Exome-Seq sample preparation guidelines

Genomic DNA for target/exome sequencing

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

Prepare the DNA following your favorite extraction method, even if it is strongly recommended to use commercial column-based protocols.

Recommended commercially available kits

DNA extraction kits (catalog number)	Blood*	Tissue (incl. tumors)	Cultured cells
Qiagen DNaesy Blood & Tissue Kit (69504)	X	X	
Qiagen Gentra Puregene Cell Kit (158722)			X
Qiagen Gentra Puregene Blood Kit (158445)	X		
Qiagen QIAmp DNA Blood Midi Kit (51183)	X		

* Use preferentially EDTA or ACD as anticoagulant. Heparin can inhibit Taq Polymerase.

DNA Quantity and Integrity

Submit at least 500 ng of DNA per sample, min concentration 20 ng/µL in min volume 20uL 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.
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 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. Each plate must be labeled with a plate identifier indicated in the **Sample Spreadsheet**.
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.

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

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.