MASSEY UNIVERSITY
COLLEGE OF SCIENCES
TE WĀHANGA PŪTAIAO

MGS NEXT GENERATION SEQUENCING SERVICES

Next Generation sequencing using Illumina MiSeq™ Instrumentation Massey Genome Service

CUSTOMER SERVICE REQUIREMENTS

DOCUMENT OUTLINES

- The procedures for clients to follow from submission of an enquiry via email, through to delivery of the data.
- Service specific information on:
  - Applications provided by the service
  - Illumina MiSeq™ run options provided by the service
  - Sample preparation, quality assessment requirements and quality standards
  - Sample delivery requirements
  - Sample & Library QC checks carried out by the service
  - Storage and retention of samples
  - Sequencing run report

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SUBMISSION PROCESS

1. Submission of Email Enquiry

Please submit an email enquiry to the Massey Genome Service (MGS) regarding your project at mgs-manager@massey.ac.nz. The enquiry provides the MGS service team with an outline of your project requirements, so the services can make informed decisions regarding your project design and requirements. Please include the following information in your email enquiry:

- Client's Name
- PI Name
- Client's Organisation/Institute Name
- Organisation/Institute paying for the Project (if different from Organisation/Institute)
- Client's phone number
- Client's email address
- Additional client's/contacts
- Person who will be downloading the data when the project is completed
- Project Title
- Description of Project and Objectives – Aim of the project:
  - What are the scientific aims of the project, and the desired outcomes?
  - What do you want the data to tell you?
- Request for project advise on the following:
  - Genomics - Sample requirements and platform choice
  - Bioinformatics - Data analysis
- Experimental Plan (if known):
  - Describe the basic experimental plan including sample numbers
  - Should include library type and number/type of Illumina MiSeq™ runs, if known
- Bioinformatics analysis required (Yes/No)
- Do you require the Express Service or the Standard Service?
  Express Service – Priority is placed on providing a faster turnaround time for the project. Data will be delivered to the client within 1-2 weeks, depending on the size of the project, from the date of delivery of the samples to the service. There is an additional 30% premium on the cost associated with the Express Service and this additional cost is stated in the Project Plan/Quotation for each project.
  Standard Service – There is no priority placed on providing a fast turnaround time for the project. Data will be delivered to the client within 5-8 weeks, depending on the size of the project, from the date of delivery of the samples to the service.

You will receive an e-mail response back from the MGS Service Team within 2 working days regarding your enquiry. The service team may ask for more information to be supplied and a decision will be made as to the best approach to take for your project.

The MGS Service Team will put together a Project Plan and quotation and will email this through to you for review. Should you wish to proceed with the Project then please sign the Project Plan/Quotation and email this back to the MGS Service Team at mgs-manager@massey.ac.nz, along with an electronic copy of a Purchase Order (PO) for payment. You will need to raise the PO with your organization's accounts department. For External Clients i.e. projects not funded by Massey University, the Project Plan and quotation will be accompanied by a Service Contract, which also needs to be signed, dated and an electronic copy returned to the MGS Service Team at mgs-manager@massey.ac.nz.

2. Sample Preparation and Sample Quality Data Requirements

Upon acceptance of the Project Plan/Quotation (and Service Contract for External Clients) you will be asked to prepare your samples, and send data regarding the quality of your samples to the MGS. The service will assess the quality of your samples before you send them to the service. This is a preliminary check of sample quality. The service will also carry out sample QC checks when the samples arrive at the facility.
Please refer to the section "Sample Preparation, Quantification and Quality Requirements" for the quality data to supply and for details on determining the quality of your samples.
Please do NOT send your samples to MGS until you have been notified by MGS to do so. If your quality data does not meet the standards for sample quality you will be informed on procedures to take to improve the quality of your samples.

3. Sample Delivery

You will be asked via e-mail to send your samples by courier to MGS on dry ice for RNA samples or ice packs for DNA samples. If you are local to the service you are welcome to hand deliver your samples. **You will be e-mailed a copy of the "Sample Submission Form", which must be completed, and a printed copy sent with your samples and electronic copy sent via email to mgs-manager@massey.ac.nz.**
Please refer to "Sample Delivery Information" for instructions on how your samples should be sent.

4. Sample Quality Assessment and Report

When the samples arrive at the MGS, a quality and quantification assessment will be carried out on the samples, before the service proceeds with the library preparation.
Please refer to **"Sample and Library QC Checks Performed by the Service"** for details on quality and quantification assessment MGS disclaimer, which will state why your samples do not meet our quality standard. This document MUST be signed and return to MGS via email at mgs-manager@massey.ac.nz, authorizing the service to either continue or not to continue with library preparation on the samples. You will incur the cost of the sample quality assessment whether or not you decide to continue. Should your samples meet the services quality standard, then the service will continue with the library preparation.

5. Library Preparation

MGS will proceed with the preparation of your libraries. Once the libraries have been prepared, a quality and quantification assessment will be carried out on the libraries, before the service proceeds with the sequencing of the libraries.
Please refer to "Sample and Library QC Checks Performed by the Service" for details on quality and quantification assessment of the libraries. If the libraries do not meet our quality standards you will be sent a "Library Quality Report" containing an MGS disclaimer, which will state why your libraries do not meet our quality standards. This document MUST be signed and return to MGS via email at mgs-manager@massey.ac.nz, authorizing the service to either continue or not to continue with the sequencing of the libraries. You will incur the cost of library preparation whether or not you decide to continue. Should your libraries' meet the services quality standard, then the service will continue with the library preparation.

6. Sequencing of Libraries

The MGS will carry out the sequencing of the libraries once you send authorization to MGS to do so. The run quality is monitored in real-time, which monitors signal intensities, base quality scores, fluidics and imaging. Analysis is performed during the run to save on downstream analysis time. Primary analysis generates quality-scored base calls from the raw image files, which contain base calls per cycle.
Please refer to "Sample Preparation, Quantification and Quality Requirements" for Illumina MiSeq instrument run specifications and quality standards.

7. Data Delivery

Data is delivered to you in a hard drive via overnight courier service along with a "Sequencing Run Report" and "Data Quality Report" containing the following:
- Sample quality information
- Library quality information
- Illumina MiSeq™ run quality
- Data quality information

The data is delivered in fastq format and the sequencing reads are quality trimmed to 0.01 probability.
Please note, data will NOT be delivered to customers until a PO has been received by MGS for payment.

MGS SERVICE CONTACT INFORMATION

Massey Genome Service
Xiaoxiao Lin
Massey Genome Service
c/- Institute of Fundamental Science
Massey University
Private Bag 11-222
Palmerston North, 4442
New Zealand

Phone: Laboratory: +64 6 9518735 or +64 6 951 9080 (external)
Laboratory: ext 85735 or Office: ext 86050 (internal)
E-mail: x.x.lin@massey.ac.nz

SERVICES PROVIDED

The MGS Next Generation Sequencing Services provides two service delivery options:

- **Express Service –** Priority is placed on providing a faster turnaround time for the project. Data will be delivered to the client within 1-2 weeks, depending on the size of the project, from the date of delivery of the samples to the service. There is an additional cost associated with the express Service and this additional cost is stated in the Project Plan/Quotation for each project.
- **Standard Service –** There is no priority placed on providing a fast turnaround time for the project. Data will be delivered to the client within 5-8 weeks, depending on the size of the project, from the date of delivery of the samples to the service.

Applications

The MGS Next Generation Sequencing Service offers the following applications:

- **Whole Genomic Sequencing (WGS) of small genomes e.g. bacterial, fungal and viral.**
  Microbial sequencing targets single microbes in contrast to metagenomics, with the aim to discover of genetic variations.
  - ***de novo* sequencing**
    DNA sequencing and assembly of novel genomes.
  - **Re-sequencing**
    DNA sequencing and mapping to a reference to check for gene variations.

- **Whole Metagenomic Sequencing**
  Metagenomics is the study of genetic material isolated from microbial communities. This technical advancement has initiated the trend of sequencing multiple samples in different conditions or environments to explore the similarities and dissimilarities of the microbial communities.

- **16S Metagenomic Sequencing**
  Metagenomic studies can be carried out using the prokaryotic 16S ribosomal RNA (rRNA) gene. The 16S rRNA gene is ~1500 bp and contains 9 hyper-variable regions, which can be sequenced for phylogenetic classification. The protocol for 16S rRNA sequencing targets the V3 and V4 hyper-variable regions, generating an amplicon of ~459 bp, although the length will vary depending on the organisms. This methodology can be adapted and used to target various regions of the 16S gene, or any other gene of interest.

- **Custom Amplicon Sequencing**
  Custom amplicon sequencing or targeted DNA sequencing allows the researcher to utilize the specificity of PCR in order to target the genes of their choosing. Targeted DNA sequencing utilizes the ability to generate deep sequencing coverage of a target gene or gene region, to identify genes expressed at lower levels that may possibly have been missed by other sequencing methods.

- **RNA Sequencing (Library preparation only – including sample and library QC)**
  - Gene Expression analysis
  - Transcriptomics
  - Total RNA Sequencing

Refer to the Illumina website at http://www.illumina.com/systems/miseq.ilmn for further information

Illumina MiSeq™ Run Options

Illumina MiSeq™ Platform	**Illumina MiSeq™ V2 (2x 250 base PE)[1]**	**Illumina MiSeq™ V2 (2x 150 base PE)[1]**	**Illumina MiSeq™ V2 (2x 25 base PE)[1]**	**Illumina MiSeq™ Micro V2 (2x 150 base PE)[1]**	**Illumina MiSeq™ Nano V2 (2x 150 base PE)[1]**	**Illumina MiSeq™ Nano V2 (2x 250 base PE)[1]**	**Illumina MiSeq™ V3 (2x 75 base PE)[2]**	**Illumina MiSeq™ V3 (2x 300 base PE)[2,]**
Output	~6-7.5 Gb per run	~3-4.5Gb per run	~750-850Mb per run	~1.0-1.2Gb per run	~250-300Mb per run	~450-500Mb per run	~3-3.8Gb per run	~12-15Gb per run
Number of reads	~24-30 million paired-end reads per run	~24-30 million paired-end reads per run	~24-30 million paired-end reads per run	~ 6.7-8 million paired-end reads per run	~ 1.6-2 million paired-end reads per run	~1.6-2 million paired-end reads per run	~44-50 million paired-end reads per run	~44-50 million paired-end reads per run
MiSeq™ instrument Data Quality[3]	75% bases higher than Q30 at 2X250 bp	80% bases higher than Q30 at 2X150 bp	90% bases higher than Q30 at 2X25 bp	80% bases higher than Q30 at 2X150 bp	80% bases higher than Q30 at 2X150 bp	75% bases higher than Q30 at 2X250 bp	85% bases higher than Q30 at 2X75 bp	70% bases higher than Q30 at 2X300 bp

NOTES:

1. Version 2 Illumina MiSeq™ chemistry.
2. Version 3 Illumina MiSeq™ chemistry.
3. Illumina Quality Specifications.

NOTE: The Illumina Micro and Nano Illumina Miseq™ kits are suitable for genomic sequencing projects where there are only a small number of small genomes to be sequenced, and also for small scale custom amplicon sequencing projects that require less reads.

Refer to "Sample Preparation, Quantification and Quality Requirements" as a guide for which run lengths are suitable for each application.

SAMPLE PREPARATION, QUANTIFICATION & QUALITY REQUIREMENTS

ILLUMINA MISEQ™ SEQUENCING

Application	Type of Sample to Supply	Illumina MiSeq™ Read lengths recommendations	Method of Library Preparation	Minimum Amount Required	Minimum Concentration Required	Buffer
Whole Genomic DNA Sequencing (WGS) of small genomes (De novo assembly)	Fungal, bacterial, viral and smaller complex genomes	Illumina MiSeq™ V2 (2x 250 base PE)* or Illumina MiSeq™ V3 (2x 300 base PE)!	Illumina TruSeq™ DNA PCR-Free Library Preparation	1.5µg DNA (350 bp insert) or 2.5µg DNA (550 bp insert)	20ng/µl	10mM Tris-HCl, pH8.5 or molecular grade water
Whole Genomic DNA Sequencing of small genomes (Re-sequencing)	Fungal, bacterial, viral and smaller complex genomes	Illumina MiSeq™ V2 (2x 250 base PE)* or Illumina MiSeq™ V3 (2x 300 base PE)!	Illumina TruSeq™ DNA PCR-Free Library Preparation	1.5µg DNA (350 bp insert) or 2.5µg DNA (550 bp insert)	20ng/µl	10mM Tris-HCl, pH8.5 or molecular grade water
			Illumina TruSeq™ DNA Nano Library Preparation	120ng DNA (350 bp insert) or 220ng DNA (550 bp insert)	10ng/µl	
			Illumina Nextera™ Flex DNA Library Preparation	1-500ng DNA	10ng/µl	
			Illumina Nextera XT™ DNA Library Preparation	1.5ng DNA	0.2ng/ µl	
Whole Metagenomic Sequencing	Microbial environmental samples containing a population of microbes	Illumina MiSeq™ V2 (2x 250 base PE)* or Illumina MiSeq™ V3 (2x 300 base PE)!	Illumina TruSeq™ DNA PCR-Free Library Preparation	1.5µg DNA (350 bp insert) or 2.5µg DNA (550 bp insert)	20ng/µl	10mM Tris-HCl, pH8.5 or molecular grade water
			Illumina TruSeq™ DNA Nano Library Preparation	120ng DNA (350 bp insert) or 220ng DNA (550 bp insert)	10ng/µl	
			Illumina Nextera™ Flex DNA Library Preparation	1-500ng DNA	10ng/µl	
			Illumina Nextera XT™	1.5ng DNA	0.2ng/ µl	

			DNA Library Preparation			
PCR Amplicon Sequencing	Large Amplicons ≥ 2Kb	Illumina MiSeq™ V2 (2x 250 base PE)* or Illumina MiSeq™ V3 (2x 300 base PE)!	Illumina Nextera™ Flex DNA Library Preparation Illumina Nextera XT™ DNA Library Preparation	-500ng DNA 1.5ng DNA	10ng/µl 0.2ng/ µl	10mM Tris-HCl, pH8.5 or molecular grade water
Custom Amplicon Sequencing	Amplicons ≤ 550bp	Illumina MiSeq™ V2 (2x 150 base PE)* or Illumina MiSeq™ V2 (2x 250 base PE)* or Illumina MiSeq™ V3 (2x 300 base PE)! (Run length is dependent on the size of the amplicons being sequenced)	Illumina Two Step PCR Amplicon Approach using the Illumina Nextera XT ™ DNA Primers for the second PCR step. Client is responsible for carrying out the first PCR step using custom made Illumina tailed primers (Additional information is provided upon scoping of the project)	25µL of the amplicon generated from the first PCR step	5ng/µl	10mM Tris-HCl, pH8.5 or molecular grade water
Metagenomic 16S Amplicon Sequencing V3-V4 hypervariable region	Genomic DNA or PCR Amplicon containing the 16S gene (Amplicon size = ~459 bp)	Illumina MiSeq™ V2 (2x 250 base PE)*	16S V3-V4 Dual-Index Sequencing on the MiSeq Illumina Sequencing Platform#	5-50ng DNA	5ng/µl	10mM Tris-HCl, pH8.5 or molecular grade water
RNA Sequencing Gene Expression Analysis (Transcriptomics)	Total RNA	Illumina MiSeq™ V2 (2x 150 base PE)* or Illumina MiSeq™ V3 (2x 75 base PE)!	Illumina TruSeq™ Stranded Total RNA Library Preparation Illumina TruSeq™ Stranded mRNA Library Preparation	1.5µg Total RNA 1.5 µg Total RNA	200ng/µl 200ng/µl	10mM Tris-HCl, pH8.5 or molecular grade water

NOTES:

* Version 2 Illumina MiSeq™ chemistry. Refer to “Illumina MiSeq™ Run Options” for details.
! Version 3 Illumina MiSeq™ chemistry. Refer to “Illumina MiSeq™ Run Options” for details.
Reference: http://aem.asm.org/content/79/17/5112

IMPORTANT NOTES:

Preparing samples ready for the Illumina Nextera™ Flex DNA Library preparation or Illumina Nextera™ XT DNA Library preparation, please do not send the samples in a buffer which contains EDTA. EDTA is known to be an inhibitor in enzymatic reactions and could inhibit the enzymatic fragmentation used in the Illumina Nextera ™ DNA Library preparation and Illumina Nextera™ XT DNA Library preparation methods.

The minimum product size for the Illumina Nextera™ XT DNA Library preparation is 300 bp. This is to ensure even coverage across the length of the DNA fragment. An expected drop off in sequencing coverage about 50 bp from each distal end of a fragment may be seen. This is because the transposon tagmentation enzymatic reaction used to randomly fragment the product, cannot add an adapter right at the distal end of a fragment. This enzymatic clipping of PCR primers avoids wasted sequencing output on non-informative bases that do not contain genomic inserts. If you wish to sequence the genomic loci contained within a PCR primer, simply design your amplicons to be ~100 bases larger than the desired insert to be sequenced.

Preparation of DNA Samples

It is recommended that clients use column based purification methods to extract the genomic DNA. DNA samples should be treated with RNase prior to purification on the column. Drying the column after the wash step with a second spin is essential to remove excess ethanol, which will interfere with library preparation. Avoid the use of organic extraction methods, such as phenol or Trizol, which can interfere with the enzymatic reactions used during the library preparation protocols.

The client MUST quantitate the Genomic DNA or PCR Amplicon and assess the quality of the DNA prior to the delivery of samples for library preparation and Illumina MiSeq™ sequencing.
~150ng of genomic DNA samples must be run on a 0.5-1% agarose gel with a high molecular weight ladder. MGS will NOT run a gel when the samples arrive at the service.
PCR Amplicons must be run on either a 1-2% agarose gel with a ladder containing similar band sizes to your products, or bioanalyzer e.g. Agilent 2100 Bioanalyzer, if the client has access to this instrumentation, using either the Agilent DNA 7500 or Agilent DNA 12000 Labchip Kit.

Alternatively, MGS provide a LabChip Service. Refer to the MGS website for details. For the LabChip Service clients can provide Genomic DNA or PCR Amplicons to the MGS and the service will run them using the PerkinElmer GX Touch HT Instrument using:

- DNA High Sensitivity Assay – PCR Amplicons
- Genomic DNA Assay – Genomic DNA

The client must supply MGS with an electronic copy of the 1-2% agarose gel image, or the summary report from the bioanalyzer, and the fluorometer e.g. Qubit concentration readings and spectrophotometer OD260/280 ratio and OD260/230 ratio readings.

Quality Standards

High quality genomic DNA should run as a high molecular weight band on a 1-2% agarose gel, with the majority of DNA greater than 10Kb in size and with minimal lower molecular weight smearing. If the majority of the DNA is below 10Kb or smearing is visible, this suggests that the DNA is degraded or lower molecular smearing can be indicative of the presence of RNA contamination. Please refer to Figure 1 below. The MGS will not accept your samples for sequencing if visible smearing is seen.
The spectrophotometer OD260/280 ratio should be 1.8-2.0; OD260/230 ratio should be 2.0-2.2

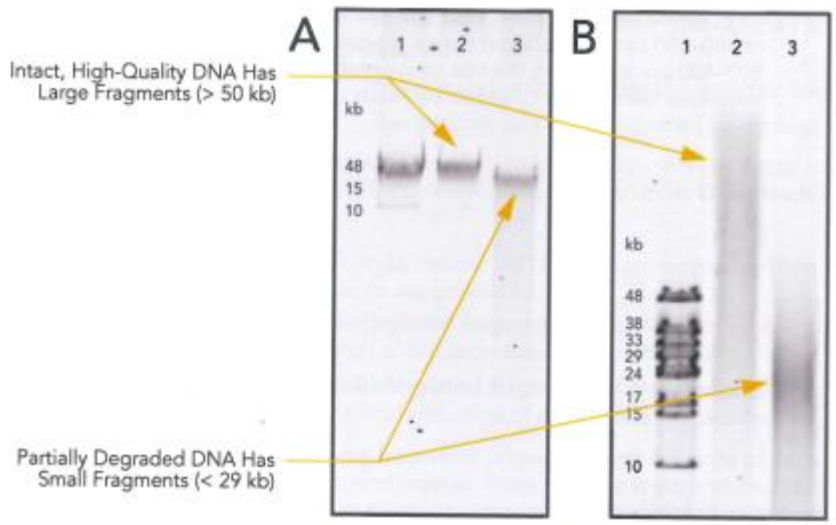

Figure 1 shows agarose gel analysis of two genomic DNA samples. Approximately 200ng of sample was loaded per lane. Figure 1A is a 0.6% standard agarose gel stained with ethidium bromide. Figure 1B is a higher-resolution Pulse Field Gel, which more clearly shows the difference in quality and integrity.

Lane 1 – Lambda Mono Cut DNA marker (NEB, N3019S)
Lane 2 – Intact high quality genomic DNA sample
Lane 3 – Partially degraded genomic sample

High quality PCR amplicons should be seen as a single clean band at the expected size when run against a ladder with bands of a similar size to that of the product. No extra bands or smearing should be seen which will indicate the presence of contamination or degradation. No PCR primer bands should be seen.

Preparation of RNA Samples

Total RNA should be extracted using a column purification method. Organic extraction methods, such as phenol or Trizol, are discouraged for the purification of total RNA. Tissues with a high fat content and certain embryonic tissues require an organic step to remove the excess amount of these other bio-molecules. For this we recommend the use of one of the following Qiagen kits: Qiagen RNeasy Lipid Tissue Mini Kit, Qiagen RNeasy microarray tissue mini kit, or Qiagen miRNeasy mini kit. All three of these kits include an organic step prior to column purification. Residual DNA can also be removed during the purification process when using any of these three kits.

Total RNA samples must be run on a bioanalyzer e.g. Agilent 2100 Bioanalyzer using the Agilent RNA 6000 Nano Labchip Kit, if the client has access to this instrumentation. Alternatively run the total RNA on a formaldehyde 1% agarose gel with an RNA 6000 ladder and measure the integrity of the RNA upon staining with ethidium bromide.

Alternatively, MGS provide a LabChip Service. Refer to the MGS website for details. For the LabChip Service clients can provide Total RNA or mRNA to the MGS and the service will run them using the PerkinElmer GX Touch HT Instrument using the Standard RNA Assay.

It is very important to use high-quality RNA as the starting material. The use of degraded RNA can result in a low yield, over-representation of the 3’ ends of the RNA molecules, or failure of the library preparation. A DNase step must be included as part of the RNA isolation method. The presence of DNA contamination may result in an underestimation of the amount of RNA used.

The client must supply MGS with an electronic copy of the formaldehyde agarose gel image, or summary report from the bioanalyzer, and the fluorometer e.g. Qubit concentration readings and spectrophotometer OD260/280 ratio and OD260/230 ratio readings.

Quality Standards

Bioanalyzer Trace: An RIN or RQS value greater than 8 is required, or greater than 7 for plant RNA and rRNA ratio (28S/18S or 23S/16S) of 1.5 to 2.5.
Formaldehyde 1% Agarose: High quality RNA shows a 28S rRNA band at 4.5Kb that should be twice the intensity of the 18S rRNA band at 1.9Kb. Both kb determinations are relative to a RNA 6000 ladder. mRNA will appear as a smear from 0.5-12Kb.
The spectrophotometer OD260/280 ratio should be 1.8-2.2; OD260/230 ratio should be 2.0-2.2

Client Prepared Libraries

MGS will accept client prepared libraries using Illumina compatiable library preparation methods. However, an MGS disclaimer will be included in the MGS Project Plan, as we are not able to guarantee the quality or quantity of data generated from client prepared libraries.

Client prepared libraries must be run on a bioanalyzer e.g. Agilent 2100 Bioanalyzer, if the client has access to this instrumentation, using either the Agilent DNA 1000 or Agilent DNA High Sensitivity Labchip Kit. If the client does not have access to a bioanalyzer then MGS will QC check the libraries upon arrival at our facility.

The client must supply MGS with the summary report from the bioanalyzer.

Quality Standards for Libraries prepared using Illumina Kits

High quality libraries should have a clean single peak as follows with no noticeable adapter dimer or PCR primer peaks:
~470-670 bp for libraries prepared using the Illumina TruSeq™ DNA PCR-Free or Illumina TruSeq™ DNA Nano Library Preparation methods.
~250-350 bp for libraries prepared using the Illumina TruSeq™ RNA or Illumina TruSeq™ Stranded Total RNA Library Preparation methods.
~175-700 bp for libraries prepared using the Illumina Nextera™ or Illumina Nextera™ XT Library Preparation methods.
~570-600 bp for libraries prepared for 16S amplicon sequencing of the V3-V4 rRNA hyper-variable region.

SAMPLE & LIBRARY QC CHECKS

MGS will run client samples on the Qubit® Fluorometer or PerkinElmer Victor Plate Reader using the Quant-IT dsDNA HS Assay, as a quantification check before library preparation. The service will also run client and service prepared libraries on the Qubit® Fluorometer or PerkinElmer Victor Plate Reader, and the PerkinElmer LabChip® GX Touch HT instrument, as a quality control check before sequencing on the Illumina MiSeq™ instrument.

Qubit® Fluorometer and PerkinElmer Victor Plate Reader

The following Invitrogen® assays are run:

- Quant-iT™ HS DNA Assay or Quant-iT BR DNA Assay – For genomics DNA and PCR amplicon samples, and MGS and client prepared libraries
- Quant-iT™ RNA Assay – For Total RNA and mRNA samples#

NOTE: # The Quant-iT™ HS DNA Assay is run to determine the percentage of DNA contamination for RNA samples. % contamination should be ≤ 10%.

PerkinElmer GX Touch HT Instrument

The following PerkinElmer LabChip® assays are run:

- High Sensitivity DNA Assay – MGS and client prepared libraries.

If the samples, client prepared libraries or service prepared libraries do not meet our quality or quantification standards, you will be required to sign an MGS disclaimer should you wish to continue with the processing of the samples and/or libraries.
Refer to "Submission Process", for details.

Summary of QC Requirements

Customer Requirements	MGS Services QC Checks
Genomic, Metagenomic and PCR Amplicon samples# - 1-2% Agarose gel - Spectrophotometer or Fluorometer to measure concentration and Spectrophotometer to measure OD260/280 and OD230/260 ratios	**Genomic, Metagenomic and PCR Amplicon Sequencing samples** - Qubit® Fluorometer or PerkinElmer Victor Plate Reader
Total RNA and Small RNA samples# - Bioanalyzer (if you have access) - Formaldehyde 1% Agarose gel (if you do not have access to Bioanalyzer) - Fluorometer to measure concentration and Spectrophotometer to measure OD260/280 and OD230/260 ratios	**Total RNA and Small RNA samples** - Qubit® Fluorometer or PerkinElmer Victor Plate Reader
Libraries - Service will accept prepared libraries from clients however an MGS disclaimer will be included in the Project Plan which the client needs to sign before proceeding with the Project	**Libraries** - PerkinElmer GX Touch HT instrument using LabChip® DNA High Sensitivity Assay - Qubit® Fluorometer or PerkinElmer Victor Plate Reader

NOTE: # MGS provide a LabChip Service. Refer to the MGS website for details.

SAMPLE DELIVERY REQUIREMENTS

Samples are to be delivered to MGS via courier at the clients own expense. Within New Zealand samples are to be delivered by domestic overnight courier service for express next day delivery, and international deliveries are via international courier services arranged by the client. Samples are to be delivered on dry ice in liquid form for RNA samples or on ice packs in liquid form for DNA samples. If international deliveries are made on dry ice then it is a requirement that the dry ice is replenished during shipment to prevent thawing of samples.

Samples MUST be delivered to the following address for Illumina MiSeq™ sequencing:
Massey Genome Service
Massey University
c/- IFS Inwards Goods
Science Tower A, Level 1,
Columbo Road
Turitea Campus
Palmerston North, 4410
New Zealand
Attention: Xiaoxiao Lin

Samples may be hand delivered to the service if you are situated locally and are able to do so.

Samples MUST be put into either 0.5mL or 1.5mL screw cap or flip lid microtubes, wrapped with parafilm. Label the tube(s) with the client party contact, NZGL contract number, sample name, total amount of sample (µg) delivered and volume (µl) if in liquid form. Sample tubes MUST be put into a 50mL falcon tube(s) and packed with clean tissue for safe delivery to prevent/minimize possible breakages. Seal the 50mL falcon tube(s) with parafilm. If samples are being sent in dry form the falcon tube(s) MUST also be bubble wrapped.

International Deliveries

International customers when sending DNA or RNA samples for sequencing MUST send with your sample(s) our Import Permit for importing materials of a biological nature into New Zealand. This must be attached securely to the outside of the parcel so that it is available for customs inspection, along with written details as to the content of the parcel. Please write the following note to be sent with the import permit: "Purified DNA (or RNA), non-hazardous, non-infectious, and non-viable."

New Zealand has very strict import regulations, and the import permit must be present with the sample(s) for customs inspection. The biological import permit is updated regularly, so each time the client comes to send samples please e-mail x.x.lin@massey.ac.nz for Illumina MiSeq™ sequencing to ask for the most recent permit. Once courier arrangements have been made, please e-mail through tracking information along with the following information:

- Client's organisations name, address and your contact details.
- Name of the courier company the client is using and their contact details.
- Date the parcel was sent.

Refer to the section "Sample Preparation, Quantification & Quality Requirements", for the total amount and concentration of sample to supply.

STORAGE & RETENTION OF SAMPLES & DATA

Storage of Samples

All samples and client prepared libraries upon entering the facility are unpackaged and checked for breakages. If tubes are broken, then the client will be contacted immediately and will be asked to resend the samples or client prepared libraries.

All DNA samples and prepared libraries are stored in a -20^{o}C freezer, and all RNA samples are stored in a -80^{o}C chest freezer. The -20^{o}C freezer is temperature monitored and temperature recorded daily.

Retention and Return of Samples

All samples that enter the facility, both DNA and RNA, and prepared libraries are stored for a period of at least 1 year. After which the client will be notified via e-mail, asking whether they want the samples returned to you and if not the service will dispose of the samples. Libraries prepared by the service will not be returned to clients.

Should the client request the samples to be returned, then they will be sent the MGS “Authorization for Return of Samples Form”, to be filled in, signed and returned. The samples will be released to the client within 3 working days of receiving the signed “Authorization Form”.

Storage of Data

All data is stored on the Illumina BaseSpace server, which excludes image files.
All data is stored locally in the Illumina MiSeq™ instruments, which includes images for a period of 1-2 months. All data is backed up to hard drives, including images, for long term storage.

Delivery of Data

The raw and quality checked data in fastq format, a “Sequencing Run Report” and “Data Quality Report” will be sent to clients on completion of the Project, subject to the terms and conditions of the MGS Project Plan. MGS will notify you when the data ready.

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