

# **GUIDELINES for correct completion of SAMPLE SUBMISSION FORM**



## **ILLUMINA SEQUENCING**

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## I. HOW TO MAKE A SUBMISSION

Submission is simple – just fully complete the submission form and send it with required attachments to [post@sequencing.uio.no](mailto:post@sequencing.uio.no), wait for our reply that your submission is accepted, then bring or ship your samples to the address advised by the NSC staff in their reply (either OUS or UiO, shipping addresses are at the back of the submission form).

### Required attachments (documenting sample size and integrity)

Unless agreed with us in advance, you are required to provide both:

1. **Our submission form**  
(<http://www.sequencing.uio.no/forms/samplesubmissionforms.html>)
2. **Gel image or BioAnalyzer (or equivalent method) traces of your samples / libraries.**

**DNA:** For genomic DNA samples, an agarose gel showing high MW DNA with no degradation is most appropriate (indicate relevant marker sizes, and amount of sample loaded). For ChIP samples, input DNA must be shown.

**RNA:** A BioAnalyzer trace/list of RIN numbers is preferred. Agilent Bioanalyzer RNA Integrity Number (RIN) should be > 7. Gel images showing rRNA are also acceptable, in which case samples should have a 28S/18S ratio >1.6.

**LIBRARIES:** For prepared libraries, a bioanalyzer trace or equivalent is most appropriate.

Send all documents in a single mail, when fully complete. If you need a price quote in order to obtain a purchase order number, contact us to obtain one on [post@sequencing.uio.no](mailto:post@sequencing.uio.no) – do not send an incomplete form and the purchase information later.

### Sample purity requirements

**Note that purity measurements (spectrophotometer absorbance) must be documented for each sample in the [sample information table](#) (section 3 / download excel template for 96-well PCR plate submissions). Different requirements apply for ChIP samples - please see the application-specific note below.**

**DNA and RNA samples:** The 260/280 ratio should fall in the range 1.8-2.1 and the 260/230 ratio within 1.8-2.4.

## II. GENERAL SAMPLE SUBMISSION INFORMATION

### Sample library preparation capacity of the NSC.

You may perform sample library preparation in your own lab, or you may submit samples to us for preparation. Subject to our agreement, you may also send trained personnel to perform the preps under our supervision. We operate pipetting robots to maximize throughput, and continually aim to increase the number of samples we can prepare as a service. However, not all protocols are suitable for automation, so we are in some cases obliged to ask that you contribute manpower when the number of samples exceeds our capacity. You should contact us **before submission** if you have in excess of the following sample numbers:

<i>Sample prep type</i>	<i>Number of samples / submission</i>
DNA	96
RNA	96
ChIP / low-input DNA	48
Small RNA	24
16S	940

These limits are subject to revision as we add to our automation capabilities. Higher sample numbers may be handled, but require our prior agreement. Ensure you have downloaded our most recent guidelines to guarantee you are viewing the most up-to-date information from [www.sequencing.uio.no](http://www.sequencing.uio.no).

### Sample storage

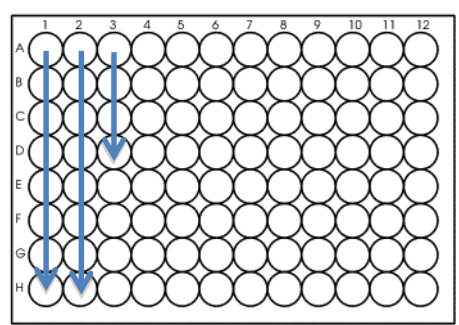
We will store your submitted samples for a period of 3 months following submission. Samples cannot be returned to users except in exceptional circumstances. We store your completed libraries for at least 1 year following project completion to allow further sequencing if requested.

### Accepted buffers

Except where specified (see Table 2 below), do not use buffers containing EDTA, as this will inhibit the enzymatic steps of sample library preparation. If you must concentrate your samples to achieve the recommended minima below, please bear in mind that the final concentration of buffers will affect downstream performance. Do not submit samples in >10mM Tris or other concentrated buffer. We do not accept lyophilized or precipitated samples. *Samples delivered in inappropriate buffers will either be returned to the user or subject to additional cleanup costs of 1000 NOK/sample at our discretion.*

### Accepted tubes/plates and labeling.

**Each tube/plate must be marked with the user's name and date, in addition to sample name. When submitting >16 samples, use 96-well plates (filling in columns – see figure below). Lower numbers of samples should be submitted in 1.5 ml Safelock Eppendorf tubes.** Do not submit samples in 0.5 ml tubes or 8-well strips. We do not accept precipitated/dried samples.



Correct layout for filling samples in 96-well PCR plates (i.e. filled by columns from left side, not rows).

Do not leave empty wells or gaps!

### Sample concentrations.

We do not insist that you equalise the concentration of all samples in your submission. However, your samples will be processed faster if you do so, and we encourage this practice. Please see the Table 2 below for required sample amounts and accepted buffers.

### PhiX blending

It is standard practice to spike Illumina sequencing runs with 1% PhiX library, which acts as a reference for sequencing performance. The level of PhiX blend must be increased for low-diversity samples, up to 50%, at the expense of yield. If you are concerned that the use of PhiX will cause problems for your downstream data analysis, it is your responsibility to tell us this and request an alternative solution.

**Failure to follow these guidelines voids any guarantees on library preparation or run yields.**

### III. ILLUMINA SEQUENCING AVAILABLE AT THE NSC

#### Choice of sequencer and read length

Users of the NSC may choose to have their samples run on MiSeq, NextSeq-500, HiSeq 3000/4000/X or NovaSeq systems. Note that the HiSeq X can only be used for whole-genome sequencing applications. We are happy to advise on the most appropriate system for your needs, based on read length available, output yield, budget and queue time. The tables below provide a simplified guide to the choices available – users are advised to check [www.illumina.com](http://www.illumina.com) for the most up to date information:

**Table 1: Typical read lengths and yields of Illumina sequencing instruments**

#### SHORT READS

Machine	MiSeq (per run)	NextSeq Mid-output (per run)	NextSeq High-output (per run)	HiSeq 3/4000 (lane)	HiSeq 3/4000 (lane)	NovaSeq (4 flowcell sizes)
Read length(s)	50 bp SR	75 bp PE	40 bp PE / 75 bp SR	50 bp SR	75 bp PE	100 bp SR / 50 bp PE
No. Reads per end (M)	12-15	80-130	3-400	250-300	250-300	650-4,000
Yield (Gb)	0,5-0,75	12-20	20-30	13-15	40-46	65-400

#### LONG READS

Machine	MiSeq (per run)	NextSeq Mid-output (per run)	NextSeq High-output (per run)	HiSeq 3/4000 (lane)	HiSeq X (lane)	NovaSeq (4 flowcell sizes)
Read length(s)	300 bp PE	150 bp PE	150 bp PE	150 bp PE	150 bp PE	150 / 250 bp PE
No. Reads per end (M)	15-25	80-130	3-400	250-300	330-375	650- 10,000
Yield (Gb)	9-15	25-39	75-120	80-90	100-110	200-3000

SR = single read, PE = paired end

The above tables do not detail an exhaustive list of services offered. Most machines run additional reagents giving lower output and shorter read lengths than listed above.

Yields are anticipated yields per run / lane based upper limits of Illumina specified yields, and do not constitute guaranteed amounts, which can be lower.

#### Reagents held in stock.

Only the following reagents / read lengths are routinely in use and held in stock. All others must be ordered specifically at your request, or on submission, which may take 1-2 weeks.

NovaSeq SP flowcell: 150 bp PE

NextSeq: 75 bp SR

MiSeq: 300 bp PE

#### Data output performance guarantees

## The Norwegian High Throughput Sequencing Centre

If your samples are submitted in accordance with these guidelines and meet our quality and quantity requirements, we provide the performance guarantees below. Note that these are our minimal output criteria and we routinely expect greater output. Should we fail to meet these targets, we will run additional sequencing at no cost to you or offer a discount as appropriate. In the case of multiplexed samples, we cannot guarantee equal distribution of reads between different indexes, but we do run a qPCR assay to achieve this as best possible.

<b>System/reagents</b>	<b>Reads per end per lane/run*</b>	<b>Quality</b>
NovaSeq SP flowcell	400,000,000	as per Illumina specifications (see URL below):
NovaSeq S1 flowcell	800,000,000	as per Illumina specifications (see URL below):
NovaSeq S2 flowcell	2,000,000,000	as per Illumina specifications (see URL below):
NovaSeq S4 flowcell	5,000,000,000	as per Illumina specifications (see URL below):
HiSeq 3000 / 4000 / X	150,000,000	as per Illumina specifications (see URL below):
NextSeq mid-output	65,000,000	as per Illumina specifications (see URL below):
NextSeq high-output	200,000,000	as per Illumina specifications (see URL below):
MiSeq V3	10,000,000	as per Illumina specifications (see URL below):
MiSeq V2	6,000,000	as per Illumina specifications (see URL below):

<https://emea.illumina.com/systems/sequencing-platforms/novaseq/specifications.html>

<http://www.illumina.com/systems/hiseq-x-sequencing-system/performance-specifications.html>

<https://www.illumina.com/systems/sequencing-platforms/hiseq-3000-4000/specifications.html>

<https://www.illumina.com/systems/sequencing-platforms/nextseq/specifications.html>

[http://www.illumina.com/systems/miseq/performance\\_specifications.ilmn](http://www.illumina.com/systems/miseq/performance_specifications.ilmn)

\* Read (cluster) number passing Illumina's default filter in a single-read run (MiSeq and NextSeq). Read numbers for HiSeq refer to a single lane (HiSeq runs consist of 8 lanes). NovaSeq read numbers refer to full flowcells, but in some cases it is possible to order half- or quarter- flowcells, in which case read numbers will be reduced accordingly. The numbers above should be doubled for paired end runs. We do not distinguish between reads mapping to a reference sequence and those that do not.

*Exceptions:* Data guarantees are not valid for long-insert libraries (insert size > 500 bp) and low-diversity samples (e.g. PCR amplicons, restriction digestion fragments, RAD libraries), which require dilution and blending with control library using Illumina technology. Note that if your submitted samples do not meet our quality or quantity criteria, we may still be willing to attempt sample prep and sequencing, but our performance guarantee no longer applies and you will be liable for all expenses incurred regardless of the output amount, also in the event that no sequence data can be generated.

## IV. APPLICATION-SPECIFIC NOTES

### DNA preps

Users may choose between library preparation by transposon tagmentation (Nextera™ Flex) or by sonication and adapter ligation reagents (TruSeq™ Nano, TruSeq™ PCR-free, or SMARTer ThruPLEX® low-input). Our favoured default procedure employs the Nextera™ Flex reagents, entailing 6 cycles PCR, which allows a rapid turnaround and is relatively insensitive to DNA input amounts (see table 2 below). Where DNA amounts allow ( $\geq 2 \mu\text{g}$ ), PCR-free preps are recommended when the organism to be sequenced has high or low GC content. When limiting sample amounts are available (100 pg – 50 ng), SMARTer ThruPLEX® preps should be selected. TruSeq™ Nano reagent is a legacy product that in most cases has been superseded by the Nextera™ Flex reagent, but is still available.

Note that PCR-free libraries, due to being partially single stranded, do not store well. PCR-free libraries over 2 months of age must be re-QC'd before sequencing, and may not retain sufficient activity to provide data. PCR-free libraries older than 6 months are unlikely to be sequence-able.

DNA for sequencing should be treated with RNase before final cleanup and submission.

### RNA-seq.

We offer TruSeq™ stranded RNA-seq sample prep as a service, which employs poly-T beads to enrich the polyadenylated fraction of mRNA. **If the use of poly-T beads is not appropriate for your sample, it is your responsibility to inform us of this.** Alternative kits/procedures also exist for rRNA depletion, but these are not offered as a standard service by the NSC. We may consider performing these as part of a research collaboration – please inquire.

*For bacterial RNA / rRNA depleted eukaryotic RNA:* You may perform rRNA depletion yourself and submit the rRNA-depleted or mRNA-enriched samples to us to enter the RNA-seq prep at the appropriate stage in the protocol. It is critical that the RNA is in this case supplied only in water. We can also perform RNA-seq on total RNA without RNA depletion in this way, although you must be prepared that the majority of reads will be derived from rRNA.

Note that we cannot guarantee successful prep of rRNA-depleted samples, as it is usually impossible to distinguish successfully depleted samples from degraded RNA. These library preps are therefore performed at the user's risk.

*We strongly recommend* that RNA be prepared by procedures that include a DNase digestion to remove contaminating DNA.

### ChIP samples.

It is not necessary to provide gel or Bioanalyzer documentation of ChIP sample size or quality, nor 260/280 or 260/230 ratios. However, it is necessary to document the size of input material used prior to immunoprecipitation. If possible, please provide a concentration of the ChIP sample determined by a fluorometric method such as the Qubit system from Invitrogen, as this will greatly increase your chances of obtaining a high-quality sequencing

library. Do not use a Nanodrop instrument. **Note that ChIP samples must not contain salmon sperm DNA or other nucleic acid blocking agents.**

#### **DNA for 16S library prep:**

Samples for 16S prep must be submitted in 96-well plates, with up to 94 samples per plate (to allow us to add positive and negative controls during amplification). We use the Fadrosch et al. ([Microbiome](#), 2014 Feb 24;2(1):6. doi: 10.1186/2049-2618-2-6.) protocol to prepare amplicon libraries of the V3-V4 region of the prokaryotic 16S rRNA gene. This region can be used to characterize the microbial diversity on genus or species levels in e.g. clinical samples or other sample types, containing sufficient microbial DNA. The procedure employs the 319F and 806R primers, but incorporate the modified 806R recommended by the Earth Microbiome Project, thus the 16S amplification primer sequences are as follows:

319F forward primer: 5' ACTCCTACGGGAGGCAGCAG 3'  
806R reverse primer: 5' GGACTACNVGGGTWTCTAAT 3'

The libraries contain in-read dual indexes and heterogeneity spacers, enabling multiplexing of up to 570 samples and sequencing on MiSeq 300 bp paired end reads with only 10% PhiX spike-in. Up to 190 samples are recommended to be sequenced on one Miseq run. A positive control sample (ZymoBIOMICS Microbial Community DNA standard II, Zymo Research) and a negative control sample (H<sub>2</sub>O) will always be included. For DNA input requirements- check Table 2. We will provide demultiplexed files from read 1 and read 2 for each sample in fastq, and upon request and for an additional fee, QIIME2 or MOTHUR analysis reports.

**Note:** The user is responsible for ensuring submitted DNA samples contain sufficient microbial DNA. If unsure of this, please contact us and we can perform a small test with a few samples. Although we will attempt to re-prepare any samples that fail in a first round of library preparation, we cannot promise successful library prep for all samples, and will contact you in the case samples do not amplify before proceeding to sequencing. We employ normalization to achieve even yields from all samples, but are unable to guarantee read thresholds per sample except by prior arrangement, which may entail additional costs. Unless agreed in advance, runs delivering >10,000 reads (for 94 or fewer samples per run – proportionally lower if more samples) for 95% of submitted samples will be considered successful and additional sequencing will only be performed at the user's expense.

#### **Small (micro) RNA.**

Total RNA must be prepared by a method that retains small RNAs. Various vendors provide kits and protocols with adaptations to retain small RNAs. Avoid precipitating RNA, as this reduces the content of small RNAs.

Due to large variations in miRNA amounts in different tissues, success of miRNA preps is not guaranteed. Users submitting total RNA will therefore be billed for reactions consumed even in the event we are unable to prepare libraries. In the event that preps from total RNA are unsuccessful, users may attempt to enrich the small RNA fraction by PAGE or column fractionation and submit this instead, but be sure to note this in the submission form.



**WGA- and WTA- amplified samples.**

Whole-genome amplified (WGA) samples are frequently problematic, so will only be sequenced at the risk of the submitter.

**V. SAMPLE REQUIREMENTS – DNA/RNA SAMPLES**

As a general rule, **more DNA/RNA is better**, so please provide more than the recommended sample amounts listed below if you can. Using the minimum amount increases the chances of failed preps. We may also be willing to attempt library preparation with amounts lower than the given minima, but this must be agreed with us in advance of submission, and you must nonetheless pay the cost of consumed reagents in the event of prep failure.

**DNA/RNA samples requiring full sample library preparation:**

Table 2: Amounts, volumes and buffers accepted for samples requiring library prep.

Sample Type	Min. Amount	Recommended Amount	Max. Volume	Accepted Buffers.
Genomic DNA (Nextera Flex prep)*	2 ng*	200 – 1000 ng	60 µl	10 mM Tris (TE also OK if conc. > 40 ng / µl)
Genomic DNA (TruSeq Nano, 350 bp insert)	0.2 µg	1 µg	130 µl	10 mM Tris, TE
TruSeq Nano, 550 bp insert	0.4 µg	2 µg	130 µl	
Genomic DNA (PCR-free prep)	2 µg (measured by Qubit, NOT nanodrop)	10 µg (measured by Qubit, NOT nanodrop)	130 µl (min. concentration 30 ng / µl)	10 mM Tris, TE
DNA for 16S prep	200 ng / sample (Min. 10 ng/µl in 20 µl)	200 – 2000 ng	100 µl	10 mM Tris
mRNA	0.5 µg total RNA	1 – 10 µg total RNA	50 µl	TE, 10mM Tris, H <sub>2</sub> O
	or purified mRNA /rRNA-depleted RNA / bacterial RNA	or mRNA purified from 1-10 µg starting total RNA (10 – 400 ng)	5 µl per 1 µg starting material	H <sub>2</sub> O

Sample Type	Min. Amount	Recommended Amount	Max. Volume	Accepted Buffers.
ChIP/ low-input DNA	0.1 ng (see note on p4)	1 – 10 ng	20 µl	5-10 mM Tris
Small RNA	0.2 µg total RNA <i>or</i> Purified small RNAs (preferred)	2 – 5 µg tot. RNA <i>or</i> Purified from 2-5 µg starting tot. RNA	10 µl  10 µl	H <sub>2</sub> O  H <sub>2</sub> O
PCR amplicons	200 ng	1 µg	100 µl	10 mM Tris

\* **For Nextera submissions**, samples on any one plate submitted should all fall within the following ranges of DNA amounts: 2-20 ng, 20-50 ng, 50-100 ng or **100-1000 ng (optimum)** in a maximum volume of 60 µl. If sample concentrations span these ranges, samples of similar concentration should be grouped and submitted on separate plates. Concentrations must be determined by a fluorometric method such as Invitrogen's Qubit, not spectrophotometric methods such as Nanodrop. It is not recommended to submit less than 100 ng DNA except in the case of small (e.g. bacterial) genomes.

**Note: Samples with high genomic DNA concentrations (>1 µg/µl)** cause problems with our automation due to their viscosity, so are not acceptable. Please dilute your samples to under this limit and re-check their concentration. **Do not send DNA or RNA samples, even at high concentrations, in less than 5 µl, as this makes use of automation or multichannel pipettes difficult.**

### Sample Multiplexing (a.k.a. indexing / barcoding)

Do not forget to request indexing of samples unless you wish each sample to occupy a separate lane of Illumina sequencing. It is important you specify which samples should be run together in each lane if this will affect your experiment.

The sample prep kits we use automatically provide indexing capacity for the pooling of 96 samples (24 for miRNA preps). Pooling of up to 384 samples is possible, but may incur additional reagent costs.

Other companies also offer indexing kits that are Illumina-compatible, and we will sequence these at your request. However, we do not offer library preparation using other systems as a service. We do consider requests to perform such preps on a collaborative basis, so please inquire to the contact email address below.

## VI. SAMPLE REQUIREMENTS – USER-PREPARED LIBRARIES

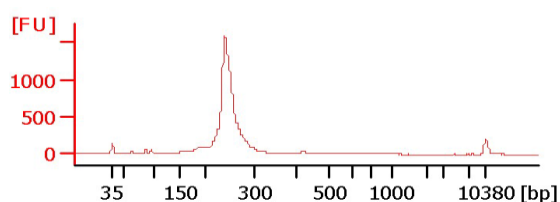
**We will test the quality and performance of your libraries by qPCR prior to clustering. If preparing your own libraries, please:**

1. Pay attention to index compatibility when preparing your libraries as follows:
  - i. all samples to be run in a single lane **MUST** have different indexes.
  - ii. Compatible indexes must be chosen. e.g. - for 2 samples per lane, Illumina indexes 6 & 12 or 5 & 19. The following rule applies to choosing appropriate index combinations: Each index sequencing cycle, irrespective of index read length, must contain at least one base from each of the following two pairs: (G/T) and (A/C). For more information on choosing compatible index combinations, please see [www.illumina.com](http://www.illumina.com).
  - iii. Provide full information on the indexes you have used in the sample table – eg.

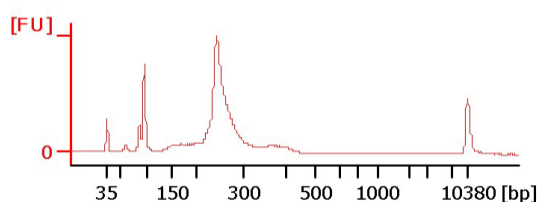
**Table 3: Format for defining indexes used**

	Index name	Index Seq
Example single index	TruSeq index 1	ATCACG
Example dual index	D701-D502	ATTACTCG-ATAGAGGC

2. Submit only in 10 mM Tris buffer
3. Provide Bioanalyzer (or equivalent) profiles of your completed libraries. In particular, it is essential that you demonstrate that adapter dimers are not present in your library preparations. Examples are provided below:



Acceptable library, showing minor amounts of adapter dimer ca. 120 bp.



Library with high levels of adapter dimer. Cannot be sequenced unless adapters are removed.

**Note that for long-insert libraries (>500 bp), and samples to be run on HiSeq 4000 or HiSeq X, dimers must be COMPLETELY eliminated.**

4. Measure the concentration by Qubit or similar fluorometric method. Nanodrop is not adequate for concentration measurements.

5. Provide measures of purity based on a spectroscopic method such as Nanodrop (A260/A280, and also A260/230 ratios). Acceptable ranges are defined on the first page.
6. Minimum concentrations as defined in the following table should be met (sizes are based on total size of insert and adapters – not insert size alone):

**Table 4: Minimum concentrations accepted for prepared libraries**

Mean / Modal fragment size	Minimum concentration (Qubit)	Minimum volume to send
150 bp	1.0 ng/μl	10 μl
200 bp	1.3 ng/μl	10 μl
250 bp	1.7 ng/μl	10 μl
300 bp	2.0 ng/μl	10 μl
400 bp	2.6 ng/μl	10 μl
500 bp	3.3 ng/μl	10 μl
600 bp	4.0 ng/μl	10 μl

The minimum amounts given above are for a single lane of sequencing, so if you require multiple lanes, please multiply accordingly. Performance of your library is also critical – it is not uncommon to require 5 times the minimal amounts given above in order to achieve full sequence output capacity, so provide more if you can. If your sample concentrations fall just below the given minima, please get in touch as we may still be able to run your samples if you do not require maximum sequence yields. *For experienced users only:* If you are submitting “ready to sequence” libraries or pooled libraries diluted to 10 nM, please indicate this information in the comments section of the *sample information table*. For samples destined for the HiSeq 4000 or HiSeq X, we cannot accept samples diluted below 4 nM.

The following minimal requirements apply for pools to be sequenced on Novaseq.

**Table 5. Minimum concentrations and volumes accepted for pools for Novaseq**

Flow cell	XP workflow		Standard workflow	
	Pool molarity [nM]	min. vol. [μl]	Pool molarity [nM]	min. vol. [μl]
Novaseq SP	10	10	10	50
Novaseq S1	10	10	10	50
Novaseq S2	10	10	10	70
Novaseq S4	10	15	10	160

If we determine by qPCR that your libraries have relatively poor performance and we cannot achieve recommended clustering concentrations, yet we believe we can still obtain data, we will contact you before a run commences. In this instance, the run will only be performed at your own risk, and our data output guarantees will not apply.

## VII. SECTION-BY-SECTION GUIDE TO COMPLETING THE SUBMISSION FORM

### Section 1: General Sample Information

Enter text/check boxes as required. Provide buffer information for the buffer your samples are delivered to us in, not the buffer they may originally have been extracted in. Note that different DNA amounts are required if you are submitting DNA for the NSC to prepare libraries (often called “sample prep”) on your behalf, or if you have prepared libraries yourself.

If your submission consists of more than one sample type (e.g. both DNA for PCR-free library and RNA for miRNA library construction), please complete two separate copies of the submission form.

### Section 2: Sample prep requested

Please tick the appropriate box for your needs (mandatory if requesting that the NSC performs sample prep). This section should be left blank if you have prepared your own libraries.

**Paired end library insert sizes.** For DNA samples, average insert sizes can be delivered at either 350 bp or 550 bp (valid for TruSeq DNA preps, for Nextera DNA FLEX the insert size is ca. 450 bp). For low numbers of samples (<8), library insert sizes can be tailored in the range 100-700 bp, but only using DNA preps that employ PCR. For RNA samples, we employ fragmentation conditions that produce insert sizes with a range of approximately 130-310 bp (median approx. 190 bp).

### Section 3. Sample Information table

Complete the table, paying attention to the information below. If you have >16 samples, please submit in 96-well plates and complete the *Excel* template table instead, available for download here: [http://www.sequencing.uio.no/forms/sample\\_table\\_96\\_template.xlsx](http://www.sequencing.uio.no/forms/sample_table_96_template.xlsx)

#### Sample Name

Names may ONLY consist of letters, numbers and the hyphen (-). NOT UNDERSCORE (\_). Do not use any other characters, including æ, å, ø spaces or period. Character limit = 16. A simple numbering is recommended in addition to more complex name details (e.g. 1-abc, 2-

abc). Please also write the submitters name on individual tubes / plates – we can receive hundreds of samples in a week.

### Sample Type

Detail the type of sample you are submitting, NOT the prep type you wish performed / have performed.

\* Please choose from the following abbreviations. Use your own if none are appropriate, but provide an explanation:

LIBRARY	= Ready-prepared libraries
gDNA	= genomic DNA
Tot. RNA	= total RNA
mRNA	= purified mRNA (poly-T enriched or rRNA-depleted)
cDNA	= cDNA
mi-RNA	= purified short RNAs (please specify length)
SeqCap	= captured/enriched DNA
ChIP	= chromatin immunoprecipitate
meDIP	= methyl DNA immunoprecipitate
BS DNA	= bisulfite converted DNA
amplic	= PCR amplicons

§ If combining multiple samples per lane, please indicate which samples may be combined together. If you have already performed sample library prep and Illumina-compatible indexing, also detail which index codes (manufacturer, index position in adapter, and index sequence) were used for each sample.

† If you have used any whole-genome amplification techniques that attach nucleic acid adapters/linkers to your DNA, provide details here (including sequence). In addition, if you have used restriction enzyme digestion of your sample, this should be noted here. Both of the above will result in many sequences starting with the same bases, which requires special handling during Illumina sequencing.

### Section 4. Sequencing Information Required

If you are unsure/did not receive a recommendation from us as to which kind of sequencing is appropriate, please get in touch with us using the email address below, or complete the *project request form* available at <http://www.sequencing.uio.no/forms/>. The type and length of sequencing have a major effect on the cost of your project, and sequencing of your project cannot begin without this information.

Please also give a brief description of your project goals, which will help us ensure you get the most appropriate sequencing type and length. Norwegian projects involving human subjects are required to provide a REK number.

### Section 5. Data Delivery:

Please select your preferred data delivery method. For non-sensitive data, it is usually convenient to select a download option. Sensitive data can only be delivered on hard drives or through secure networks. If you have a secure network available to which we may upload the data, please contact us to arrange access.

#### **Notes on sequence and quality score output files**

Output files can be up to 1 Tb in size, depending on the run type and length. Sequence is output as a file containing the sequences of all clusters in the sample and the associated quality scores in fastq format (see [http://en.wikipedia.org/wiki/FASTQ\\_format](http://en.wikipedia.org/wiki/FASTQ_format)). In the case of paired-end reads, the base calling software outputs two files for each sample (one for each end). These files are sorted, so that the nth read in the first file originates from the same cluster as the nth read in the second file.

**Data storage policy:** Image files are not stored, and other associated run data will be deleted soon after the data is delivered. **Fastq files along with reports will be stored for a period of 2 months from the date of the delivery mail, after which they will be deleted.**

#### **Section 6. Contact, Billing & Delivery details (All fields mandatory):**

We require a single person to act as our contact regarding sample preparation. This person must also be capable of providing information on billing & data delivery, but you are required also to enter the name of the relevant purchasing official to whom the bill should be addressed. **SUBMISSIONS WITHOUT A VALID PURCHASE ORDER NUMBER WILL BE REJECTED.** Should you require an estimate in order to generate this number, please get in touch with us at the address below. If your institution does not use an order number system, then please enter “not applicable”.

Note that additional information is mandatory for internal OUS orders.

**Registering us as a service provider:** The NSC is not a legal entity, and you will therefore not find us in the Brønnøysund Register. Your invoice will be sent from either Oslo University Hospital or the University of Oslo, depending on which node will sequence your samples (if this is unclear, please contact us on [post@sequencing.uio.no](mailto:post@sequencing.uio.no) to clarify). Banking details can be provided if necessary.

**Pricing:** Our prices vary in accordance with USD:NOK exchange rates at the time reagents are purchased for your project. Please contact us for an estimate for your project. We provide quotes, usually valid for 30 days, which you can use to raise a purchase order number for your submission.

#### **Section 7. Sample Delivery Information**

Please submit the completed submission form by email prior to making your delivery. During seasonal holiday periods, we may not accept submissions – please check

## The Norwegian High Throughput Sequencing Centre

[www.sequencing.uio.no](http://www.sequencing.uio.no) for news prior to sending samples. Provide carrier information, or if delivering in person, please check that we are available to receive you.

DO NOT send samples by registered post or TNT carrier (they may be delayed for several days in internal post before we are notified that our signature is required to collect the package).

A map can be found here:

<http://www.sequencing.uio.no/contact/>

Detailed directions can be provided by mail upon request.

### **About the NSC:**

The NSC is a collaborative venture between Oslo University Hospital and the University of Oslo. General inquiries should be addressed to our common email address [post@sequencing.uio.no](mailto:post@sequencing.uio.no).

If you have already received instructions on which node to use, the individual nodes can be reached as follows:

#### **OUS Node**

Tel: +47 2211 9724 / 2301 6419

Email: [ous-seq@sequencing.uio.no](mailto:ous-seq@sequencing.uio.no)

#### **UiO Node**

Tel: +47 2284 4446

Email: [cees-seq@sequencing.uio.no](mailto:cees-seq@sequencing.uio.no)