Non-Human Target Resequencing sample preparation guidelines

Genomic DNA for target resequencing (hybridization)

IGATech offers nucleic acids extraction 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 effect on the successful of the experiment. Poor quality DNA can determine the presence of duplicated (e.g. clonal) reads and consequent insufficient coverage.

DNA Quantity and Integrity

Submit minimum 1 µg of DNA per sample, at minimum concentration of 20 ng/µL in minimum volume of 20µL with most of the fragments >20Kbp (perform a 0.8% agarose gel check to control for possible degradation*). Degraded gDNA may affect the quality of the final libraries, leading to over-fragmentation of DNA and insufficient libraries complexity*.

For challenging samples (low inputs or FFPE specimens) please contact us to determine the feasibility of the processing.

For this pooled application DNA normalization is pivotal and DNA must be provided to same concentration for all samples (±20%). Failure in using normalized DNA will result in high sample-to-sample sequencing yield variation, hampering high-quality results*.

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.

DNA Purity

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. TE buffer).

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.

Shipping

For batches of <24 samples, send samples in 1.5 or 2 mL Eppendorf tubes sealed with parafilm (***0.5 mL / 0.2 mL as well as strips tubes will not be accepted***). 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. Each plate must be labeled with a plate identifier indicated in the **Sample Spreadsheet**.

Plates must be one of the following:

- MicroAmp Optical 96-Well Reaction Plate' (Thermo Fisher™)
- Eppendorf 96-Well twin.tec™ PCR Plates (Eppendorf™)
- If not possible to provide such plate types, please inquire and IGATech can supply them.

Dry ice shipment is the preferable method to avoid evaporation and DNA degradation. If the latter is not available, cold pack (*e.g.* Blue ice) can be used instead if the duration of shipment does not take too long. 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.

If samples are of different origin, such as tissue type, method of extraction or age of the specimen, significant differences in concentration of samples can occur and result in different efficiencies during library construction. Please, organize plates by creating homogeneous groups and report all information in the **Sample Spreadsheet**.

If any of these conditions is not satisfied IGATech may reject samples processing.

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

*Quantification and normalization procedures can be applied at additional costs. Please inquire.