Carrying out PCR amplification from gDNA, reading and analysis of the 24 markers**	Genomic DNA (human only)

*All PCR products must have been amplified with one of the fluorochrome-labeled primer. Accepted fluorochromes are FAM, VIC, NED and PET.

**For the identification of human cell lines, the kits used include the following markers:
GenePrint® 10: AMEL, CSF1PO, D13S317, D16S539, D21S11, D5S818, D7S820, TH01, TPOX, vWA.

GenePrint® 24: Amelogenin, D3S1358, D1S1656, D2S441, D10S1248, D13S317, Penta E, D16S539, D18S51, D2S1338, CSF1PO, Penta D, TH01, vWA, D21S11, D7S820, D5S818, TPOX D, YS391, D8S1179, D12S391, D19S433, FGA, D22S1045.

Sample Plating

- For the microsatellite marker genotyping service, 96-well plates can be submitted full.

  For the read only and reading +analysis options (options 1 and 4), if a plate is not full, type 1 ultrapure water (or another accepted solution) must be added to each well empty. See the section Accepted Solutions in Empty Wells.

- For the human cell line identification service, two wells per plate must be left empty for the addition of controls, wells H11 and H12.

- Samples must be placed in plates as indicated in the Sample Submission.

Plates and Adhesive Films

Recommended 96-well Plates

Half-skirt clear 96-well plates

Examples:

- Thermo-Fast 96 PCR detection plate with flat deck; Life Technologies, Cat#AB1400L
- 96-well plate, standard semi-skirted, clear; FroggaBio, Cat#SS-96S

Unaccepted 96-well Plates

- 96-well cell culture plates
- No-skirt 96-well PCR plates
- Full-skirt 96-well PCR plates
- Opaques 96-well PCR plates

Recommended Adhesive Films

- Clear adhesive film
  Example: Adhesive PCR Films; Thermo Fisher Scientific, Cat#AB0558
- Aluminum adhesive film
  Example : Adhesive PCR Foil Seals; VWR International, Cat#60941-074

Requirements

Samples Volume and Concentration

Sample type	Option	Volume (concentration)	Arrival temperature at the laboratory
PCR product in formamide	1 et 4	10 µL	Frozen
PCR product*	2 et 3	10 µL	Frozen
Genomic DNA	5 et 6	10 µL (10 to 50 ng/µL)	Frozen

*For options 2 and 3, if the volume of 10 µL is not respected for each sample, the plate will be automatically rejected.

It is recommended to quantify the DNA using a fluorometric method for double-stranded DNA, for example the PicoGreen method, and not UV absorbance.

It is the customer's responsibility to provide samples of good quality and sufficient quantity.

Solutions Accepted in Empty Wells

Each empty well must have the same volume as a well that contains a PCR product by adding one of the solutions below:

- Hi-Di™ formamide
- EDTA 0.2 mM
- Ultra pure water type 1 (MilliQ water)
- UltraPure™ DNase/RNase-Free Distilled Water

Identification

All plates must be clearly identified, and the identification must match exactly what is indicated in the Sample Submission.

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 cause 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 lefthand 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" on the lefthand menu.
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 "Genotyping" as the service type and "Microsatellite analysis (fine mapping) - ABI 3730xl" as the technology.
   - Choose the desired option in the "Microsatellite analysis (fine mapping) - ABI 3730xl" section.
   - Click on the "Add new samples" tab to submit the samples.
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 be printed. However, two copies are required.

Package Preparation

It is recommended to seal the plates for genotyping microsatellite markers with an adhesive aluminum film and to protect them from light (example: wrap the plate with aluminum foil). Any exposure of the plate to light may reduce the quality of the results.

For plates for human cell line identification, a clear adhesive film can be used.

Plates must be properly sealed and sent on dry ice.

The package must contain enough dry ice to keep the samples frozen until they arrive at their destination. If the samples thaw during transportation, it can cause the seal on the plates to unstick, which may cause a loss of sample volume or cross contamination.

If the package contains heavy items that can damage the contents during transportation (ex.: block of dry ice) it is recommended to protect it from those impacts. The plates must be placed in a transport-resistant container.

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
Internet site: https://genomequebec.com/en/technological-services/centre-dexpertise-et-de-services-2/

Additional Information

Transmission of results

An email is sent as soon as the results are available.

The reading and analysis results, if applicable, are directly accessible via the Nanuq web application.

The raw data files generated by the 3730xl DNA Analyzer are in .fsa format.