NATIONAL GENOMICS INFRASTRUCTURE

1373:17

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Category	NGI -» User Contacts

A summary describing the different sections in the document was included, new methods were added in Table 1 with more explanations, updated run policy for different kits, more clear information about how to deliver samples.

Sample requirements for genomics projects

USER GUIDE: General information about sample requirements and shipping information (NGI-Stockholm)

We encourage ALL users, especially NEW USERS, to read this document BEFORE preparing your samples.

1. Information that you will find in this document

1- OUR DIFFERENT METHODS (library preparation, sequencing). Table 1 and Table 2.

2- QUALITY CONTROL OF STARTING MATERIAL. Section 2.1.

3- RE-RUN POLICY. Section 2.2.

4- SPECIFIC CONSIDERATIONS FOR DNA / RNA SAMPLES / LIBRARIES MADE BY USER. Sections 3.1, 3.2 and 3.3.

5- PREPARING SAMPLES FOR DELIVERY, SAMPLE INFO FILES, SHIPMENT. Section 4.

2. Our different methods (library preparation, sequencing)

The most common library preparation methods run at NGI Stockholm are listed in **Table 1**. We also provide only sequencing services if you prefer to send us ready-made libraries (libraries made by user). The different sequencing alternatives are listed in **Table 2**.

Table 1. **Most common library preparation methods at NGI-Stockholm and their specific sample requirements.**

Method	Input material	Concentration (ng/ul)	Volume (ul)	Min amount (ng)	Quality
Illumina TruSeq PCR-free (180 / 350 bp)	DNA	50 - 300	20-100	2200	Gel recommended
Illumina TruSeq PCR-free (670 bp)	DNA	50 - 300	20-100	4400	Gel recommended

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SMARTer DNA (Rubicon, complex genome, e.g. eukaryote)	DNA	1 - 10	15-100	40	Gel recommended
SMARTer DNA (Rubicon, small genome, e.g. bacteria)	DNA	0.2 - 10	15-100	2	Gel recommended
SMARTer DNA (Rubicon, ChIP-seq)	DNA	0.2 - 10	15-100	1	Bioanalyzer
SMARTer DNA (Rubicon, FFPE)	DNA	20 - 50	15-100	200	Gel recommended
10X Chromium (non-validated)*	DNA	5 - 20	20-100	100	Gel recommended
RAD-Seq**	DNA	16-100	16-170	200	Gel recommended
Illumina TruSeq mRNA (poly-A selection)***	total RNA	30 - 100	15-100	>400	Bioanalyzer (RIN ≥8)
Illumina TruSeq total RNA (RiboZero) standard kit human, mouse, rat	total RNA	100 - 250	20-100	>1000 *	Bioanalyzer (RIN ≥8)
Illumina TruSeq mRNA (no selection/depletion)	mRNA	10 - 25	15-100	100	Bioanalyzer (RIN ≥8)
Illumina TruSeq Small RNA	total RNA	250 - 400	15-100	1000	Small RNA Bioanalyzer on total RNA****
SMARTer Pico (non-validated, incl. FFPE, mammalian)*****	total RNA	0,03 - 10	12-30	0,3	Bioanalyzer (RIN ≥8)

Note! For non-validated methods a planning meeting is required. Please contact us via support@ngisweden.se

*** Input depends on genome, the values in Table 1 are for human. Official recommendation is > 50 kb average molecular size but shorter size can work depending on the organism and analysis (Longranger or SuperNova).**

**** Input DNA must be EcoRI digested. Gel recommended (preferably digested / undigested samples side-by-side).**

***** We recommend >2000ng but can start with a lower amount as stated in the list above.**

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Sample requirements for genomics projects

****** Small RNA Bioanalyzer on total RNA extract is recommended to verify that small RNAs are present.**

******* Input amount recommended by the manufacturer, but undetectable levels could work. Concentration should be measured with Qubit. Includes a ribosomal depletion step.**

Table 2. Summary of sample requirements for **libraries made by user**.

Sequencing Platform	Input Material	Concentration (nM)	Volume (ul)	Quality
MiSeq	Illumina compatible library	10	20-100	Bioanalyzer
NovaSeq SP/S1/S2/S4 (separate lanes)	Illumina compatible library	10	20-100	Bioanalyzer
NovaSeq SP/S1/S2/S4 (entire flow cell)	Illumina compatible library	10	70-200*	Bioanalyzer

*** Divide pool into two wells with max. 100 ul in each well.**

2.1 Quality control of starting material

Before sending your samples to us, perform a Quality Control (QC) on them and verify that you are providing the required amount, concentration, volume and other quality parameters. This is in order to minimize wasting valuable time sending replacement samples and doing extra quality controls and to reduce communication for trouble-shooting purposes. **We prefer that you send enough material for at least two library preparation attempts.**

Samples with missing information will not be processed.

When your samples arrive at NGI Stockholm, we will perform a **Reception Control (RC)** of all submitted samples; your Project Coordinator will contact you if we find any deviations from the specified requirements (**Table 1**).

IMPORTANT NOTE! NGI will NOT concentrate or pool individual samples. In some special cases, we could dilute your samples. **Projects with samples that do not meet our requirements will take longer time since this causes extra laboratory processing and communication with the user.**

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Sample requirements for genomics projects

2.2 Re-run policy

RNA and DNA samples that pass Reception Control

If samples pass RC, we will do up to two library preparation attempts if the amount of material allows so. If the sample should for some reason not produce a library, you will not have to pay for the preparation.

IMPORTANT NOTE: for **Rubicon Thruplex**, **small RNA** (mate-pair, not listed), RAD-Seq and **non-validated protocols**, we perform only one library preparation attempt (no re-runs unless there is a technical issue). For **RNA-RiboZero**, we sell individual reactions only for the standard kit which is **HMR** (human, mouse rabbit); for non-standard RiboZero kits a whole kit should be ordered (smallest kit has 48 reactions) and re-preps can be done only if there are enough reactions left (columns) or if you order additional kit/s.

RNA and DNA samples that fail Reception Control

If a sample fails RC and if you choose to proceed with a failed sample (after being contacted by a Project Coordinator), we will do only one library preparation attempt (with the exception of a technical failure, if this occurs NGI will make an additional library preparation attempt if amount allows so at no extra cost). If the library preparation is of low quality or concentration and you want to perform sequencing anyway, you will be charged for the cost even if you do not get enough sequencing data.

Libraries made by user that pass Reception Control

If libraries made by user pass RC, we will sequence the library pool to achieve the requested amount of sequence data per lane. However, no guarantees are given regarding relative amount of reads among the libraries in the pool.

Libraries made by user that fail Reception Control

If libraries made by user fail RC and you choose to proceed with sequencing of failed libraries (after being contacted by a Project Coordinator), we will not give any guarantees regarding the yield or quality of the sequencing and you will be charged for the sequencing cost regardless of the data generated. We will sequence these libraries only once (unless there is a technical issue).

Replacement samples

Users can send 1 (ONE) replacement for any sample or library made by user that fails RC, immediately after RC is done (maximum 2 months after arrival of the samples) and before any further laboratory processing. If the user would like to send a replacement sample **after library preparation, this must be done as a different project** and he / she will pay for the corresponding library preparation and sequencing.

In the case of specific projects run by our Applications Team, where no RC is performed, please contact us via support@ngisweden.se.

We will not pool, or concentrate samples, so if you need to do adjustments to the already submitted samples we will send the plate(s) back to you. **When the replacement sample(s) have been received, we will do a new RC.** In special cases, you can be asked to provide a replacement sample after library preparation (e.g. if the library preparation failed due to a technical issue and you only provided material for 1 library attempt).

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Sample requirements for genomics projects

3. Specific considerations

3.1 Specific considerations for DNA samples

For DNA-seq we offer several different library preparation protocols depending on the input amount and application. We recommend you to run gel electrophoresis to verify that genomic DNA is of high molecular weight.

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 (10 mM Tris-Cl, pH 8.5).

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, see **Table 1** above.

Library construction for DNA samples with low amount of starting material

For challenging samples where it is not possible to get sufficient amount of starting material for Illumina TruSeq PCR-free library preparation, we offer the **SMARTer DNA HT Dual Index kit** from Takara (old name Rubicon Thruplex).

Based on the sample type, we have four main tracks:

1. Genomic DNA for eukaryotes and metagenomes
2. Genomic DNA for prokaryotes
3. ChIP-seq samples
4. FFPE samples

The generated library 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.

De novo projects: 10X Genomics Chromium Genome

For *de novo* projects, we offer 10X Genomics Chromium Genome library preparation (run by our Applications Development Team, non-validated method). It is highly important that the **DNA is of high molecular weight and of good quality**. In those cases where NGI will perform DNA extractions from samples that are dissolved, please indicate the **exact composition of the corresponding buffer.** Contact us via support@ngisweden.se for more information.

We also continue offering **Nextera mate-pair** library prep from Illumina. You are welcome to contact us for a project discussion.

Sample requirements for genomics projects

3.2 Specific considerations for RNA samples

For RNA-Seq it is recommended to include a minimum of **3 biological replicates**. More replicates gives a higher power than deeper sequencing. **DNase treatment** is strongly recommended, especially for library preparation protocol using ribosomal depletion, since this is the only way of ensuring that there is no genomic DNA contamination of the library.

All RNA samples should:

- be quality controlled using **Agilent Bioanalyzer** or equivalent instrument (e.g. Tape Station / Fragment Analyzer). When sending small RNA (total or enriched), Small RNA Bioanalyzer is recommended.
- 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 (10 mM Tris-Cl, pH 8.5).
- have an OD 260/280 ratio of >2.

If Bioanalyzer or equivalent traces are not provided, we can carry out the analysis at a charge of 250 SEK per sample.

RNA samples consisting of non-total RNA
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. In those cases where NGI will perform RNA extractions from samples that are dissolved, please indicate the **exact composition of the corresponding buffer.** Contact us via support@ngisweden.se for more information.

There are three main tracks for preparing RNA libraries:

1. Library construction for poly-A enrichment, **Illumina TruSeq Stranded mRNA (poly-A selection)**
NOTE: We recommend submitting **2000 ng total RNA** to ensure optimal sample diversity and consequently data quality. However we can start library preparation with down to 200 ng total RNA, see **Table 1** above.

2. Library construction for ribosomal depletion, **Illumina TruSeq Stranded total RNA (RiboZero).** The standard kit uses probes compatible with human, mouse and rat samples.
IMPORTANT: Special kits for other species have a different policy (we do not sell individual reactions, please contact us).
NOTE: We recommend submitting **2000 ng total RNA** to ensure optimal sample diversity and consequently data quality. However we can start library preparation with down to 500 ng total RNA, see **Table 1** above.

3. Library construction for small RNA-seq, **Illumina TruSeq Small RNA. Note: only 48 different indexes available for this method.**

Low-input library preparations alternatives are available (run buy our Applications Development Team, non-validated methods). Contact us (support@ngisweden.se) for more information.

If you have a different starting material, such as cDNA, please contact us for a project discussion.

3.3 Specific considerations for libraries made by user

Libraries made by user must to be pooled by the user before delivery (= one well per pool, except when sequencing on a NovaSeq entire flow cell as specified in Table 2). 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 file 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(s).** For an example see Figure 3, below.

If Bioanalyzer traces are not provided, we can carry out the analysis at a charge of 250 SEK per sample.

- qPCR may only be used for concentration measurement of libraries from PCR-free protocols. In all other cases, we recommend Qubit.
- **Position indexes** with at least a couple of bases inbetween, we demultiplex using 1 mismatch
- **Custom read 1 and index primer** should be supplied at **minimum 100 uM and 15 ul volume.**
- **If sequencing on NovaSeq, make sure that your samples do not contain indexes starting with GG.**

NOTE: Low diversity libraries made by user need to be loaded with lower concentration (because PhiX should be added along with the libraries to allow for data processing by the Illumina software during sequencing), thus the sample yield can be lower than what we guarantee. Please specify in the order if the library is of low diversity. NGI can not be held responsible for any data loss related to the the quality of the library.

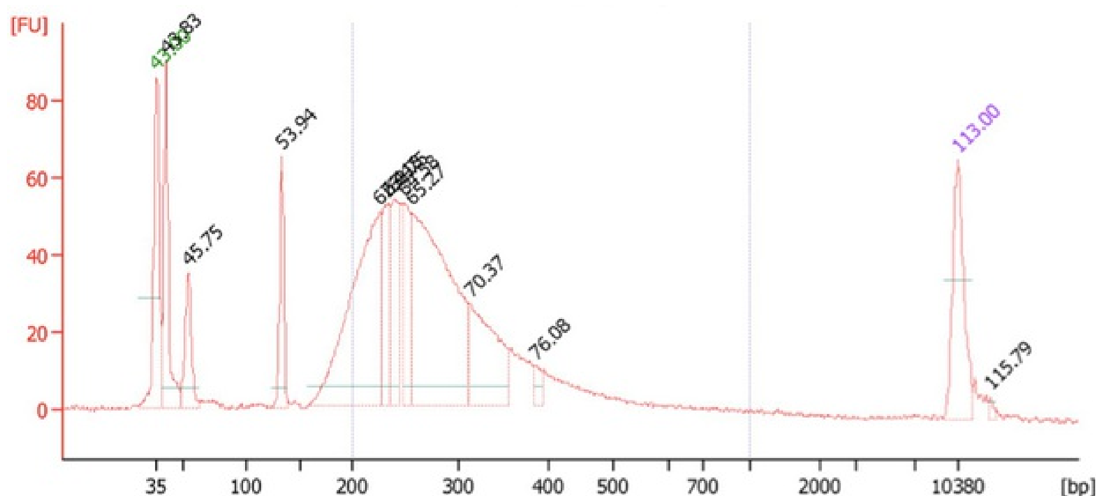

Figure 3. Example of a Bioanalyzer trace with a significant adapter dimer peak (at approximately 130 bp). The adapter dimer will probably cluster better than the library in this case, increasing the risk that the sequence data will be mostly adapter sequences rather than from the library.

4. How to prepare your sample plate and ship your samples

Before delivering your samples to NGI-Stockholm (loaded on the 96-well plate provided by us), please fill out the

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Sample Information (doc 1372, for RNA or DNA samples) or **Library Information** (doc 1401, for libraries made by user) **Excel file.** One of the the above files is sent to the email address specified in the order together with the project agreement. Send all sample- and project-related documents by email to support@ngisweden.se **BEFORE** sending the sample plate. If you have not received the appropriate form or plate, please request one by sending a message to the aforementioned e-mail address.

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

4.1 How to load and prepare your plate or deliver other types of samples

For details about specific sample requirements for the different applications, see section 1.

- Only send samples in the **colored plate** provided by NGI. Our robotic system is calibrated only for those plates.
- Make sure the **plates are sealed properly** with the aluminium seal provided by us. NGI does not take responsibility for improperly sealed plates.
- **Load samples in columns** starting with column 1 row A. For several plates, or replacement samples, please make sure you change the plate number in the sample information according to the barcode.
- Make sure that the 96-well plate is **labeled with the correct project-specific barcode** provided by us after processing of the order (the first plate to be delivered to us is PXXXXXP1, second plate is PXXXXXP2).
- When sending tissues or samples that can not be loaded on a plate, please contact us via support@ngisweden.se, so we can instruct you how to prepare and label your sample(s) in the best way.

Nanodrop often overestimates the concentration of a sample. If you measure with Nanodrop, we recommend that you send in twice the amount stated in Table 1, in order to make sure the minimal amount is met. Qubit gives a more accurate quantification and is preferred.

4.2 Shipping your samples

- **DNA samples and libraries made by user**: deliver on regular ice. Place your plate in a plastic bag to protect the seal from being broken by the ice.
- **RNA samples**: deliver on dry ice. Place your plate in a plastic bag to protect the seal from being broken by the ice.

Please make sure you use PRIORITY shipping, mark the package in an appropriate way indicating COLD DELIVERY (so it is properly handled at NGI upon arrival, please refer to doc 1460 Plate Information, sent to you with the agreement). If possible, send the package at the beginning of the week to make sure the package does not get stuck somewhere over the weekend. **Please do not send samples without confirming with us first!**

NOTE: If you stack several plates on top of each other, you should put some material between them to avoid the risk of wells penetrating the seal on the plate below.

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Sample requirements for genomics projects

1372:4 Sample information
Related document

1401:7 Library information
Related document

1203:18 Reception control
Related document

1203-18_Sample_requirement_summary
Attachment