GENOTYPING BY SEQUENCING

Empowering Australian Genomics

Large scale discovery and genotyping of genetic polymorphisms for all species.

Genotyoing-by-sequencing (GBS) data can be used in the following studies:

- Model and non-model organisms lacking reference
- Genome or genotyping arrays
- Genetic, QTL and association mapping
- Population structure and phylogeny

GBS workflow at AGRF

Our GBS workflow is carried out in two stages.

Stage 1 GBS Establishment Service

To enable efficient library preparation, we offer two levels of establishment services for any new project prior to batch processing.

Standard Establishment Service

Determines a suitable enzyme combination.

Eight combinations of 6 and 4bp site restriction enzymes in a double digest are trialed and analysed to determine the best combination for GBS library construction that will lead to the maximum potential for SNP detection.

Premium Establishment Service

Determines a suitable library preparation method and estimates useful tag frequency for project design.

Includes a standard establishment phase, followed by preparation of individual sample libraries with two different Pippin Prep size selected insert sizes (60 and 95 bp wide).

Sequencing is performed on a MiSeq flow cell with the data analysed for tag number / polymorphic frequency over the samples tested. This output is used to tune the optimal coverage of the genome in the batch processing stage to suit your scientific question and budget.

Stage 2 Batch Processing

Following the establishment of the optimal library preparation method, GBS project samples are processed in batches of 48, 96, 144 and 192 multiplex sets which enables very high sequencing economies.

These are sequenced on the Illumina NextSeq 500 platform using single or paired 150 cycle sequencing.

DNA quality

DNA should be high molecular weight (>15 Kb), digestible and free of RNA as assessed by gel electrophoresis.

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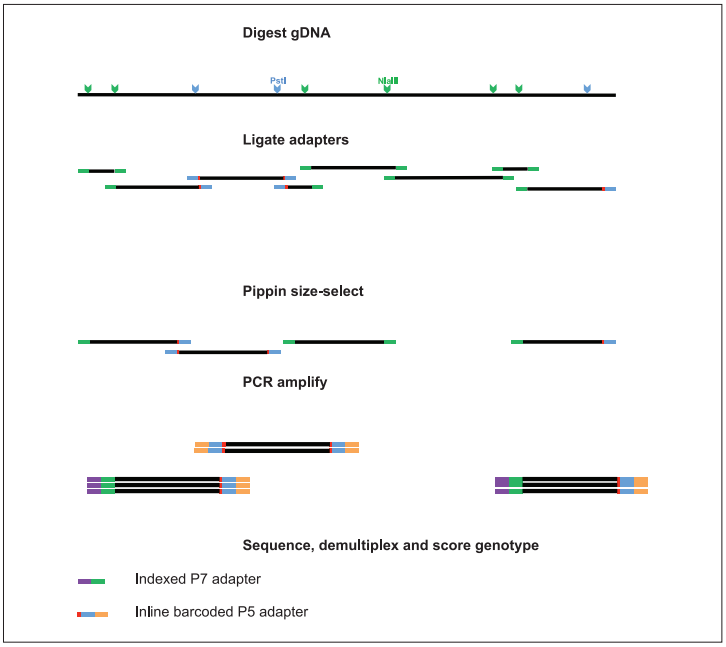
Optimised to improve efficiency

AGRF's method is a variant of the double digest RAD (ddRAD) protocol, and is optimised to improve efficiency and reproducibility of genotype calls while minimising cost.

AGRF GBS library preparation



Data delivery

Users receive de-multiplexed FastQ sequence files for individual samples and tab-delimited files of all the sample alleles in the project for the set of loci sampled.

Further add-on options include:

- Genetic linkage mapping including JoinMap, OneMap, R/QTL
- Population genetic analysis including structure and GenePoP
- Phasing included PHASE, fastPHASE, Beagle and Plink

DNA extraction service

With our DNA extraction service we may be able to provide suitable genomic DNA from your samples for GBS. However, our routine DNA extraction processes cannot guarantee direct recovery of DNA at >30ng/ul from some sample types (especially plant, insect, feathers, blood spots). If you intend to use the AGRF DNA extraction service, we suggest that a compatibility assessment is organised well in advance.

Contact adelaide@agrf.org.au to arrange free testing.

Our customised approach

We know every project is unique. That is why we offer customised and tailor-made solutions for analysing every individual research topic.

Get in touch with us to find out more on how we can help develop the right solution and customise data analysis for your project.

Our funding partners

AGRF is a not-for-profit organisation supported by the Commonwealth Government infrastructure schemes administered through Bioplatforms Australia.

These schemes include NCRIS, EIF, Super Science Initiative CRIS and NCRIS 2.

NCRIS
National Research Infrastructure for Australia
An Australian Government Initiative

BIOPLATFORMS AUSTRALIA

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