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Brighton Genomics: Requirements for Submission of Biological Samples (purified nucleic acids or DNA sequencing libraries)

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Quantification of samples prior to submission

DNA/RNA samples should be quantified using fluorimetric methods (e.g. Qubit, Quant-iT, Picogreen for dsDNA and Ribogreen for RNA). We do not recommend the use a Nanodrop Spectrophotometer for quantification of nucleic acids submitted for NGS.

Please send samples at the required concentrations (indicated below).

The quality/purity of your sample should be estimated using spectrophotometric methods (e.g. Nanodrop spectrophotometer). The minimum requirements for DNA/RNA are specified below under the *Genomic DNA* and *Total RNA* sections.

Pictures of agarose gels and/or electropherograms (from Agilent BioAnalyser or Agilent TapeStation) should be provided with your sample. Calculated concentration of nucleic acids should also be provided.

Genomic DNA (gDNA)

DNA should be RNA-free and free of other contaminants (A260/280 ratio > 1.8). Samples have to be purified after RNase treatment (we recommend Riboshredder RNase blend (https://www.cambio.co.uk/43/204/15/products/riboshredder-rnase-blend/). The genomic DNA should be minimally degraded. Assess the gDNA quality by loading 2 μl on 0.8% agarose gel alongside a 1 kb molecular size marker. A high molecular weight band above 12 kb should be visible with very little or no smear underneath (which is a sign of degradation). DNA should be resuspended in 10 mM Tris pH7.5 (not EDTA containing buffers such as TE.) Minimum requirement: at least 1 μg of DNA at a concentration of 50-100 ng/μl and a minimum volume of 20 μl.

Total RNA

All RNA samples should be treated with DNase and purified prior to submission. We suggest Turbo RNase-free DNase enzyme (Life Technologies cat. No AM1907). Integrity of RNA should be assessed through an Agilent Technologies Bioanalyser or Agilent TapeStation. The RNA integrity number (R.I.N.) (or R.I.N.^{e} in case of the TapeStation 4200) should be ≥ 7 . If you don't have access to a Bioanalyser or TapeStation, samples should be assessed for integrity by loading an aliquot on a 1.2% agarose gel (the 28S rRNA band should be 1.5-2-fold brighter than the 18S rRNA band). Quality control should also be performed spectrophotometrically (on a Nanodrop the A260/280 should be ≥ 1.9).

Minimum amount required: at least 1.2 μg of RNA sample should be provided at a minimum concentration of 20 ng/μl.

For small RNA library preparation a minimum of 2 μg total RNA (preserving the small RNA species, for example, using Qiagen miRNeasy or Life Technologies mirVana kits) should be provided at 200 ng minimum concentration.

The RNA should be dissolved in RNase free water and shipped on dry-ice.

Sequencing libraries

User-provided libraries should be provided in Illumina EBT buffer at 5-10 nM concentration and minimum volume of 20µl. Use the following formula to calculate the concentration of your libraries:
nM = (ng per uL of DNA x 1,000,000) / (Number of base pairs x 660).

Note: On receipt of samples we will perform additional Quality Control tests prior to sequencing and will contact you if there are any issues with sample quality.