BS-Seq sample preparation guidelines

Genomic DNA for WGBS-Seq and RRBS-Seq. Input Requirements.

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

DNA Quantity

Submit 300 ng of DNA per sample at a minimum concentration of 20 ng/µL.

Please note that fluorimetry-based quantification (*e.g.* Qubit, plate-reader) assays are more accurate than absorbance-based methods (*e.g.* Nanodrop), which might overestimate the quantity.

DNA Purity

For WGBS-seq experiments, resuspend DNA in low-EDTA TE buffer or Nuclease-free Water.

In case of RRBS-seq application, DNA must be submitted in Nuclease-free Water.

DNA samples must be free of contaminating proteins, RNA, organic solvents (including phenol and ethanol) and salts.

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.

DNA Integrity

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

Control DNA on 0.8% agarose gel to check for integrity. 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.

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 or more 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.

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.

Summary of best practice and quality requirements for NGS
260/280>1.8 and 260/230>1.8
Quantify by dsDNA-specific fluorimetry (Qubit/fluorimeter)
Avoid repeated freeze-thaw cycles (arrange aliquots)
Do not expose to high temperatures (>65 Cº) especially for HMW DNA
Store the DNA in stabilizing buffer (Tris-HCl pH 8.0-8.5)
Avoid EDTA (conc. > 0.1 nM)
DNA must be RNA-free (RNAse treatment is strongly recommended)
DNA must be free of phenol and polyphenols