MiSeq sample submission guide

Next-Generation Sequencing service

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

WESTERN SYDNEY UNIVERSITY

Research

Metagenomics 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.
- Custom primer projects which require case-specific optimisation **must be discussed with the NGS Facility staff** prior to sample submission.
- 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.
- Pre-constructed customer libraries and or PCR products, must be submitted on separate plates from genomic DNA samples, the nature of the samples to be submitted must be discussed with the NGS Facility staff.
- To submit sample information i.e. amplicon choice, 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/miseq_sample_submission_form
- Download and fill in the following form:
    - MiSeq sample submission sheet.
- Please fill in the following forms online:
    - MiSeq 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/miseq_sample_submission_form
- Print out the following completed forms to submit with your samples:
    - MiSeq sample submission form and MiSeq 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.

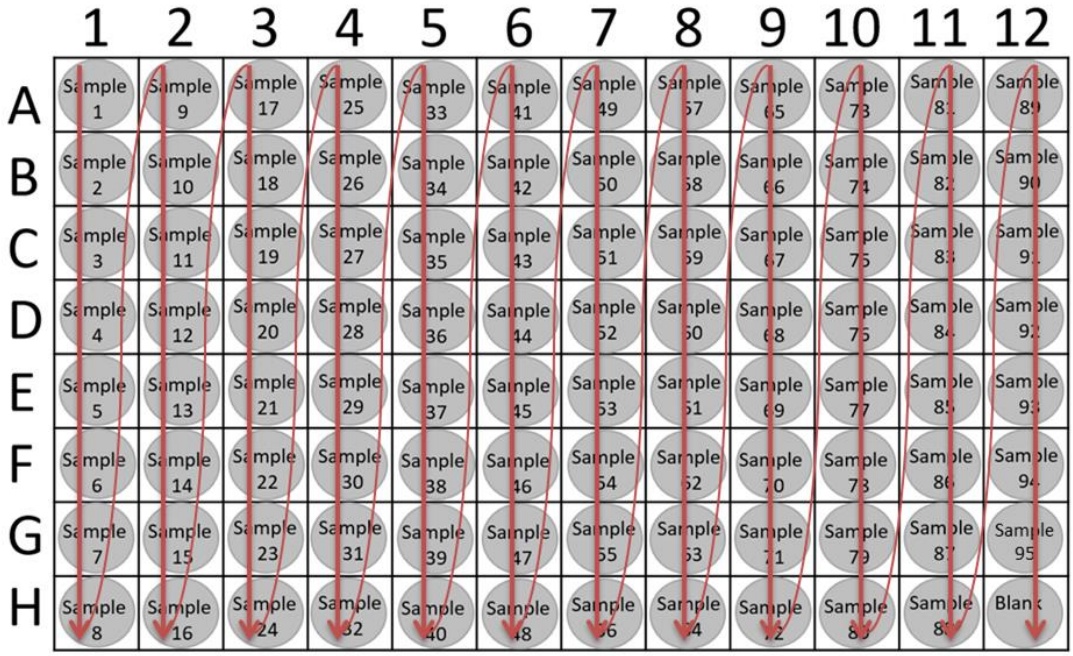
- If submitting more than 25 samples, an Eppendorf fully skirted 96-well plate (Eppendorf Cat No. 003128648), sealed with flat caps (Eppendorf Cat No. 0030124847), or storage foil (Eppendorf Cat No. 0030127889) must be used (These are available from the WSU NGS Facility on request). Please follow the 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). Elution of DNA in buffers other than NFH_2O may necessitate a purification step*. 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 sequencing run

- Please ensure that samples are of similar concentrations to enable optimal normalisation.
- 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.
- It is preferable that all submitted samples are between 30-50 ng/µL.
- DNA concentration **should not be below** 20 ng/µL (If the DNA conc. is below 20 ng/µL, please submit 50 µL of DNA).
- The **minimum** volume for each sample is 30µL.
- If the DNA concentration of samples across the plate is < 20ng/µL, and the total volume of DNA is sufficient (~50 µL), a purification and concentration step* will be done before QC.

*** 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.**

Customer prepared amplicon library sequencing run

The Illumina overhang adapter sequence **must** be added to locus-specific sequences for the region to be targeted (please contact us for further guidance at ngs@westernsydney.edu.au).

Input material	Concentration*	Minimum Volume	Purity	Buffer
≥150ng	≥10ng/µL	30 µL	Purified PCR free of primers/primer dimers.	NFH_2O

* Concentration must be assessed using fluorescence-based quantification method (Qubit or Picogreen).
^ 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 compound contamination of nucleic acids.
NFH_2O (Nuclease-Free water).

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.
- Gel images demonstrating successful PCR amplification of the submitted samples, **must** be uploaded with the online sample submission forms. Please note – if a gel image is not supplied as proof of amplification, the NGS facility cannot be held responsible for sample failure during amplification and or sequencing.

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 samples*."

"*Paired-end 16S rRNA community sequencing was performed using the Illumina MiSeq® 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.

Metagenomic primer sets

Bacterial primers -16S

The primer pair 341 forward and 805 reverse, amplifies the V3-V4 regions of the 16S gene.

Name	Sequence
341	5’ CCTACGGGNGGCWGCAG
805	5’ GACTACHVGGGTATCTAATCC

The primer pair 515 forward and 806 reverse, amplifies the V4 region of the 16S gene.

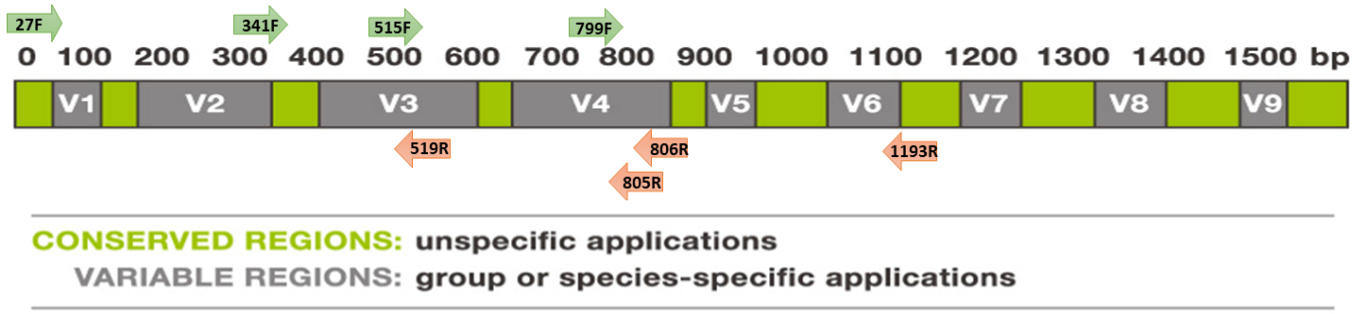
Name	Sequence
515	5’ GTGCCAGCMGCCGCGGTAA
806	5’ GGACTACHVGGGTWTCTAAT

The primer pair 27 forward and 519 reverse, amplifies the V1-V2-V3 regions of the 16S gene.

Name	Sequence
27	5’ AGAGTTTGATCMTGGCTCAG
519	5’ GWATTACCGCGGCKGCTG

The primer pair 799 forward and 1193 reverse, amplifies the V5-V6-V7 regions of the 16S gene.

Name	Sequence
799	5’ AACMGGATTAGATACCCKG
1193	5’ ACGTCATCCCCACCTTCC



Fungal primers - ITS

The primer pair fITS7 forward and ITS4 reverse, amplifies the internal transcribed spacer ITS2 region.

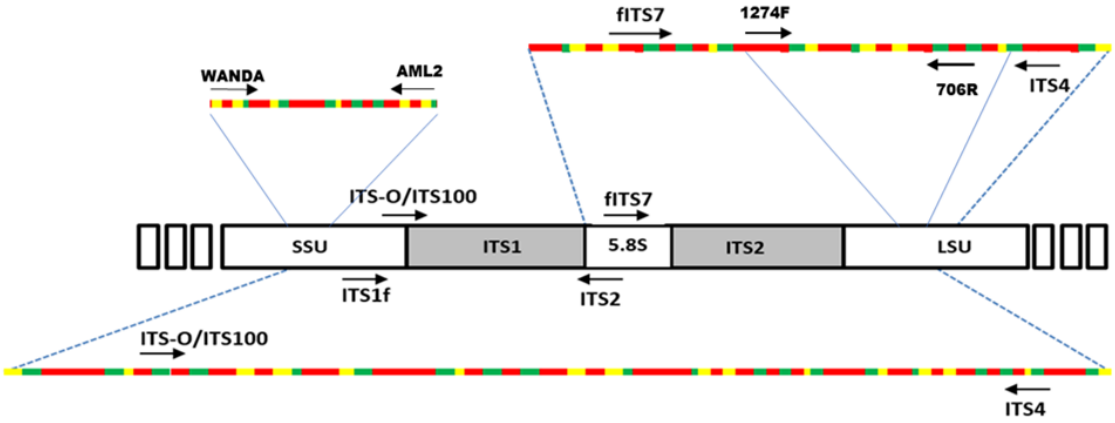
Name	Sequence
fITS7	5’ GTGARTCATCGAATCTTTG
ITS4	5’ TCCTCCGCTTATTGATATGC

The primer pair ITS1oo forward and ITS4 reverse, amplifies the internal transcribed spacer ITS1 and ITS2 regions.

Name	Sequence
ITS1oo	5’ GGAAGGATCATTACCACA
ITS4	5’ TCCTCCGCTTATTGATATGC

The primer pair ITS1 forward and ITS2 reverse, amplifies the internal transcribed spacer ITS1 region.

Name	Sequence
ITS1	5’ CTTGGTCATTTAGAGGAAGTAA
ITS2	5’ GCTGCGTTCTTCATCGATGC



Arbuscular mycorrhizal fungi (AMF) primers

The primer pair WANDA forward and AML2 reverse, amplifies the AMF NS31 (18S gene) and SSU gene regions.

Name	Sequence
WANDA	5' CAGCCGCGGTAATTCCAGCT
AML2	5' GAACCCAAACACTTTGGTTTCC

Eukaryote primers - 28S

The primer pair 1274 forward and 706 reverse, amplifies the D3-D5 region of the large subunit of the 28S gene.

Name	Sequence
28S (1274)	5' GACCCGTCTTGAAACACGGA
28S (706)	5' GCCAGTTCTGCTTACC

Protist primers -18S

The primer pair Euk_1391 forward and EukBr reverse, amplifies the V9 region of the small subunit of the 18S gene.

Name	Sequence
18S (Euk_1391)	5' GTACACACCGCCCGTC
18S (EukBr)	5' TGATCCTTCTGCAGGTTCACCTAC

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.
- ☐ Perform a PCR amplification of the submitted samples and take a gel photo. The photo **must** be supplied with the online sample submission.
- ☐ Dispense a minimum of 30µL of each sample into clearly labelled plates or tubes (if <24 samples). Please see pages 1 and 2 for recommended plastic ware.
- ☐ Go to the MiSeq submission page to download and fill in the MiSeq Sample 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/miseq_sample_submission_form
- ☐ Fill in the online MiSeq submission form with your details (please print a copy of this form to include with your shipped samples. Upload a copy of your MiSeq Sample Submission Sheet and gel photo when requested during 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 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.