McDonnell Genome Institute
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Submitting Prepared Libraries for Sequencing on Shared Lanes on Illumina NovaSeq X Plus (2x150)

Minimum per submission = 150M (2x150) Paired-End (PE) reads (45Gb) targeted

Up to 48 libraries (index pairs) allowed per 150M PE reads (45Gb) targeted

Library QC Service Options:

1. Final Library QC – Individual libraries or pools are submitted to the GTAC Illumina library prep lab. We provide an Agilent BioAnalyzer or Tapestation trace and Qubit concentration estimate. These values are used to estimate molarity to pool (if requested) prior to sequencing. With this service, we will send you QC results and confirm you wish to proceed prior to sequencing. If a high proportion of adapter/primer fragments are present and sufficient mass has been provided, we will recommend a size-selecting AMPURE XP bead purification and perform this step if requested.
2. 10x Library QC and qPCR – Individual prepared 10x libraries are submitted to the GTAC 10x Genomics lab. We provide Agilent BioAnalyzer trace and Qubit concentration estimate, and pass libraries to the sequencing lab for qPCR quantification and sequencing.
3. Direct to sequencing (qPCR only) – Individual libraries or pools are submitted directly to the GTAC Illumina sequencing lab for qPCR and sequencing (no additional QC steps). Minimum target per library or pool (per tube) = 30M PE reads.

   Customers will be charged for additional qPCR if libraries/pools are submitted at concentration exceeding 30nM by qPCR (accepted range by qPCR = 1.5nM-30nM).

   We recommend submitting libraries at 5nM-20nM with 10nM being optimal to avoid falling outside of quantitative range for qPCR.

Accurate reporting of average size (fragment length) of libraries is critical for accurate quantification. **Please request our Final Library QC service if you have not confirmed the average size of your libraries or pools.**

If libraries are mass and/or volume limited, they should be submitted for Final Library QC services to be pooled prior to qPCR and sequencing.

For “Direct to Sequencing” service, libraries or pools should be submitted in ≥30µl at 5-20nM.

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Indexing Requirements and Recommendations:

Due to the number and complexity of library types submitted for Illumina sequencing, there are limitations on index combinations and indexing strategies allowed in shared lanes of NovaSeq X Plus flow cells. All i5 and i7 index sequences, regardless of length, are required to have a minimum hamming distance of 3 (must differ from all other index sequences in the shared lane by at least 3 bases). The i5 and i7 index sequences are compared separately for hamming distance, and also checked for duplicate pairs. Pools that contain libraries that do not meet these criteria will not be permitted in shared lanes. Commercial kits generally adhere to this hamming distance requirement, but please contact us if you have questions about your index sequences or this policy.

The preferred indexing strategy for Illumina sequencing at the GTAC@MGI is 10-base unique dual indexing (UDI) (with minimum hamming distance between index sequences = 3). We can accommodate other index lengths in shared lanes so long as they meet minimum hamming distance requirements and available cycles. Submitting libraries with index sequences shorter that 10 bases in length may result in delayed processing in our sequencing queue due to increased difficultly meeting the minimum hamming distance requirement when combined with other libraries in a shared lane.

We advise using UDIs as this is considered *best practice*s for sequencing on Illumina instruments that use patterned flow cells and "ExAmp" or "XLEAP" chemistry, including the NovaSeq. Single-indexed libraries and libraries incorporating a combinatorial indexing strategy should not be sequenced on the NovaSeq platform. A phenomenon called *index hopping* is known to occur on these sequencing platforms. Without UDIs, this phenomenon will result in misassignment of reads to incorrect samples. We do not currently prohibit customers from submitting libraries with combinatorial dual indexing, but advise using UDIs to mitigate the potential effects of *index hopping*. Below are some documents that explain this issue in greater detail.

https://www.10xgenomics.com/blog/sequence-with-confidence-understand-index-hopping-and-how-to-resolve-it

https://www.illumina.com/content/dam/illumina-marketing/documents/products/whitepapers/index-hopping-white-paper-770-2017-004.pdf

https://support.illumina.com/bulletins/2018/08/understanding-unique-dual-indexes--udi--and-associated-library-p.html