The use of the InnoScan® 710 scanner and Mapix® software with CytoSure™ microarrays

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Introduction

The Innoscan710 scanner from Innopys® is a widely used microarray scanner capable of scanning microarray slides with a resolution of 3 µm. It has two high performance lasers with an excitation of 532nm and 635nm and is available with sophisticated feature extraction software named Mapix®. With this capability the Innoscan710 scanner should be compatible with OGT's CytoSure™ range of microarray slides, reagents and analysis software. This application note tests the use of Innoscan710 scanner and Mapix with a variety of OGT slides which detect chromosomal copy number variation.

Material and Methods

A variety of OGT CytoSure slides were used according to OGT protocols (*www.ogt.com*). Briefly, in the case of the CytoSure Embryo Screen arrays, DNA was first amplified using the PicoPLEX™ kit (Rubicon). All other slides used 1µg of starting DNA. The DNA was then labelled using the CytoSure Genomic DNA Labelling Kit and purified with the supplied CytoSure columns. Sample DNA was labelled with Cy3 and Reference DNA with Cy5. After purification, the DNAs were pooled and dried in a SpeedVac™ until dry. The DNA was resuspended in water, CotI, 5x blocking agent (Agilent) and 2x High RPM hybridization buffer (Agilent) and hybridised to OGT's microarray slides.

After hybridization, the slides were washed and scanned with the InnoScan 710 microarray scanner. Innopsys has developed an auto-setting function dedicated to oligo slides, either 8x60k or 4x180k. Using these slide configurations, all the scan parameters are automatically adapted to slide format to get optimal signals in compliance with OGT QC metrics. The Oligo 8x60k and Oligo 4x180 scan configurations are available from *Mapix version 7.4.0*.

Following scanning, the image was feature extracted using the Innopsys Mapix software. This was carried out by importing the GAL file supplied with the CytoSure array and feature extracting all the arrays on the slide simultaneously. The resulting .gpr files generated were then imported into CytoSure Interpret Software. Normalisation was carried out in the software using the Segmentation-based normalisation. The process is summarised in **Figure 1**.

Results

The CytoSure ISCA v2 8x60k arrays are used for cytogenetics research. They contain probes spaced throughout the genome and also some extra probes in areas of the genome recommended by the International Standards for Cytogenomic Arrays (ISCA) consortium (now called ClinGen). A CytoSure ISCA v2 8x60k slide was hybridised with

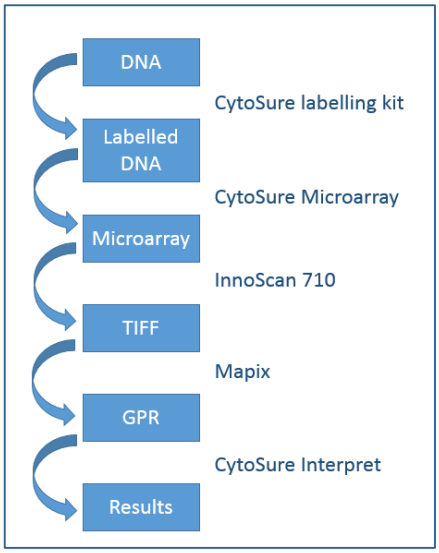

Figure 1. Summary of the process used in this study

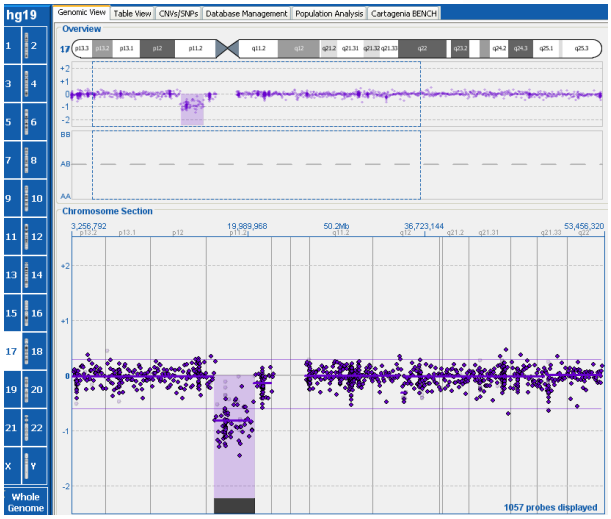

Figure 2: Detection of a 3.6Mb deletion on chromosome 17 in sample NA18324

DNA samples with a wide range of sized chromosomal gains and losses, ranging from large, such as NA00072 with a ~25Mb deletion, to small intragenic deletions. The results are shown in the Table below and in **Figure 2**.

The quality metrics produced were good, with Derivative Log Ratios Spread (DLRs) being below 0.2 except for the NA04517 sample (**Table 1**). All the expected aberrations were detected except for NA04517 in which there are few probes for the GALC gene on the v2 array and the DLR was 0.22 potentially interfering in the detection of this very small aberration.

CytoSure 4x180k ISCAv2 array

The CytoSure 4x180k ISCA v2 arrays are again arrays that are designed for cytogenetic research based on recommendations provided by ISCA. They have a larger probe set with approximately 180k probes. The performance of the InnoScan710 when scanning CytoSure ISCA v2 4x180k slide was assessed. All arrays gave good or excellent DLRs (**Table 2**).

	Sample	DLRs	gSig	rSig
B1	Male/Female	0.21	1727	1371
B2	Male/Female	0.18	1555	1638
B3	Male/Female	0.20	2166	1924
B4	Male/Female	0.23	1698	1555

Table 2: CytoSure 4x180k ISCA v2 results, where the arrays have been scanned with an Innoscan scanner and feature extracted using Mapix.

8x60k CytoSure Embryo Screen array

The 8x60k CytoSure Embryo Screen arrays are optimised for analysing DNA that has been amplified by the Rubicon PicoPLEX kit. This enables chromosomal aberrations to be identified in DNA

	Sample	Aberration	DLRs	gSig	rSig	Detection
B1 (2_4)	NA22624	arr 11p12p11.2(40433344-46031324)x1	0.15	3099	2292	Yes
B2 (1_4)	NA18324	arr 17p11.2(16697859-20294610)x1	0.16	2472	3300	Yes
B3 (2_3)	NA13434	Exon 3-4 *PLP1*	0.17	2576	3194	Yes
B4 (1_3)	NA14117	arr 5p15.33p14.3(68519-22367289)x1	0.17	2372	3093	Yes
B5 (2_2)	None	N/A	N/A	N/A	N/A	N/A
B6 (1_2)	NA06937	10pter>10p1	0.17	3057	2833	Yes
B7 (2_1)	NA04517	*GALC* deletion	0.22	3110	2585	No
B8 (1_1)	NA00072	arr 4p16.3p15.2(1618261-25512740)x1	0.16	2744	3253	Yes

Table 1: Results from the CytoSure ISCA v2 8x60k arrays

extracted from a single cell. In this experiment 50pg of DNA was amplified using the PicoPLEX kit. The amplified DNA was then labelled and hybridised as described in the CytoSure Embryo Screen protocol. The slide was scanned using the Innopsys scanner, feature extracted using Mapix and imported into CytoSure Interpret Software.

Analysis was carried out using the PGS interpretation module and the results shown in **Table 3** and **Figure 3**. All arrays gave good signal to noise ratios (SNR). The DLRs were well below 1.0 which is classed as 'excellent' for PicoPLEX amplified material and is sufficient to detect aberrations of 15Mb and above.

	Sample	Aberration	DLRs	gSig	rSig	Detection
B1 (2_4)	Male/Female		0.60	4312	5487	Yes
B3 (2_3)	NA14117	arr 5p15.33p14.3(68519-22367289)x1	0.63	4555	3828	Yes
B5 (2_2)	NA00072	arr 4p16.3p15.2(1618261-25512740)x1	0.64	3716	5740	Yes (manual)
B7 (2_1)	NA01921	arr 21q11.2q22.3(13583116-46921373)x3	0.73	4508	4463	Yes

Table 3: The embryo screen results.

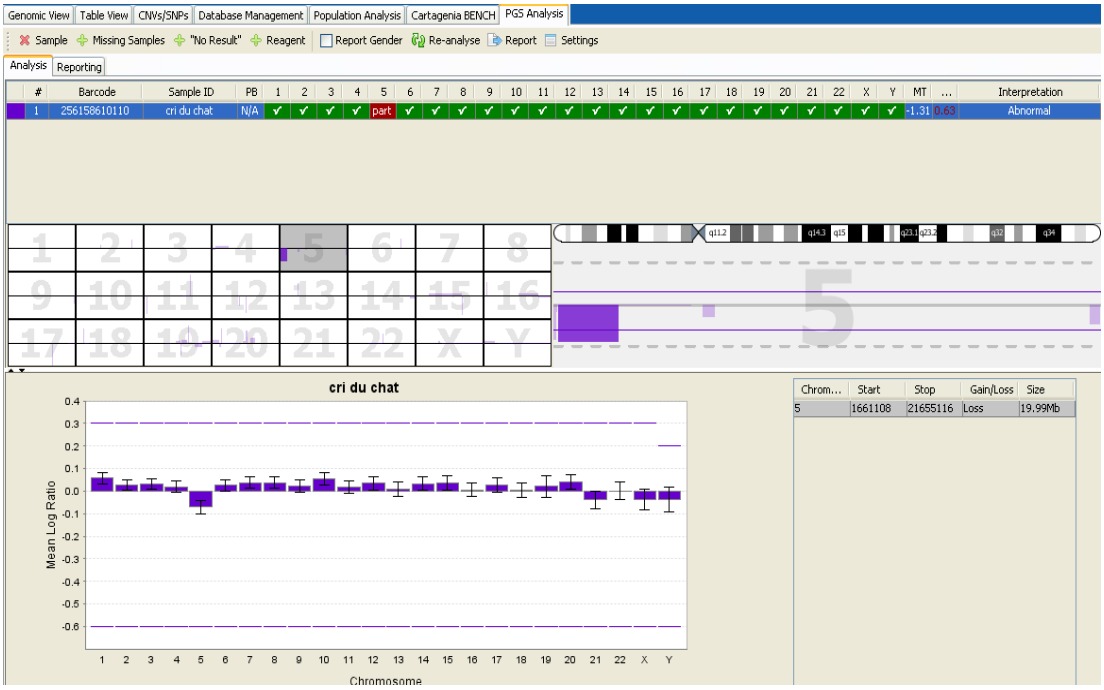

Figure 3: Detection of a 15Mb deletion on chromosome 5 in sample NA14117, using a CytoSure Embryo Screen array.

1x1M CytoSure Medical Research Exome array

The CytoSure Medical Research Exome array is a 1x 1 million spot array which contains probes targeted to exons of medically-relevant genes of interest. NA13434 DNA was labelled and hybridised to the array using the standard protocol and scanned using the Innopsys scanner. Results are shown in **Table 4** and **Figure 4**.

Conclusions

The InnoScan 710 scanner can be used effectively to scan OGT's CytoSure slides to detect chromosomal copy number variation. The Mapix software feature extracts the scanned image and can be coupled with the CytoSure Interpret Software to analyse the results in a fast efficient process. It is recommended to use either, the oligo 8x60k or oligo 4x180k dedicated scanner auto-settings adapted to CytoSure 8x60k and CytoSure 4x180k slides, respectivelly.

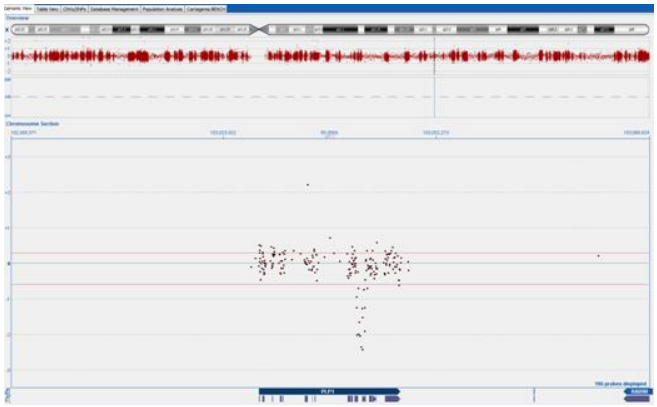

Figure 4: Detection of an aberration within the PLP1 gene using the 1x1M CytoSure Medical Research Exome array.

	Sample	Aberration	DLRs	gSig	rSig	Detection
B1	NA13434	*PLP1* deletion in exon 3 and 4	0.225	1785	1006	Yes

Table 4. CytoSure Medical Research Exome array results

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