



SERVICE GUIDE

Illumina Mouse Methylation BeadChip (MMB) Service

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SVG2206MMB

Service Guide:

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1.0 Overview

At AGRF, we use the Illumina array technology to deliver high-quality data for mouse methylation studies. Our high-throughput and semi-automated process produces the highest quality data. Using the Illumina Infinium methylation technology, AGRF offers the Infinium Methylation BeadChip providing quantitative methylation measurements at the single CpG site level, offering high resolution for studies of epigenetic changes.

2.0 Mouse Methylation BeadChip (MMB)

The Illumina Mouse Methylation BeadChip features >285k methylation sites per sample at single-nucleotide resolution and enables epigenetic analysis of virtually all murine strains used in research laboratories, including wildtype, knockout, transgenic and other types of engineered mice. The BeadChip provides balanced coverage of CpG islands, translation start sites (TSS), enhancers, imprinted loci, gene body regions, repetitive element regions, lamin attachment domains and hyper-methylated regions in cancer. The Global genome coverage enables diverse studies. The possible applications include epigenome-wide association studies, xenograft experiments, developmental biology studies, analysis of mouse models of disease, preclinical validation studies and more.

AGRF's Illumina Mouse Methylation BeadChip service includes DNA QC assessment of submitted samples, Illumina processing and all reagents. The bisulfite conversion is also included in the service.

3.0 Submission Types and DNA Requirements

The array supports genomic DNA derived from sample types including blood, solid tumours and fresh frozen tissue. The total amount of DNA required per sample is 750ng total DNA, at 50-100ng/μl in a volume of 15-30ul. Please note, although the Illumina published guides indicate the minimum input amount for the bisulfite conversion for the Mouse Methylation BeadChip is >250ng/sample, AGRF does request additional DNA for the DNA QC Assessment.

If you do have samples below the requested amount, please let us know as we may be able to accommodate. This may involve omitting of the DNA Quality Control (QC) assessment, or lower input amount into the bisulfite conversion for methylation studies. Please contact your Account Manager to discuss your options.

Illumina have conducted experiments of the MMB performance with increasing amounts of DNA. The findings were the amount of probes passing detection p-value <0.05 increases when increasing amounts of DNA were applied to the array. When NIH3T3 cell line DNA is run on the array, from 50 ng to 1,000 ng, the probe success rate increases from 0.85 to 0.97. The average beta values are roughly the same, but the beta values at CpG islands (CGI) and partially methylated domain (PMD) sites do not stabilise unless over 250ng. Based on this, AGRF recommends 500ng input into the bisulfite conversion, where possible and at least 250ng as a minimum. See table below.

Cell Type	Input DNA	Success Rate	Median beta values		
			All CpGs	CGI CpGs	PMD solo CpGs
NIH3T3	50ng	0.85	0.83	0.64	0.48
NIH3T3	100ng	0.89	0.84	0.69	0.43
NIH3T3	250ng	0.94	0.86	0.72	0.33
NIH3T3	500ng	0.96	0.86	0.73	0.35
NIH3T3	500ng	0.96	0.86	0.73	0.35
NIH3T3	1000ng	0.97	0.86	0.72	0.35

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After we receive your samples, we will quantify them in our lab. We will provide quantitative results for DNA concentration and feedback regarding your samples within 5 days of receiving your DNA. If we note something of concern, we will work with you to correct any issues in DNA preparation and shipping before moving forward with your DNA samples.

Based on AGRF's experience processing >100,000 samples for the Infinium assay, we find that samples conforming to the following requirements are more likely to provide high quality array data.

- Quantify DNA using a dsDNA-specific method such as the PicoGreen method. Optical density (OD) quantification, such as nanodrop is considered suboptimal and can often overestimate the total DNA concentration of a sample.
- We do not recommend normalising samples below 50ng/ul. Samples may need to be concentrated by AGRF by speedycap to achieve the required minimum input concentration of 50ng/ul into the assay, however this may also concentrate any salts within the buffer.
- DNA must be pure, intact and of high molecular weight ($\geq 20\text{Kb}$). DNA must be free of RNA and contaminating nucleic acids from other individuals or species.
- OD 260/280 must be between 1.8–2.0.

Samples bisulfite converted by clients can be submitted for the service. Please note, a volume $\geq 5\mu\text{l}$ is needed. AGRF will not perform any quality control assessment on bisulfite converted samples and has no guarantee on the overall data quality produced. The AGRF service does recommend input amount into bisulfite conversion is 250-500ng total DNA and the Zymo EZ Methylation kit.

4.0 Sample Submission and Shipping

DNA samples must be shipped to AGRF in tube or 96 well, V-bottom plates and be clearly labelled and sealed.

- < 24 samples – 1.5mL tube
- > 24 samples – 96 well plate

For tube submissions our preferred tube format is 1.5mL snap cap tubes and tube samples can be shipped at room temperature.

Plate Format Submission Requirements:

- Array samples down the column (not across the row).
- Ensure the seal / strip cap is thoroughly closed on the plate prior to shipping.
- Label your plate with your Contract ID & Name.
- We recommend shipping on dry ice to avoid potential cross contamination of liquid between wells during transit due to air pressure changes in aeroplanes.

Online sample submission via the AGRF Sample Submission Portal needs to be completed prior to sample shipment and a copy of the sample receipt should accompany your samples.

Please include sample ID, tube ID (if different) and gender, sample concentration and volume on sample submission template, along with tumour/normal indication, if applicable.

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If you are sending via Australia Post, please use the following address:

AGRF
Level 13, Victorian Comprehensive Cancer Centre
305 Grattan Street
Melbourne VIC 3000

If you are using a courier, please use our Loading Dock address:

AGRF
VCCC Loading Dock
14 Flemington Road
North Melbourne VIC 3051
(note our loading dock is open from 8am to 4pm weekdays)

5.0 Turnaround Time

< 288 samples	5 weeks	from QC or receipt of reagents
< 576 samples	7 weeks	from QC or receipt of reagents
< 864 samples	10 weeks	from QC or receipt of reagents
< 1,152 samples	12 weeks	from QC or receipt of reagents

6.0 Sample Storage

Samples are stored with AGRF for three months after you receive your data. If you require your samples to be returned to you post-processing, please let your Account Manager know at the time of quoting. Please note that a fee will be charged for return of samples. If return of DNA has been requested, at the completion of processing we will return your samples by post, either by Australia Post satchel at ambient temperature or if required, on dry ice via courier. Both options do incur a charge. If return of samples has not been requested, we will discard your samples three months after your data delivery.

7.0 Data Quality

Data performance for Mouse Methylation BeadChip is >96% of CpG sites detected ($p=0.05$). Values are expected for typical projects, based on non-cancer samples and recommended sample input amounts of high-quality DNA.

The Mouse Methylation BeadChip achieves > 98% reproducibility for technical replicates and shows a high correlation with whole-genome bisulfite sequencing data.

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8.0 Data Output

The complete BeadChip files, together with the GenomeStudio analysis files and exported data files are provided as part of the service. All files provided include:

- BeadChip files
- Genome Studio Project File (.bsc format)
- Sample Sheet (.csv format)
- Samples Table (.txt format)
- Manifest file (.bpm format)
- Project Report (.pdf format) containing a summary of project and control dashboard.

9.0 Quality Statement

All works carried out during the course of the project have followed the strict requirements of ISO17025. AGRF LTD has National Association of Testing Authorities (NATA) accreditation for Molecular Analysis under Healthcare, Pharmaceutical and Media Products. Our staff follow Standard Operating Procedures, which define their responsibilities and provide guidance on achieving standards; compliance is monitored at regular reviews and internal audits. The work was supervised by a person with relevant qualifications and was checked while in progress and upon completion to ensure that it met the necessary ISO17025 standards.

AGRF is an illumina Certified Service Provider (CSPro) for the Infinium Genotyping service. The Illumina CSPro program is a collaborative service partnership ensuring illumina's best practices are adhered to, ensuring that scientific methods and data quality are always optimized and maintained.