NovaSeq sample submission guide

Next-Generation Sequencing service

Contact: NGS team Email: ngs@westernsydney.edu.au

WESTERN SYDNEY UNIVERSITY

Research

NovaSeq sequencing requirements

Please read the following submission guidelines carefully and prepare your samples accordingly.

Failure to follow submission guidelines may result in the rejection of the submitted samples or might incur additional costs.

Prior to submission

- Samples are accepted on a 'first-come, first-serve' basis, unless otherwise indicated. Samples and submission form must be received before samples are added to the queue for library preparation.
- Due to variations in sample processing time, we require that you allow **three months** from receipt of samples to data acquisition.
- Customers should organise collection of any left-over samples within **six months** of receiving their sequencing results. Samples not collected after this period will be disposed of due to limited available storage.
- To submit sample information i.e. sample ID and well placement, please go to our online submission site page https://www.westernsydney.edu.au/research/centralised_research_facilities/next_generation_genome_sequencing_facility/novaseq_sample_submission_form
- Download and fill in the following form:
    - NovaSeq sample submission sheet.
- Please fill in the following forms online:
    - NovaSeq sample submission sorm.
    - Use the Unimutual research project cost calculator 2021 form to evaluate the dollar value of the samples being submitted.
- Upload your completed forms to our online submission page https://www.westernsydney.edu.au/research/centralised_research_facilities/next_generation_genome_sequencing_facility/novaseq_sample_submission_form
- Print out the following completed forms to submit with your samples:
    - NovaSeq sample submission form and NovaSeq sample submission sheet.

All fields in the sample submission form must be completed. Incomplete submissions will not be accepted.

Format of submitted samples

The preferred format for all sample submissions are Eppendorf 96-well fully skirted PCR plates.

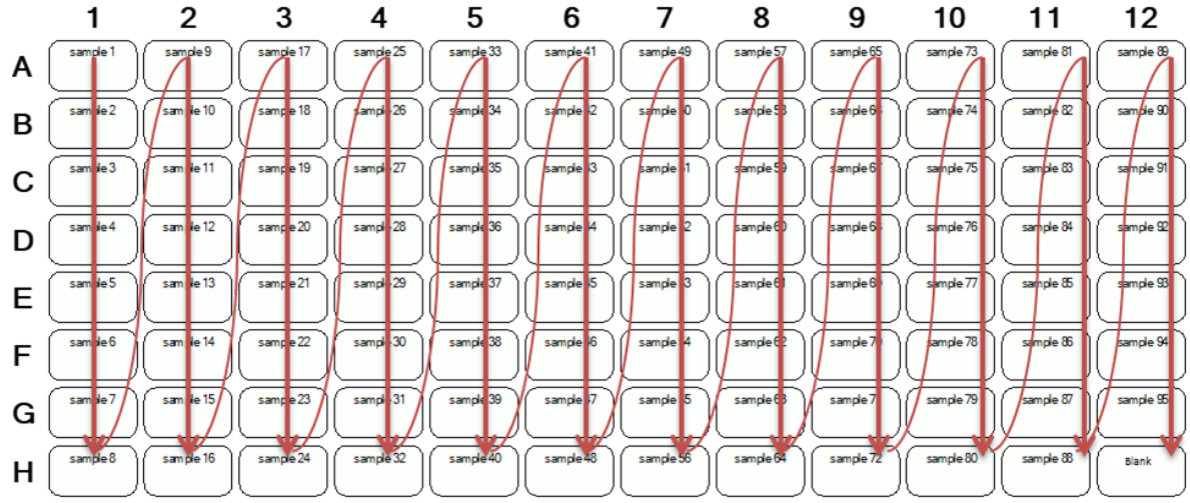
- If submitting more than 25 samples, an Eppendorf fully skirted 96-well plate (Eppendorf Cat No. 003128648), sealed with flat caps (Eppendorf Cat No. 0030124847), or storage foil (Eppendorf Cat No. 0030127889) must be used (These are available from the WSU NGS Facility on request). Please follow the plate layout detailed below, ensuring there are no empty wells within the plate other than the required blank at position H12.
- If submitting less than 25 samples, 0.2ml PCR tubes in strips of 8 format (Eppendorf Cat No. 951010022) are preferred.

Please note: If well gaps occur, each gap will be charged as a standard library preparation.

A fee for sample transfer will be charged for > 24 samples not submitted in Eppendorf fully skirted 96-well plates.

Required 96-well plate format

The plate layout below must be followed to conform with our EpMotion automated pipetting systems.



Note: *Plate layout must be followed to conform with the EpMotion automated pipetting systems, otherwise samples will be* ***rejected****.*

Labelling

- Samples in 0.2 ml tubes must be labelled "customer initials - sample number" (e.g. BH–1 or BH1).
- Samples submitted in plates must have the label on the front rim of each plate with "customer initials – plate number – date" (e.g. BH–P1–20190616 or BH– Plate 1–16/6/2019).
- Once sample submission has been completed, please arrange physical sample submission with the lab staff. Please email ngs@westernsydney.edu.au to arrange a date and time.

Preparing samples for submission

Please verify the volume of each sample before submission and fill in the sample sheet. **Failures in quantity and volume assessment may lead to rejection of the samples or incur extra costs** (The facility offers several services to precisely assess the quality of samples before submission, our staff are happy to help you choose from the available options. Please ask for the QC sample submission form and further information).

- Please ensure that samples are of similar concentrations to enable optimal normalisation.
- Refer to tables below for the minimum concentration required for your sample preparation.
- The minimum volume for each sample is 30µL.

DNA sequencing					
Genome re-sequencing using TruSeq® DNA PCR-free kit					
Input material	**Concentration (ng/µl)***	**OD 260/280^**	**OD 260/230~**	**Buffer**	**Comments**
1.5µg	≥20ng/µl	1.8-2.0	>1.8	NFH_2O	Double-stranded, contaminant free 350bp insert (2x 125bp run).
Genome re-sequencing using Nextera™ DNA Flex kit					
Input material	**Concentration (ng/µl)***	**OD 260/280^**	**OD 260/230~**	**Buffer**	**Comments**
1µg	≥50ng/µl	1.8-2.0	>1.8	NFH_2O	Double-stranded, contaminant free.

** Concentration must be assessed using fluorescence-based quantification (Qubit or Picogreen).*
NFH_2O (Nuclease-free water)

Targeted sequencing (Exome and Custom Enrichment)					
Input material	**Concentration (ng/µl)***	**OD 260/280^**	**OD 260/230~**	**Buffer**	**Comments**
3µg	≥100ng/µl	1.8-2.0	>1.8	NFH_2O	Double-stranded, contaminant free.

ChIP-Seq and MBD-sequencing					
Input material	**Concentration (ng/µl)***	**OD 260/280^**	**Customer QC**	**Buffer**	**Comments**
45ng	≥1ng/µl	1.8-2.0	Fragmented DNA (~200-600bp) checked for enrichment by qPCR	NFH_2O	Do not use salmon sperm DNA as a blocking agent during immunoprecipitation.

Transcriptome sequencing

Standard Illumina® protocol					
Input material	**Concentration (ng/µl)***	**OD 260/280^**	**OD 260/230~**	**Buffer**	**Quality (RIN) RNA Integrity Number**
1.5µg	≥50ng/µl	1.8-2.0	>1.8	RNase-free water	>8

Strand-specific RNA-Seq Ribo-Zero including rRNA Depletion					
Input material	**Concentration (ng/µl)***	**OD 260/280^**	**OD 260/230~**	**Buffer**	**Quality (RIN) RNA Integrity Number**
1.5µg	≥50ng/µl	1.8-2.0	>1.8	RNase-free water	All RINs (including degraded RNA samples derived from FFPE sources (formalin-fixed, paraffin-embedded) accepted.

Small RNA discovery					
Input material	**Concentration (ng/µl)***	**OD 260/280^**	**OD 260/230~**	**Buffer**	**Quality (RIN) RNA Integrity Number**
3µg total RNA or 150ng purified small RNA	≥200ng/µl total RNA or ≥10ng/µl purified small RNA	1.8-2.0	>1.8	RNase-free water	>8 RNA isolation kits, which enrich the population of miRNAs should be used.

Customer prepared libraries			
Volume	**Concentration (qPCR)**	**Buffer**	**Comments**
20µL	≥10nM	NFH_2O	Concentration **must** be supplied with submission of libraries (assessed by qPCR only). Whilst we aim to produce high quality sequencing data, the customer is responsible, and liable for clustering and/or sequencing failures. Barcodes **must** be included with sample submission form (non-Illumina barcodes are not supported).

** Concentration must be assessed using any quantification method. Fluorescence-based quantification (Qubit or Picogreen) is preferred.*

^ a low 260/280 ratio (below 1.8) indicates protein contamination of nucleic acids.

~ a low 260/230 ratio (below 1.8) indicates organic or carbohydrate compounds contamination of nucleic acids.

§ DNA-free RNA sample – DNase treatment for RNA sample is required.

NFH_2O (Nuclease-free water).

Acknowledgement and authorship agreement

The NGS facility must be acknowledged in any research output (including publications, conference presentations or conference posters) resulting from the data generated by the NGS facility. A typical acknowledgement and method section wording is provided below.

"*The authors would like to acknowledge the Next-Generation Sequencing facility (NGS) of Western Sydney University for the processing and sequencing of our DNA/RNA samples*."

"*Sequencing was performed using the Illumina NovaSeq® platform at the Western Sydney University's Next-Generation Sequencing Facility (Richmond, Australia)."*

Authorship

If our standard in-house pipeline is modified by staff they must be acknowledged as co-authors on publications, conference presentations or conference posters resulting from data generated by the NGS facility. In accordance with the Western Sydney University's Research Code of Practice which defines the conditions for authorship. Please see the link provided specifically: Part C - Authorship and Attribution sections 22 and 23 for author responsibilities (https://policies.westernsydney.edu.au/view.current.php?id=00166).

Instances where acknowledgement of the WSU NGS facility should be situated within the methods section.

- When DNA samples are submitted to the NGS facility which meet our standards as per the sample submission guideline document.
- DNA samples are pushed through the sample processing pipeline without alteration to in-house protocols from clean-up through to data acquisition by customer.

Instances where acknowledgement of the WSU NGS facility should be situated within an acknowledgement section.

- Where submitted DNA does not comply with submission standards (as per the sample submission guidelines), thereby requiring extra processing to increase quality and or quantity.
- Where there is significant modification to our in-house sample processing pipeline to increase sequencing output.

Instances where authorship is suitable.

- If staff are required to design, test and implement new sample processing protocols and in-house pipelines when dealing with challenging samples. As specified under part C (22 and 23) of the Western Sydney University's Research Code of Practice which specifies that all contributors are given due recognition.

Shipping of samples

- Ensure samples are shipped in an appropriate Eppendorf fully skirted 96-well plate (Eppendorf Cat No. 003128648) and sealed with flat caps (Eppendorf Cat No. 0030124847). The WSU NGS facility cannot take responsibility for sample leakage and or cross-contamination occurring during shipping.
- Please be aware that we recommend samples are shipped to us via courier service. We do not recommend posting samples by Australia Post, as prolonged delivery delays have previously occurred.

Checklist for sample submission

- ☐ Obtain a quote - contact us via email at ngs@westernsydney.edu.au
- ☐ Accept our quote via email. Sign and submit the "Fee for Service Contract" and return this to us.
- ☐ Check the quality and quantity of your samples to ensure they meet the required submission criteria.
- ☐ Dispense a minimum of 30µL of each sample into clearly labelled plates or tubes (if <24 samples). Please see page 1 for recommended plastic ware.
- ☐ Go to the NovaSeq submission page and download the NovaSeq sample submission sheet.xlsx and fill in this form. Print out a copy of this form to include with your shipped samples. https://www.westernsydney.edu.au/research/centralised_research_facilities/next_generation_genome_sequencing_facility/novaseq_sample_submission_form
- ☐ Fill in the online NovaSeq submission form with your details. Upload a copy of your NovaSeq sample submission sheet.xlsx when requested in your online submission.
- ☐ Indicate by selecting the box if you would like your samples returned to you after sequencing.
- ☐ Use the Unimutual research project cost calculator 2021 form to evaluate the dollar value of the samples being submitted https://www.westernsydney.edu.au/__data/assets/pdf_file/0008/1770335/Guideline-for-the-Valuation-of-Controlled-Environment-Contents_2020_1.pdf
- ☐ Prepare and pack your samples for shipping. Include your printed copy of the sample submission form and sample submission sheet.
- ☐ Read the NGS facility's acknowledgement and authorship agreement section (page 4).
- ☐ Ship to the following address:

The Next-Generation Sequencing Facility.
Western Sydney University – Hawkesbury Campus.
Building L9, R1.14-1.18
50 Bourke St,
Richmond, NSW 2753.