

# Detection of Mosaicism by Array CGH

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## Introduction

- Reports from the literature suggest that array CGH is a technique which allows the detection of low-level mosaicism in a more sensitive manner than other techniques including traditional cytogenetic analysis (1-3). It is also suggested that aCGH, which utilises genomic DNA from uncultured peripheral blood, can more accurately define the true level of mosaicism in peripheral blood samples than traditional techniques which require the selective stimulation of cells (1-3).
- Some examples of low-level mosaicism detected by aCGH have been reported. Menten *et al* (2006) reported the detection of monosomy 7 at 8% (1), Cheung *et al* (2007) detected a case of trisomy 8 at 7% (2), whilst Ballif *et al* (2006) reported the detection of trisomy 9 at levels as low as 3% (3). However, the limit at which low-level mosaicism can be detected by aCGH has not been accurately defined, and indeed may be platform and/or laboratory specific. Furthermore, European best practise guidelines have recommended that individual laboratories establish the levels of mosaicism detectable 'in-house' (4).
- This study aimed to determine the level of mosaicism detectable by the Newcastle Laboratory, using the CytoChip ISCA 4x44K (v 2.0) oligonucleotide array. 'Artificial' mosaics were created using patients with a number of previously characterised abnormalities of varying size. These included segmental deletions and duplications as well as whole chromosome aneuploidy.

## Results

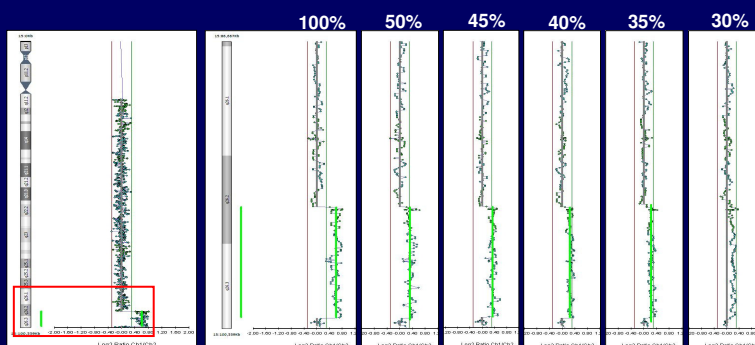
**A**

	chromosome	size	features	LEVEL OF MOSAICISM (%)									
				100	50	45	40	35	30	25	20	10	
<b>LOSS (MONOSOMY)</b>	X	155Mb	2694				✓			[✓]	[✓]	×	
<b>LOSS</b>	3p	7.6 Mb	116	✓	✓	✓	✓	✓	✓	[✓]	×		
<b>LOSS</b>	18q	1.9 Mb	25	✓	✓	✓	✓	✓	[✓]	×	×		
<b>LOSS</b>	2q	100 Kb	24	✓	✓	✓	✓	✓	✓	✓	×		×
<b>LOSS</b>	16p	860 Kb	10	✓	✓	✓	✓	×	×	×	×		
<b>LOSS</b>	3p	270 Kb	4	✓	×	×	×	×	×	×	×		
<b>GAIN (TRISOMY)</b>	10	135Mb	1889				✓			[✓]	[✓]	×	
<b>GAIN</b>	15q	5.4 Mb	114	✓	✓	✓	✓	✓	✓	×	×		×
<b>GAIN</b>	2q	850 Kb	14	✓	✓	×	×	×	×	×	×		×
<b>GAIN</b>	15q	215 Kb	13	✓	✓	✓	×	×	×				
<b>GAIN</b>	15q	330 Kb	7	✓	×	×	×	×	×				

(A) Table showing the level of mosaicism (%) detected by aCGH for a range of losses and gains. ✓ indicates 'called' copy number change and × indicates abnormalities not 'called'. [✓] indicates incomplete 'calls' for example where an abnormality has only been partially detected. Some levels of mosaicism were not investigated.

(B) Oligonucleotide aCGH analysis of chromosome 15 from a patient showing a 5.4 Mb, 114 feature copy number gain. Red box indicates the duplicated region magnified for 100, 50, 45, 40, 35 and 30 percent mosaicism. Green line indicates copy number gain 'called' by the analysis software.

**B**



## Methods

- Four patients with confirmed multiple abnormalities were used in this study.
- 'Artificial' mosaics were created by diluting DNA from these patients with sex-matched normal reference DNA (Promega).
- Oligonucleotide aCGH was performed using the whole-genome CytoChip ISCA 4x44K (v2.0) (BlueGnome, Cambridge, UK) microarray. This platform provides both genome-wide and targeted coverage.
- Data was analysed using BlueFuse Multi 2.3 analysis software (BlueGnome, Cambridge, UK). Mean DLRS (derivative log ratio spread) : 0.13 (range 0.11-0.15)

## References

- (1) Menten B *et al* (2006) J Med Genet 625-633
- (2) Cheung SW *et al* (2007) Am J Med Genet Part A 143A: 1679-1686
- (3) Ballif BC *et al* (2006) Am J Med Genet Part A 140A: 2757-2767
- (4) Vermeesch JR *et al* (2007) Eur J Hum Genet 15: 1105-1114

## Conclusion

- These results demonstrate an inverse correlation between the levels of mosaicism that can be detected by aCGH and the number of features within the area of imbalance. That is, regions of copy number change containing fewer features are more difficult to detect at lower levels than those with more features. Therefore, as the number of features increases, the sensitivity of the technique to detect low-level mosaicism also increases.
- There are two results within this data set, which do not fit this trend (18q loss/30%, 2q gain/45%). These results can be explained by differences in the DLRS emphasising that the quality of the experiment and levels of 'noise' are extremely important in determining the levels of mosaicism detectable by aCGH (data not shown).
- The lowest level of mosaic whole chromosome aneuploidy was detected at 25%.
- The lowest level mosaic segmental copy number change was detected at 30%.
- Partial detections are useful as they highlight areas of imbalance which, upon further inspection of the flanking areas should reveal the true extent of the copy number change.
- In cases of known or suspected abnormalities, the level of mosaicism detected may be much lower because abnormalities which are not automatically called by the software, may be investigated manually for regions of copy number change.