APPLICATION NOTE

KaryoLite® - A rapid single cell screening assay to simultaneously detect aneuploidies for all chromosomes

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Aneuploidy Detection In Preimplantation Genetic Screening (PGS) Research

Introduction

The number of women utilizing assisted reproductive technologies has steadily increased over the past 10 years. In order to improve the outcome of pregnancies, Preimplantation Genetic Screening (PGS) has been suggested to identify embryos prior to implantation that appear to have the greatest chances for a successful pregnancy. PGS is typically performed on blastomeres, blastocysts or polar bodies, and offers information on the aneuploidy state of embryonic DNA. As PGS is performed on a small number of cells, or even a single cell, whole genome DNA amplification (WGA) is required prior to screening for chromosomal aneuploidies. Methods commonly used to identify these chromosomal abnormalities include array (aCGH) and FISH analysis. Both technologies however are hampered by cost, throughput, or in case of FISH, the limitation of screening several regions of the genome simultaneously.

Here we describe a workflow that combines whole genome amplification with a cost-effective high-throughput method using KaryoLite BoBs™ for aneuploidy detection in blastomere research samples.

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DNA Amplification using PicoPLEX™ WGA and KaryoLite BoBs™ Analysis

Reliable DNA amplification is crucial for the accurate detection of chromosomal aneuploidies when starting with low amounts of DNA. For this study we used the PicoPLEX WGA (whole genome amplification kit) as it yields a reproducible representation of all sequences with low allele drop out, and can be used on blastomere cells, blastocyst cells and polar bodies.

In this study, 117 DNA samples derived from single cell blastomeres at day 3 (Repromeda, Brno, Czech Republic) were amplified by use of the PicoPLEX WGA kit. Cells were generally processed by WGA within 2 hours of extraction. In case of a delay cells were placed at -80°C prior to further processing. WGA steps were performed according to the manufacturer's recommendations. WGA samples were then stored in 15µl final buffer at -80°C until use.

To then assess the aneuploidy status of each sample, 240ng of amplified and purified DNA was fluorescently labeled and analyzed using KaryoLite BoBs (BACs-on-beads). KaryoLite BoBs utilizes a new concept of composite beads having DNA from three different BAC clones coupled on each bead type. The composite clone format expands the region of chromosomal DNA interrogated by each bead. The assay is fluorescence based and uses encoded multiplex beads which have been coupled to BAC derived DNA from defined loci on all 24 chromosomes (Figure 1). This allows detection of aneuploidies on all chromosomes in a single well of a 96-well microtiter plate with results in as little as 16 hours.

KaryoLite BoBs assays were performed using either the standard protocol with a 16h hybridization, or a modified protocol using a 4h hybridization. Assays were performed in duplicate and analyzed with beta BoBsoft® 2.0, using the ratio algorithm and computed references mode. Results were then compared with analyses previously obtained by aCGH.

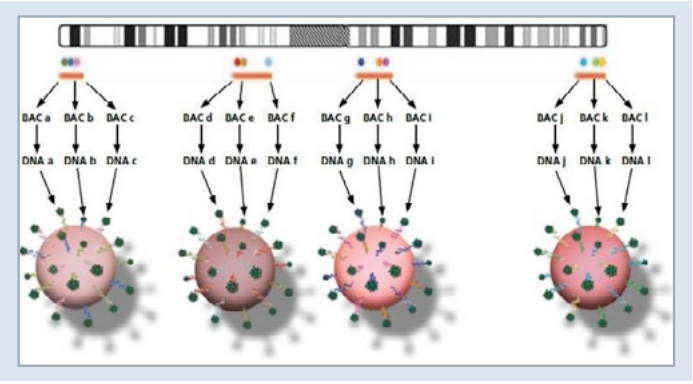

Figure 1. Diagram illustrating the coverage of a single chromosome with KaryoLite BoBs™

Table 1. Comparison of aCGH and KaryoLite BoBs results

aCGH			KaryoLite BoBs			
ID	Karyotype	Sex	ID	Karyotype	Sex	%CV
A1	-16	XX	A1	-16, +12	XX	9.62
2	N	XY	2	-2	XY	13.7
3	N	XX	3	N	XX	6.36
4	N	XX	4	N	XX	7.4
5	-18	XY	5	-18 -19	XY	5.48
6	N	XY	6	N	XY	5.83
7	N	XX	7	N	XX	5.12
8	-7	XX	8	-7	XX	7.43
B1	N	XY	B1	N	XY	8.36
2	N	XX	2	-15 +16	XX	7.21
3	N	XX	3	+16p	XX	7.84
4	N	XX	4	N	XX	7.56
5	-9q	XX	5	-9 -22	XX	7.76
6	-14, +15	XY	6	-14 +15	XY	8.5
7	+17pterq21.1, -17q21.1qter	XX	7	-1p	XX	8.39
8	N	XY	8	N	XY	6.63
9	N	XX	9	N	XX	7.81
10	-2q14.3qter	XX	10	-2q -6 +21	XX	10.5
C1	-5pterp15.31, -19	XX	C1	-19	XX	6.62
2	complex aneuploidy		2	-5 -11 -20 -21		13.9
3	-8p	XX	3	-8p -21	XX	7.18
4	-5q12.3.qter, +16	XX	4	+16 +19	XX	8.7
5	-16	XY	5	-16	XY	6.32
D4	complex aneuploidy		D4	COMPLEX		41.7
5	N	XX	5	+14	XX	6.81
6	-1q21.3qter, -4	XX	6	-1 -4	XX	8.43
E1	N	XX	E1	N	XX	5.09
2	N	XX	2	N	XX	8.61
3	-6, -13	XY	3	-6 -13	XY	5.91
4	N	XX	4	+22	XX	8.24
5	complex aneuploidy		5	COMPLEX		13.9
6	13	XX	6	+9 +13 +19	XX	8.21
F1	+9pterq34.11,-9q34.11qter; -11pterq23.2;+11q23.2qter	XY	F1	+9 -11	XY	10.1
2	-Xq21.32qter,-12	--	2	-12	--	8.75
3	-18	XY	3	-18	XY	8.71
7	-19	XX	7	-2 -19	XX	6.38
8	18	XX	8	-14 +18 -19 -22	XX	6.18
9	21	XY	9	21	XY	6
G1	4	XY	G1	+4	XY	9.52
2	2	XX	2	+2	XX	7.5
4	complex aneuploidy	--	4	COMPLEX	--	20.4
5	-16	XX	5	+8 -16	XX	8.17
H1	2	XY	H1	+2	XY	6.24
3	N	XX	3	N	XX	9.01
4	complex aneuploidy		4	+8 +12*		18.4
5	+8q21.3qter	XX	5	+8 +15 -19	XX	6.07
I2	1	XY	I2	+1	XY	8.9
3	N	XX	3	N	XX	6.78
4	-9q, +20q	XX	4	-9	XX	6.07
5	-9q	XY	5	+13	XY	7.15
J2	+9, -18	XY	J2	+9 -18	XY	7.36
3	N	XX	3	+19	XX	8.14
4	+1q31.1qter, +12, -22	XX	4	+12 -22	XX	6.68
K1	-1, -2, +10, +21	XY	K1	-2 +8 +21	XY	16.9
3	+1pter p13.3, -9q	XY	3	-14	XY	8.81
4	N	XX	4	N	XX	8.12
L2	-1, -4, -5, -17	XY	L2	-1 -4 -5 -17	XY	12.5
M1	N	XX	M1	N	XX	9.42
2	-X	X	2	-19 -20 -X	X	5.86
3	N	XX	3	N	XX	8.42
4	6	XX	4	+6	XX	7.15
7	-16	XX	7	-16	XX	8.44
N1	-1, -8, -15, -17, -21, -22	XY	N1	-1 -21 -22*	XY	16
3	complex aneuploidy		3	-2 -15		16.1
4	-8p	XY	4	N	XY	7.62
6	-15	XX	6	+14 -15 -17 +19	XX	12.7
O1	-15	XY	O1	-15	XY	6.76
2	-Yq11.222qter, -16	XY	2	-16	XY	7.45
4	complex aneuploidy	XX	4	-7 -17*	XX	25.6
5	-10q23.33qter	XX	5	+10	XX	7.45
P1	-20	XX	P1	+7 -20	XX	8.31
2	N	XX	2	N	XX	9.01
3	N	XX	3	N	XX	8.22
Q4	-X	Y	Q4	-X	Y	8.76
5	N	XX	5	+19	XX	16.94
6	+1, -12	XX	6	+1 -12	XX	12
7	complex aneuploidy	XX	7	COMPLEX	XX	18.3
R1	-6, -22	XX	R1	-6 -22	XX	10.5
S1	complex aneuploidy	XY	S1	+14 +20*	XY	15.3
2	-16	XX	2	-16 -22	XX	11
3	-4p	XY	3	N	XY	10.2
T1	-1, -6, -8, -17	XX	T1	-6 -8 -17	XX	15.4
3	N	XX	3	N	XX	9.44
4	-15q26.1qter, -20, -Yq11.221qter	XY	4	-20	XY	10.5
5	-15, -21	XX	5	-15 +17 -21	XX	9.68
7	+15q26.1qter, -18	XX	7	+15 -18	XX	8.88
U1	chaotický profil	XY	U1	-5 -12 +13 -22*	XY	16
3	-9, -13	XY	3	-9 -13	XY	10.5
4	+Y	XYY	4	N (+Y)	XYY	7.5
6	16	XY	6	+16 -18	XY	9.75
V3	chaotický profil	XX	V3	+15	XX	11.4
4	-1q, +2p, -5q, -16q	XY	4	-1 -13	XY	11.1
W2	-X, +11	X	W2	+11 -X -18	X	9.95
3	N	XX	3	N	XX	6.31
4	-9, -10	XY	4	-9 -10	XY	9.91
X3	+8q12.1qter, -22	XX	X3	+8 -22	XX	10.2
5	16	XY	5	+16	XY	8.24
7	-1, -3, -22	XY	7	-1 -3 -22	XY	11
Y1	5	XX	Y1	+5	XX	9.59
2	+9p, -9q, +12, +13	XY	2	+13	XY	10.5
3	+8, +11	XY	3	+8 +11 -20	XY	11.4
Z1	-14	XX	Z1	-14	XX	7.19
6	11	XX	6	+11	XX	7.02
7	-22	XX	7	-22	XX	6.66
9	+13, +22	XY	9	+22	XY	10.1
AB2	-5	XX	AB2	-5 +7 +22	XX	6.58
3	-3	XX	3	-3 -13	XX	8.94
CD1	+2pterq32.1,-2q32.1qter	XX	CD1	+2	XX	7.6
2	+18q22.1qter	XY	2	N	XY	7.93
3	-X	X0	3	-X +19	X0	8.34
5	-1,-4	XY	5	-1 -4 +22	XY	8.11
EF1	-18	XX	EF1	-18 -20 +22	XX	7.63
2	+3, +6, -7, +8, +9, +10, +15, +16, -17, -19, +20, +21, -22	XX	2	-7 +8*	XX	17.3
3	-1, +16, +17	XX	3	-1 +16 +17	XX	8.8
GH2	+9pterq31.2, -9q31.2qter, -16	XY	GH2	+9 -16	XY	9.13
3	-15, +16	XY	3	-15 +16 +19 +22	XY	8.93
6	-15	XY	6	-15	XY	8.04
7	N	XX	7	N	XX	7.29

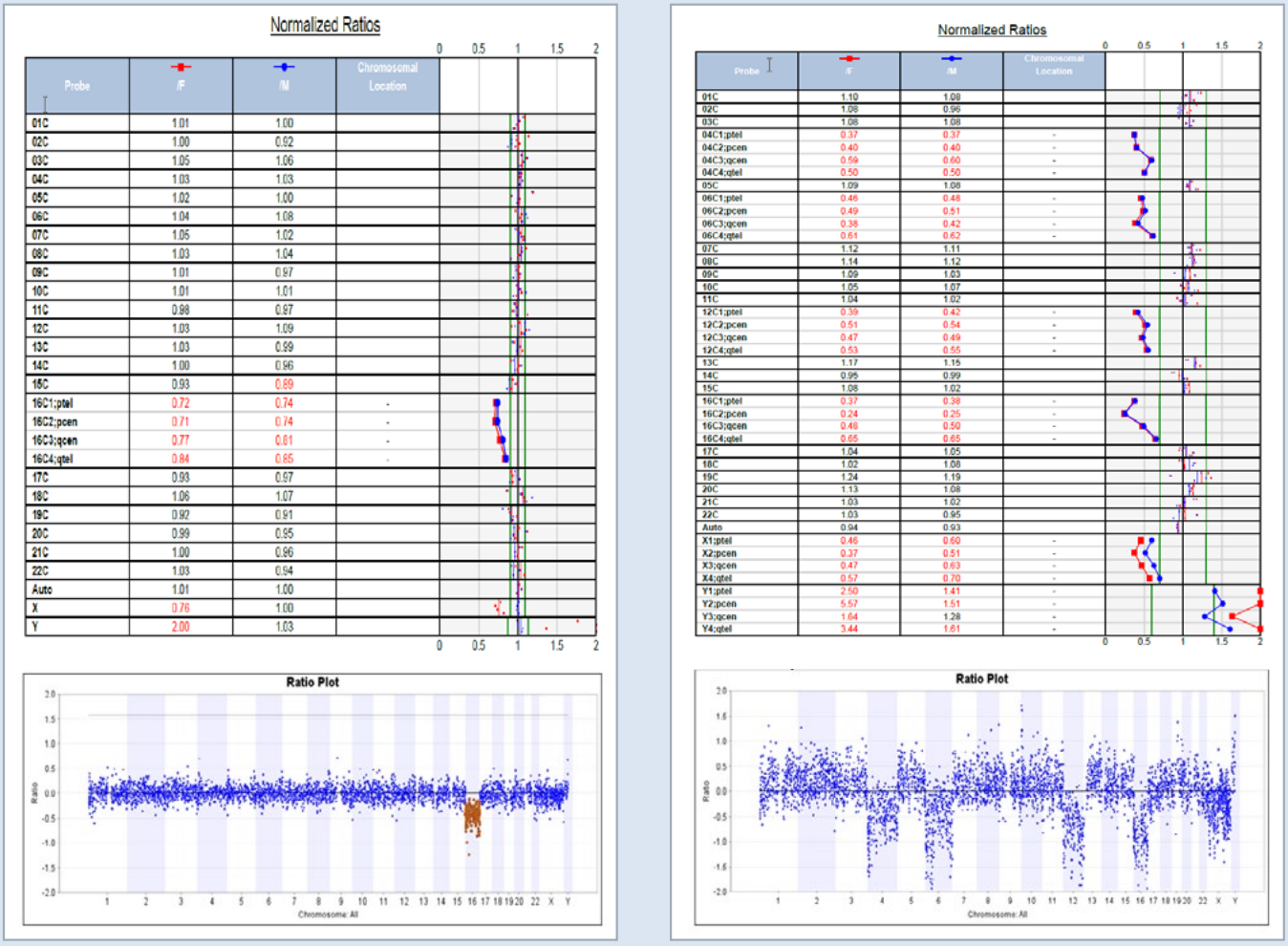

Figure 2. Examples of BoBSoft 2.0 and corresponding aCGH output files is shown for samples G4 and C5

Results

Out of the 117 samples, 16 samples were found to be normal, 87 indicated whole chromosome aneuplodies, 9 arm specific aneuploidies, and 5 sex chromosome aneuploidies. The concordance between the aCGH and KaryoLite BoBs results was found to be 94% with 9 out of the 117 samples showing deviations between the two technologies (Table 1). Examples of BoBSoft 2.0 and corresponding aCGH output files are shown in Figure 2 for samples G4 and C5. The 6% discordance found between the two technologies used in this study is mainly due to regional bias or partial chromosome aneuploidy.

Conclusion

In summary, these results show KaryoLite BoBs to be a sensitive, multiplex, high throughput and so cost-effective assay that can detect chromosomal aneuploidies from single cell blastomere samples, and demonstrate KaryoLite BoBs as a useful tool in preimplantation genetic screening research.

PRODUCT	ITEM NUMBER
KARYOLITE BOBS*	4501-0010
PICOPLEX WGA* – WHOLE GENOME AMPLIFICATION**	4504-0010
BOBSOFT V2.0 – DATA ANALYSIS SOFTWARE	5012-0202
LUMINEX 200 WITH XPONENT SOFTWARE	1014-0020

*This product is RUO – Research Use Only. Not for use in diagnostic procedures.

** PicoPLEX™ WGA Whole Genome Amplification kits are manufactured by Rubicon Genomics.

KaryoLite BoBs™ and PicoPLEX™ WGA are for Research Use Only.

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