Aneuploidy diagnosis in single cells using array CGH

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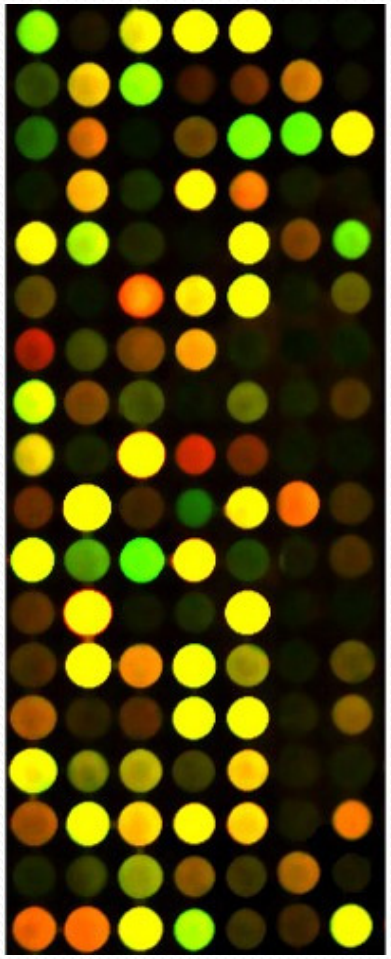
Minimum requirements of array CGH technology for PGS

- Capacity to simultaneously analyse the ploidy status of all chromosomes – 15-30% of aneuploidies are not detected using a standard 9 chromosome FISH probe panel
- Rapid so as to fit in with clinical treatment
- Accurate and reliable diagnostic outcome
- Simple to use and interpret
- Low cost so that large numbers can be processed

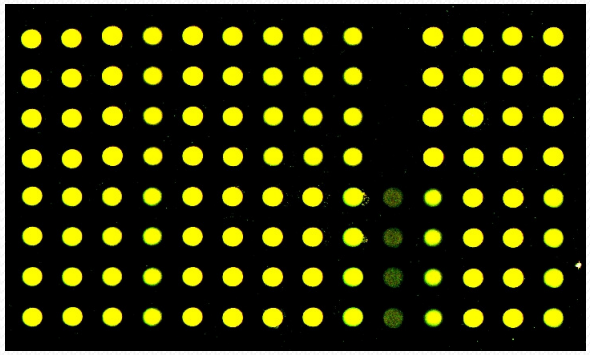
Array CGH platforms



- BAC (Bacterial Artificial Chromosome) arrays
- Oligonucleotide arrays
- SNP arrays
- Chromosome library arrays **(RHS array)**

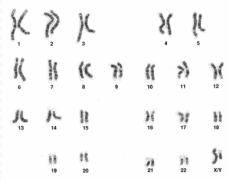
The RHS Array

- Diagnosis of every chromosome,
- on single cells,
- within 24 hours



- Contains probes for the 22 human autosomes, X, Y, blank, positive & negative controls each replicated 4 times
- Employs ‘DOP’ on ‘DOP’ hybridization

Principles of the RHS Array



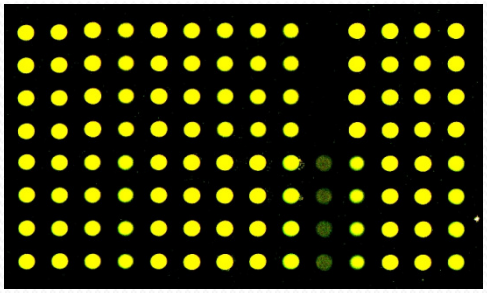
Individual microdissected human chromosomes

Unknown test cell

Euploid male reference cell

DOP-PCR amplified

Probe bound to array

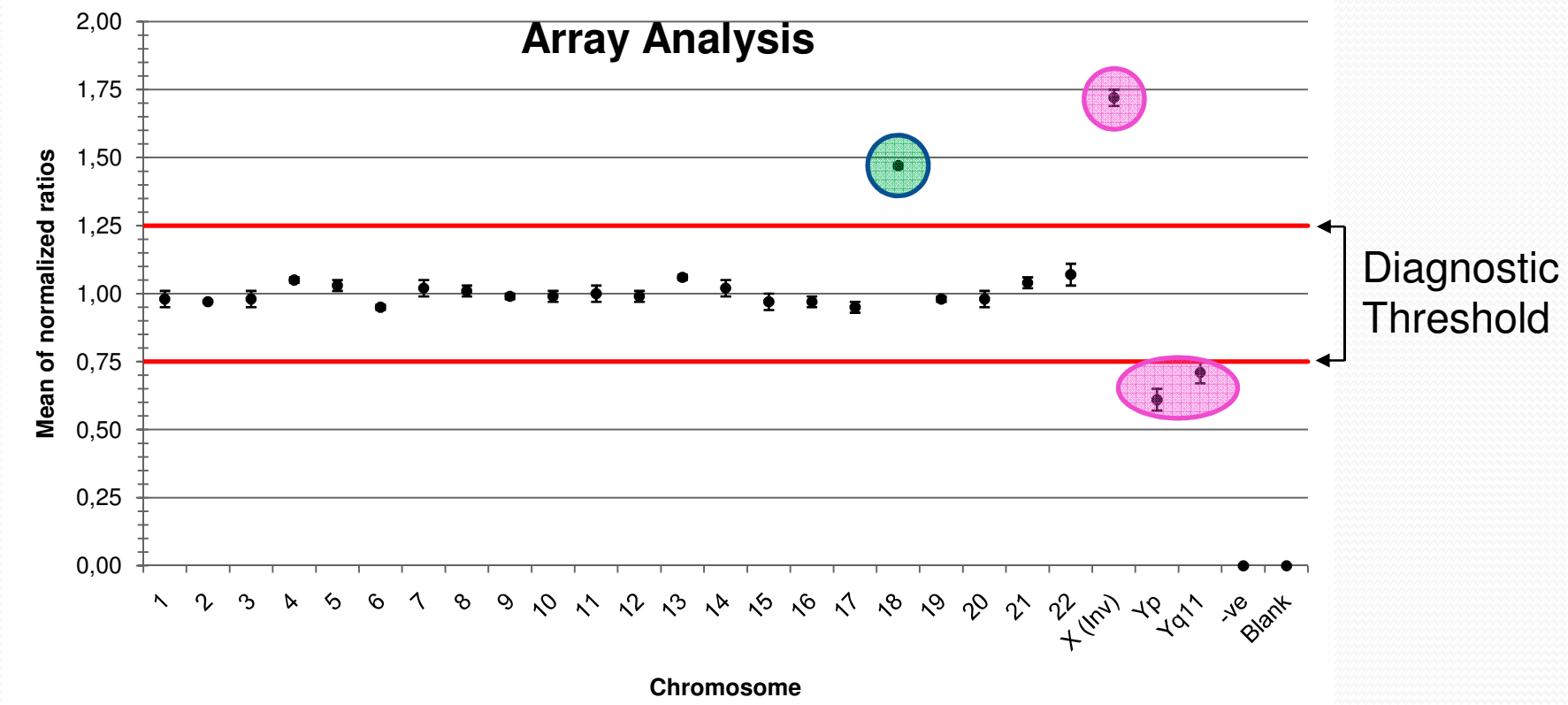


Any DOP-PCR bias
is shared by all components
of the test

Array resolution

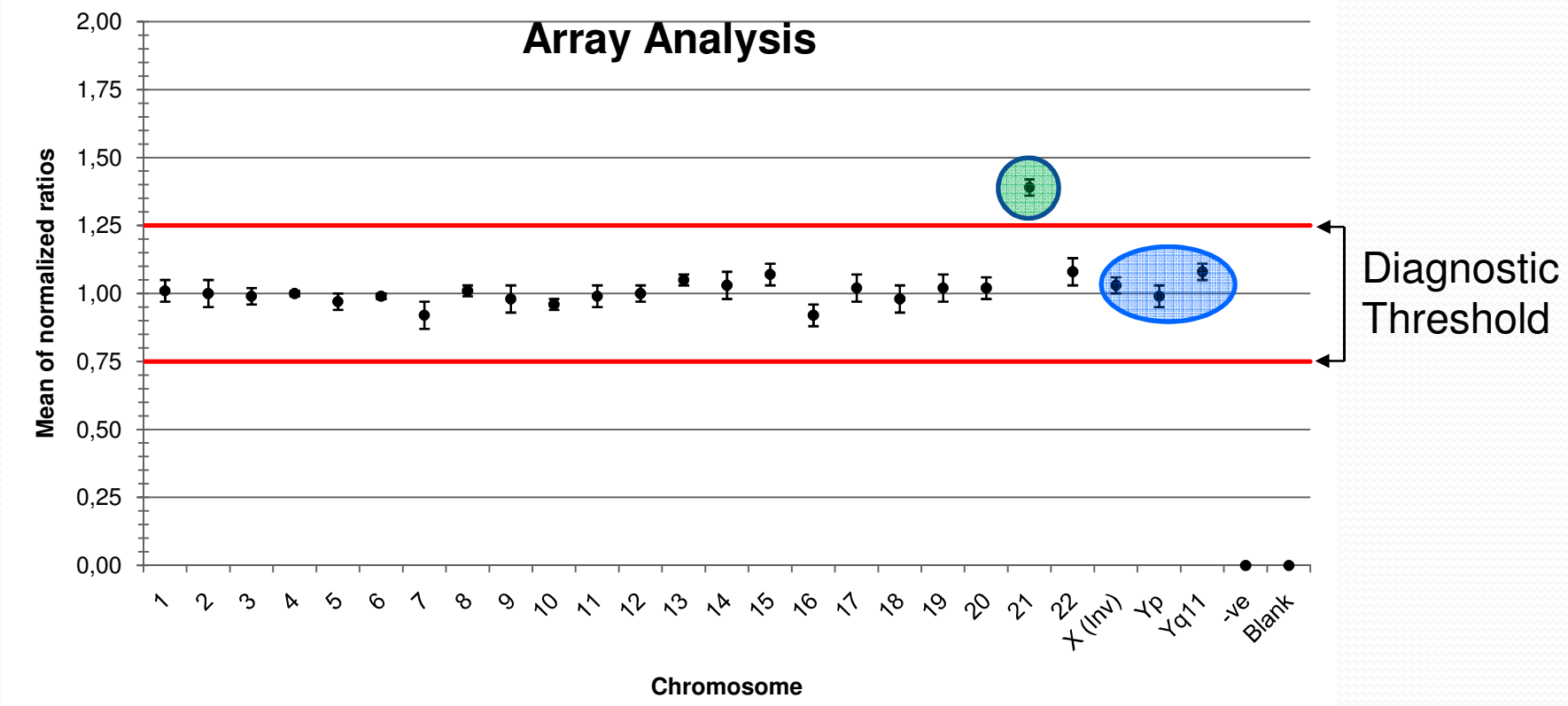
- The RHS array looks no further than aneuploidy but:
  - the principle could be applied to smaller regions, and;
  - the DOP products can be used in other tests eg diagnosis of single gene disorders as with other WGA methods

Aneuploid Diagnosis: Trisomy 18



Female Trisomy 18 single fibroblast cell (47,XX+18) versus normal male

Aneuploid Diagnosis: Trisomy 21



Male Trisomy 21 single blood lymphocyte (47,XY+21) versus normal male

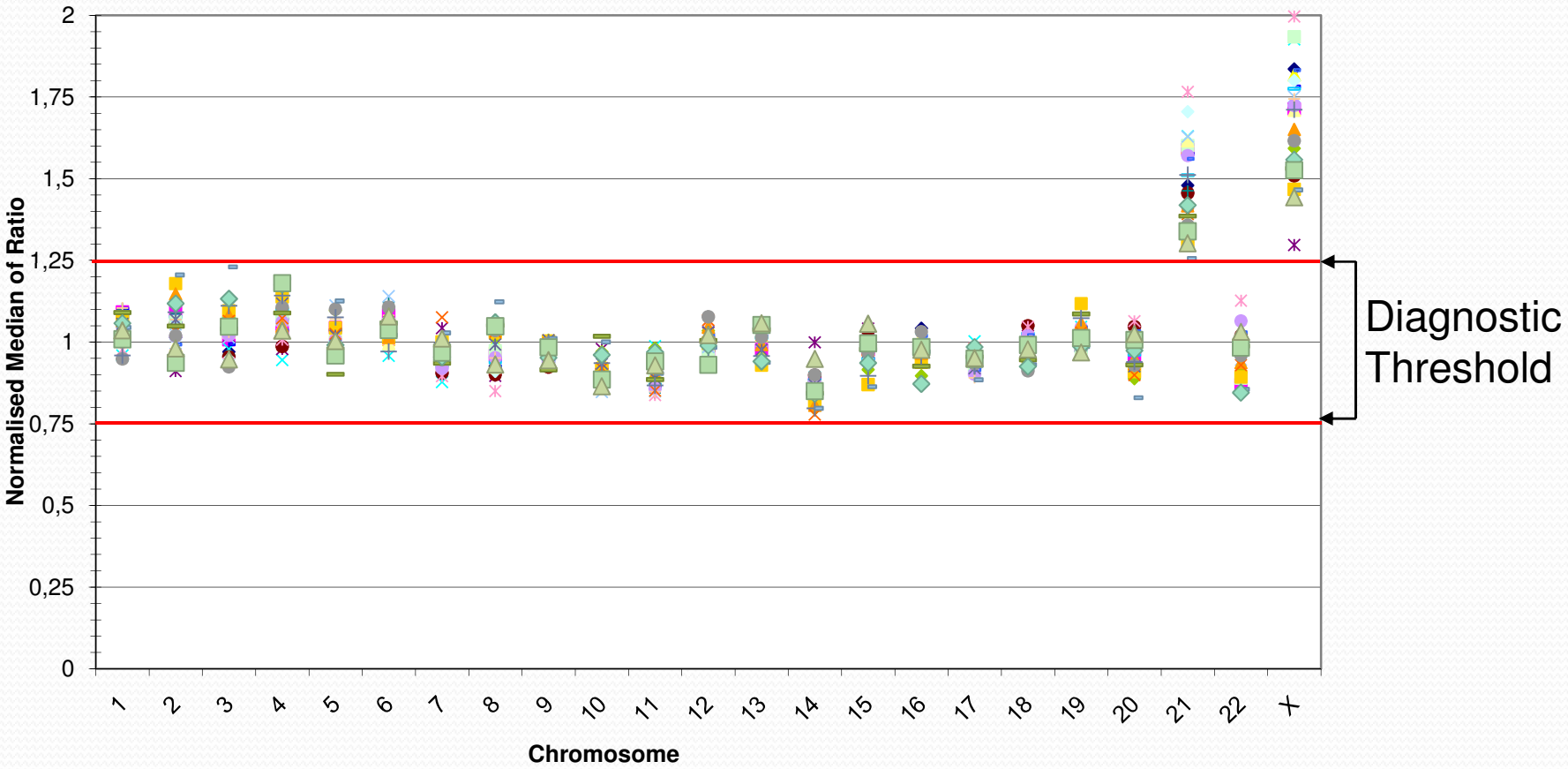
Intra and inter-assay variability

Repeat hybridisations of single cell DOP-products:

Karyotype	Number of cells	Number of correct karyotypes
46,XY	2	9/9, 7/7
47,XY,+9	1	4/5 (false negative, 9 = 1.23)
47,XY,+13	1	3/3
47,XY,+15	3	8/8, 6/6, 11/11
47,XX,+18	1	7/7
48,XY,+2,+21	1	9/9
47,XX,+21	2	5/5, 31/33*
45,X	1	9/9
47,XXY	1	6/6
47,XYY	2	2/2, 3/3
47,XXX	1	2/2

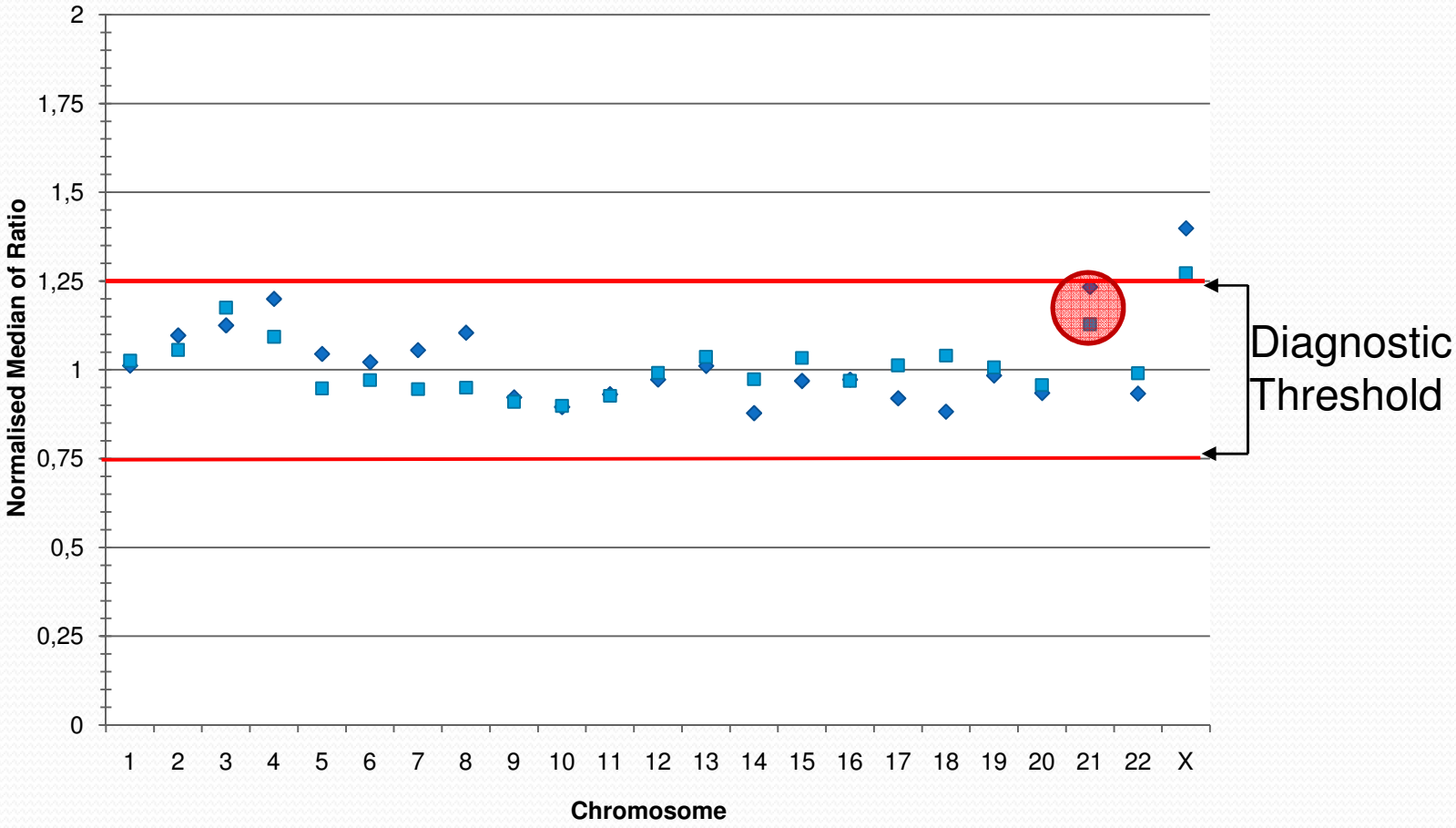
Intravariability study: 47,XX,+21

(n=31 hybridisations, 1 cell)

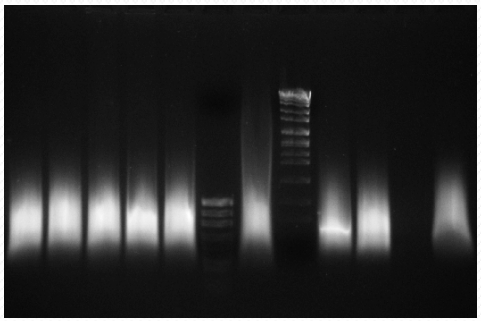


Intravariability study: 47,XX,+21

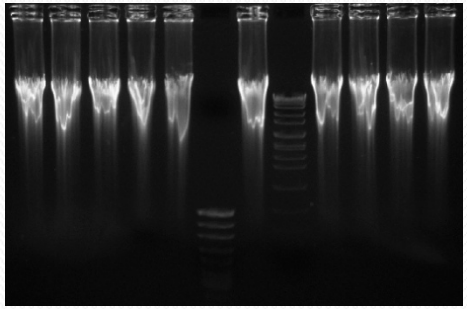
False Negatives: n=2 hybridisations, 46,XX

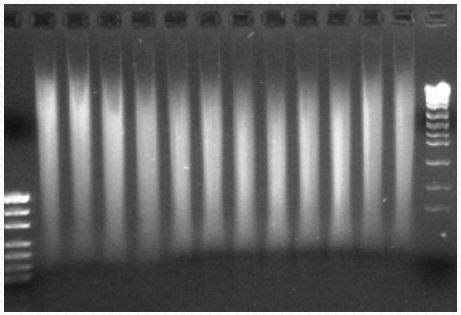


Do WGA methods differ?

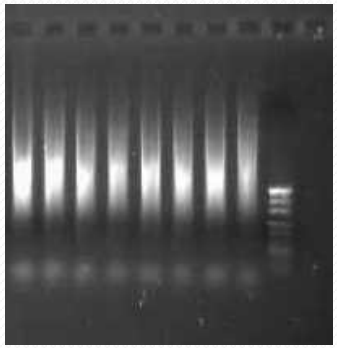


GenomePlex (Sigma)



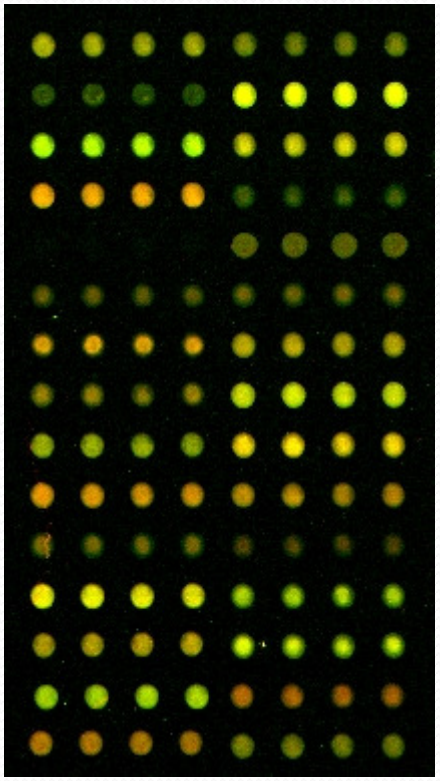


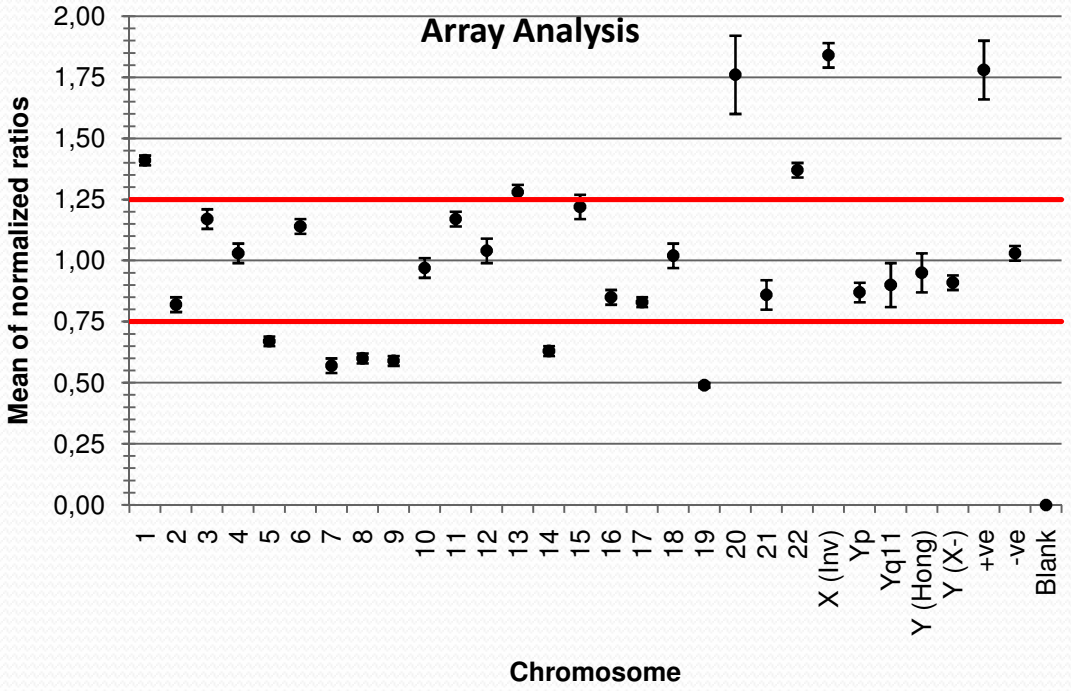
Repli-G (Qiagen)
pre-heat fragmentation post-heat fragmentation



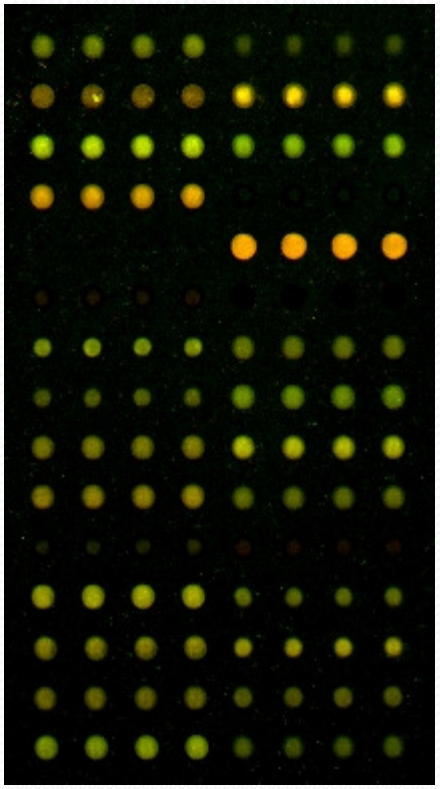
RHS DOP

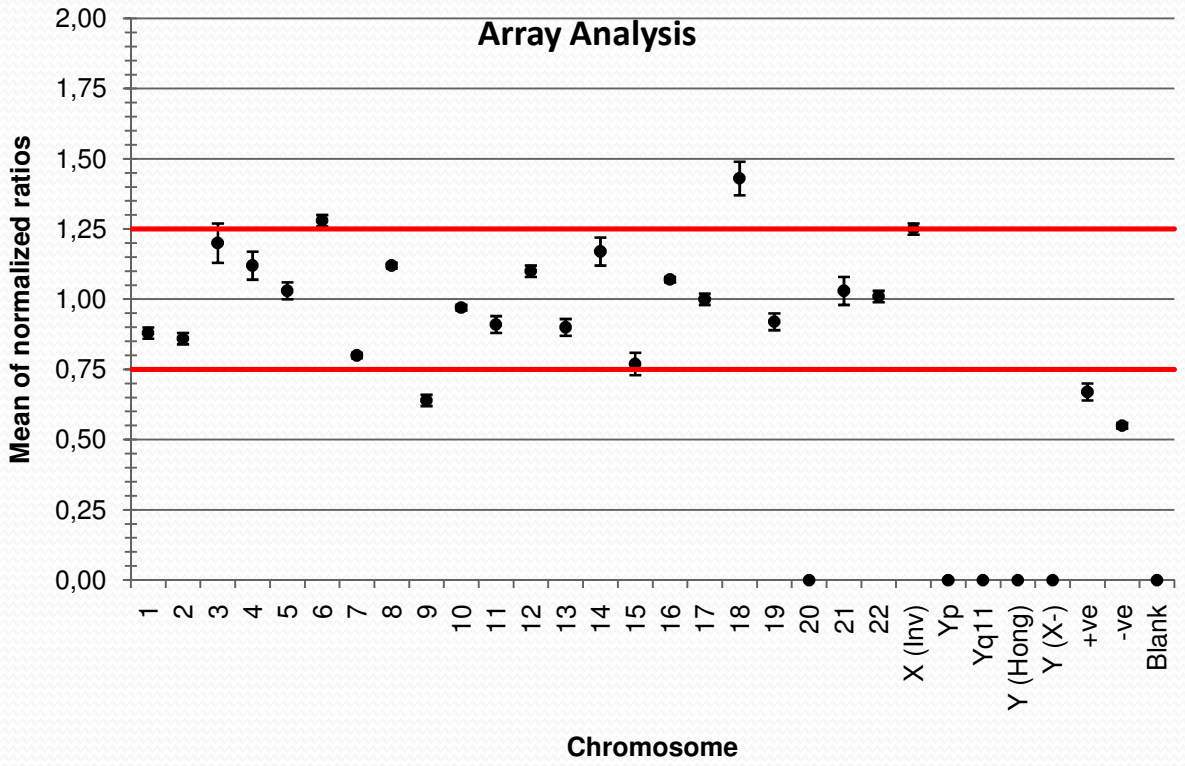
GenomePlex kit results





Repli-G kit results





PGS study

- 8 aneuploid embryos from 3 patients
- 1 biopsied blastomere diagnosed by FISH

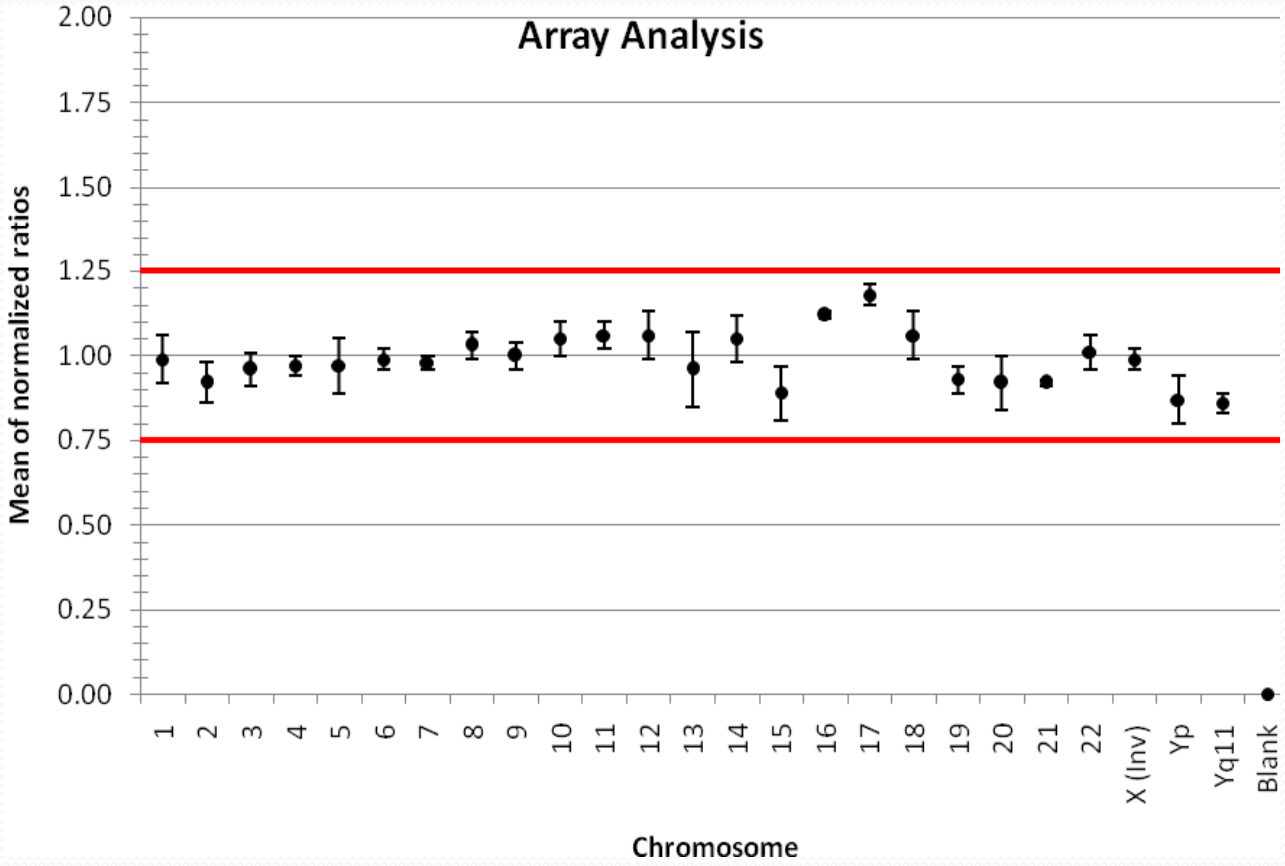
  - X, Y, 13, 18, 21 in first round

  -15, 16, 22 in second round
- Aneuploid embryos succumbed for 18-24 hours
- Zona dissolved using acid tyrodes and 26 blastomeres transferred to PCR tubes
- 18 of 26 (70%) of blastomeres produced a DOP-PCR product

Embryo 5 (7 blastomeres)

FISH cell 5a = 46,XY,+13

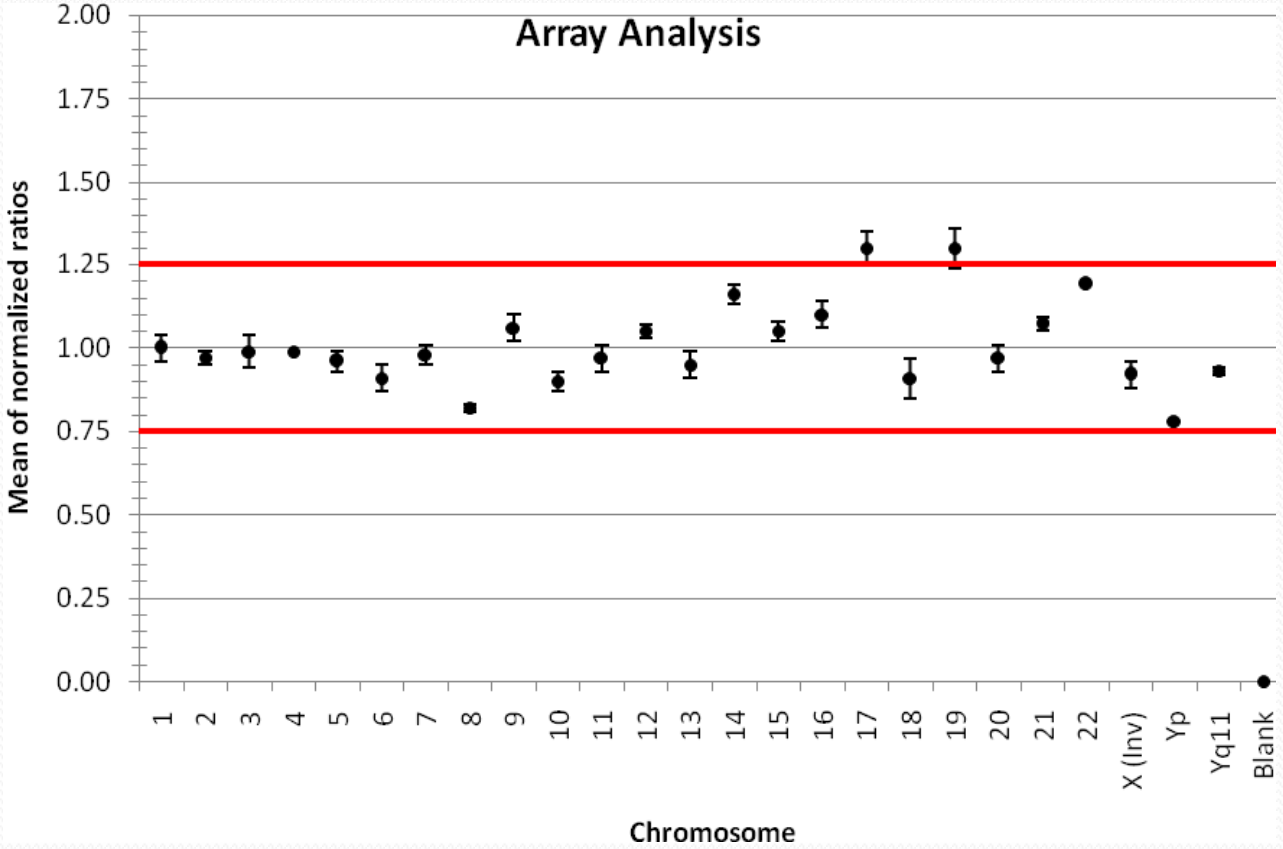
aCGH cell 5b = 46,XY



Embryo 5 (7 blastomeres)

FISH cell 5a = 46,XY,+13

aCGH cell 5c = 48,XY,+17,+19



Overview of Embryo 5

Method	Blastomere	Diagnosis
FISH	a	47,XY,+13
CGH	b	46,XY
CGH	c	48,XY,+17,+19
CGH	d	46,XY
CGH	e	48,XY,+19,+20
CGH	f	46,XY
CGH	g	46,XY
CGH	h & i	failed PCRs

Embryo results

Embryo	FISH result	aCGH results
1	45,XX,-15	45,XX,-4
2	46,XY,-15,+16	47,XY,+1 45,XY,-10,-15,+19 48,XXY,+1,-3,+4,-12,+19
3	46,XX,+16	51,XYY,+5,-6,+12,+14,+19,+22 43,XX,-1,-6,-9,+10,-11,-12,-17 1 x failed PCR
4	46,XX,+13,-18	46,XXY,+13,-14,-19 47,XXY,+13,-14,+16,-19 4 x failed PCR
6	44,XY,-21,-22	42,XY,-3,-9,-19,-22 43,XY,-3,-19,-21 1 x failed PCR
7	45,XX,-15	46,XX,-9,+17
8	44,XX,-15,-21	48,XXY,+17 48,XX,+16,+22 47,XX,+17

Embryo results

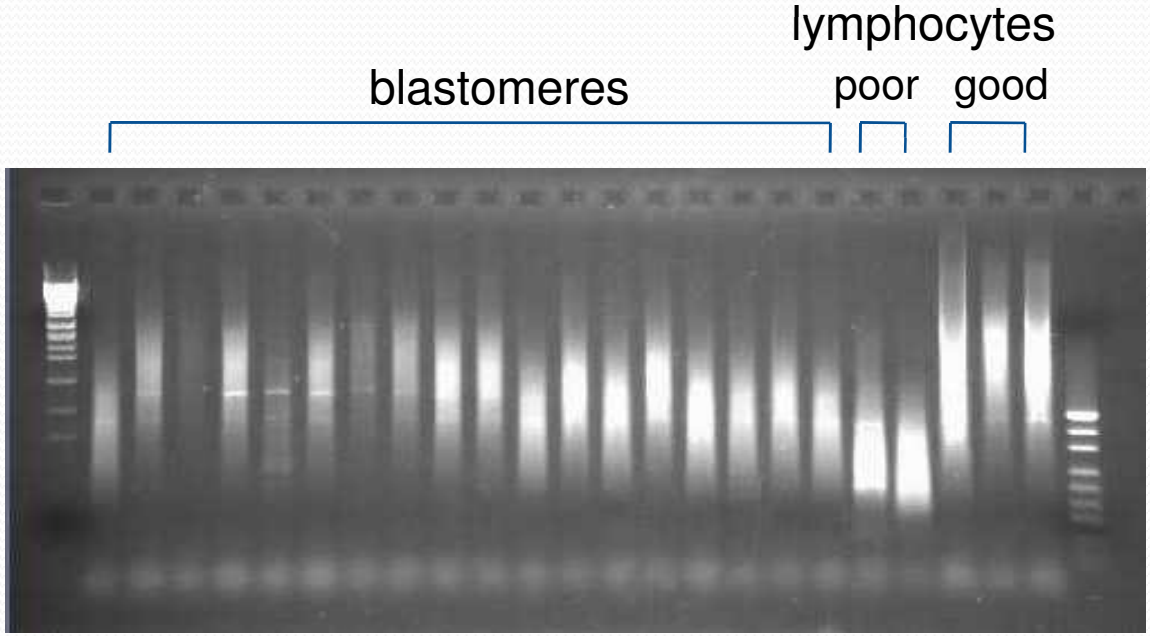
Embryo	FISH result	aCGH results
1	45,XX,-15	45,XX,-4
2	46,XY,**-15**,+16	47,XY,**+1** 45,XY,-10,**-15**,**+19** 48,XXY,**+1**,-3,+4,-12,**+19**
3	46,XX,+16	51,XYY,+5,**-6**,+12,+14,+19,+22 43,XX,-1,**-6**,-9,+10,-11,-12,-17 1 x failed PCR
4	46,XX,**+13**,-18	46,XXY,**+13**,**-14**,**-19** 47,XXY,**+13**,**-14**,+16,**-19** 4 x failed PCR
6	44,XY,**-21**,**-22**	42,XY,**-3**,-9,**-19**,**-22** 43,XY,**-3**,**-19**,**-21** 1 x failed PCR
7	45,XX,-15	46,XX,-9,+17
8	44,XX,-15,-21	48,XXY,**+17** 48,XX,+16,+22 47,XX,**+17**

Summary of FISH/array CGH results on aneuploid embryos

- All 8 embryos were mosaic
- Mean concordance of non-affected FISH chromosomes with array CGH was 75% (range 62.5-82.5%)
- Mean concordance of affected FISH chromosomes with array CGH was 17%
- Additional non-FISH chromosome aneuploidies seen in 15/18 blastomeres (83%)
- Complex abnormalities (> 3 chromosomes) seen in 6/18 blastomeres (33%)
- The data is similar to that reported for FISH re-analysis of aneuploid embryos

Caveats on embryo data

- High level of mosaicism
  - cascading errors in 1st, 2nd and 3rd cleavage divisions?
- DNA degradation prior to PCR is likely
  - DOP-PCR was performed on poor quality blastomeres



Strategies to validate array CGH prior to clinical application

- Hybridise DOP-PCR products using metaphase CGH to confirm results – separate the array performance from the PCR
- Analyse surplus frozen IVF embryos donated to research – young donors, not all aneuploid
- Validate using a range of clinical cell types ie first and second polar bodies and blastocyst