

THE FETUS AS A PATIENT

# Microarrays in Prenatal Diagnosis

EUGENE PERGAMENT, MD, PhD, FACMG  
Northwestern Reproductive Genetics, Inc.

# Past Paradigm Shifts in Obstetrical Genetics

- Preimplantation genetic diagnosis (90s)
- Non-invasive approaches
  - Maternal serum AFP for ONTD (80s)
  - Ultrasound (80s)
  - Triple/Quad screen for DS (80s-90s)
  - First trimester screening for DS & Tri 13/18 (2000)
- Invasive approaches
  - Amniocentesis (70s)
  - Fetoscopy (70s)
  - Skin biopsy (80s)
  - CVS (80s)
  - PUBS (80s-90s)

## Future Paradigm Shifts in Obstetrical Genetics

- Will not be procedural
- Will originate from the molecular genetics laboratory
- Two paradigm shifts
  - High resolution array genomic hybridization
  - Non-invasive prenatal diagnosis

# First Era of cytogenetics (50s-70s)



1

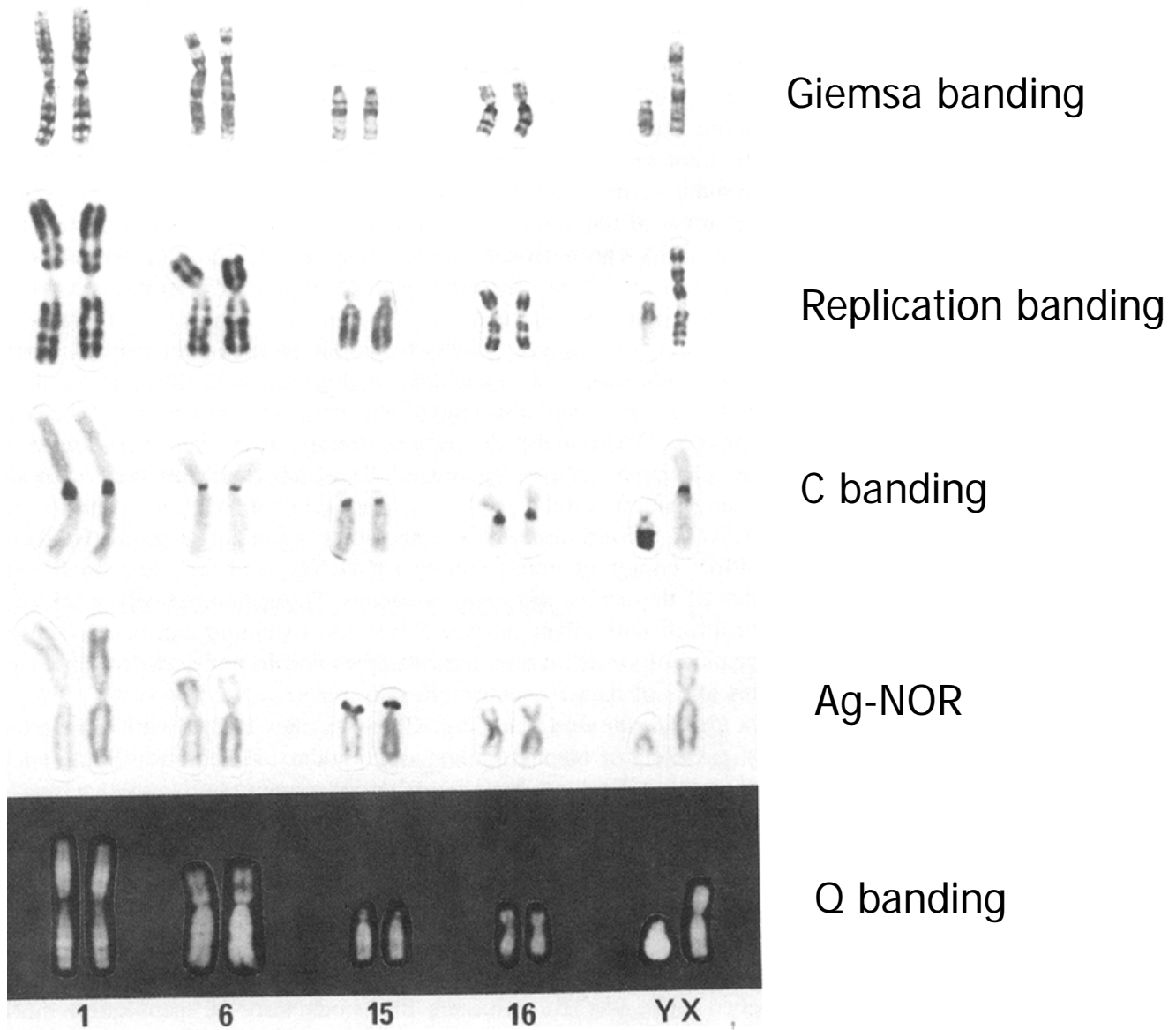
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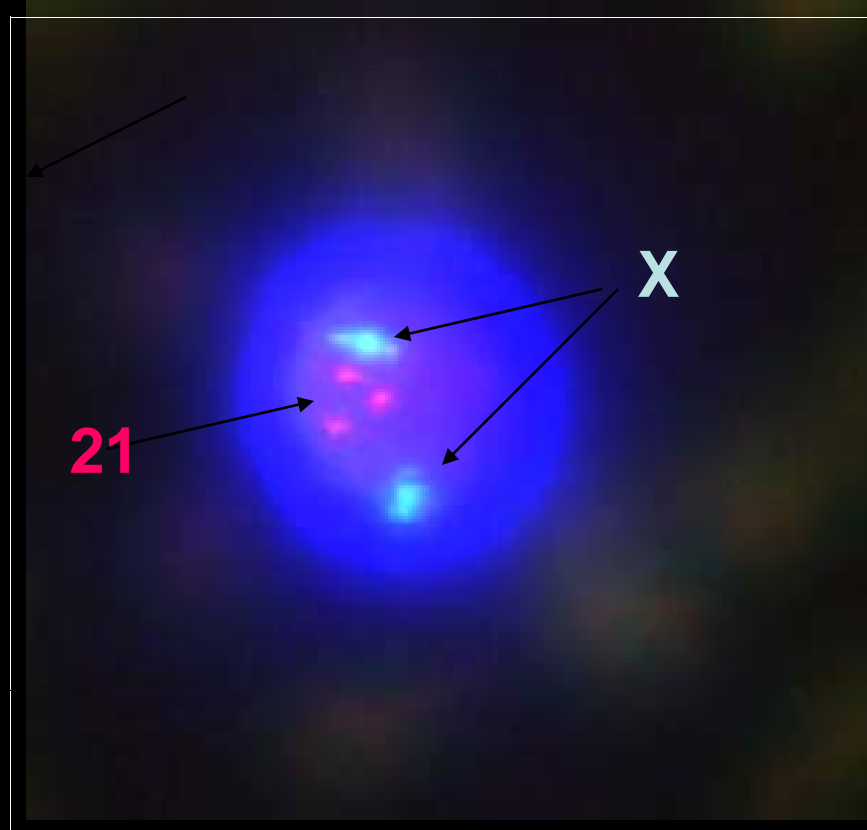
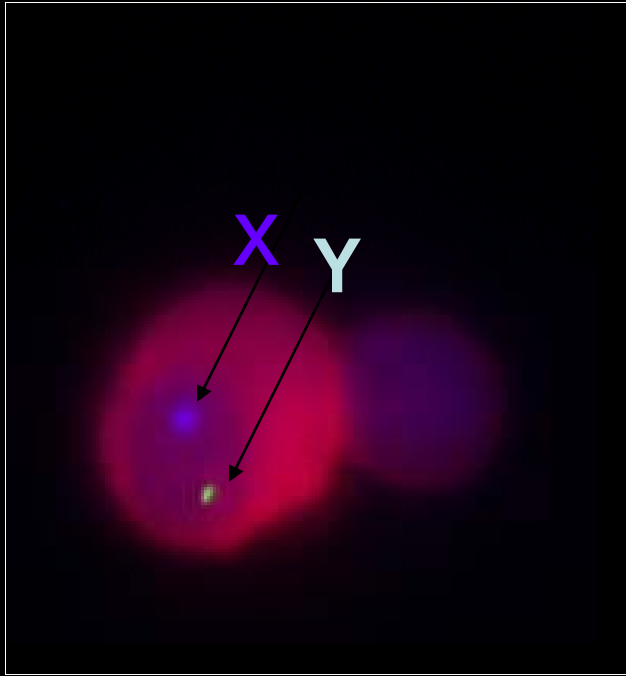
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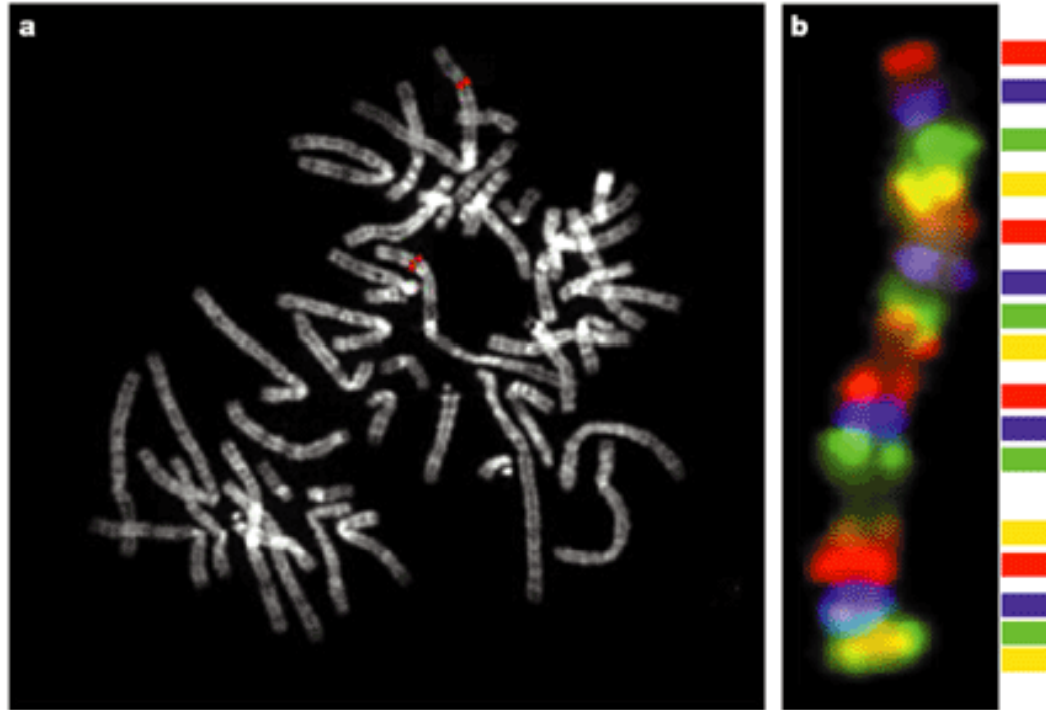
# Second era of cytogenetics (70s-2000s)



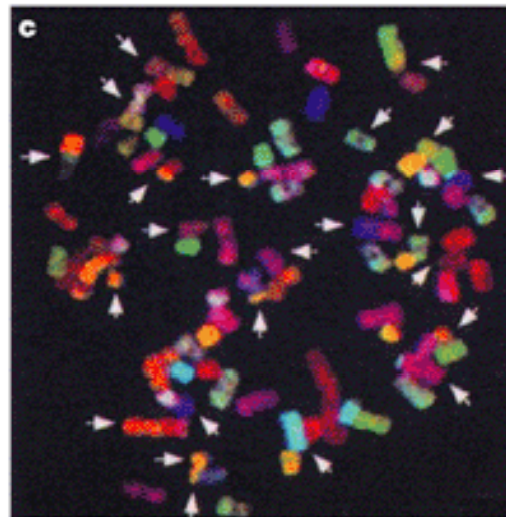
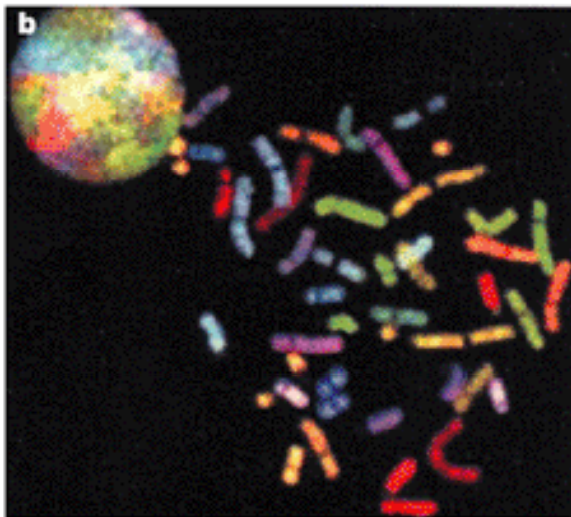
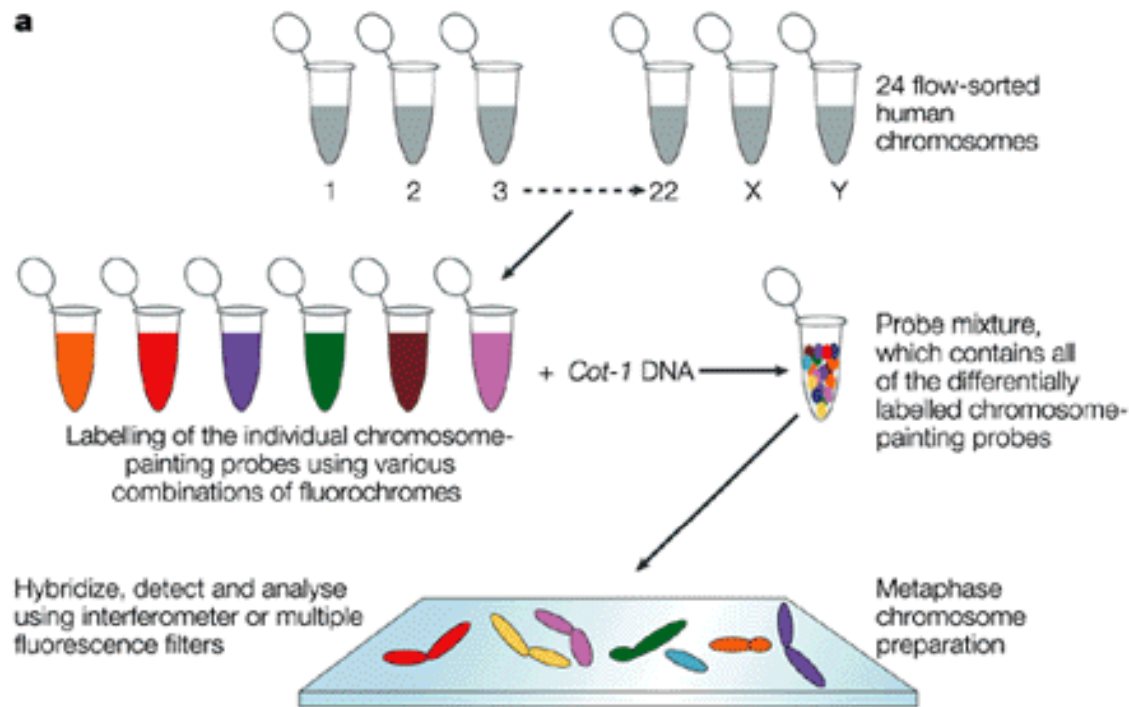
# FISH

Fluorescence in situ hybridization





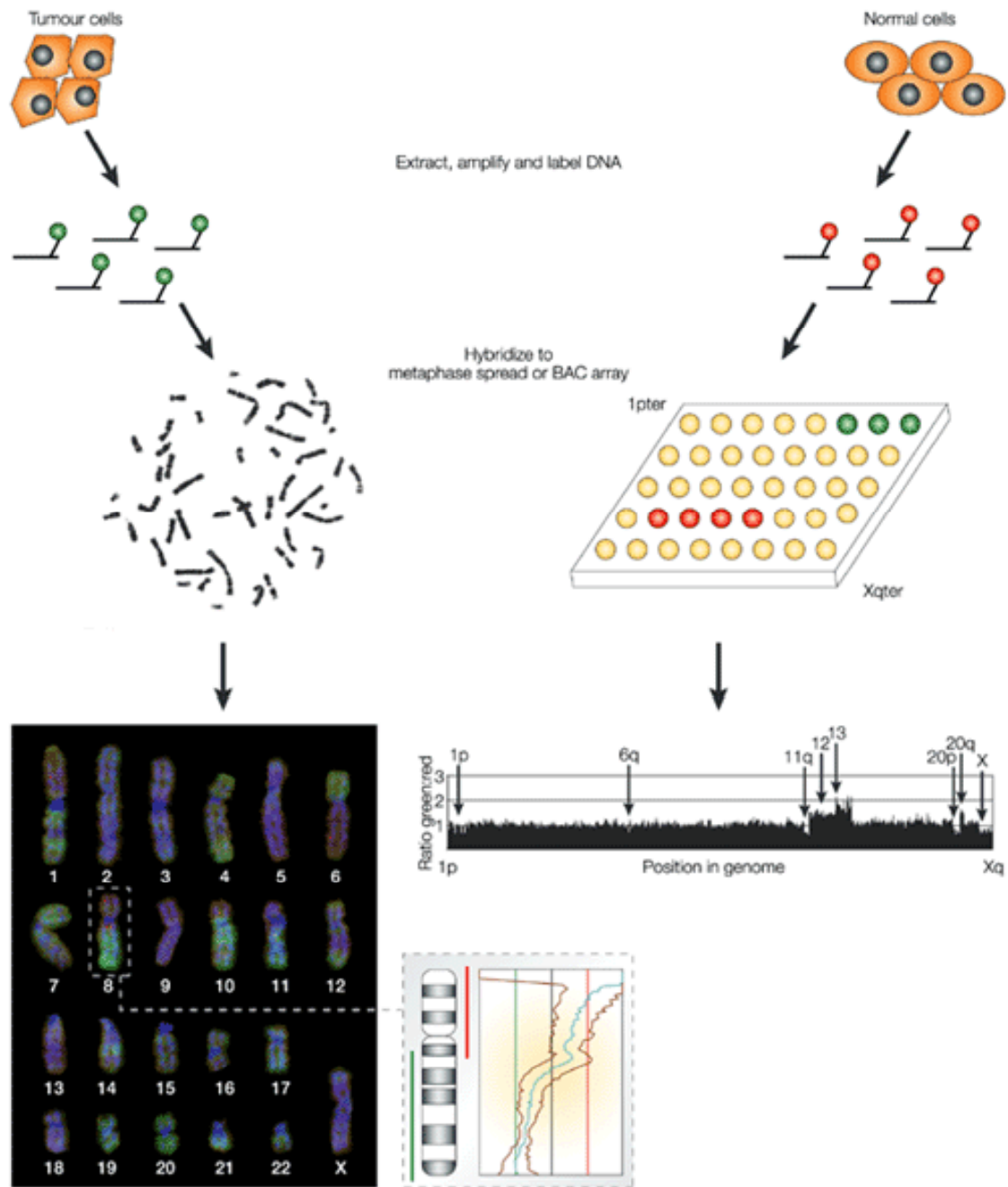
- a. FISH produces a fluorescent signal (red) at the sites of a specific DNA sequence; in this case, a 150-kb segment of chromosome
- b. Several probes, each corresponding to a defined genomic segment, can be simultaneously analyzed and ordered with respect to each other using multicolor FISH



Spectral karyotyping and multicolor-FISH paint each human chromosome in one of 24 colors.

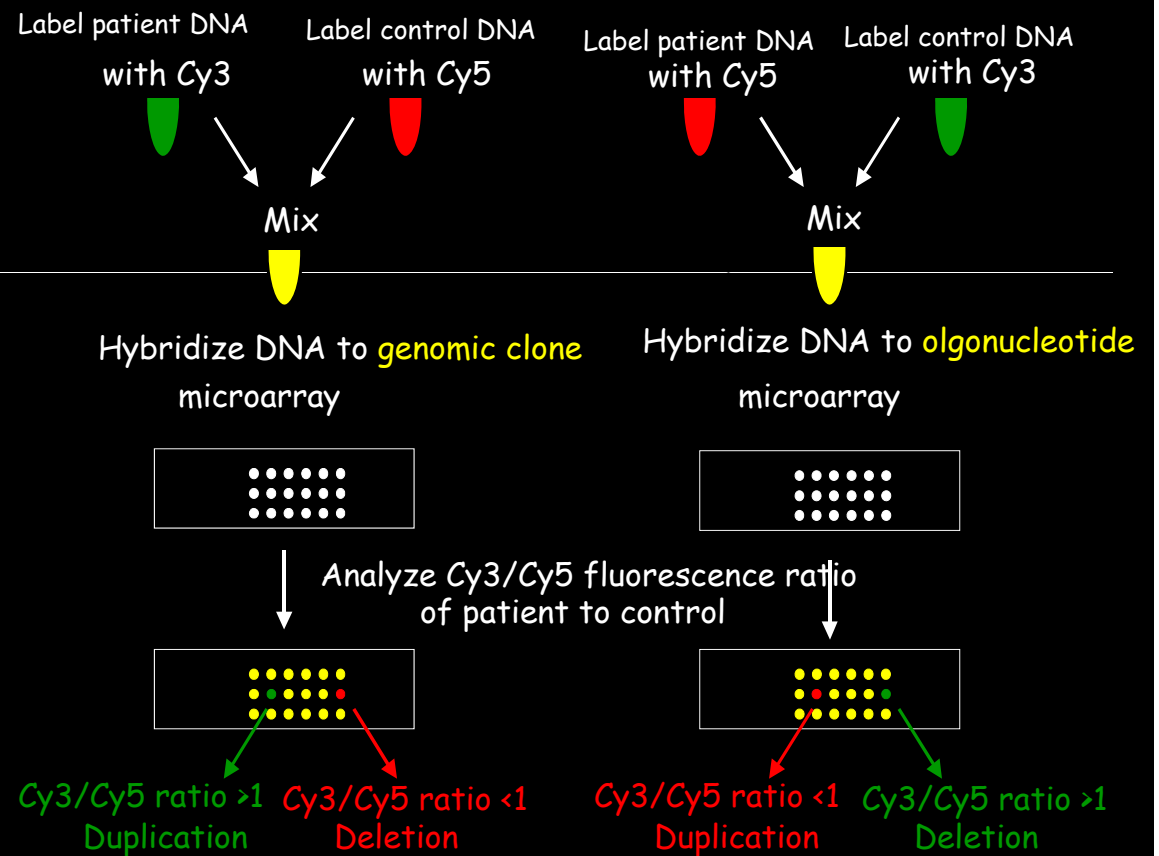
# COMPARATIVE GENOMIC HYBRIDIZATION (C G H)

- Identifies chromosomal gains and losses in a **single** hybridization procedure
- Effectively reveals any DNA sequence copy number changes (i.e., gains, amplifications, losses and deletions) in a particular specimen and maps these changes on normal chromosomes



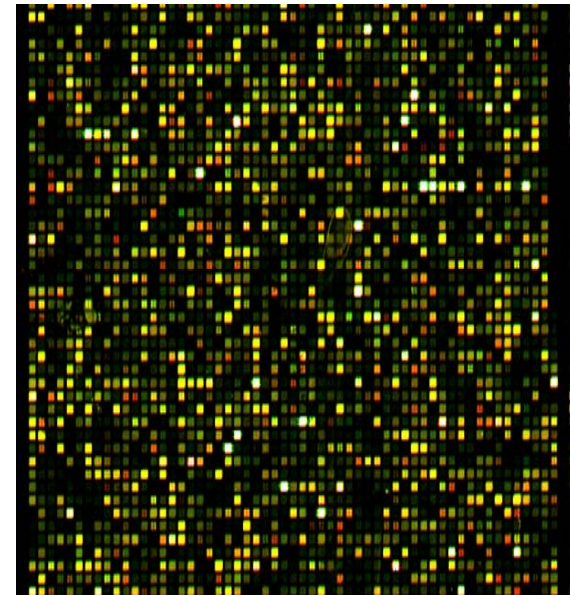
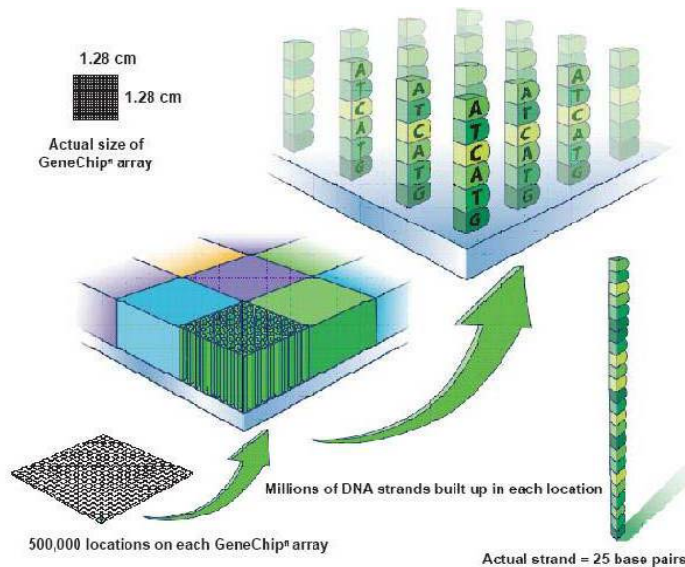
# Array CGH

( aCGH )



# Oligonucleotide-Based Microarrays

**Oligonucleotides are synthesized directly on slides**



**Short target sequences pretested for hybridization efficiency**

**Resolution limited only by number of oligonucleotides**

# Copy Number Variation (CNV)

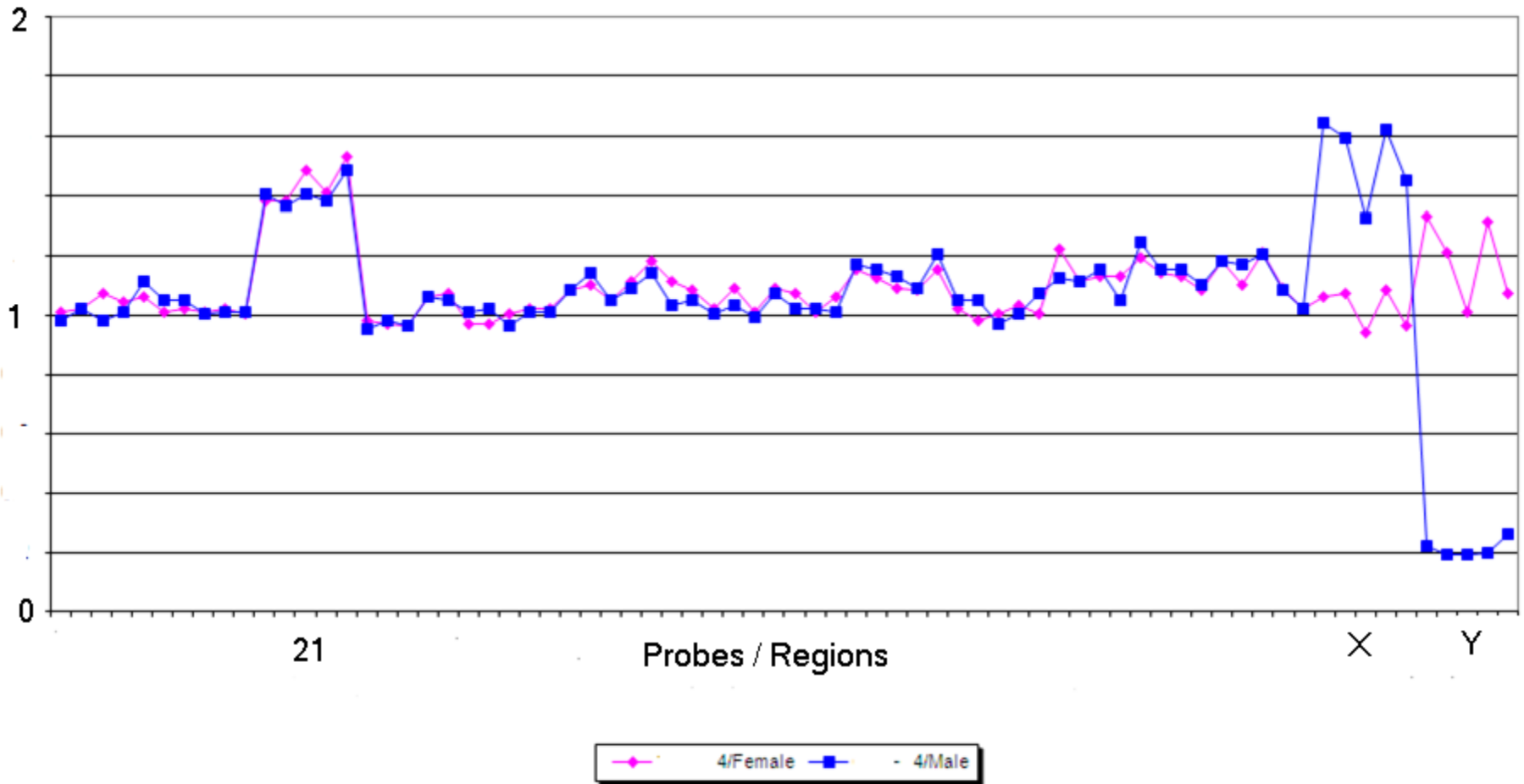
A CNV is a DNA segment (usually larger than 1 kb) present at an altered copy number in comparison with a reference genome

## Examples:

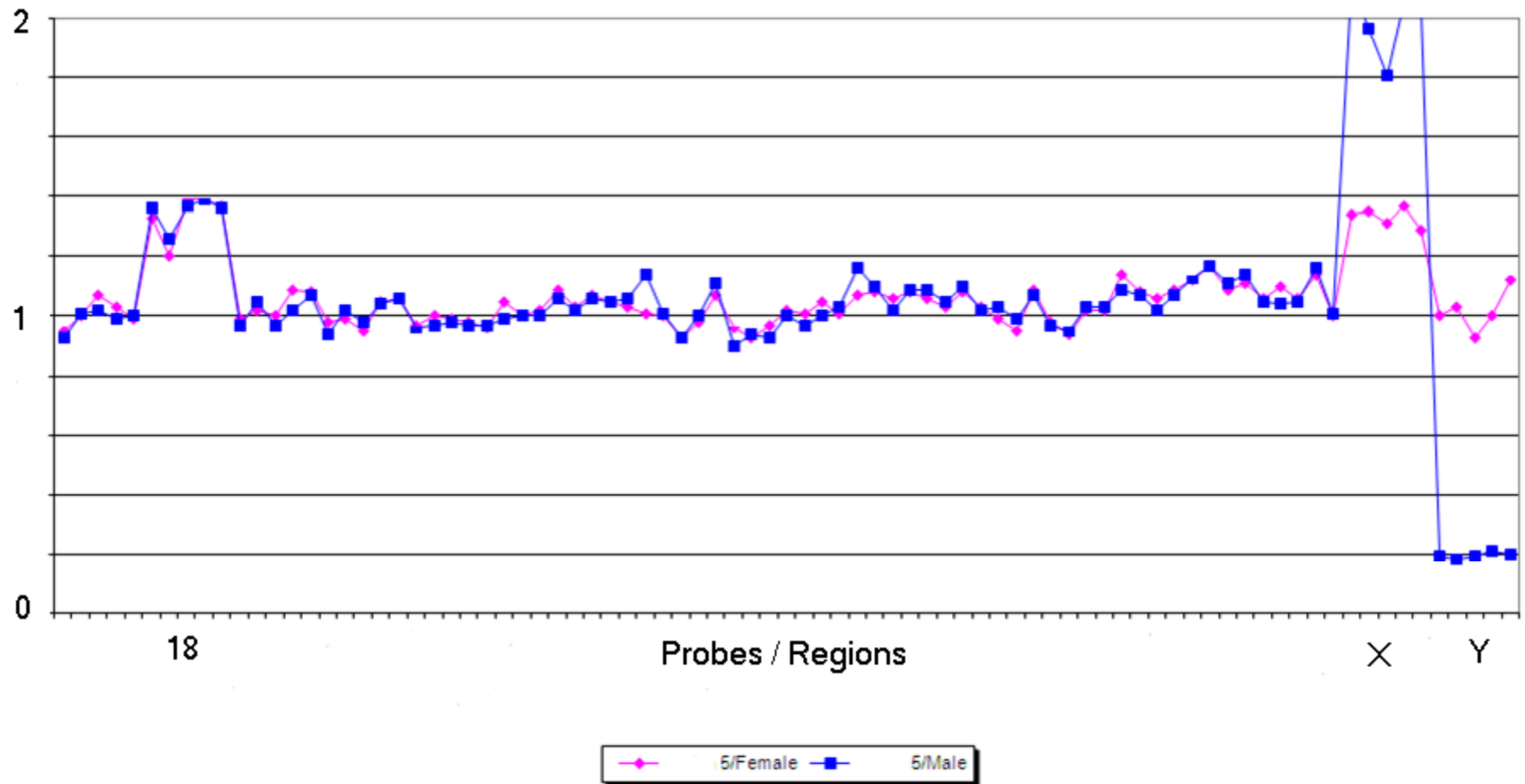
- Whole chromosome aneuploidy
- Segmental Aneuploidy
  - Deletions
  - Duplications
- Copy number polymorphisms



# Trisomy 21 Female

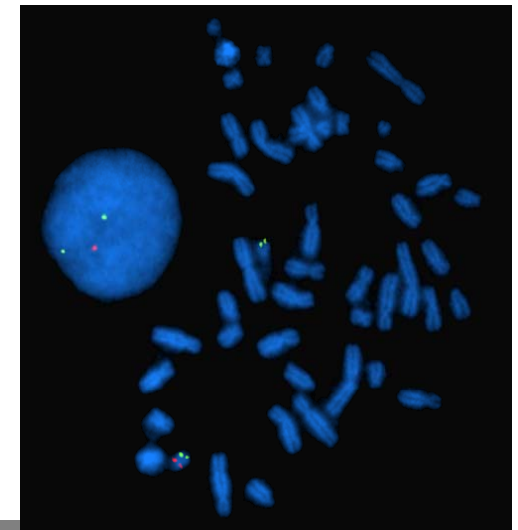
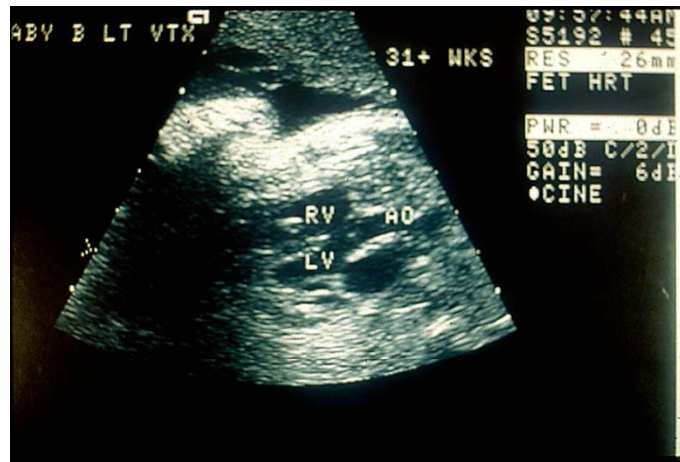
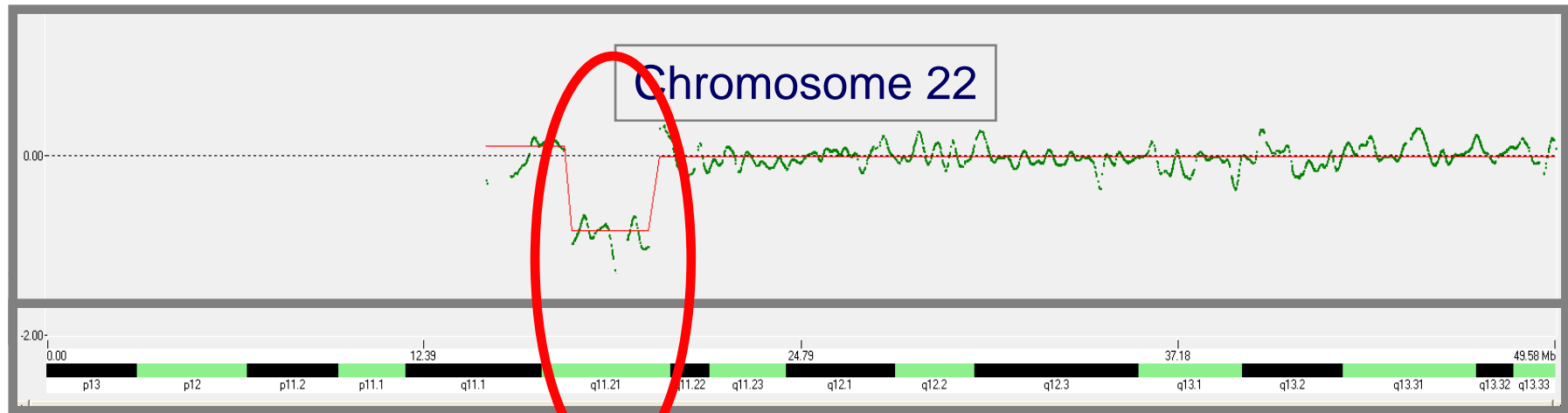


# Trisomy 18 Female XXX

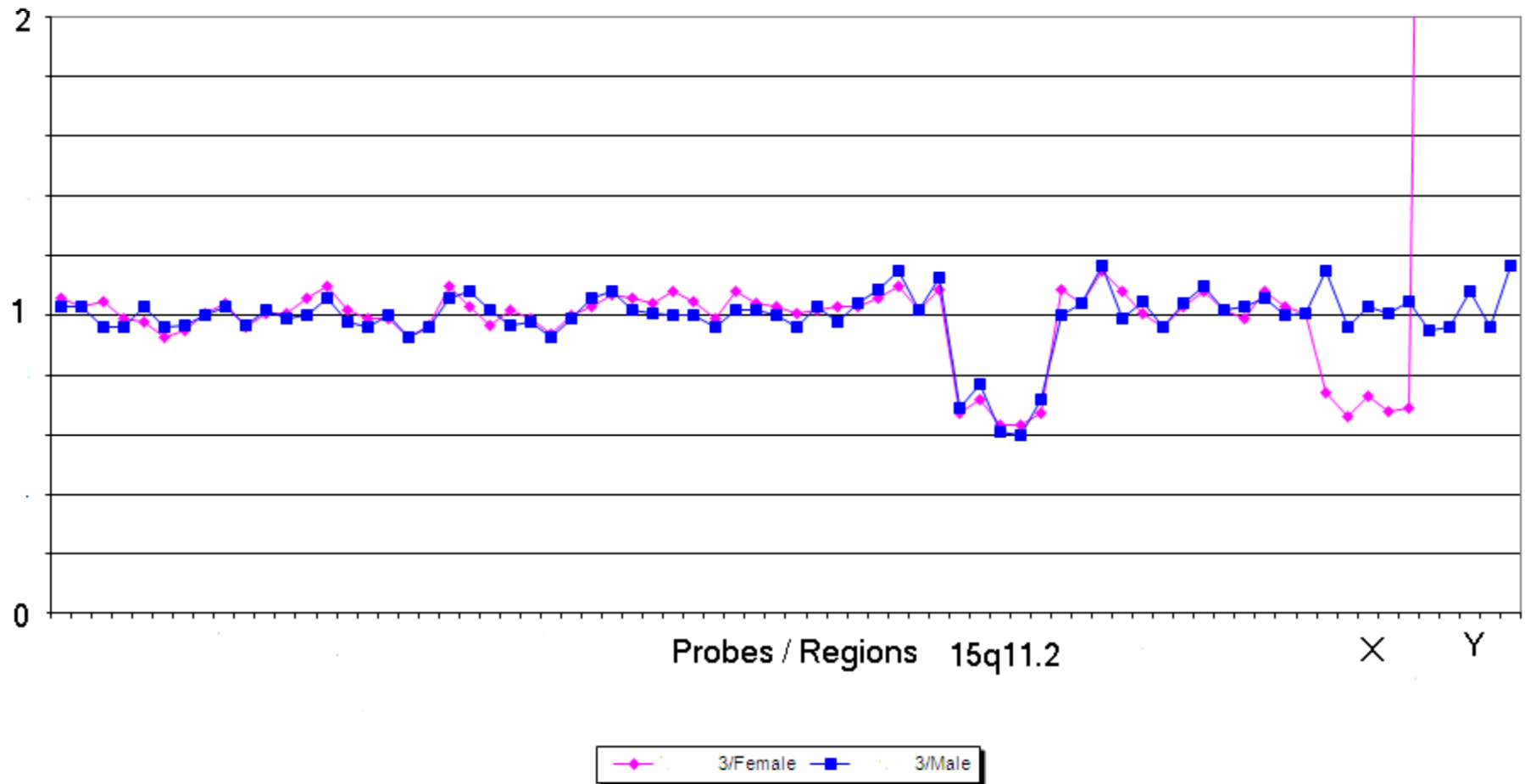




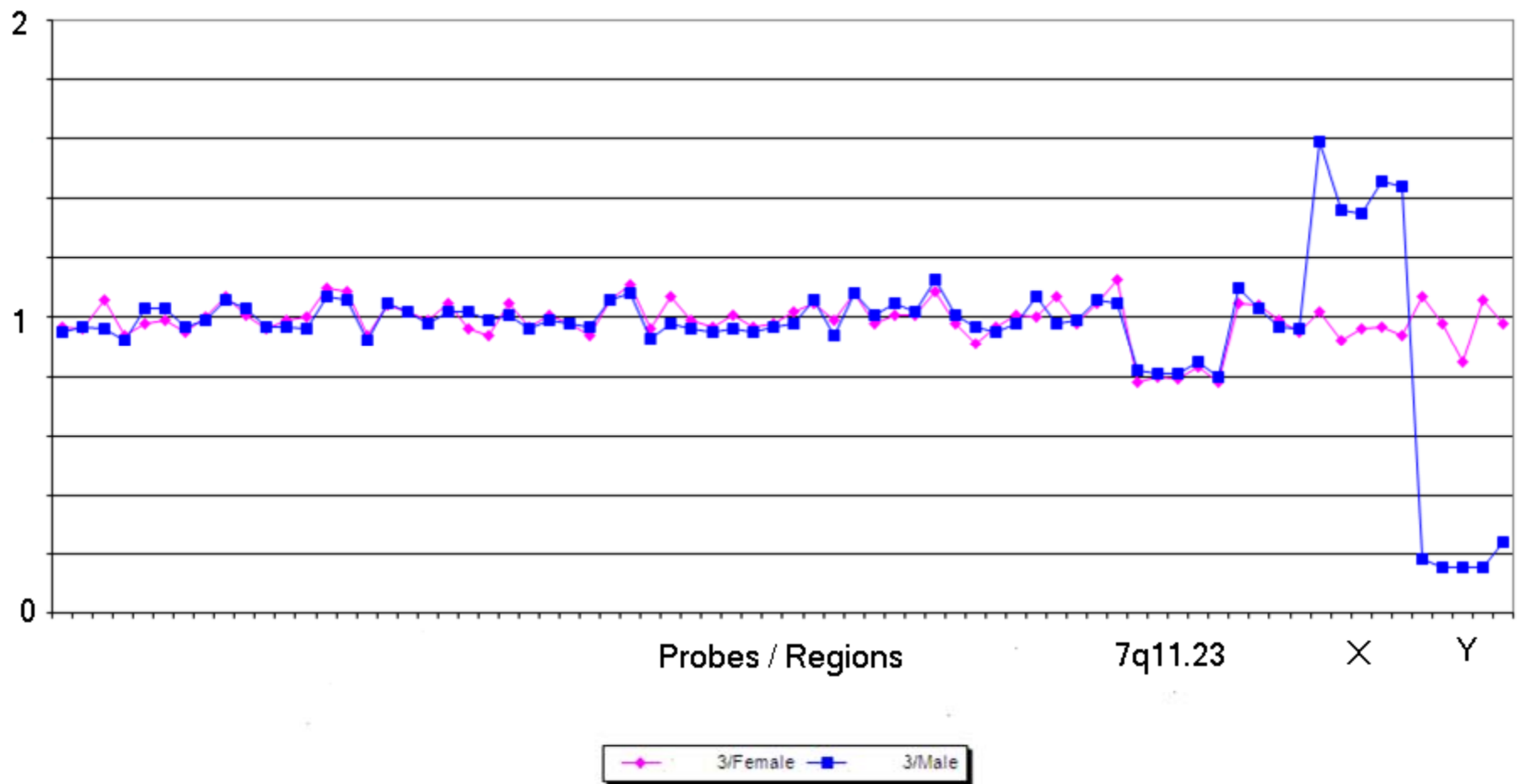
# 22q Deletion



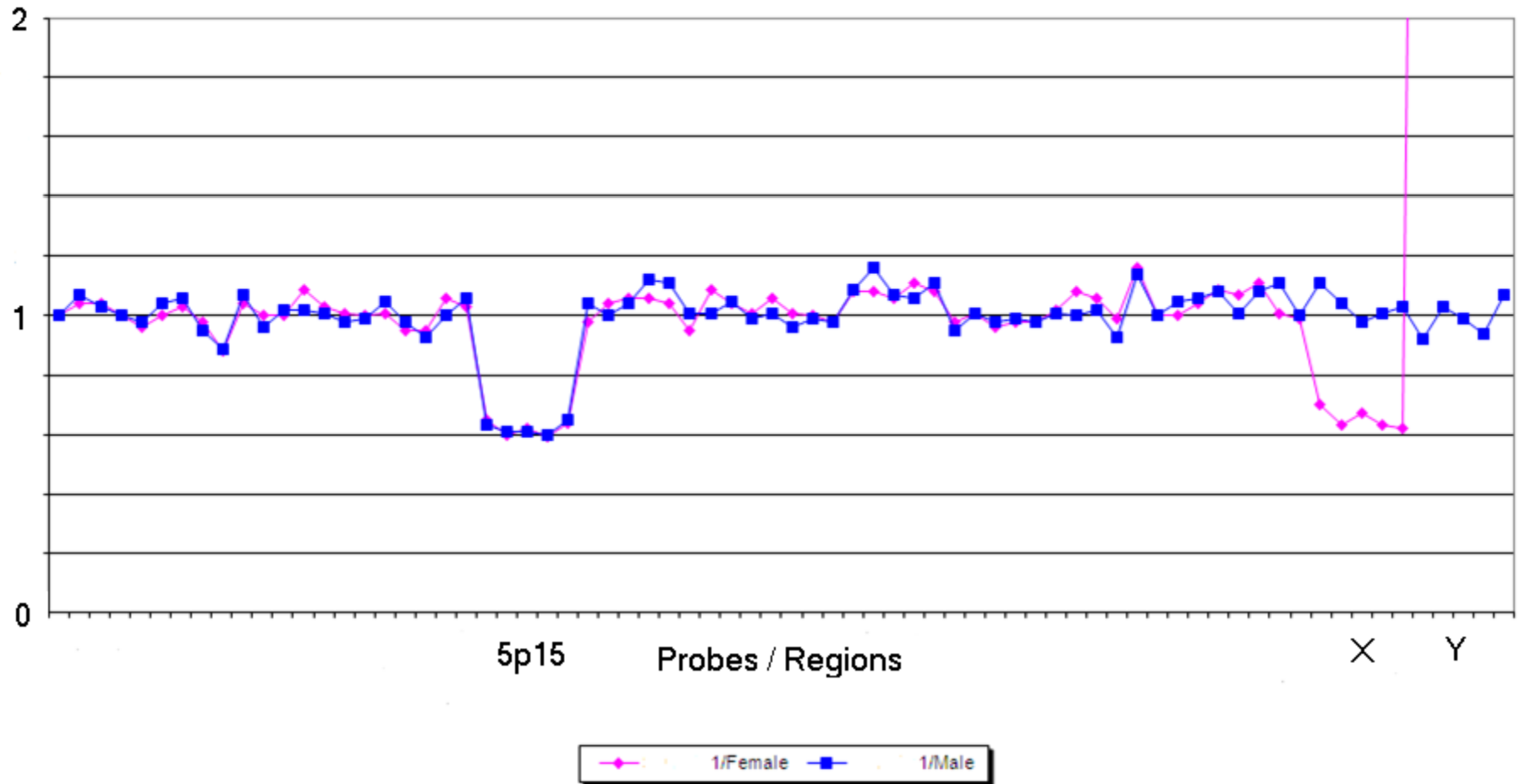
# 15q11.2 Deletion Male

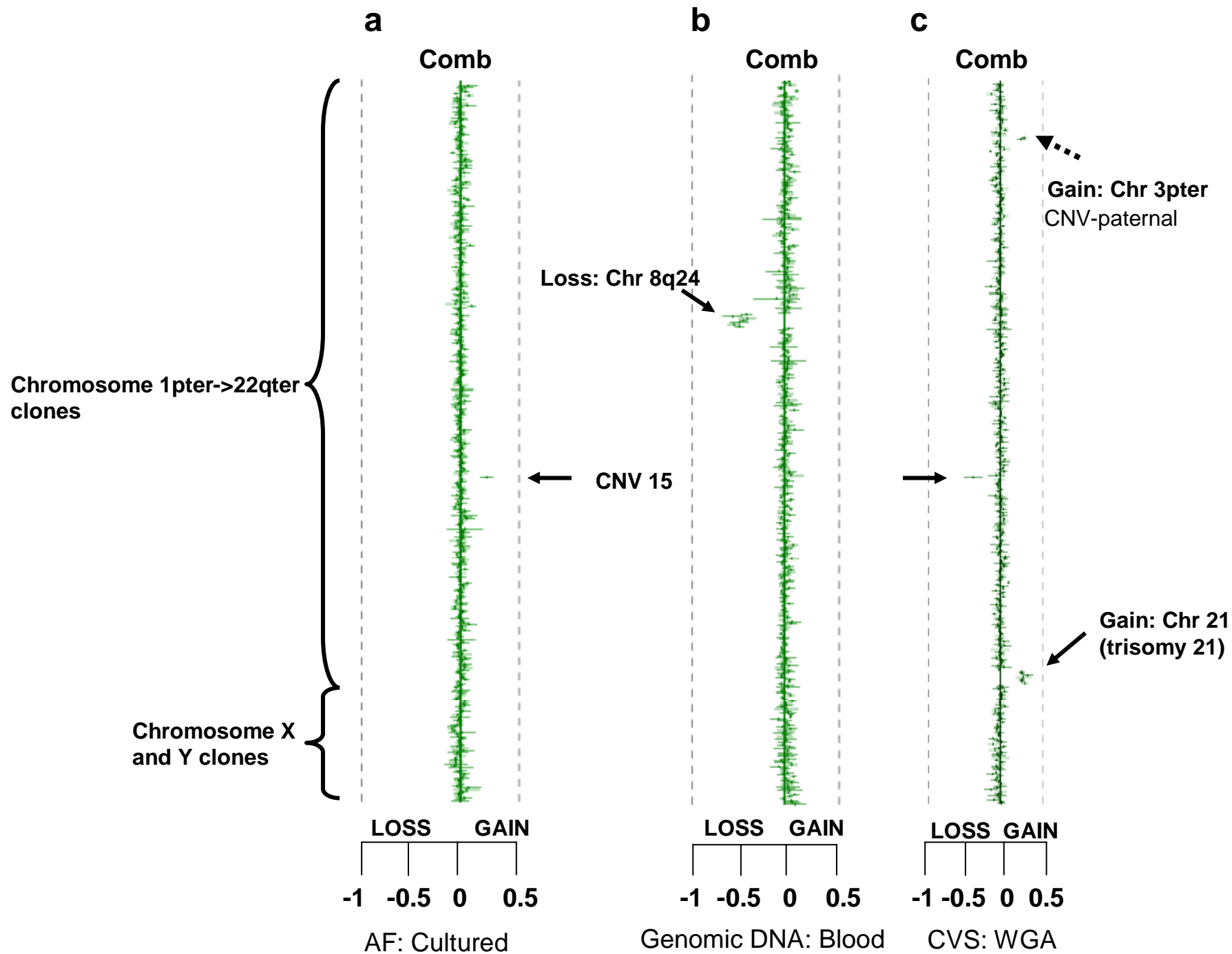


# 7q11.23 Deletion Female



# 5p15 Deletion Male





Prenatal Disorders	Frequency, 1 in n live births	Rate of detection via gain-losses
<b>DiGeorge</b>	<b>4,000</b>	<b>95%</b>
<b>Williams-Beuren</b>	<b>7,500</b>	<b>95%</b>
<b>Prader-Willi</b>	<b>10,000</b>	<b>70%</b>
<b>Angelman</b>	<b>15,000</b>	<b>85%</b>
<b>Smith-Magenis</b>	<b>15,000</b>	<b>90%</b>
<b>Wolf-Hirshhorn</b>	<b>50,000</b>	<b>95%</b>
<b>Cri-du-chat</b>	<b>30,000</b>	<b>99%</b>
<b>Langer-Giedion</b>	<b>200,000</b>	<b>95%</b>
<b>Miller-Dieker</b>	<b>200,000</b>	<b>90%</b>

# Potential Issues of aCGH for Prenatal Diagnosis

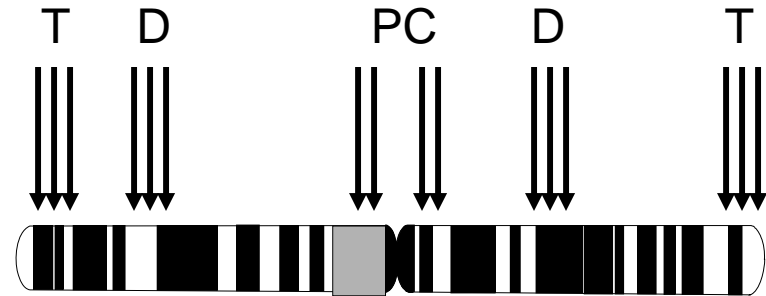
1. What should a prenatal diagnostic array contain?

***Is it better to use an array that is “targeted” to regions that are known to be involved in human disease or one that provides more or less uniform coverage across the entire genome (known as “Whole Genome Array”)?***

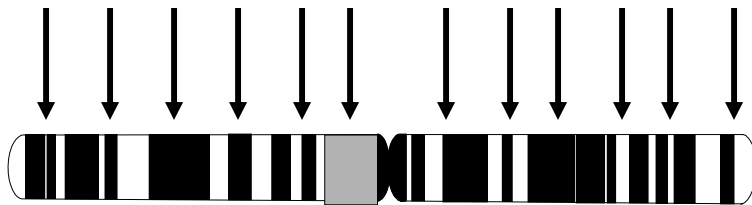
2. Distinguishing benign and pathogenic CNVs

3. Appropriate Counseling

- 450 – 1000 Oligos per array
- 4-5 oligos per region of interest
- 43 unique pericentric regions
- 41 unique telomere regions:
- Specific disease loci



Targeted Array



Whole Genome Array

- Uniform distribution of oligos along the entire length of each chromosome
- Density of distribution of oligos varies

# Criteria for Disease Loci on Prenatal Microarray

1. A significant proportion of cases caused by deletions or duplications
2. Disease of significant clinical importance
3. Spectrum of disease understood
4. Associated with ultrasound findings

## Post Natal Arrays For Children With Normal Conventional Karyotype, Dysmorphic Features And /Or Developmental Delay

<b>High Resolution Arrays</b>		<b>N</b>	<b>% with significant CNV</b>
DeVries 2005	Whole genome tiling-path	100	10
Friedman 2006	100kb	100	11
Menten 2006	1.0Mb	140	13.6

### **Targeted Arrays**

Poss 2006	Targeted	121	9.6
Aylor 2006	Targeted	1200	7.0
Schaeffer	Targeted	1500	5.6

## Prenatal Diagnosis Comparing Whole Genome aCGH versus Targeted aCGH\*

<b>Category</b>	<b>Whole Genome Array</b>	<b>Targeted Array</b>
Clinically relevant CNV	2.7%	0.9%
Benign CNV	8.8%	8.0%
Unclear clinical relevant CNC	0.5%	0.5%

\* Coppinger et al., 2009

# Prenatal Diagnosis Based on High Resolution Array CGH\*

<u>Category</u>	<b>Abnormal US</b>	<b>AMA Family History</b>
1. Clinically relevant CNV	<b>3.9%</b>	<b>0%</b>
2. Benign CNV	<b>8.3%</b>	<b>12%</b>
3. Unknown clinical significance	<b>0.6%</b>	<b>0%</b>
Total number of cases	<b>155</b>	<b>25</b>

\* Coppinger et al., 2009

# Use of Array CGH in Prenatal Diagnosis

<b>Indication</b>	<b>Author</b>	<b>Cases</b>	<b>Pathological CNV</b>	<b>Benign CNV</b>	<b>Unknown</b>
<b>US ABN</b>					
	Kleeman et al	<b>50</b>	<b>2%</b>	<b>6%</b>	
	Tyreman et al	<b>106</b>	<b>9%</b>	<b>11%</b>	<b>12%</b>
<b>NT&gt;3mm</b>					
	Schou et al	<b>100</b>	<b>0%</b>		
<b>US+AMA</b>					
	Van den Veyver et al	<b>300</b>	<b>5%</b>	<b>13.3%</b>	<b>1%</b>

In Progress.....

**NIH MULTICENTER  
CLINICAL TRIAL  
ON POTENTIAL USE OF  
MICROARRAYS  
IN  
PRENATAL DIAGNOSIS**

# Advantages of Molecular Karyotyping by aCGH

1. Higher resolution independent of the ability of the cells to grow and/or generate good metaphase spreads
  - a. Standard karyotype: 5Mb resolution
  - b. aCGH: 1 Mb to 100 or less kb resolution
2. Direct mapping of aberrations to the genome sequence
3. Amenable to automation and quality control procedures
4. Higher throughput and shorter reporting times

**Better and Cheaper**

QUESTION?????????

CONVENTIONAL  
CHROMOSOME ANALYSIS

VS

MICROARRAY ANALYSIS

**Suppose: Two types of a chromosome abnormality (CA):**  
**1) All CA based on conventional karyotyping and,**  
**2) All microdeletion syndromes (MDS)**

Then the risk of a CA at time of CVS: NL:CA:MDS

Patient risk in absence of any information:

$$1:1/50:1/50 = 50:1:1 \text{ or } \mathbf{1 / 26}$$

If the fetal karyotype is normal, the final risk:

$$1:0:1/50 = 50:0:1 \text{ or } \mathbf{1 / 51}$$

If microarrays detects 90% of the MDS, the final risk:

$$1:0:1/500 \text{ or } 500:0:1 \text{ or } \mathbf{1 / 501}$$

If patient only had microarrays without karyotype, the final risk:

$$1:1/500:1/500 \text{ or } 500:1:1 \text{ or } \mathbf{1 / 251}$$