Sample Submission Guidelines

1. For Genomic DNA, Targeted Sequencing, or Mate Pair Library:

DNA quantities required per assay:

Ideally, the concentration of the DNA should be 20-100 ng/µl. Also, please provide between 20 µl to 50 µl total, including at least 3-4 µl extra for QC purposes.

- Illumina Genomic DNA (Intact Genomics' standard assay): 1-2 µg of high molecular weight DNA.
- Illumina Genomic DNA – Nano (for limiting samples): 100-200 ng of high molecular weight DNA.
- Agilent SureSelect Exome v5: 3 µg of high molecular weight DNA preferred and recommended; 200 ng acceptable for limiting samples.
- Agilent SureSelect Custom Captures: 3 µg of high molecular weight DNA preferred and recommended; 200 ng acceptable for limiting samples.
- NextEra Mate Paired Libraries: 4-5 µg of high molecular weight DNA.

Considerations regarding DNA samples:

If the customer is currently utilizing a method successfully for isolating DNA, Intact Genomics recommends they continue to utilize the same method for this project. If quality or purity issues arise with the samples, Intact Genomics may provide alternative recommendations.

- The ratio of the absorbance (A260/A280) should be between 1.7 and 1.9.
    - Ratios greater than 1.9 are indicative of RNA contamination
    - Ratios less than 1.7 are indicative of protein contamination
- Samples should be treated with RNase to remove RNA contamination. Please clean-up samples after RNase treatment.
- Please quantify samples utilizing PicoGreen along with either a Qubit Fluorometer or plate reader. As spectrophotometers and Nanodrop can estimate quantities inaccurately.
- Dilute samples utilizing nuclease-free water or 10 mM Tris-HCL pH 8.0 and note the buffer type on the submission form.
- Please run 100 ng aliquots of each DNA sample on a 1% agarose gel in order to check the integrity of the sample. Please provide a labeled gel picture when samples are submitted. The gel should show little/no smearing and should have a single band above the highest marker band.

2. For RNA:

Recommended RNA quantity/concentration:

Please provide at least 1 µg total RNA. The concentration of RNA should be between 50-500 ng/µl. We would like to have 3-4 µl extra for QC purposes. If the RNA concentration is >500 ng/µl, please dilute it before sending.

Considerations regarding RNA samples:

- The success of an RNA sequencing project primarily depends upon the quality of the RNA received. Before sample submission, Intact Genomics strongly recommends to treat RNA samples with RNase-free DNase to remove gDNA contamination. Please clean-up samples after DNase treatment.
- Please ensure that the A_{260}/A_{280} is between 1.8 and 2.1.
    - Ratios less than 1.8 often indicate protein or DNA contamination

  (Please remove protein contamination by re-extraction with phenol:chloroform:isoamyl alcohol and other contaminations may be removed by EtOH precipitation.)
    - Ratios greater than 2.1 often indicate residual guanidine thiocyanate or beta-mercaptoethanol
- Quantify samples by using spectrophotometer, RiboGreen or NanoDrop. If quantify by NanoDrop, please provide a copy of the results.
- Dilute samples with nuclease-free water or 10 mM Tris-HCL pH 8.0 and note the buffer type on the submission form. **Please avoid EDTA.**

3. For 16S/Amplicon:

DNA quantity:

The volume of the samples should be between 10 µl and 20 µl each and the concentration should be between 2 ng/µl and 10 ng/µl. Please measure the DNA by intercalating dye/fluorometer (*e.g*. PicoGreen/Qubit, Life Technologies or Quantus, Promega).

NanoDrop and other spectrophotometers are not acceptable due to reliability concerns for quantifying smaller DNA concentrations.

Once received, Intact Genomics will quantify the DNA by utilizing a fluorometer. Regardless of previous measurements done by the customer, the quantification performed by Intact Genomics will be considered as the gold standard. The customer will be informed if the samples fail to meet these set QC measures, prior to proceeding with sequencing.

4. For Customer Prepared Libraries:

DNA quantity:

Please provide a minimum library volume of 10 ng for QC. Additional volume is preferred if possible. The libraries should be submitted at concentrations equal to or greater than the following:

- 5 ng/µl as measured by Qubit
- 15 ng/µl as measured by Nanodrop.

Considerations regarding upstream library preparation:

- Please provide the expected size of your library if possible. A gel image may be helpful.
- Because the same index number from different library kits do not always refer to the same sequence, please provide **all index sequences.**
- The ratio of the absorbance (A_{260}/A_{280}) should be between 1.7 and 1.9
- Please dilute samples in nuclease-free water or in 10 mM Tris-HCL pH 8.0 and note the buffer type on the submission form. **EDTA should be avoided.**