

24sure

pre-implantation chromosomal aneuploidy screening

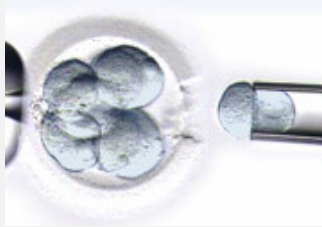
the technical development of single cell
analysis technology

Introduction to BlueGnome

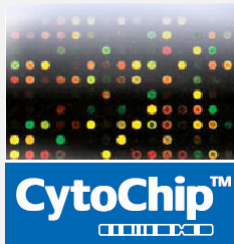


- BlueGnome is
 - Dedicated solely to production of clinical microarray solutions
 - Europe's largest manufacturer of clinical microarrays
 - 100+ labs/20+ countries

Introduction to BlueGnome



PGS



24Sure



Prenatal



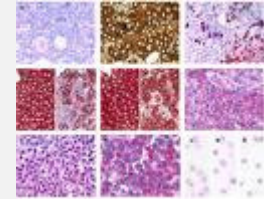
Focus
Constitutional



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

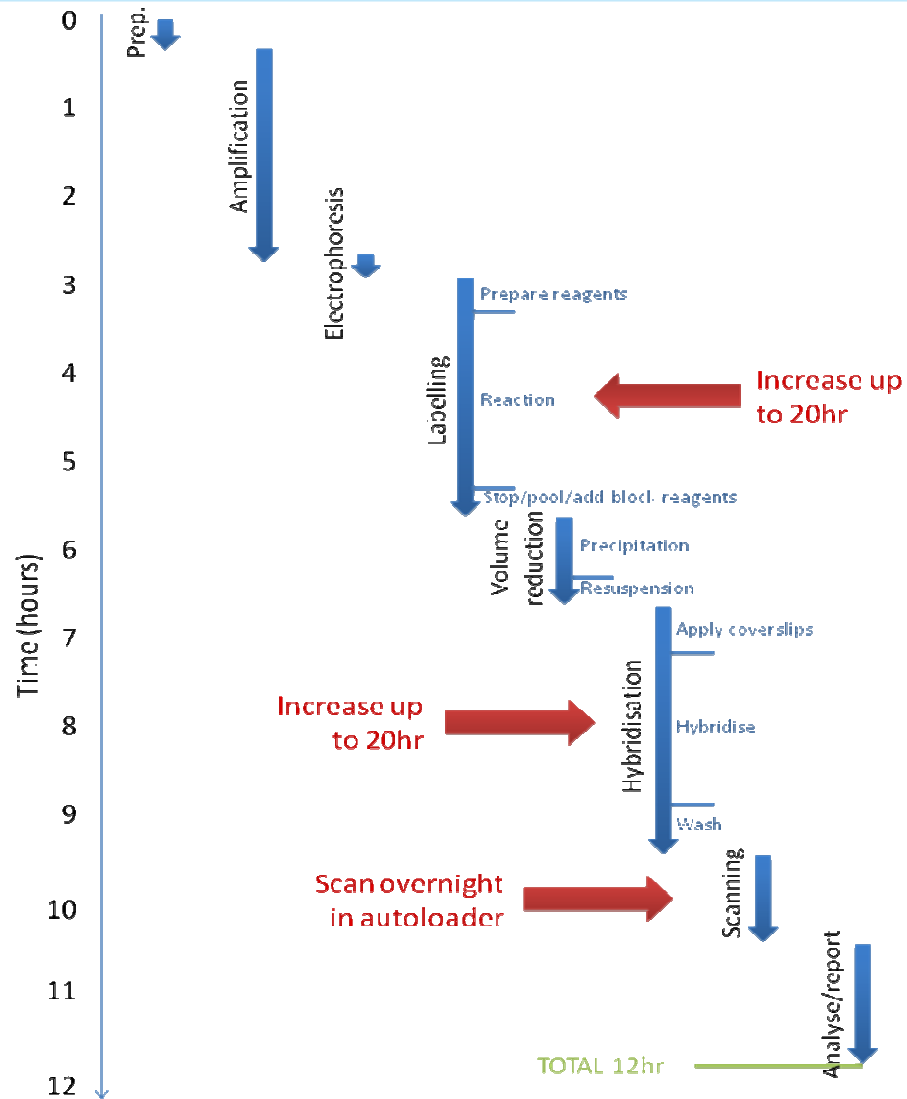
24sure BAC microarray

- 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?

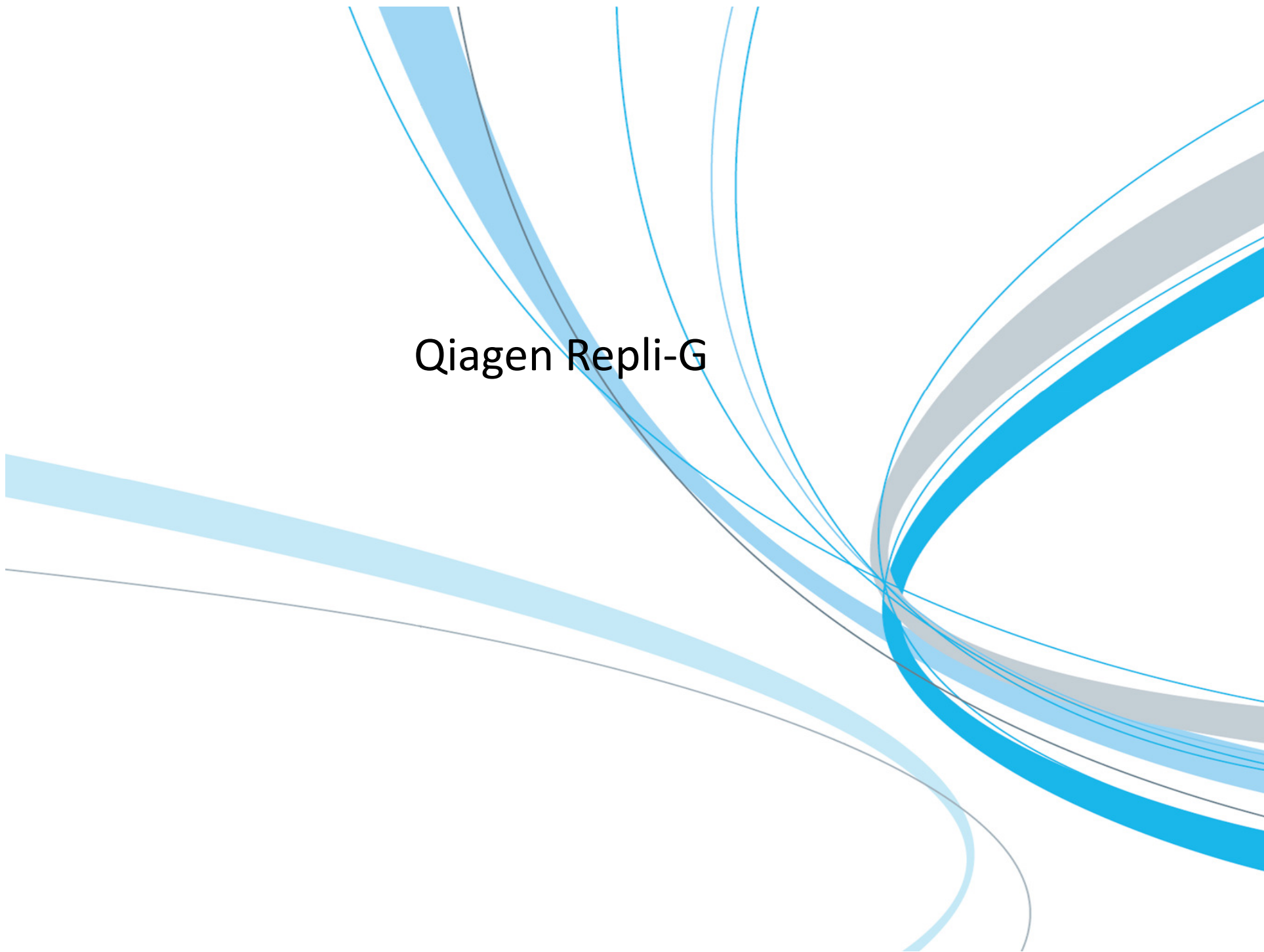
The image features a central text question, "Which amplification technology?", set against a white background. The text is surrounded by several thick, curved, overlapping bands in shades of light blue, medium blue, and grey. These bands originate from the left side of the frame and curve towards the right, creating a sense of motion and depth. The lines are smooth and fluid, suggesting a dynamic or evolving process.



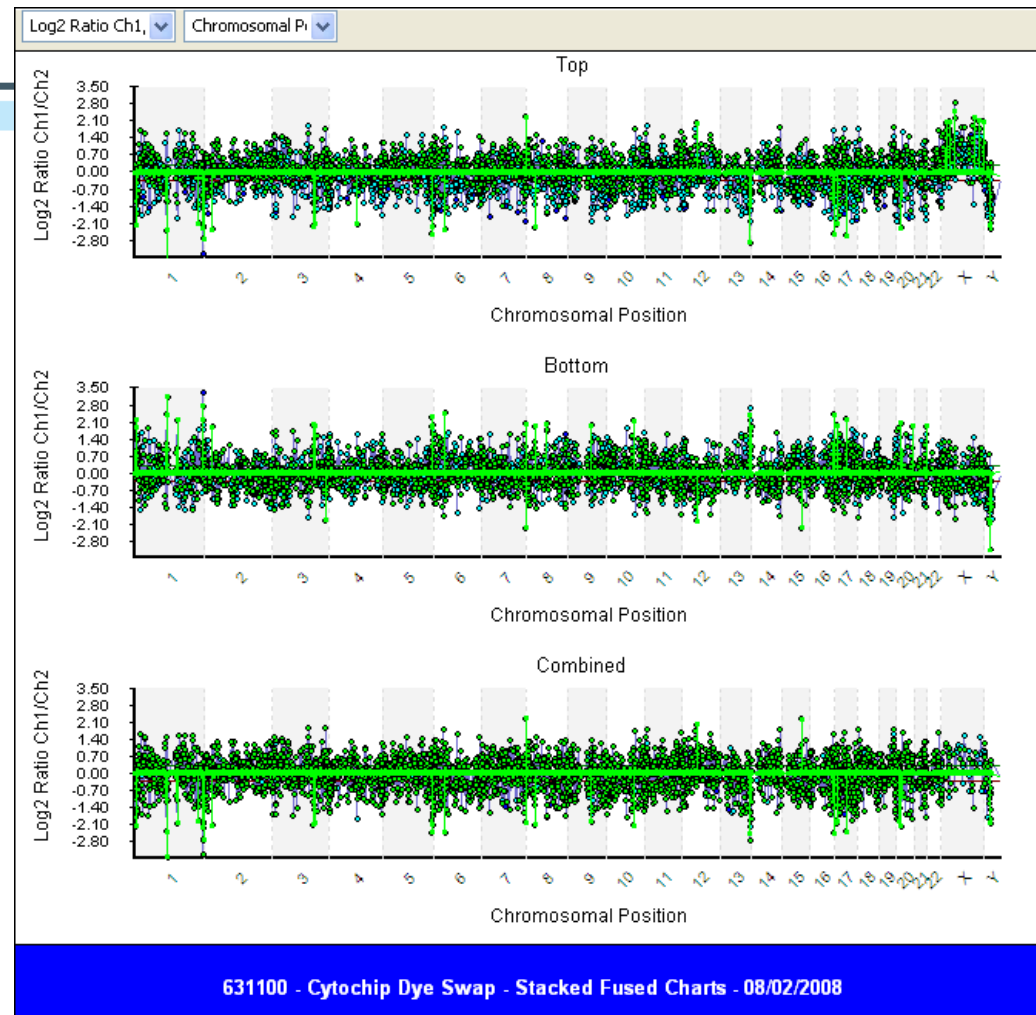
Initial testing of BAC arrays focused on
amplification technology using control cell
samples

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

Qiagen Repli-G

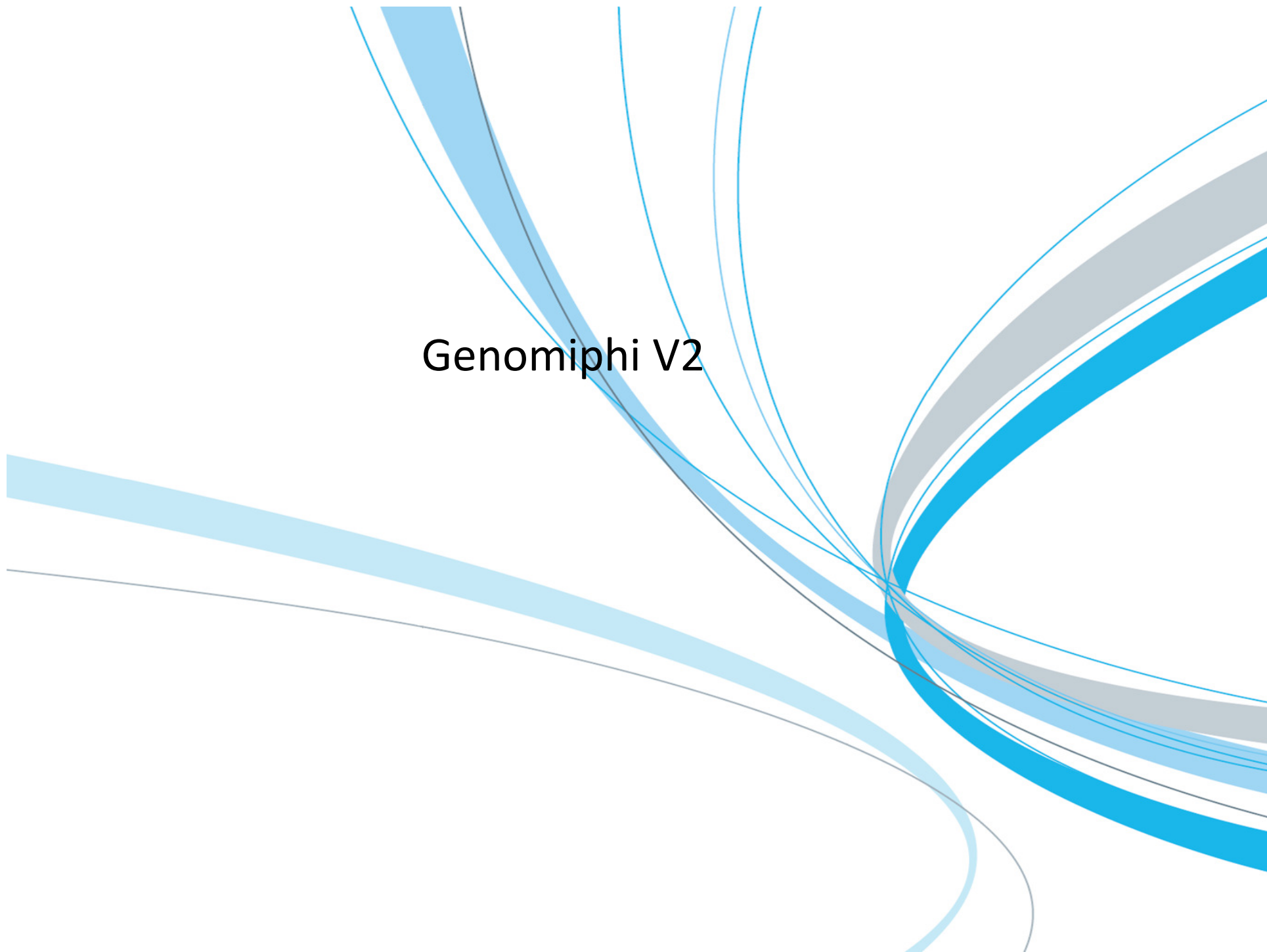


Repli-G

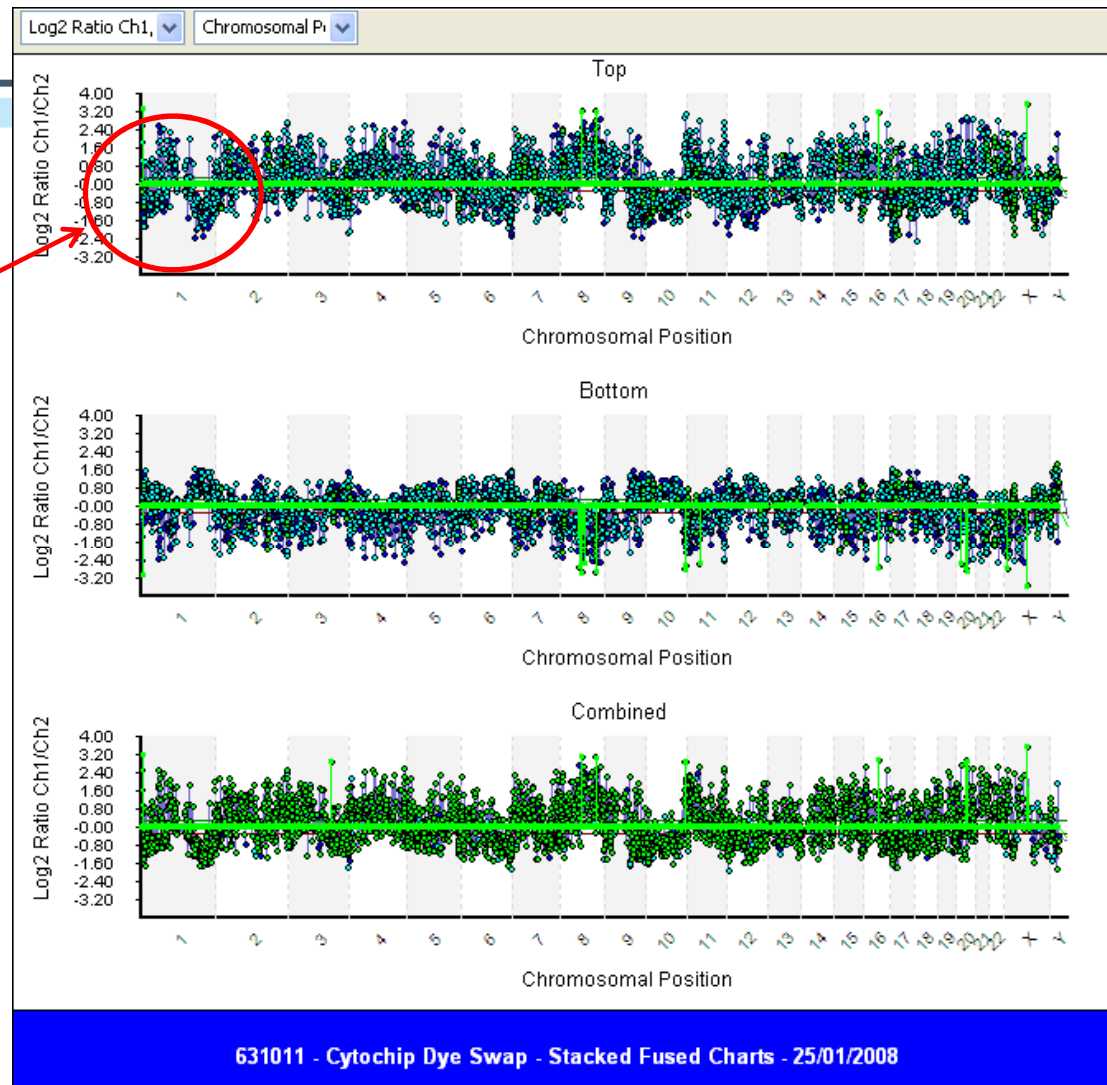


- Hybridisations showed high noise levels
- Fast, cost effective, easy to use
- Difficult to QC for specific amplification after simple (gel) QC since negative controls amplified as well as positive controls.

Genomiphi V2



Genomiphi v2



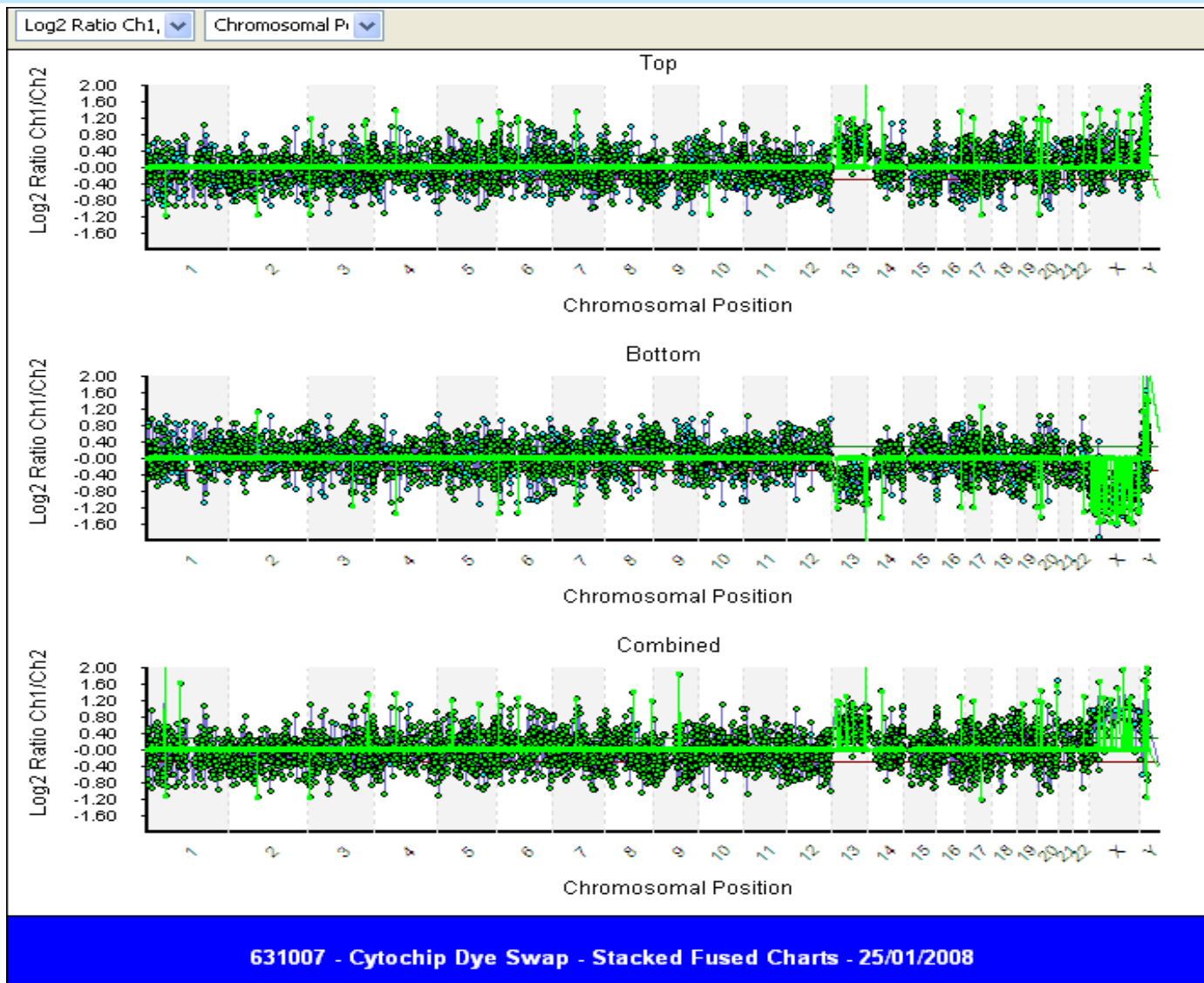
Wave seen on
Chromosome 1

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

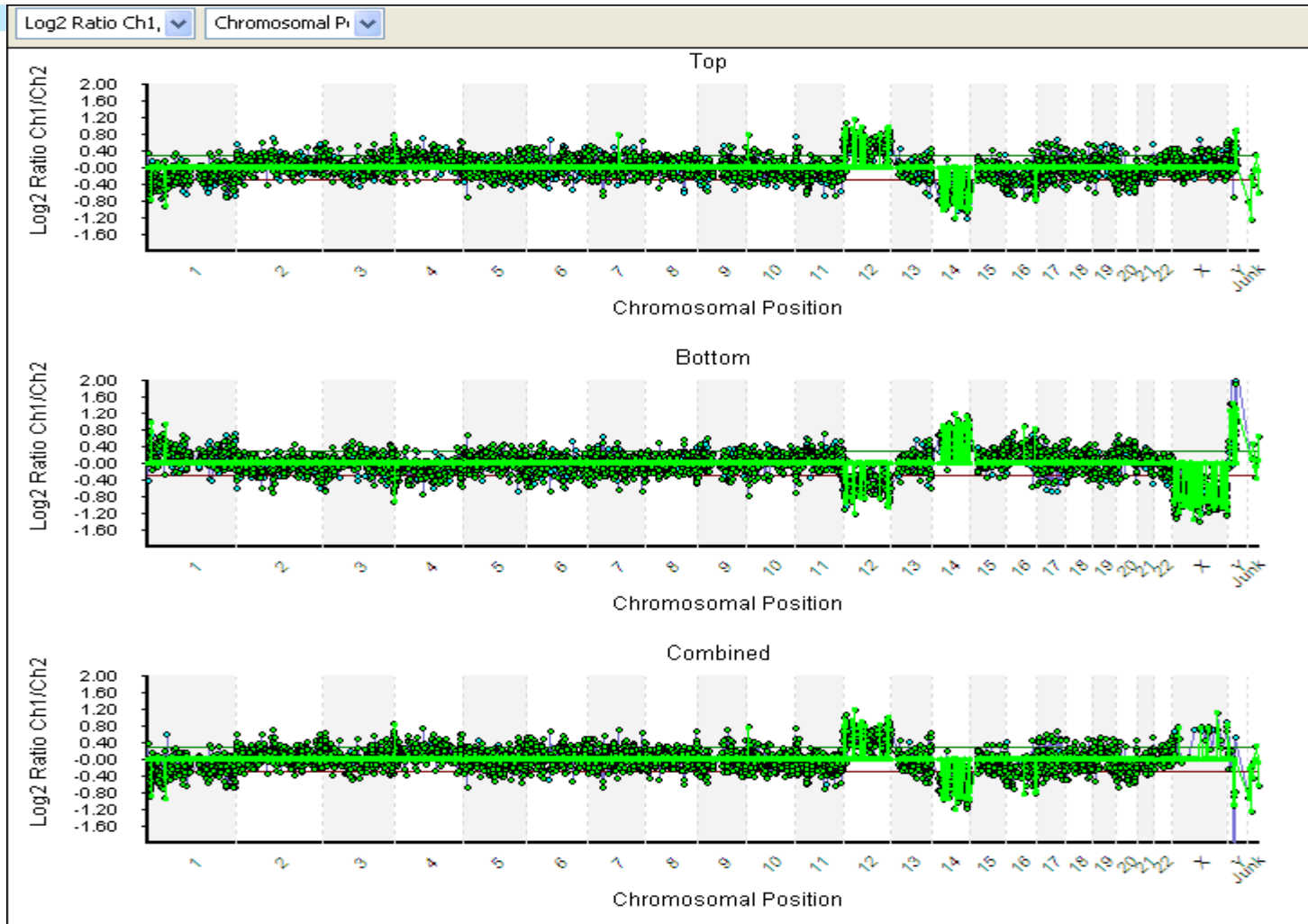
Sigma WGA4 - GenomePlex

The image features a central text label 'Sigma WGA4 - GenomePlex' in a bold, black, sans-serif font. The background is a white canvas with several abstract, flowing lines. On the left side, there are three thick, light blue curved bands that sweep downwards and towards the center. On the right side, there are three thick, vibrant blue curved bands that sweep upwards and towards the center. Interspersed among these are several thin, grey curved lines that also flow towards the center. The overall composition is dynamic and modern, with a clear focus on the central text.

Trisomy 13 – GenomePlex - single cell known abnormality

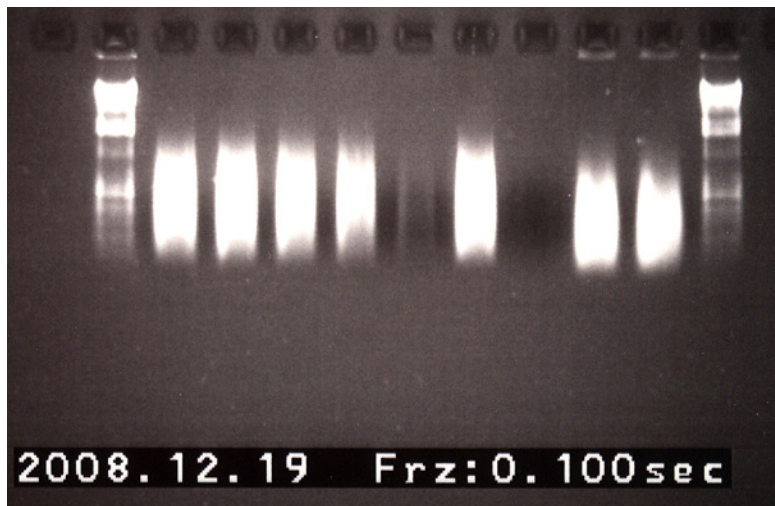


Single cell +12, -14



24sure - QC checkpoint - amplification

Cell 1
Cell 2
Cell 3
Cell 4
Cell 5
Cell 6
Negative
Positive 1
Positive 2

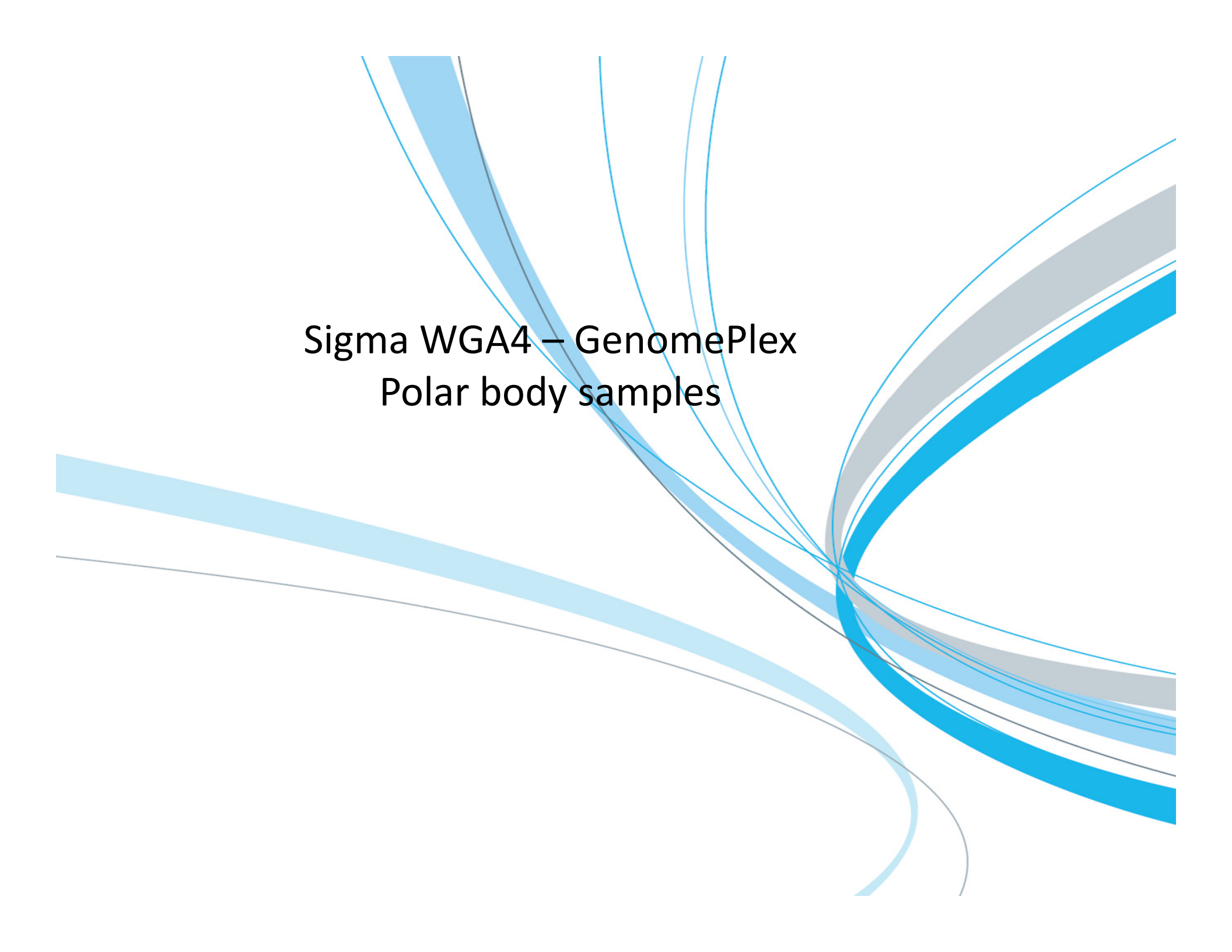


- Cells 1, 2, 3, 4, 6 successfully amplified
- Cell 5 failed to amplify
- Negative control (water) correctly failed to amplify
- Positive controls (100 pg female genomic DNA) amplified successfully
- All amplifications labelled and hybridised

Sigma WGA4 GenomePlex

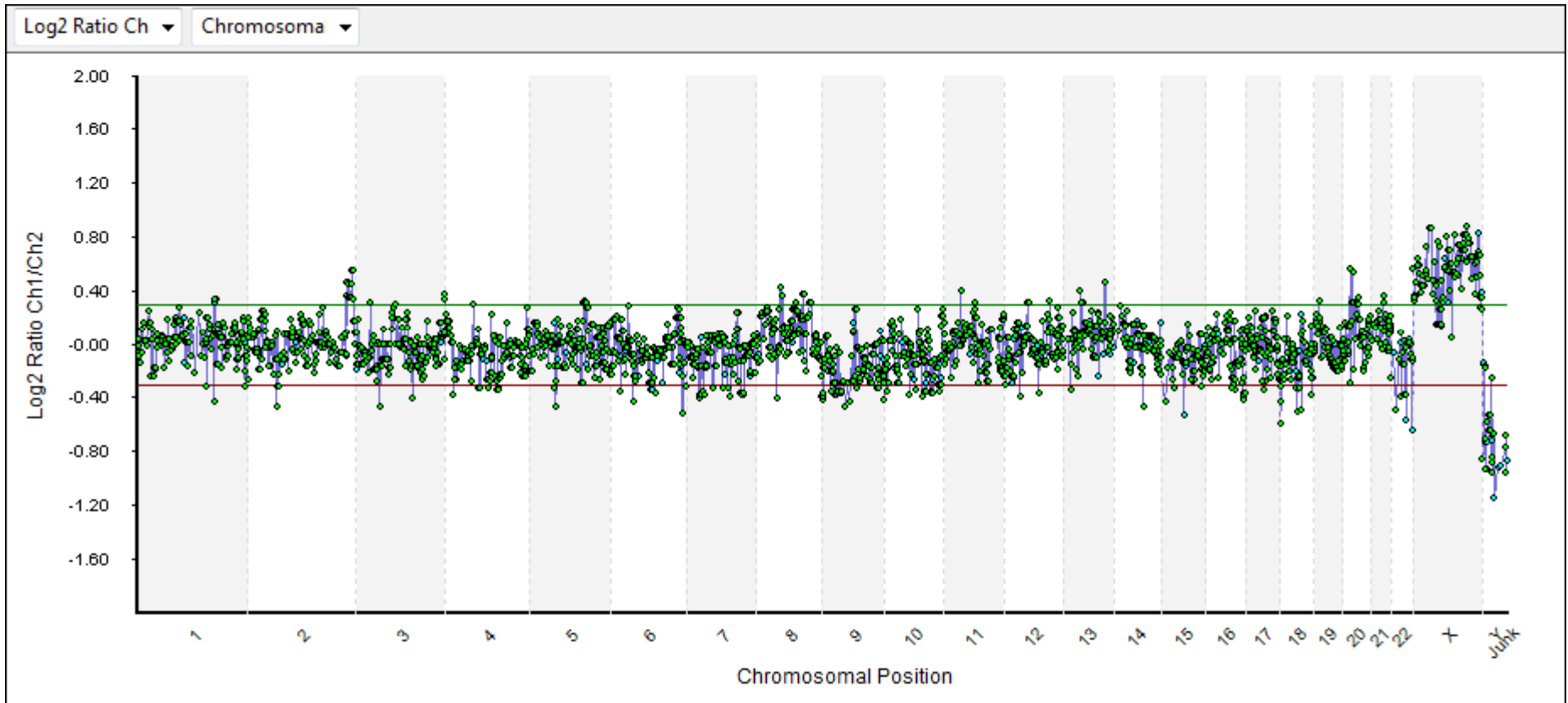


- Easy to QC
- Low noise
- Long protocol
- High ADO
- Confirmation of aneuploidy with other technologies (FISH, markers, Karyomapping, single cells of cell lines of known abnormality)

The background features several thick, curved lines in shades of light blue and grey, sweeping across the frame from the top and left towards the bottom right. The lines vary in thickness and curvature, creating a sense of motion and depth. The text is centered in the upper-middle portion of the image.

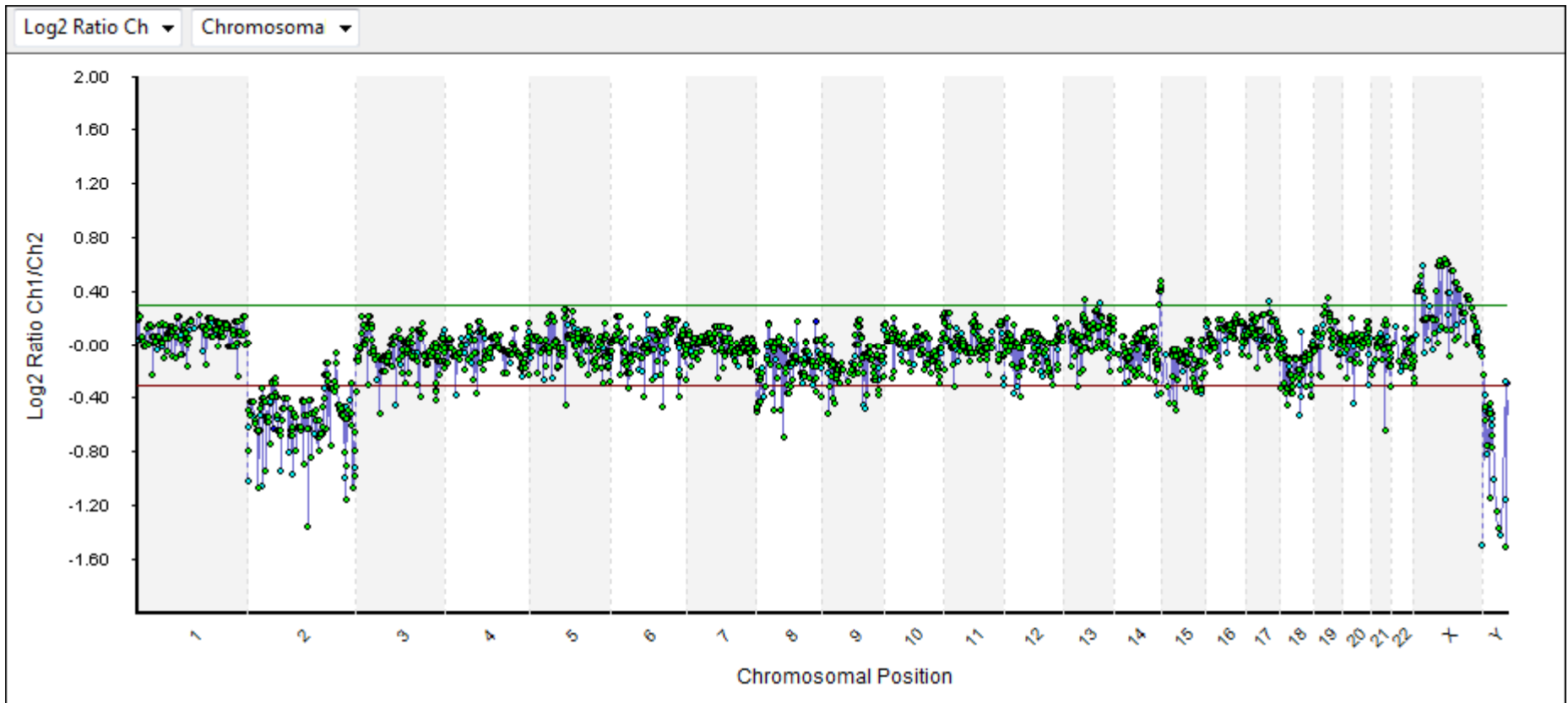
Sigma WGA4 – GenomePlex
Polar body samples

euploid

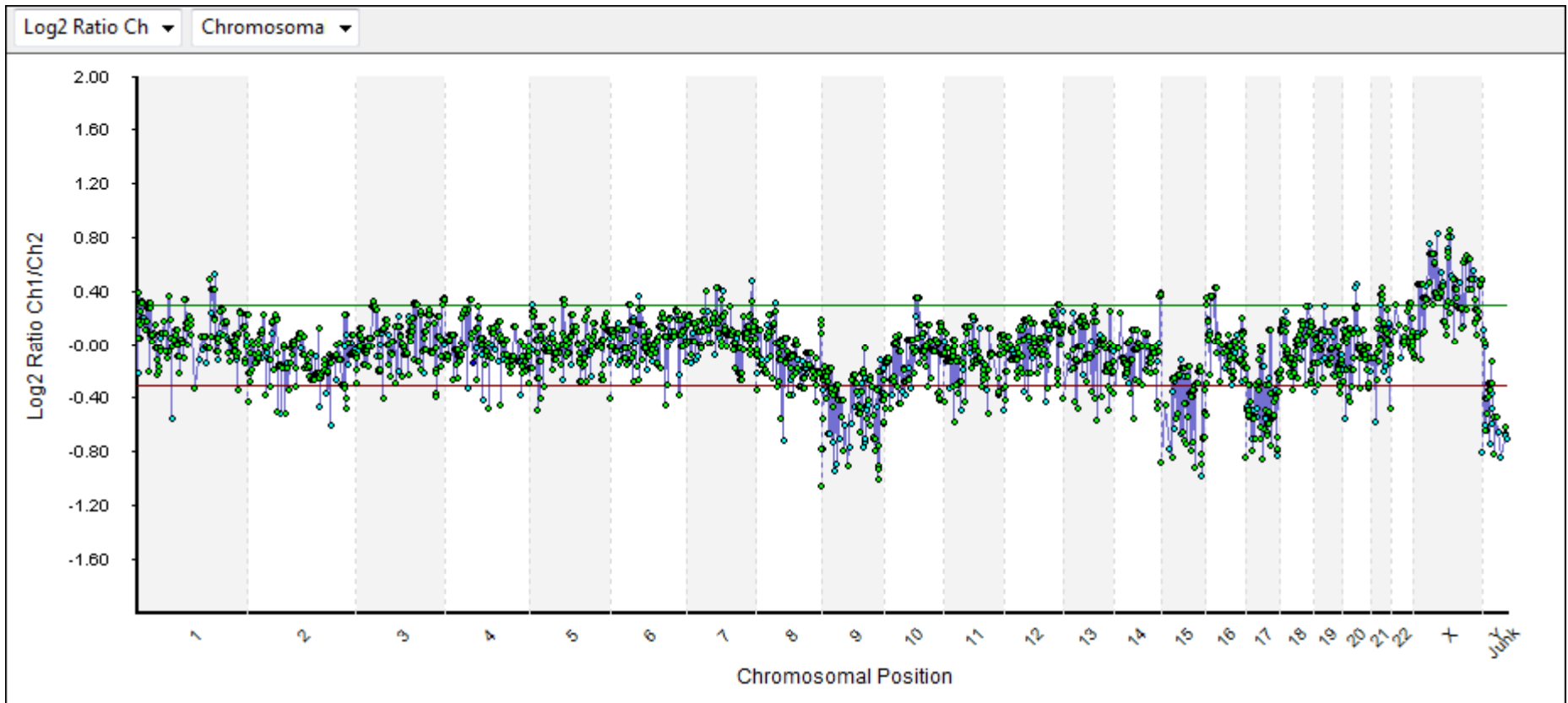


001024A - BG22121-03 - Cytochip Top Subarray - Fused Chart - 14/04/2009

aneuploid



005047A - BG25682-13 - Cytochip Top Subarray - Fused Chart - 14/04/2009



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%

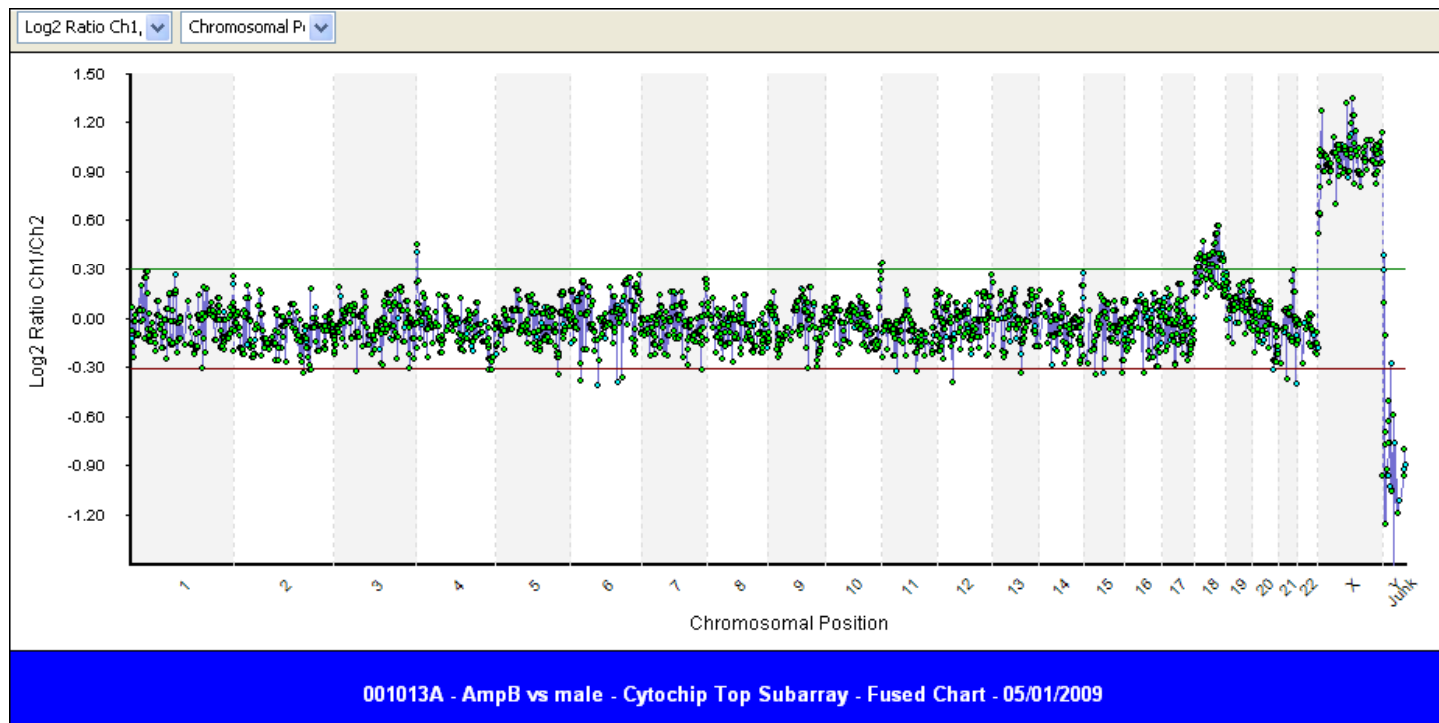
Aneuploid results reported for cells 56%
Percentage cases where all cells were aneuploid - 19%

Successful hybridizations per amplified product 94%

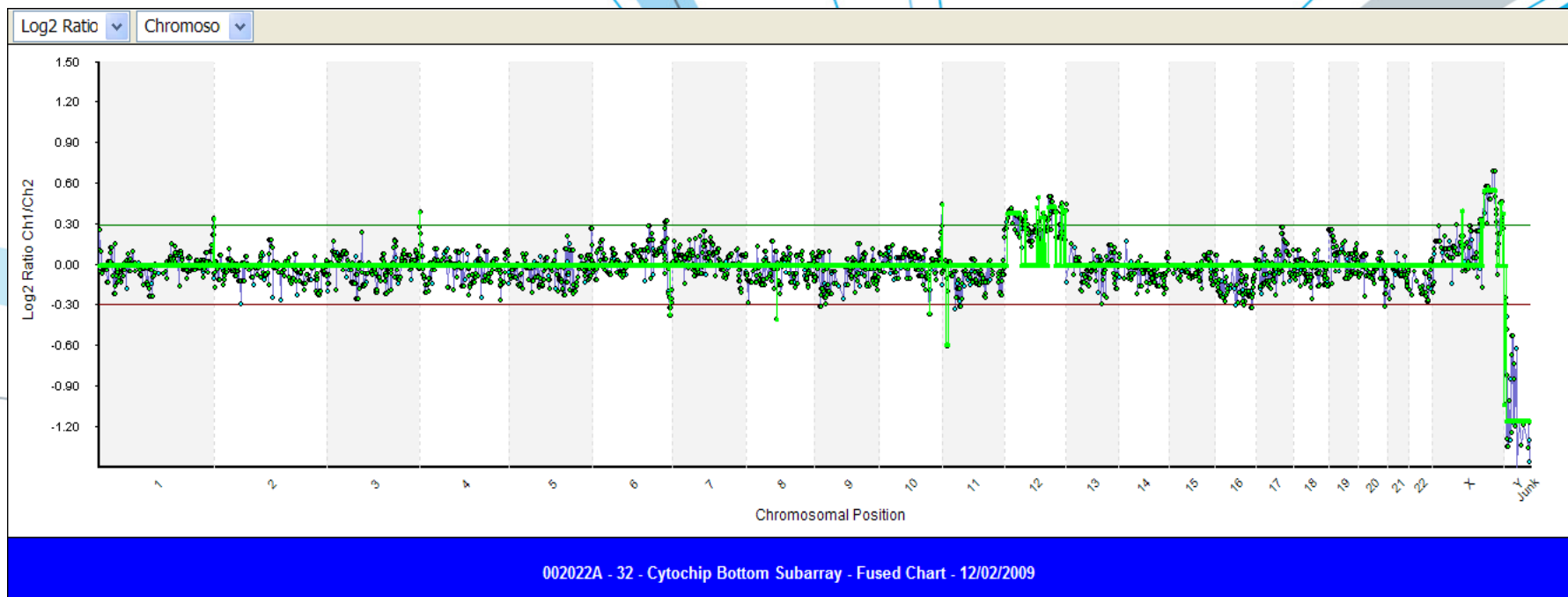
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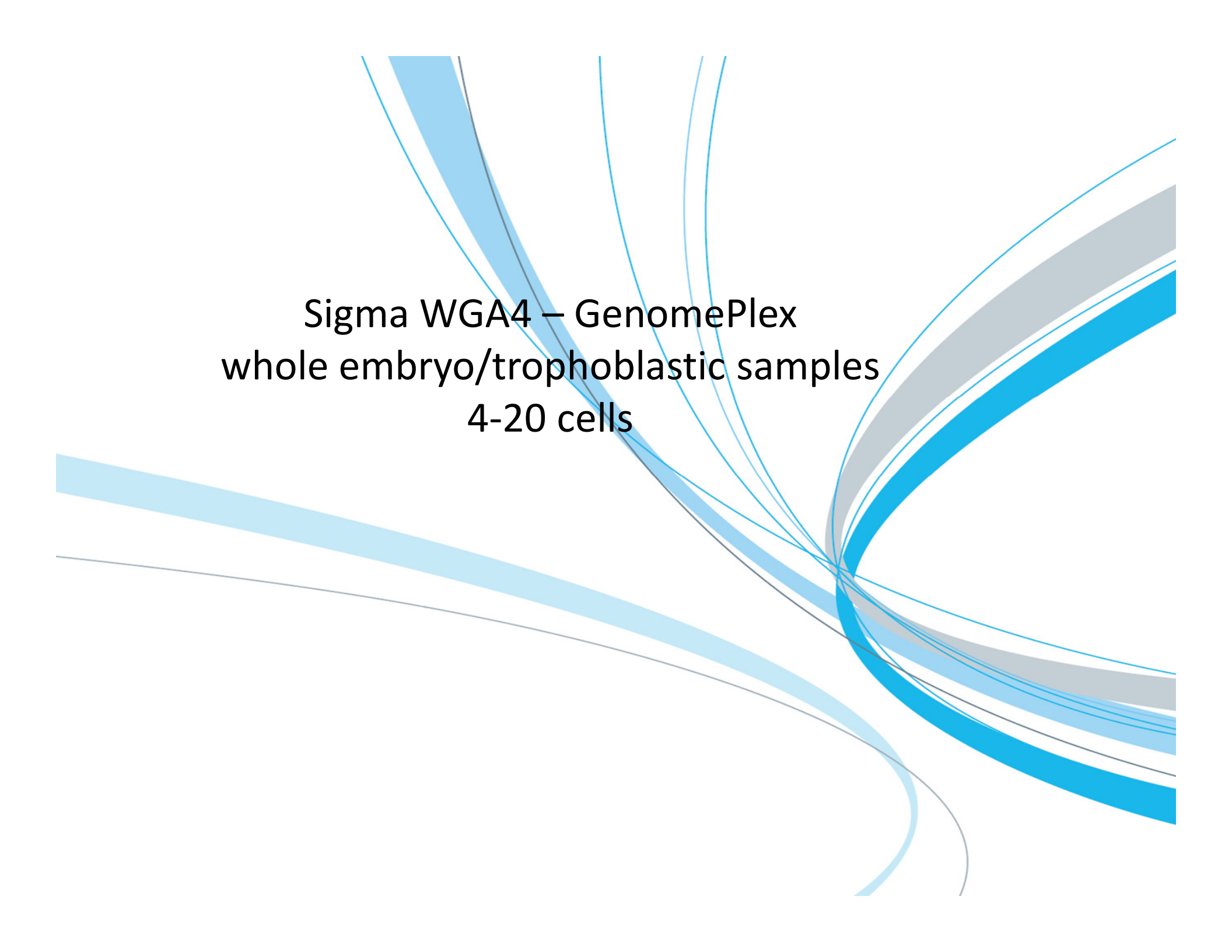
Sigma WGA4 – GenomePlex
Blastomere samples

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



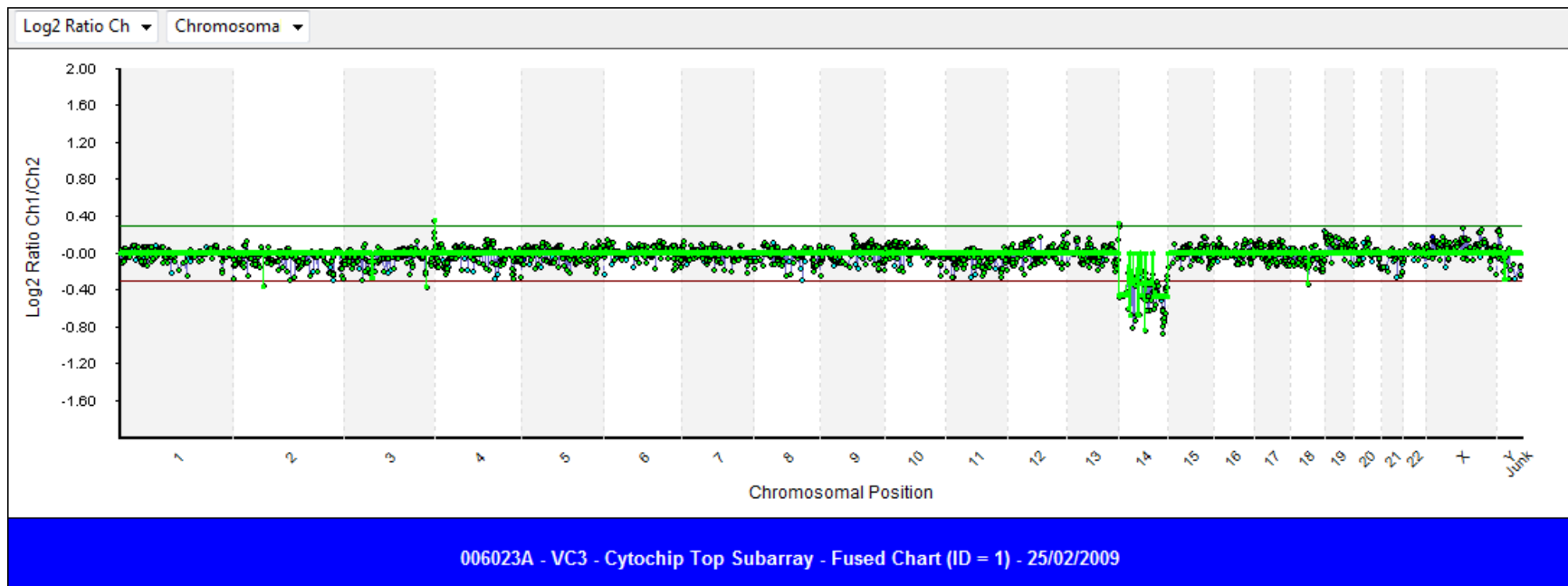
Single cell showing Trisomy chr12, duplication ~25Mb chrXq



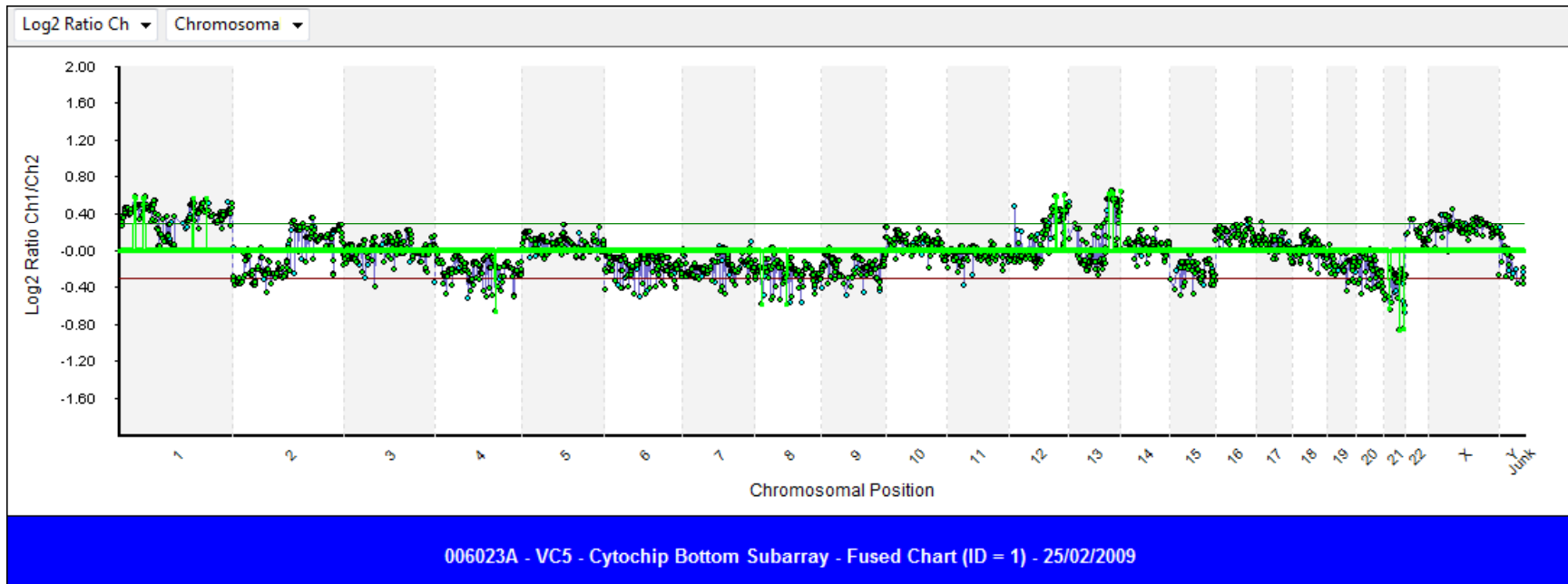
The background features several thick, curved lines in shades of blue and grey, sweeping across the page from the top and left towards the bottom right. The lines vary in thickness and color, creating a dynamic, abstract composition.

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

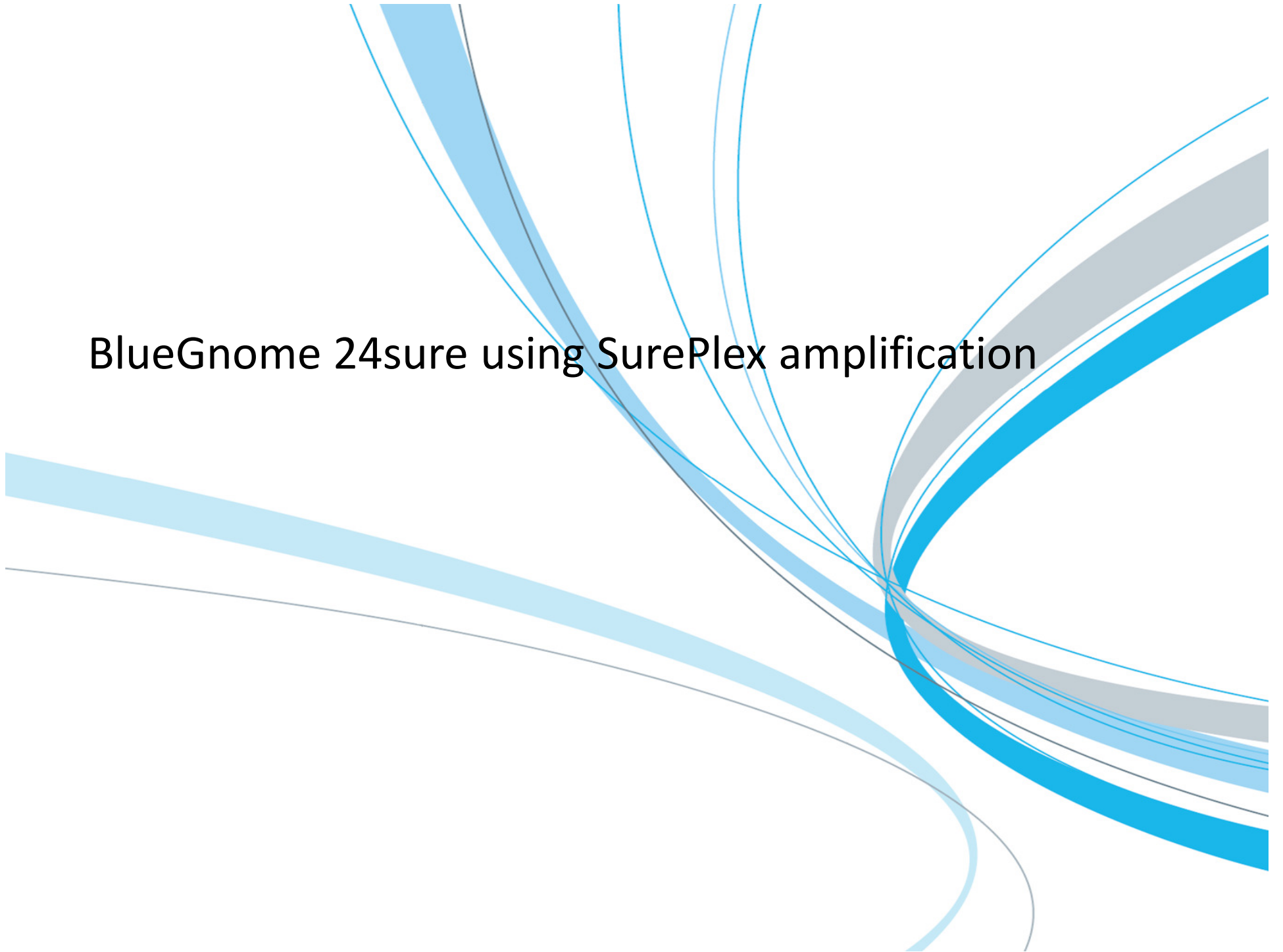
Multiple cell biopsy



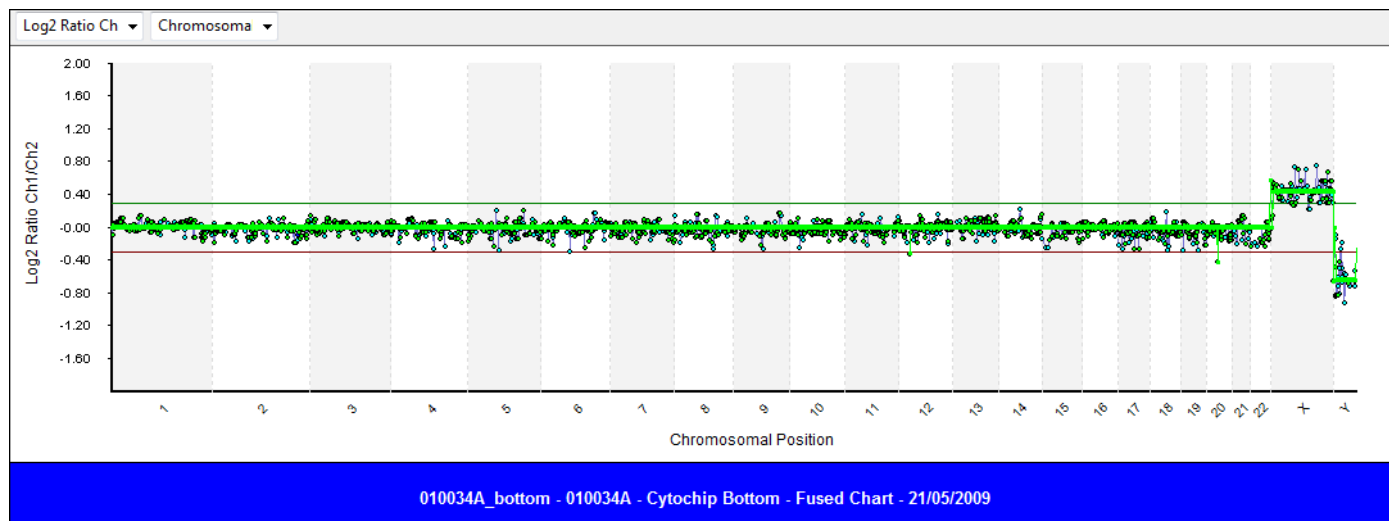
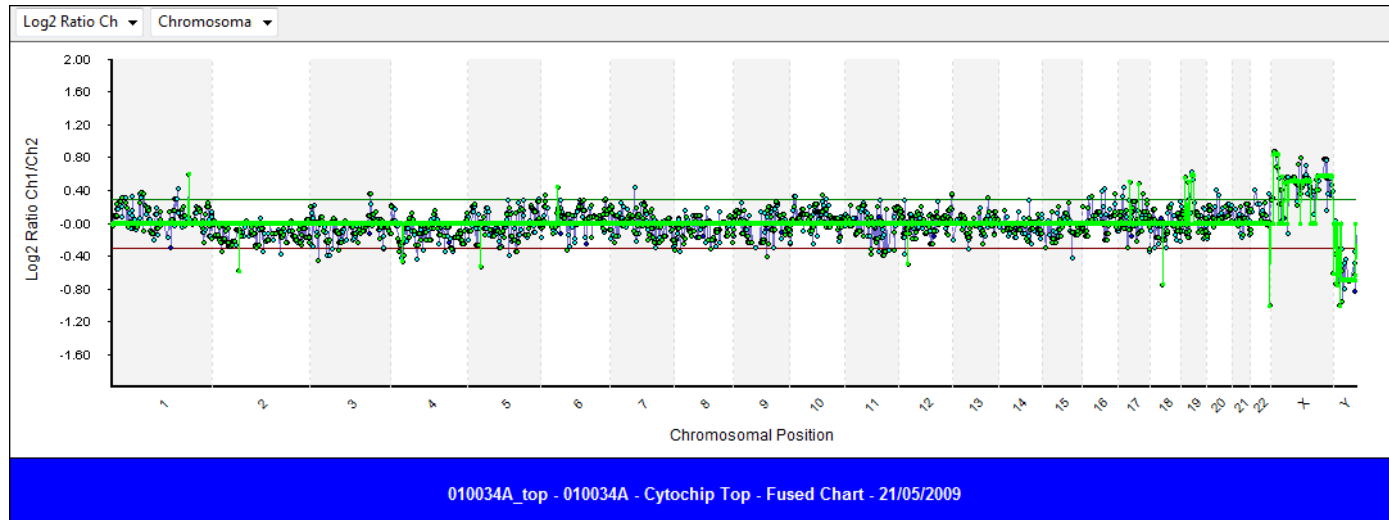
Multiple cell biopsy



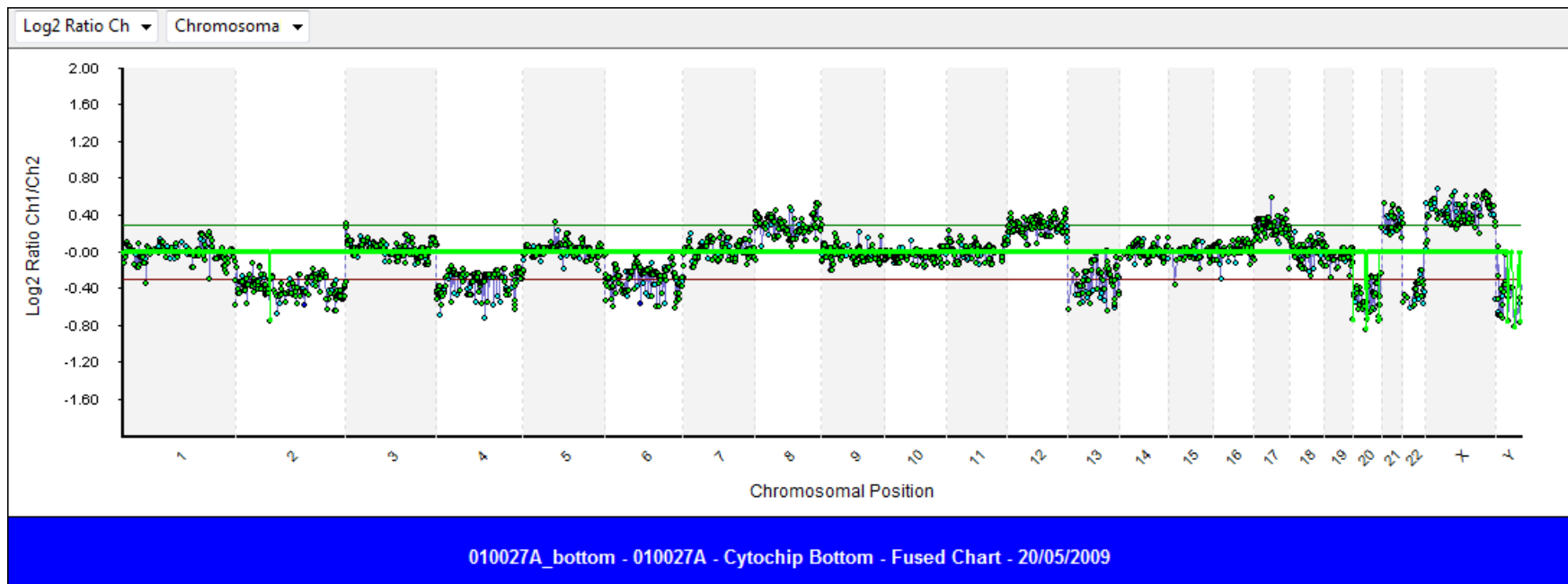
BlueGnome 24sure using SurePlex amplification



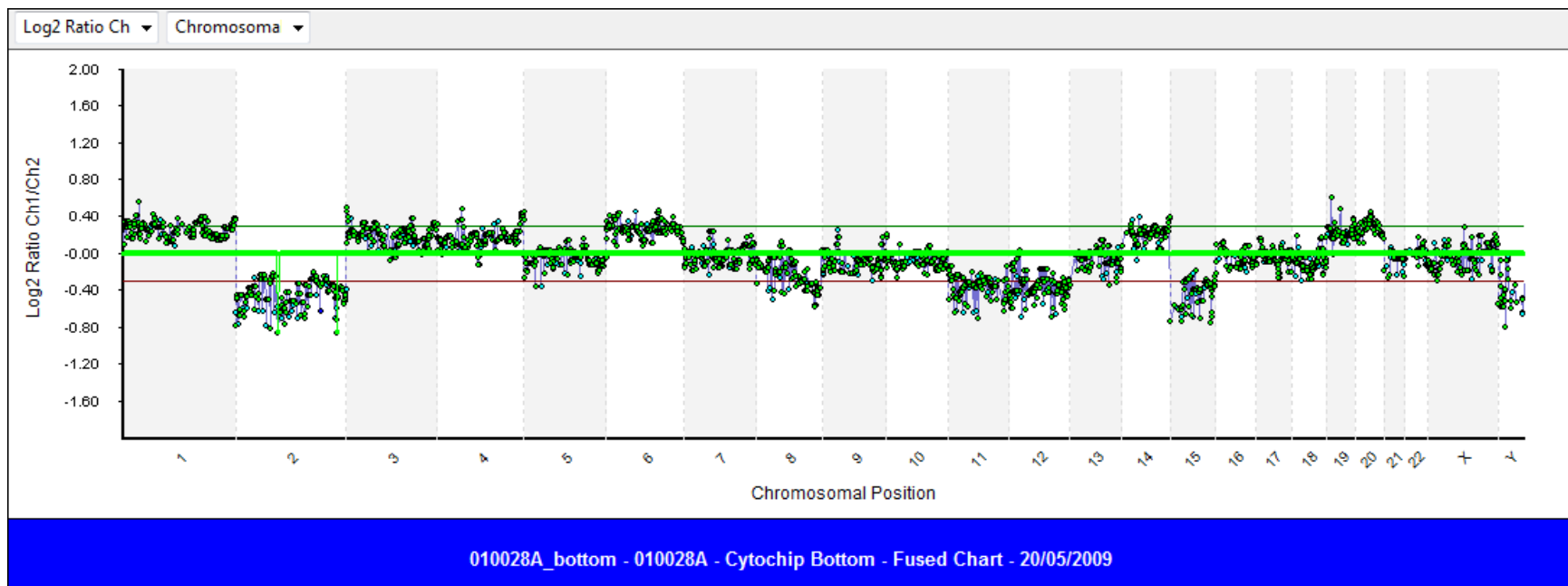
15pg Sureplex female sample – top array
Promega DNA / bottom array SurePlex
amplified control DNA



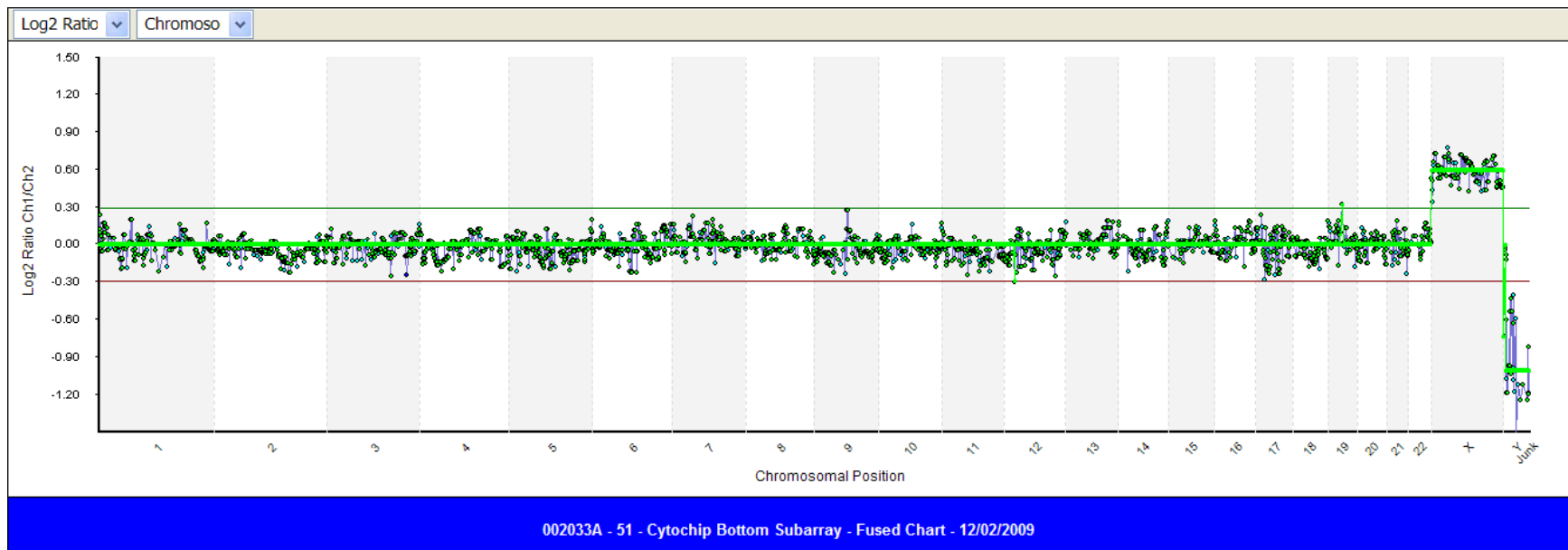
SurePlex - Blastomere single cell sample



SurePlex - Blastomere single cell sample

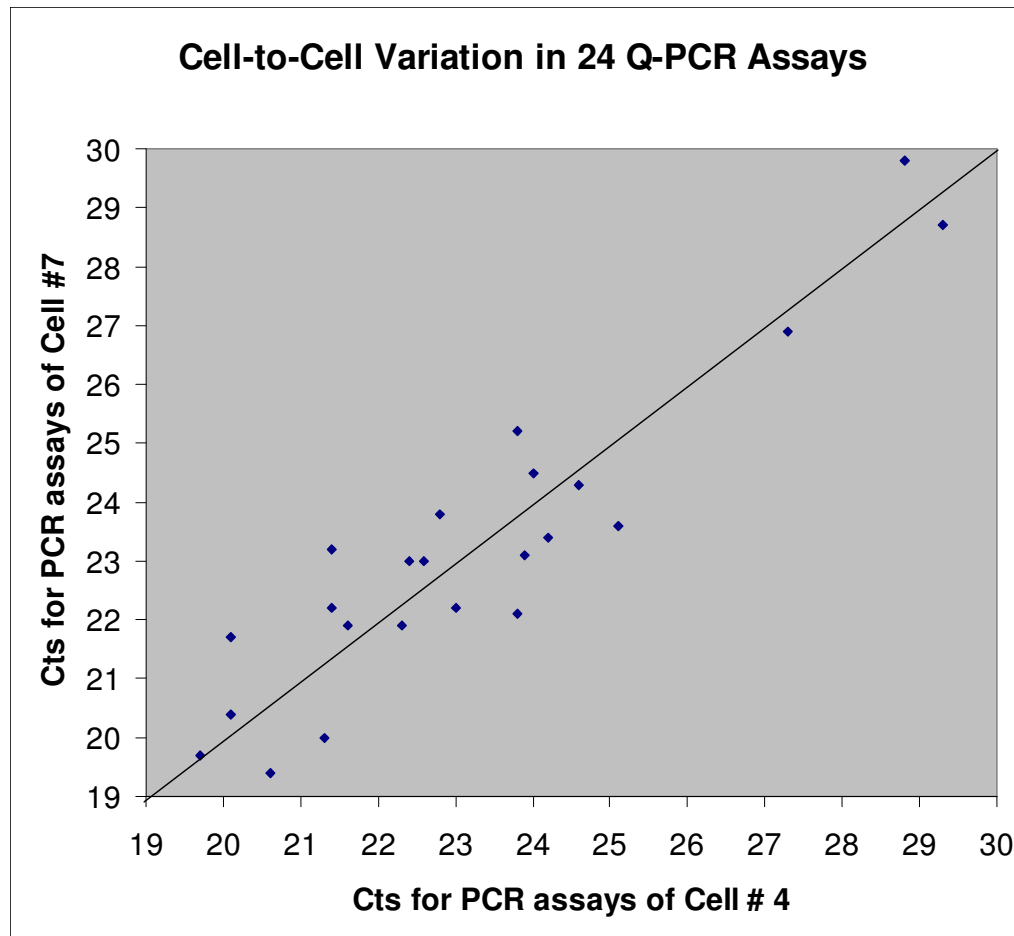


SurePlex - Blastomere single sample



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

Reproducibility of SurePlex



Results courtesy of Rubicon Genomics

ADO of SurePlex



	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

- 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

24sure

pre-implantation chromosomal aneuploidy screening