New and Developing Technologies for Genetic Diagnostics
National Genetics Reference Laboratory (Wessex)
Salisbury, UK - July 2010

BACs on Beads™

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Human Genetics Laboratory
Jacobi Medical Center

EINSTEIN
Albert Einstein College of Medicine
OF YESHIVA UNIVERSITY

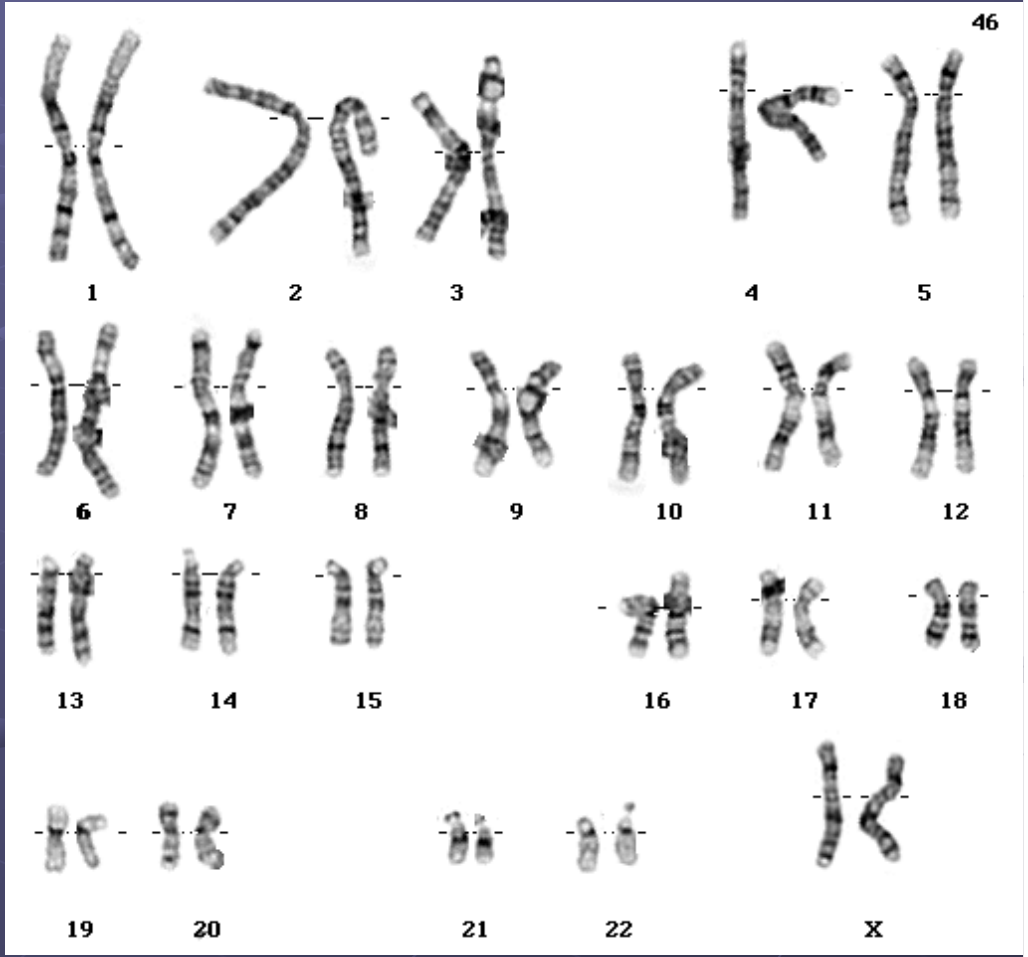
a new high throughput, cost effective technology for the rapid detection of fetal microdeletions and aneuploidies

Disclosure:

This study was supported by a research grant provided by PerkinElmer Inc.

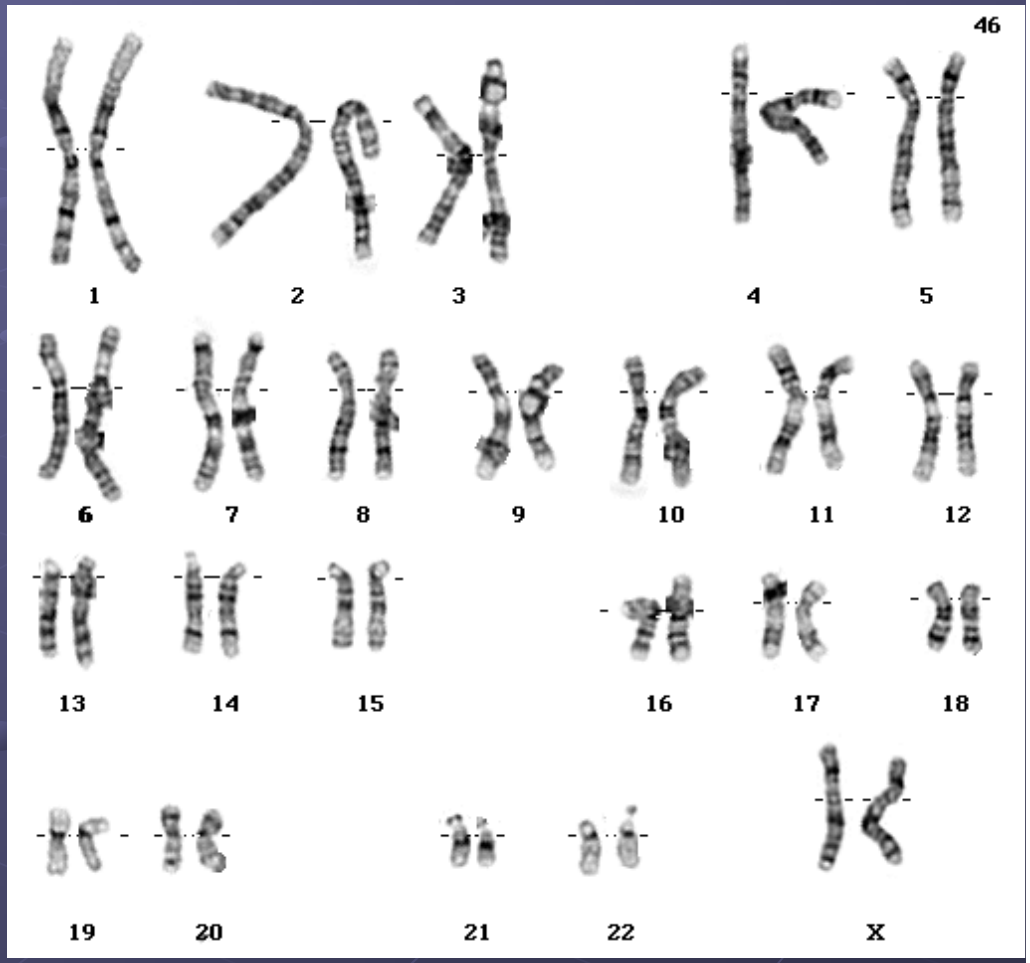
Cytogenetics

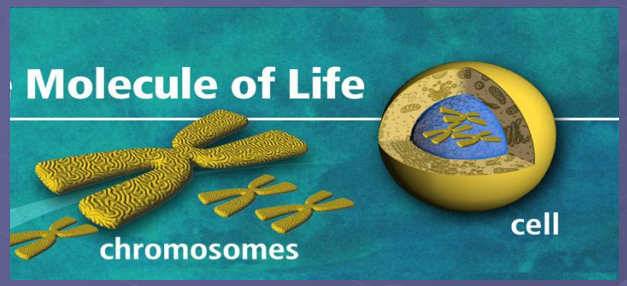
- Staining to look at banding pattern
- **Can** detect structural changes



Cytogenetics- BUT 3 issues

- TIME: 1- 2 weeks for results
- Small deletions & duplications will be missed (<5Mb)
- Living Tissue required





Molecular Cytogenetics

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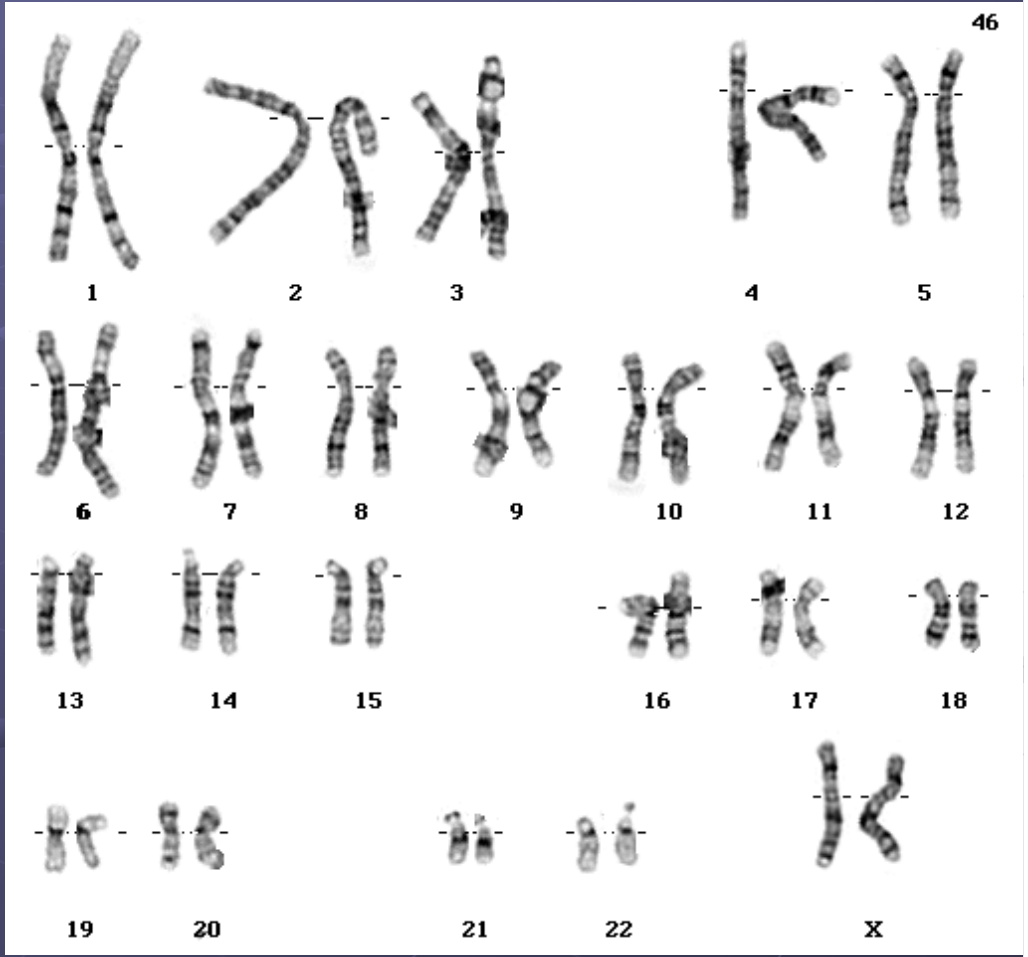
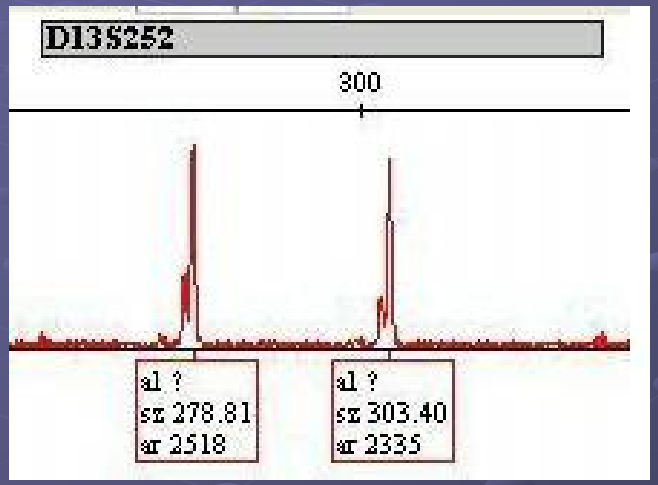
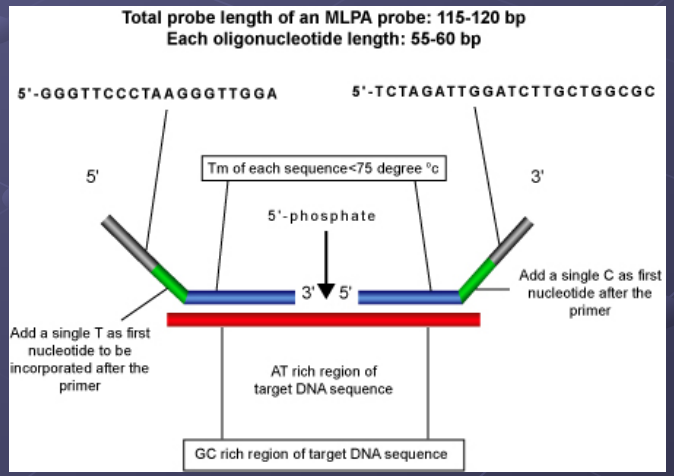


Rapid Testing

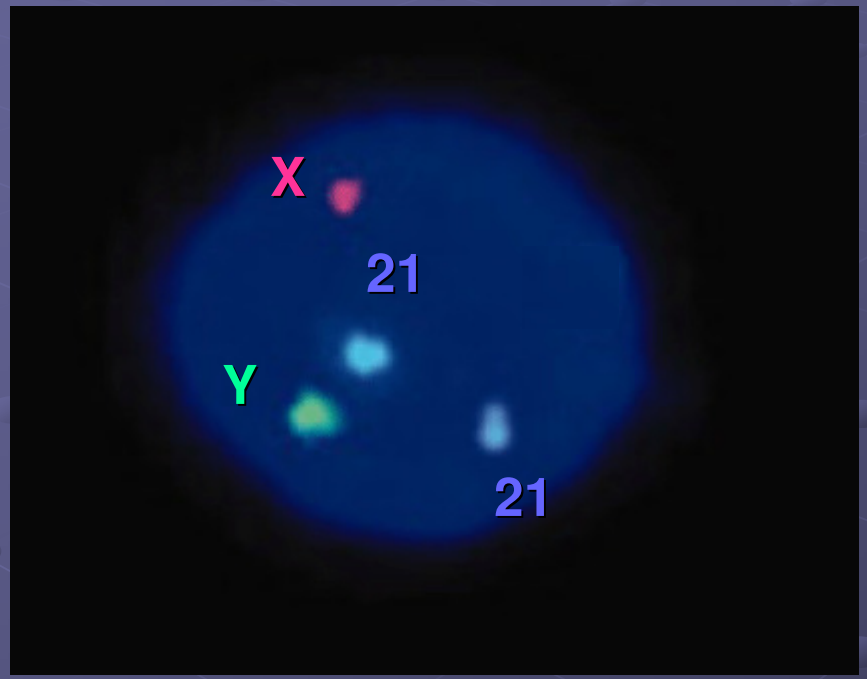
- Quantitative Fluorescence-Polymerase Chain Reaction (QF-PCR)



- Multiplex Ligation-Dependent Probe Amplification (MLPA)



FISH



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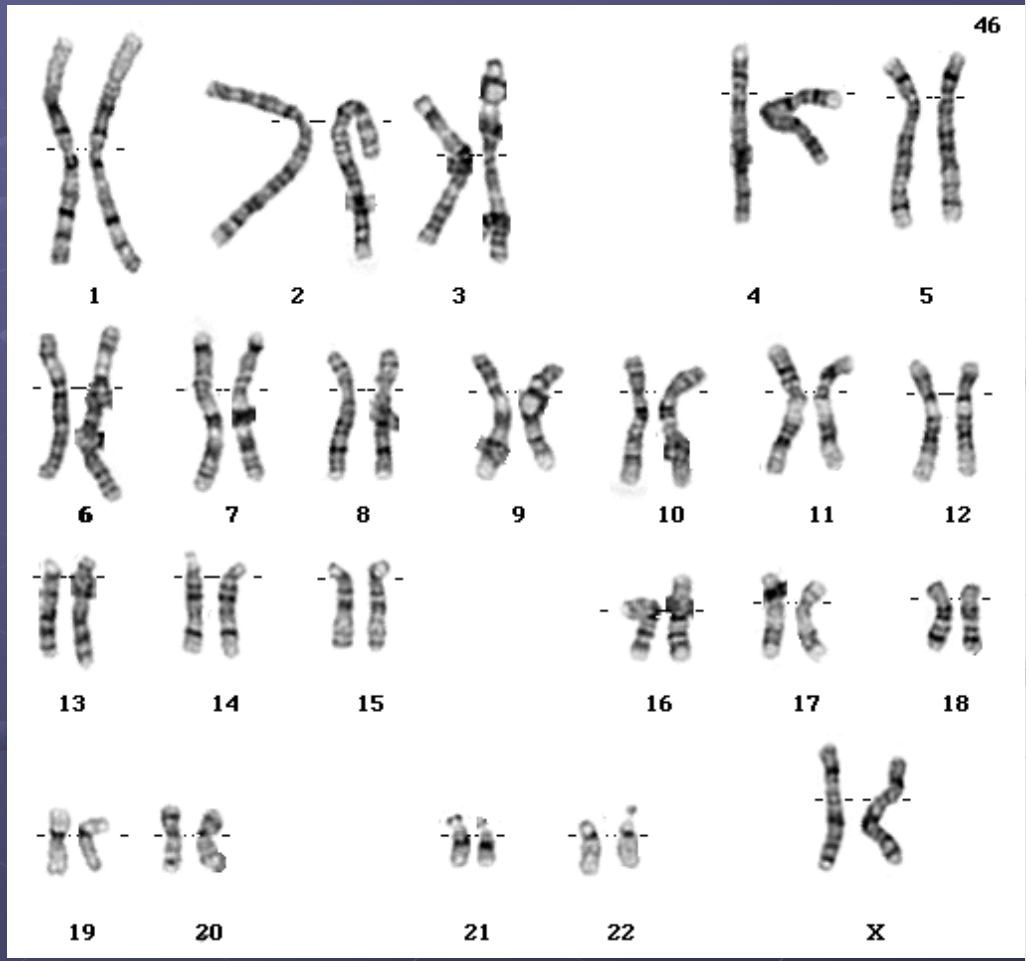
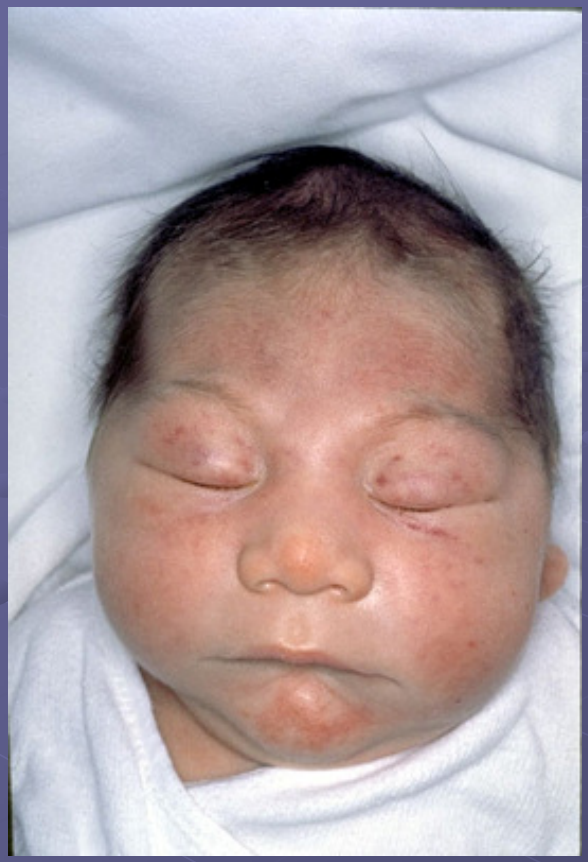
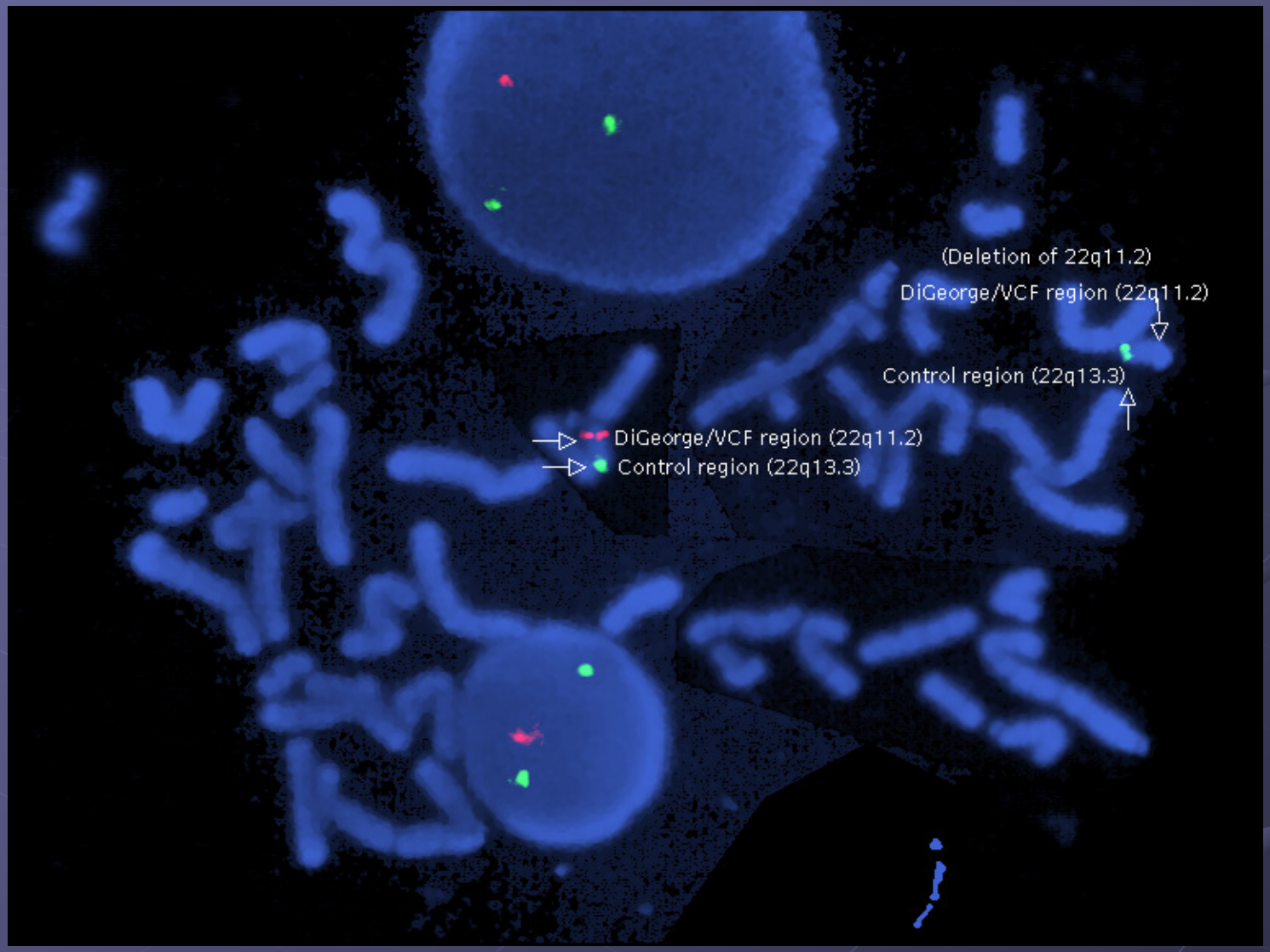


Microdeletions matter: *Miller-Dieker Syndrome*



- deletion of 17p13.3
- Approximately 1 in 25,000 births
- Prenatal manifestations: none, IUGR, polyhydramnios
- Postnatal manifestations: mental retardation, seizures, death before age 2.

WILL BE MISSED ON KARYOTYPE
AND
MAY BE MISSED ON ULTRASOUND


(Deletion of 22q11.2)
DiGeorge/VCF region (22q11.2)
Control region (22q13.3)
DiGeorge/VCF region (22q11.2)
Control region (22q13.3)

FISH

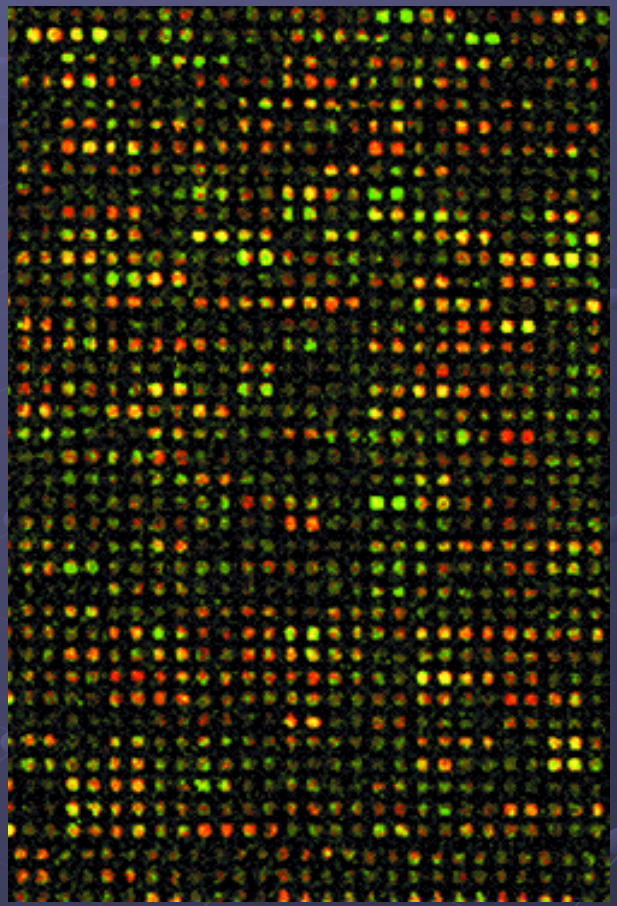
- Quick answer (24-48 hours)
- But…
  - Labor intensive
  - **YOU MUST KNOW WHAT YOU ARE LOOKING FOR**

NEXT GENERATION

MICROARRAYS

Microarrays

- BACs
  - Bacterial Artificial Chromosomes
    - 150 to 200 kilobases
- Oligonucleotide
  - Short DNA molecule
    - 25 to 85 base pairs



Microarrays

- **Benefits**
  - Pick up problems not seen on routine cytogenetics or detailed prenatal sonography
  - Do NOT need living cells (e.g. stillbirth)
- **Disadvantages**
  - Price (justified)
  - Must be able to validate all regions/probes
  - May pick up unexpected findings and/or variants that are of unknown significance

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  - Pick up problems not seen on routine cytogenetics or detailed prenatal sonography
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ACOG COMMITTEE OPINION

NUMBER 446, NOVEMBER 2009

Array Comparative Genomic Hybridization in Prenatal Diagnosis

Recommendations:

Conventional karyotyping remains the principal cytogenetic tool in prenatal diagnosis.

Targeted array CGH, in concert with genetic counseling, can be offered as an adjunct tool in prenatal cases with abnormal anatomic findings and a normal conventional karyotype, as well as in cases of fetal demise with congenital anomalies and the inability to obtain a conventional karyotype.

Targeted array CGH may be useful as a screening tool; however, further studies are necessary and are underway to fully determine its utility and its limitations.

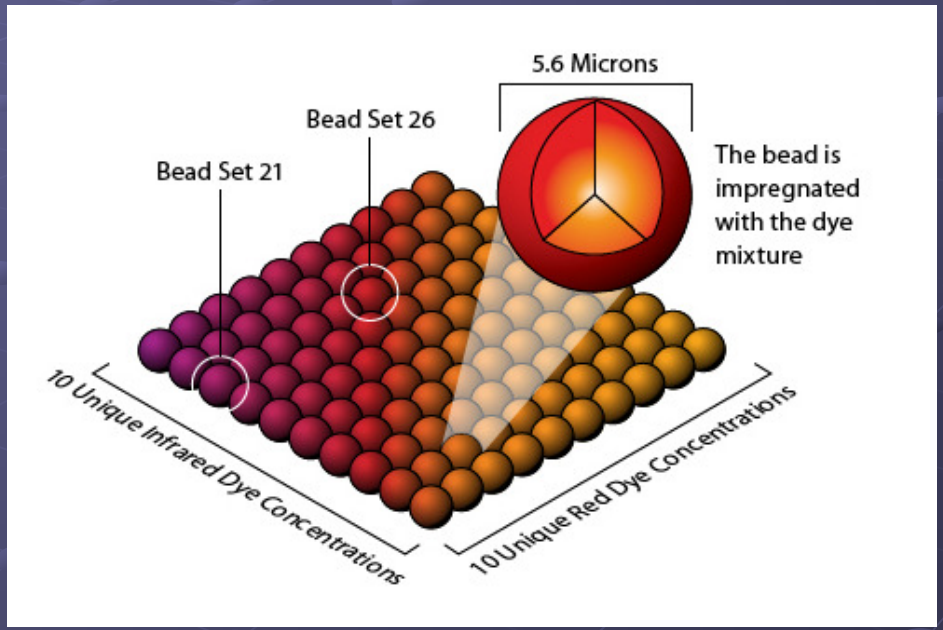
How to bring molecular cytogenetic technology to patients now so that it is accessible to ALL pregnant women???

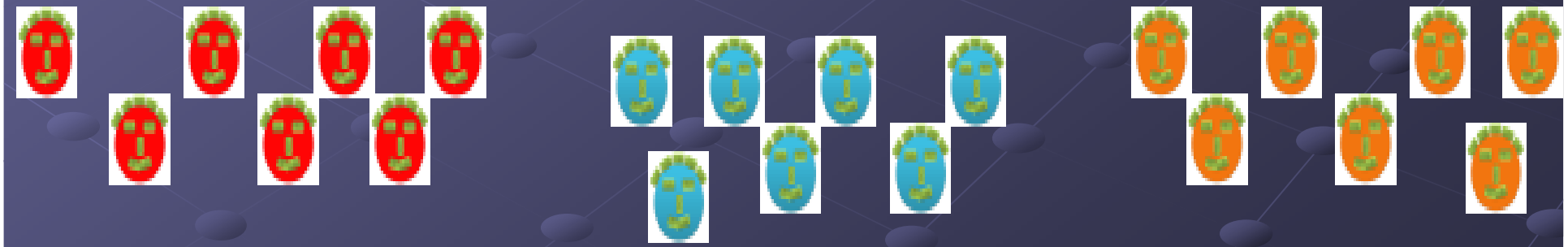
Design Criteria

- Disorders
  - Significant Morbidity and Mortality
  - Diseases should be relatively common
  - Majority of cases actually caused by deletion
  - May be missed on detailed sonography
- Platform
  - Low cost
  - High throughput
  - Available in clinical laboratories
  - Flexible – can adjust disorders depending on setting
  - Rapid result
  - Can easily validate any result

BACs on Beads™

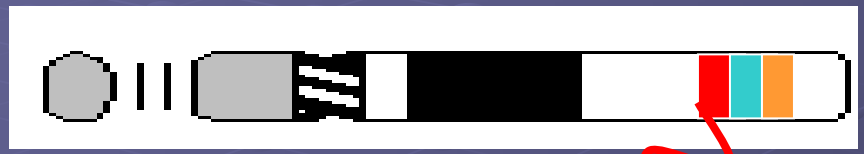
- xMAP (Luminex®) beads

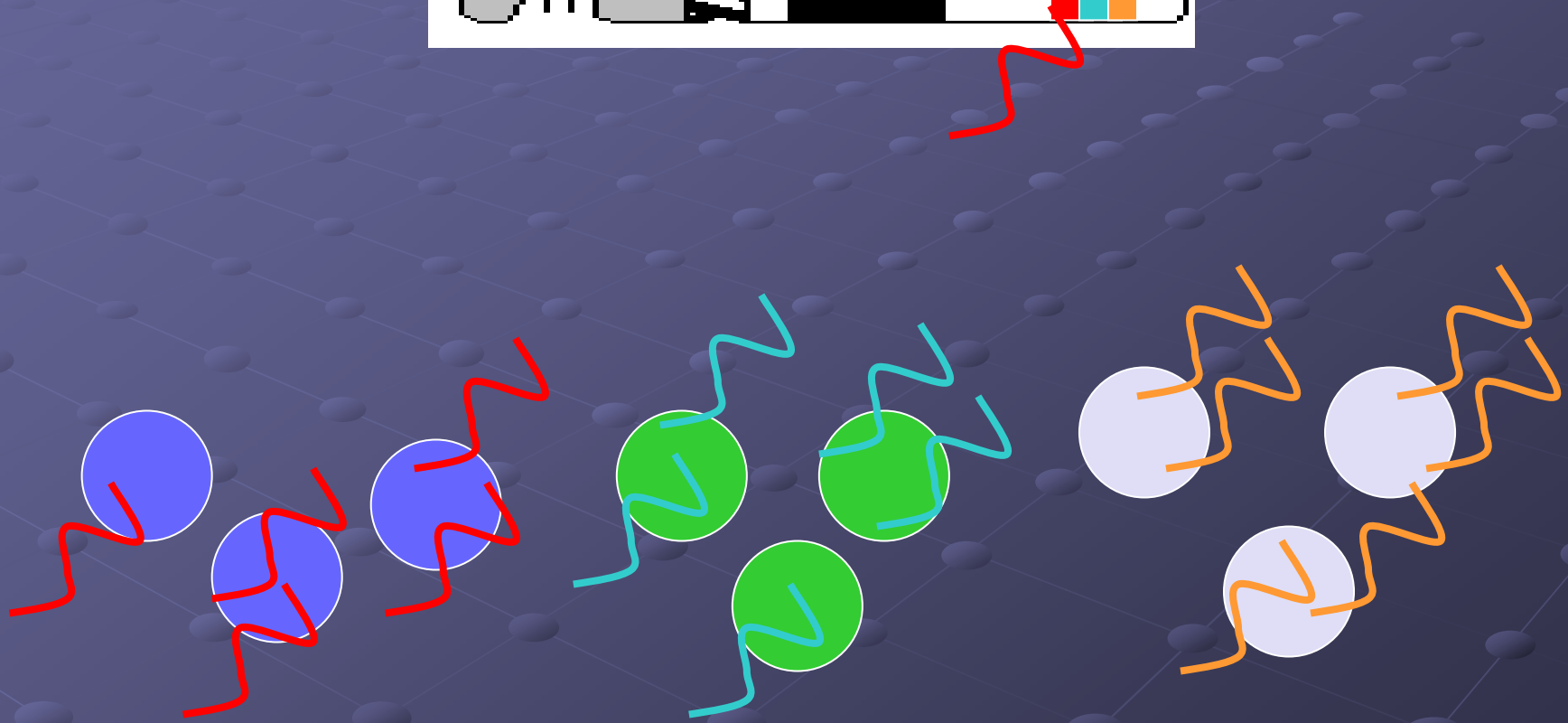




Chromosome 21-DSCR

BAC derived DNA is immobilized on xMAP (Luminex®) beads

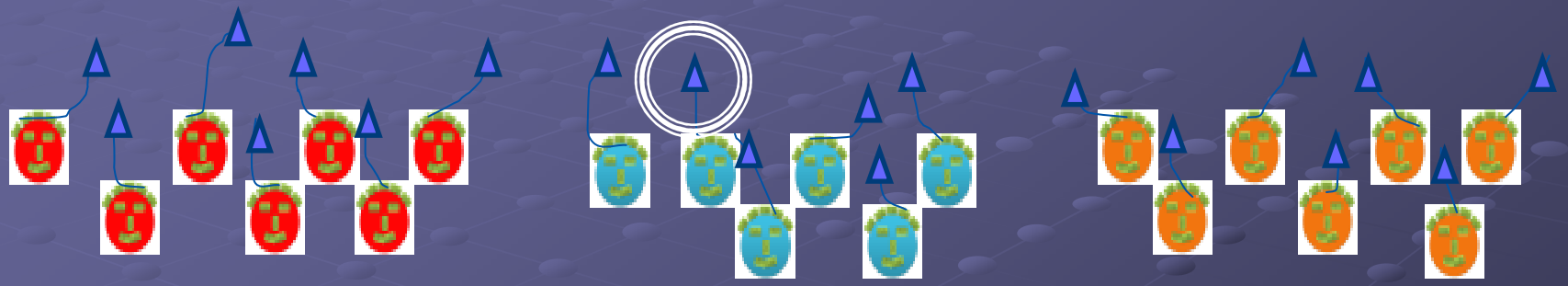




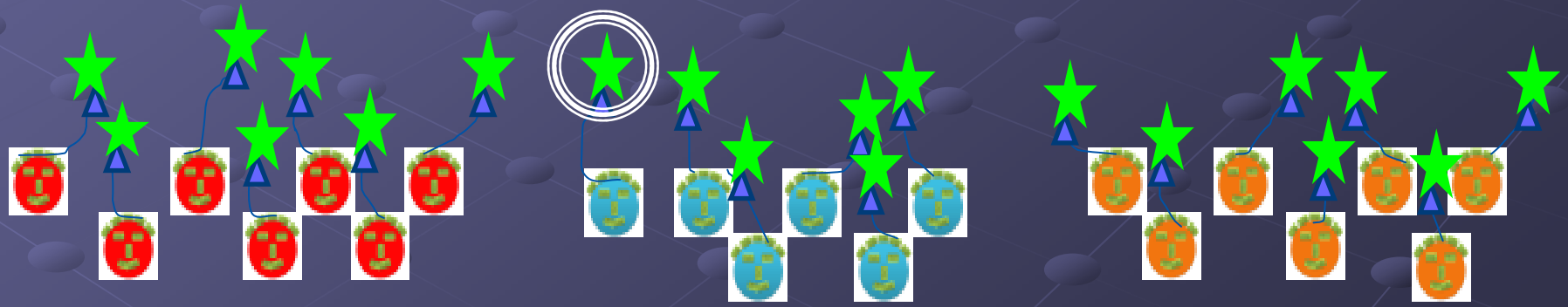
VCFS – 22q region

CAN USE SAME PROCESS FOR ANY DELETION or DUPLICATION ON ANY CHROMOSOME

Add DNA to Beads – controls and test samples

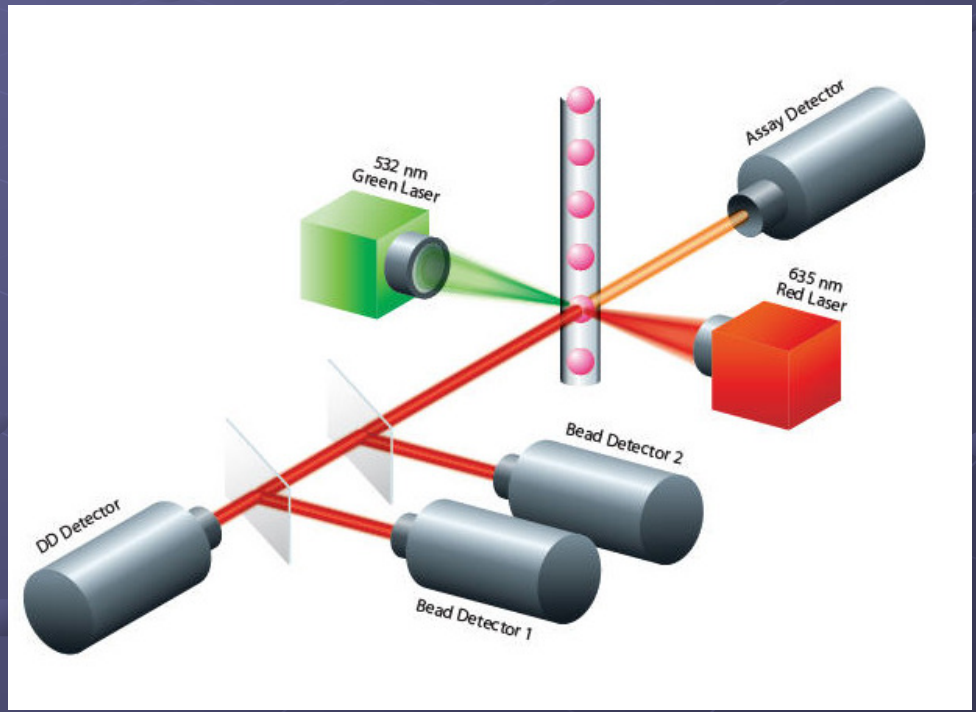


Add Fluorescence to DNA



BACs on Beads™

- Beads are read in the Luminex reader



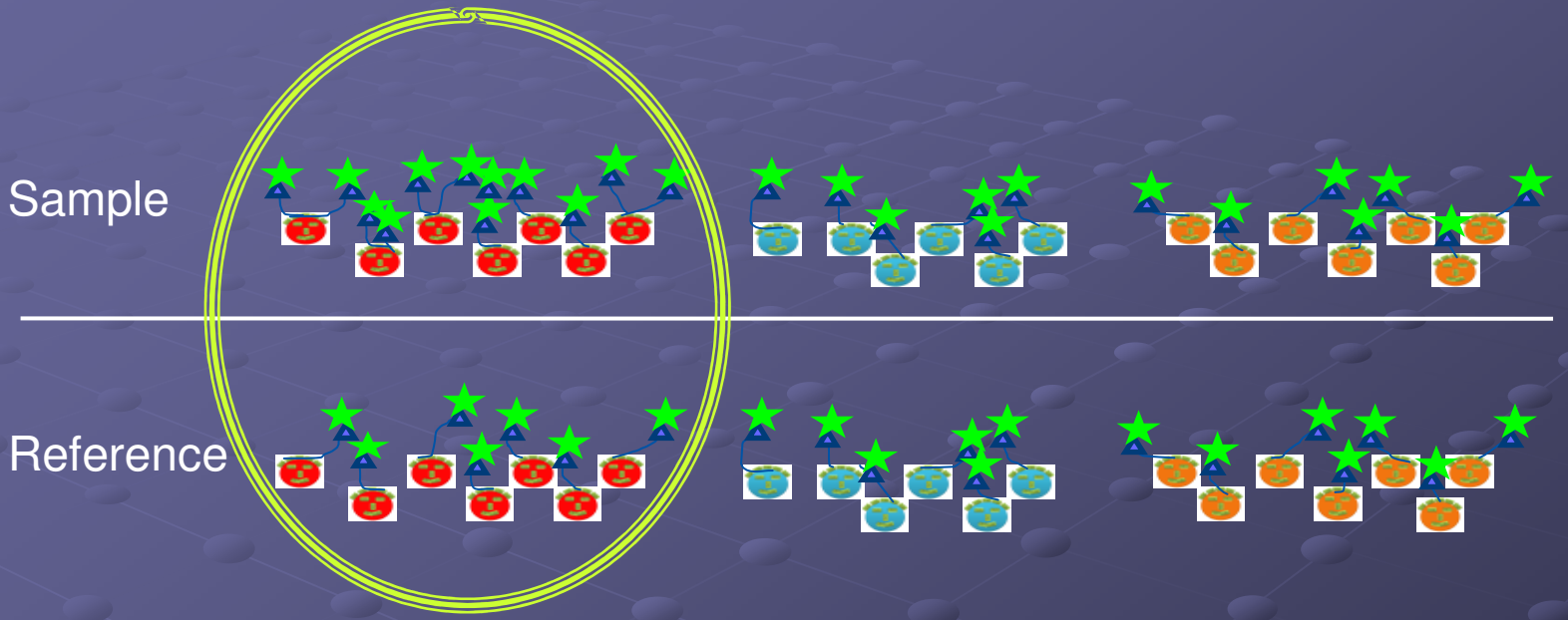
Ratiometric Analysis – Normal Sample

Sample

Reference

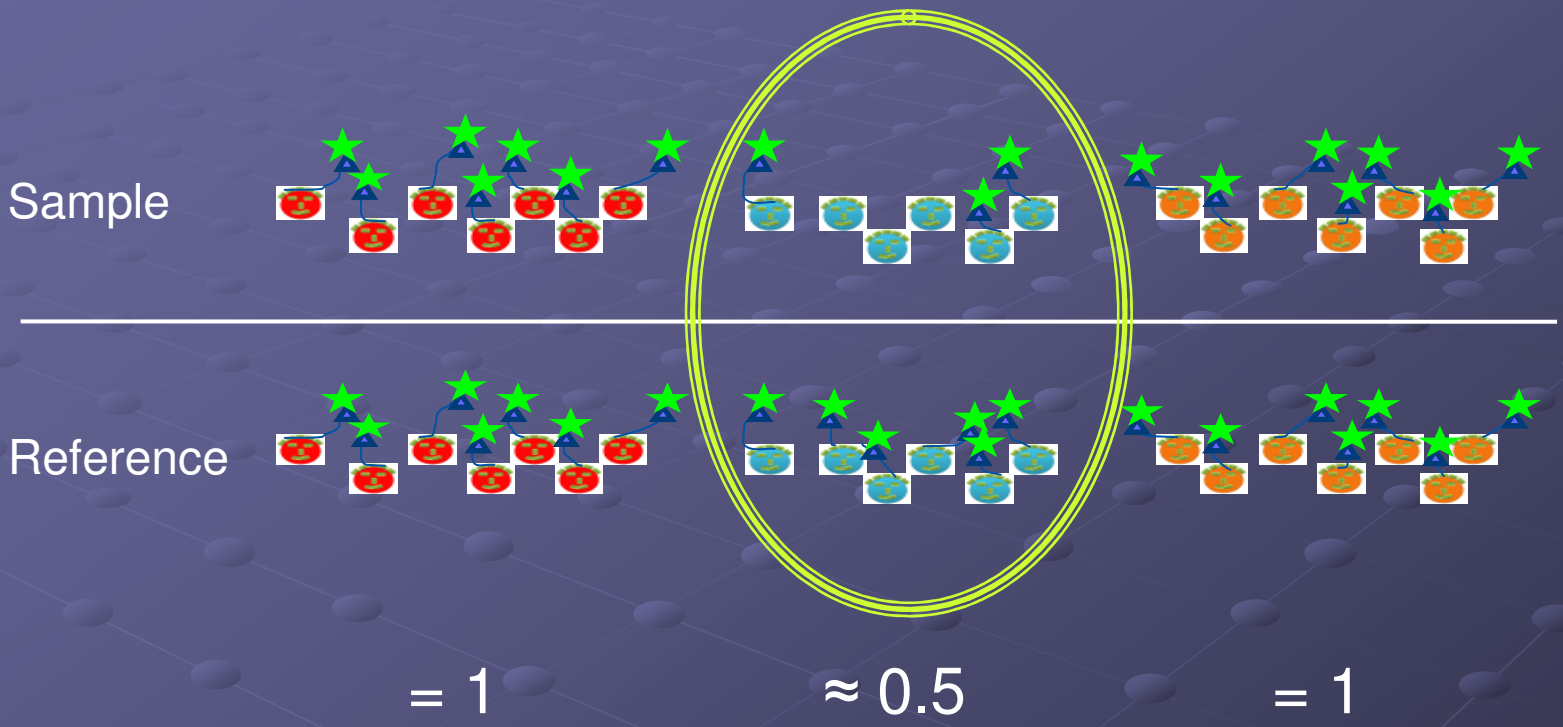
= 1 = 1 = 1

Ratiometric Analysis – Chromosomal Gain



≈ 1.5 = 1 = 1

Ratiometric Analysis – Chromosomal Loss



It’s all about ratios!!!!

Disorders

- Common trisomies – 13, 18, 21
- Sex aneuploidies (X and Y chromosome)
- Microdeletion syndromes

Microdeletion Syndromes

- DiGeorge syndrome
- Williams-Beuren syndrome
- Prader-Willi syndrome
- Angelman syndrome
- Miller-Dieker syndrome
- Smith-Magenis syndrome
- Wolf-Hirschhorn syndrome
- Cri du Chat syndrome
- Langer-Giedion syndrome
- DiGeorge Syndrome 2

OVERALL – occurs 1/1600 deliveries

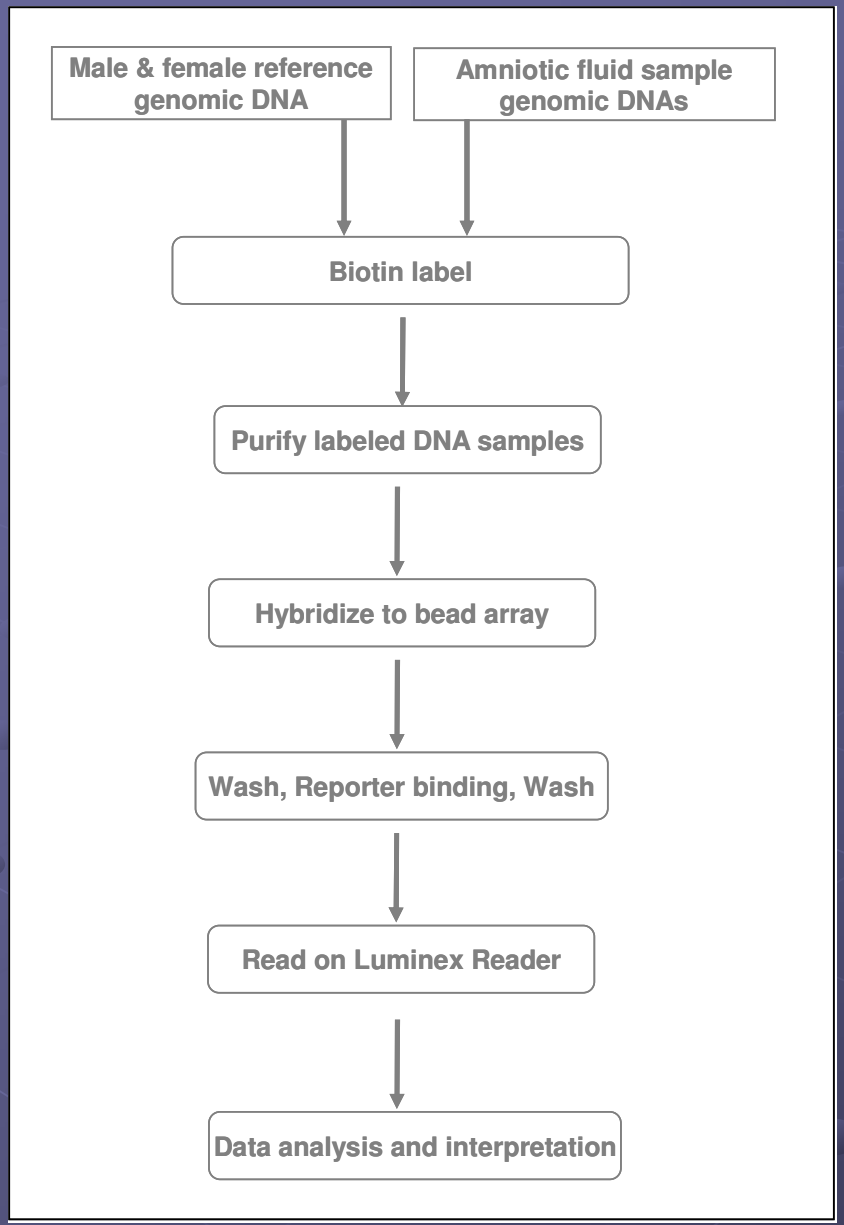
Significance of 1/1600 Deliveries

- Down Syndrome occurs 1/800 deliveries
- With 4 million deliveries in the US/year
  - Approximately 5700 DS births per year
  - Approximately 2500 microdeletion syndromes per year

Would increase detection of serious disorders associated with mental retardation or birth defects by approximately 40%

Platform Summary

- 4-8 probes per disorder
- Relatively small amount of DNA compared to CGH (125 nanograms vs 10 micrograms)
- Turn around time: 24-48 hours
- Price comparable between QF-PCR and FISH BUT with additional information of microdeletions
- Clinical FISH probes are available to confirm all findings
- No additional procedures required beyond amniocentesis



- "significant" deflections = all the probes or all but one of the probes deflects beyond two standard deviations of the reference sample

From R&D to Clinical Study

1) Validation Study - Microdeletions and Aneuploidies

2) Prospective Data to confirm performance of BoBs in real time, in the field

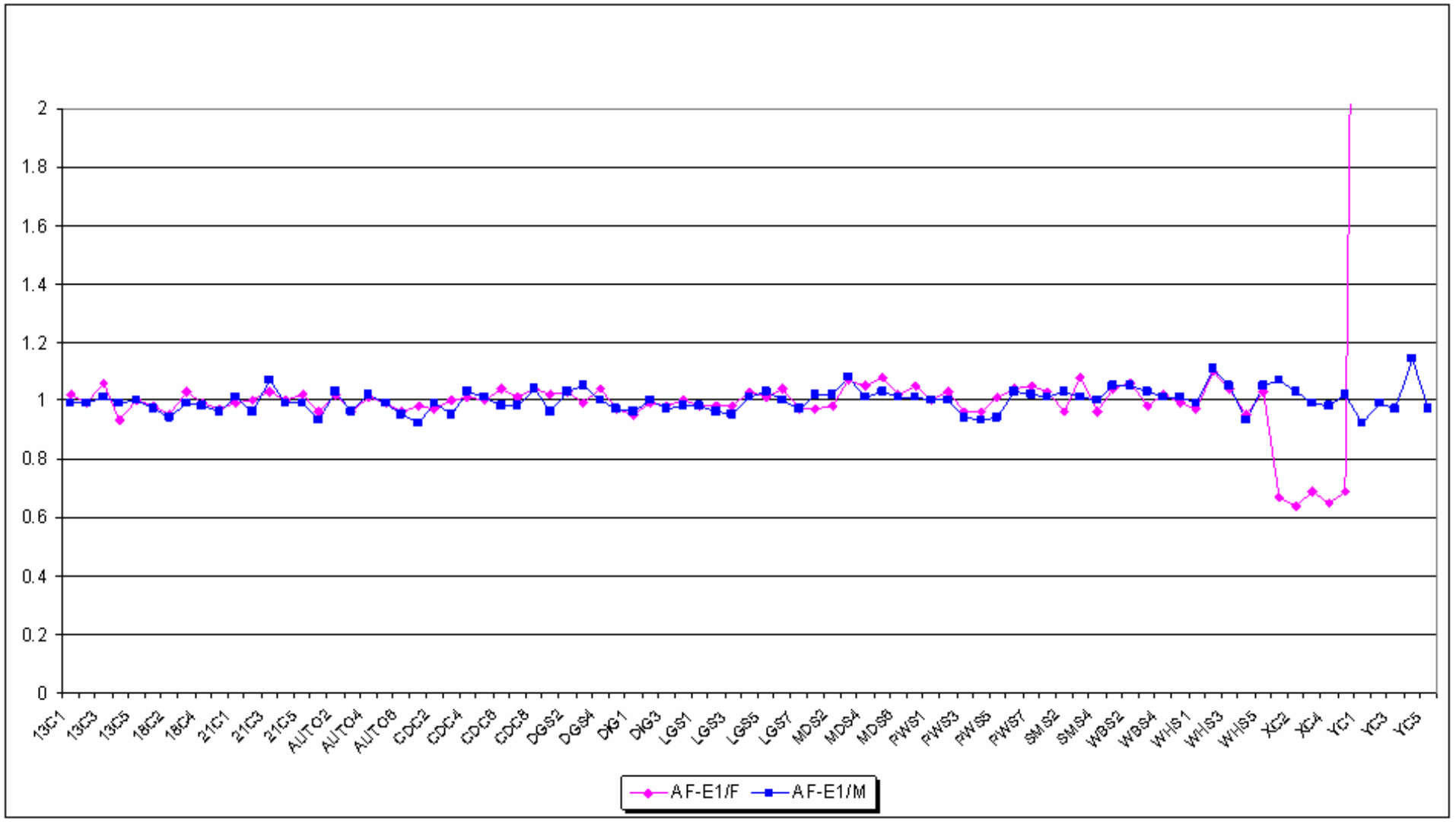
How to validate relatively rare disorders?

- Amniocytes (and other cells) removed from amniotic fluid by centrifugation
- Fluid samples then ‘spiked’ with cells from known deletion syndromes and aneuploidies (200,000 cells/2mL)
- Proxy for natural samples

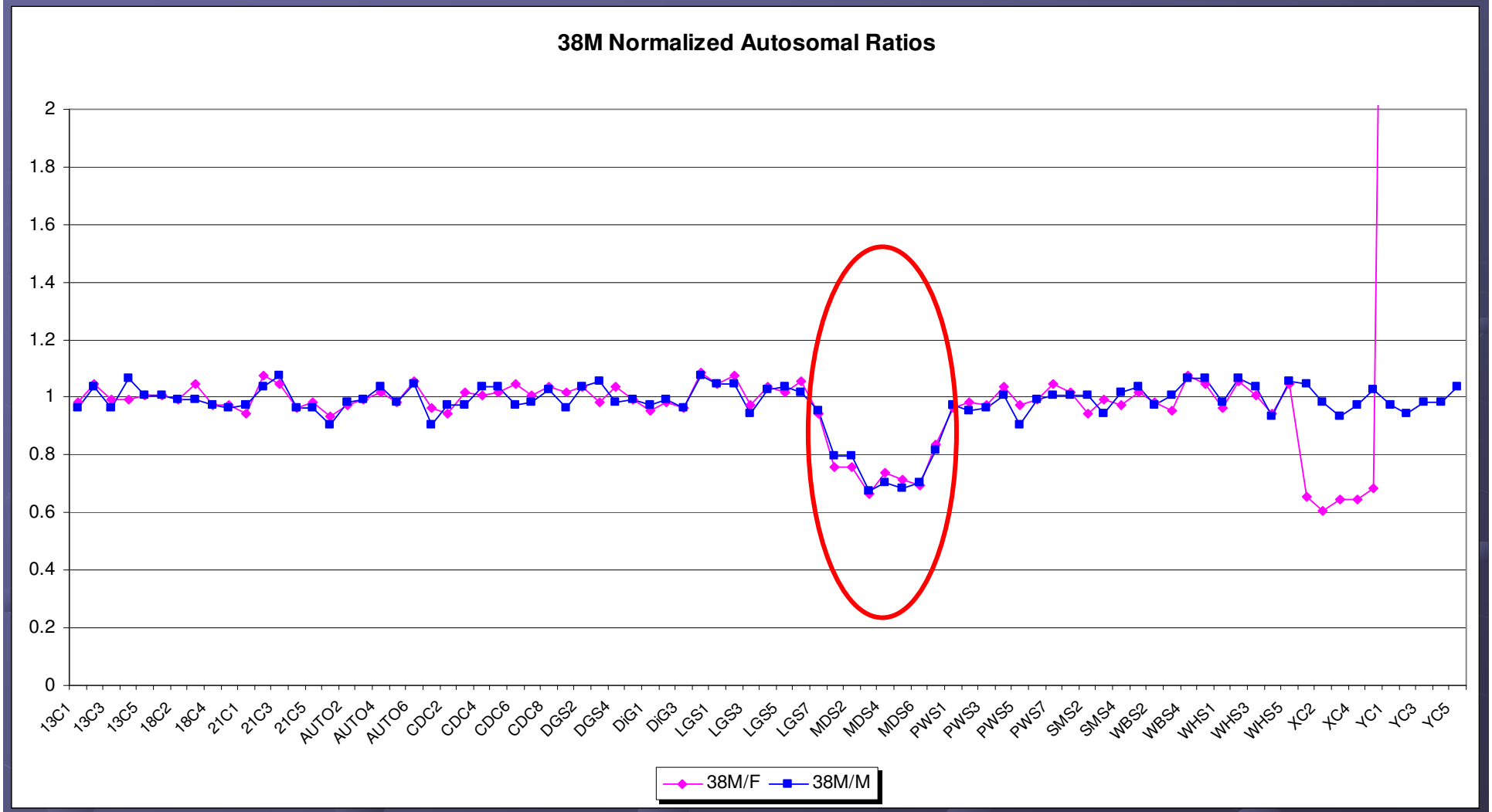
Results

- *Aneuploidies and Microdeletions*
  - At least 2 different cell lines per disorder
  - 48 samples
  - All samples were identified correctly

Data Output – Normal Study



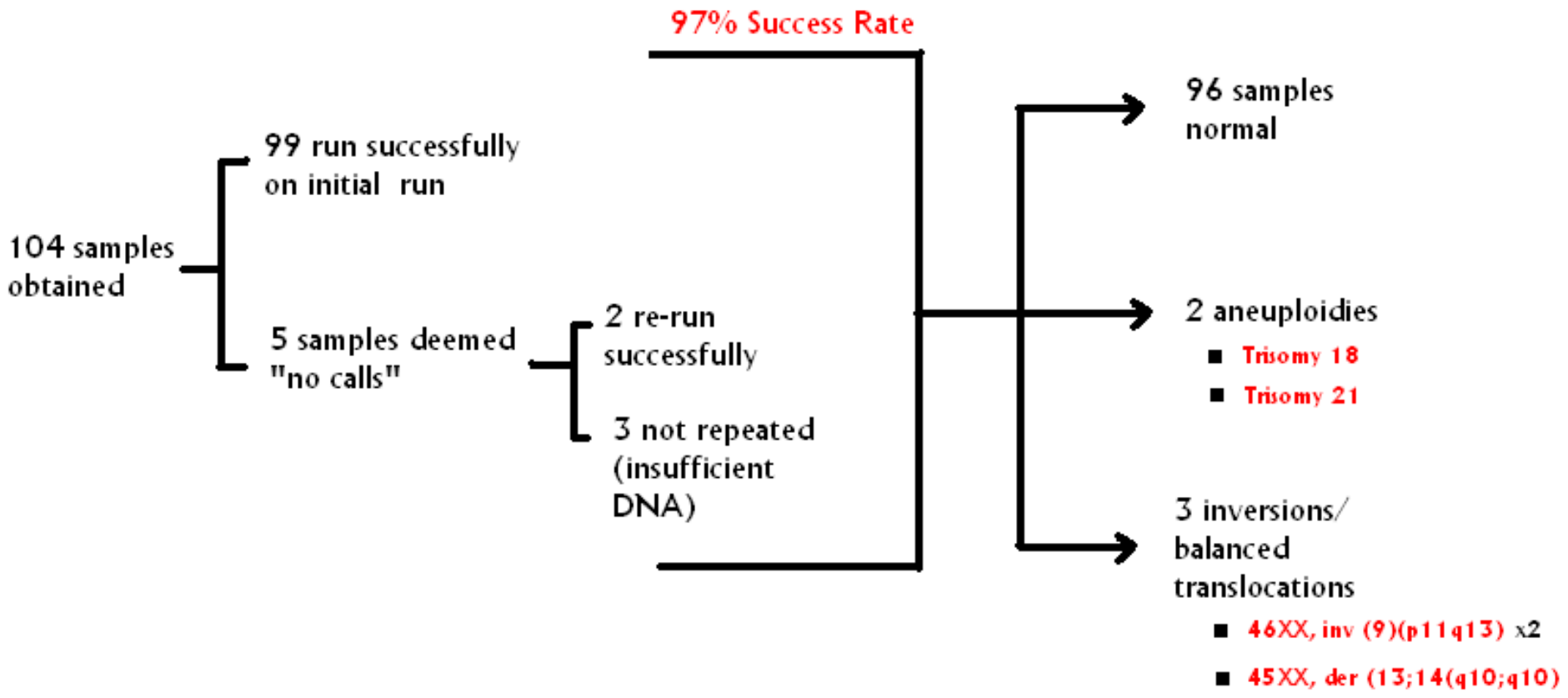
Miller-Dieker Syndrome

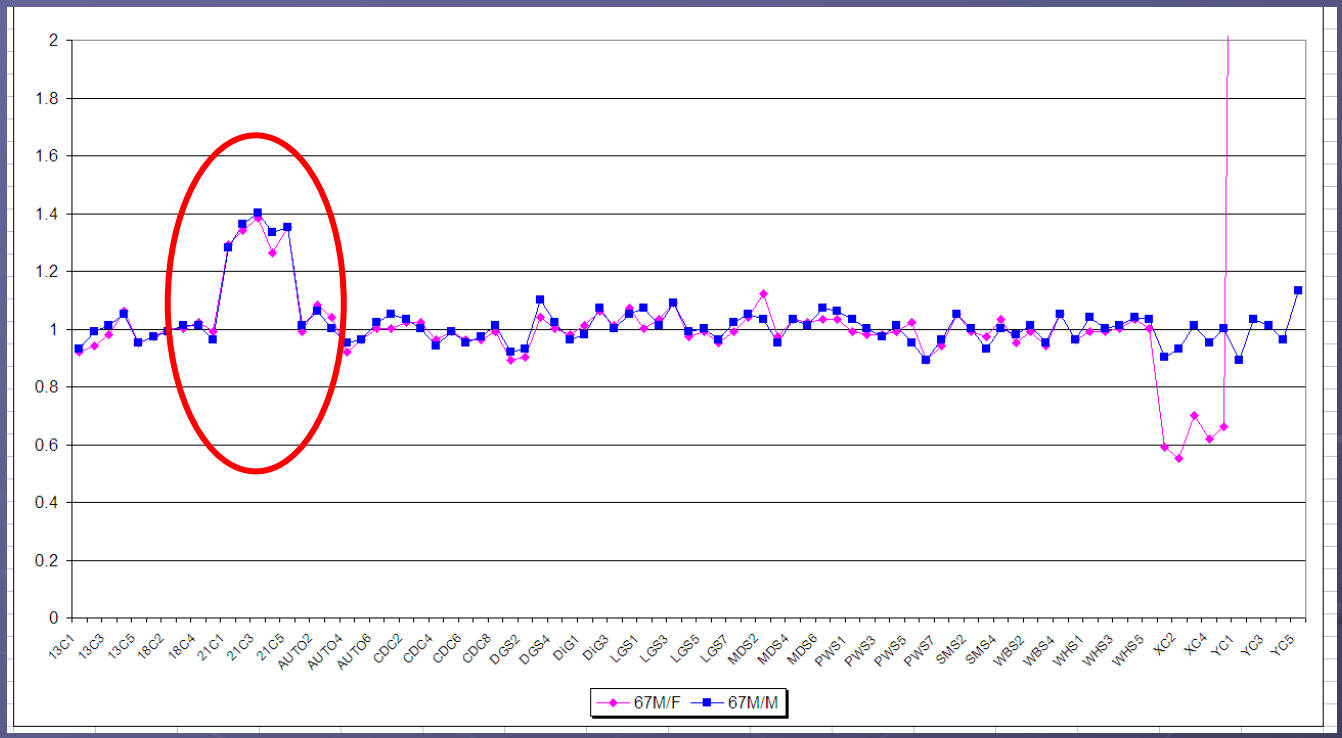


Prospective Study

- Multicentered study
  - 5 mls of amniotic fluid collected from women undergoing clinically indicated amniocentesis under an IRB approved protocol
  - Results confirmed with:
    - routine cytogenetics performed on the amniotic fluid sample
    - newborn follow-up data

Prospective Multicentered Study

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0.8
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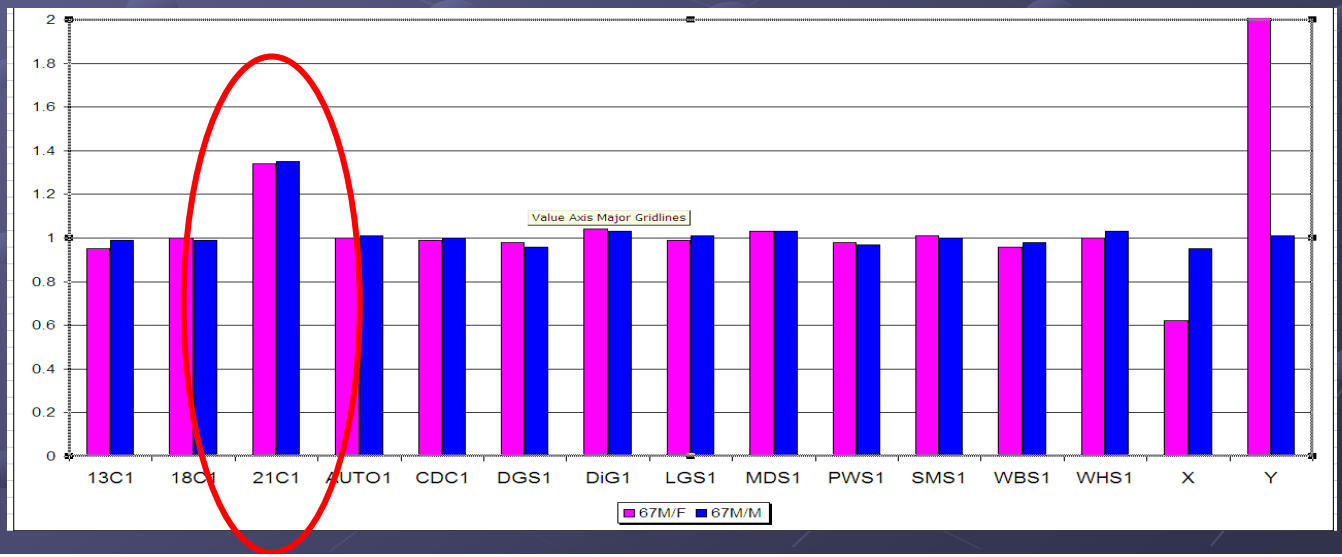
67M/F
67M/M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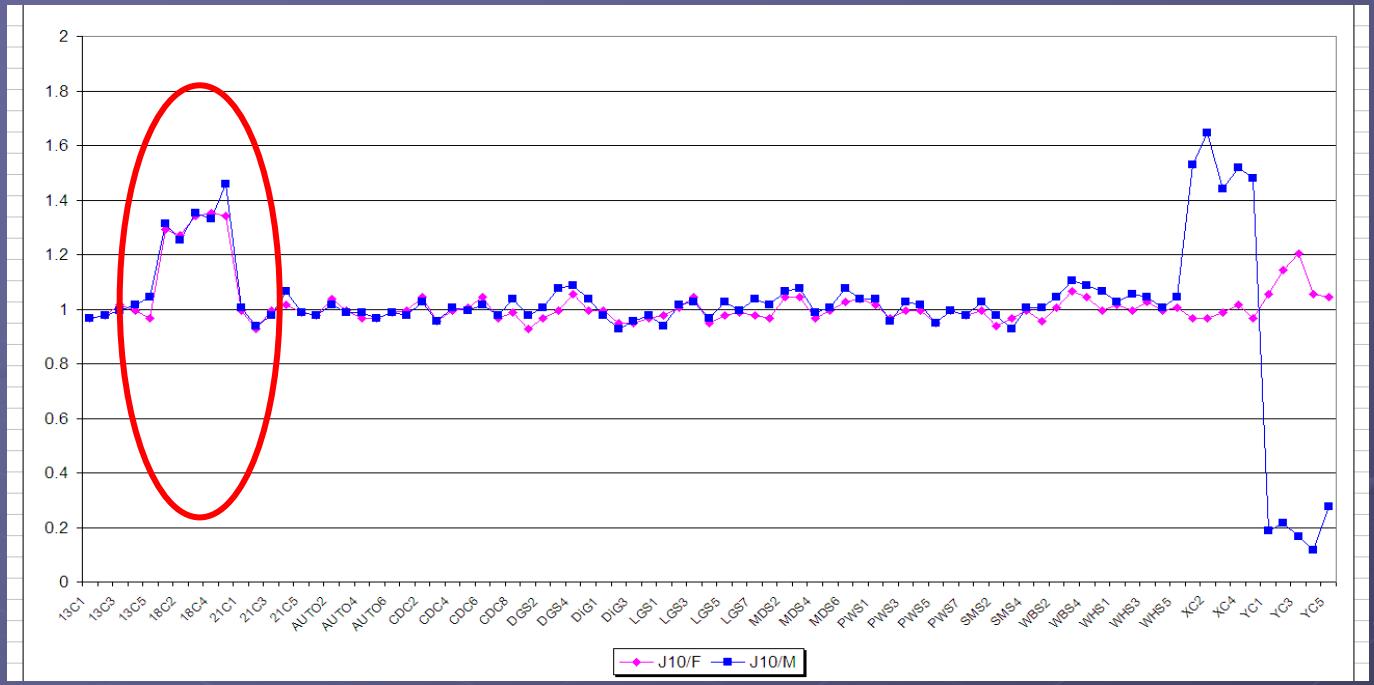
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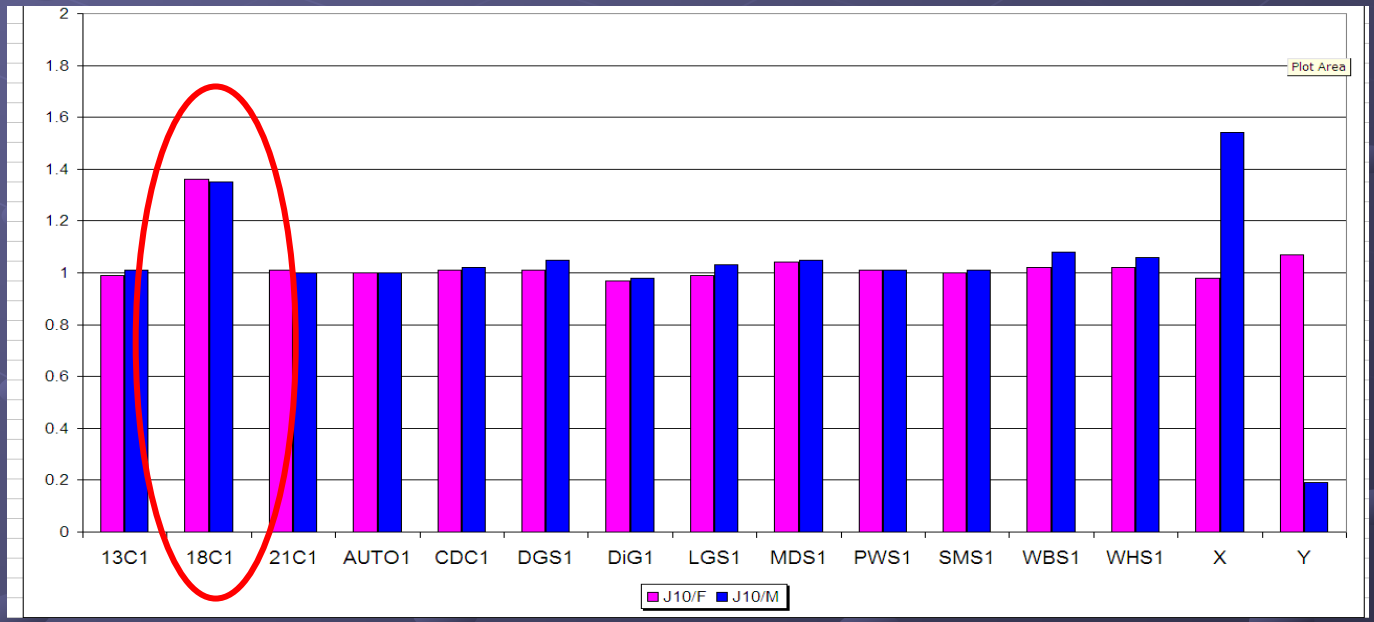
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18C1
21C1
AUTO1
CDC1
DGS1
DiG1
LGS1
MDS1
PWS1
SMS1
WBS1
WHS1
X
Y
J10/F
J10/M

Assay Limitations

- A negative result does not fully exclude the diagnosis of any of these syndromes (other etiological mechanisms for the disorders such as point mutations cannot be excluded)

- Rigorous criteria calling a sample “positive” means that there may be false negatives

- Such as other quantitative molecular approaches, balanced translocations or inversions cannot be detected

Assay Advantages

- all regions covered by the assay have been validated AND associated with significant newborn morbidity and mortality
- quick turn-around time
- relatively small DNA requirement
- does not require a blood sample from either parent
- bead-based platform already used effectively in other clinical settings

Develop a New Approach

- **Benefits**
  - Pick up problems not seen on routine cytogenetics or detailed prenatal sonography
  - Do NOT need living cells (e.g. stillbirth)
- **Disadvantages**
  - Price (justified)
  - Must be able to validate all regions/probes
  - May pick up unexpected findings

The Future…

- BoBs should be made available to ALL women who are undergoing invasive testing
- There is potential that BoBs could be ‘diagnostic’
  - However – at this point, MUST await prospective data – SCREENING ONLY
  - ONLY ACT ON CONFIRMATION of FINDINGS BY FISH

The BoBs Team

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  - Dr. David Garry
  - Dr. Brian Wagner
- **Division of Reproductive Genetics, Montefiore Medical Center**
  - Dr. Susan Klugman
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- **North Shore Long Island Jewish Health System**
- **Bronx Lebanon Hospital Center**
  - Dr. Aleksandr M. Fuks
  - Sara Said-Delgado