DNA-Seq Sample preparation guidelines

Genomic DNA for DNA-Seq

IGATech offers nucleic acids extraction service, and we can set up a dedicated extraction workflow for your specific substrate. Please enquire.

General best practice and quality for DNA:

- *260/280>1.8 and 260/230>1.8*
- *Quantification made by dsDNA-specific fluorimetry (Qubit/fluorimeter)*
- *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*

Submit minimum 250 ng of DNA per sample at a minimum concentration of 10 ng/µL, min volume 25µL; 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.
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 1mM EDTA - but consider 10mM Tris-HCl as best buffer for HMW DNA stability or 10mM Tris-HCl + 0.1 mM EDTA.

Shipping

For batches of <24 samples, send samples in 1.5 mL or 2 mL Eppendorf tubes sealed with parafilm (***0,5 mL and 0.2 mL tubes as well as strips will be not accepted***). The tubes must have on the vial top, a clear and permanent sign (or a thin label) with a **progressive number** corresponding to information specified in the **Sample Spreadsheet**.

For batches of >24 samples, send samples in a skirted 96-wells plate, sealed with adhesive/heat-sealed aluminum foil **ONCE YOU HAVE PERFORMED** a fluorimetry-based quantification and normalized the DNA quantities. DNA concentration across samples should be even within each plate (+/-20% of average). In case internal normalization service is waived, IGAtech is not responsible for the quality of the data in terms of per-sample coverage.

Each plate must be labeled with a plate identifier and accompanied with the **Sample spreadsheet** containing information on sample names and sample position. **Feel free to add extra columns to Sample Spread Sheet to include all available metadata of samples, if the analysis service has been requested**. This information will be used to run several statistical tests, as well as labeling/coloring of outputs in our standard analysis.

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.

IMPORTANT: We remind you that human-derived samples must be anonymized. Therefore, we cannot accept samples that come along with personal identification data (name and surname, fiscal code, etc.). Supplementary data related to the study such as prognosis, biometrics values, age, sex and other information not directly associated to an individual can be provided with no limitation (please use the Sample Spreadsheet available in the Documents section).

If human samples are provided in the form of tissue or body fluid, please fill out the Human samples clearance form and return a signed copy.