McDonnell Genome Institute
A program of WashU Medicine

The Genome Technology Access Center (GTAC)

DIRECT-TO-SEQUENCING SUBMISSION GUIDELINES

Submitting Prepared Libraries for Sequencing on Shared Lanes

Illumina NovaSeq X Plus · 2 × 150 bp paired-end

150M PE reads	Up to 48	10M PE reads
minimum per submission (45 Gb)	libraries (index pairs) per 150M PE reads	additional units available

■ Submission Specs

Sequencing is available as **paired-end (PE) reads targeted**. The minimum per submission is **150M (2×150) PE reads (45 Gb) targeted** with up to **48 libraries (index pairs)** allowed. Minimum individual target per library or pool (per tube) = 30M PE reads unless requesting Final Library QC services.

qPCR concentration window

Accepted range by qPCR: 1.5 nM – 30 nM. Libraries or pools submitted above 30 nM will be charged for additional qPCR. We recommend submitting libraries at 5–20 nM **(10 nM optimal)** to stay within the quantitative range for qPCR.

Don't know your average fragment length?

Request **Final Library QC** service if the average size of the library or pool is unknown. If libraries are mass- or volume-limited, submit for Final Library QC. Our team will pool prior to qPCR and sequencing.

■ Library QC Service Options

	Direct to Sequencing	Final Library QC	10x Library QC + qPCR
Where it goes	GTAC Sequencing Lab	GTAC Library Prep Lab	GTAC 10x Genomics Lab
What you get	• qPCR quantification	• Agilent BioAnalyzer or TapeStation trace • Qubit concentration • Pooling on request • AMPure XP size-selection when needed	• Agilent BioAnalyzer trace • Qubit concentration • qPCR quantification
Best when…	• Libraries or pools have passed full QC • Within the recommended concentration range	• Library size is unknown • Pooling assistance required • Libraries are mass/volume limited	• Library size is unknown • Additional review is desired

■ Indexing Requirements & 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.

Hamming distance ≥ 3, required

All **i5 and i7 index sequences**, regardless of length, must differ from every other index sequence in the shared lane by **at least 3 bases**. The i5 and i7 sequences are compared separately and also checked for duplicate pairs. Submitted pools that don't meet these criteria will not be permitted in shared lanes. Commercial kits generally comply — contact us with questions about specific index sequences.

PREFERRED INDEXING STRATEGY

The preferred indexing strategy at GTAC@MGI is **10-base unique dual indexing (UDI)** with a minimum Hamming distance of 3. Other index lengths can be accommodated in shared lanes provided they meet the Hamming distance requirements. **Submitting libraries with index sequences shorter than 10 bases may delay processing** due to the difficulty of meeting the minimum Hamming distance when combined with other libraries in a shared lane.

AVOID SINGLE & COMBINATORIAL INDEXING ON NOVASEQ

UDIs are considered best practice for Illumina instruments that use patterned flow cells and ExAmp / XLEAP chemistry which includes the NovaSeq. A phenomenon called index hopping is known to occur on these platforms; without UDIs it can result in misassignment of reads to incorrect samples. We accept combinatorial-indexed libraries for sequencing however strongly recommend UDIs to minimize index hopping risk. Single-indexed libraries may be accommodated on shared lanes however finding compatible libraries that satisfy Hamming distance requirements can be challenging and may result in longer turnaround times. The resources below explain the issue in further detail.

■ Further Reading

- 10x Genomics — Sequence with confidence: understand index hopping and how to resolve it
  https://www.10xgenomics.com/blog/sequence-with-confidence-understand-index-hopping-and-how-to-resolve-it
- Illumina — Index hopping white paper (770-2017-004)
  https://www.illumina.com/content/dam/illumina-marketing/documents/products/whitepapers/index-hopping-white-paper-770-2017-004.pdf
- Illumina Support — Understanding unique dual indexes (UDI) and associated library prep
  https://support.illumina.com/bulletins/2018/08/understanding-unique-dual-indexes--udi--and-associated-library-p.html

Questions about a submission?

Talk to the GTACatMGI Project Development team. We routinely help investigators design the most informative sequencing and analysis strategy at minimal cost.
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