De novo - sample preparation guidelines

Genomic DNA for *de novo* projects

IGATech offers nucleic acids extraction **(including high molecular weight DNA)** service and we can set up a dedicated extraction workflow for your specific substrate. Please enquire.

Standard Illumina short-read sequencing

Submit minimum 500 ng of DNA per sample, 5 µg for PCR-free libraries.
Please note that fluorimetry-based quantification (*e.g.,* Qubit, plate-reader) assays are more accurate methods than absorbance-based methods (*e.g.,* Nanodrop), which might overestimate the quantity.
Resuspend DNA in 10 mM Tris-HCl pH 8.5 (standard elution buffer of most commercial column-based extraction kits); water is accepted as an alternative (no EDTA must be present in the solution – *e.g.*, no TE 1X buffer 1mM EDTA). Consider 10mM Tris-HCl as the best buffer for HMW DNA stability (or 10mM Tris-HCl + 0.1 mM EDTA). The A260:A280 and A260:A230 ratios for DNA samples should be > 1.8. Use of DNA with lower ratios may result in low amplification yield.

Ship samples in a cold pack (*e.g.,* Blue ice). Organize the packaging in a way to avoid damaging and dispersion during the shipment: place tubes and plates in smaller plastic bags or boxes. Put a separator between stacked plates to avoid perforations of adhesive foils and leakage.

It is **MANDATORY** to send us the compiled **Sample Spreadsheet**, both with the shipped parcel and via e-mail. To be able to properly track and safeguard your samples send us the Tracking Number via e-mail.

Oxford Nanopore long-read sequencing

For small genome sequencing (bacteria, yest):
Submit minimum of 3 µg of DNA at a minimum concentration of 50 ng/µl.

For large genome sequencing (plants, animal, fungi):
Submit minimum of 6 µg of DNA at a minimum concentration of 50 ng/µl.
For high quality assembly, consider sending 10ug.

Please use fluorimetry-based methods for DNA quantification (Qubit, picogreen, *etc*). Otherwise, if using absorbance quantification, consider sending at least 3 times more than the standard specifications.
Samples must be chemically pure with Nanodrop spectrometer 260/280 nm ratios between 1.8-2 and 260/230 nm ratios between 1.7-2.2.
Resuspended DNA in 10 mM TRIS (pH=8.0-8.4). Please avoid using detergents and surfactants in the resuspension buffer.
DNA integrity is a critical point for successful library preparation. Most of the fragments should be >30Kbp. PFGE is the recommended system to assay high molecular weight DNA integrity. We can perform this in our facility; however, we encourage our customers to assess the integrity of their DNA before sending the DNA. You can assess lack of smear below 30Kb also with standard gel electrophoresis. Perform a 0.8% agarose gel check to control for possible degradation, run should contain also a marker of at least a 20 kb size (*e.g.,* GeneRuler 1 kb Plus DNA Ladder or Lambda DNA/HindIII Digest Marker; suggested is also a lane with undigested Lamdba phage DNA [48kb *e.g*., NEB N3011S]). Please run the electrophoresis slowly (*e.g.,* at 80V depending on setup).

For difficult samples, especially plant DNA with hard-to-remove contaminants (*e.g.,* some polysaccharides), we recommend carrying out a high-salt/phenol/chloroform cleanup. Please note that this protocol often leads to a loss of 50% of the sample.

Send DNA samples in 1.5 or 2 mL Eppendorf tubes sealed with parafilm (0.5 mL / 0.2 mL tubes will not be accepted), and send samples in a cold pack (*e.g.,* Blue ice).

It is **MANDATORY** to send us the compiled **Sample Spreadsheet**, both with the shipped parcel and via e-mail. To be able to properly track and safeguard your samples send us the Tracking Number via e-mail.

Hi-C-Sequencing

Send flash frozen (liquid nitrogen) tissue in dry ice box. Please use Falcon tubes (50mL) labelled with a progressive number. Use only a progressive number as defined on the **Samples Spreadsheet** (Label columns). PLANT TISSUES IN PLASTIC BAGS OR WRAPPED IN ALUMINIUM FOILS OR IN ANY OTHER PLASTIC TUBES WLL NOT BE ACCEPTED.

Plants:
Send a minimum of 5g of flash frozen (liquid nitrogen) tissue (young leaves) in dry ice box.

Cells:
Send a minimum of 10^7 cells. Procedure: pellet cells and wash with PBS, centrifuge, remove supernatant and flash frozen in liquid nitrogen. Store at -80°C and ship pellets on dry ice.

It is **MANDATORY** to send us the compiled **Sample Spreadsheet**, both with the shipped parcel and via e-mail. To be able to properly track and safeguard your samples send us the Tracking Number via e-mail.

For other tissue types please enquire.

On-site extraction service

Plants.
Send flash frozen (liquid nitrogen) tissue (young leaves) in dry ice box.
Please use Falcon tubes (50mL) labelled with a progressive number. Use only a progressive number as defined on the **Samples Spreadsheet** (Label columns). PLANT TISSUES IN PLASTIC BAGS OR WRAPPED IN ALUMINIUM FOILS OR IN ANY OTHER PLASTIC TUBES ARE NOT ACCEPTED.

- Min. 10 g for HMW-DNA extraction via nuclei isolation (for Nanopore sequencing)
- Min. 2g for standard DNA extraction (for Illumina short reads sequencing)

For other sampling material please enquire.

It is **MANDATORY** to send us the compiled **Sample Spreadsheet**, both with the shipped parcel and via e-mail. To be able to properly track and safeguard your samples send us the Tracking Number via e-mail.

Recommended extraction and clean-up protocols for plants:

https://www.qiagen.com/us/resources/download.aspx?id=cb2ac658-8d66-43f0-968e-7bb0ea2c402a&lang=en

https://www.protocols.io/view/high-molecular-weight-gdna-extraction-after-mayjon-khkct4w

https://www.pacb.com/wp-content/uploads/2015/09/Experimental-Protocol-Guidelines-for-Using-a-Salt-Chloroform-Wash-to-Clean-Up-gDNA.pdf

Recommended extraction and clean-up protocols for bacteria:

- QIAGEN genomic-tip 100G for HMW-DNA (up to 150Kb)
- QIAamp DNA Mini kit (up to 50kbp)

Recommended extraction and clean-up protocols for blood:

- QIAGEN Mag-Attract

For other tissue types please enquire.