

Arrays for clinical diagnosis: a diagnostic laboratory perspective

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Sourcing of appropriate positive and negative controls

- **Problem for all genetic labs**
- **Also EQA providers**

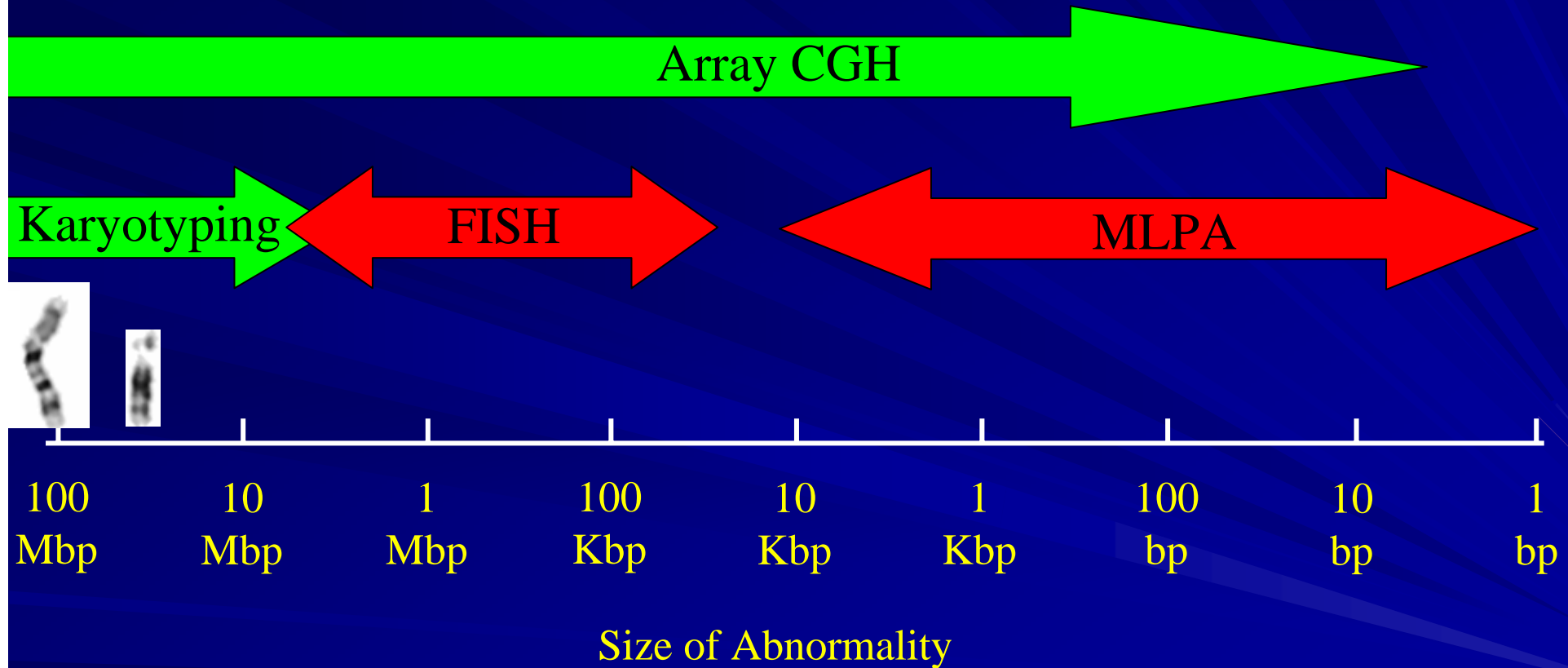
Need reference materials for:

- **Validation of test/methods**
- **New diagnostic tests**
- **Normal controls**
- **Rotating controls**
- **Rare disorders currently no positive controls**

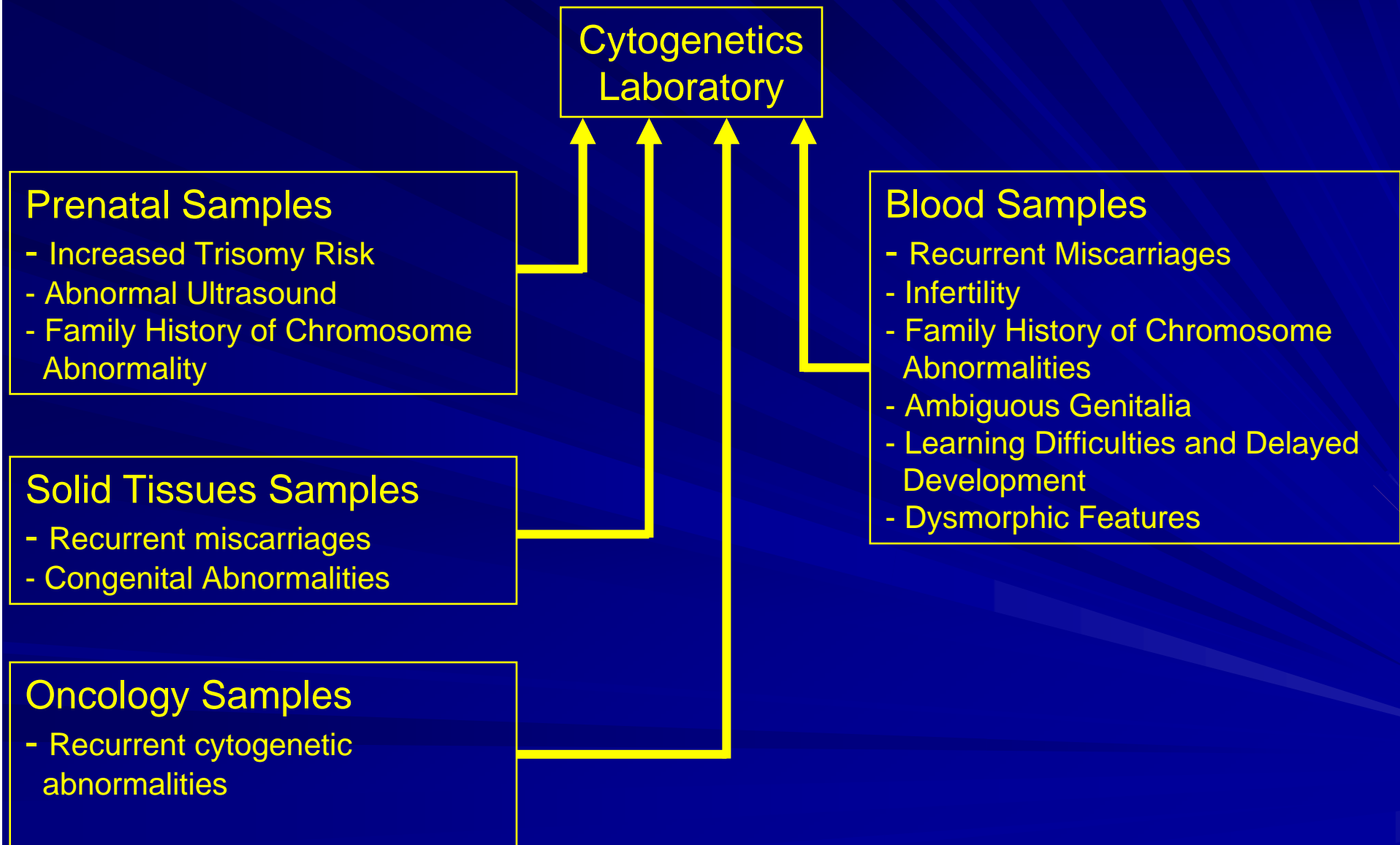
Cytogenetics

- Whole genome analysis
- Constitutional and Haemato-Oncology (leukaemias)
- New technologies e.g. MLPA, Microarrays
- Overlap of technologies

Chromosome Abnormalities



Referrals to Cytogenetics Laboratories



Referrals to Cytogenetics Laboratories

Cytogenetics Laboratory

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graph BT; A["Prenatal Samples - later?"] --> C["Cytogenetics Laboratory"]; B["Solid Tissues Samples"] --> C; D["Oncology Samples"] --> C; E["Blood Samples"] --> C;
```

Prenatal Samples – later?

- Increased Trisomy Risk
- Abnormal Ultrasound
- Family History of Chromosome Abnormality

Solid Tissues Samples

- Recurrent miscarriages
- Congenital Abnormalities

Oncology Samples

- Recurrent cytogenetic abnormalities

Blood Samples

- Recurrent Miscarriages
- Infertility
- Family History of Chromosome Abnormalities
- Ambiguous Genitalia
- Learning Difficulties and Delayed Development
- Dysmorphic Features

Key

Samples suitable for microarrays

Potential future sample group

How many laboratories?

700 cytogenetic labs in Europe

Cases per year in UK

- UK 2004 54,000 Blood samples a year
 - 34,000 Prenatal
 - 11,500 Diagnostic leukaemia (excludes follow up)
- 20% MCA/MR blood samples - Microarrays
 - 10,800 Normal control DNA

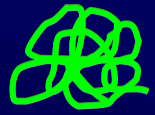
Microarray workload currently where technique established

- UK - 600 per year
- Dutch – 700 per year
- Belgium – 500 per year

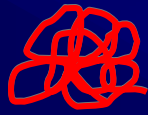
Controls within commercial kits?

Array Comparative Genome Hybridisation

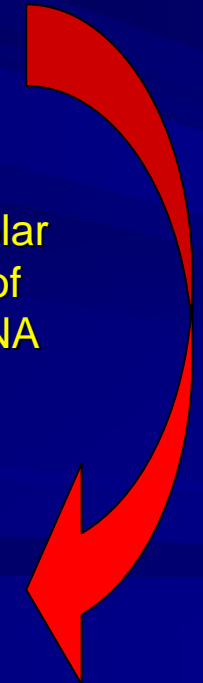
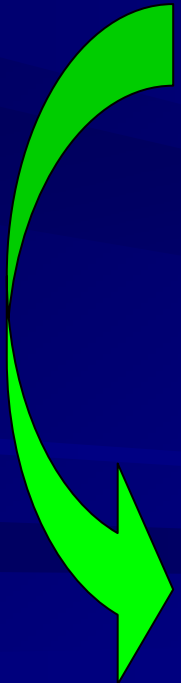
Patient DNA



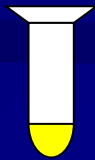
Normal Control DNA



Label DNA with different fluorescent dyes

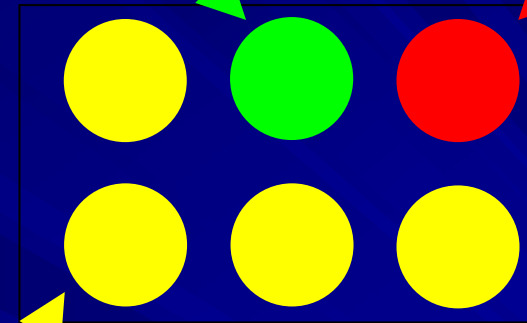


Mix equimolar amounts of labelled DNA

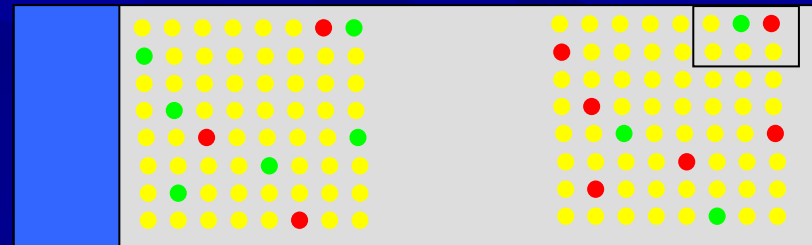


Patient:Control Ratio = 1.5:1
Duplication

Patient:Control Ratio = 0.5:1
Deletion



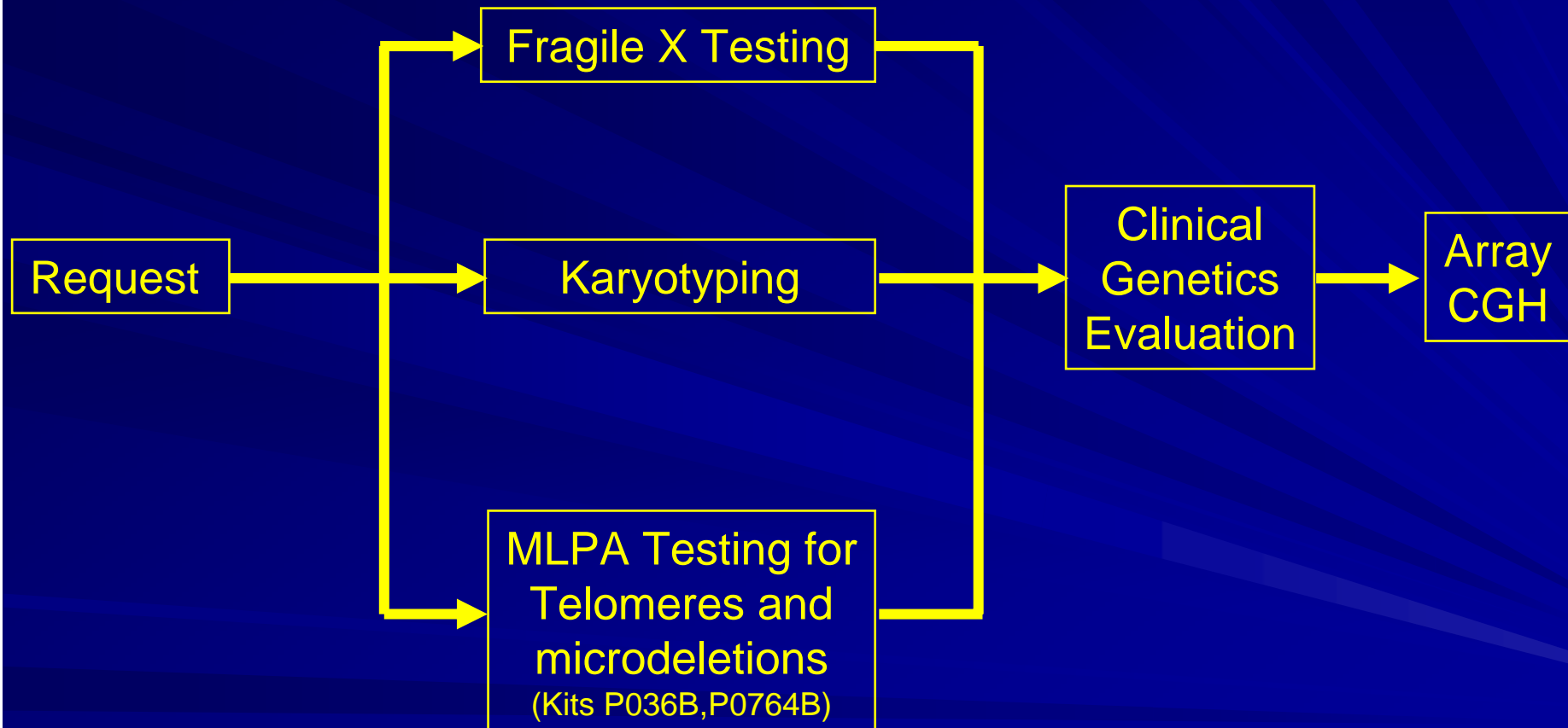
Patient:Control Ratio = 1



Apply DNA mix to glass slide with high-density array of different DNA probes with known location in the human genome

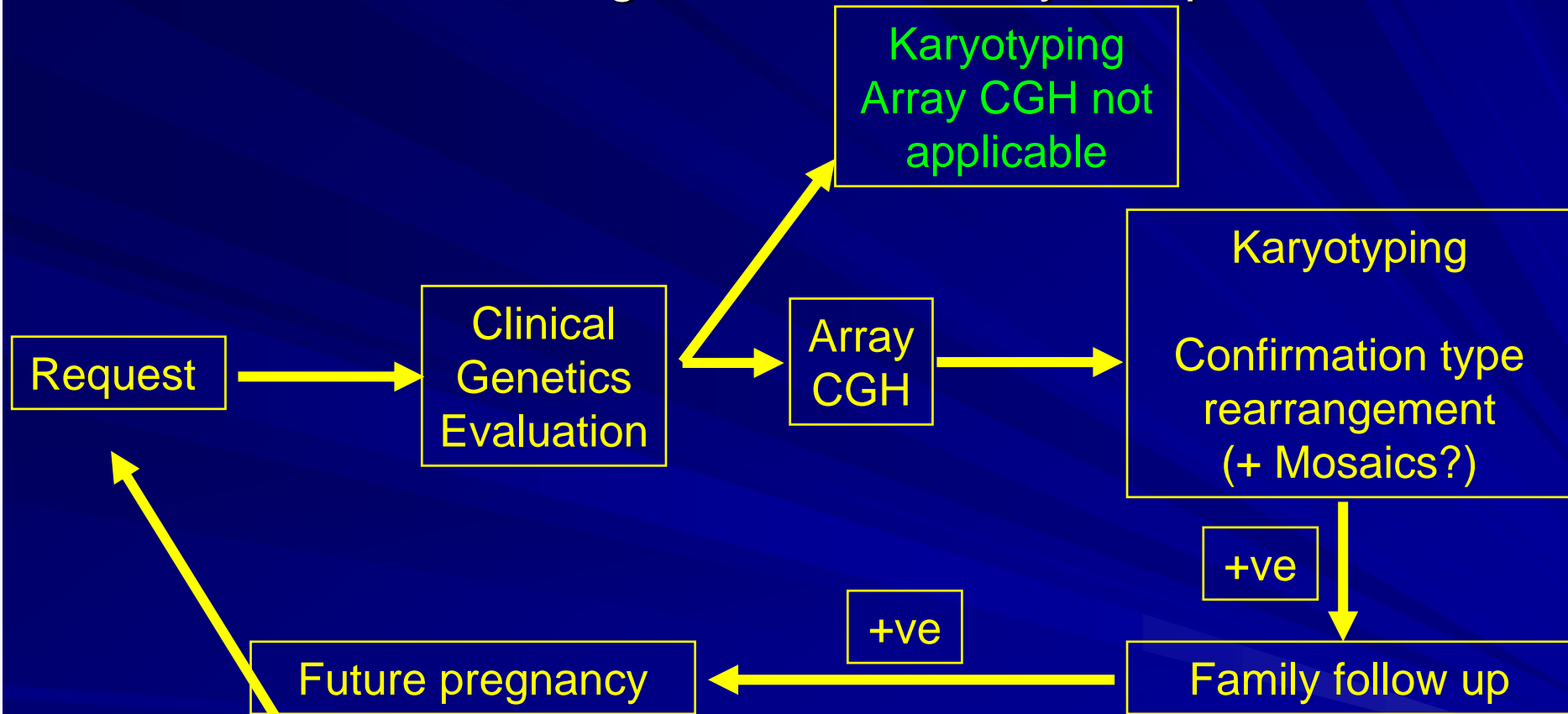
Screening Strategy

Patients with learning difficulties and dysmorphic features



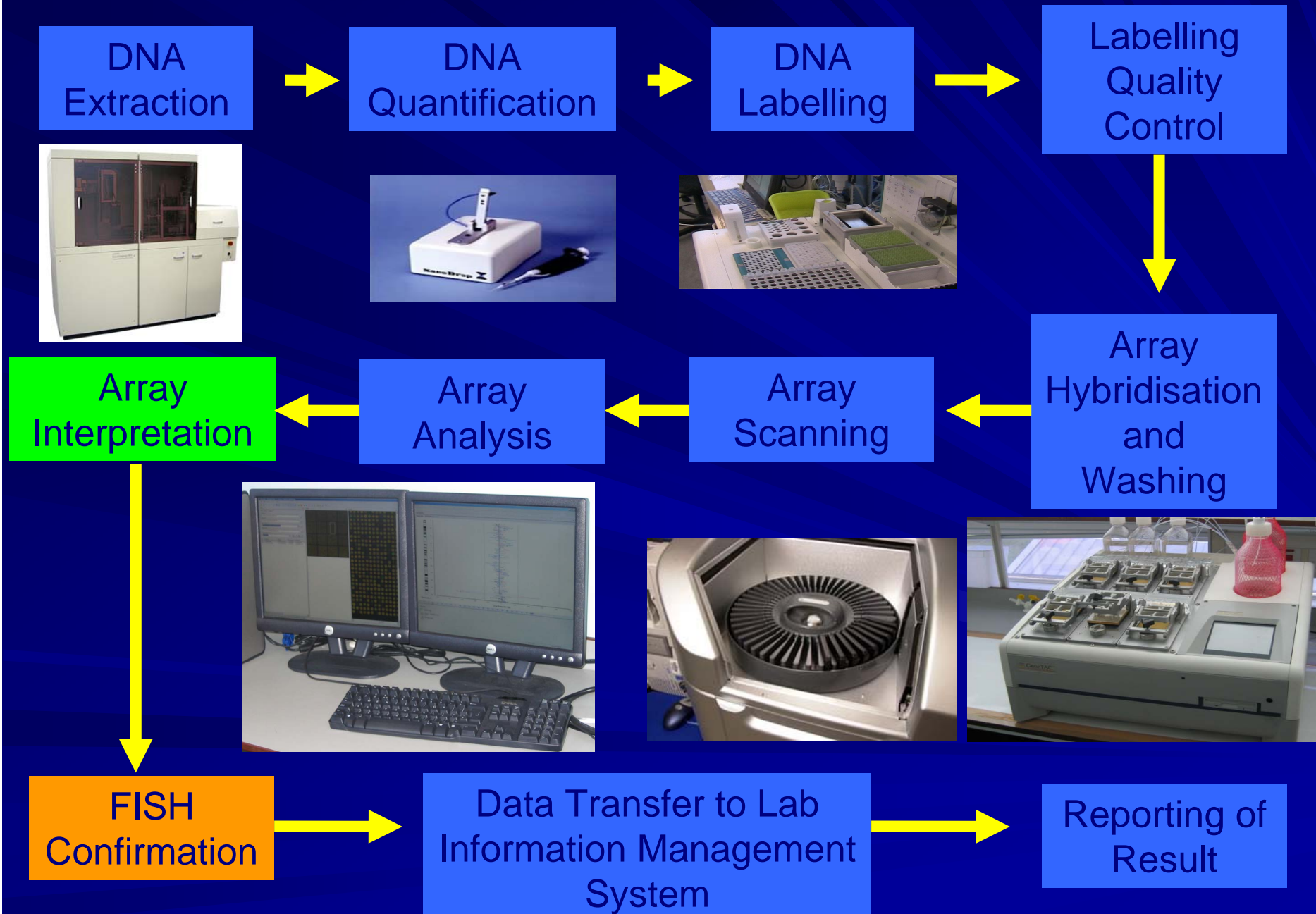
? Future Screening Strategy

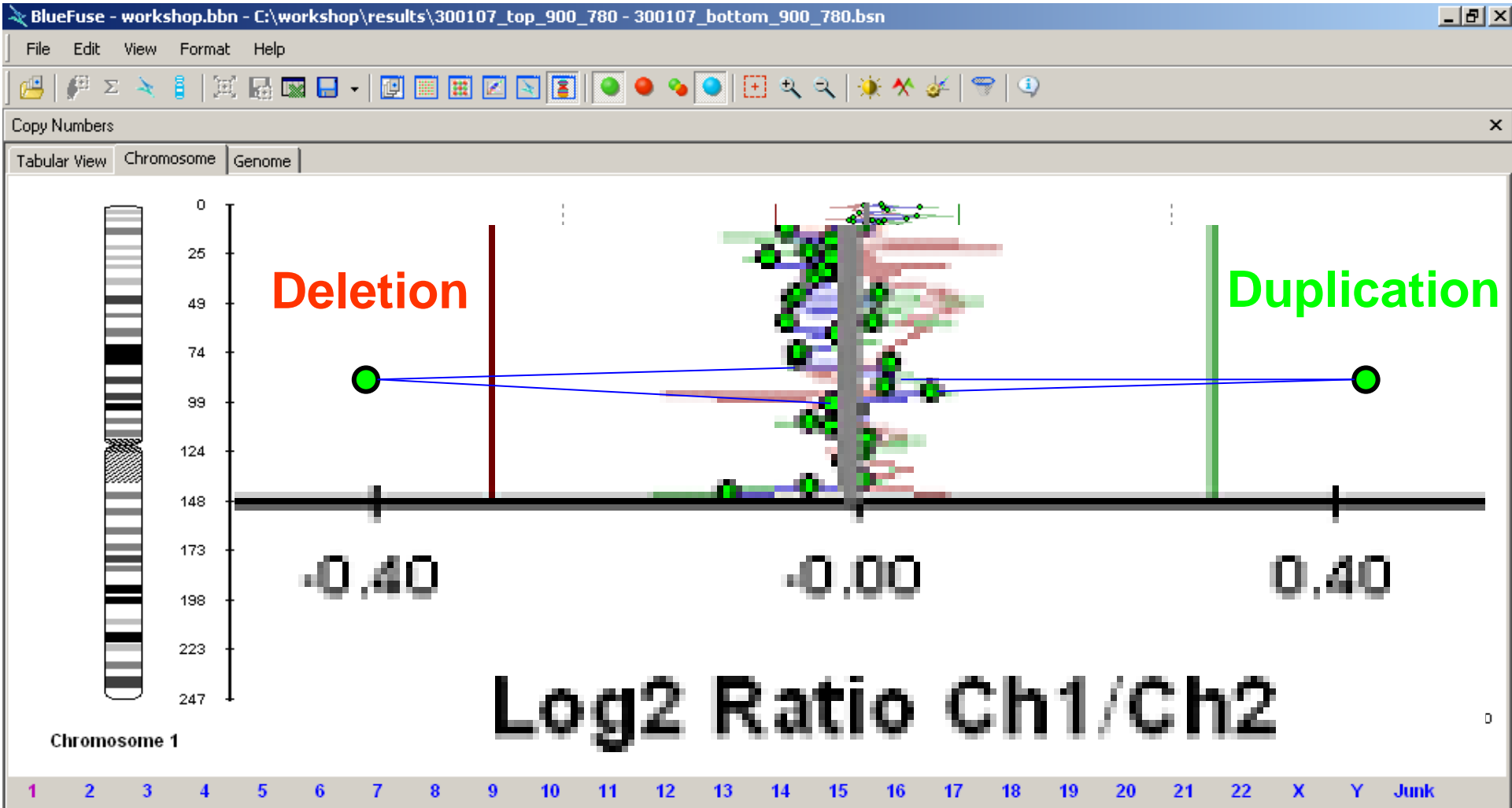
Patients with learning difficulties and dysmorphic features



Dependant on cost of microarrays and detection rate.

Array Workflow





Dye Swap



300107_top_900_780.bgn



300107_top_900_780 - 300107_bottom_900_780.bsn

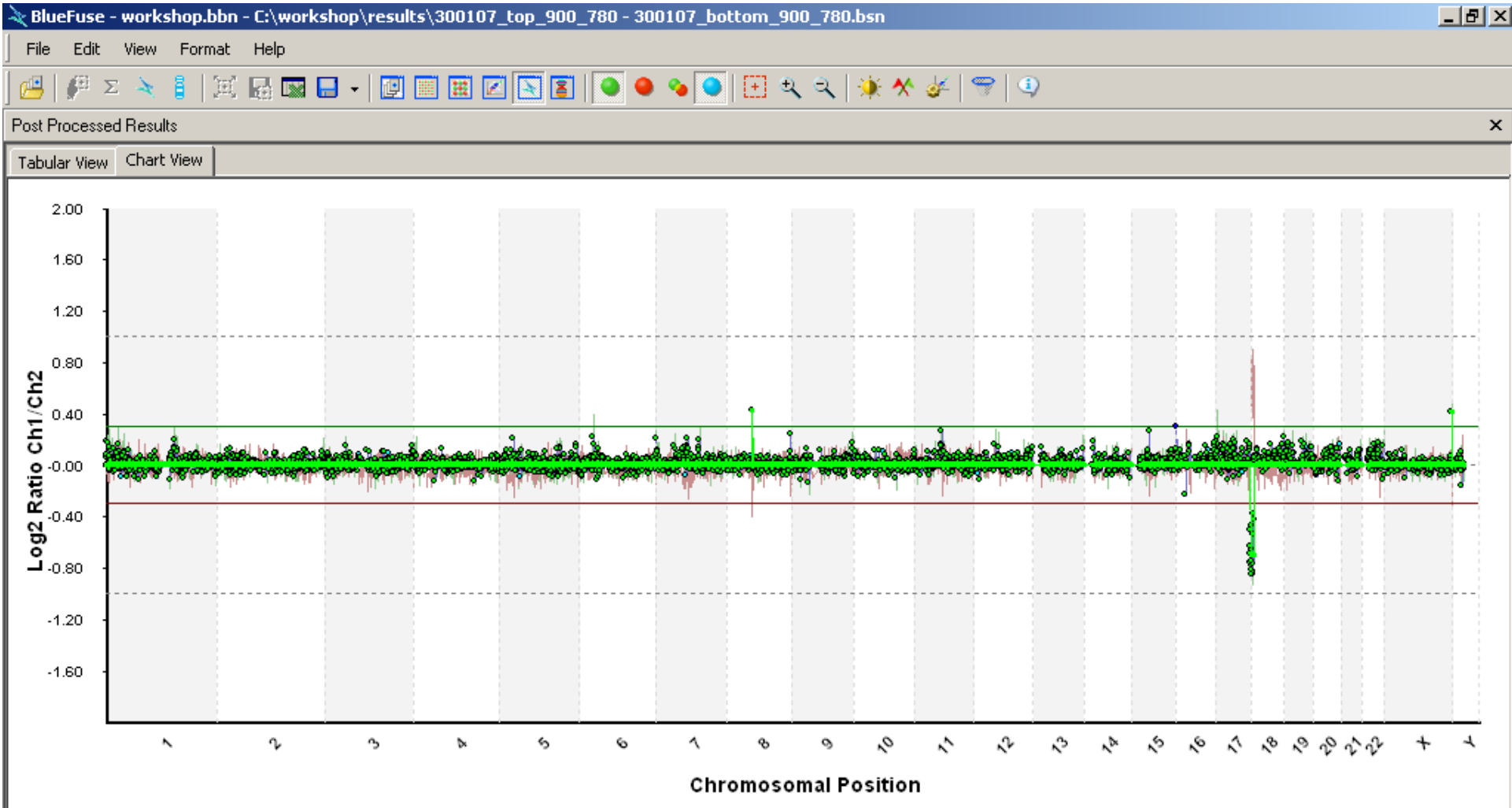


300107_bottom_900_780.bgn

SD of Autosome: 0.071

% Inclusion: 99.89

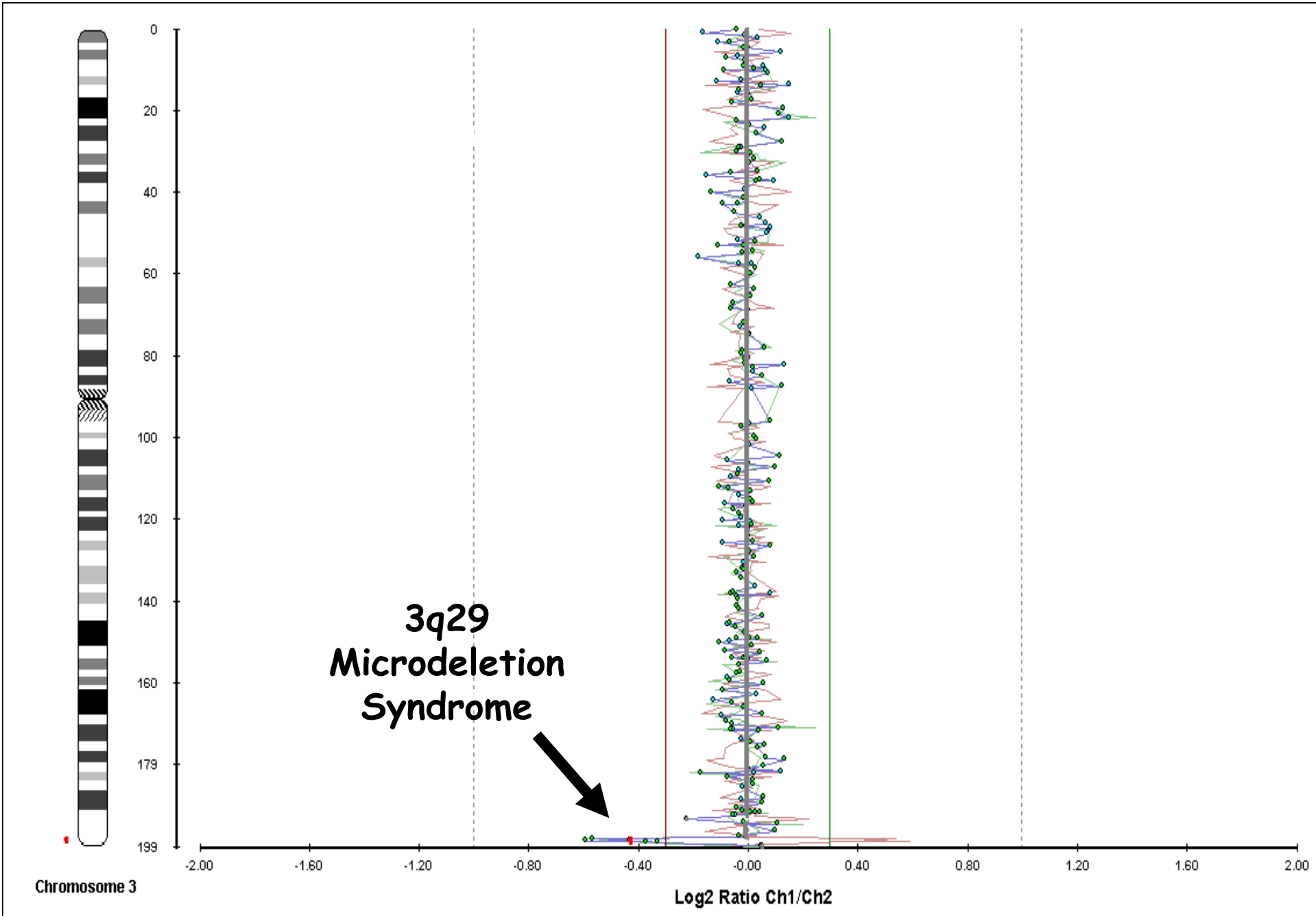
Courtesy of Eddy Maher, South East Scotland Cytogenetics Service



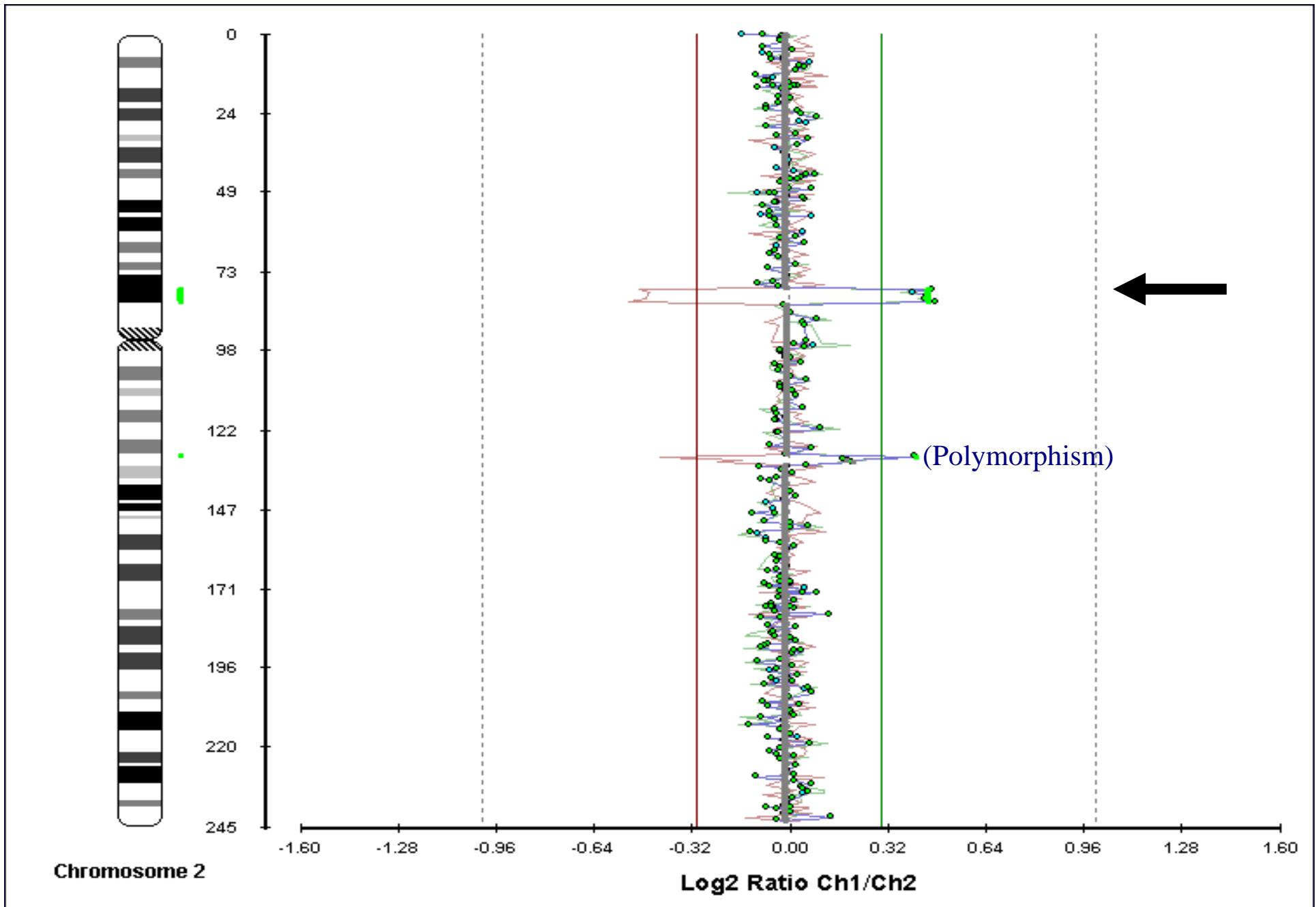
Post processing actions differ from current settings!

CytoChip Post Processing

Courtesy of Eddy Maher, South East Scotland Cytogenetics Service

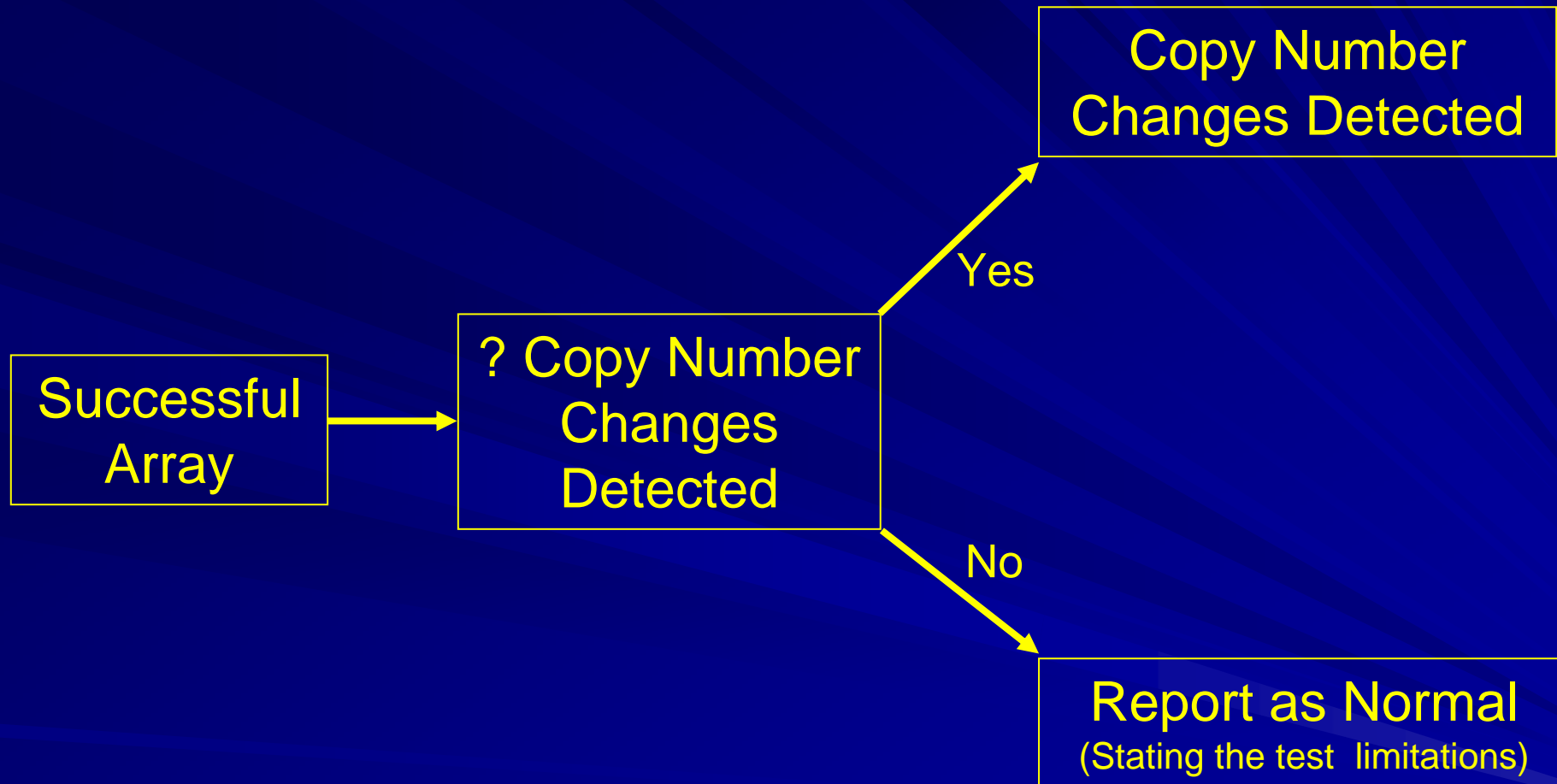


Courtesy of Eddy Maher, South East Scotland Cytogenetics Service

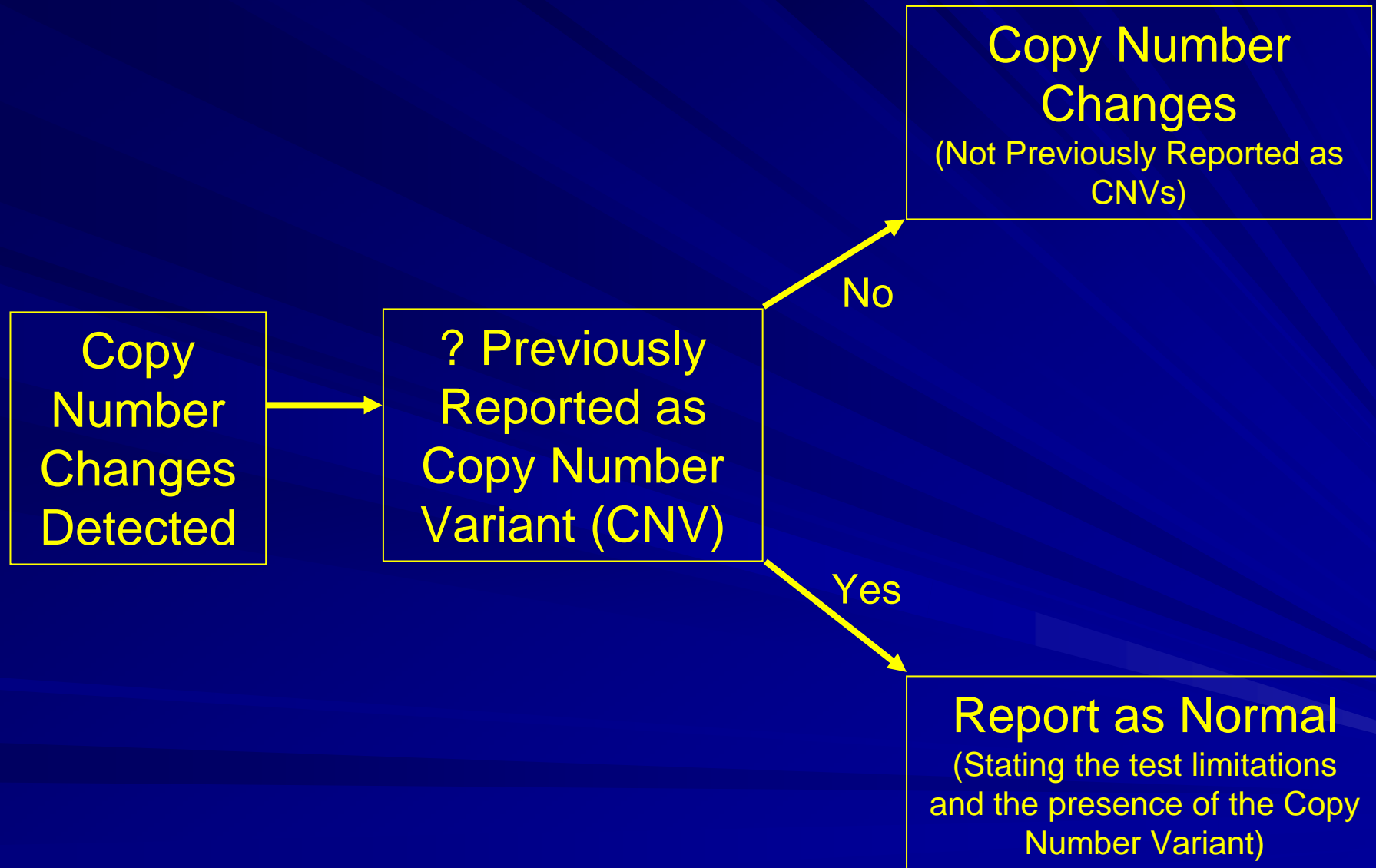


Courtesy of Eddy Maher, South East Scotland Cytogenetics Service

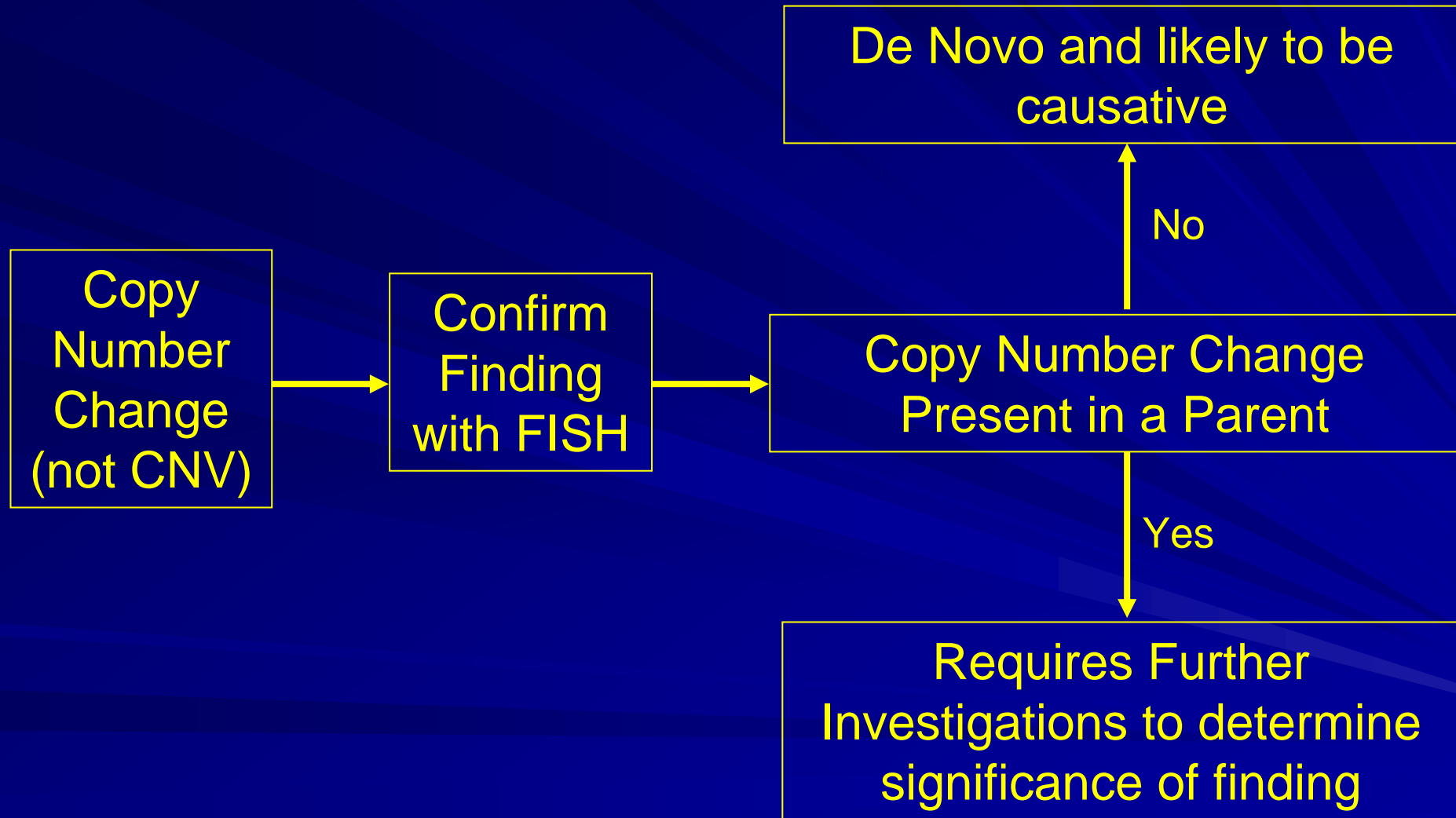
Reporting Flowchart - 1



Reporting Flowchart - 2



Reporting Flowchart - 3



Reference Materials

What do labs require?

- Reference Materials that don't change profile frequently. Whole genome checked
- ✓ Minimum 1 year –ideal 2-3 years. Population profile given against all 3 platforms (BAC, Oligo, SNP)
- ✓ All CNVs + size given - against all 3 platforms
- ✓ For CNVs need start and end bp codon
- ✓ Which genome map version profile mapped against.
- Source Material
 - Cell line– ?chromosome evolution
 - Amplified DNA- ?no changes to DNA coding.

Which Reference Materials?

Microarray:- Whole genome checked

- Normal male with known CNVs
- Known abnormal duplication
- Known abnormal deletion
- MECP2 duplication or X chromosome anomaly
- Normal female with known CNVs
- Mosaic – low level

N.B. Normal controls would also be applicable for QF-PCR and MLPA techniques

Which Reference Materials?

Microarray:- Whole genome checked

■ Normal male

- ✓ Single or pooled individual(s)?
- ✓ European population for CNVs?

■ Known abnormal duplication

Or abnormal with duplication and deletion (autosome)

■ Known abnormal deletion

■ MECP2 duplication or X chromosome anomaly

X chromosome duplication and deletion

■ Normal female with CNVs

■ Mosaic low level

Options

- Whole genome checked on BAC, Oligo and SNP arrays platforms!!!
- ✓ Same control for all three platforms
- ✓ Best Resolution array optimal for RMs
- ✓ ?Minimum one call for BAC array control.

Acknowledgements

- Eurogentest partners
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