CytoSure™

A Sysmex Group Company

CytoSure™ Array Handbook (8x15k and 8x60k formats)

CytoSure Genomic DNA Labelling Kit

Oxford Gene Technology — The Molecular Genetics Company™

Founded by Professor Ed Southern, Oxford Gene Technology (OGT) provides world-class genetics research solutions to leading clinical and academic research institutions.

- **CytoSure™ arrays** — Class-leading products and services offering the complete array solution for clinical genetics research

- **Cytocell® FISH probes** — High-quality products for the detection of gene rearrangements related to inherited genetic disease and cancer

- **SureSeq™ NGS products** — Delivering comprehensive, high–quality targeted sequencing products to clinical and academic researchers.

For more information, visit www.ogt.com.

Contents

Introduction

This handbook is for use with CytoSure 8x60k and 8x15k arrays using the CytoSure Genomic DNA Labelling Kit. For information about specific CytoSure products, visit www.ogt.com.

Array synthesis

The microarrays are synthesised on the glass using an *in-situ* ink-jet printer ensuring high quality microarrays. They are designed to be used in two-colour experiments i.e. one sample is labelled with one fluorescent dye (Cy3) and another sample is labelled with another fluorescent dye (Cy5). Usually one sample is the DNA of interest and the second sample is a reference (or 'normal') DNA.

There are eight arrays per slide. It is intended that the arrays are hybridised as independent hybridisations, so that eight array CGH experiments can be simultaneously conducted per slide. These hybridisations should be carried out together.

OGT CytoSure microarrays are manufactured by Agilent using SurePrint™ technology and can be hybridised as Agilent CGH arrays (www.agilent.com). The scanner requirements are dependent upon microarray format and the feature extraction software. It is recommended that the 8x60k arrays are scanned at 2 or 3 µm and the 8x15k at 5 µm. However, if using the CytoSure Interpret Feature Extraction Software provided by OGT the 8x60k arrays can be scanned at 5µm.

For further information, consult the CytoSure Interpret Feature Extraction Software User Guide or contact OGT at products@ogt.com.

CytoSure Interpret Software

CytoSure Interpret Software is a powerful, easy-to-use package for the analysis of aCGH data, enabling translation of data into meaningful results. CytoSure Interpret Software offers a large combination of features that allow the choice of standardised data analysis (using the Accelerate Workflow) or customised, user-defined data analysis. The functionality allows effortless identification of both copy number variation (CNV) and loss of heterozygosity (LOH)*. CytoSure Interpret Software is provided exclusively with OGT's extensive range of CytoSure aCGH arrays.

For further information, consult the CytoSure Interpret Software User Guide or contact OGT at products@ogt.com.

Genomic DNA labelling

The CytoSure Genomic DNA Labelling Kit (cat. no. 020020) and CytoSure HT Genomic DNA Labelling Kit (500040) are optimised for use with oligonucleotide arrays. They use significantly reduced quantities of dye than alternative labelling kits, yet retain a very high labelling efficiency and produce good array results with low derivative log ratio spreads (DLRS). The labelling protocol is fast and straightforward.

* When used in conjunction with arrays containing SNP probes

This is a low-throughput protocol and the CytoSure DNA Genomic DNA Labelling Kit (cat. no. 020020) is required.

For further information, contact OGT at products@ogt.com.

Automation products

OGT supplies the Labefficiency™ range of automation products throughout Europe and recommends SciGene® automated products in the US to streamline the microarray process. Automation solutions improve test reproducibility and lower costs, while saving hours of valuable hands-on time. Such solutions provide researchers with a powerful way to automate the entire microarray workflow, from DNA labelling and purification, through hybridisation, to array washing and drying. The following systems can be used:

- ArrayPrep® Target Preparation — for automated labelling and hybridisation set-up
- Labefficiency Hyb Oven — for automation compatible hybridisation
- Little Dipper® Microarray Processors — for washing and drying steps
- LabEfficiency O_3Zone Workspace and Filter — for protecting fluorescent dyes from damaging ozone levels, from array washing to scanning

For further information, contact OGT at products@ogt.com.

CytoSure Services

CytoSure Services from OGT — provider of the world's leading high-throughput array comparative genomic hybridization (aCGH) service — offers a flexible and cost-effective aCGH processing solution to suit your specific requirements.

CytoSure Services deliver:

- Immediate and cost-effective access to high-resolution aCGH chromosome analysis
- Fast, reproducible and consistent high-quality results, without the costs associated with setting up aCGH in your laboratory
- The capacity to fulfil your entire current and future constitutional cytogenetic testing requirements
- Peace of mind through complete sample tracking and expertise at every step of process
- An industry-leading aCGH analysis package with full complementary training and support.

For further information, visit www.ogt.com or contact OGT at services@ogt.com.

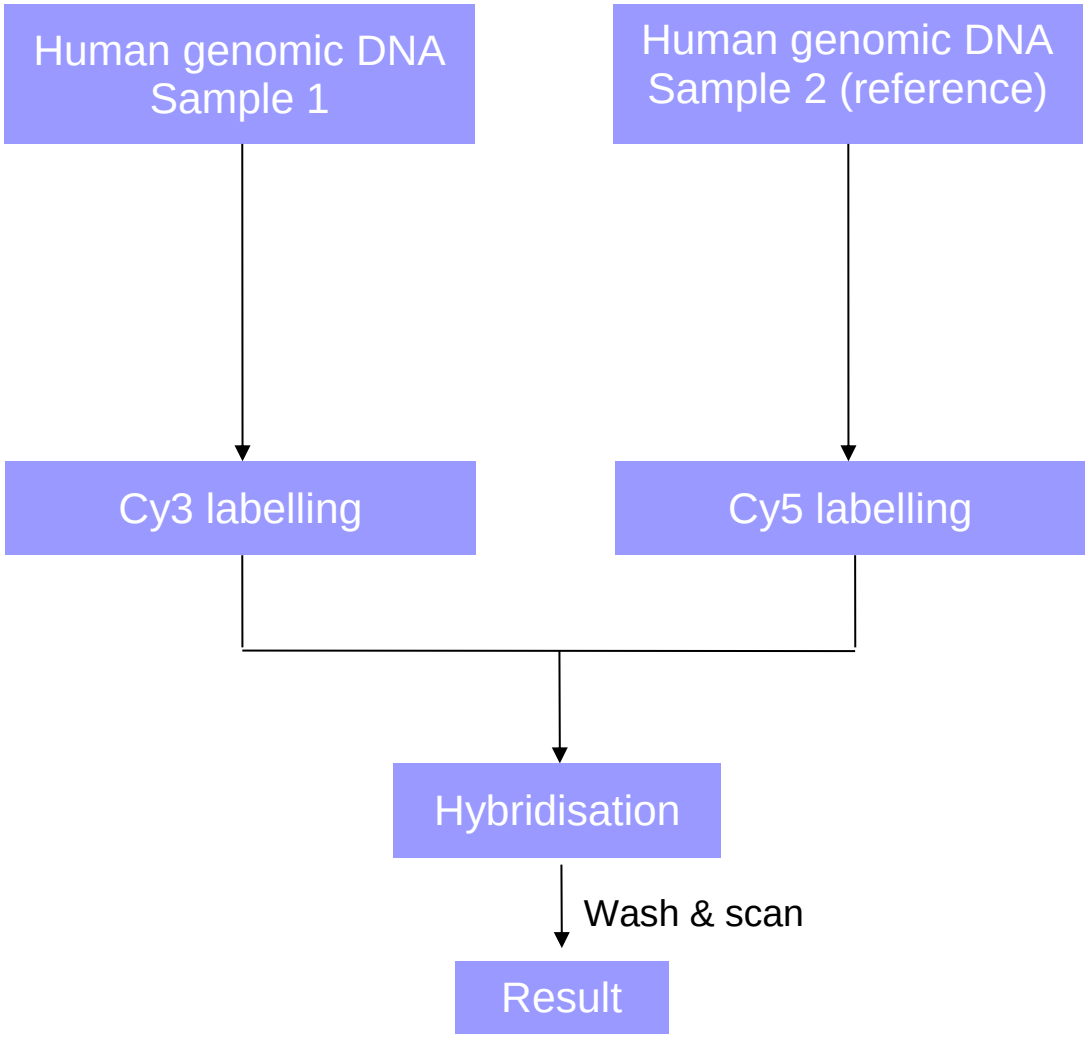


Figure 1: Overview of the microarray procedure

Pack contents

CytoSure Constitutional v3 Array (cat. no. 020045)

Component	Contents
Slides	8x60k format oligonucleotide microarray slides (eight arrays with ~60,000 probes per slide)
CD-ROM	PDF protocol booklets, MSDS for labelling kit reagents, XML and GAL pattern files for feature extraction, CytoSure Interpret Software executable file

CytoSure ISCA v2 Array (cat. no. 020040)

Component	Contents
Slides	8x60k format oligonucleotide microarray slides (eight arrays with ~60,000 probes per slide)
CD-ROM	PDF protocol booklets, MSDS for labelling kit reagents, XML and GAL pattern files for feature extraction, CytoSure Interpret Software executable file

CytoSure ISCA +SNP Array (cat. no. 020052)

Component	Contents
Slides	8x60k format oligonucleotide microarray slides (eight arrays with ~60,000 probes per slide)
CD-ROM	PDF protocol booklets, MSDS for labelling kit reagents, XML and GAL pattern files for feature extraction, CytoSure Interpret Software executable file

CytoSure Aneuploidy Array (cat. no. 020024)

Component	Contents
Slides	8x15k format oligonucleotide microarray slides (eight arrays with ~15,000 probes per slide)
CD-ROM	PDF protocol booklets, MSDS for labelling kit reagents, XML and GAL pattern files for feature extraction, CytoSure Interpret Software executable file

CytoSure Molecular Testing Array A (cat. no. 020062)

Component	Contents
Slides	8x60k format oligonucleotide microarray slides (eight arrays with ~60,000 probes per slide)
CD-ROM	PDF protocol booklets, MSDS for labelling kit reagents, XML and GAL pattern files for feature extraction, CytoSure Interpret Software executable file

CytoSure Haematological Cancer +SNP Array (cat. no. 020070)

Component	Contents
Slides	8x60k format oligonucleotide microarray slides (eight arrays with ~60,000 probes per slide)
CD-ROM	PDF protocol booklets, MSDS for labelling kit reagents, XML and GAL pattern files for feature extraction, CytoSure Interpret Software executable file

CytoSure Custom Designs (cat. no. 020018)

Component	Contents
Slides	8x60k or 8x15k format oligonucleotide microarray slides designed to your specification
CD-ROM	PDF protocol booklets, MSDS for labelling kit reagents, XML and GAL pattern files for feature extraction, CytoSure Interpret Software executable file

CytoSure Genomic DNA Labelling Kit (cat. no. 020020)

Component	Contents
Labelling kit	Random primers, reaction buffer, nucleotide mix, Cy3-dCTP, Cy5-dCTP, Klenow, control λ DNA, water

Storage

The microarrays should be stored in a dehumidified chamber within a light-tight box. The arrays can be stored for up to 3 months. The CytoSure Genomic DNA Labelling Kit should be stored at –20°C. The Purification Columns should be stored at room temperature (15–25°C).

Safety

Take care when handling the microarray slides to prevent them from breaking. If the slides do break, ensure that protective equipment is used to prevent injury.

Handling of slides should be carried out by trained laboratory staff in accordance with good laboratory practice, using the correct protective equipment such as laboratory coats, safety glasses and gloves. Any chemicals used are potentially hazardous. Please refer to the manufacturer's MSDS for specific information.

Use of compressed nitrogen should be conducted by trained staff only using the correct procedures.

Sample preparation and experimental design

Sample preparation

The genomic DNA should be prepared and purified to a standard where the A_{260}/A_{280} ratio exceeds 1.8. Ideally the A_{260}/A_{230} ratio should also exceed 1.5. The final volume of the sample should be 18 µl. At least 1.0 µg of DNA is required and similar starting amounts of sample and reference DNA should be used.

Experimental design

Two samples labelled with different fluorescent dyes can be hybridised to a single array. Therefore it is recommended that one of these samples is a reference sample that can be compared to the test sample. Eight arrays are printed on each slide. Therefore eight separate hybridisations should be carried out in parallel. Prepare eight samples and eight references.

Equipment and reagents required

Labelling of genomic DNA for use on OGT microarrays

Required (not supplied)

- PCR block with heated lid (required for the standard protocol) or heating block (for the digestion protocol in Appendix 1)
- CytoSure Genomic DNA labelling kit (OGT, cat. no. 020020)
- Microcentrifuge

Optional (not supplied)

- NanoDrop™ spectrophotometer
- CytoSure Sample Tracking Spike-ins (tubes) (OGT, cat. nos. various)

Hybridisation and washing of OGT arrays

Equipment for the hybridisation of 1” × 3” microarrays

OGT recommends that the Labefficiency system or Agilent SureHyb™ system is used in conjunction with the gasket slides. A hybridisation oven with a rotisserie is also recommended (e.g., Labefficiency — Hyb Oven, cat. nos. oven: 800010; Labefficiency — Hyb Oven Rotator: 800020, or Agilent, cat. nos.: oven: G2545A; rotisserie: G2530-60029).

- Labefficiency — Hyb Oven Chambers (OGT, cat. no. 800030) or SureHyb hybridisation cassette (Agilent, cat. no. G2534A)
- Gasket slides (OGT, cat. no. 500010)
- Glass dishes and rack for washing the slides
- Magnetic stirrer
- A microarray scanner capable of scanning 1” x 3” glass slides. The scanner requirements are dependent upon microarray format and the feature extraction software.

It is recommended that the 8x60k arrays are scanned at 2 or 3 µm and the 8x15k at 5 µm. However, if using the feature extraction solution provided by OGT, the 8x60k arrays can be scanned at 5 µm.

- Dry nitrogen (recommended)
- Forceps
- Vacuum dessicator (e.g., SpeedVac®)

Recommended reagents (not supplied)

- Oligo aCGH Hybridization Kit (OGT, cat. no. 500014)
- Human Cot-1 DNA® (Life Technologies, cat. no. 15279-011)
- Wash buffers (see table, next page)

Wash 1	Volume (1 litre)	Wash 2	Volume (1 litre)
20x SSPE	25 ml	20x SSPE	5 ml
20% N-lauroylsarcosine	0.25 ml	20% N-lauroylsarcosine	0.25 ml
Water (Milli-Q or similar)	975 ml	Water (Milli-Q or similar)	995 ml

Store wash solutions at room temperature (15–25°C).

Alternatively, ready-to-use Agilent Wash 1 (OGT, cat. no. 500015) and Agilent Wash 2 (OGT, cat. no. 500015) may be used.

Protocol: Running OGT arrays

General procedures

- It is highly recommended that powder-free gloves are used throughout this protocol.
- OGT arrays should be handled by the edges of the glass slide. The array should not be touched.
- CyDye™ is light sensitive and the labelled target or hybridised slides should not be exposed to light. Please refer to the CyDye manufacturer's recommendations.
- The arrays are on the same side of the slide as the 'Agilent' labelled barcode.
- The water used should be molecular biology grade, DNase-free (sterile, 18.2 Ω).

Labelling genomic DNA for use on OGT microarrays (non-restriction–digested DNA)

- This protocol requires the use of a PCR block with a heated lid. If this is not available, use the restriction digestion protocol in Appendix 1.
- Prepare all eight samples and all eight references together. Master mixes of reagents reduce pipetting steps and improve accuracy.
- Use ~1 µg of non-amplified DNA.

Labelling the target

IMPORTANT: Remove the random primer and reaction buffer from the freezer and defrost on ice. The dyes and enzymes should be removed from the freezer immediately before use. Defrost both on ice. Then spin in a centrifuge for 5 to 10 seconds to drive contents off of walls and lid.

The use of spikes is optional. For more information on the spikes, see Appendix 2.

- **No spikes**: The genomic DNA samples should be in ~18 µl volume. If less, then make the volume up to 18 µl with water.

- **Using spikes**: The genomic DNA samples should be in ~13 µl volume. If less, then make the volume up to 13 µl with water.

1. Set up the reaction tubes as shown in the table below:

   Note: If using spikes, the spikes should only go in the sample tubes. Use a different spike for each sample. To ensure that the correct spike is added it is recommended that this step is independently verified. Use the TE buffer provided for the reference.

	Sample	Reference
DNA	13 µl (18 µl without spikes)	18 µl
Spikes (optional)	5 µl	–
Random primer	10 µl	10 µl
Reaction buffer	10 µl	10 µl

2. Mix by flicking the tubes, then microcentrifuge for 15 s.

3. Denature in a PCR block with heated lid at 99°C for 20 min.

   IMPORTANT: Ensure that the tubes are correctly sealed to prevent evaporation

4. Immediately place on ice for 5 min.

5. Microcentrifuge for 15 s.

6. On ice, add the following to the tubes:

	Sample	Reference
dCTP labelling mix	10 µl	10 µl
Cy3-dCTP	1 µl	–
Cy5-dCTP	–	1 µl
Klenow	1 µl	1 µl

7. Mix by flicking the tubes followed by a brief (<10 s) spin in a microcentrifuge.

8. Incubate at 37°C for 2 h.

9. Incubate at 65°C for 10 min, then place on ice for 5 min.

10. Microcentrifuge for 15 s.

Purifying the labelled target

1. Prepare the two purification columns (supplied in the CytoSure Genomic DNA Labelling Kit) by vortexing the resin briefly.

2. Loosen the cap one-quarter turn and snap off the bottom closure.

3. Place the column in a collection tube (supplied).

4. Pre-spin the column for 1 min at 2,000 x *g*.

5. Remove the spin column from the tube and discard the eluate.

6. Place the column in a fresh microcentrifuge tube.

7. Remove the lid from the column, add the sample to the centre of the resin and centrifuge at 2,000 x *g* for 1 min. The final volume should be ~45 µl.

8. If a NanoDrop™ spectrophotometer is available, it is recommended that 1.5 µl of material is used to measure the absorbance at 260 nm, 550 nm and 650 nm. The amounts of target that should be obtained are shown in the table below.

Approximate amounts (from a 1.0 µg labelling)	**Cy3**	**Cy5**
DNA	6 µg	5 µg
Dye	250 pmol	200 pmol

Note: These are minimum amounts. If the figures are higher, it is still possible to proceed.

9. Combine the labelled targets in one tube.

10. The labelled targets should then be dried to ~16 µl. This may be done using a vacuum dryer such as a SpeedVac. Dry on low heat in the dark. If over-dried, then resuspend the pellet in 16 µl of water by gently pipetting up and down.

    The labelled targets should be used immediately for hybridisation. If this is not possible, targets should be stored at –70°C in the dark.

Hybridisation of arrays with labelled target

OGT recommends the use of the Labefficiency hybridisation system or Agilent SureHyb hybridisation equipment. If other hybridisation equipment is used, adjustments to this protocol may be required.

1. Prepare the 10x Blocking Agent by adding 1,350 µl water to the 10x Blocking Agent tube (supplied with the Oligo aCGH Hybridisation Kit).

2. Leave at room temperature (15–25°C) for 60 min.

   IMPORTANT: After use, store the Blocking Agent at –20°C for no more than 2 months.

3. Remove the slide box from its packaging and store slides in a dehumidified chamber. The slides should be stored in a light-tight box until use.

4. When ready for use, remove the slides from the box. Return unused slides to dehumidified chamber.

Note: Wear clean powder-free gloves at all times when handling the microarrays. Handle in a low-dust laboratory.
Note: The arrays are printed on the same side of the slide as the 'Agilent'-labelled barcode.

5. Set up the hybridisation. The precise method of hybridisation will depend on the hybridisation equipment available to the user.

   Note: OGT recommends the Labefficiency hybridisation system or Agilent's SureHyb equipment, in which each of the eight hybridisations is carried out in a 45 µl volume.

6. Prepare the following in a separate, clean tube. Shake the hybridisation buffer before use and take extra care when pipetting the viscous hybridisation buffer. The use of a master mix is recommended as suggested in the table below.

	Volumes required for each 45 µl hybridisation mix	**Volumes for master mix (8x)***
Cy3 and Cy5-labelled genomic DNA	16 µl	–
Cot-1 (1 mg/ml)	2 µl	18 µl
Agilent 10x Blocking Agent	4.5 µl	40.5 µl
Agilent 2x HiRPM Hybridization Buffer	22.5 µl	202.5 µl

* Excess reagents have been included as the solution is viscous.

7. Add 29 µl of the master mix to each sample and mix thoroughly by pipetting.

8. Denature the target at 94°C for 3 min.

9. Incubate at 37°C for 30 min.

10. Quickly spin down in a microcentrifuge for 10 s.

11. Place an Agilent SureHyb Gasket into an Agilent Chamber base or Labefficiency — Hyb Oven Chambers.

12. Immediately pipette 45 µl of the first hybridisation mix onto the first chamber of the gasket slide.

13. Dispense the other 7 hybridisation mixes (45 µl each) onto the remaining 7 chambers of the gasket slide (Figure 2). Record which sample goes in which chamber.

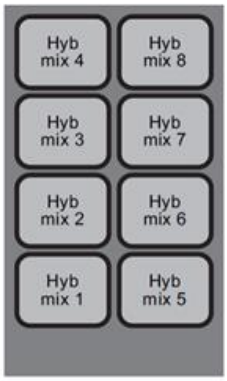


Figure 2: Gasket slide layout

14. Place an OGT array onto the Gasket slide.

    IMPORTANT: Ensure the array is placed with the array side facing down and in contact with the hybridisation mix.

    Note: The arrays are printed on the same side of the slide as the 'Agilent'-labelled barcode.

15. If using the Agilent SureHyb system, place the clamp assembly on the slide and tighten the thumbscrew firmly. If using the Labefficiency assembly, place the cover over the slides and tighten the thumbscrews firmly.

    Some bubbles should form. These bubbles should be moving. If they are not, tap the chamber on the bench.

16. Place the hybridisation chamber in the hybridisation oven (ideally with a rotisserie). Hybridise at 65°C for 22 h in a light-tight container. Fit the slides vertically and rotate the chambers at a speed of 20 rpm.

    IMPORTANT: Many fluorescent dyes used for microarrays are light sensitive. Minimise exposure of hybridised microarrays to light. Please refer to the dye manufacturer's recommendations.

Washing and scanning of arrays

Before starting, decide if the Agilent stabilisation wash is going to be used (see Appendix 3).

1. Pre-warm Wash 2 and containers to 37°C.

2. Ensure that all glass dishes have been cleaned thoroughly with deionised distilled water. Acetonitrile may also be used. Wear clean powder-free gloves and change gloves frequently.

3. Place Wash 1 in two glass dishes (one for disassembly and one for first wash).

4. Place the slide in the disassembly bath, gently prise the gasket slide from the CytoSure array under the surface of the buffer. Transfer the slide quickly to the Wash 1 bath.

   Note: The wash solution should be changed every 4 slides.

5. Place a stirring flea in the Wash 1 bath.

6. Stir at room temperature for 5 min.

7. Remove the rack and blot on a paper towel. Place quickly into the pre-warmed Wash 2 bath.

8. Stir at 37°C for exactly 1 min.

9. Remove the rack, blot and blow dry using air or nitrogen.

10. Scan the slide immediately.

11. Insert the slide into the scanner. Refer to the scanner manufacturer's instruction booklet and safety information for instructions about the correct use of the scanner.

Scanner Requirements
The scanner requirements are dependent upon microarray format and the feature extraction software. It is recommended that the 8x60k arrays are scanned at 2 or 3 µm and the 8x15k at 5 µm. If using the CytoSure Interpret Feature Extraction Software provided by OGT, the 8x60k arrays can be scanned at 5 µm. If using an Agilent scanner and Agilent Feature Extraction Software or GenePix® scanner and GenePix software, the following procedures are advised.

Agilent scanner
Insert the slide into the Agilent slide holder, with the array side facing up. The non-barcoded edge should be placed into the slide holder first. The slide should be scanned with the green laser (~532 nm) and the red laser (~633 nm). It is recommended to scan at 2 µm for an 8x60k array although 3 µm resolution is acceptable and 5 µm for an 8x15k array resolution. The whole slide should be scanned.

GenePix scanner
The slides should be inserted into the scanner array side facing down. The non-barcoded edge should go into the scanner first. The slide should be scanned with the green laser (~532 nm) and the red laser (~633 nm). It is recommended to scan at 2 µm resolution for an 8x60k array and 5 µm for an 8x15k or 4x44k array. The whole slide should be scanned.

Visualisation of scanned array images

On printing, each oligonucleotide on an OGT array is assigned a unique feature number. The identity of the feature number on the array is given the design files which can be found on the CD-ROM that accompanies the microarrays. As different scanners display the image of the slides differently, the position of the feature number will vary according to the scanner.

Agilent scanner (Agilent image analysis software)

Figure 3 shows how the features are displayed on the screen using an Agilent scanner.

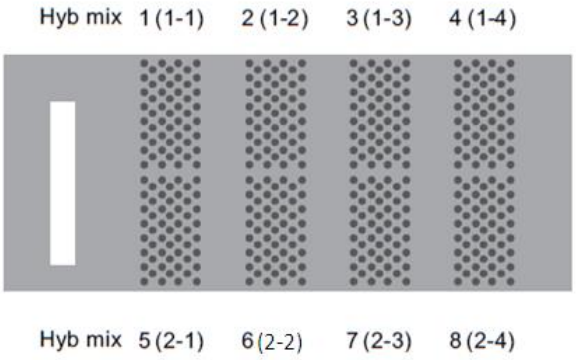


Figure 3: OGT microarray positioned on an Agilent scanner

GenePix scanner

Figure 4 shows how the features are displayed on the screen using the GenePix scanner.

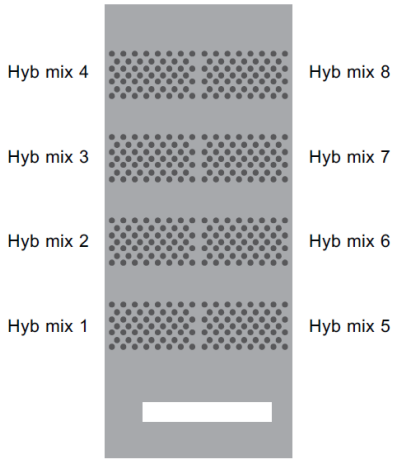


Figure 4: OGT microarray positioned on a GenePix scanner

Protocol: Feature extraction

OGT feature extraction software

Please refer to the CytoSure Interpret Feature Extraction User Guide. This is provided on the CD-ROM accompanying the microarrays.

Agilent and GenePix feature extraction software

Please refer to the Agilent and GenePix Feature Extraction User Guide that is provided on the CD-ROM accompanying the microarrays.

Data analysis

CytoSure Interpret Software, supplied with OGT arrays, is a comprehensive array CGH data analysis and results interpretation package.

The files generated by the feature extraction software can be loaded into the CytoSure Interpret Software by going to “File” and selecting “Import”. Once loaded into the software, the results are displayed in a graph with the y-axis being red (Cy5)/green (Cy3).

Normalisation: ISCA and Constitutional arrays

On importing the file into the software, a dialog box appears providing the option to normalise.

If you are using Agilent software we recommend that you do not use this option as the data has already been normalised during feature extraction. For other feature extraction software packages such as GenePix we recommend that the normalisation option in CytoSure Interpret Software is used.

Normalisation: Cancer arrays

On importing the file into the software, a dialog box appears providing the option to normalise.

If you are using Agilent software we recommend that you do not use this option as the data has already been normalised during feature extraction. For other feature extraction software packages such as GenePix we recommend that the normalisation option in CytoSure Interpret Software is used. In addition, for samples containing a lot of aberrations an additional normalisation step is available in CytoSure Interpret Software v4.4 and above. This is a segmentation-based method and is suitable for all sample types but particularly beneficial for complex samples. To apply the segmentation-based method, go to ‘Tools’ and in the drop-down menu select ‘Apply Segmentation Normalisation’ (Figure 5).

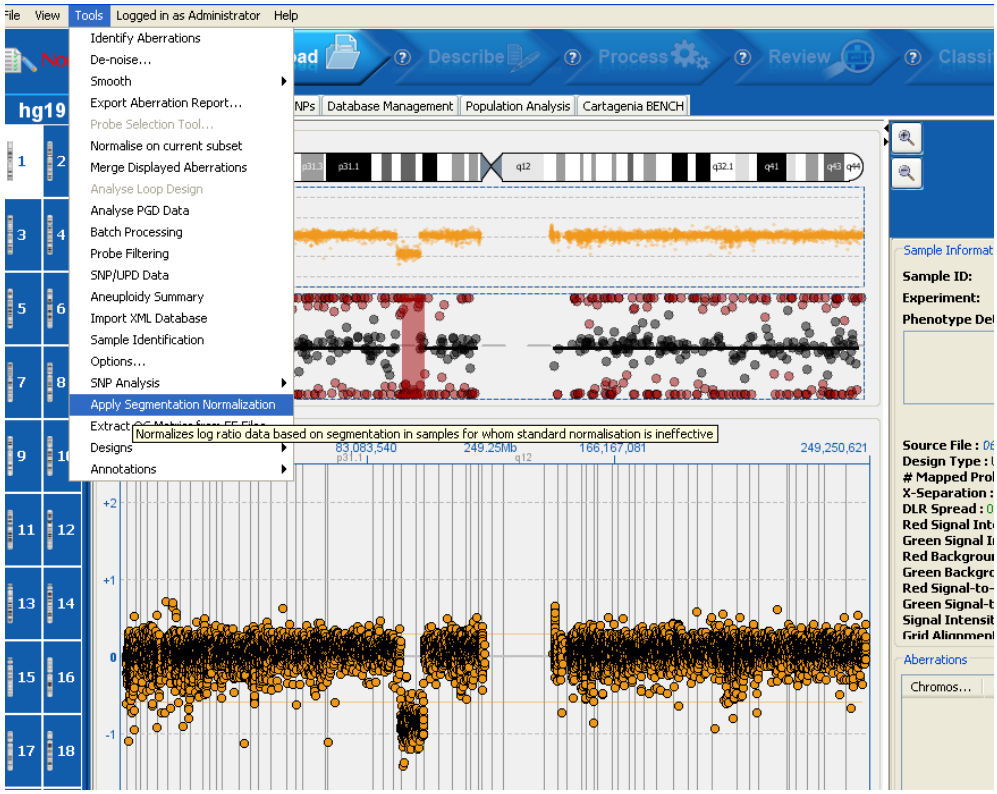


Figure 5: Segmentation-based normalisation in CytoSure Interpret Software

LOH detection

Several CytoSure arrays allow detection of both copy number changes and regions with loss of heterozygosity (LOH). For accurate detection of LOH, the default settings in CytoSure Interpret Software may need to be changed, it is important that these values are checked before starting data analysis. After loading the data, navigate to the CNVs/SNPs tab and click on the 'Options' button (Figure 6).

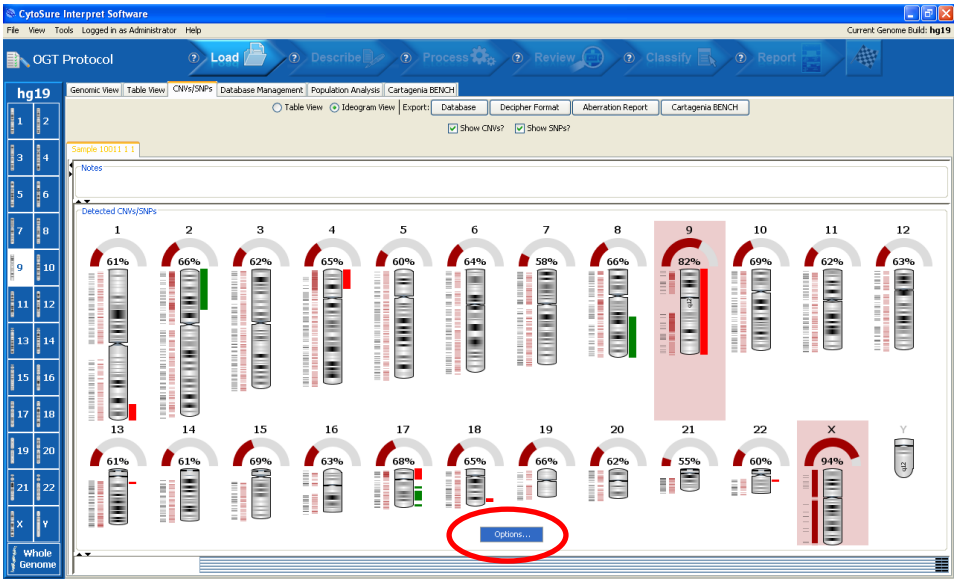


Figure 6: Screenshot of the CNV/SNPs window in CytoSure Interpret Software

In the pop-up window that appears, click on the 'LOH Calling' tab and change the 'Only display LOH regions with scores above:' and 'Only report LOH regions with scores above:' to the recommended values provided in Table 1 (Figure 7).

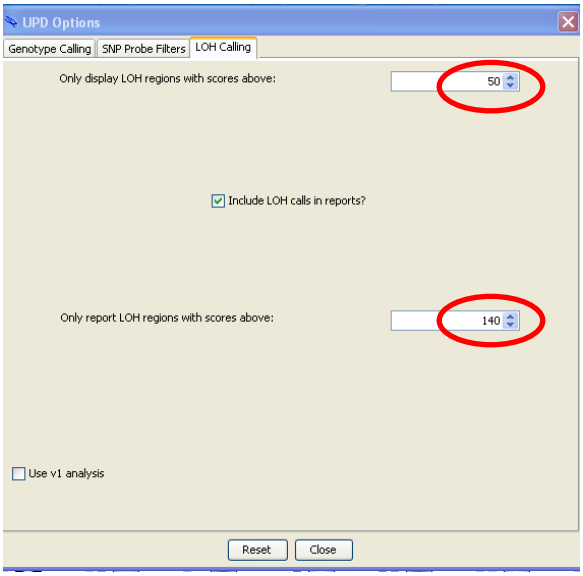


Figure 7: The LOH calling dialog box in CytoSure Interpret Software

Array	Cat. Number	Display LOH with scores above:	Report LOH with scores above:
CytoSure ISCA +SNP (8x60k)	020052	50	140
CytoSure Haematological Cancer +SNP (8x60k)	020070	50	140

Table 1: The recommended LOH analysis values for CytoSure arrays. These values ensure that the correct regions of LOH are displayed and reported for the combined CN and LOH arrays.

Aneuploidy array initial analysis

The CytoSure Aneuploidy array allows simplified detection of chromosome imbalances. CytoSure Interpret Software incorporates a dedicated aneuploidy feature that rapidly tests to see if there are any aneuploidies in the sample. In genomic view, click "Tools", followed by "Aneuploidy Test". This provides a rapid method for averaging all probes on each chromosome and plotting a box and whisker plot. The plot shows the following:

- The position of the mean
- The lower quartile (25th percentile)
- The maximum value
- The median (50th percentile)
- The upper quartile (75th percentile)
- The minimum value

See Figures 8 and 9 for details. The user can zoom into regions on the plot by dragging the mouse icon over the relevant region.

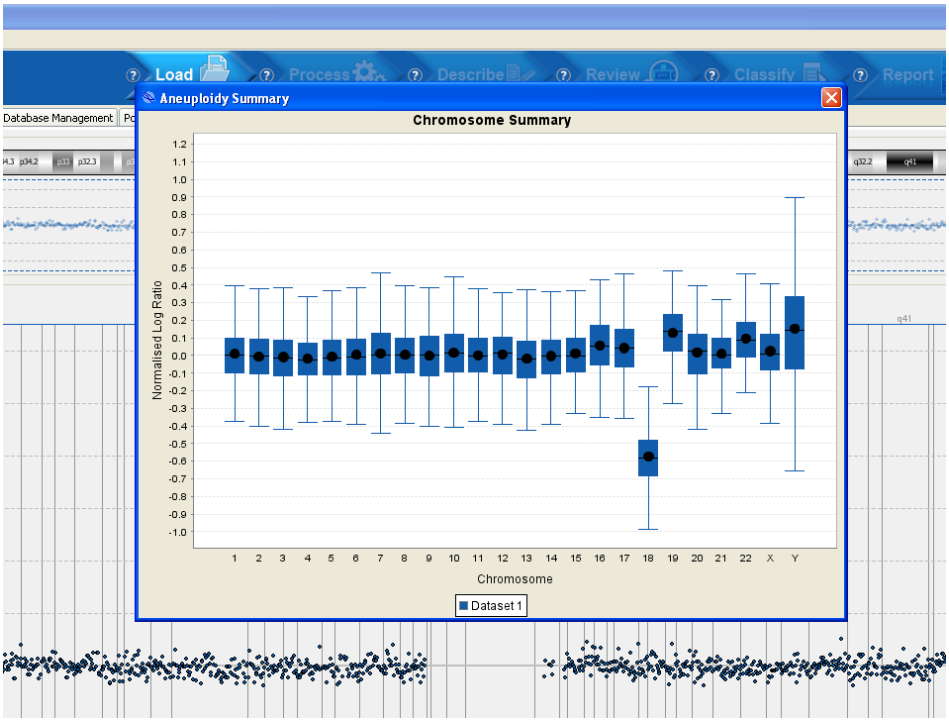


Figure 8: Screenshot of the Aneuploidy Summary Window in CytoSure Interpret Software

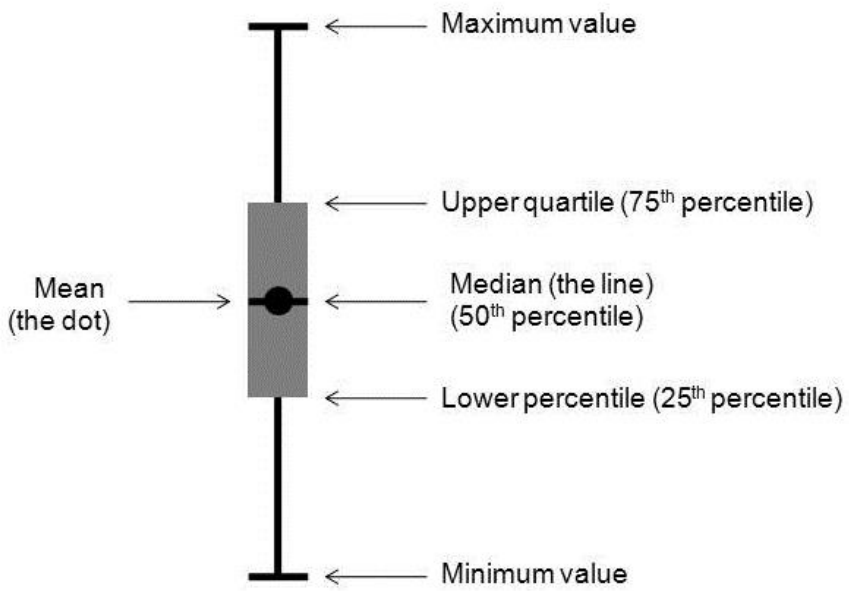
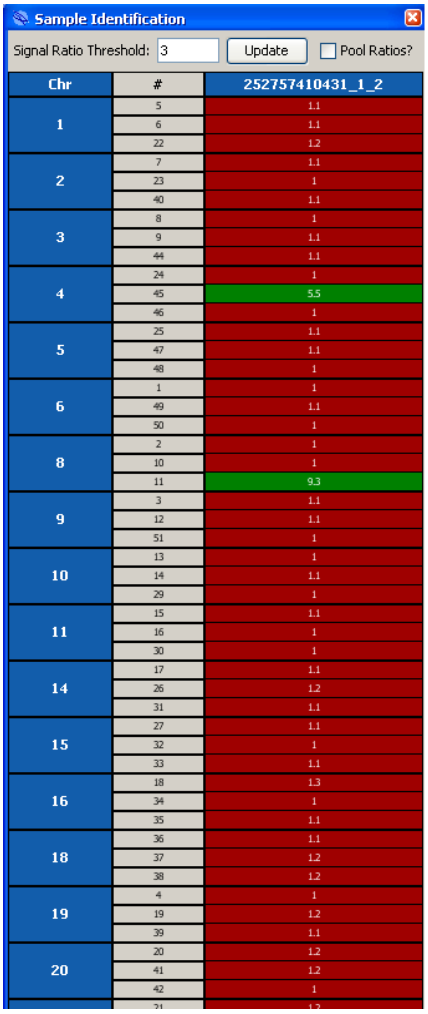


Figure 9: The different values on the box and whiskers plot

CytoSure Sample Tracking Spike-ins

If CytoSure Sample Tracking Spike-ins are included in the design of the array and are included in the labelling reaction, the user can verify that the correct sample has been placed on the correct array. CytoSure Interpret Software (v3.4.2 and above) displays the signals from the spike probes in a table (Figure 10). The table can be accessed via “Tools” followed by “Sample identification”. Appendix 2 provides further details about the spikes.

Furthermore, the spike’s identity can be entered into the software at the Sample Details stage (click on “Other” tab, Figure 11). The software will then automatically detect if the correct spike has increased intensity on the array and will report a match in the QC metrics.



Chr	#	252757410431_1_2
1	5	1.1
	6	1.1
	22	1.2
2	7	1.1
	23	1
	40	1.1
3	8	1
	9	1.1
	44	1.1
4	24	1
	45	5.5
	46	1
5	25	1.1
	47	1.1
	48	1
6	1	1
	49	1.1
	50	1
8	2	1
	10	1
	11	9.3
9	3	1.1
	12	1.1
	51	1
10	13	1
	14	1.1
	29	1
11	15	1.1
	16	1
	30	1
14	17	1.1
	26	1.2
	31	1.1
15	27	1.1
	32	1
	33	1.1
16	18	1.3
	34	1
	35	1.1
18	36	1.1
	37	1.2
	38	1.2
19	4	1
	19	1.2
	39	1.1
20	20	1.2
	41	1.2
	42	1

Figure 10: Spike identification

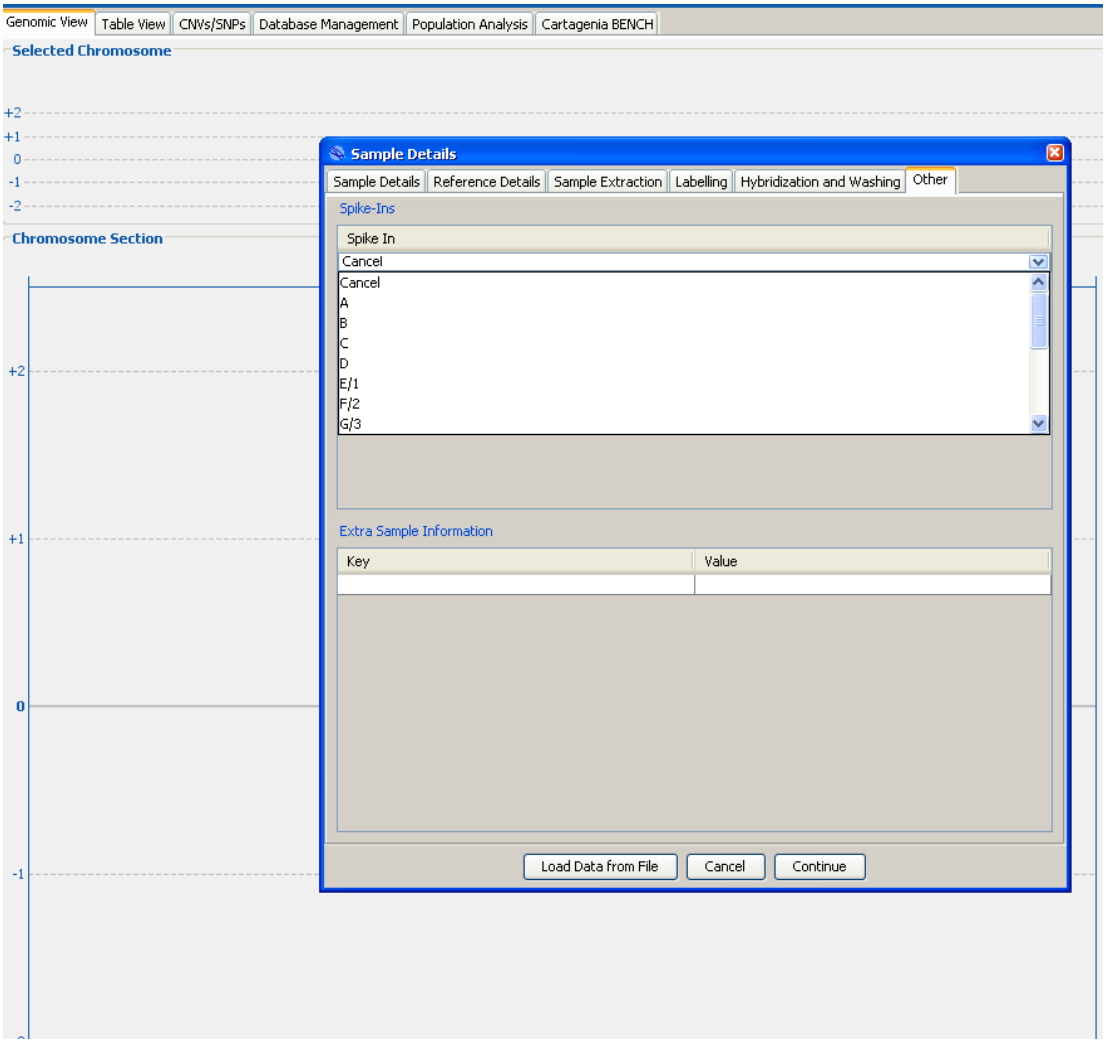


Figure 11: Entering spike information (requires CytoSure Interpret Software v3.4.2 or above)

Troubleshooting

Array hybridisation

Black holes on array	Ensure the correct volume of hybridisation solution has been used. Take care not to spill any of the hybridisation solution when setting-up the array. Check the gasket slide seal has not leaked, if it has report the failure to support@ogt.com
Fluorescent smears across the slide	Contamination with fluorescent material during washing. Ensure clean gloves are worn and forceps are cleaned with water before and between washes. Any wash containers used should be cleaned with acetonitrile and copious amounts of deionised, distilled water. Contamination from the nitrogen or air used to dry the microarrays prior to scanning. Change the source of nitrogen or air used. The arrays have dried out during the hybridisation or wash procedure. Remove slides from gasket slides under Wash 1. Carry out a final acetonitrile wash for 1 minute at room temperature.
Low/no signals on the array	Poor DNA labelling; check labelling using a NanoDrop. If not available use a UV spectrophotometer. Check that the correct side of the array has been scanned. Check correct side of the array has been used in the array hybridisation.
Low Cy5 signal	Ensure slides are scanned immediately after washing. Enclose scanners in a box with ozone scrubbers (such as from Labefficiency). Cy5-labelled targets were exposed to light. Cover with foil or use amber microcentrifuge tubes.
Saturated features	Reduce the PMT setting on the microarray scanner.
High DLRS values	Check the quality of the DNA on a high percentage agarose gel for degradation. If the DNA is degraded, shown by a smear on the gel, re-extract the sample.
High background	The most common cause of high background is contaminated wash dishes. Ensure clean gloves are worn and forceps are cleaned with water before and between washes. Any wash containers used should be cleaned with acetonitrile and copious amounts of deionised, distilled water.

Appendix 1: Digestion protocol

If a PCR block with a heated lid is not available, it is necessary to digest the sample beforehand using the following protocol.

Restriction digestion of genomic DNA

Use ~1 µg of non-amplified genomic DNA. Prepare all eight samples and all eight references together. Master mixes of reagents can reduce pipetting steps and improve accuracy.

1. Digest the genomic DNA with the restriction enzymes *Rsa*I and *Alu*I:

	Sample	Reference
DNA	X µl	X µl
Restriction enzyme buffer (10x)	1.8 µl	1.8 µl
Water	To total volume of 18 µl	To total volume of 18 µl
*Alu*I (10 U/µl)	0.5 µl	0.5 µl
*Rsa*I (10 U/µl)	0.5 µl	0.5 µl

2. Incubate at 37°C for 2 h.

3. Inactivate the enzymes by heating at 65°C for 20 min.

4. Genomic DNA digestion should be checked. We recommend the Agilent Bioanalyzer with the DNA 12000 chip. Run 1 µl of the sample on a DNA 12000 chip following the manufacturer's instructions. Fragment size should peak at ~200 bp.

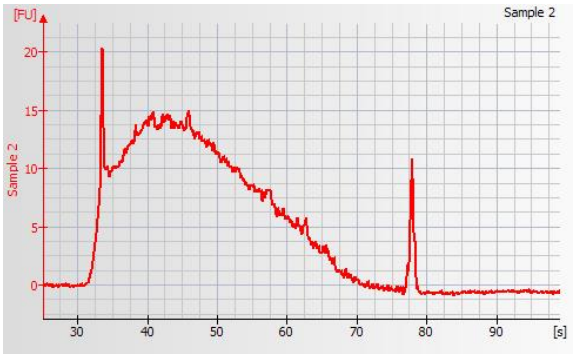


Figure 12: Bioanalyzer trace of genomic DNA digested with *Rsa*I and *Alu*I

Labelling the target

1. The restriction digested genomic DNA samples should be in ~18 µl volume.

2. Follow the protocol from "Protocol: Running OGT arrays", page 11.

IMPORTANT: The denaturation step (step 3) should be changed to 95°C for 3 min.

Appendix 2: CytoSure Sample Tracking Spike-ins

The purpose of CytoSure Sample Tracking Spike-ins is to reduce the possibility that the user places the wrong sample on a particular array, resulting in a mix-up of the results.

The spike-ins are short PCR products amplified from human genomic DNA. The products have been cloned and sequenced to ensure that there is no contaminating human DNA present in the sample arising from non-specific PCR amplification. They are supplied ready to use.

The spike-ins are used by incorporating 5 µl in the sample labelling reaction. No spike-in should be included in the reference labelling reaction. Each sample should ideally have a different spike-in. If desired, more than 1 spike-in can be used per sample. These spike-ins will then be labelled and will hybridise to specially designed oligonucleotide probes present on the arrays.

The user can then verify that the correct sample has been placed on the correct array. CytoSure Interpret Software helps by reporting the signals from the spike probes. The report can be accessed by going to “Tools”, and selecting “Sample Identification”.

The spikes will react with the probes as follows:

Spike-in name	Spike-in cap colour	Spike-in probes
A	Yellow	Chr1_22
B	Amber	Chr3_9
C	Red	Chr3_44
D	Purple	Chr4_45
E	Black	Chr5_48
F	Green	Chr6_50
G	Beige (transparent)	Chr6_1
H	Orange	Chr8_11

The identity of the spike-in is displayed in CytoSure Interpret Software.

Appendix 3: Optional ozone stabilisation of dyes

Materials required (not supplied)

- Stabilization and Drying Solution (OGT, cat. no. 500016)
- Acetonitrile (Sigma, cat. no. 271004)

Procedure

IMPORTANT: Wear gloves, laboratory coat and safety glasses for this procedure and carry out in a fume hood.

1. Before starting the wash, warm the stabilisation solution to 40°C in a water bath, allowing sufficient head space for expansion.

2. Once the precipitate is dissolved, allow the solution to cool to room temperature before use.

3. When preparing the wash solutions (Washing and scanning of arrays, page 15), pour the acetonitrile and the stabilisation solution into suitable containers.

4. Once Wash 2 has been completed, dip the slide into the acetonitrile 3 times.

5. Dip the slide into the stabilisation solution 3 times.

6. Using clean forceps, remove the microarray and blow dry with dry nitrogen. Proceed with step 10, page 16.

Alternatively, ozone levels in the working environment can be minimised using the Labefficiency — O_3Zone Filter (OGT, cat. no. 800050) and the Labefficiency — O_3Zone Workspace (OGT, cat. no. 800040), both available from OGT. A slide barrier to protect the slide from ozone is available from Agilent (cat. no. G2505-60550).

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Oxford Gene Technology, Begbroke Hill, Woodstock Road, Begbroke, Oxfordshire, OX5 1PF, UK.

Oxford Gene Technology (Operations) Ltd. Registered in England No: 03845432
Begbroke Hill, Woodstock Road, Begbroke, Oxfordshire, OX5 1PF.

Tel: +44(0)1865 856826 (US: 914-467-5285)

Email: products@ogt.com Technical support email: support@ogt.com

Web: www.ogt.com

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Ordering information

Product	Contents	Cat. no.
Genome-wide arrays		
CytoSure Constitutional v3 (8x60k)	Microarray with eight arrays of ~60,000 spots; CytoSure Interpret Software	020045
CytoSure Constitutional v3 (4x180k)	Microarray with four arrays of ~180,000 spots; CytoSure Interpret Software	020046
CytoSure Constituional v3 +SNP (4x180k)	Microarray with four arrays of ~180,000 spots; CytoSure Interpret Software	020047
CytoSure ISCA v2 (8x60k)	Microarray with eight arrays of ~60,000 spots; CytoSure Interpret Software	020040
CytoSure ISCA v2 (4x180k)	Microarray with four arrays of ~180,000 spots; CytoSure Interpret Software	020041
CytoSure ISCA v2 (4x44k)	Microarray with four arrays of ~44,000 spots; CytoSure Interpret Software	020042
CytoSure ISCA UPD (4x180k)	Microarray with four arrays of ~180,000 spots; CytoSure Interpret Software	020050
CytoSure ISCA +SNP (4x180k)	Microarray with four arrays of ~180,000 spots; CytoSure Interpret Software	020051
CytoSure ISCA +SNP (8x60k)	Microarray with eight arrays of ~60,000 spots; CytoSure Interpret Software	020052
CytoSure Syndrome Plus v2 (2x105k)	Microarray with two arrays of ~105,000 spots; CytoSure Interpret Software	020019
CytoSure Aneuploidy (8x15k)	Microarray with eight arrays of ~15,000 spots; CytoSure Interpret Software	020024
CytoSure Embryo Screen Array (8x60k)	Microarray with eight arrays of ~60,000 spots; CytoSure Interpret Software	020044
Chromosome-specific arrays		
CytoSure Chromosome X (4x44k)	Microarray with four arrays of ~44,000 spots; CytoSure Interpret Software	020015
CytoSure Chromosome X Array (2x105k)	Microarray with two arrays of ~105,000 spots; CytoSure Interpret Software	020021
Gene-focused arrays		
CytoSure Medical Research Exome Array (1x1M)	Microarray with one array of ~1,000,000 spots; CytoSure Interpret Software	020100

CytoSure Molecular Testing Array A (8x60k)	Microarray with eight arrays of ~60,000 spots; CytoSure Interpret Software. Content focus: neuromuscular disorders	020062
CytoSure Molecular Testing Array B (4x180k)	Microarray with four arrays of ~180,000 spots, CytoSure Interpret Software. Content focus: in-born metabolic disorders	020061
CytoSure Molecular Testing Array C (4x180k)	Microarray with four arrays of ~180,000 spots, CytoSure Interpret Software. Content focus: mental retardation and autism	020060
CytoSure DMD (4x44k)	Microarray with four arrays of ~44,000 spots; CytoSure Interpret Software	020023
CytoSure Custom Molecular Testing Array (various formats)	Your choice of array design and format; CytoSure Interpret Software	020018
Cancer arrays		
CytoSure Haematological Cancer +SNP Array (8x60k)	Microarray with eight arrays of ~60,000 spots; CytoSure Interpret Software	020070
CytoSure Cancer +SNP Array (4x180k)	Microarray with four arrays of ~180,000 spots; CytoSure Interpret Software	700090
CytoSure Consortium Cancer +SNP Array (4x180k)	Microarray with four arrays of ~180,000 spots; CytoSure Interpret Software	020071
Custom arrays		
CytoSure Custom Designs	Your choice of array design and format; CytoSure Interpret Software	020018
Genomic DNA labelling		
CytoSure Genomic DNA Labelling Kit	24 reactions: clean-up columns, dyes, nucleotide mix, random primers, enzyme, collection tubes	020020
CytoSure HT Genomic DNA Labelling Kit	96-Well Plate format for 96 reactions: 96-Well Purification Plate, dyes, nucleotide mix, random primers, enzyme	500040

Analysis software		
CytoSure Interpret Software	Class-leading microarray analysis software provided with CytoSure arrays. Email products@ogt.com to request your demo version	020022
CytoSure Interpret Feature Extraction Module	Microarray feature extraction module, compatible with CytoSure Interpret Software versions 4.4 and above	030022

Services		
CytoSure Services	Outsourced array processing	Enquire

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Notes

Notes

Contact us

Oxford Gene Technology,
Begbroke Hill,
Woodstock Road,
Begbroke,
Oxfordshire
OX5 1PF, UK

T: +44(0)1865 856826; (US: 914-467-5285)
E: products@ogt.com; W: www.ogt.com

Technical support: E: support@ogt.com
T: +44(0)1865 856826

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