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.

The quality of the DNA sample can have a significant impact on the success of the experiment. Poor quality DNA can determine the presence of duplicated (e.g. clonal) reads and consequent insufficient coverage. In long-molecule and linked-reads sequencing, poor quality DNA can heavily impact the final results. Protein contamination, nicks in the double-stranded DNA are major causes of experiments with data quality below expectation. Also good estimation of DNA integrity for any long-range application (mate-pairs, PacBio sequencing, Chromium linked-reads) is a critical point for successful library preparation. PFGE is the recommended system to assay high molecular weight DNA integrity. We can perform this in our facility, however we encourage our customer to assess the integrity of their DNA (if possible) before sending the DNA.

General best practice and quality for DNA:

- *260/280>1.8 and 260/230>1.8*
- *Quantification made by dsDNA-specific fluorimetry (Qubit/fluorimeter)*
- *Avoid repeated freeze-thaw cycles (use aliquots)*
- *Do not expose to high temperatures (>65 C)**
- *Store the DNA in stabilizing buffer (Tris-HCl pH 8.0-8.5)*
- *Do not provide DNA with EDTA (conc. > 0.1 nM)*
- *DNA must be RNA-free (so RNAse treatment is strongly suggested)*
- *Does not contain phenol, polyphenols*

**Especially for HMW DNA*

Submit minimum 2 µg of DNA per sample. 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. Quality of the DNA should be 260/280 > 1.8 and 260/230 >1.8.

DNA must be resuspended in 10mM Tris-HCl pH 8.5 (standard elution buffer of most commercial column-based extraction kits); water is accepted as an alternative (**NO high concentration of EDTA must be present in the solution** – e.g. no TE 1X buffer has 1mM EDTA - but consider 10mM Tris-HCl as best buffer for HMW DNA stability or 10mM Tris-HCl + 0.1 mM EDTA.

Mail DNA samples in 1.5 or 2 mL Eppendorf tubes sealed with parafilm (0.5 mL / 0.2 mL tubes will not be accepted).

If you have 24 or more samples, please put them in a 96-well skirted plate sealed with adhesive/heat-sealed aluminum foil. The tubes must have, on the vial top, a clear and permanent sign (or a thin label) with a progressive number of the mailed samples and the customer's name (at least the initials).

Send DNA samples in a cold pack (*e.g.* Blue ice) or dry ice. Do not ship plates without secondary containment as these may crack when placed directly on dry ice.

Application-specific quantities and sizes

- Standard paired-end libraries

  A minimum of 2 µg of DNA is required (conc. > 20 ng/µL), with molecules > 10kbp

- Genomic DNA for Chromium linked-reads

  A minimum quantity of 2 µg (conc. > 20 ng/µL); mol size >50kbp, best with >100kbp

- Gel-free mate-pairs libraries (up to 3kbp)

  A minimum of 5 µg is required (conc. > 20 ng/µL), molecules must be >20kb

- Gel-plus mate-pairs libraries (4-10kbp)

  A minimum of 10 µg is required (conc. > 20 ng/µL), majority molecules must be >30kb

- PacBio libraries*
    - 3kbp library: min. 5 µg (conc. > 100 ng/µL); mol. size > 20kbp
    - 3-10kbp library: min. 5 µg (conc. > 100 ng/µL); mol. size > 20kbp
    - 10-20kbp library: min. 20 µg (conc. > 100 ng/µL); mol. size > 40kbp
    - >20kbp library: min. 40 µg (conc. > 100 ng/µL); mol. size > 50kbp

**Low-ETDA buffer can also be used (10mM Tris-HCl , 0.1nM EDTA, pH 8.0-8.4)*

Plant tissue for gDNA extraction or *in situ* Hi-C libraries

In situ Hi-C
Send a minimum of 5g of flash frozen (liquid nitrogen) tissue (young leaves) in dry ice box.

HMW DNA extraction
Send a minimum of 10g of frozen fresh tissue (young leaves) in dry ice box.

Regular DNA extraction
Send a minimum of 1g of frozen fresh tissue (young leaves) in dry ice box.

Please, do not forget to send us compiled **Samples Spreadsheet** and the **Customer registration form**, together with shipped samples as well as via e-mail.