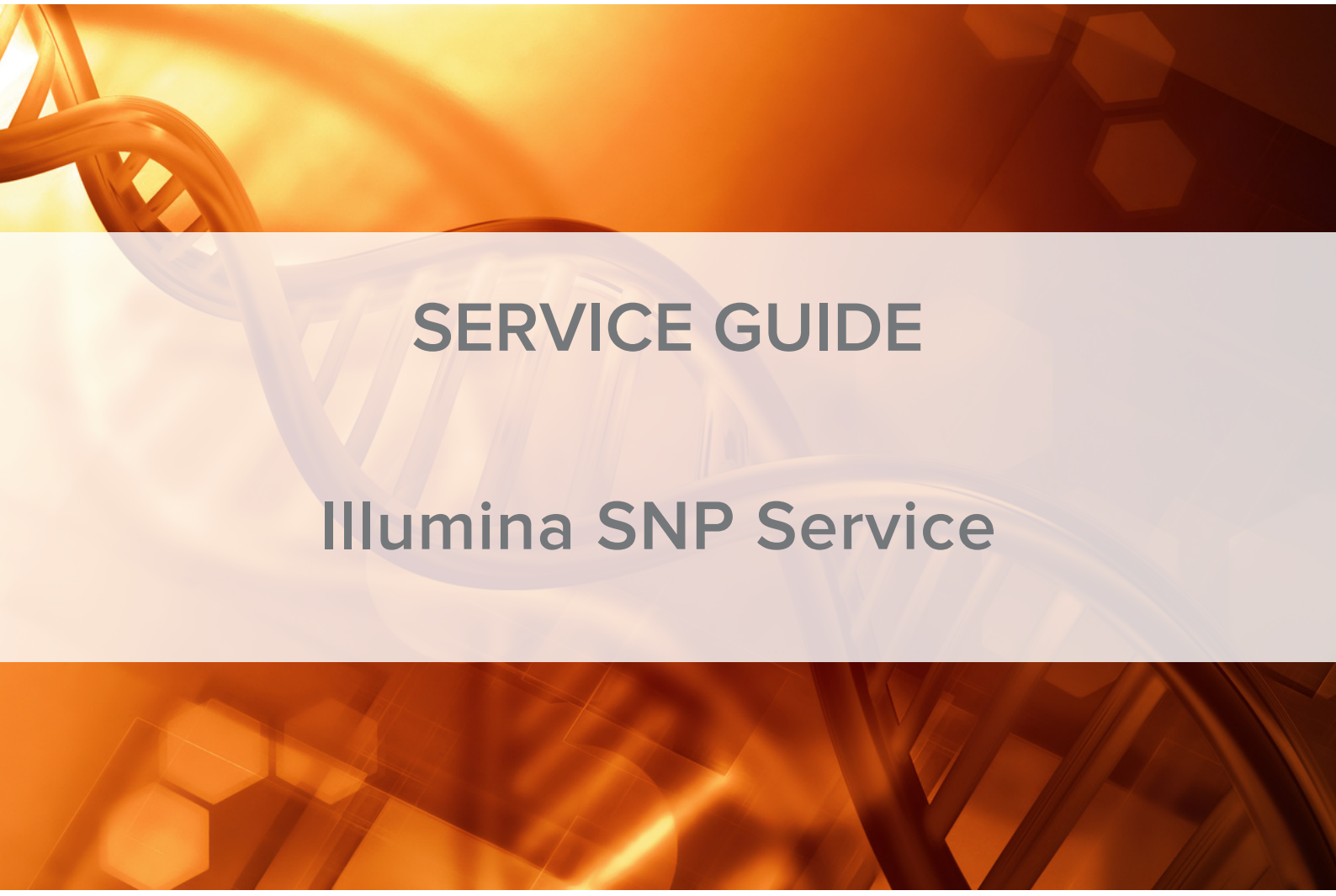

SERVICE GUIDE

Illumina SNP Service

CustomerCare@agrf.org.au
1300 247 301
www.agrf.org.au

SVG2404SRL

Service Guide
Illumina SNP Service

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1.0 Overview

AGRF utilises Illumina's array technology for genotyping studies. Our high-throughput and semi-automated process, with integrated Laboratory Information Management System (LIMS) produces high-quality data, delivered in a timely fashion. AGRF offers array services for human (including cytogenetic arrays) and non-human organisms, including both animal and plant species, using Illumina Infinium genotyping technology.

Illumina's array-based genotyping can be used for different purposes, including:

- Association studies
- Linkage analysis
- Copy Number Variation (CNV)
- Identification of haplotypes
- Breed identification
- Genomic selection and much more.

AGRF has provided genotyping services with Illumina array technology since 2008. The laboratory has genotyped over 180,000 samples across more than 4,000 projects. Accredited as a Medical Testing laboratory according to the ISO15189 standard by the National Association of Testing Authorities (NATA), the AGRF Illumina SNP array service ensures the highest quality data.

Previous project work has involved studies in human genetic disease, oncology (ovarian, breast, melanoma and brain cancers), and population wide studies of Schizophrenia, Alzheimer's, childhood allergies, and Diabetes. AGRF's projects come from a diverse range of fields, including the research community, cytogenetics, agriculture, and personal genomics, and encompass a wide range of sample types.

2.0 Processing Options

AGRF offers the following processing options in our Illumina SNP Array workflows:

Human Infinium Arrays

Human Genotyping Arrays are typically used for whole-genome or targeted genotyping of human samples for genome-wide association studies (GWAS), precision medicine research, cytogenetics, pharmacogenomics, translational research, disease association studies to detect common variants, CNVs, and more.

Arrays offered include, but are not limited to:

- Global Screening Array (GSA) and the following predesigned booster content as an add-on to the array. Booster content, including:
    - ◊ Multi-disease, Psych, Cytogenetics, Direct-To-Consumer Booster and Confluence Oncology Booster
- Global Diversity Array (GDA) and the following predesigned booster content as an add-on to the array. Booster content, including:
    - ◊ Neuro, Confluence Oncology, Carrier Screening, Enhanced PGx and Cytogenetics
- Cytogenetic Arrays, including Cyto850K and CytoSNP-12
- Any of the Illumina catalogue arrays, including:
    - ◊ Omni Array family
    - ◊ Core Array family

Agrigenomics Genotyping Arrays

For projects requiring whole-genome selection studies at low or high density, for DNA fingerprinting, marker-assisted breeding and consortia-developed microarrays, agrigenomics genotyping arrays can offer a solution. Please contact AGRF to discuss options for a SNP array for your species of interest.

Custom Genotyping Arrays

For species and projects where we do not have an array to suit your project needs, custom genotyping arrays are available. With a minimum sample requirement of 1152, the iSelect Custom BeadChip can be tailored for project requirements, with SNP selection according to need.

3.0 Submission Types and DNA Requirements

Illumina SNP arrays support genotyping and CNV calling for 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 offer FFPE restoration as a service using the Illumina FFPE restoration kit. Please discuss with your Account manager for a quote.

Table 1: Requested DNA for service

Requested DNA concentration for service	Requested minimum volume per sample	Requested total DNA per sample
50 - 100 ng/µl	15 - 30 µl	750 ng

If samples are below the requested amount, please contact us, as we may be able to alter workflows to accommodate this. These alterations may involve omitting the DNA QC assessment or lowering the Illumina recommended 200 ng total DNA input amount. From internal AGRF trials with control DNA (Genome in a bottle NA12878), AGRF has successfully produced data with SNP call rates between 98-99% and LogRDev <0.3, utilising inputs as low as 25 ng of total DNA. Data generated was from pure, intact of high molecular weight control DNA and comparable results may not be achieved on lower-quality DNA.

Based on our experience processing >180,000 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/ul. Samples may need to be concentrated by AGRF by vacuum concentration to achieve the required minimum input concentration of 50 ng/ul. This may also concentrate any salts within the buffer which can impact the quality of data generated.
- DNA must be pure, intact, and of high molecular weight (≥ 20Kb). DNA must be free of RNA and contaminating nucleic acids from other individuals or species. In our experience, different buccal swab and saliva collection kits can produce varying data, outside of the expected >99% SNP call rate and Log R Deviation <0.3. Table 2 lists kit types which have been extracted by AGRFs extraction facility and processed by AGRF on Illumina SNP arrays.

Table 2: Average Call Rates and LogRDev for varying swab and saliva kits. Product specifications for Global Screening Array >99.0% average Call Rate and < 0.30 average Log R deviation.

Swab and Saliva Kit Type	Average SNP Call Rate	Average Log R Deviation
Small Dry Generic Swab	95.22%	0.30
DNA Genotek Oragene Swabs: ORAcollect•Dx OCR-100	99.24%	0.19
Copan FLOQswabs: hDNA Free FLOQSwabs® 50C013D01	98.46%	0.22
Copan eNAT swab: eNat® 6U073S01	99.41%	0.19
DNA Genotek Oragene Saliva: Oragene•Dx OG-610	99.54%	0.21

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

4.0 AGRF Recommendations for Whole Genome Amplification

It is not recommended to submit whole-genome amplified (WGA) samples. WGA samples have an unpredictable success rate. However, if you must submit WGA samples, the following observations may be helpful:

- Unlike gDNA samples, the final concentration of WGA samples does not correlate to sample quality. After WGA, it is possible to have a high DNA concentration that is not representative of the original DNA sample—particularly if the starting concentration was low or if contaminating DNA was present.
- The best metric for correlating WGA sample quality is the final quality/quantity of the sample before amplification. It is recommended to use a minimum of 10 ng of gDNA for the WGA reaction. (Better results have been observed with ≥ 50 ng of gDNA).
- If a combination of WGA and non-WGA samples are submitted as a single batch, it is recommended the sample sets be separated for analysis, due to potential differences in the clustering of the SNPs for allele calling.

WGA samples must be clearly marked on the electronic sample submission.

5.0 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. Our clinical extractions are NATA accredited.

6.0 How to Submit Samples

6.1 Online Submission

- Login to the 'My AGRF Hub' at www.agrf.org.au.
- 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.
- ≤23 please complete tube submissions.
- ≥24 Please complete plate submissions, (an additional handling charge per sample will occur if tubes are used).
- We recommend shipping plates that are heat-sealed, or strip-cap sealed on dry ice.
- Submit the form and print the submission receipt to be included with your sample package.

7.0 Shipping

Samples must be shipped to AGRF in tubes or 96 well 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 as part of your quoted services or you can use our free shipping between nodes once a week service. For information on this service go to Free Shipping.

Post/send/deliver samples to the addresses below:

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

8.0 Turnaround Time

Samples undergo a DNA Quality Control assessment upon receipt at AGRF, and a DNA QC Report will be provided once complete. The typical turnaround time expected from samples receipt is 2-6 weeks. Please note larger sample sets, less commonly processed array types and arrays with custom or booster content may have a longer turnaround time. Please contact AGRF to discuss the expected turnaround time for your specific project

9.0 Data Quality Control

All Illumina Infinium SNP arrays have a set of internal control probes designed to support quality control of the assay's performance. High quality samples generally yield genotype calls with average SNP call rates of >99% and LogRDev <0.3. 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. Arrays with custom or booster content may generate data <99% SNP call rate. 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. AGRF Quality Control assessment, in addition to review of the internal control probes, includes gender checks, when applicable.

10.0 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)
- CNMetrics Report (.csv format) {Includes LogRDev values}
- DNA Report (.csv format) {Includes SNP call rate values}
- Final Report (.txt format), containing SNP and genotype data
- Text File (.xlsx format) {Summary file of data}
- PLINK output file
- Reproducibility and Heritability report (.txt format) {when applicable}
- Illumina manifest file (.bpm format)
- Different manifest options are available for different arrays depending on genome build
- Illumina cluster file (.egt format)

Illumina do not supply a commercial cluster file for the custom arrays, or GSA and GDA with booster content, including the GSA with multi-disease booster (GSA-MD). A cluster file will be created by AGRF using your own sample set by cluster amongst the samples.

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

11.0 Downstream Data Analysist

Our Bioinformatics team is well-equipped to handle your downstream data analysis. Please speak with your Account Manager about organising a quote for additional bioinformatics analysis including:

- quality control
- data formatting CNV analysis
- custom bioinformatics

Our copy number analysis service uses the DNACopy package, and we provide visualisation of the karyotype for each sample.

CNV pipeline outputs:

- Karyotyping: BAF/logR plots provided per sample as a whole genome plot and a tiled image with one figure per chromosome.
- CNV: DNACopy outputs are provided, and the unfiltered results are merged for easy viewing in a heatmap. Gain/loss counts are provided in cases where batch calling is not appropriate.

For those requiring a custom analysis we offer interpretation of the data structure, impact of covariates, population structure and family studies, tumour purity estimation, genome imputation and more. Please contact AGRF for a quote for additional data analysis.

12.0 Sample Storage and Sample Returns/Discards

Samples are stored with AGRF for 3 months after you receive your data, unless arrangement has been made. 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 time frame, samples will be automatically discarded.

13.0 Quality Statement

All clinical works carried out by AGRF follow the strict requirements of ISO15189. AGRF Ltd is accredited by the National Association of Testing Authorities (NATA) in the field of Medical Testing (Scope: Investigation of constitutional genetic variants - Diagnostic Testing. Whole exome sequencing studies for inherited (germline) DNA/RNA changes). Staff and analysis processes follow Standard Operating Procedures, which define responsibilities and quality checks to achieve reported standards. Compliance is monitored at regular reviews and during internal audits. The work is supervised by a person with relevant qualifications and checked while in progress and upon completion to ensure that it meets the necessary ISO15189 standards.

Non-clinical works are performed following the strict requirements of ISO17025: 2005. AGRF Ltd is accredited in the field of Biological Testing (Scope: DNA Analysis) according to the ISO17025: 2005 standard by the National Association of Testing Authorities (NATA). Staff and analysis processes follow Standard Operating Procedures, which define responsibilities and quality checks to achieve reported standards. Compliance is monitored at regular reviews and during internal audits. All work is supervised by a person with relevant qualifications and is checked while in progress and upon completion to ensure that it meets the necessary ISO17025: 2005 standards.