Analysis of the compatibility of the InnoScan® 910 scanner with Agilent SurePrint G3 CGH arrays

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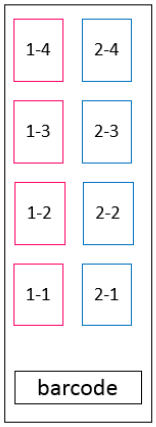
Introduction

Array CGH analysis to determine chromosomal Copy Number Variation (CNV) is becoming a routine laboratory technique on cytogenetics research and diagnostics. Rapid and accurate analysis of patient samples is essential for identification of genome instability. At the Toulouse Purpan Hospital, the cytogenetics research lab has tested the use of Innopsys' InnoScan 910 scanner with Agilent SurePring G3 Human CGH microarray. Agilent high density (HD) oligo slides contain features arranged onto a hexagonal grid, the features are sized of 30µm with a nominal offset of the even rows of 31.75µm. This configurations needs for high resolution scanning in order to assure the adequate fluorescence signal detection.

The InnoScan 910 scanner is a high resolution, low noise and autofocusing microarray scanner dedicated to HD slides. Its optical system allows for simultaneous detection of 532 nm and 635 nm signals at a resolution up to 1µm/pixel. In addition of its high resolution and sensitivity, the InnoScan 910 scanner provides advantages in terms of speed and ease of use. Innopsys has developed a specific scan configuration for HD oligo slides, either in 8x60K or 4x180K format.

To validate the use of the InnoScan 910 scanner with Agilent 8x60K arrays, the service for medical genetics at Toulouse Purpan Hospital tested 4 male and 4 female samples with known chromosome abnormalities. Both, quality control and the detection of expected abnormalities were considered as critical points for the validation process.



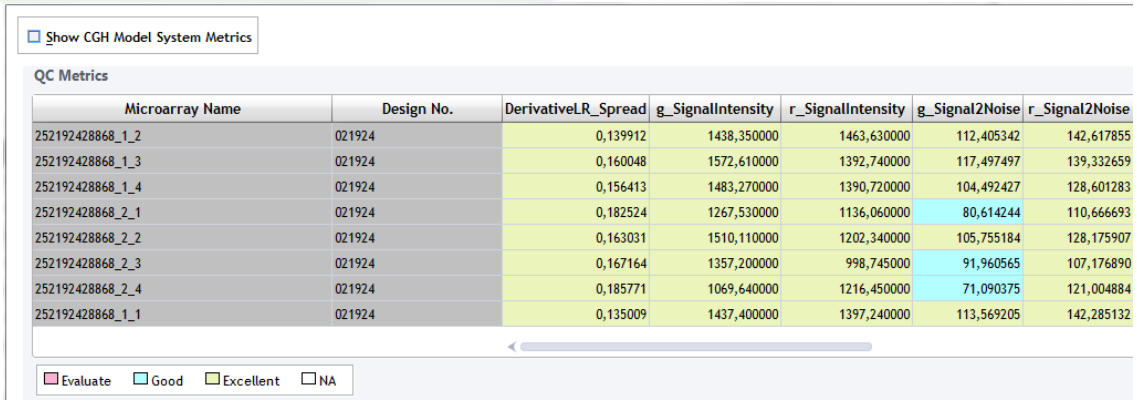
Block number	Test (Cy3 labeling)	Control (Cy5 labeling)
1-1	Female 1	Female 2
1-2	Female 2	Female 3
1-3	Female 3	Female 4
1-4	Female 4	Female 1
2-1	Male 1	Male 2
2-2	Male 2	Male 3
2-3	Male 3	Male 4
2-4	Male 4	Male 1

Figure 1.Slide layout and Dye-swap design

Material and Methods

Genomic DNA was isolated from blood samples following the recommended operational procedures of the vendor. In order to diminish dye incorporation and detection biases, purified DNA samples were combined in a dye-swap design, in which samples are labeled with both Cy5 and Cy3 dyes. Equivalent quantities of Cy3-labelled and Cy5-labelled independent samples were mixed in the hybridization reaction (Figure 1). Hybridization mixtures were then incubated with Agilent 8x60K oligo arrays according to manufacturer instructions (version 7.4, August 2015).

After hybridization and washing steps, slides were immediately scanned in an Innopsys InnoScan 910 microarray scanner. Innopsys has developed an automating settings mode dedicated to high density oligo arrays. Using the Oligo 8x60K slide configuration in an auto-



Microarray Name	Design No.	DerivativeLR_Spread	g_SignalIntensity	r_SignalIntensity	g_Signal2Noise	r_Signal2Noise
252192428868_1_2	021924	0,139912	1438,350000	1463,630000	112,405342	142,617855
252192428868_1_3	021924	0,160048	1572,610000	1392,740000	117,497497	139,332659
252192428868_1_4	021924	0,156413	1483,270000	1390,720000	104,492427	128,601283
252192428868_2_1	021924	0,182524	1267,530000	1136,060000	80,614244	110,666693
252192428868_2_2	021924	0,163031	1510,110000	1202,340000	105,755184	128,175907
252192428868_2_3	021924	0,167164	1357,200000	998,745000	91,960565	107,176890
252192428868_2_4	021924	0,185771	1069,640000	1216,450000	71,090375	121,004884
252192428868_1_1	021924	0,135009	1437,400000	1397,240000	113,569205	142,285132

Figure 2. Cytogenomics QC criteria screenshot. In green, those parameters considered as "Excellent, on blue, those considered as "Good".

settings mode, the scan is automatically done under the appropriate scanner parameters in order to get optimal signals to be compliant with Agilent's QC metrics. The 16-bit images were imported and analyzed in Agilent Cytogenomics© software (v2.9.2.4).

Due to the dye-swap design, for each sample only those events present in both Cy3- and Cy5-label results were considered as real chromosomal CNVs.

Results and discussion

QC metrics

All of the QC metrics were within the "Excellent" or "Good" thresholds of the Agilent Cytogenomics QC criteria (Figure 2). The minimum and maximum DLR values were 0.135 and 0.185, respectively. These results prove the compatibility of the InnoScan 910 scanner with Agilent Cytogenomics software.

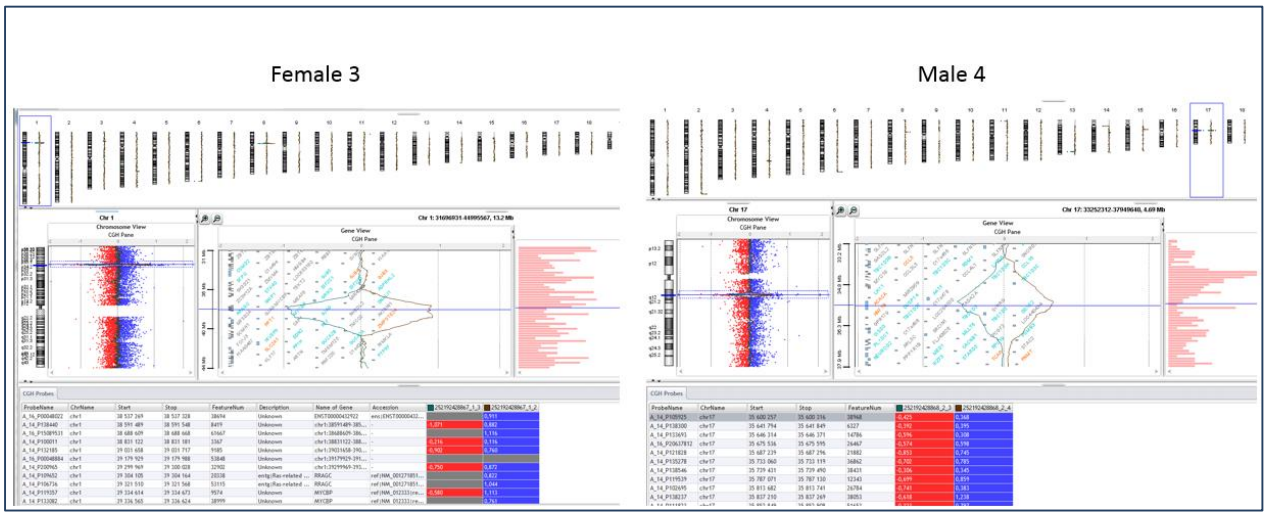


Figure 3. Dye-swap analysis. Representative data analysis on CytoGenomics software is shown for the *Female 3* and *Male 4* patients. Only those opposite events in a multi-sample analysis are considered as significant.

The results show also the compliance of the image signals obtained by using the Oligo 8x60K scan configuration with the recommended metrics needed to assure high quality results.

Dye-swap analysis and data validation

Due to their chemical proprieties Cy3 and Cy5 do not have the same incorporation rate nor the same fluorescence activity. Even when normalization procedures can diminish these biases, to assure the reliability of the results, one can chose for the dye-swap experiment design.

In a dye-swap design the purified DNA is divided into two samples. Each of one is labelled with a different dye, either Cy3 or Cy5. The Cy3- and Cy5-labelled samples are combined with independent Cy5- and Cy3-labelled samples, respectively. At the end, the sample is hybridized in two arrays of the slide (Figure 1). To analyze the data, the two arrays corresponding to a patient are confronted in a multi-sample analysis. Only those opposite events (deletion and duplication) are considered as significant (Figure 3).

Final results were compared with validated chromosomal modifications for each sample (Table 1). All the expected modifications were found, some new modifications appeared, but these are certainly due to the genomic design of the slide. All together the results were proved compatibility of the Innopsys scanner to analyze Agilent oligo G3 CGH arrays.

Conclusion

Array CGH analysis to determine Copy Number Variation is becoming a routine laboratory technique to better define breakpoints of cytogenetic rearrangements, and to identify or confirm aberration. The current analyze proved for the compatibility of the Innopsys InnoScan 910 scanner in scanning Agilent Surprint G3 CGH arrays. Using the Oligo 8x60K slide configuration the scan of high density arrays became automatized and easy to use.

Table 1. Result validation

Sample	Expected modifications	Found modifications
Female 1	del 4q35.2 (850 kb)	del 4q35.2 (624-707 kb) dup 15q11.1-q11.2 (2 Mb) dup 17q21.3 (134 kb)
Female 2	del 13q12.12 (473 kb)	del 13q12.12 (421 kb)
Female 3	dup 22q11.21q11.23 (2.5 Mb)	dup 22q11.21-q11.23 (2.51 Mb)
Female 4	dup 1q21.21- 1q21.2 (5.5Mb) dup 21q21.1 (314 kb)	dup 1q21.1 – q21.2 (4.29 Mb) del 15q11.1 –q11.2 (2Mb) dup 20p12.3 (210 kb) dup 21q21.1 (375-439kb)
Male 1	del 15q13.3 (663 kb)	del 15q13.3 (538 kb) del 8p11.22 (127-183 kb) dup 11p15.4 (114-145 kb)
Male 2	dup 15q11.2 15q13.3 (10.2Mb)	dup 15q11.1 – q13.3 (12.2 Mb)
Male 3	del 13q33.1q33.2 (1.58 Mb)	del 13.33.1 – q33.2 (1.4 Mb)
Male 4	dup 17q12 (1.3 – 1.6 Mb)	dup 17q13 (1.4 Mb)

Disclamers

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Acknowledges

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