



aCGH for CNV Q & A Sheet

1. Introduction

1.1 What is aCGH?

Array Comparative Genomic Hybridisation (aCGH) is a microarray based technique to detect genomic copy number changes (gains or losses) in DNA. It has become an important tool to enable studies on genetic variation, to understand associations with disease and for studies to gain an insight into tumourigenesis.

1.2 How does aCGH differ from single-nucleotide polymorphism (SNP) based methods of looking at Copy Number Variation (CNVs)?

aCGH typically uses 60mer probes to directly and accurately detect both known and *de novo* CNVs, SNP arrays use 25mer polymorphic probes which are used as surrogates for CNVs, with the idea being that any deleted or amplified region that contains a SNP can be detected. Novel SNP platforms also use copy number non polymorphic probes which have been designed to cover *known* CNVs including CNVs void of SNPs.

aCGH utilizes a two-color assay, in which two DNA samples—a test and a reference—are labeled with different fluorescent dyes and then co-hybridized to the array; copy number is assessed by the relative signal intensity of the two samples detected by each probe. SNP based platforms on the other hand, hybridize just one sample per array, calling CNVs by comparison to a reference data file or on-chip controls.

2. Sample DNA Preparation

2.1 How much genomic DNA do I need?

The array comparative genomic hybridisation (aCGH) array experiment requires between 500 ng and 3µg of genomic DNA as starting material depending on the array format and density. We recommend supplying 500ng-1µg of extra DNA (at a concentration of >50ng/µl) to allow for QC checks (see question 2.5).

2.2 What is the recommended quality and purity of DNA samples supplied?

DNA samples should be free of contaminating proteins, carbohydrates, organic solvents, ethanol and salts associated with DNA isolation procedures. An effective measure of DNA purity is the ratio of absorbance readings at 260 and 280 nm and the 230nm reading. The ratio of A260 to A280 values should fall in the range of 1.8–2.1 and the A260 to A230 ratio should be above 1.8. These ratios should be determined using a spectrophotometer (e.g. NanoDrop). DNA must be suspended in high quality DNase-free water or TE buffer or the recommended elution buffer.

If DNA is contaminated and does not meet the required QC metrics then samples should be re-purified using either; ETOH/ sodium acetate precipitation or the final steps of the DNeasy Blood and Tissue Kit - Qiagen p/n 69504.

Genomic DNA should also be intact with minimal degradation. At OGT we will also check the quality of the genomic DNA by gel electrophoresis. Degraded samples can be more challenging to label and may require extra DNA. Please inform OGT if your samples are from FFPE samples as this will alter the processing procedure.



2.3 Can you recommend a commercially available kit to isolate genomic DNA?

Agilent recommend the following kits for extraction of high quality genomic DNA: DNeasy Blood and Tissue Kit - Qiagen p/n 69504, and for FFPE samples - DNeasy Blood and Tissue Kit - Qiagen p/n 69504 or QIAmp DNA FFPE tissue kit - Qiagen p/n 56404. When you use these please follow the Agilent recommended protocol (<http://www.chem.agilent.com/en-us/products/instruments/dnamicroarrays/genomicdnaenzymaticlabelingkit/pages/default.aspx>) or http://www.chem.agilent.com/en-US/Search/Library/_layouts/Agilent/PublicationSummary.aspx?whid=52010&liid=4579 as this differs to the manufacturers protocol, especially for FFPE sample isolation.

2.4 Do I need to provide a reference sample?

OGT can supply the reference sample. In most cases this will be a commercially available pooled DNA sample (Promega). This can either be sex matched with your sample or sex mis-matched, depending on your analysis requirements. There can be additional costs associated with OGT supplying the reference.

2.5 What quality control procedures do you employ upon arrival of my samples?

Prior to initiating the analysis we will subject your samples to a DNA quality control (QC), using gel electrophoresis, to assess DNA integrity. We also run the samples on a NanoDrop UV spectrophotometer to confirm quality metrics as described in question 2.2 above.

2.6 Does the aCGH protocol offer dual or single colour labelling?

The aCGH protocol uses dual colour labelling protocols so that sample and control genomes can be compared on the same array. The method enables net gains or losses to be detected and quantified by direct comparison of hybridization signal from sample and control/reference. This eliminates inter-array variability and results in tighter data.

2.7 Does the protocol permit the labelling of non-human samples?

Yes. The protocol can be used for mouse, rat and chicken genomes on Agilent Catalogue array designs. Custom design arrays can be made for other organisms where there is sufficient publicly available sequence information. The labeling protocol will label any type of genomic DNA.

3. Agilent aCGH/CNV microarray

3.1 What release of the human genome is used for the aCGH for CNV probes?

Probes are currently annotated against NCBI build 36 (UCSC hg18, March 2006) for human. Probes are annotated against NCBI build 36 (UCSC mm8, February 2006) for mouse. Probes are annotated against HGSC build 3.4 (UCSC rn4, November 2004) for rat. Probes are annotated against HCSC galGal3 (WASTL v2.1, May 2006) for chicken.

3.2 What areas of the genome do the aCGH arrays cover?

Comprehensive probe coverage spans coding and non-coding regions, with emphasis on known genes, promoter regions, miRNAs, disease, pseudoautosomal and telomeric regions. Probe design and selection have been carefully optimised and validated and ensure the most accurate quantification of copy number changes of all microarray platforms ⁽¹⁾. This contributes to Genefficiency offering maximal sensitivity and genome coverage. For a comprehensive comparative analysis of the array performance please refer to the following paper <http://www.ncbi.nlm.nih.gov/pubmed/19995423>



3.3 Which species does the aCGH array cover?

The current aCGH array portfolio includes human, mouse, rat and chicken.

3.4 How many arrays are on an aCGH slide?

This depends on the format of the slide. Currently there are catalogue designs for 1x1M 1x244K, 2x400K or 2x105K, 4x180K or 4x44K, 8x60K and 8x15K. Therefore, between one and eight different samples can be hybridised simultaneously. Custom aCGH arrays can be designed and manufactured using the above formats based on your experimental design and content requirements, providing maximum flexibility, the ability to run samples on the same array and cost effectiveness

3.5 How many probe replicates are included on the aCGH arrays?

Apart from some replicate control probes, most probes are unique on the array in order to minimize the probe spacing and therefore maximize the resolution of the array. For example the 400K SurePrint G3 Human CNV 2x400K microarray has a set of 1000 probes present in triplicate. All remaining probes on the array are unique to allow comprehensive genome coverage. Curtis *et al* showed that in comparison to other microarray platforms for CNV analysis, Agilent probes have the highest sensitivity and lowest inter and intra array variation ⁽¹⁾.

3.6 What technology is employed to print Agilent arrays?

Agilent's oligonucleotide microarrays are fabricated using ink jet in-situ printer technology (IJISS) developed by Rosetta Inpharmatics and Agilent Technologies. This SurePrint technology is rapid and versatile and enables fast turnaround of array fabrication. It also utilises a robust, well characterised phosphoramidite chemistry which combines to provide a robust and highly sensitivity platform. A high coupling efficiency enables *in-situ* synthesis of long oligonucleotides.

3.7 How does the aCGH platform deal with tumour samples?

Tumour samples are often affected by stromal contamination. Curtis *et al* investigated this in both aCGH and SNP microarray platforms and found that the benefits of direct competitive hybridization of the aCGH platform were significant and this resulted in a higher sensitive of detection and more robust results ⁽¹⁾.

3.8 How good is the aCGH platform at calling copy number changes?

The aCGH platform from Agilent demonstrates the best discrimination between whole chromosome gains or losses compared to any other microarray based method of calling CNVs⁽¹⁾. In the same study the Agilent aCGH platform also outperformed other microarray platforms for detecting subchromosomal gains or losses.

4. aCGH for CNV Genefficiency profiling service

4.1 What are the benefits of the OGT Genefficiency Service?

OGT offers the Genefficiency service on the Agilent platform. You can use standard off the shelf arrays for whole genome copy number detection or customise your content from our library of >24 million high resolution CNV probes spanning the entire genome to provide more comprehensive genome coverage. The Agilent aCGH arrays locate *more* chromosomal breakpoints at *higher* resolution, and quantify copy number changes *more accurately* than any other method ⁽¹⁾. OGT have the capacity to run >1,000 samples per week with >30 QC checks on each sample. OGT



delivers high quality data quickly. Our dedicated, experienced scientists are available for consultation and support throughout your project providing you with the ability to get results fast.

4.2 **Is my project high throughput or low throughput?**

Your project will be classified as High Throughput if you have >47 samples. Samples should be provided in a 96 well plate in rows E-H, leaving well H12 empty. Well H12 is used for the internal control.

4.3 **What experience do OGT have in running high throughput aCGH studies?**

OGT has recently been named by Agilent as the world's first High-Throughput Microarray Certified Service Provider confirming OGT as the leading choice for large-scale outsourced microarray studies. OGT's facility completed a 22,000 sample, array comparative genomic hybridisation (aCGH) copy number variation (CNV) study⁽²⁾ for the Wellcome Trust Case Control Consortium. This was the largest ever CNV study to date, generating almost 2 billion data points to exacting quality requirements, in a short timeframe of only 20 weeks. Additional major studies are underway.

4.4 **What in-process QC checks are performed during the sample processing?**

At OGT, we pride ourselves on rigorous quality control (QC) at every step of the microarray process regardless of sample throughput. We provide a good laboratory practice (GLP)-like system comprising:

- 20 sample QC steps
- >30 in-process QC checks
- 15 sign-off/validation steps.

These include standard Agilent QC metrics: - initial sample QC metrics, post-labelling QC metrics, and slide QC metrics. In addition, with every experiment an internal control sample is run alongside as an in-process control. This sample has well established QC metrics and enables us to validate the processing at every stage of the protocol. Furthermore, we record all equipment and reagents that are used throughout the protocol and measure ozone and temperature levels where applicable. Click [here](#) for details of Geneefficiency's QC processes – where [>30 checks are made on each precious sample](#)

4.5 **Can OGT help with the experimental design?**

Yes. This element is included in our custom microarray consultancy. The project specifics are usually discussed via a teleconference before recommendations are made.

4.6 **Can OGT help to develop a custom array design?**

Should you require a custom array design, we would generate a draft design following a detailed discussion to determine your requirement. Once completed, the draft design will be sent to you with a software viewing package so that you can review the content, make any changes and finally approve the design before being uploaded for manufacture.

4.7 **Which reagents do I need to supply for the aCGH for CNV microarray service?**

You will only need to supply extracted and purified genomic DNA that meets OGT's QC metrics (See questions 2.1 and 2.2 above). All labelling, dye removal, hybridisation, wash buffers are supplied with the protocols utilised by our in-house highly experienced scientists.



4.8 How do I send my samples?

DNA samples should be shipped on ice. Most customers send their samples on dry ice but this is not a requirement. However, we would like to avoid excessive numbers of freeze thaw cycles. Please make sure the package is labelled appropriately using our pre-formatted labels and sample tracking forms.

4.9 When do I send my samples?

On receipt of a purchase order the Account Manager for your project will send out a sample submission form and labels for shipping. This will include your project and customer identification.

4.10 Where do I send my samples?

Please send your samples to:

Sample Manager

Oxford Gene Technology, Begbroke Science Park,
Sandy Lane, Yarnton, Oxford OX5 1PF UK

T: +44 (0)1865 856861 F: +44 (0)1865 842116

E: sample.manager@ogt.co.uk

4.11 What data will I receive from OGT?

This is dependent on the level of work requested. In general, you will receive a full report outlining the design of the microarray, if applicable, the molecular biological work that has been carried out, and a summary of the results outputted from our bespoke laboratory information management (LIMS) system. In addition, you will also receive the raw text output files from feature extraction, the feature extraction QC report and TIFF images of the hybridised array areas. If requested we can also supply .cgh files which can be uploaded into OGT's CytoSure™ Interpret software or a database of all pre-processed arrays ready for population analysis within the CytoSure Interpret software. (See question 4.13 below).

Data analysis is offered by our team of experienced bioinformaticians, as a basic, standard or advanced data analysis package. Please enquire for additional information.

4.12 How do I receive the data?

The data is sent to you via CD/DVD, or if required a secure FTP site can be provided to allow the transfer of data. If data files are very large then an external hard drive will be sent.

4.13 Does OGT have a software package to analyse aCGH for CNV data?

Yes, OGT has developed the CytoSure Interpret software for aCGH for CNV - for the translation of aCGH data into meaningful results. The features allow you the choice of standardized data analysis or customized data analysis. Click [here](#) for more information.

4.14 What does OGT do with the data?

Your data is held on our secure server for up to 3 months. This data can be held for longer if specifically requested by you.

4.15 What about unused sample?

The unused portion of any samples will be disposed of 6 months after completion of the project. Any un-used material may be returned if specifically requested.



4.16 How long will the service take?

This is dependent on the size of the project and the level of service required. OGT aims to complete the hybridisation within 1-2 weeks of receipt of the sample.

4.17 I still have additional questions, who do I contact?

Please contact us directly for further information.

E: genomicservices@ogt.co.uk

T: +44(0)1865 856823

For further information visit www.ogt.co.uk/genefficiency or contact us directly at:

E: genomicservices@ogt.co.uk

T: +44(0)1865 856823

References

1. Curtis, C *et al* BMC Genomics. (2009) Dec 8;10:588
2. Conrad, D.F. *et al* (2009) Nature doi:10.1038/nature08516

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