



UPPSALA
UNIVERSITET

Appendix 3 to Agreement on Commissioned Activities /Collaboration Project

Instructions for Sample Delivery

The amount and quality of sample needed depend on the type of library(ies) that are going to be constructed. In turn, library quality is directly correlated to quality and quantity of the starting material.

All details concerning the type of library preparation and sequencing strategy for a given project must be discussed with the NGI-UGC staff prior to the sample delivery.

Partner shall assess quality and quantity of the nucleic acid sample before submitting to NGI-UGC. We highly recommend **low TE** as the sample solvent.

AMOUNT OF STARTING MATERIAL REQUIRED IN THE PROJECT

Partner should be aware of the fact that spectrophotometers of NanoDrop and NanoVue type usually overestimate the nucleic acid concentration. All measurements will be verified by NGI-UGC on the Qubit system.

All communication within the project is done via Zendesk, an interface that NGI uses for communication with all users.

A separate Sample Submission Form, as well as the Agreement document and Quotation (Appendix 1 to the Agreement) will be sent to the Principal Investigator/Contact Person of Partner's project by the NGI-UGC project coordinator. The filled form as well as the signed agreement must be submitted as a print-out together with the samples, as well as an electronic version sent within the same Zendesk communication thread.

PacBio

See separate document: "Sample preparation instructions for use on the PacBio instruments". Please submit your samples together with a picture from agarose gel, or a pdf-file with Bioanalyzer results. If a gel picture is

submitted, please specify the information of amount of loaded nucleic acid and include size of fragments in ladder. Optical absorption ratios (260/280 and 260/230) must be measured and sent to NGI-UGC before submitting the samples.

Nanopore

Purity and integrity of the DNA submitted will highly affect the sequencing results. Contaminants extracted along with the DNA, or extraction carry-overs, may block the nanopores during sequencing, resulting in lower yield.

We have found that predicting the Nanopore sequencing results with optical absorption ratios (260/280 and 260/230) cannot be done. Each project design is therefore best discussed in detail with NGI-UGC staff before start.

However, some general guidelines may be considered:

- Concentration measurements that agree between NanoDrop and Qubit may be indicative of good extraction purity.
- Some kit extraction methods have given good results previously. For high molecular weight DNA, Circulomics Nanobind-series kits are currently our first methods of choice.
- DNA is best long-term stored in low TE at -80°C. High levels of EDTA can disturb the library preparation reactions.
- One library loaded on one flow cell requires 2µg of starting DNA. However, if shearing and/or size selection is required, up to 6 µg is required per flow cell.
- Sequence read length will to a high degree depend on the length of the input material. Avoid freeze-thaw cycles. Samples may be stored at 4°C for some period of time. The degree of gDNA fragmentation will be assessed by NGI-UGC staff using FemtoPulse during sample input QC.
- Sequence read length will also highly depend on DNA integrity. A DNA repair step is included in most sample preparations, but this salvages long DNA molecules only to a certain extent. If you suspect that your DNA has a high degree of nicks, please notify NGI-UGC, and we can include an extra repair step prior to project start.
- Long sequence reads generally comes at the expense of yield during nanopore sequencing.

Ion S5XL

- AmpliSeq Human Exome sequencing: 50-100 ng DNA.
- AmpliSeq Human or Mouse Transcriptome sequencing: 50-100 ng RNA (depending on quality).

- AmpliSeq custom panels: 10-100 ng DNA, depending on the panel.
- Amplicons: 10 - 100 ng. Make sure that the size difference among amplicons do not exceed 50 bp. Maximum amplicon length that is possible to sequence is 600 bp.

SHIPMENT AND SAMPLE DELIVERY

Samples received without submission forms will not be processed.

- Always use dry ice for shipment of RNA samples for any type of sequencing technology.
- For PacBio applications, amplicons exceeding 2 kbp and High Molecular Weight genomic DNA must be shipped solid-frozen on dry ice.
- For Nanopore applications, samples must always be shipped cold. If samples are not frozen, ship on ice. If samples are frozen, samples must be shipped on dry ice.
- For Ion applications, all DNA samples (including amplicons) must be shipped or submitted on ice.
- If samples are brought to NGI-UGC by Partner's co-workers, always use ice-filled containers.
- Mark the parcel with "**Kylvara**" stamp ("Chilled package").

Do not ship samples on Thursdays, unless using same-day or overnight delivery courier service, and never ship on Fridays or the day before a holiday (<https://mp.uu.se/en/web/info/anstallning/arbetstid>).

If the samples are dispatched by a **courier service**, Partner must contact NGI-UGC at least one day in advance before the pick-up to ensure that NGI-UGC has dedicated personnel on site to accept delivery (use 070-4250285, or the Zendesk communication thread). Courier service must be aware that the samples can only be delivered to NGI-UGC between 9:00 - 16:00. If the courier cannot get in contact with NGI-UGC personnel, the package should be delivered to the BMC goods reception. For BMC goods reception opening hours, please consult their web site: (https://www.bmc.uu.se/Campus+management/Unit+for+Technical+Services/The+Goods+reception/Paket_och_kurir/?languageId=1).

NGI-UGC address(for personal/courier service delivery):

Uppsala University, Uppsala Genome Center
 BMC, Entrance C11
 Husargatan 3
 752 37 Uppsala

BMC goods reception address (for postal service delivery):

Uppsala Genome Center

Husargatan 3

Ref. Box 815

751 23 Uppsala

Please do not write any recipient names on the package; address it only to the Uppsala Genome Center and clearly mark it with storage temperature.

Personal deliveries are accepted **only** by agreement. Use BMC entrance C11 and call 070-4250285 upon arrival.