

SNP GENOTYPING



With state of the art facilities in both Europe and North America, Almac Diagnostics is the leading Genomics Services provider to academia, biotech and pharma companies. Our expert team will put your investigation on the fast track to results.

SNP GENOTYPING

The ability to type tens of thousands of SNPs on a single array format has opened up new horizons for genomics researchers.

Relevant to a wide range of studies from genome wide linkage analyses to population association studies, this approach has become increasingly popular due to the robust, reproducible results that are enabled by the high-density array format.

Almac Diagnostics is an authorised Affymetrix service provider and offers the full range of SNP Genotyping assays.

QUALITY FOCUS

As the first Affymetrix service provider in the world to gain ISO 17025 accreditation for our Gene Expression and Bioinformatics services, Almac Diagnostics is the partner of choice for researchers who value high quality, usable results.

Our commitment is to provide the highest quality service, both inside and outside the lab.

EXPERT SCIENTIFIC TEAM

Employing over 50 PhDs, our project teams include specialist molecular biologists, world class bioinformaticians and expert, customer focused, field based support staff.

Each customer receives the attention of a dedicated Project Specialist who will oversee the project.

From advice on the optimal experimental design through to provision of actionable insightful results, the Almac Diagnostics team will ensure that each project is tailored to achieve your precise objectives.

WORLD CLASS BIOINFORMATICS

Almac Diagnostics has invested significantly in its bioinformatics capability. The latent potential of the most up to date technical resources available is realised by a world class team of experts in a range of bioinformatics disciplines.

Our Bioinformatics scientists will work with you to recommend the most appropriate strategy to identify the maximum potential in your data for further investigation.

All experiment data is returned to you in a user friendly MIAME compliant, HTML formatted CD-ROM.

SAMPLE PREPARATION

SHIPMENT OF SAMPLES

Genomic DNA (gDNA) samples to be processed should be shipped in a 96-well PCR plate format. The plates should be tightly sealed with strip caps or adhesive PCR film. It is recommended that the samples are frozen before shipment. Ideally, each plate of samples should be wrapped in an insulating material (such as bubble wrap) before packaging on dry ice in a secure container for transport.

We accept DNA samples which have been resuspended / eluted in either reduced EDTA TE Buffer (10 mM Tris-HCl, pH 8.0, 0.1 mM EDTA, pH 8.0), or nuclease-free water.

DNA CONCENTRATION / VOLUME REQUIREMENTS:

- 1µg of DNA is required for GeneChip® Human Mapping Assay
- Minimum concentration of 100ng/µl
- Minimum volume 10µl

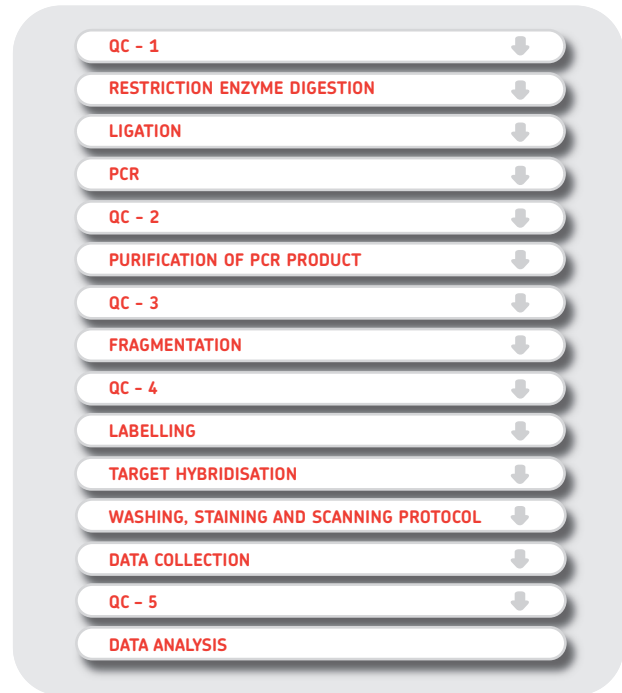
DNA QUALITY REQUIREMENTS:

To ensure high quality results, DNA should be:

- Double-stranded (not single-stranded)
- Free of PCR inhibitors e.g. high concentrations of heme (from blood), chelating agents (i.e. EDTA) and salts
- Uncontaminated by other human genomic DNA sources, or genomic DNA from other organisms
- Intact and not highly degraded

It is recommended that the approximate size of prepared gDNA is assessed on a 0.8% agarose gel using an appropriately sized DNA ladder as a sizing control. High quality gDNA is expected to produce a major band of approximately 10–20Kb. This gel will also demonstrate that the sample is intact and not highly degraded. It is also recommended that gDNA samples are quantified using a spectrophotometer.

PROCESSING



QC - 1

Genomic DNA samples received are quantified using the Eppendorf Biophotometer and must have a **minimum** concentration of 100ng/µl in a volume of 10µl. The A260/280 ratios should read between 1.7 and 1.9. Should any of your samples fail to meet these QC requirements your Project Specialist will contact you to advise on the best way to proceed.

RESTRICTION ENZYME DIGESTION

250ng of Genomic DNA (gDNA) is digested using a restriction enzyme specific to each particular Mapping Assay.

LIGATION

During this process specialised adaptor molecules, specific to the restriction enzyme in question, are ligated to the ends of digested DNA fragments produced in the previous step.

PCR

The copy number of the ligated DNA fragments is amplified using a standard PCR reaction. A generic primer supplied by Affymetrix is used to amplify ligated DNA fragments ranging in size from 250-1100bp (200-1100bp for 500K Assay and 250-1000bp for 10K Assay).

QC - 2

The products of the PCR stage of the mapping assay are assessed using the Agilent 2100 Bioanalyser to ensure appropriately sized PCR fragments have been amplified (see previous section).

PURIFICATION OF PCR PRODUCT

The samples are then cleaned in order to purify and concentrate the PCR product for the next stage of analysis. Samples are quantified and their volume adjusted.

QC - 3

PCR products are quantified using the Eppendorf Biophotometer. The 10K assay PCR products must have an **absolute minimum** concentration of 600ng/μl in order to provide 20μg of material for fragmentation. The 500K assay PCR products must have an **absolute minimum** concentration of 2000ng/μl in order to provide 90μg of material for fragmentation.

FRAGMENTATION

The purified PCR products (90μg for the 500K Assay and 20μg for the 10K Assay) are fragmented. The reaction generates fragments less than 180bp in size for the 500K Assay and approximately 50bp in size for the 10K Assay.

QC - 4

The products of fragmentation are assessed using the Agilent 2100 Bioanalyser to ensure appropriately sized fragmentation products have been generated (see previous section).

LABELLING

The labelling process takes approximately 4 hours and the samples are end labelled using terminal deoxynucleotidyl transferase.

TARGET HYBRIDISATION

The entire fragmented, labelled sample is used to prepare a hybridisation cocktail which is added to the appropriate array and hybridised for 16 hours.

WASHING, STAINING AND SCANNING PROTOCOL

The array is washed and stained on the GeneChip® Fluidics Station 450 using the appropriate fluidics protocol. Once completed the array is inserted into the Affymetrix autoloader carousel and scanned using the GeneChip® Scanner 3000 7G.

DATA COLLECTION

Mapping array data is generated through 3 files (.DAT, .CEL & .CHP).

QC - 5

Mapping array QC checks performed include:

- B2 oligo performance
- Hybridisation control genes
- Call rates
- Concordance with reference genomic DNA 103
- MDR-MCR: to monitor sample contamination

DATA ANALYSIS

Scanned images are analysed and a number of quality control parameters should be met:

- Batch import of pedigree information for sample annotation, gender identification and Mendel-error checking
- Export into MERLIN and GeneHunter compatible formats
- Virtual Array feature allows the 100K set to be managed as a “single array” data set



PUT YOUR INVESTIGATION ON THE FAST TRACK TO RESULTS:

- STATE OF THE ART FACILITIES
- BIOINFORMATICS CENTRE OF EXCELLENCE
- COMPREHENSIVE SERVICE PLATFORM
- EXPERT SUPPORT ON EXPERIMENTAL DESIGN
- DEDICATED PhD LED PROJECT TEAMS
- HIGH QUALITY COST EFFECTIVE OUTSOURCING
- GLOBAL PRESENCE

For European Enquiries:

Almac Diagnostics
19 Seagoe Industrial Estate,
Craigavon, BT63 5QD,
United Kingdom

T +44 (0)28 3833 7575
F +44 (0)28 3839 8676

For American Enquiries:

Almac Diagnostics
801-1 Capitola Drive,
Durham NC 27713,
United States of America

T +1 (919) 294 0230
F +1 (919) 544 8420

E diagnostics@almacgroup.com

Diagnostics
Sciences
Clinical Services
Clinical Technologies
Pharma Services

www.almacgroup.com