Genomics and Microarrays: Novel Methodologies and Objectives

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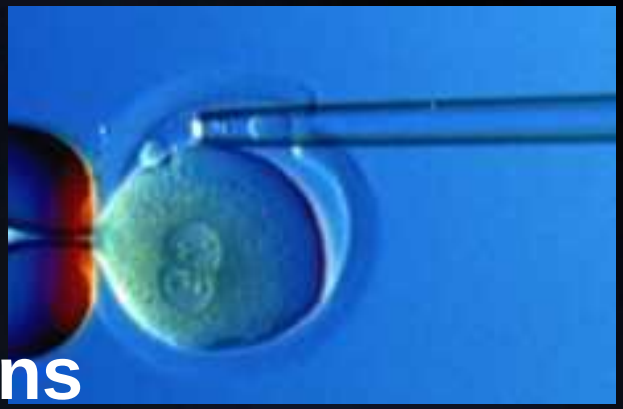
Indications for Genetic Assessment of Embryos

- Aneuploidy screening (PGS)
- Structural chromosome aberrations (PGD)
- Single gene disorders (PGD)
- Mitochondrial disorders (PGD)

Cell Types - Biopsy

- **Polar Bodies**
  - Meiosis I errors
  - Meiosis II errors
  - Structural aberrations
  - X-linked disorders



- **Blastomeres**

Biopsy a Better Cell Type?

- Trophectoderm

New Technologies- Microarrays

- **Single nucleotide polymorphisms (SNPs)**

T

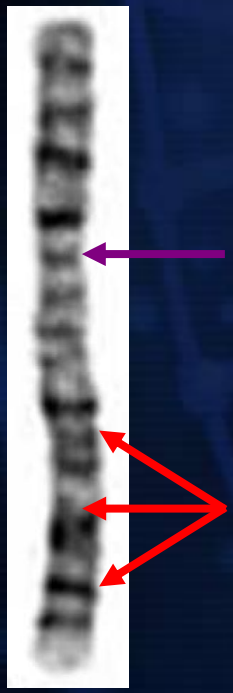
5’-ACTGGGAATCCCGAAGTGTGC TGATTACA-3’

  - **Normally occurring genetic variant**
  - **~11,000,000 estimated to be in human genome**
  - **Stable in >1% of the human population**

    - **More dense**
      - **Various density arrays**
        - **Up to ~ 1,000,000 genomic hits**

- **CGH**
  - **Less dense**
  - **Ratio**
  - **Oligonucleotides or BACs**
    - **~ 42,894 genomic hits (~ Exonic (16404), Intronic (19805), Intergenic (6685))**

FISH vs Microarrays

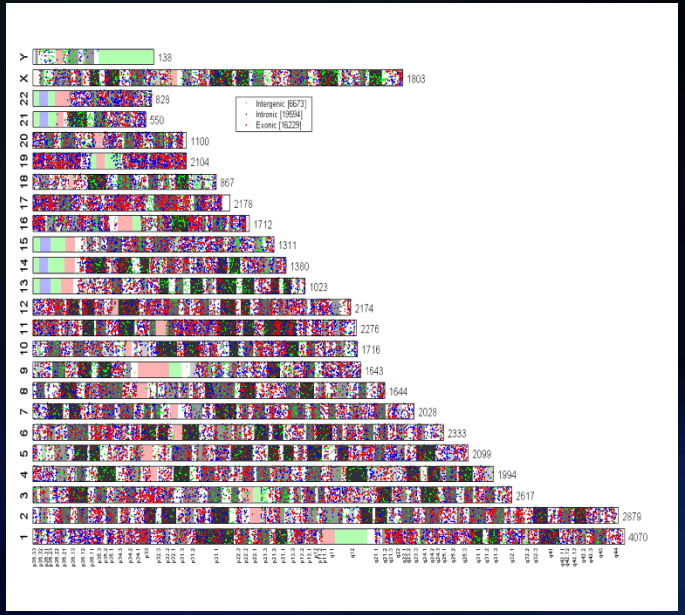


FISH: hybridization of a long oligonucleotide to a peri-centromeric or locus specific location

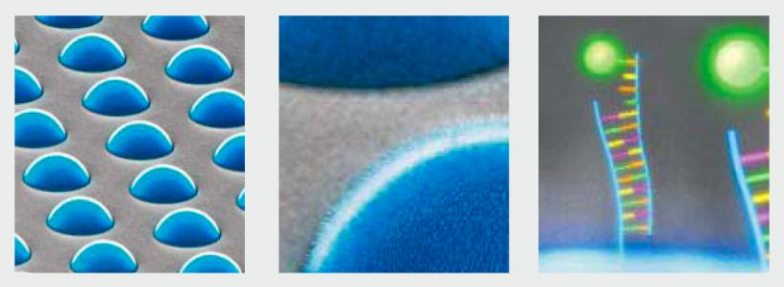
- detection via microscopy
- analysis and interpretation via microscopy

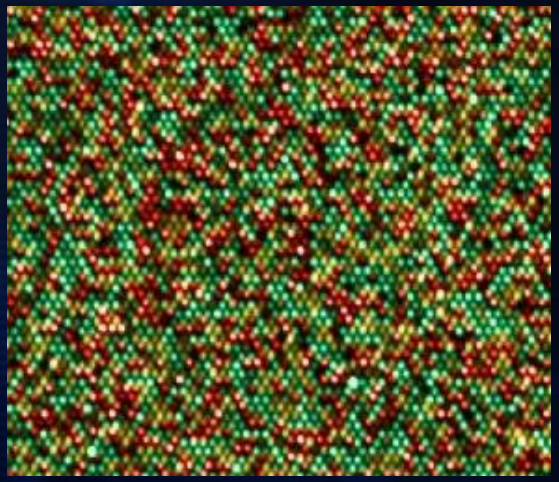
SNP or CGH: DNA markers throughout the chromosome

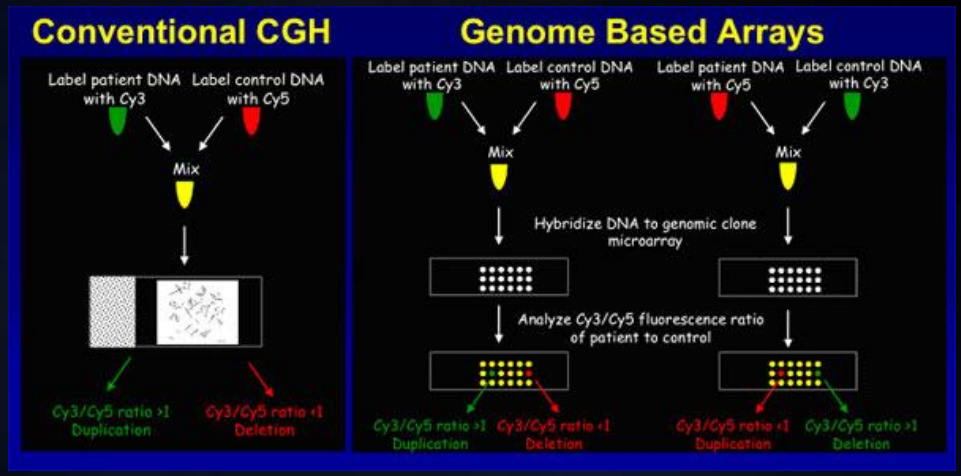
- detection via microarray and scanner
- analysis and interpretation via algorithm



SNP and CGH Microarrays







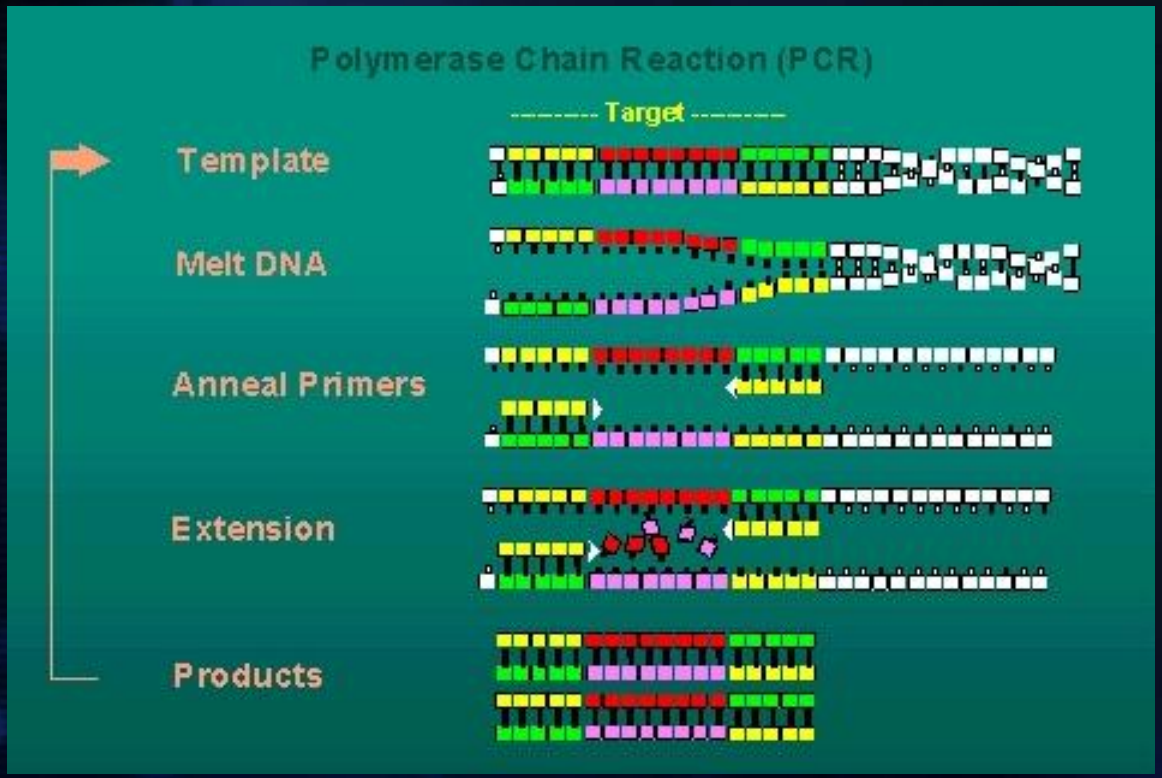
SNP Arrays vs CGH Arrays

Genetic Diagnostics and Screening	SNP	CGH
23/24 chromosome aneuploidy	X	X
Copy number variations (CNVs)	X	X
Structural chromosome imbalances	~1.5kb	~.1-10mb
Genome-wide scans	X	
What embryo implanted?	X	
What partner provided the extra chromosome?	X	
Single gene disorders	X	
Mitochondrial mutations	X	
Uni-parental disomy	X	
Copy neutral event	X	

Validation -Materials and Methods

- n = 802 single cells / blastomeres (110 day-3 abnormal embryos) and 34 cell lines
- Embryo biopsy of a single cell – laser
  - Blastomere
- Modified whole genome amplification (WGA)

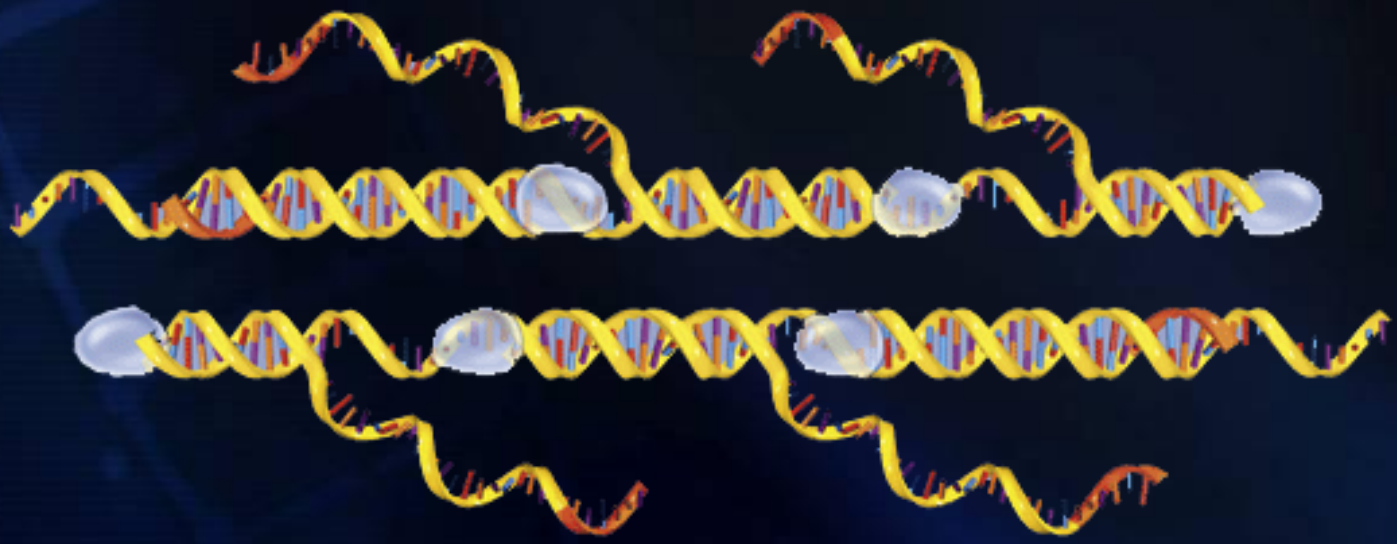
Experimental Problem to Overcome



- ~ 6 picograms DNA
- Need 200ng for microarray analysis
- PCR
  - Artifacts
  - Amplification
  - Allele dropout

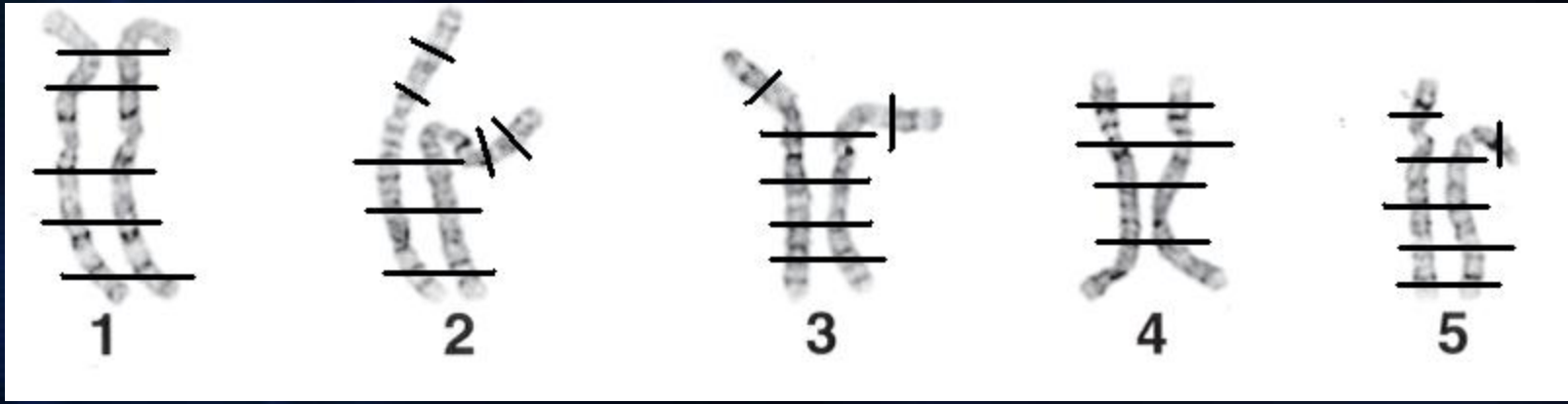
Modified Whole Genome Amplification (WGA)

- Home Brew



Materials and Methods

- **Invariant DNA genomic loci identified by PCR to ensure the entire genome was amplified**



Materials and Methods

- TaqMan PCR to ensure heterozygous allele amplification

- Microarrays
  - Illumina HumanHap370 ~370,000 SNPs
  - Illumina Cyto-12 ~ 320,000 SNPs

Materials and Methods

- **Two-channel intensity values – high-resolution copy-number profile**
  - **– Identifies copy number variations (CNVs)**
- **Genome-Wide scans / Genotyping**
  - **Identifies SNP Genotypes**
  - **Genome-Wide Scan Analysis**
    - **Single Gene Disorders**
    - **Complex Genetic Disorders**
- **Bioinformatics**
  - **deCode genetics Disease Minor Professional, Illumina BeadStudio, GenomeStudio and KaryoStudio software**

Results

- DNA yields of ~ 300 ng / ul – 4 hr rxn
- In many cases, a genomic coverage > 98% (Range 30-98%)
  - Correlates with day-3 embryo quality
- Heterozygous allele detection rate > 90%

Results

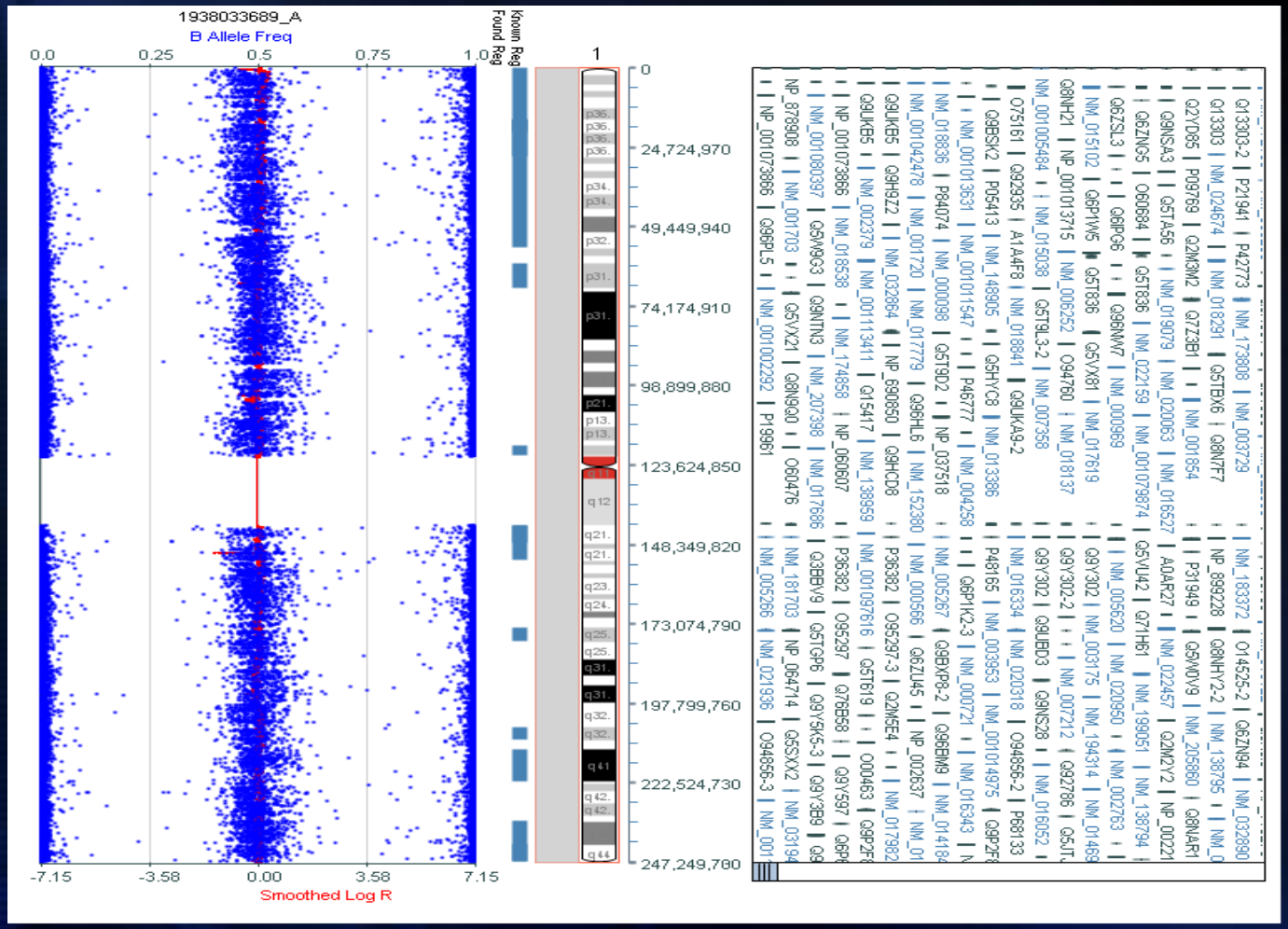
- Microarray detection rate > 90%
  (some cases > 99%)
  - (Range 30-98%)
    - Correlates with day-3 embryo quality
    - 30% detection rate still permitted aneuploidy detection

- Genotype call rate > 90%
  (some cases > 99%)
  - (Range 30-98%)
    - Correlates with day-3 embryo quality
    - 30% call rate still permitted aneuploidy detection

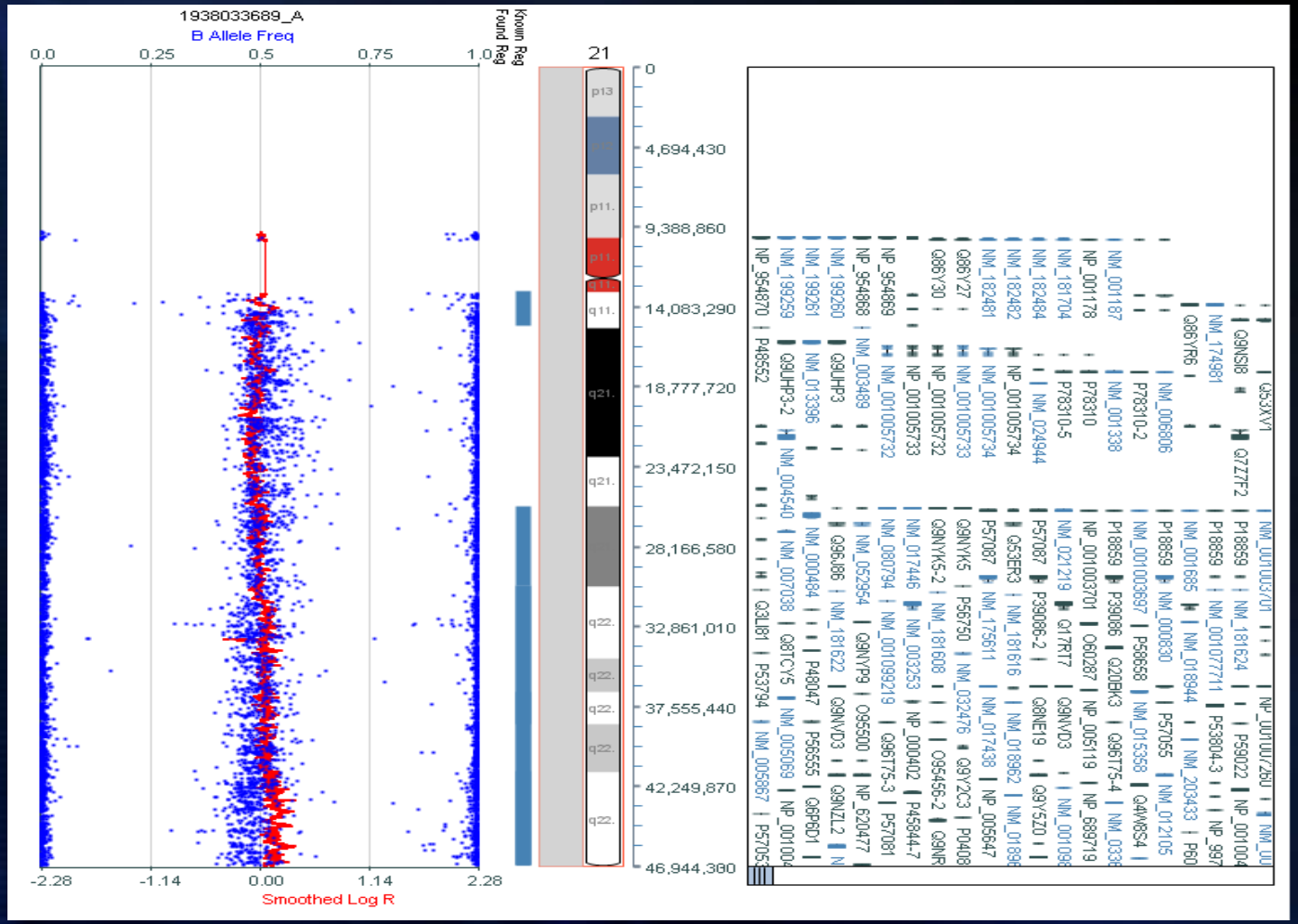
Aneuploidy Results

- 110 embryos (day-3 PGS *abnormal* for 10-chromosome FISH)
  - Aneuploid = 5%
    - 3 chromosomes
  - Mosaic diploid / aneuploid = 64%
    - Ranged from 2 – 7 chromosomes
  - Mosaic aneuploid = 23%
    - Ranged from 3 – 9 chromosomes
  - Complex mosaic = 8%
    - Ranged from 3 – 13 chromosomes

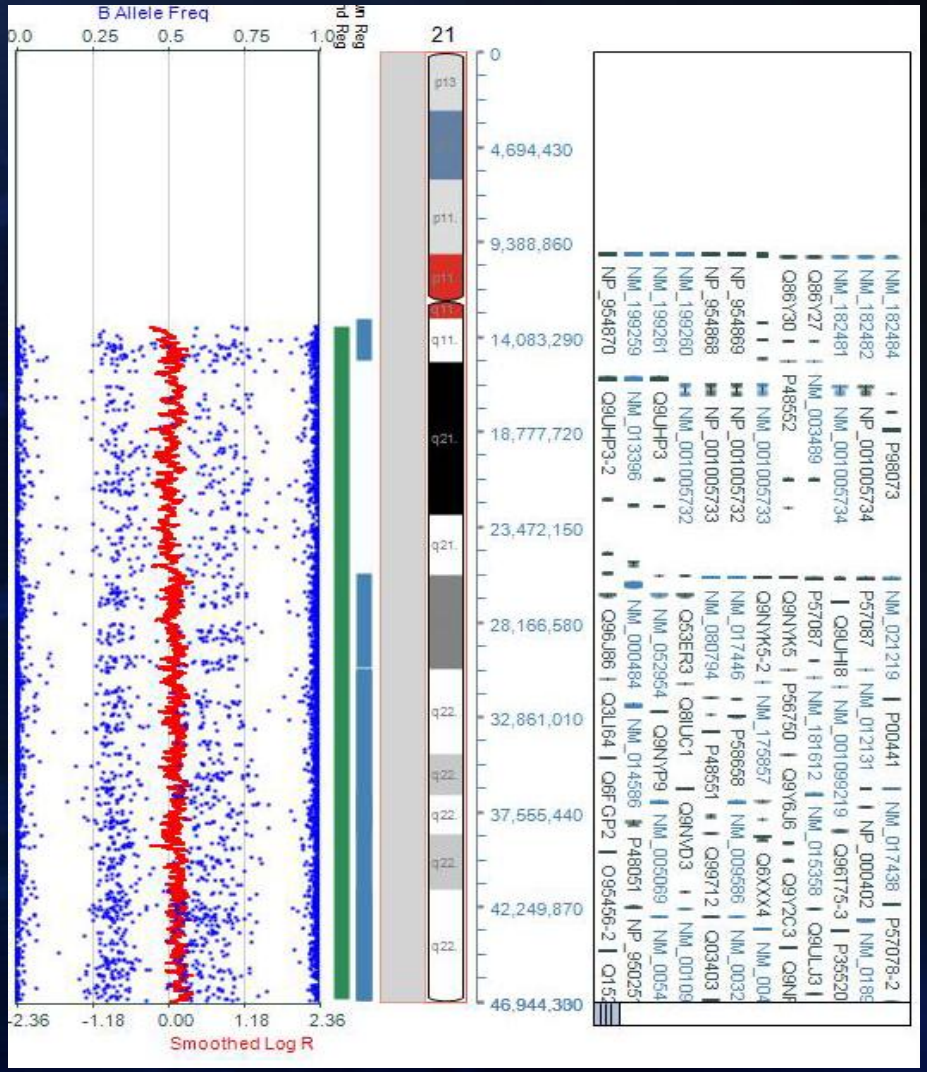
Two Copies of Chromosome 1



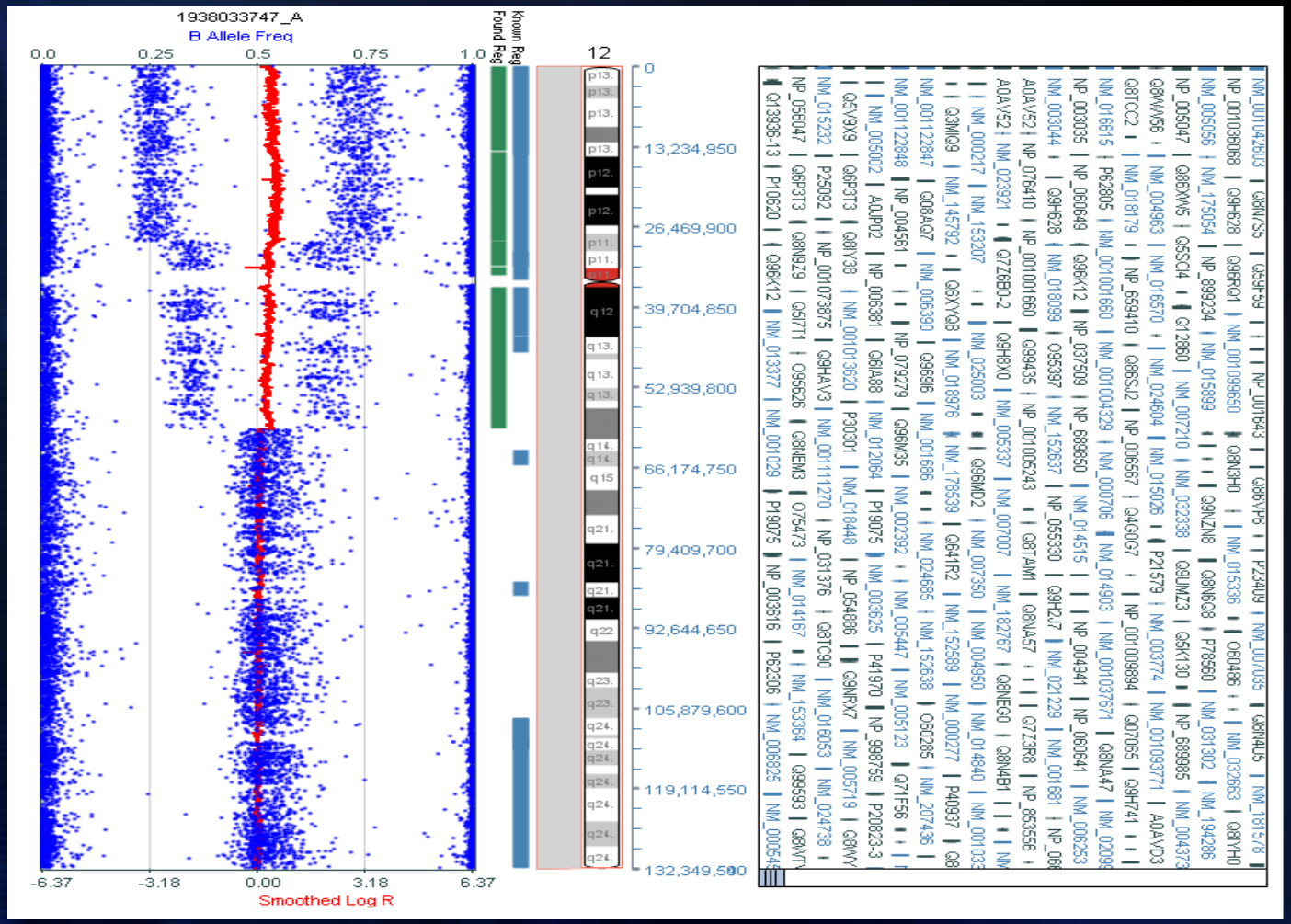
Two Copies of Chromosome 21



Three Copies of Chromosome 21



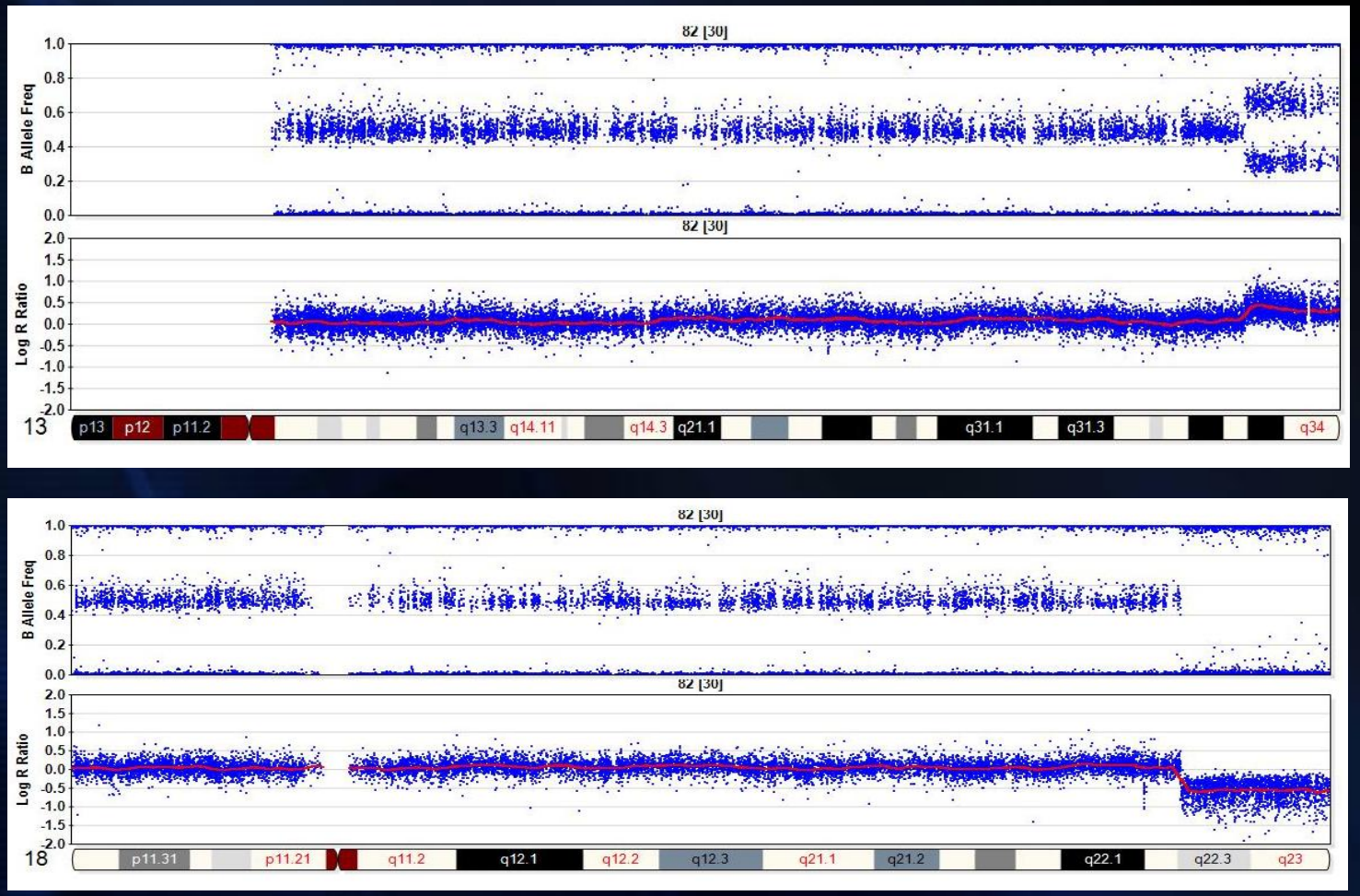
Three Copies of Chromosome 12
One is Deleted from Band q14 ?



Results

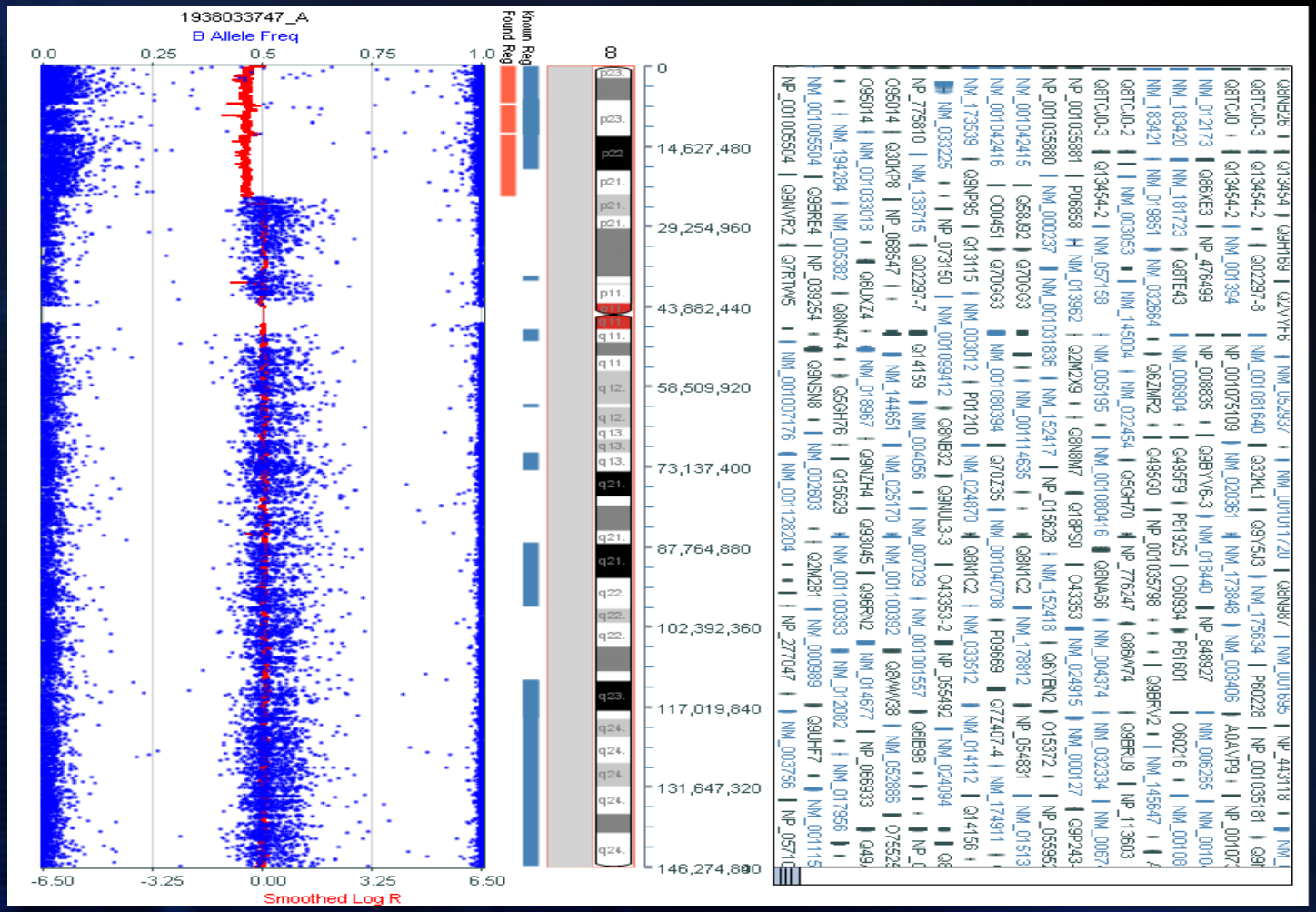
- Structural chromosome imbalances were identified from all 9 cytogenetically abnormal cell lines
  - del(8q), add(17p), del(17p), add(4q), add(9p), add(14q), dup(18p), dic(5), del(12p) and del(9p)
    - Based upon the density of the SNP microarray
    - CGH array couldn’t identify genetic imbalances

Unbalanced Reciprocal Translocation

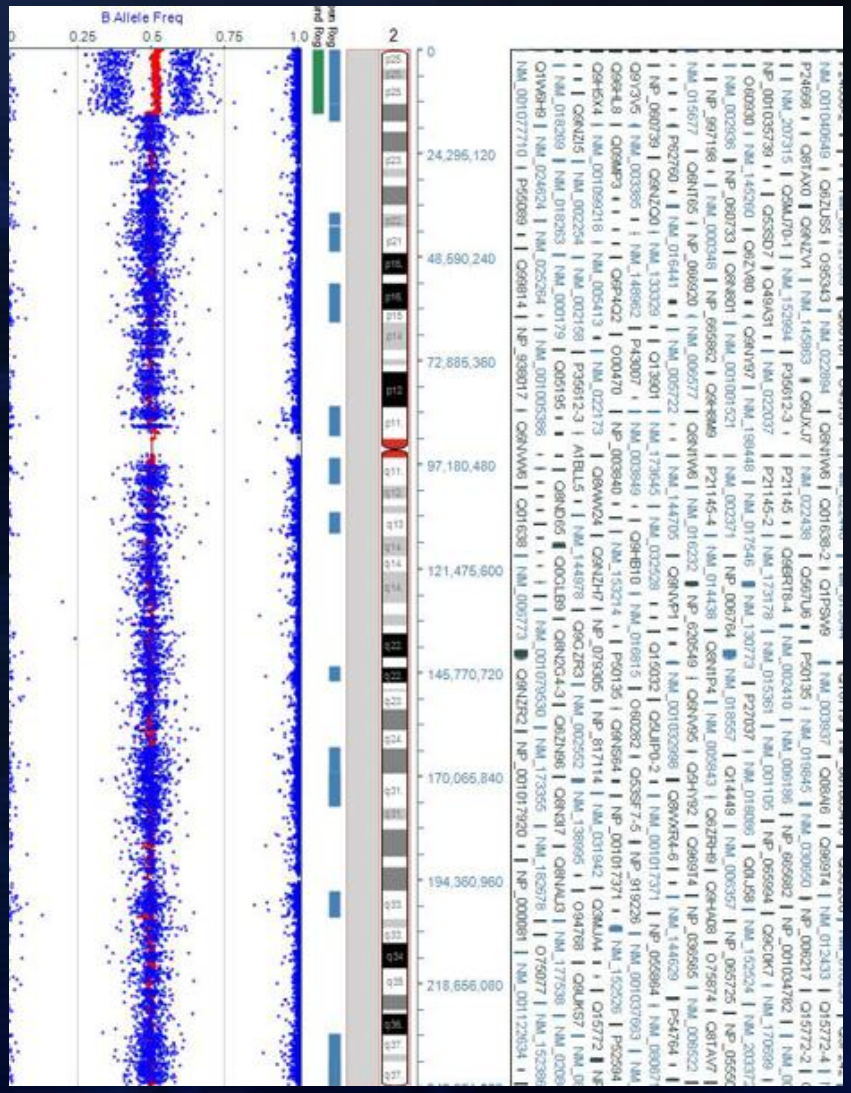


der(18)t(13;18)(q33.2;q22.3)

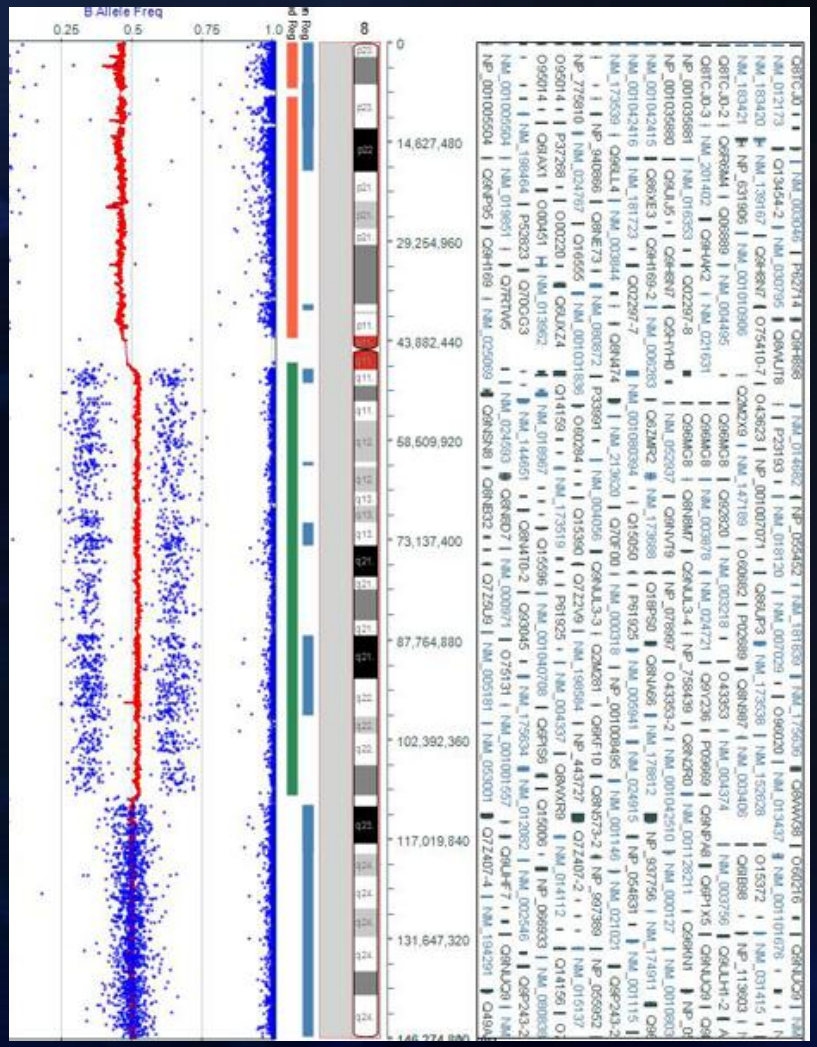
8p Deletion


1938033747_A
B Allele Freq
0.0
0.25
0.5
0.75
1.0
Known Reg
Found Reg
8
0
14,627,480
29,254,960
43,882,440
58,509,920
73,137,400
87,764,880
102,392,360
117,019,840
131,647,320
146,274,800
-6.50
-3.25
0.00
3.25
6.50
Smoothed Log R

2p Duplication



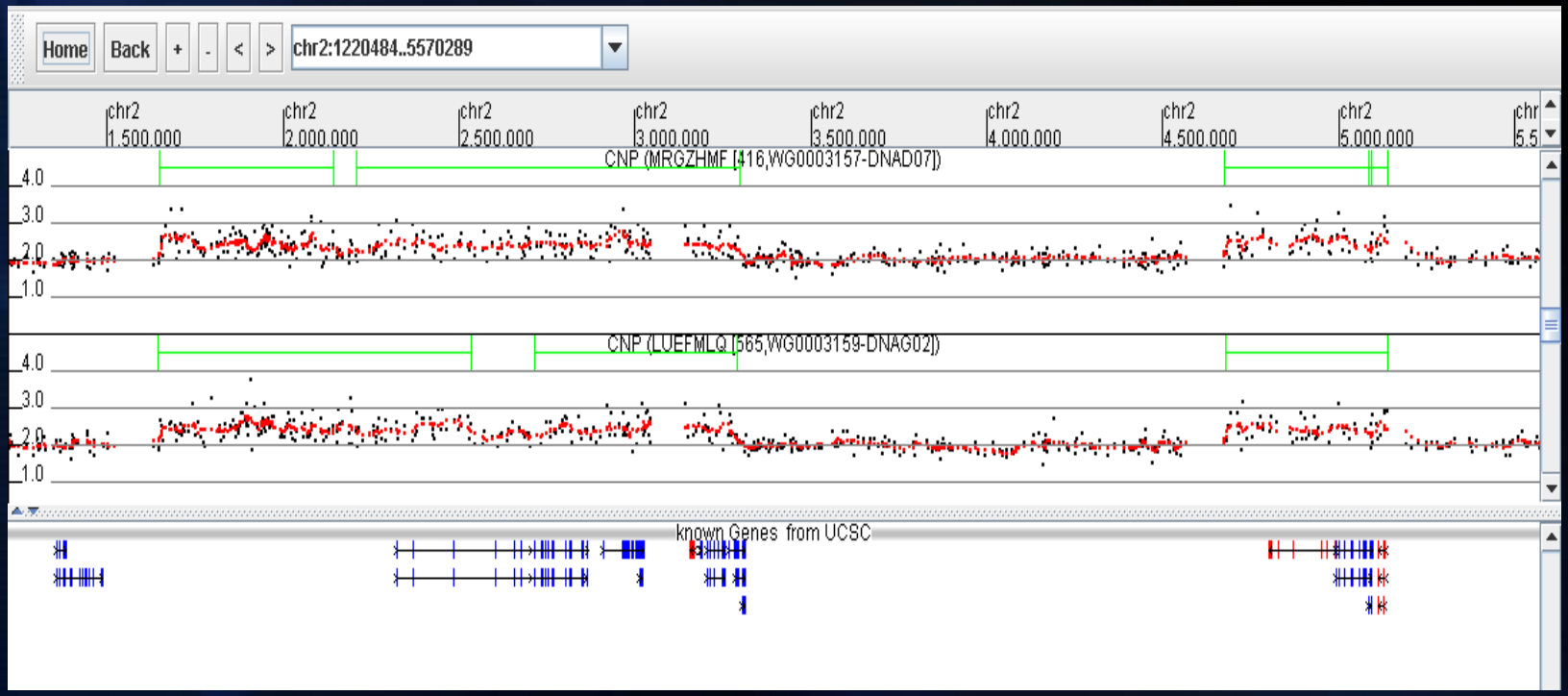
8q Trisomy and 8p Deletion?

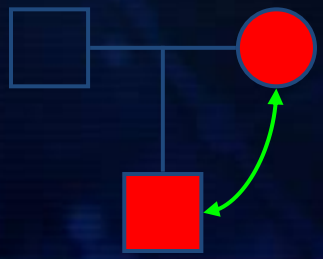


Copy Number Variations

- A high-resolution copy-number map identified CNVs in all 61 embryos and cell lines
  - Inheritable
- Segmental deletions
- Duplications

Copy Number Variations



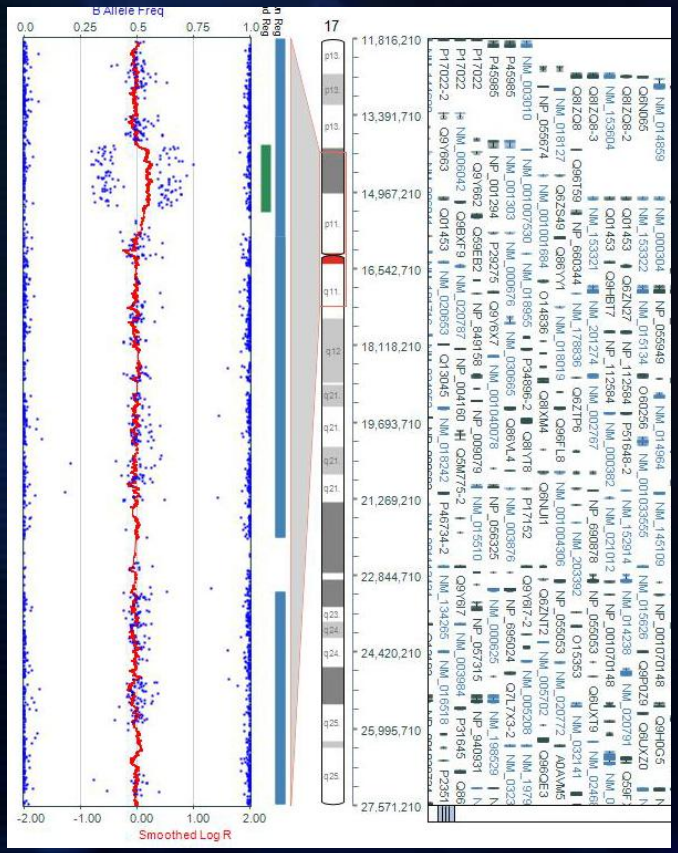


Genome-Wide Scans / Genotyping

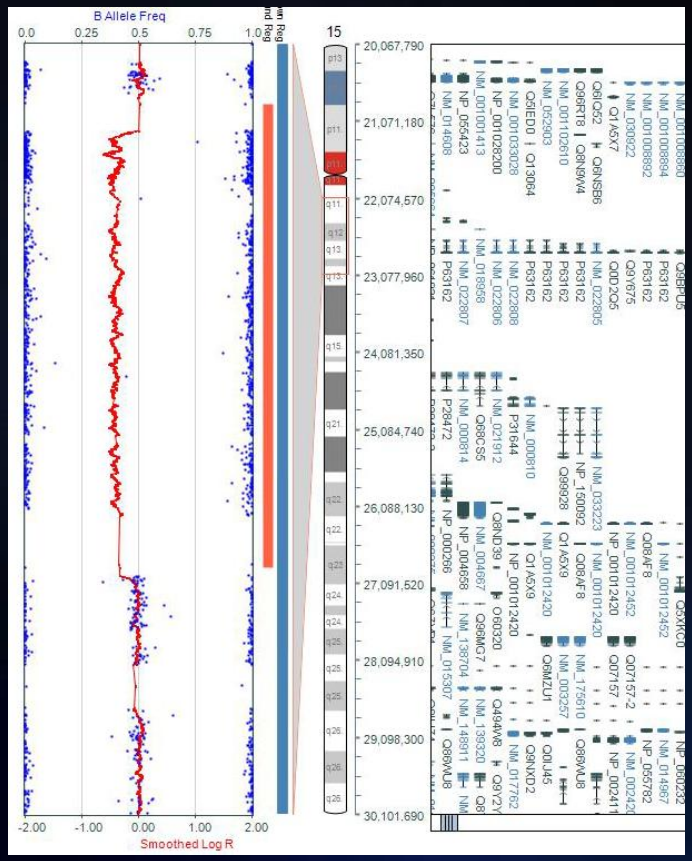
- Beckwith-Wiedmann Syndrome
- Some forms of Prader Willi / Angelman Syndrome
- DiGeorge Syndrome
- Some forms of Autism (~3%)
- Uniparental disomy
- Single gene disorders

Genome-Wide Scans / Genotyping

NF-1



Prader- Willi



Genome-Wide Scans / Genotyping

- What embryo implanted?
- What partner provided the extra chromosome in aneuploid embryos?
  - Sperm?
  - Oocyte?

Controversies for PGS

- Does aneuploidy screening work?
  - Mastenbroek S et al. *N Engl J Med*. July 2007
    - 8 chromosomes assessed
    - Showed no improvement in implantation
  - BUT
    - Maternal age (35-41)
      - We wouldn't offer PGS
    - Many 4-cell embryos biopsied
    - 20% no FISH results
    - Failed to test for chromosomes 22 and 15 (~ 25% of aneuploidy)
    - Tested for chromosome 1 ???
    - Biopsied 2 cells sometimes

Mastenbroek S et al. *N Engl J Med*. 2007;357(1):9-17.

PGS (10-Probe FISH)
n= 729 Reason for PGS = ≥ 2 miscarriages

- < 35
- CP = 35% / ET
- Miscarriage = 4%

- 35-37
- CP = 32% / ET
- Miscarriage = 8%

- 38-40
- CP = 20% / ET
- Miscarriage = 17%

- >40
- CP = 14%
- Miscarriage = 20%

SNP Microarrays n = 45 470 embryos

- 59% abnormal (277 / 470)
- 3.1 +/- 2 euploid embryos identified / patient

SNP Microarray Clinical Cases n = 45
470 embryos CP = 62%
Frozen day-5 = 7 No Transfer = 5

< 35 (n = 9)

CP = 56% (5/9) / ET

Miscarriage = 1

35-37 (n = 8)

7 consecutive miscarriages

CP = 63% / ET (5/8)

Miscarriage = 0%

38-40 (n = 12)

CP = 75% / ET

Miscarriage = 0%

>40 (n = 9)

CP = 44%

Miscarriage = 0%

*** One 44 yr old went through 3 times with neg results**

Conclusions

- Complete molecular karyotype for all 23-pairs of chromosomes
- Simultaneously determine chromosomal imbalances due to translocations, inversions, deletions or duplications
- Simutaneously determine complex genetic disorders and some single gene disorders

Conclusions

- Results available for a day-5 transfer
- Blastomeres, trophectoderm or polar bodies

Conclusions

- What embryo implanted?
- Who provided the extra chromosome?
  - Sperm?
  - Oocytes?

Laboratory

- Rasmei Pen
- Andy Benner
- Adam Kittai
- Andrew Siegel
- Chris Chipko
- Kara Nguyen
- Richard Leach
- William G Kearns

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