



SERVICE GUIDE

Illumina Human Methylation Array Service

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SVG2302MLA

Service Guide:

Illumina Human Methylation Array Service



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1.0 Overview

AGRF utilises Illumina's array technology to offer high-quality data for methylation studies. Our high-throughput and semi-automated process, with integrated Laboratory Information Management System (LIMS) produces high-quality data, delivered in a timely fashion. Using the Illumina Infinium methylation technology, AGRF provides quantitative methylation measurements at the single CpG site level, offering high resolution for studies of epigenetic changes.

AGRF has been providing services with Illumina array technology since 2008. The lab has genotyped over >135,000 samples across >3,000 projects. As an Illumina Certified Service Provider (CSP) laboratory and a Methylation array service according to the ISO17025 standard by the National Association of Testing Authorities (NATA), the AGRF Illumina Methylation array service offers the highest quality data.

Previous project work has involved epigenetic wide association studies (EWAS) in human genetic disease, oncology (breast, prostate, melanoma and tumour profiling of brain cancers), and epigenetic population wide studies of childhood health and disease. AGRF's projects come from diverse fields, including the research community, cytogenetics, and personal genomics, and encompass a wide range of sample types.

2.0 Human Methylation Analysis

Epigenetic patterns across the genome can be analysed with methylation arrays, providing valuable insights into the regulation of gene expression. DNA methylation allows cells to suppress expression of viral and non-host DNA elements, and facilitates response to environmental stimuli. Aberrant DNA methylation (hyper- or hypomethylation) and its impact on gene expression have been implicated in many biological processes and diseases. Methylation microarrays combine comprehensive coverage and high-throughput capabilities. They enable quantitative interrogation of selected methylation sites across the genome, and the high-throughput capabilities minimize the cost per sample.

3.0 Illumina Human MethylationEPIC BeadChip

With the Illumina Infinium MethylationEPIC BeadChip, >850,000 methylation sites per sample at single nucleotide resolution can be analysed, including 99% of RefSeq genes, 95% of CpG islands, high coverage of enhancer regions, and other content categories. The eight-sample format array allows multiple samples, including FFPE, to be analysed as a single experiment to deliver high-throughput power. With >98% reproducibility between technical replicates for all Illumina Infinium methylation arrays, the array produces the highest quality, reproducible data.

The array offers comprehensive genome-wide coverage and includes the following content categories:

- CpG sites outside of CpG islands
- Non-CpG methylated sites identified in human stem cells (CHH sites)
- Differentially methylated sites identified in tumor versus normal
- FANTOM5 enhancers
- ENCODE open chromatin and enhancers
- DNase hypersensitive sites
- miRNA promoter regions

AGRF's Illumina MethylationEPIC array service includes DNA quality control assessment of submitted samples, bisulfite conversion and Illumina processing and all reagents.

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4.0 Submission Types and DNA Requirements

Illumina Methylation arrays support genomic DNA derived from sample types including saliva, blood, solid tumours, fresh frozen tissue and buccal swabs. The service is compatible with the Infinium FFPE QC and DNA Restoration Kits, enabling genotyping of formalin-fixed, paraffin-embedded (FFPE) samples. Please note, AGRF does not offer FFPE restoration as a service and samples will need to be restored using the Illumina FFPE restoration kit and the restored samples submitted to AGRF for the Illumina Methylation array service.

Table 1. Requested DNA for service.

Requested DNA concentration per sample	Requested minimum volume per sample	Requested total DNA per sample
50 - 100 ng/ μ l	15 - 30 μ l	1 μ g

If you do have samples below the requested amount, please let us know as we may be able to accommodate. This may involve omitting the DNA QC assessment or lowering the input amount from the Illumina recommended 500ng total DNA for the bisulfite conversion. From internal AGRF trials with control DNA (Genome in a bottle NA12878) AGRF has successfully produced data within Illumina expectations of $\geq 96\%$ detected CpG sites ($p < 0.05$) for sample input as low as 25ng total DNA. Data generated was from pure, intact high molecular weight control DNA and results may not be achieved on DNA not of this quality.

Based on our experience processing >135,000 samples, 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/ μ l. Samples may need to be concentrated by AGRF by speedycap to achieve the required minimum input concentration of 50ng/ μ l into the assay, however this may also concentrate any salts within the buffer
- DNA must be pure, intact, and of high molecular weight (≥ 20 Kb). DNA must be free of RNA and contaminating nucleic acids from other individuals or species.

All samples received at AGRF, unless otherwise requested, will undergo a DNA Quality Control assessment by fluorometric quantitation methods and resolution on agarose gel and a DNA QC Report will be provided, prior to commencement of your project.

AGRF's service includes the bisulfite conversion of the submitted DNA sample.

5.0 Bisulfite Conversion

The efficiency and accuracy of the bisulfite conversion step, including using the final elution volume recommended by Illumina, is critical to the accuracy and performance of the Infinium Methylation assay.

Illumina has validated and recommends only the following kits from Zymo Research.

- » EZ DNA / EZ-Methylation Kits (Catalogue Numbers D5001, D5002 and D5004)
- » EZ-96 DNA Methylation Lightning MagPrep Kits (uses magnetic beads for purification) (Catalogue Numbers D5046, D5047 and D5049).

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AGRF does not accept samples which have undergone bisulfite conversion by the client as an input material for the service. The minimum volume required is 6µl of bisulfite converted DNA. AGRF will not perform any quality control assessment of the converted material and makes no guarantees of the data performance.

6.0 AGRF Recommendations for Whole Genome Amplification

It is recommended not to submit whole-genome amplified (WGA) samples. WGA samples have an unpredictable success rate.

7.0 Let AGRF Extract Your Samples for You

Avoid the hassle of extracting DNA yourself and let AGRF do this step for you. Our Extraction Service works with a wide range of DNA sources and prepares DNA to meet the requirements of our service. Please contact AGRF for a quote or assistance with your extraction.

Phone: **1300 247 301**

Email: **CustomerCare@agrif.org.au**

8.0 How to Submit Samples

Online Submission

- In the client portal, select 'Submit Samples' from the service menu.
- Select your 'Agreement ID' from the drop down menu and complete details and submission format (tube or plate).
- Complete and upload the template file.
- Submit the form and print the submission receipt to be included with your sample package.

9.0 Shipping

DNA samples must be shipped to AGRF in tubes or 96 well, half skirt plates and be clearly labelled and sealed.

- Samples in tubes can be shipped at room temperature via express post.
- For samples in plate format, we recommend shipping on dry ice to avoid potential cross contamination of liquid between wells during transit due to air pressure changes in flight.
- AGRF can organise dry ice shipment for your samples, at the cost to the client. Please ask your account manager for a quote.

Physical address (courier):

AGRF MELBOURNE
VCCC LOADING DOCK*
14 FLEMINGTON ROAD
NORTH MELBOURNE VIC 3051

Postal address (mail):

AGRF MELBOURNE
LEVEL 13, VICTORIAN COMPREHENSIVE CANCER CENTRE
305 GRATTAN STREET
MELBOURNE VIC 3000

*Note: our loading dock is open
from 8am to 4pm weekdays

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10.0 Turnaround Time

Samples undergo a DNA Quality Control assessment when received at AGRF, and a DNA QC Report will be provided once complete. The typical turnaround time expected is 3-5 weeks for this service. Please note that larger sample sets, may have a longer turnaround time.

Please contact AGRF to discuss the expected turnaround time for your specific project.

11.0 Data Quality Control

All Illumina Infinium Methylation arrays have a set of internal control probes designed to support quality control of the assay's performance. High quality samples generally yield $\geq 96\%$ detected CpG sites ($p < 0.05$) based on noncancer samples, recommended sample input amounts of high-quality DNA. Built-in Infinium controls help identify samples for which data characteristics are significantly different, and may need to be excluded as outliers from further analysis. As intensity levels may be different for any given project, or between batches of samples processed, Infinium controls are not designed to perform quality control based on specific thresholds. Instead, they are evaluated based on relative intensities.

Estimated Gender assessment using RStudio is performed.

12.0 Data Output

AGRF's service provides the following output files as standard:

- BeadChip files (contains the .idat files)
- Genome Studio Project File (.bsc format)
- Sample Sheet (.csv format)
- Samples Table (.txt format)
- Illumina manifest file (.bpm format)
- Gender estimate file (sex check) (.csv format)

Data files can be accessed from AGRF's secure Cloud site. AGRF offers complete data confidentiality, with no claim of rights to the data.

13.0 Downstream Data Analysis

Our Bioinformatics team is well-equipped to handle your downstream data analysis. Please speak to your Account Manager about organising a quote for additional bioinformatic analysis including: **Array quality control, differential methylation analysis**; and, we also offer a **custom bioinformatics service** for other common DNA methylation analyses.

Our differential methylation pipeline includes data quality control with lumi, minfi and arrayQualityMetrics. Standard probe filtering methods are applied. Differential methylation testing is conducted with LIMMA. Our pipeline includes a case-control design and we offer additional packages for complex designs and controlling batch effects. Functional analysis is provided via Reactome pathway enrichment and gene ontology analyses.

Quality control outputs:

- Raw data: Raw and processed methylation values (CSV format)
- Array quality metrics: Pre and post QC data summary figures
- Sample clustering: MDS and dendrogram of sample relations
- Variance analysis: Summary of variable probes and PCA plots
- Report: Service report with analysis details and a description of the metrics and files provided.

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Additional outputs provided for differential methylation testing:

- Heatmaps: The most variable genes and differentially methylated genes
- Volcano plots: Global methylation view
- Differential Methylation Results: One spreadsheet for each comparison tested (.csv)
- Pathways Analysis: One spreadsheet for each comparison (.csv) and an enrichment dot plot (.png)
- Gene Ontology: One spreadsheet for each comparison (.csv)

Please contact AGRF for a quote for additional data analysis.

Phone: **1300 247 301**

Email: **CustomerCare@agrif.org.au**

14.0 Sample Storage and Sample Returns/Discards

Samples are stored with AGRF for three months after you receive your data. If you wish for your samples to be returned, you must discuss this with your account manager during quoting or contact us after you receive your data. At the completion of your project, we can either:

- Return your samples by courier at ambient (please ask your account manager for a quote).
- Return samples by courier with dry ice (please ask your account manager for a quote).

If we are not notified within the specified three-month timeframe, samples will be automatically discarded.

15.0 Quality Statement

All works carried out by AGRF are performed following the strict requirements of ISO17025. AGRF LTD has National Association of Testing Authorities (NATA) accreditation for Molecular Analysis under Healthcare, Pharmaceutical and Media Products. Staff follow Standard Operating Procedures, which define their responsibilities and provide guidance on achieving standards; compliance is monitored at regular reviews and internal audits. All work is 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, and that scientific methods and data quality are always optimised and maintained.