Customer guidelines for Next-Generation Sequencing

NCCT
NGS Competence Center Tübingen
Core Facility Genomics

Core Facility Genomics shipping guidelines for library pools

(Version 2025-08-01)

A) Shipping address for library pools being sequenced on the Illumina NovaSeq6000, NovaSeqX, or NanoPore PromethION for eukaryotic samples:

Note: The NCCT Human Genetics laboratory does NOT accept genetically modified organisms (GMO) or organisms above the safety-level S1.

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Institut für Medizinische Genetik und Angewandte Genomik
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Calwerstr. 7
72076 Tübingen
Tel. Lab 07071 29-72298
ncct@med.uni-tuebingen.de

B) Shipping address for library pools being sequenced on the Illumina NextSeq500, Illumina MiSeq, NanoPore MinION or NanoPore PromethION for microbiological samples:

Note: The NCCT Microbiology laboratory does accept S1 and S2 safety-level materials. Both types of materials have to be clearly announced to the corresponding project manager

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Libraries and ready-to-sequence pools

This section covers important aspects of how to plan and ship libraries or pools for sequencing at the NCCT.

If you have experience with library-pooling and can ensure that the frozen pools are stable for at least four weeks, we recommend pooling in your own facility to speed up the process and achieve a good sample representation in the sequencing run. Indeed, when preparing the library, you will have access to more information on the input material, on the library performance, and on the most effective quantification methods to determine molarity. We do not propose to quantify extern library molarity by quantitative PCR in our facilities. We also do not propose non-equimolar pooling from external library preparation, as it complicates the troubleshooting of potential poor sample representation.

We advise to provide quality parameters in the metadata sheet to avoid submitting samples that are not suitable for sequencing. We strongly recommend using fluorescence-based methods for concentration assessment, and capillary-electrophoresis to determine fragment length. Commercial kits based on qPCR can also be used to evaluate library concentration. Prior to sequencing, our laboratory will validate experimentally concentration, and do the PhiX spike-in the final diluted pool.

Please **submit at least 20 µl of a ready-to-sequence pool**. Individual library volumes vary among protocols and depending on the success of the library preparation may be the bottleneck for pooling. If you have over 40 µl of a ready to sequence pool, we recommend only sending half of your libraries or pools since they could get damaged or lost on their way to the NCCT. Keep in mind that for all submitted pools and libraries, we will access the concentrations and fragment sizes, requiring approximately 5 µl of the submitted total volume.

In order to avoid unnecessary freeze-and-thaw cycles, we recommend doing the library quality assessment, pooling and pool quality assessment in quick direct succession. This reduces the risk of index hopping. Also please make sure that the libraries or pools do not contain any fragments below 200 nts.

If you have no experience of pooling samples, we recommend sending us over 20 µL of the original libraries. We will perform library quality control and propose a pooling strategy based on your requirements. We recommend equimolar pooling to make the troubleshooting easier if the sequencing clusters are distributed poorly between the samples. Pooling samples incurs an additional cost when compared to shipping ready-to-sequence pools.

Application	Required volume	Required molarity	Fragment length
Library Pools	> 20 µl	> 10 nM	<900 nt
10x Library Pools	> 20 µl	> 20 nM	< 600 nt

General Libraries (Illumina-Compatible)

Sample indexing enables the sequencing of different libraries within one pool. Depending on the number of sequences added to your sample sequences, you either generate single-indexed libraries or dual-indexed libraries. Indices used for dual-indexed libraries are either combinatorial dual indices or unique dual indices. Any kind of index sequence used for your samples has to be stated in the metadata sheet. We **recommend using unique dual indices** that are compatible with Illumina standard sequencing primers. If your project is incompatible with standard Illumina sequencing primers, please send the custom primers (> 20 µl) with your samples. Also indicate the primer name, sequence and concentration in the metadata sheet. We cannot assure optimal performance for custom indexed libraries.

10x Libraries / Pools for single cell sequencing

10x ready-to-sequence pools must be at least 20 nM. Please note that we will always run a full flow cell per project, as the sequencing mode is customized for 10x libraries and is not compatible with other library types. Please contact us for further information, especially if you have questions regarding library pooling.

Using a flow cell single lane

On the Illumina NovaSeq 6000, flow-cells have either two sequencing lanes (for SP, S1 and S2) or four sequencing lanes (for S4). Sequencing lanes are independent and can be loaded independently. Loading independent flow cells can be achieved using the "XP workflow" which needs to be purchased additionally to the flow cell, and thus represent an extra cost. This workflow allows using more samples per run as the indexing is independent for each lane. This workflow also requires less volume of the final library to be loaded. Finally, the XP workflow uses capillary effects instead of the instrument microfluidics. This protocol allows a less reproducible loading of the flow-cell and usually results in an output lower than the usual flow-cell specification.
In the case that the wanted output is below the capacity of all the lanes available on the flow cell, the turnaround time of the data delivery will be higher than usual, as a complementary library needs to be sequenced on the remaining lane(s).

Nanopore libraries

At the moment, we do not recommend providing with your ready-for-sequencing libraries for Nanopore sequencing.

PacBio libraries

Custom library preparation: Fragment size in the range of 12.000-20.000; concentration > 70 ng/µL; volume 10 µl in EB PacBio. In case multiple flow cells per sample/library need to be used, please scale the volumes provided. Concentration measured using a Qubit device. Please indicate the library prep kit version and protocol used for the library generation, the average library size in base pairs (bp), and used index barcodes (if applies) in the sample sheet.