

Implementation of Preimplantation Genetic Screening using 24sure™

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Background

Cheshire and Merseyside Regional Genetics Service

- Serves a population of around 2.8 million people across Cheshire, Merseyside and Isle of Man
- Based at the Liverpool Women's Hospital, one of the UK's leading maternity and Neonatal units and renowned NHS Centre of Excellence in Gynaecology and Genetics services.

Hewitt Centre for Reproductive Medicine

- Includes partners North West Fertility
- Average of 3000 treatment cycles per year (NHS and Private)
- Europe's largest single NHS IVF unit
- Main treatments include - IVF, ICSI and IUI

Why PGS?

Clinical need:

- **<10% oocytes result in delivery** (Fertility and Sterility Vol 91(4):1061-66)
- **Up to 70% of embryos are non viable** (Fertility and Sterility Vol 87(3):1033-40)
- **Up to 85% of eggs are non viable** (Fertility and Sterility Vol 87(3):1033-40)
- **Miscarriage risk increases with MA**
- **Chance of chromosomal aneuploid increases with MA**

Objective assessment of viability:

- **Morphological assessment**
- **FISH analysis of 5-9 chromosomes tried but reduced use due to clinical trials reporting limited benefit (Link: [doi:10.1093/humrep/dep476](https://doi.org/10.1093/humrep/dep476))**

Clinical opportunities:

- **To increase live birth rate**
- **To improve single egg transfer efficiency**
- **Patient choice**

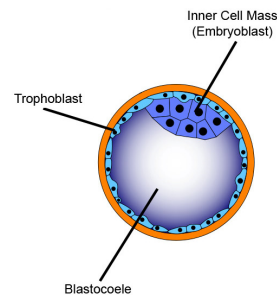
Aims of Service

- **To validate for diagnostic use the 24sure (Bluegnome, Cambridge) platform**
 - Allows the aneuploidy screening for whole chromosome changes
 - Analysis of single cells (Polar Bodies, Blastocyst) and multiple cells (Trophectodermal)
- **Test Characteristics**
 - Easily interpretable – No CNV
 - Rapid, next day result possible
 - Allows transfer of fresh embryos

Polar Body



Blastocyst

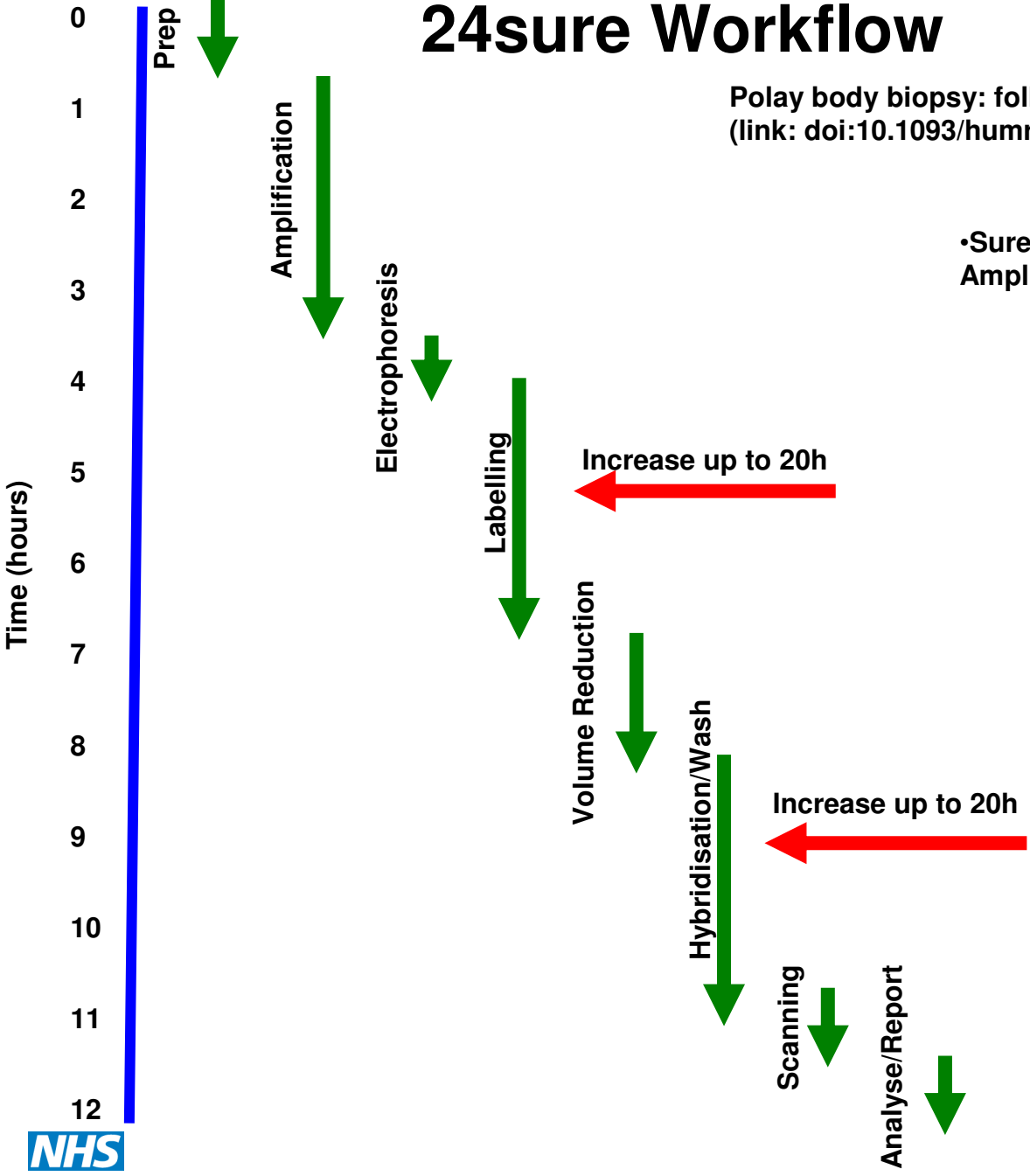


Polar body biopsy



24sure Workflow

Polay body biopsy: follow ESHRE best practice
(link: doi:10.1093/humrep/deq265)



- Sureplex Whole Genome Amplification
 - Starts with 12pg -> 2-10ug
 - Low allele drop out
- Labeling and Hybridization
 - BAC array protocol manual method
- Washing, Scanning and Analysis
 - Analysis using BlueFuse Multi

Polar Bodies in PGS

- **Advantages**
 - **No mosaicism**
 - **Easy to biopsy**
 - **The chromosomal content is reflective of the egg**
 - **Non-invasive, it does not disrupt the chromosomes of the egg itself**

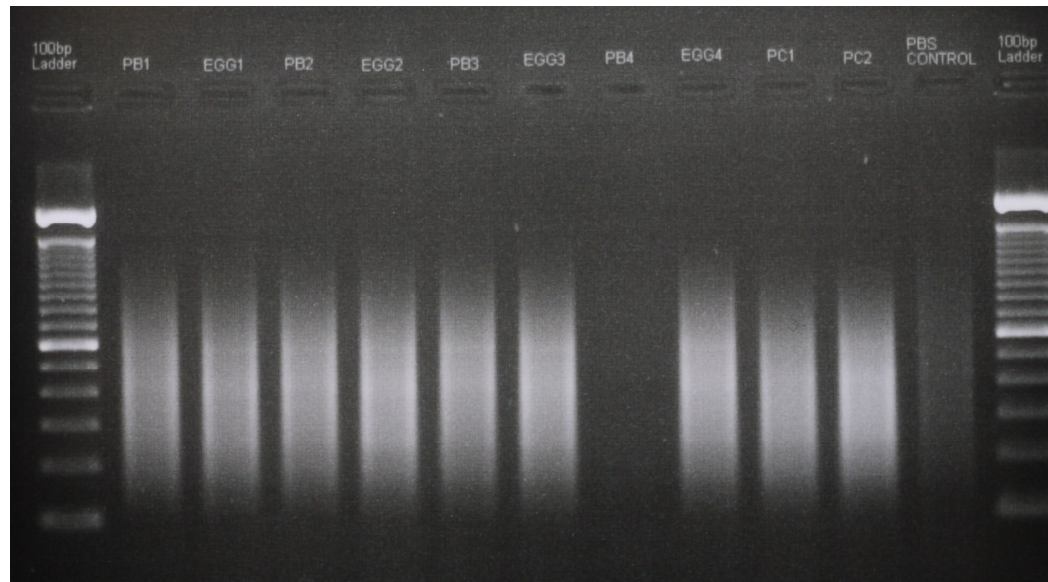
- **Disadvantages**
 - **Maternal analysis only**
 - **No post mitotic analysis**
 - **Test both polar bodies?**

SurePlex Whole Genome Amplification

Polar Bodies and Eggs amplified

Positive controls (Female reference DNA)

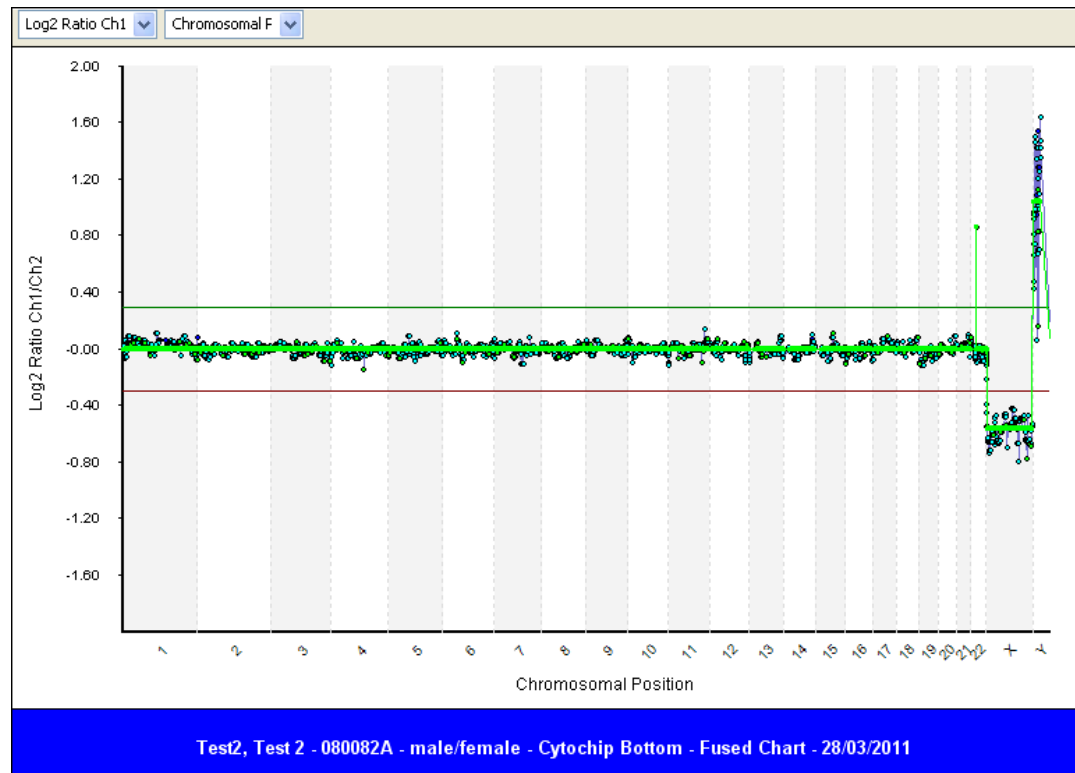
Negative control (PBS)



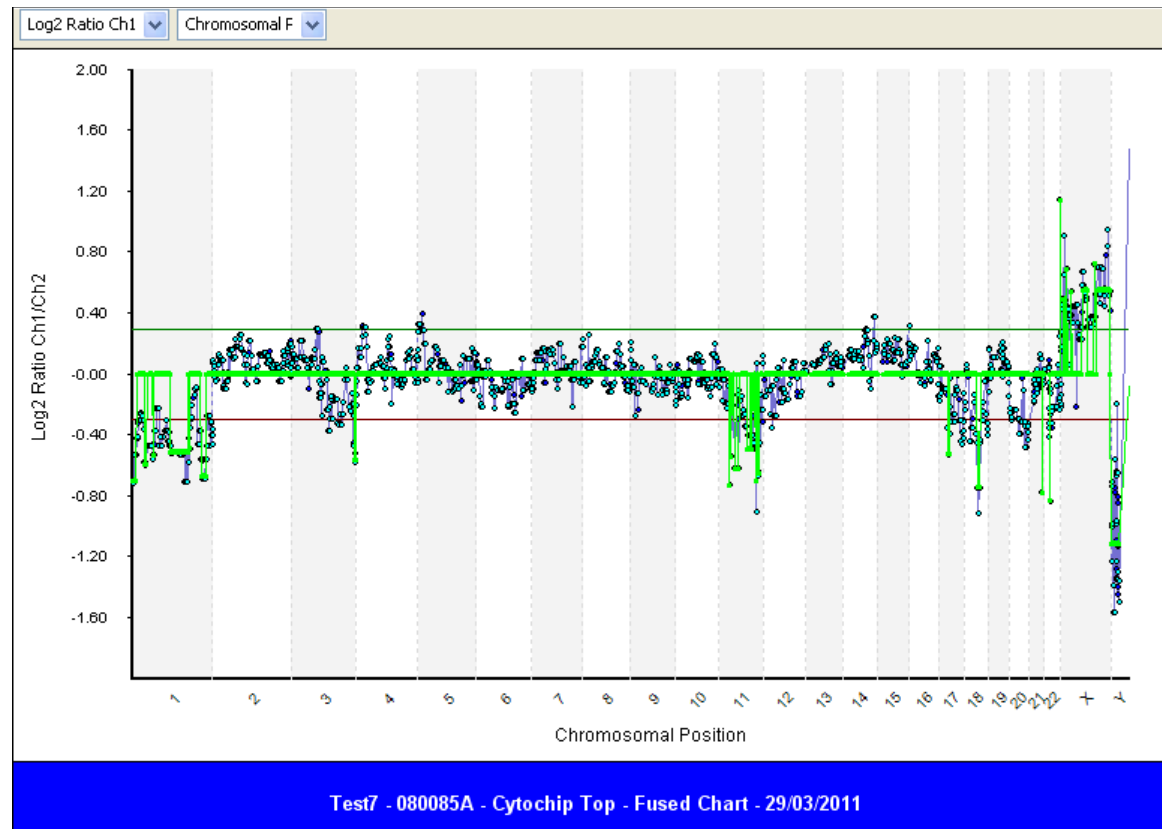
Polar body (PB4) fragmented before biopsy

Results to date

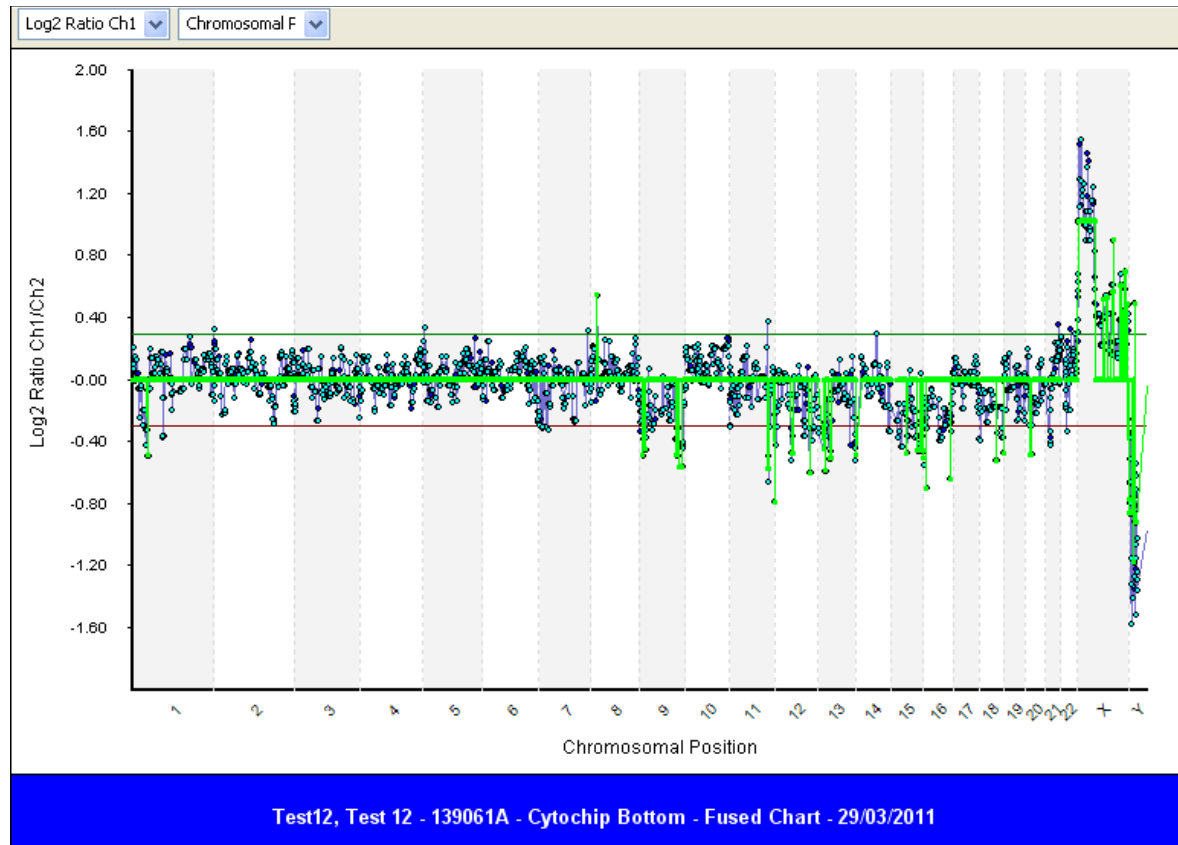
- Initial work - Dye swap experiment with amplified Female Ref DNA vs Male Ref DNA



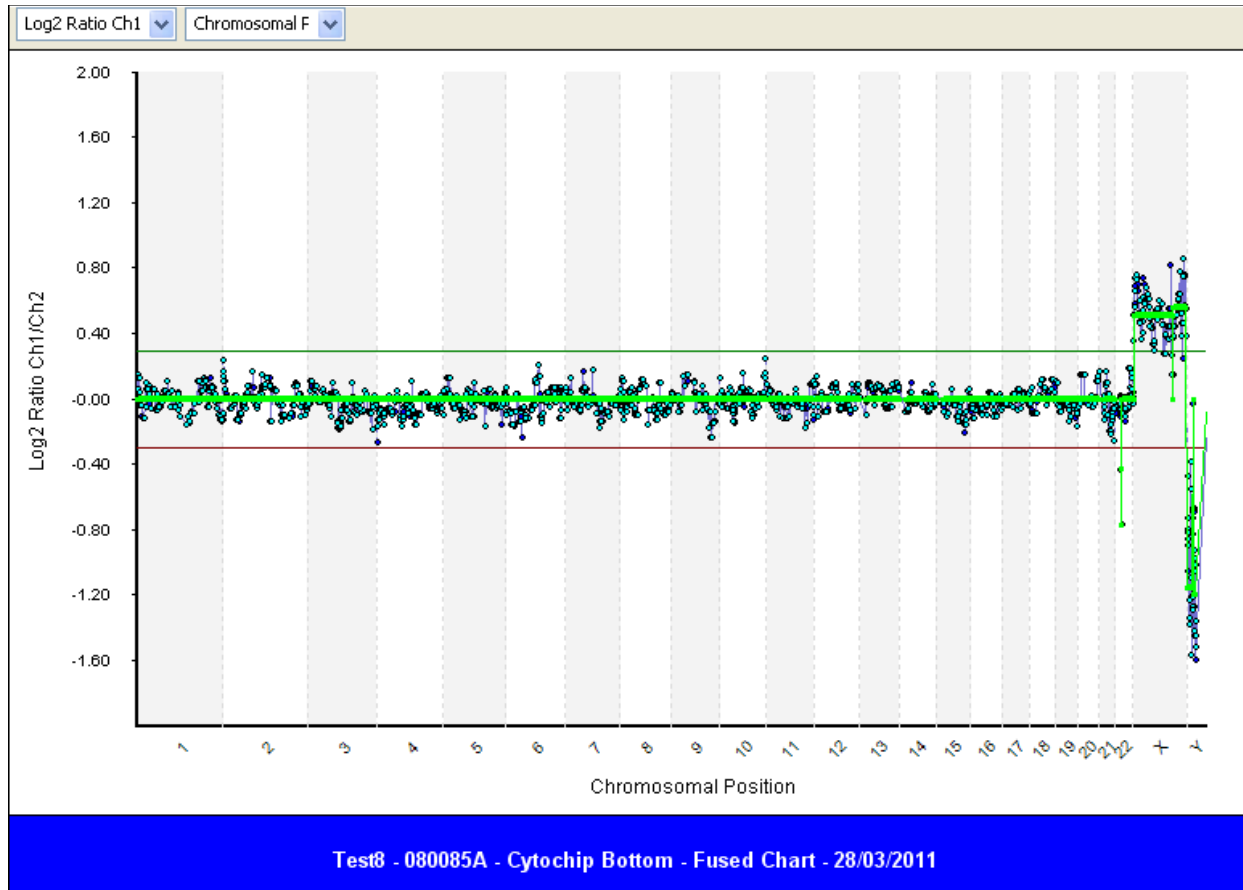
- **Polar Body – Failed QC due to low % clone inclusion**



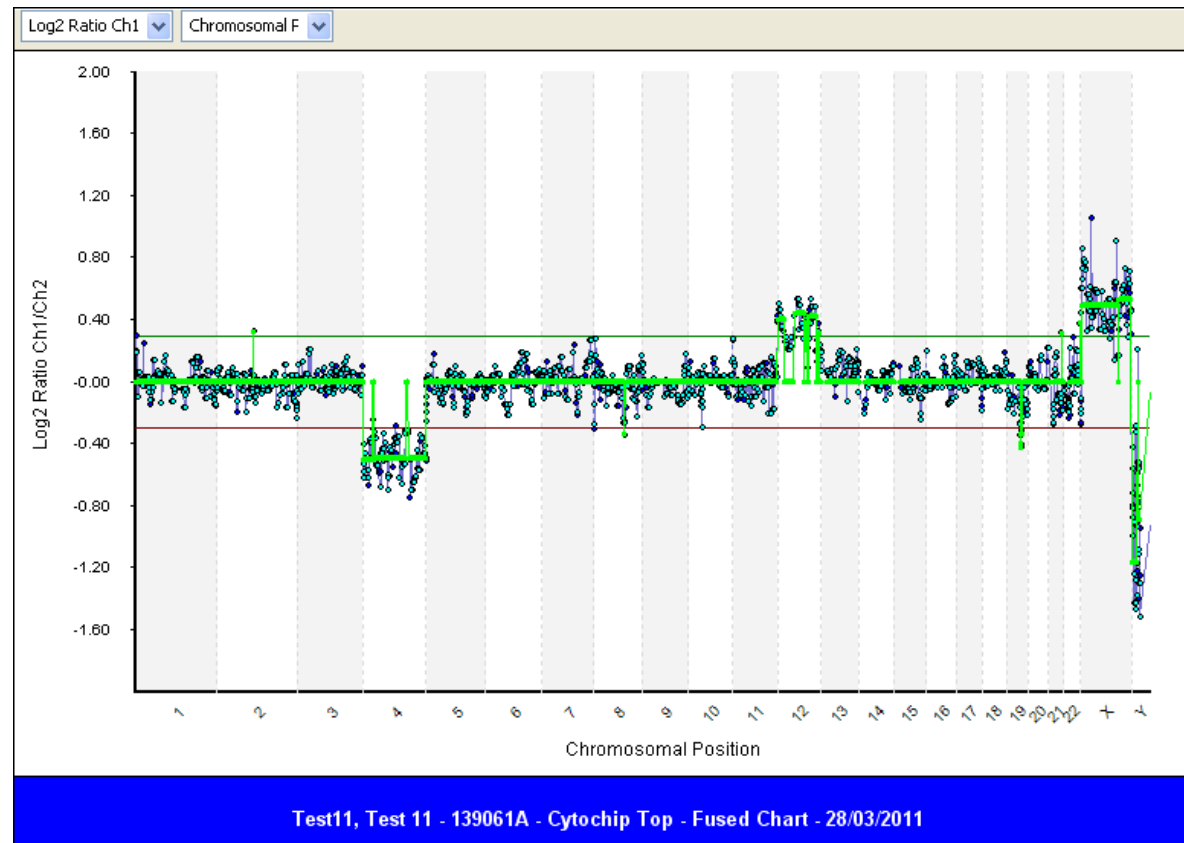
- **Polar Body – Failed QC due to poor DLR fused score**



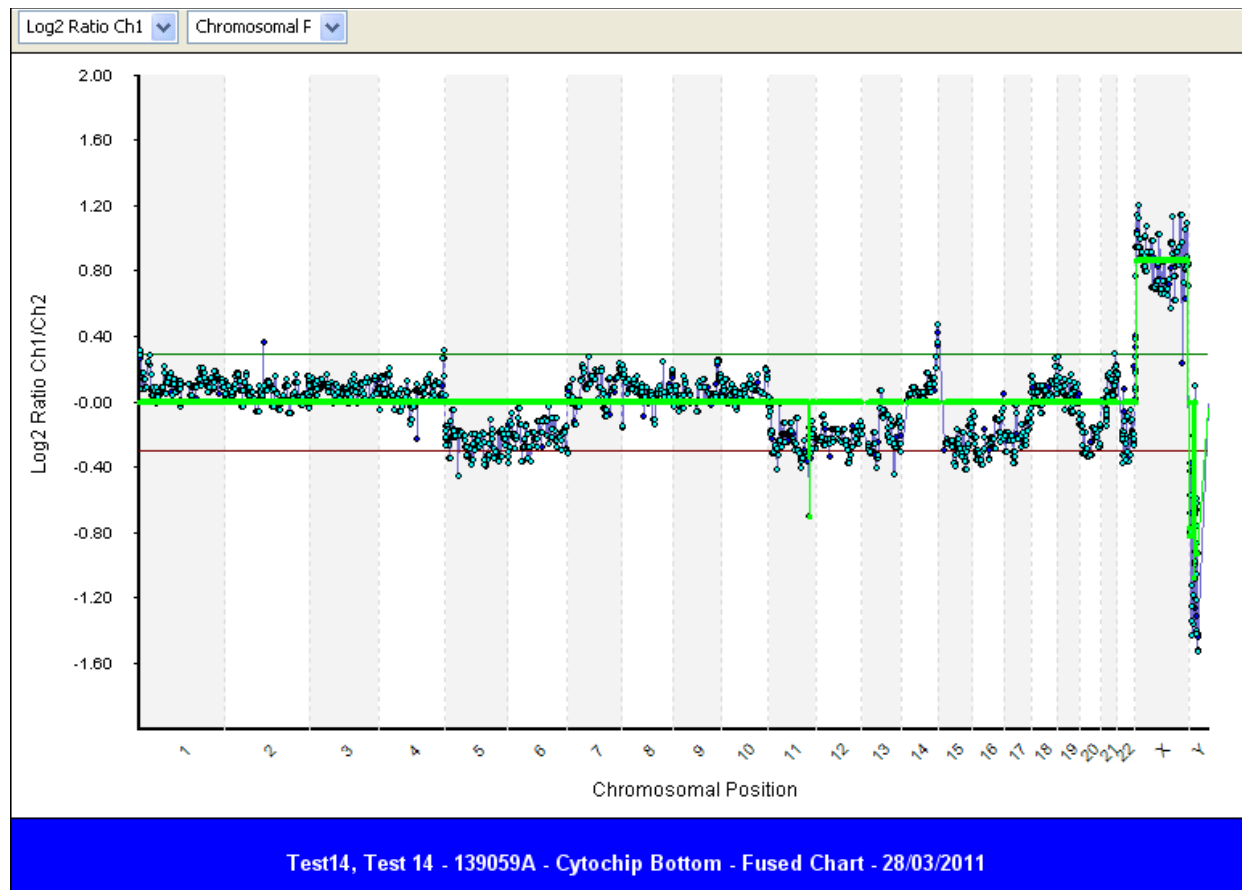
- Normal polar body



- **Abnormal polar body – Loss of chr4 and gain of chr12**



- **Polar Body – Borderline QC scores**



What is our end point?

Validated Protocol

- Consistent amplification of polar bodies with no contamination present
- Consistent labelling of cy3 and cy5 dyes and even hybridisation on array (good QC scores)
- Suitable analysis and reporting guidelines

Staffing

- Sufficient trained staff that can perform practical work, analyse, report, check and authorise reports

QMS

- Have a robust QMS in place to cover documentation, audit scheduling, QC, training issues and user satisfaction surveys

Summary

- **Successful biopsying of polar bodies**
- **Successful amplification of DNA from polar bodies**
- **Successful hybridisation to 24sure arrays**

Future work

- **Improve amplification success rate**
- **Improve QC scores of hybridisation**
- **Analyse previously identified abnormal cases from a “blind” database**
- **Ensure that the service to be offered is CPA and HFEA compliant**

Acknowledgements

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