The Centre for Applied Genomics - Next Generation Sequencing Facility

General Considerations for DNA and RNA Sample Submission

When submitting DNA or RNA samples to the NGS Facility, please ensure the guidelines given below are met. Upon sample receiving, the NGS Facility will verify that samples were received in good condition, the number and names of samples submitted to Clarity LabLink LIMS match the number and names of samples received, and that the tubes are clearly labeled. The NGS Facility will contact clients by email to notify them once samples have been received. This email will make reference to an automatically generated LIMS-ID (ex. ABC1234) specific to the order. For a quicker response time for any questions related to your project, always refer to the LIMS ID each and every time you need to contact the TCAG NGS Facility; preferably, reply to all and to the latest email you have that makes reference to the LIMS ID. Any samples received without a formal order in Clarity LabLink will not be processed.

General Submission Requirements

- Sample concentration and volume ranges should fall within the categories listed below (Table 1).
- Submit samples with clear ID labels that correspond to the ID provided on the service request form in Clarity LabLink LIMS.
- Ensure label IDs on tubes will not rub off or peel easily.
- Submit samples in 1.5ml lo-bind tubes or equivalent (for example, Eppendorf DNA LoBind Tube 1.5ml Cat. No. 022431021).
- Do not send samples in strip-tubes or plates sealed with strip-caps, as these carry a higher risk of sample cross-contamination when opened.
- If submitting samples in 96-well plates, ensure that the plastic or foil seal is securely closed around all edges and wells (Submitting samples in 1.5 ml tubes is preferred for most cases).
- Sample names listed on the sample submission form must match the name label on tubes or plate map.
- On the sample submission form, please state number of lanes required for project or number of reads per sample (if known).

- To avoid delays processing your project, we strongly recommend that you perform sample QC prior to submission. This may include but not be limited to nucleic acid quantification using a fluorometric method, checking integrity of sample on agarose gel or equivalent automated fragment analyzer, checking purity by OD260/280 and OD260/230 ratio. Specifically for RNA samples, check the integrity using an Agilent Bioanalyzer or similar instrument; if a Bioanalyzer is not available, RNA integrity can be checked on a formaldehyde 1% agarose gel, or we can check on our Bioanalyzer on a fee-for-service basis. For RNA samples that are submitted to us for library preparation, quality check on Bioanalyzer is included in the library preparation service.

DNA Requirements

- For PacBio Sequel IIe and Oxford Nanopore PromethION-24, we recommend to extract DNA using QIAgen MagAttract or Circulomics kits to obtain good quality HMW DNA and take full advantage of the long-read technology; for any other genomic applications any DNA extraction method that generates good quality, pure DNA is acceptable. If using organic methods (phenol-chloroform based methods), please ensure no organic contamination is present in the submitted sample.
- DNA can be suspended in nuclease-free water or preferably in 10mM Tris-HCl pH 7.5-8.5 (do not add EDTA, do not use DEPC-treated water).
- Quantify DNA samples using fluorometry-based method (Qubit DNA HS Assay or Picogreen).
- DNA samples should have OD 260/280 ratio of 1.8 to 2.0.
- DNA should be double stranded as most protocols will ligate double-stranded adapters to double-stranded DNA (contact facility manager if sample is single-stranded DNA or a mix of ss-DNA and ds-DNA prior to submission).

RNA Requirements

- RNA samples extracted with any method of choice that generates good quality, pure RNA are acceptable. Degraded or partially degraded RNA samples may result in failed or suboptimal libraries and biased sequencing results.
- Samples should have an Agilent Bioanalyzer RIN value of ≥ 7.0 (samples with lower RIN values may be acceptable pending discussion with Facility and method used for library preparation).
- RNA can be suspended in nuclease-free water (Do not suspend RNA samples in DEPC-treated water or buffers containing detergents).

- RNA samples should have OD 260/230 ratio of 2.0-2.2.
- The library preparation protocols we use do not include DNase treatment and we do not currently offer it as a standalone service. Please consider performing a DNase treatment prior to submitting samples to us if genomic DNA contamination in your samples is significant.

Table 1: Specifications for sample and library submission

Short-Read Sequencing	**NGS Facility recommend amount**	**Volume range**
Whole genome sequencing, metagenomics, 200-700 bp amplicons	700 - 1000 ng	20 uL - 50 uL
Metagenomics, small genomes, cfDNA	10 - 500 ng	20 uL - 50 uL
ChIP-Seq, MeDIP-Seq	2 - 20 ng	20 uL - 50 uL
Whole genome bisulfite sequencing	500 - 1000 ng	20 uL - 50 uL
Human or mouse exome sequencing	200 - 1000 ng	20 uL - 50 uL
>700-bp amplicon, small genomes (< 5 Mb), full length cDNA	1 - 5 ng	5 uL
RNA-Seq (eukaryotic poly-A mRNA)	100 - 1000 ng	20 uL - 50 uL
RNA-Seq (eukaryotic poly-A mRNA; low input)	2 - 10 ng	10 uL - 15 uL
RNA-Seq (eukaryotic rRNA-depletion RNA)	100 - 1000 ng	10 uL - 15 uL
RNA-Seq (human/murine/bacteria rRNA depletion; human globin depletion)	100 - 1000 ng	10 uL - 15 uL
microRNA / small RNA-Seq	200 - 1000 ng	10 uL
Single cell RNA-seq (<1000 cells), low input RNA-Seq (poly-A mRNA)	10 pg - 200 ng	10 uL
High throughput single cell 3' end RNA-Seq	Please inquire	20 ul
Illumina Sequencing-ready libraries	> 2 nM	20 ul
Long-read Sequencing	**NGS Facility recommend amount**	**Volume range**
Long-read Whole genome sequencing (other applications please consult with the NGS facility)	5 ug minimum	50 uL - 150 uL
PacBio or Nanopore Sequencing-ready libraries	Please inquire	20 ul
Other Standalone Services	**Recommended input amount**	**Minimum volume**
Covaris S2 - DNA	100 ng - 5000 ng	50 ul
Bioanalyzer - DNA	<= 15ng/uL	> 3 ul
TapeStation DNA	20 ng/ul to 50ng/ul	5 ul
Bioanalyzer - RNA Nano chip	> 50 ng/uL	> 3 ul
Bioanalyzer - RNA Pico chip	< 50 ng/uL	> 3 ul