QC and purification submission guide

Next-Generation Sequencing service

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

WESTERN SYDNEY UNIVERSITY

Research

QC and purification 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.
- After purification samples are eluted in 28µl of nuclease-free water.
- 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.
- Verify quantity and volume of each sample before submission and fill in the sample sheet.
- Viscous samples cannot be analysed with the BioAnalyser. Submission of viscous samples will result in their loss and no quality assessment.
- 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/QC_and_purification_sample_submission_form
- Download and fill in the following form:
    - QC and purification sample submission sheet.
- Please fill in the following forms online:
    - QC and purification sample submission form.
    - 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
- Upload your completed forms to our online submission page https://www.westernsydney.edu.au/research/centralised_research_facilities/next_generation_genome_sequencing_facility/QC_and_purification_submission_guide
- Print out the following completed forms to submit with your samples:
    - QC and purification sample submission form (SSF) and QC and purification sample submission sheet.

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

Format of submitted samples

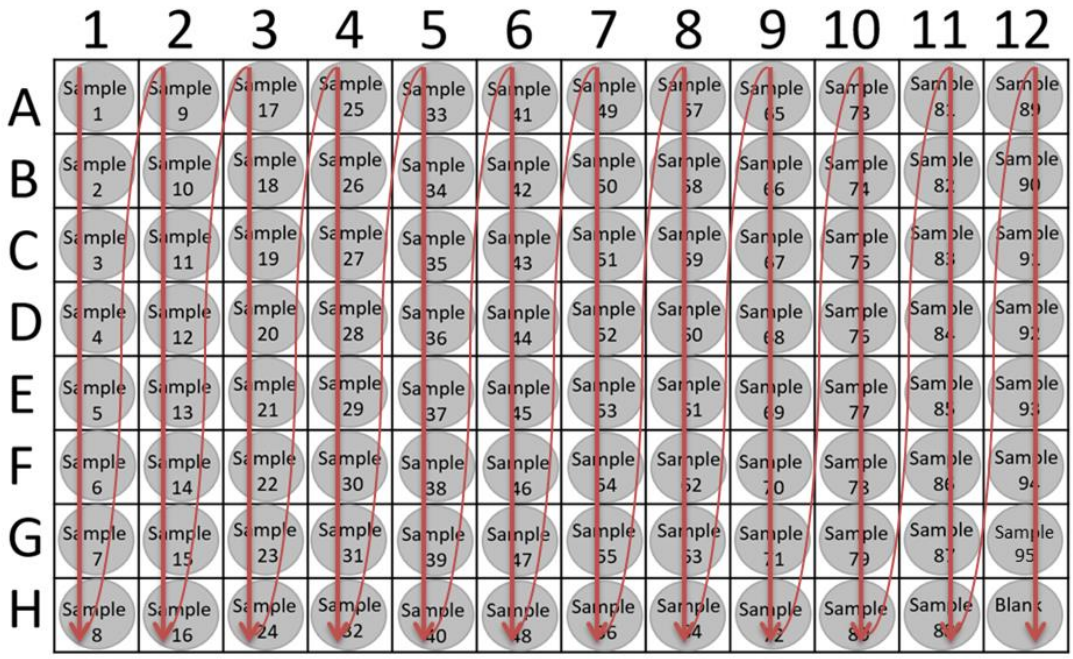
The preferred format for all sample submissions are Eppendorf 96-well fully skirted PCR plates.

- 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

Elution buffer

Please elute your samples in Nuclease-Free water (NFH_2O). If this is not possible, a tube with the elution buffer **must** be included with the sample submission, otherwise the submission will be rejected.

Genomic DNA requirements for QC and Purification

- Environmental and plant samples can be discoloured which interferes with quantification measurements and downstream assays. A purification step* will be added prior to processing these samples.
- The **minimum** volume for each sample is 30µL.

*** A concentration and/or purification step will incur an additional charge per sample, applicable to all submitted samples. Please enquire for the price prior to submission.**

LabChip and Qubit Analysis

Input volume [µl]	Concentration [ng/µl]	Elution Buffer
8	**>30** **<400**	Nuclease-free water (if different please indicate on SSF*)

BioAnalyser Analysis

BioAnalyser kit	Input volume [µl]	Concentration [ng/µl]	Elution Buffer
RNA 6000 Nano Recommended for plant and insect RNA	3	25 – 500 (total RNA)	Nuclease-free water (if different please indicate on SSF*)

TapeStation Analysis

TapeStation kit	Input volume for less than 16 samples [µl]	Input volume for more than 16 samples [µl]	Concentration [ng/µl]	Elution Buffer
DNA D1000	3	6	1 - 50	Nuclease-free water (if different please indicate on SSF*)
RNA Recommended for non-plant, non-insect RNA	3	6	25-500	

*SSF Sample Submission Form

Quality values for metagenomic samples

Metagenomic samples can often have A260/230 ratios lower than 1.8. Whilst this is not unusual with these sample types, it may be indicative of inhibitors being present in the samples and may prevent successful amplification. While we aim to produce quality sequencing data, poor A260/230 values often result in data which does not pass Illumina quality filtering.

Supporting data

- If submitting concentration values based upon Nanodrop results, please submit the concentration, the 260/280 and the 260/230 values for each sample.

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 of our DNA samples*."

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 an acknowledgement 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 the methods 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 DNA samples to ensure they meet the required submission criteria.
- ☐ Dispense a minimum of 30µL of each sample into clearly labelled plates or PCR tubes (if <24 samples). Please see pages 1 and 2 for recommended plastic ware.
- ☐ Go to the QC and Purification submission page to download and fill in the QC and Purification Submission Sheet.xlsx. Please print 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/QC_and_purification_submission_sheet.xlsx
- ☐ Fill in the online QC and purification submission form with your details (please print a copy of this form to include with your shipped samples. Upload a copy of your QC and Purification Sample Submission Sheet when requested during your online submission.
- ☐ Indicate by selecting the box if you would like your samples returned to you after processing.
- ☐ 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 and include your printed copies 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.