NeoPrep removed; Information added - Illumina does not support pooling libraries of varying insert sizes; Required custom primer concentration and volume added.

Sample requirements for genomics projects

1. Preparing and shipping samples

1. Before you send us anything, please read this document and fill out *Sample Information* (1372) or *Library Information* (1401) that you received together with the agreement and send it by email to orders@ngisweden.se. If you have not received the appropriate form, please request one by sending a message to orders@ngisweden.se.
2. First AFTER returning the documents, the barcode labelled plate/plates containing your quality controlled samples may be send to us.

IMPORTANT NOTE! Do not send/bring us samples until we have confirmed that all paperwork (order, agreement, sample Information) has been completed and we have agreed on a time for delivery.

1.1 Sample preparation

For details about specific sample requirements for the different sequencing platforms and applications, please see section 2.

- Only send samples in the by NGI provided 96-well **barcoded** colored plate. Our robotic system is currently calibrated only for those plates.
- **Please make sure the plates are sealed properly with the seal provided by us before shipping! NGI does not take the responsibility for improperly sealed plates.**
- Load samples in columns starting with column 1 row A (see Sample Information). If you deliver more than one plate, or if you send replacement samples, please make sure you change the plate number in the sample information according to the barcode.

IMPORTANT NOTE! Please be aware that using Nanodrop for concentration measurements often tend to overestimate the concentration of a sample. We measure the sample concentrations with Qubit (fluorometric analysis), which is less subject to interference from excess nucleic acids and proteins, and might give a significantly lower concentration than what is measured by a spectrophotometric method. **If you measure with Nanodrop we recommend that you send in twice the amount stated below in order to make sure the minimal amount requirement is met.**

1.2 Shipping samples

- DNA is usually shipped on regular ice while RNA has to be shipped on dry ice. Please make sure you use priority shipping and if possible send it in the beginning of the week to make sure the package won't get stuck somewhere over the weekend! **Never send samples without confirming this with us first!**
- If you stack several plates on top of eachother you should put some material between them to avoid the risk of wells penetrating the seal on the plate below.

Sample requirements for genomics projects

2. Library types for HiSeq and MiSeq Sequencing

Listed in **Table 1** below are validated library preparation methods performed by our production facility. More information for each library preparation type can be found in section 2.2 (DNA samples) and 2.3 (RNA samples). We also accept ready made Illumina compatible libraries, and the requirements for these are summarized in table 2 (more information in section 2.4).

IMPORTANT NOTE! The minimal amount specified in the tables below is the minimal amount left *AFTER* our quality control - exactly enough for two library preparation attempts. **We use AT LEAST 3µl** for reception control.
Thus the **total submitted amount** should be **at least the required amount + AT LEAST 3ul sample.**
E.g. if we request >2000ng and the concentration of the sample is 100ng/µl we will need at least 2300ng, preferably more.

IMPORTANT NOTE! NGI will NOT concentrate or pool samples. To minimize the risk of wasting valuable time sending replacement samples and doing multiple quality controls, please make sure you provide us with quality controlled samples with the required **amount, concentration, volume** and other **quality parameters. Samples with missing information will not be processed.**

2.1 Re-run policy

We will perform a quality control of all submitted samples and contact you if we find any deviations from the requirements.

Samples that pass the reception control

If necessary, we will do up to a total of two library preparation attempts for all samples that pass these criteria. If the sample should for some reason not produce a library you will not have to pay for the preparation. The exceptions are mate-pair samples.

Samples that fail the reception control

If you choose to proceed with a failed sample we will do only one attempt and charge you for the cost of library preparation regardless of the outcome.

Replacement samples

You can, if you want, send replacements for any samples that fail the reception control. However, we will not pool, or concentrate samples so if you need to do adjustments to the already submitted samples we need to send the plate(s) back to you. When the replacement samples have been received we will do another reception control.

NeoPrep removed; Information added - Illumina does not support pooling libraries of varying insert sizes; Required custom primer concentration and volume added.

Sample requirements for genomics projects

Table 1. Summary of sample requirements for validated library construction methods. For further details, see section 2.2 and 2.3 below.

Method	Input material	Conc (ng/uL)	Min vol (uL)	Min amount (ng)	Quality
Illumina TruSeq PCR-free (180 / 350 bp)	DNA	50 - 300	20	2200	Gel recommended
Illumina TruSeq PCR-free (670 bp)	DNA	50 - 300	20	4400	Gel recommended
Rubicon Thruplex DNA-seq (complex genome)	DNA	1 - 10	10	40	Gel recommended
Rubicon Thruplex DNA-seq (small genome)	DNA	0,2 - 10	10	2	Gel recommended
Rubicon Thruplex DNA-seq (ChIP-seq)	DNA	0,2 - 10	10	1	Bioanalyzer
Rubicon Thruplex DNA-seq (FFPE)	DNA	20 - 50	10	200	Gel recommended
Agilent SureSelect XT	DNA	30 - 80	15	400	Gel recommended
Nextera mate-pair, 1.5-2.5kb (gel free)	DNA	50 - 1000	20	1000	Gel recommended
Nextera mate-pair, 3-8kb (gel based)	DNA	50 - 1000	20	4000	Gel required
Illumina TruSeq mRNA (poly-A selection)	total RNA	30 - 100	15	400	Bioanalyzer (RIN ≥8)
Illumina TruSeq total RNA (RiboZero)	total RNA	100 - 250	20	1000	Bioanalyzer (RIN ≥8)
Illumina TruSeq mRNA (no selection/depletion)	mRNA	10 - 25	10	100	Bioanalyzer (RIN ≥8)
Illumina TruSeq Small RNA (Non validated)	total RNA	250 - 400	10	2000	Bioanalyzer (RIN ≥8)

Table 2. Summary of sample requirements for ready-made libraries. For further details, see section 2.4 below.

Method	Input material	Conc (nM)	Min vol (µl)	Max vol (µl)	Quality
Ready-made library	Illumina compatible library	10	20	100	Bioanalyzer

NeoPrep removed; Information added - Illumina does not support pooling libraries of varying insert sizes; Required custom primer concentration and volume added.

Sample requirements for genomics projects

2.2 DNA

All DNA samples should:

- be RNase treated
- have an OD 260/280 ratio of ca 1.8-2.0.
- be eluted in water or Tris-buffer.
- We advice you to run gel electrophoresis to verify that genomic DNA are of high molecular weight.

Standard library construction for DNA samples

The standard library preparation method for DNA samples is the Illumina **TruSeq PCR-free** library prep. Library insert sizes can be 180, 350 or 670bp. Note that the requirements are different between 180/350bp and 670bp insert size.

Library construction for DNA samples with low amount of starting material

For challenging samples where it's not possible to get sufficient amount for a PCR-free library preparation we use the **Rubicon Thruplex-FD** kits.

For Rubicon Thruplex-FD and based on the sample type we have four main tracks:
1. Genomic DNA for complex genomes
2. Genomic DNA for smaller genomes
3. ChIP-seq samples
4. FFPE samples

Insert sizes are the same as for the standard library preparation but if the amount of DNA is very low it might be necessary to use another method resulting in an average insert size of 200-400bp.

Mate-pair library construction

The preparation method used for mate-pair libraries is the **Nextera mate-pair** library prep from Illumina. Depending on the purpose of the project a discussion with the responsible lab specialist and bioinformatician is necessary to choose the most suitable mate-pair approach for each individual sample before the project can begin. **Regular re-run policies do not apply - only one attempt of each sample in library preparation**.

Buffers containing EDTA should NOT be used! A gel electrophoresis picture is needed to verify that the sample is high molecular weight. The majority of the DNA should run as a high molecular weight band greater than 50 kb in size and with minimal lower molecular weight smearing (see example in figure 2, below).

There are three main tracks for mate-pair sequencing:

1. 1.5-2.5kb gel free approach (e.g. for structural variation studies)
2. 3-5kb mate-pair with gel purification (e.g. de novo projects)
3. 5-8kb mate-pair with gel purification (e.g. de novo projects)

NeoPrep removed; Information added - Illumina does not support pooling libraries of varying insert sizes; Required custom primer concentration and volume added.

Sample requirements for genomics projects

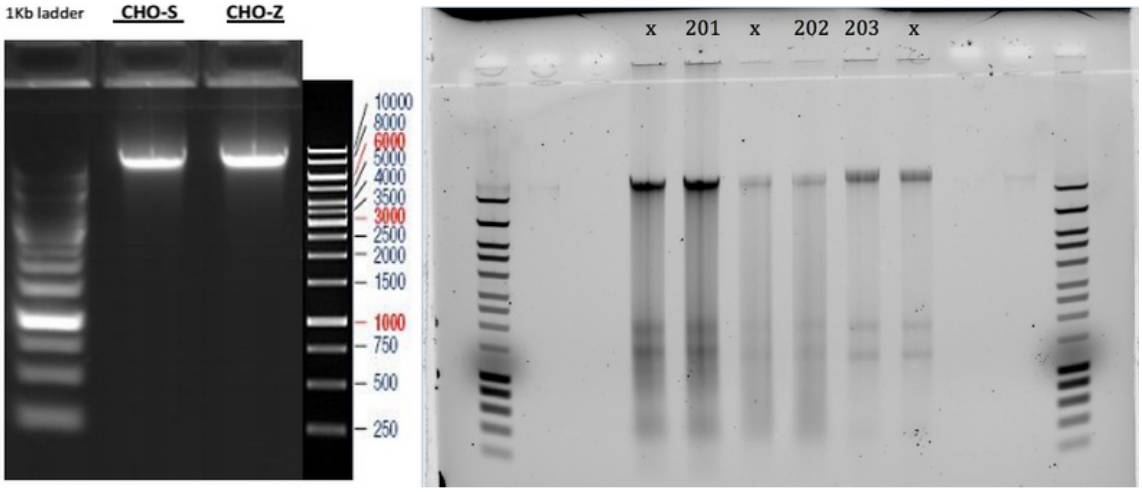

Figure 2. Examples of gels showing genomic DNA samples of both good (left) and bad quality (right) and different gel conditions.

Exome and Custom Sequence Capture

For targeted sequencing we use the **Agilent SureSelect XT** kits and for human whole exome sequencing specifically the Human All Exon V5 kit.

2.3 RNA

All RNA samples should:

- be quality controlled using Agilent Bioanalyzer or equivalent instrument.
- The RIN (RNA integrity number) must be ≥8 and electropherograms for all samples must be submitted together with the sample information.
- be eluted in nuclease free water or tris-buffer.
- have an OD 260/280 ratio of >2.

If Bioanalyzer traces are not provided, we will charge 250 SEK per sample and carry out the assay.

For other than total RNA samples
For ribosomal depleted RNA, mRNA and other types of non-total RNA samples, please provide Bioanalyzer traces and RIN values of the **original total RNA** extract.

There are two main tracks for preparing RNA libraries:

1. Library construction for poly-A enrichment, **Illumina TruSeq Stranded mRNA (poly-A selection)**
2. Library construction for ribosomal depletion, **Illumina TruSeq Stranded total RNA (RiboZero)**

Special requirements for libraries using ribosomal depletion

DNase treatment is strongly recommended since this is the only way of ensuring that there is no genomic DNA contamination of the library. The standard kit use probes compatible with human, mouse and rat samples. Other kits are available, please contact us for more information.

NeoPrep removed; Information added - Illumina does not support pooling libraries of varying insert sizes; Required custom primer concentration and volume added.

Sample requirements for genomics projects

2.4 Ready made libraries prepared by user (“Finished Libraries”)

If several libraries are made, these must be pooled before delivery (= one well per lane). Please note that Illumina does not support pooling of libraries of different insert sizes.
Insert sizes up to 650 bp; total size including adapters up to 800 bp (Bioanalyzer analysis results must be added to the library information to verify the size distribution of the library). Traces from bioanalyzer must be provided for the submitted pool(s) (not individual libraries within the pool). **Make sure that the x-axis is labeled with size and not time.** *For an example see Figure 3, below.*

If Bioanalyzer traces are not provided, we will charge 250 SEK per pool and carry out the assay.

qPCR may only be used for concentration measurement of libraries from PCR-free protocols. In all other cases, please use Qubit.

Position indexes with at least a couple of bases inbetween, we demultiplex using 1 mismatch.
Custom read 1 and index primer for Rapid and HO run should be supplied at minimum 100uM and 15 ul volume.

SciLifeLab can not be held responsible for any data loss related to the the quality of the library.
Note our re-run policy for libraries prepared by user (see Terms and Conditions in the Agreement document).

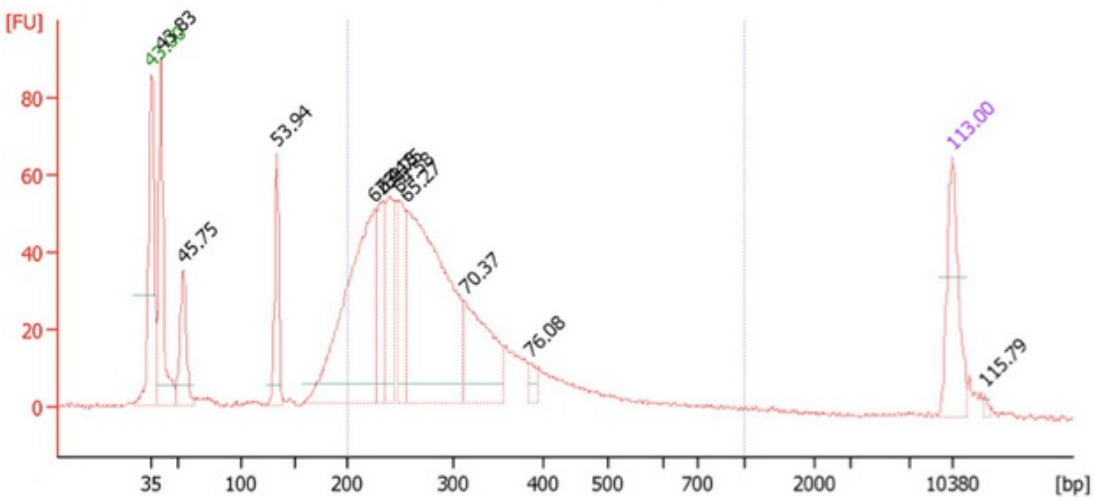

Figure 3. Example of a Bioanalyzer trace with a significant adapter dimer peak (at approximately 130bp). The adapter dimer will probably cluster better than the library in this case, increasing the risk that the sequence data will be mostly adapter sequences rather than from the library.

1372:4 Sample information
Related document

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Category NGI -» User Contacts

NeoPrep removed; Information added - Illumina does not support pooling libraries of varying insert sizes; Required custom primer concentration and volume added.

Sample requirements for genomics projects

1401:5 Library information
Related document

1203:12 Reception control
Related document