BlueGnome

24sure

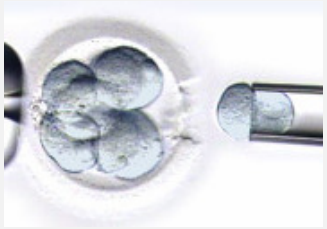
pre-implantation chromosomal aneuploidy screening

the technical development of single cell analysis technology

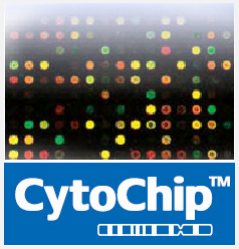
Introduction to BlueGnome

- BlueGnome is
  - Dedicated solely to production of clinical microarray solutions
  - Europe's largest manufacturer of clinical microarrays
  - 100+ labs/20+ countries

Introduction to BlueGnome

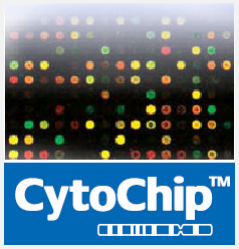


PGS



24Sure

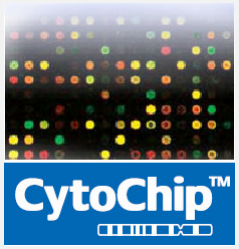
Prenatal



Focus

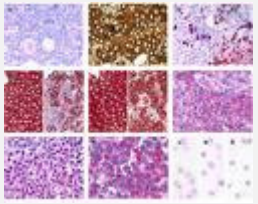
Constitutional

Postnatal



CytoChip

BAC / Oligo



Oncology

Focus

Haematology

24sure – aims of test

- To improve fertility rates for IVF
  - Aneuploidy screen for whole chromosomes / arms / large structural changes
  - Single cells (polar bodies, blastomere), multiple cells (trophoectodermal)
- Test characteristics
  - Easily interpretable – no CNP / no trait analysis / no ethically questionable results (late onset disorders, cancer predisposition, small CNV/*de novo* imbalances of unknown significance)
  - Rapid (12-24 hours)
  - Low cost per sample / start up investment

24sure - Protocol

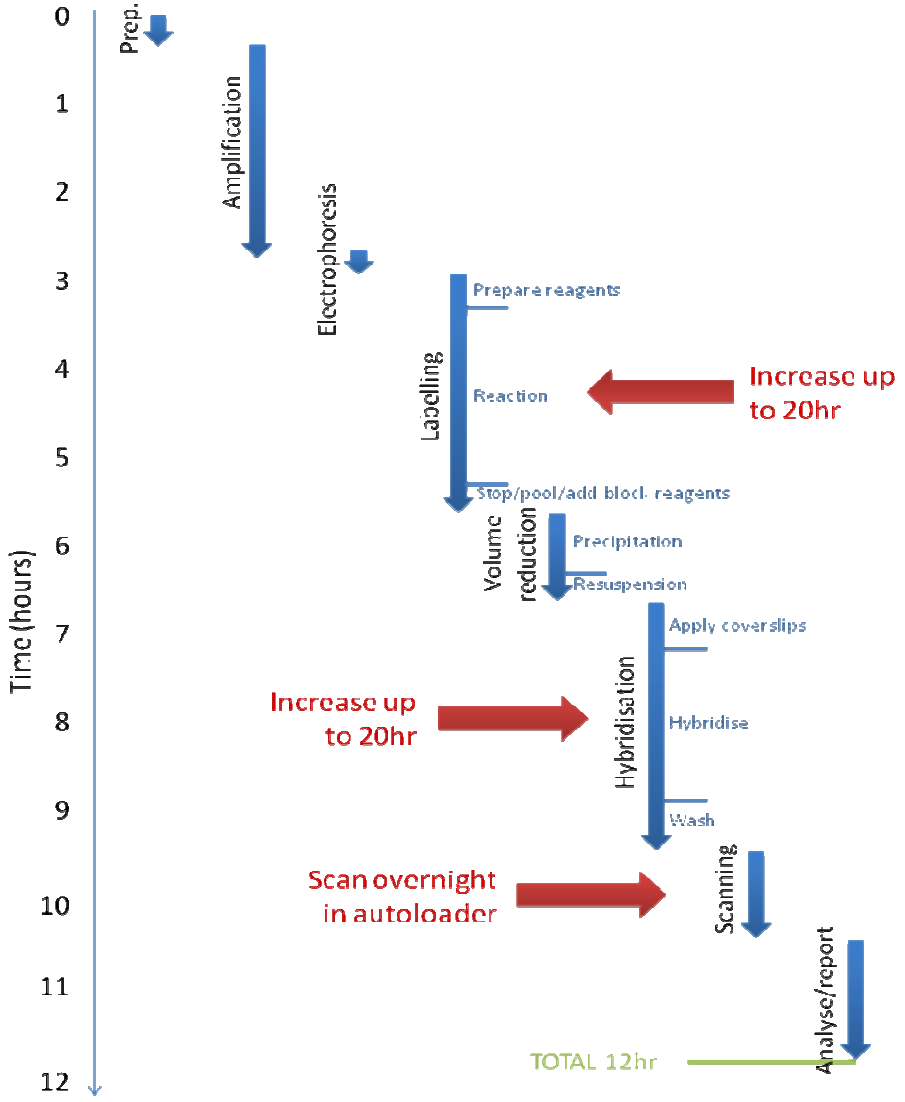
24sure BAC microarray

- 3226 BAC Clones chosen (duplicate) and optimized according to performance on over 10000 patients in a post-natal context.
- Clones designed to minimise CNP detection using BlueGnome customer in-house data of +2000 post-natal aCGH cases.

24sure – protocol

- Amplification – SurePlex (Rubicon Genomics technology)
- Labelling – BlueGnome Fluorescence labelling kit
- Arrays – 24sure
- Analysis software – BlueFuse Multi

24sure - Protocol timings



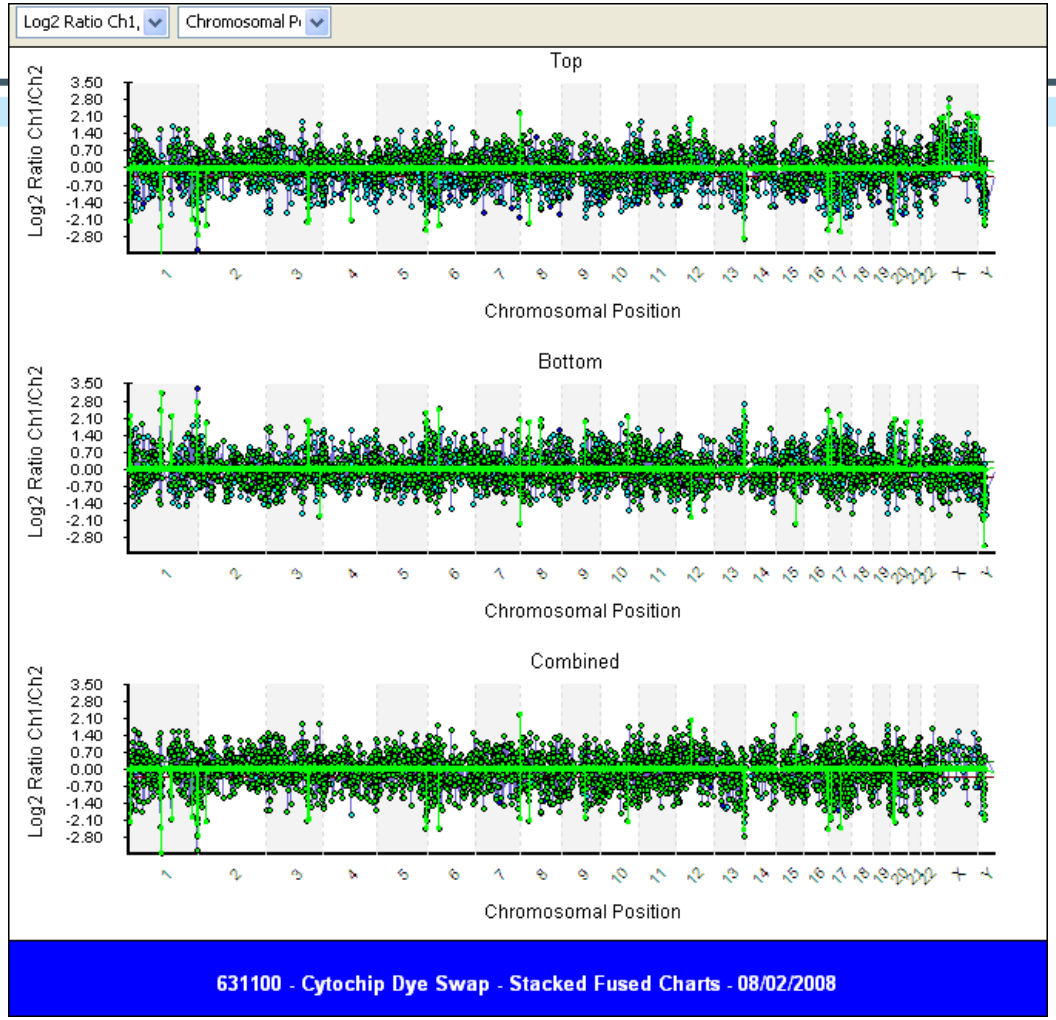
Which amplification technology?

Initial testing of BAC arrays focused on amplification technology using control cell samples

- Qiagen Repli-G
- GE Healthcare GenomiPhi
- Sigma WGA4

Qiagen Repli-G

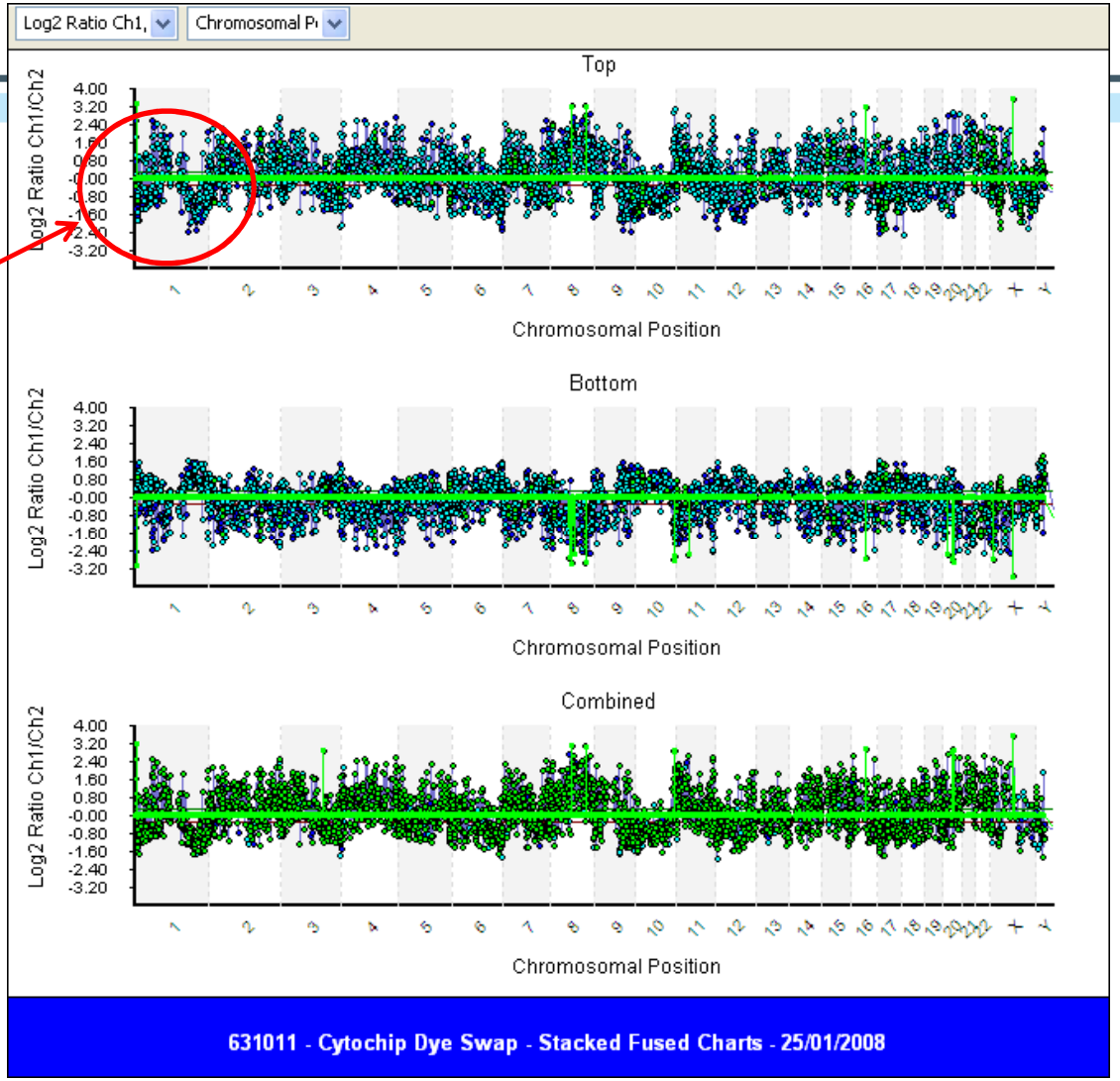
Repli-G



- Hybridisations showed high noise levels
- Fast, cost effective, easy to use
- Difficult to QC for specific amplification after simple (gel) QC since negative controls amplified as well as positive controls.

Genomiphi V2

Genomiphi v2

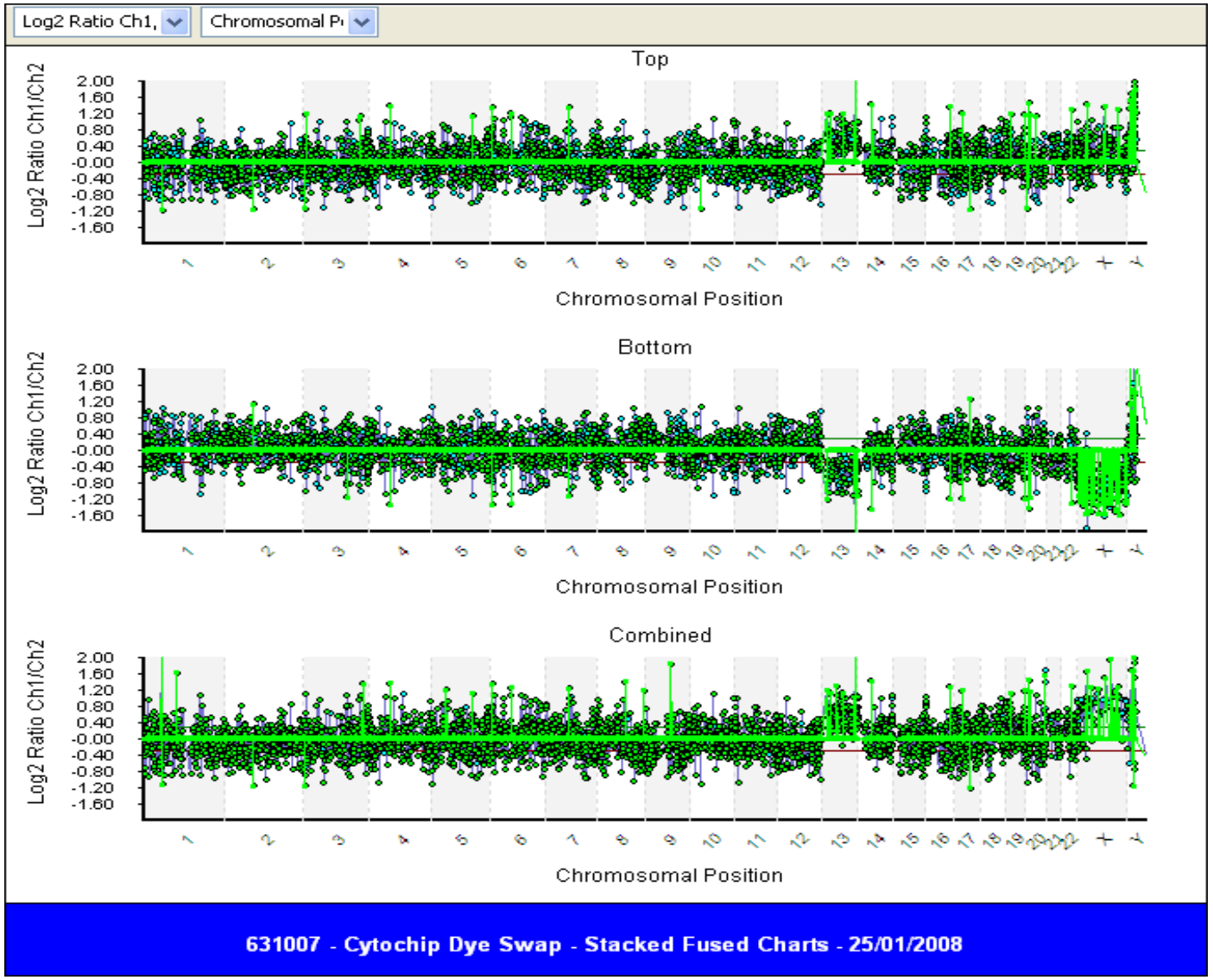


Wave seen on Chromosome 1

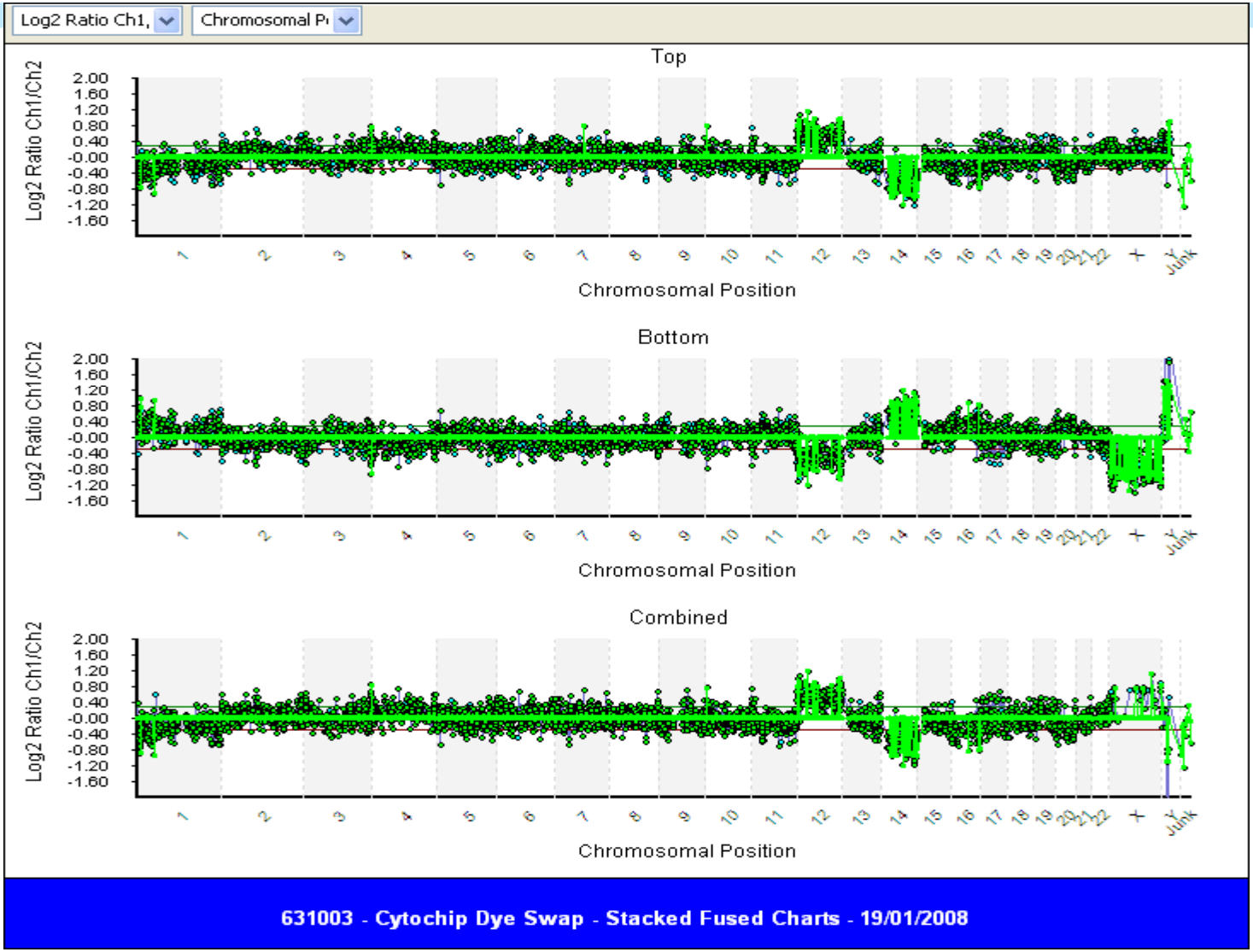
- Wave like patterns make interpretation challenging
- Easy to QC via gel

Sigma WGA4 - GenomePlex

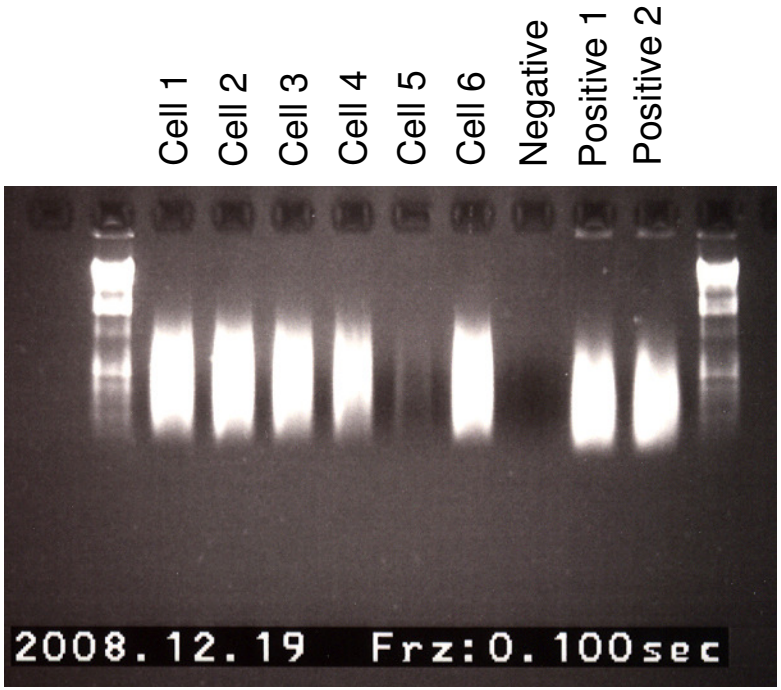
Trisomy 13 – GenomePlex - single cell known abnormality



Single cell +12, -14



24sure - QC checkpoint - amplification



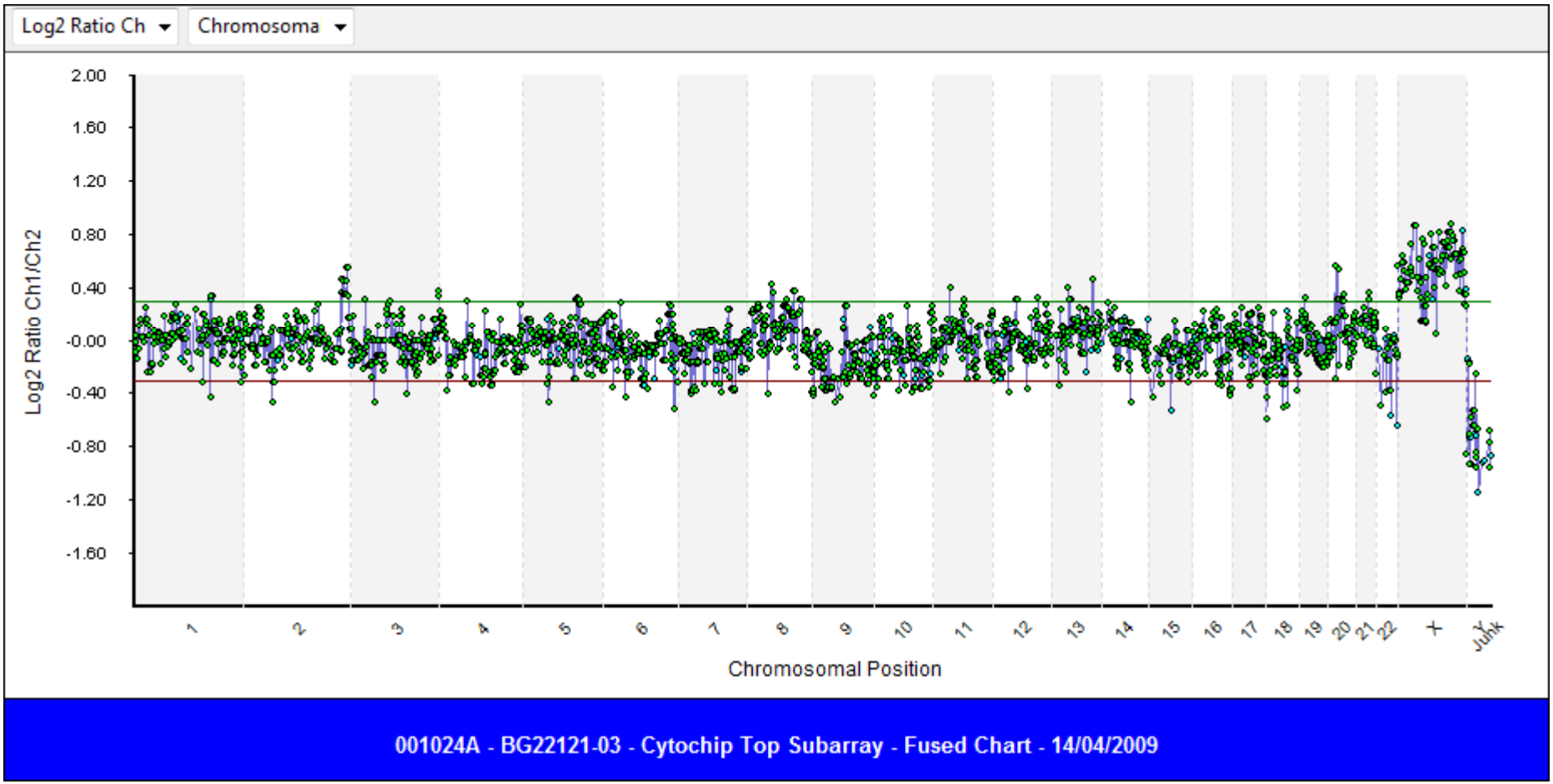
- Cells 1, 2, 3, 4, 6 successfully amplified
- Cell 5 failed to amplify
- Negative control (water) correctly failed to amplify
- Positive controls (100 pg female genomic DNA) amplified successfully
- All amplifications labelled and hybridised

Sigma WGA4 GenomePlex

- Easy to QC
- Low noise
- Long protocol
- High ADO
- Confirmation of aneuploidy with other technologies (FISH, markers, Karyomapping, single cells of cell lines of known abnormality)

Sigma WGA4 – GenomePlex
Polar body samples

euploid


Log2 Ratio Ch
Chromosoma
2.00
1.60
1.20
0.80
0.40
-0.00
-0.40
-0.80
-1.20
-1.60
Log2 Ratio Ch1/Ch2
1
2
3
4
5
6
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14
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16
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18
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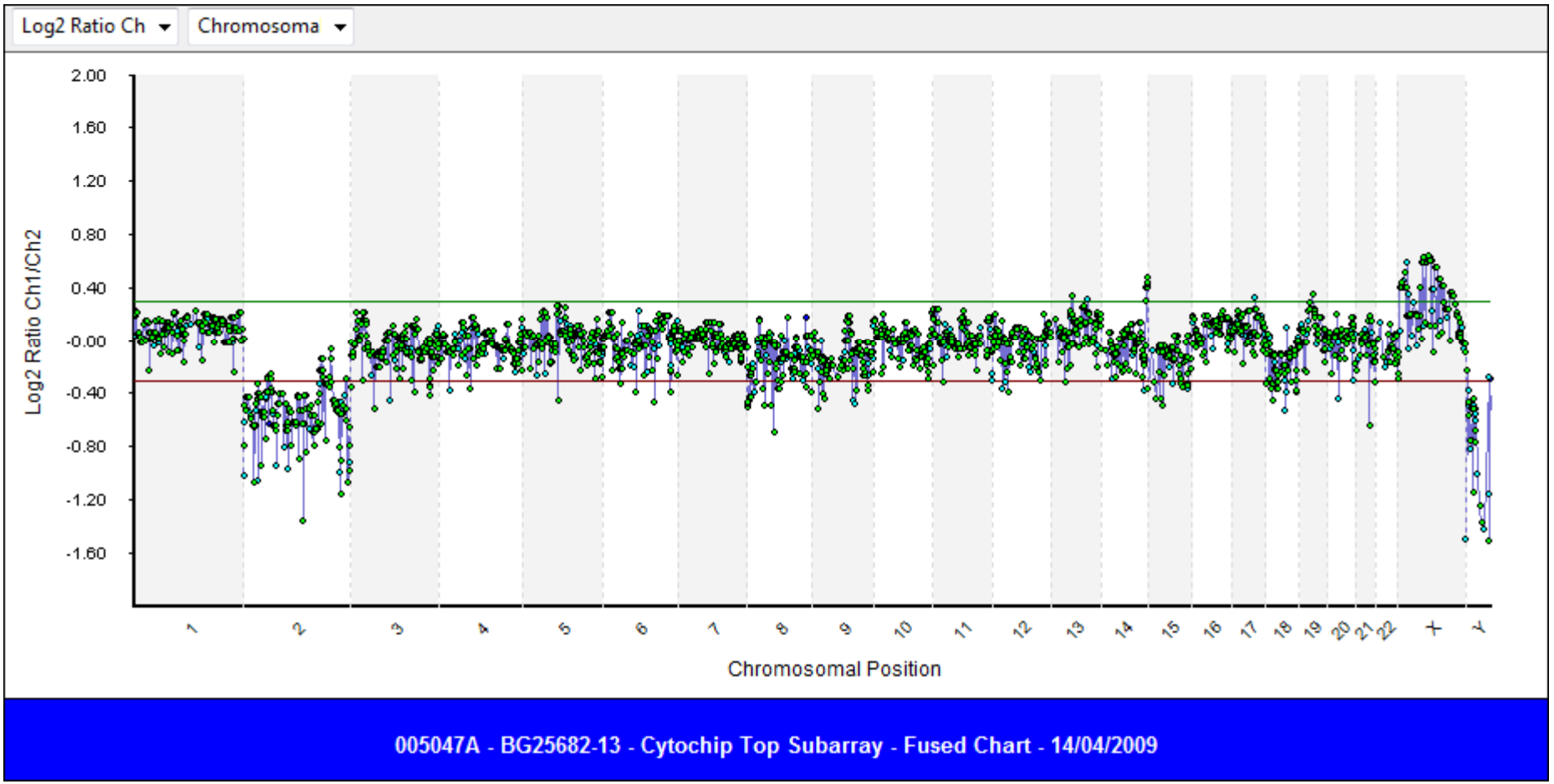
22
X
Y
Junk
Chromosomal Position
001024A - BG22121-03 - Cytochip Top Subarray - Fused Chart - 14/04/2009

aneuploid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1.60
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Log2 Ratio Ch1/Ch2
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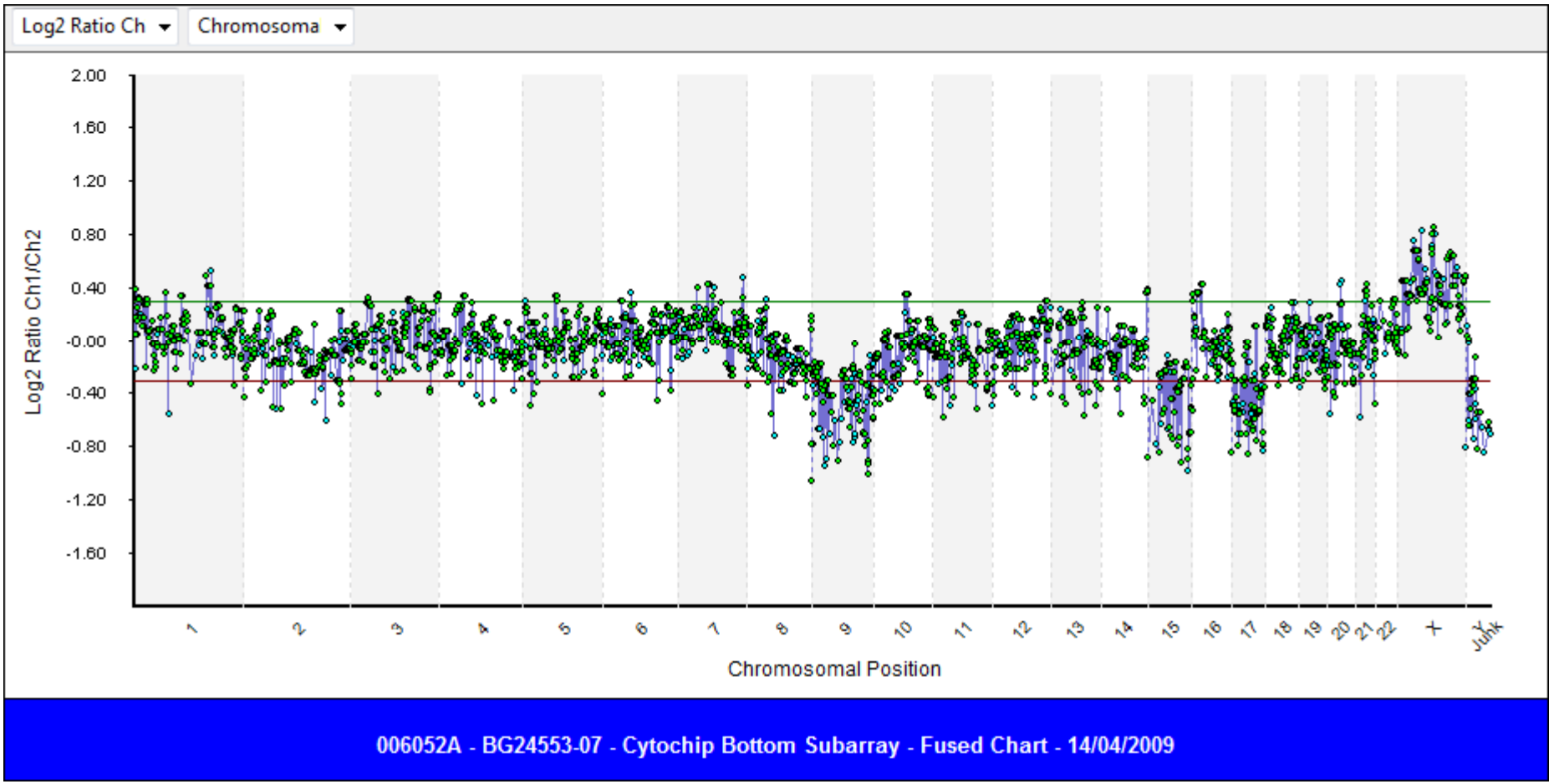
Chromosomal Position
005047A - BG25682-13 - Cytochip Top Subarray - Fused Chart - 14/04/2009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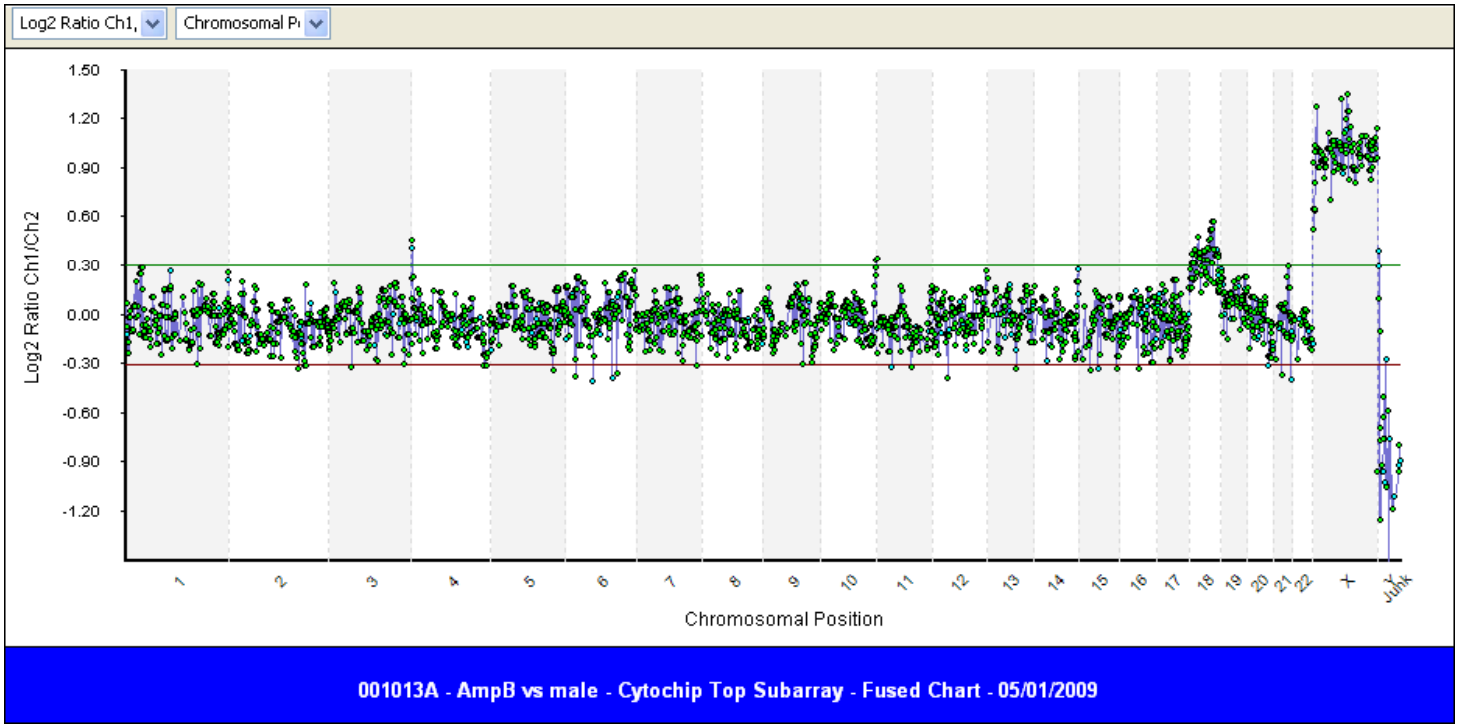
X
Y
Junk
Chromosomal Position
006052A - BG24553-07 - Cytochip Bottom Subarray - Fused Chart - 14/04/2009

24sure - Polar body samples

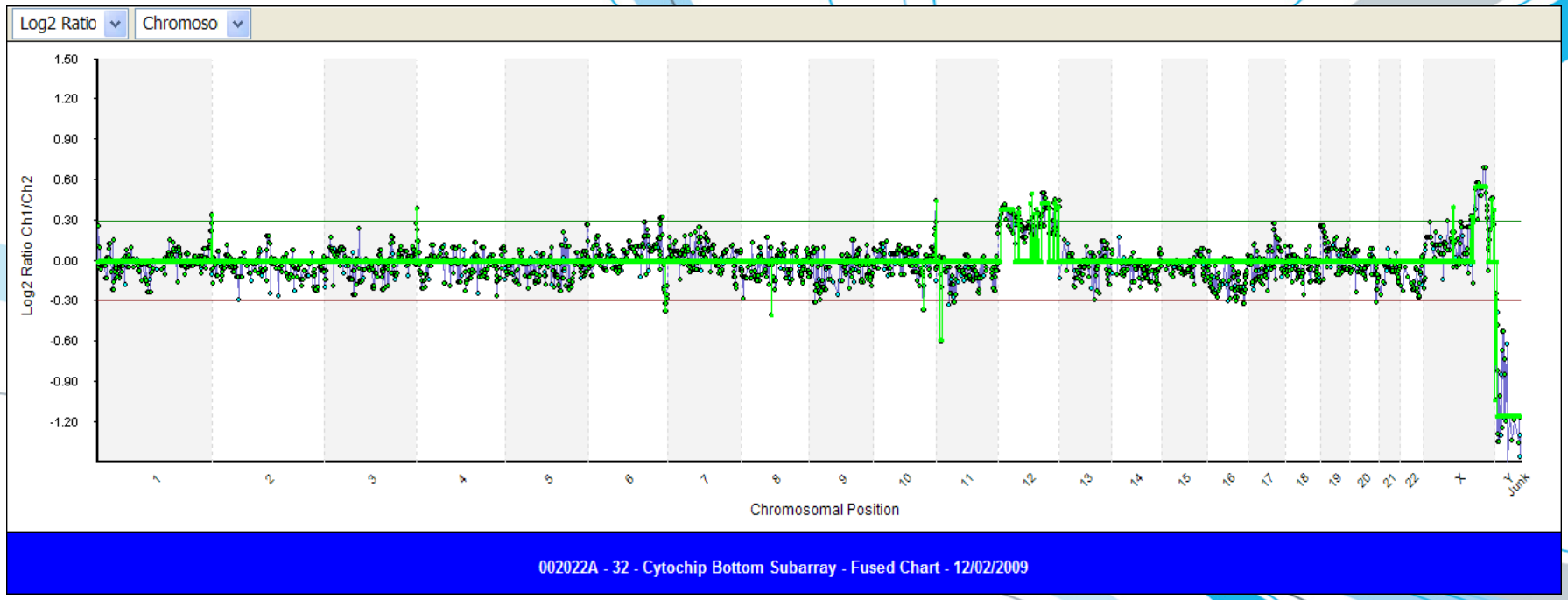
Total consented polar bodies	250
Successful amplification and hybridisation	209 (84%)
Number of cases	32
Average number of polar bodies per case	8
Euploid results reported for cells	38%
Percentage cases with one or more euploid cells - 75%	
Aneuploid results reported for cells	56%
Percentage cases where all cells were aneuploid - 19%	
Successful hybridizations per amplified product	94%

Sigma WGA4 – GenomePlex
Blastomere samples

Blastomere amplification B: female, +18 +X - confirmation with STS

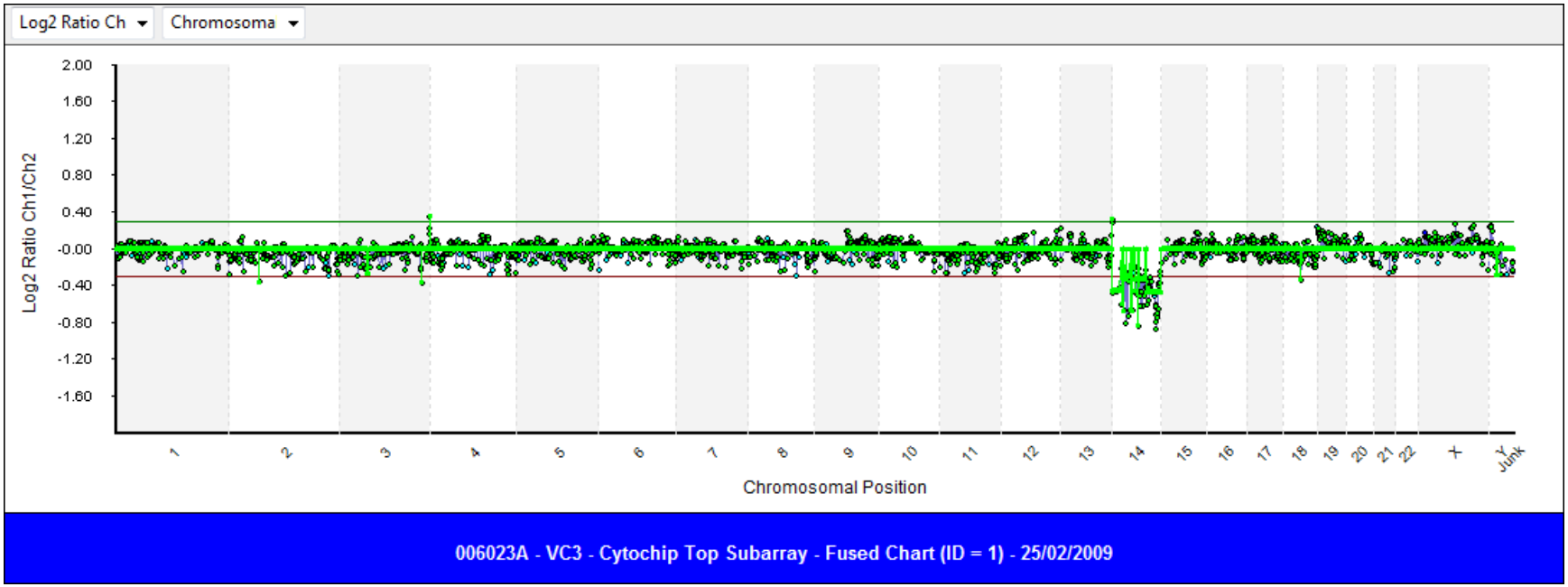


Single cell showing Trisomy chr12, duplication ~25Mb chrXq

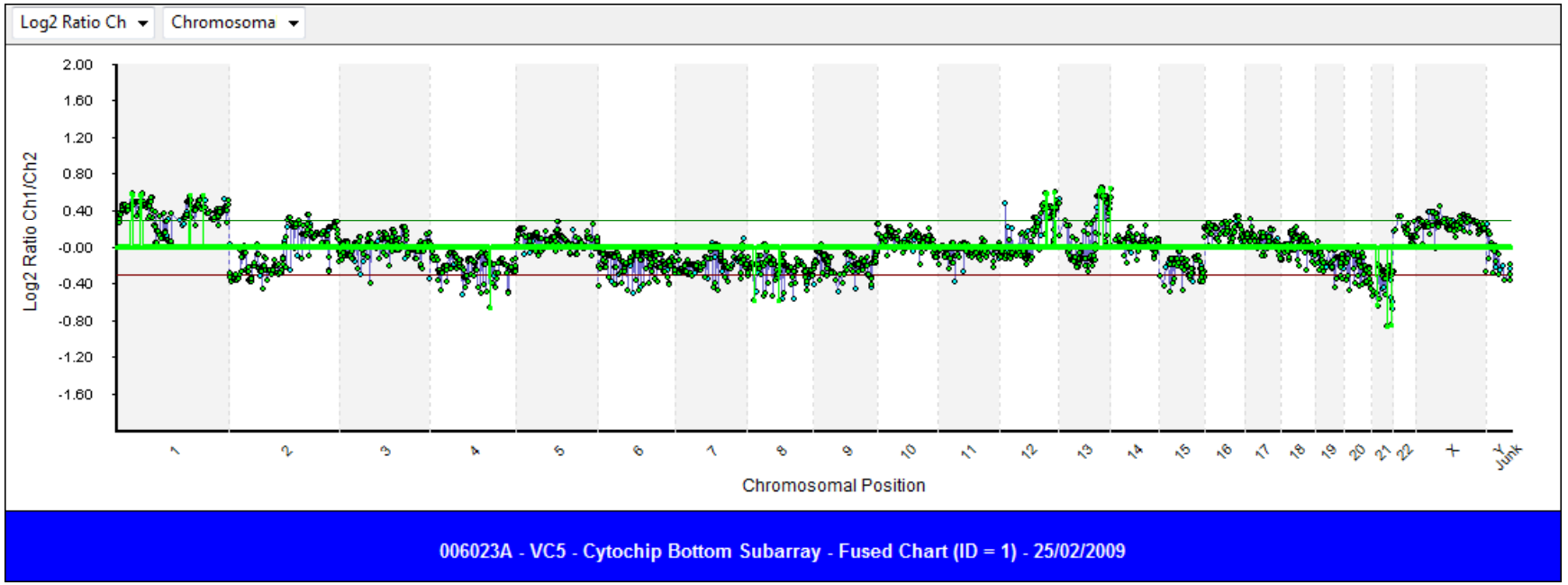


Sigma WGA4 – GenomePlex
whole embryo/trophoblastic samples
4-20 cells

Multiple cell biopsy

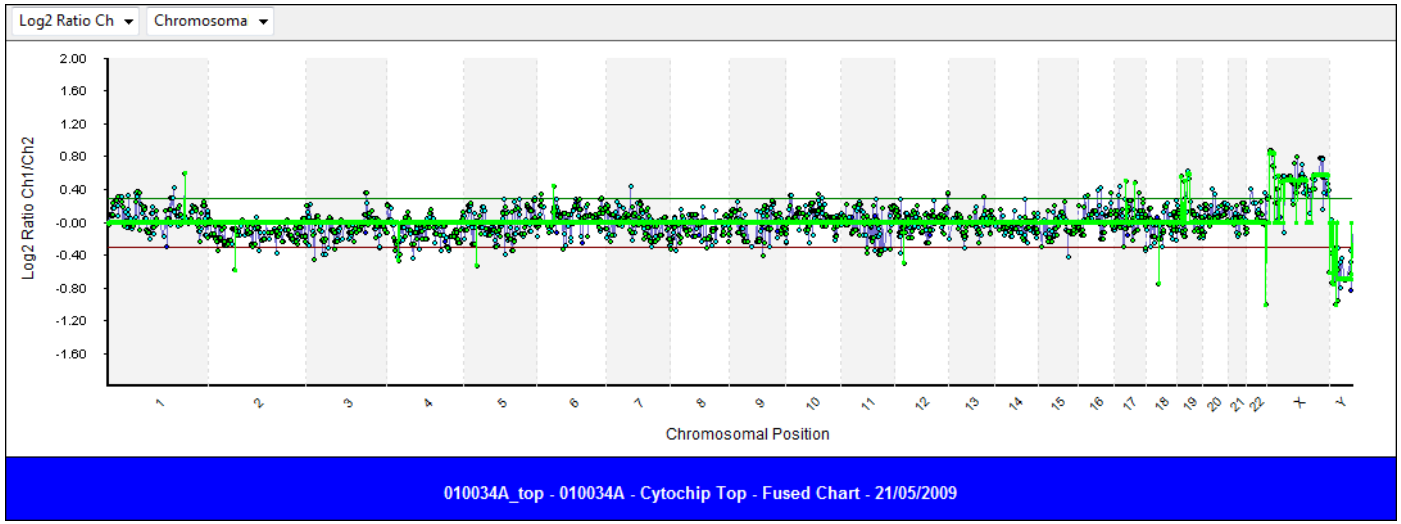


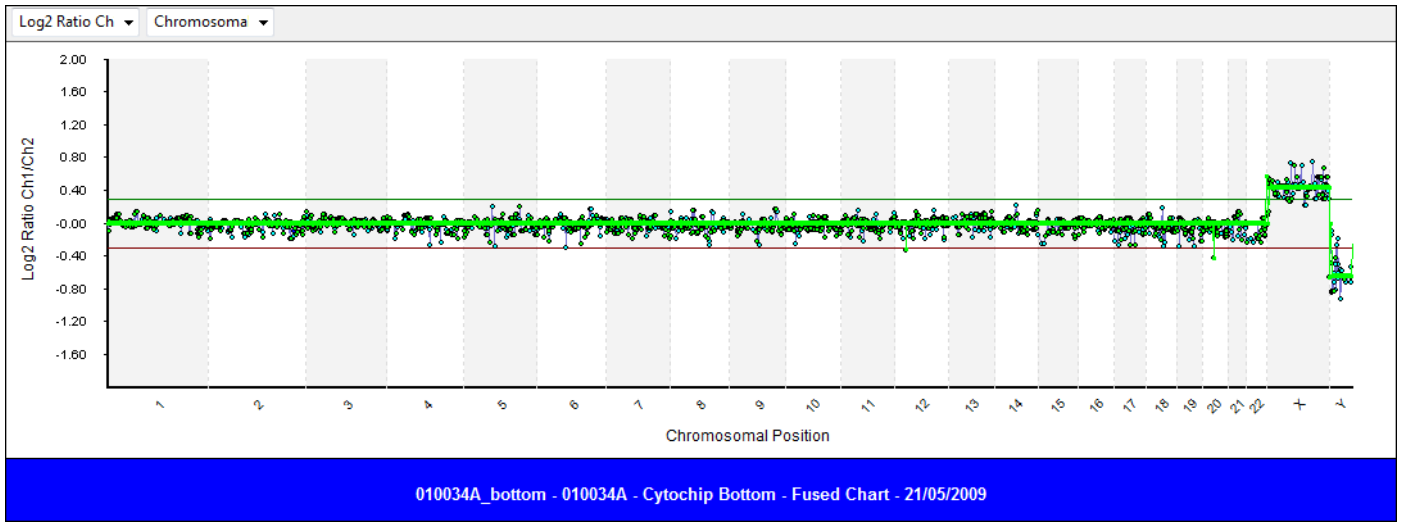
Multiple cell biopsy



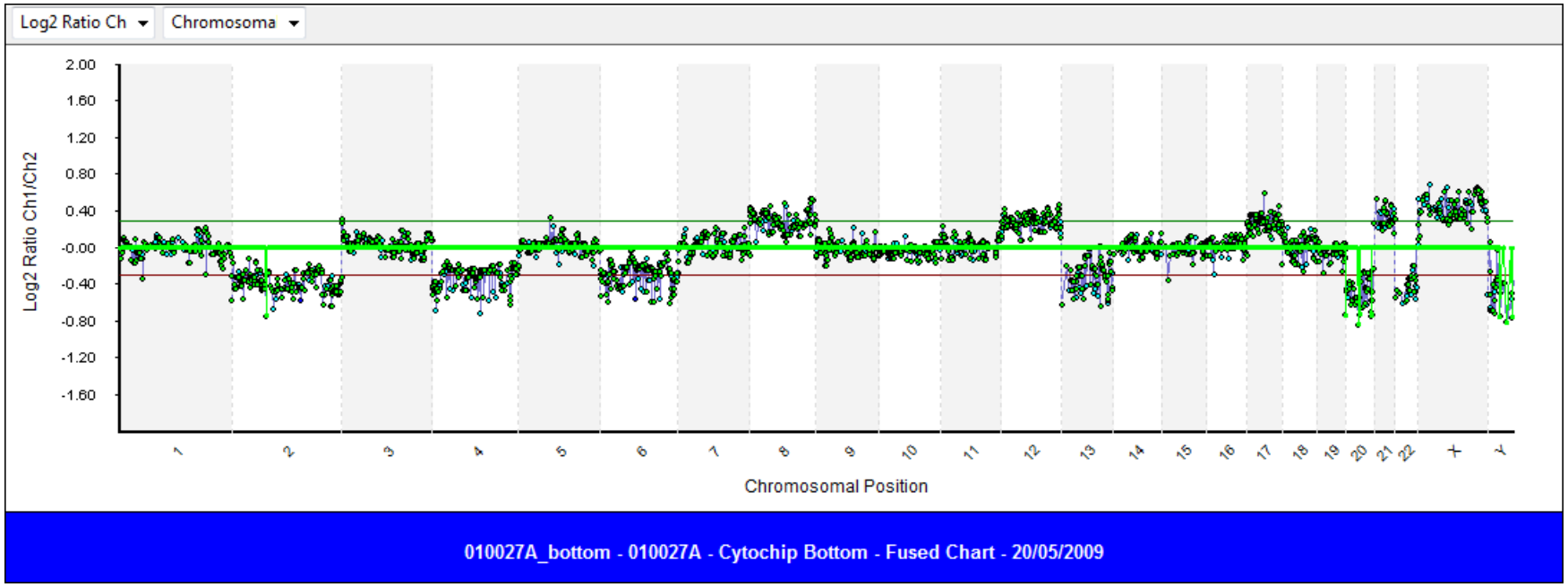
BlueGnome 24sure using SurePlex amplification

15pg Sureplex female sample – top array Promega DNA / bottom array SurePlex amplified control DNA

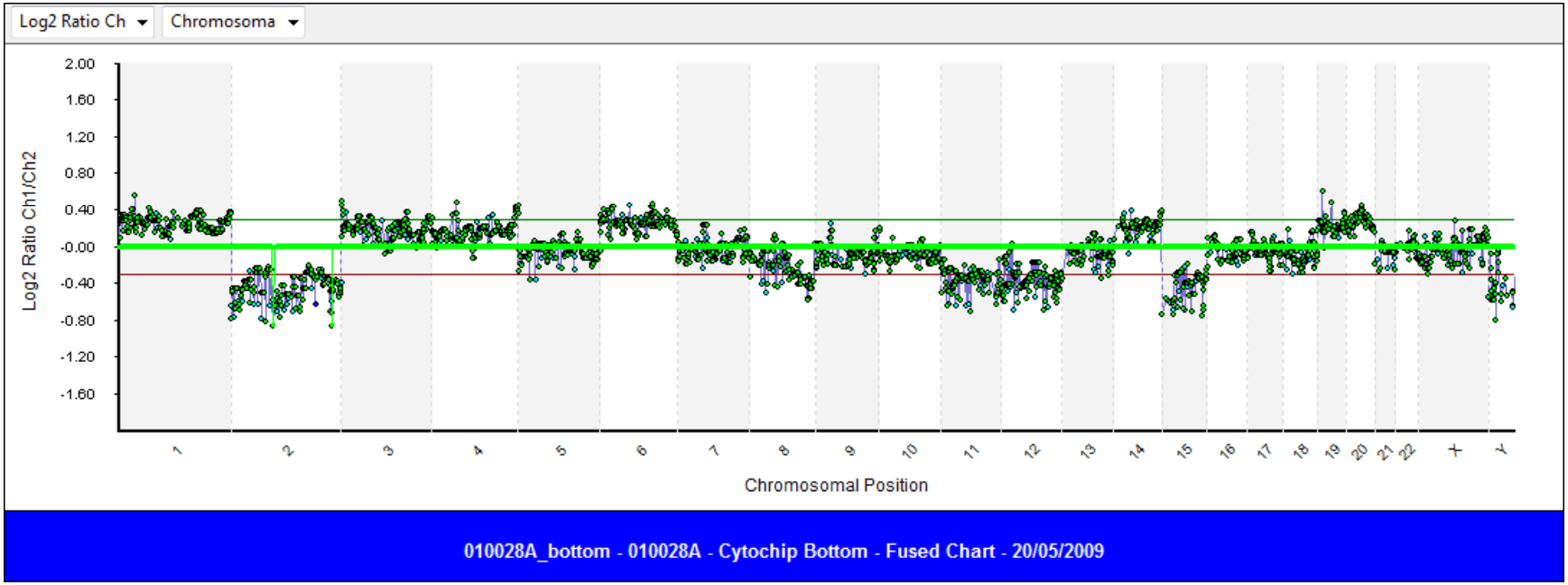




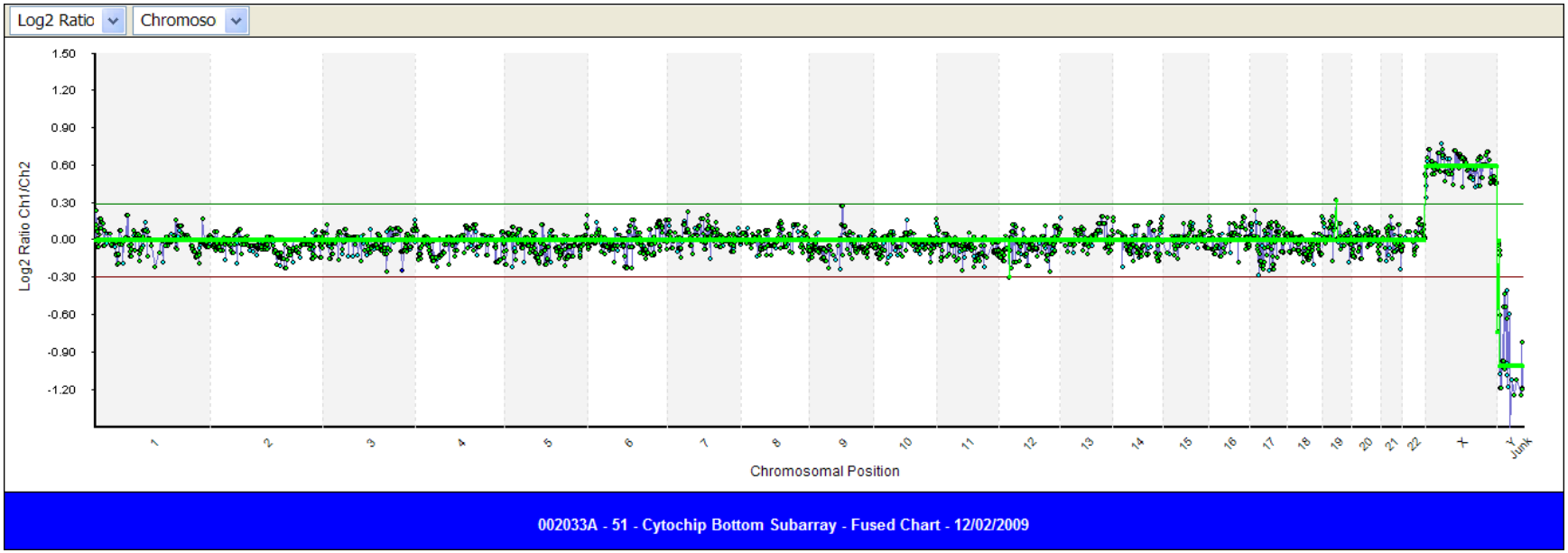
SurePlex - Blastomere single cell sample



SurePlex - Blastomere single cell sample



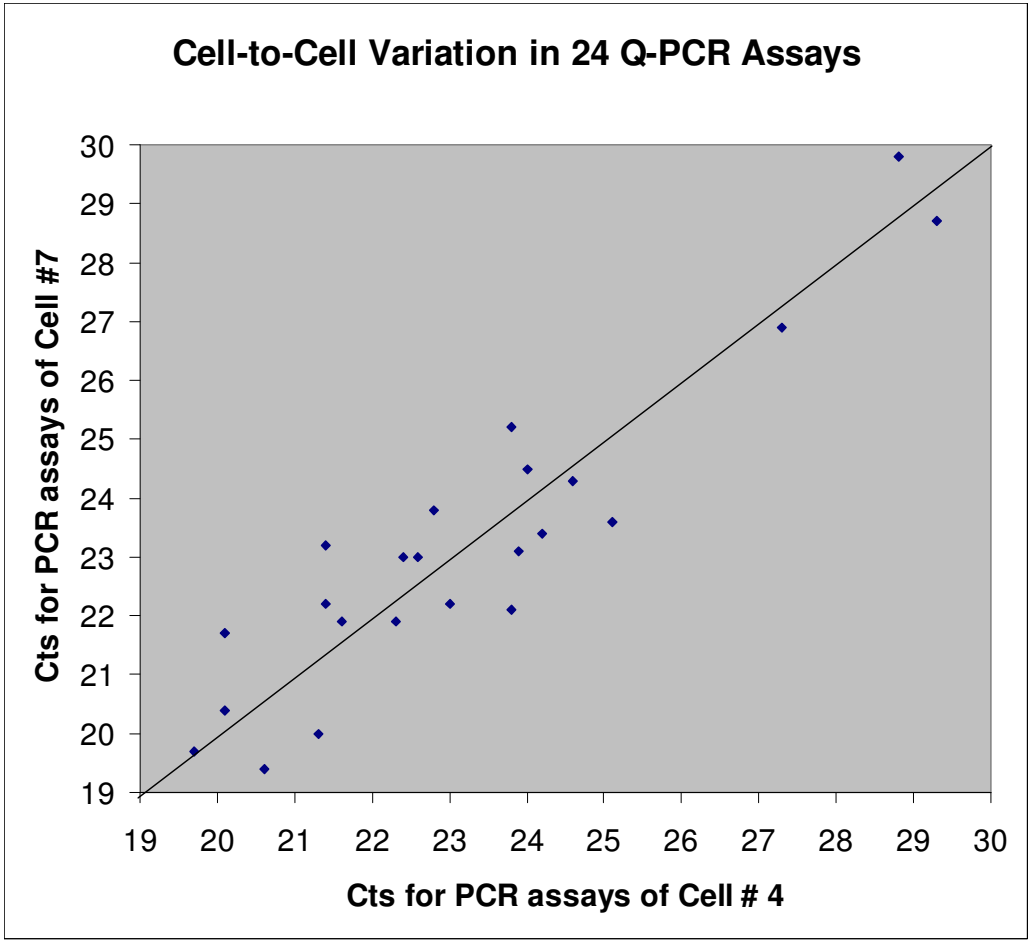
SurePlex - Blastomere single sample



SurePlex

- Easy to QC
- Low noise
- Short protocol
- Low ADO – suitable for genotyping

Reproducibility of SurePlex



Results courtesy of Rubicon Genomics

ADO of SurePlex

BlueGnome

	10 ng DNA	5 cells	single cell
drop out rate*	10%	13%	11%
avg. standard deviation of 48 assays**	-	-	1.6 cycles
ave. standard deviation of top 24 assays***	-	-	0.6 cycles

Results courtesy of Rubicon Genomics

Conclusions

BlueGnome

- 24sure has been used to reliably identify aneuploidy in single/multiple cells
- 24sure technology has been validated with a wide range of amplification protocols
- SurePlex amplification technology has been found to be most suitable for 24sure
- Clinical efficacy unknown
- Robust clinical studies are required

BlueGnome

24sure

pre-implantation chromosomal aneuploidy screening