
SNP Microarray Genetic Analyses to Determine 23-24 Chromosome Ploidy, Structural Chromosome Aberrations and Genome-Wide Scans to Identify Disease Risks from a Single Embryonic Cell
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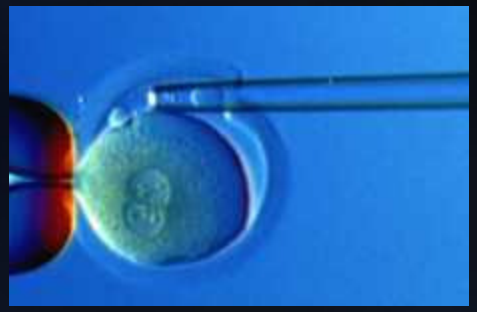
Indications for Genetic Assessment of Embryos

- 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
  - X-linked disorders



- Blastomeres

Current Benefits and Limitations

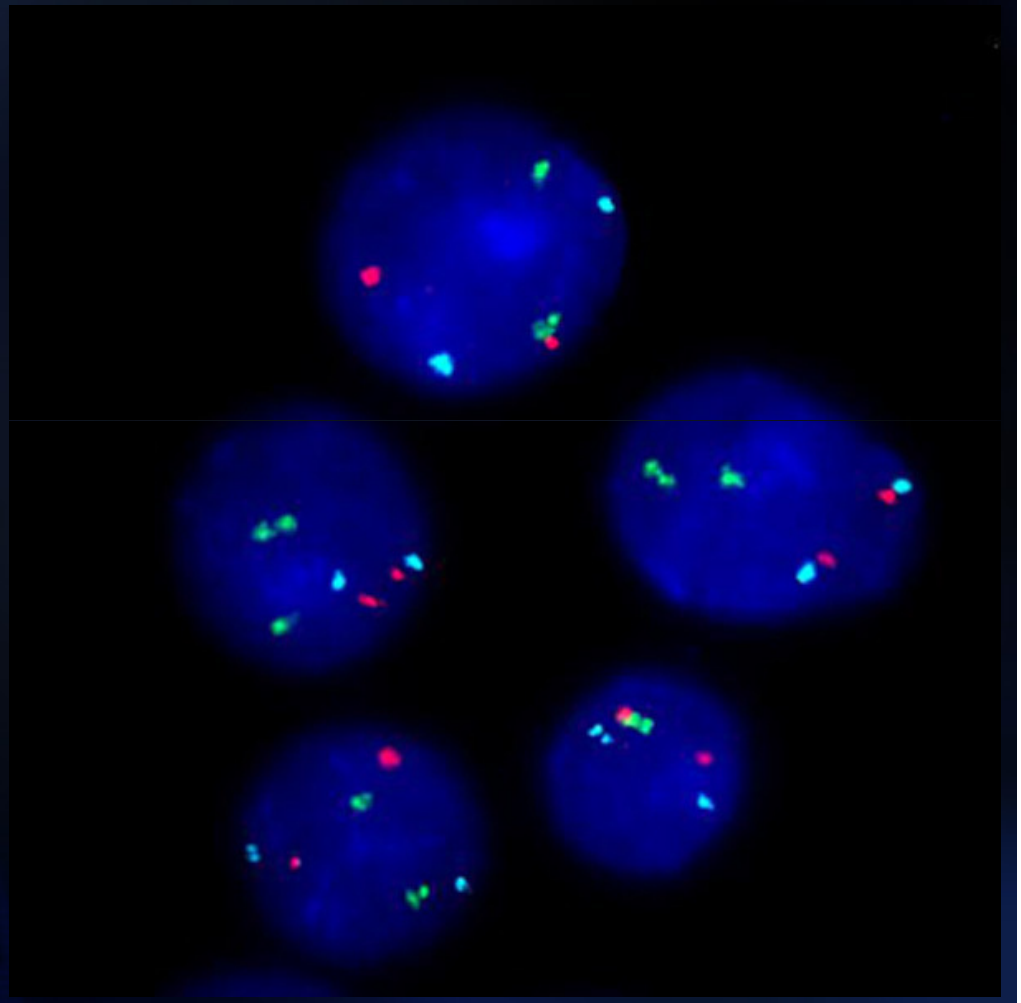
- Aneuploidy
- Structural chromosome aberrations
- Single gene disorders
- Mitochondrial disorders

Aneuploidy

- Fluorescence in situ Hybridization (FISH) on interphase nuclei
- Comparative genomic hybridization (CGH) on metaphase chromosomes

FISH Limitations

- Single "spot-check" to test for the presence of a specific chromosome
- Cells are in interphase
- Limited fluorochromes
- Reduced accuracy with additional probes
- Fixation/nuclear spreading
- ~ 10-12 chromosomes tested



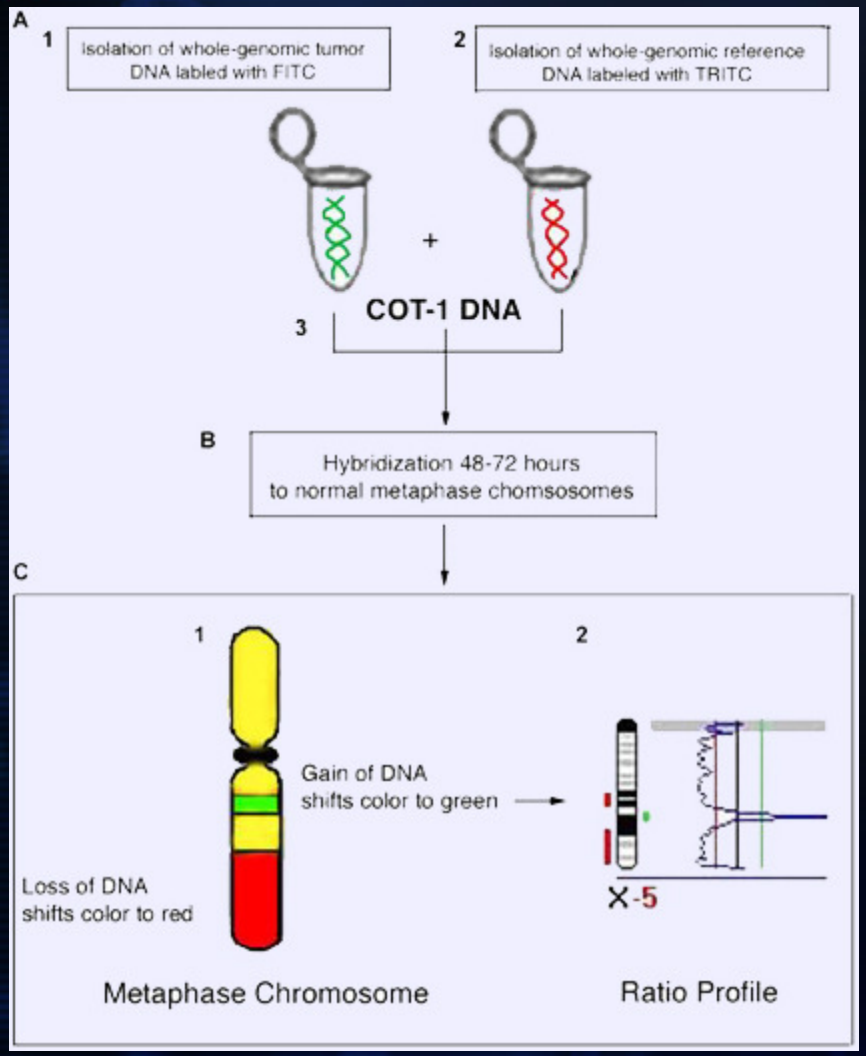
Controversies for PGS

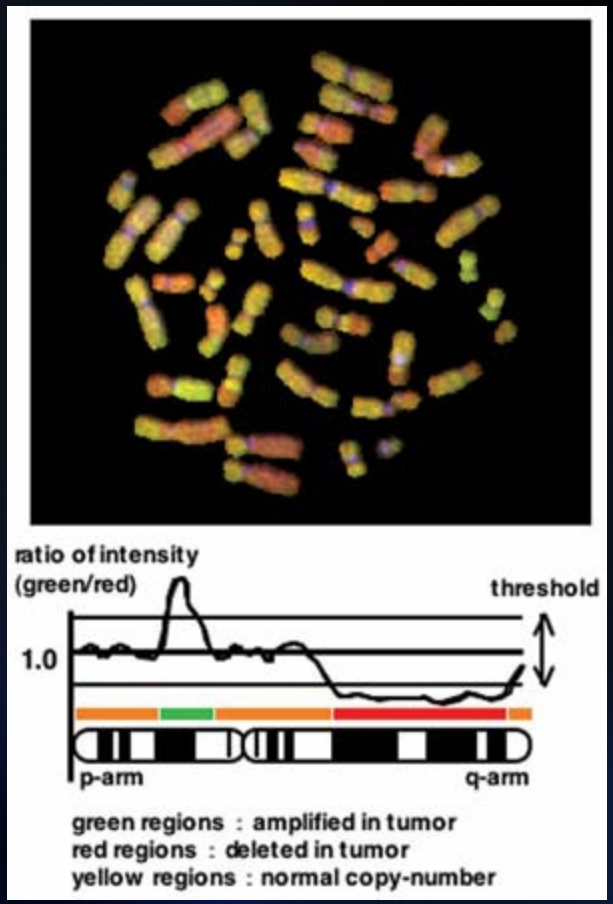
- Does aneuploidy screening work?
  - New England Journal Paper, Mastenbroek et al (July, 2007)
    - 8 chromosomes assessed
    - Showed no improvement in implantation
  - BUT ……
    - Maternal age (35-41)
      - We wouldn't offer PGS
    - Many 4-cell embryos biopsied
    - 20% no fluorescence *in situ* hybridization (FISH) results
    - Failed to test for chromosomes 22 and 15 (~ 25% of aneuploidy)
    - Tested for chromosome 1 ???
    - Biopsied 2 cells sometimes

Controversies for PGS

- PGS doesn't improve clinical pregnancy rates (gestational sac and fetal heart beat) and delivery rates
  - ASRM Practice Statement, October 2007
  - ESRE Practice Statement – July 2008
  - ACOG – May 2009
  - No prospective, randomized studies to date

CGH on Metaphase Chromosomes





CGH Benefits and Limitations

- Tests for all 23-pairs of chromosomes
- Limitations – hybridization takes ~ 3 days and requires an FET

What are the Issues / Risks of PGS?

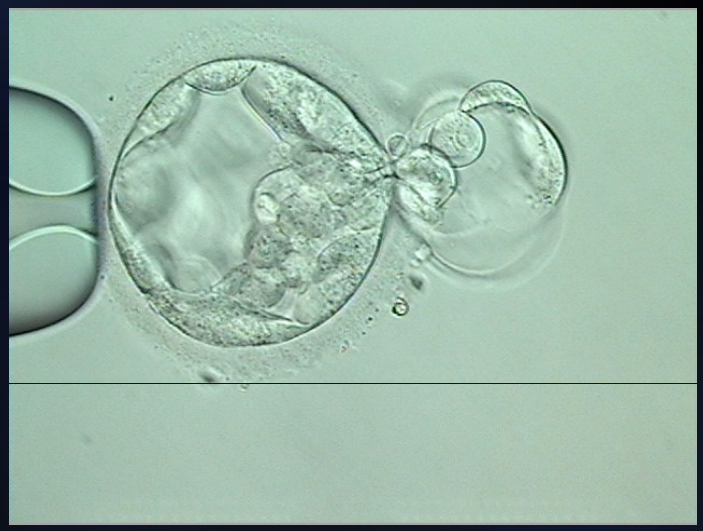
- Test Limitations
  - FISH - Limited chromosomes
  - CGH – requires an FET
- Embryo mosaicism?
- Damaging the embryo during the biopsy?
  - Reduced implantation?
  - Biochemical pregnancy?

What are the Issues / Risks of PGS?

- Embryo correction of day-3 embryo?
  - CVS – Placental aneuploid colonies

Biopsy a Better Cell Type?

- Trophectoderm



What are the Issues / Risks of Trophectoderm Biopsy and PGS?

- Mosaicism?
  - Inner cell mass
  - Trophectoderm

PGS – Trophectoderm – Clinical Trial

n = 33 cases

FISH for Chromosomes 13, 14, 15, 16, 17, 18, 21, 22, X and Y

- Patient recruitment – 3+ blasts
- Indication for PGS
- 24 hr turnaround from thaw to transfer
- 114 blasts thawed
- 78% blast survival
- 67% re-expansion
- 83% were biopsied
- 100% PGD results
- PGS (10 chromosomes) - 41% normal, 59% abnormal
- Biopsied cells (mean = 5/patient)

PGS – Trophectoderm – Clinical Trial

n = 33 cases

3 had no transfer

70% (21/30) + FHT with gestational sac (all > 12 weeks)

3% (1/30) Biochemical

6 normal deliveries

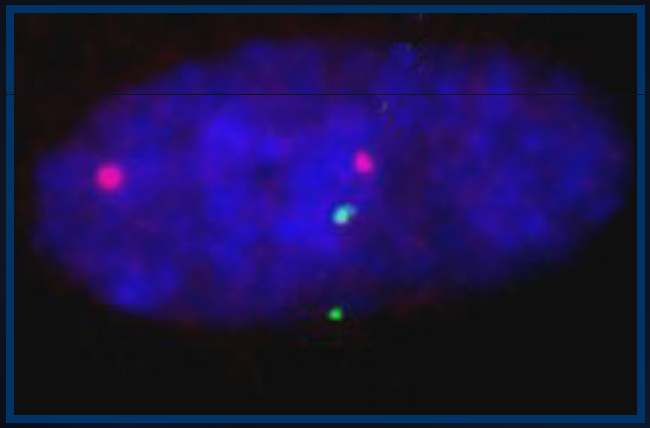
No miscarriages

Blastocyst biopsy and PGS may be a less-invasive and more beneficial option to day-3 blastomere biopsy and PGS

Structural Chromosome Imbalances

Fluorescence *in situ* Hybridization (FISH)

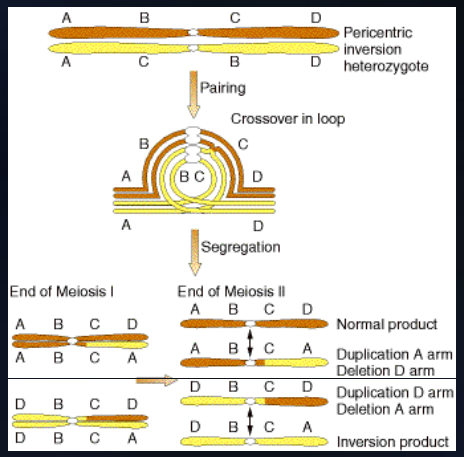
- Reciprocal translocations
  - Telomere clones
  - Genetic balance
    - May have the translocation
  - Breakpoint clones
    - No translocations

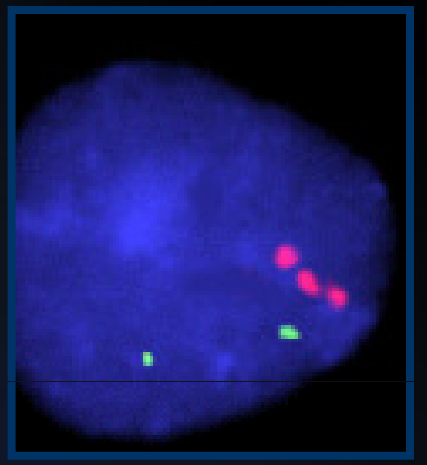


- Robertsonian translocations
  - Locus specific clones
  - Genetic balance
    - May have the translocation

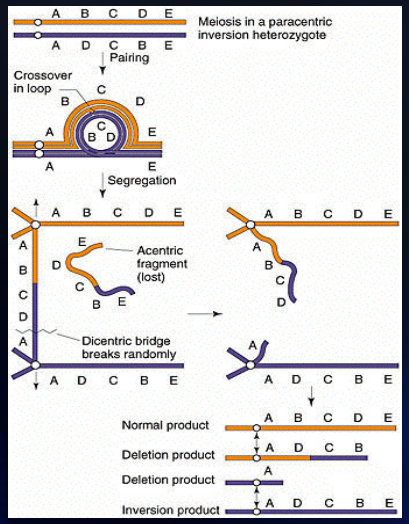
Structural Chromosome Disorder

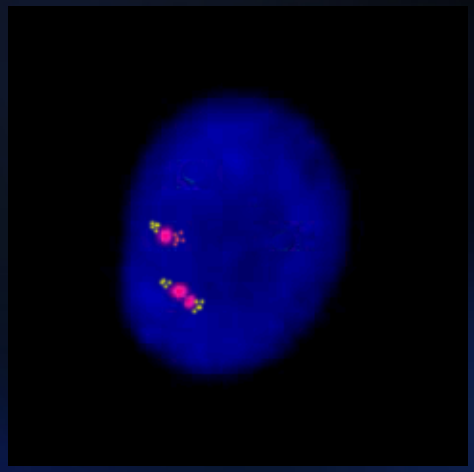
- Percentric inversions
  - Telomere probes
  - Identify duplication/deficient chromosomes in embryos





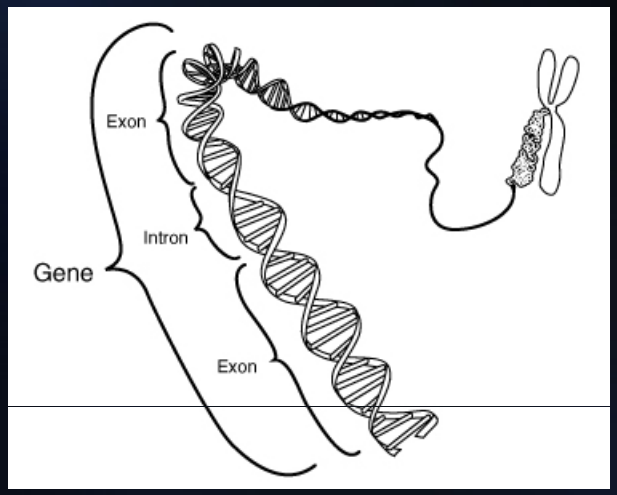
- Paracentric inversions
  - Centromere probes
  - Telomere probes
  - Identify dicentric chromosomes in embryos



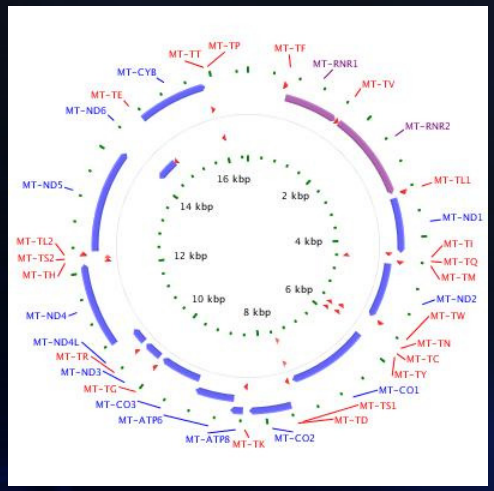


Single Gene and Mitochondrial Disorders

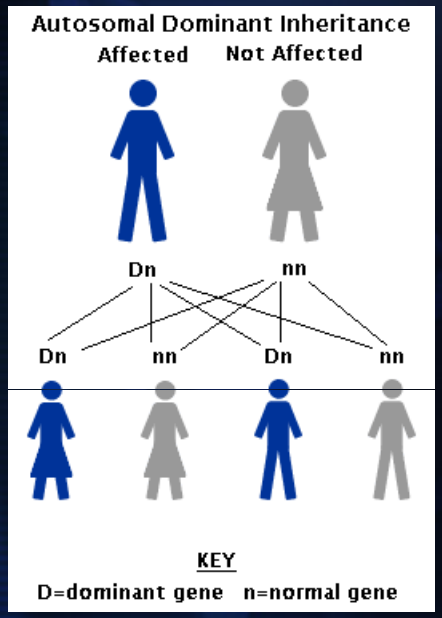
- Single gene disorders
  - PCR
  - DNA sequencing
  - Linkage analysis

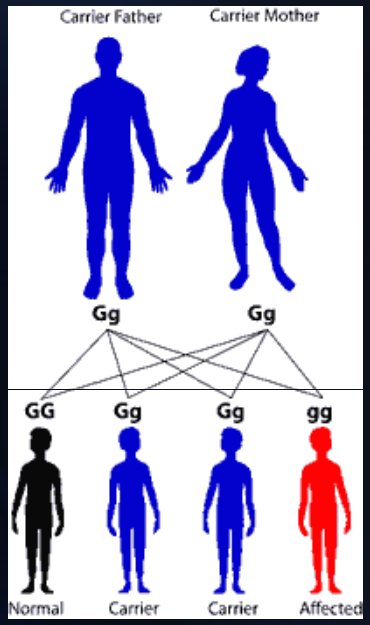


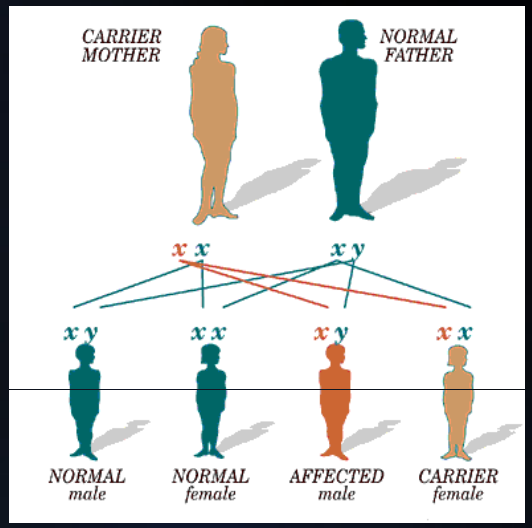
- Mitochondrial
  - Recessive



Segregation Patterns







Single Gene and Mitochondrial Analyses Limitations

- Single Gene Disorder
  - Mutation must be known
  - Allele dropout – misdiagnosis
- Mitochondrial Disorders
  - Few offered

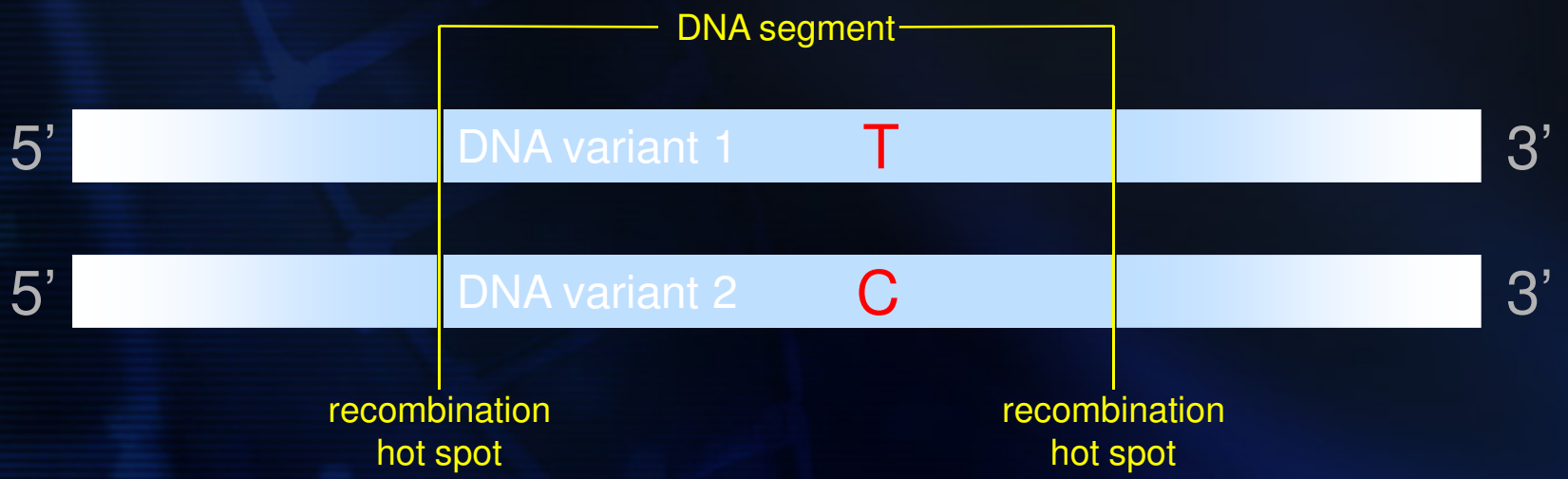
New Technologies- Microarrays

- Single nucleotide polymorphisms (SNPs)
  - 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)

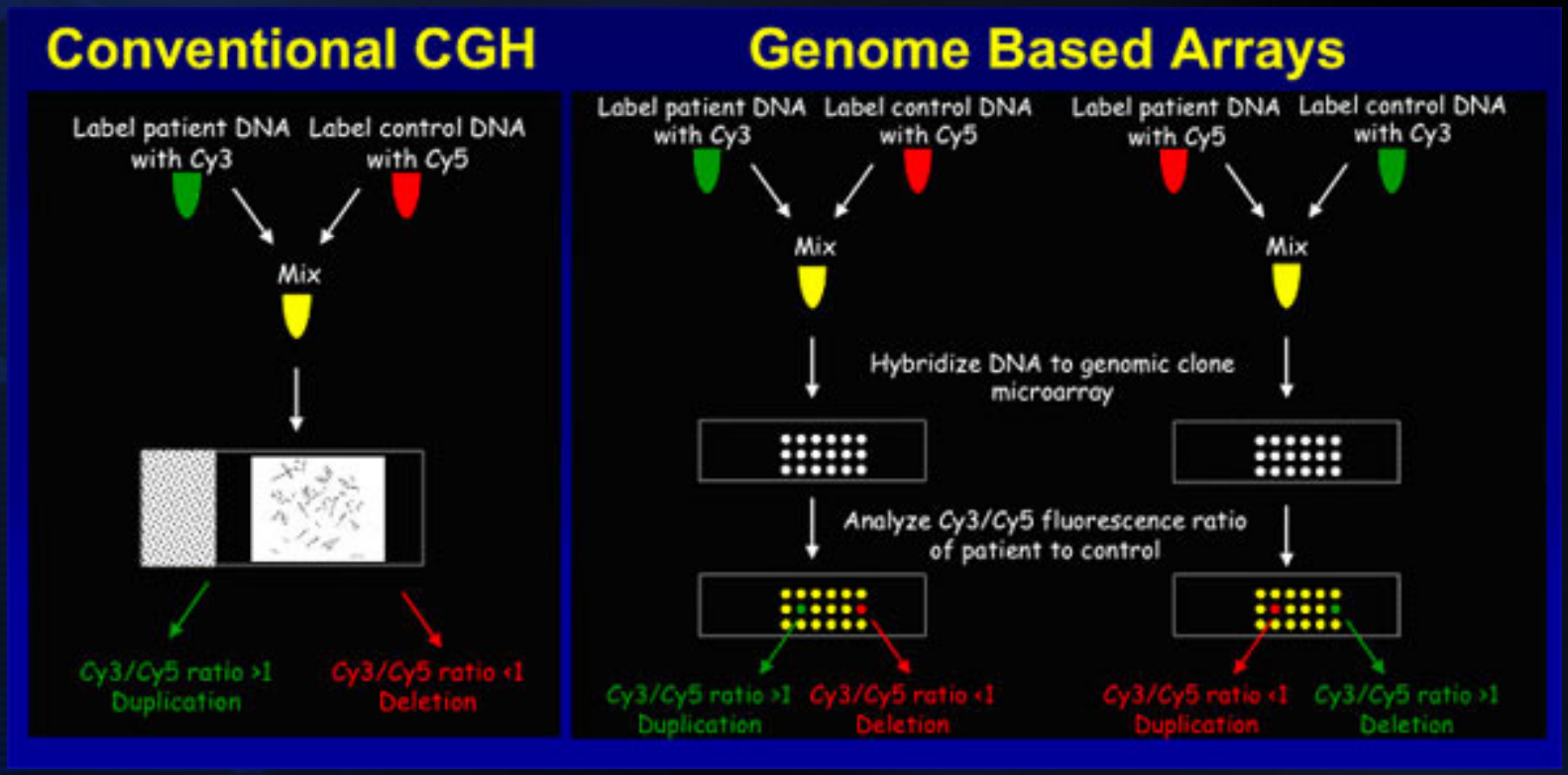
What are SNPs?

5’-ACTGGGAATCCCGAAGTGTG[T/C]GATTACA-3’

1. **Single Nucleotide Polymorphism**
2. **Normally occurring genetic variant**
3. **10,000,000 estimated to be in human genome**
4. **Stable**
5. **Used in genetics research to tag genomic segments**



CGH

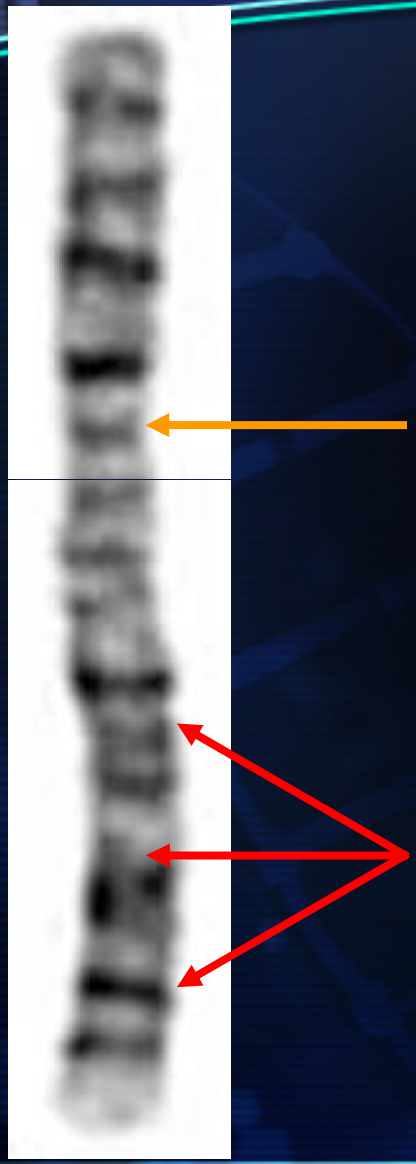

Conventional CGH
Label patient DNA with Cy3
Label control DNA with Cy5
Mix
Cy3/Cy5 ratio >1
Duplication
Cy3/Cy5 ratio <1
Deletion
Genome Based Arrays
Label patient DNA with Cy3
Label control DNA with Cy5
Label patient DNA with Cy5
Label control DNA with Cy3
Mix
Mix
Hybridize DNA to genomic clone microarray
Analyze Cy3/Cy5 fluorescence ratio of patient to control
Cy3/Cy5 ratio >1
Duplication
Cy3/Cy5 ratio <1
Deletion
Cy3/Cy5 ratio <1
Duplication
Cy3/Cy5 ratio >1
Deletion

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	

Genetic Diagnostics and Screening	SNP	CGH
Uni-parental disomy	X	
Copy neutral event	X	

FISH vs Microarrays



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

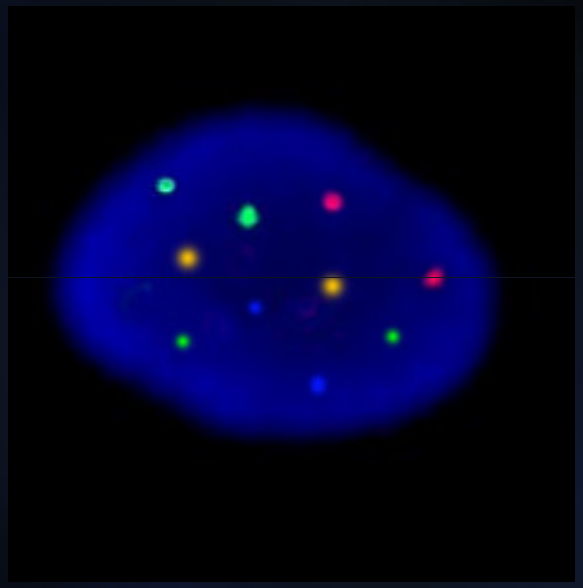
- detection via microscopy
- analysis and interpretation via human

SNP or CGH: DNA markers throughout the chromosome

- CGH is a less dense array
- detection via microarray and scanner
- analysis and interpretation via algorithm

Correlate FISH and Microarray Analysis

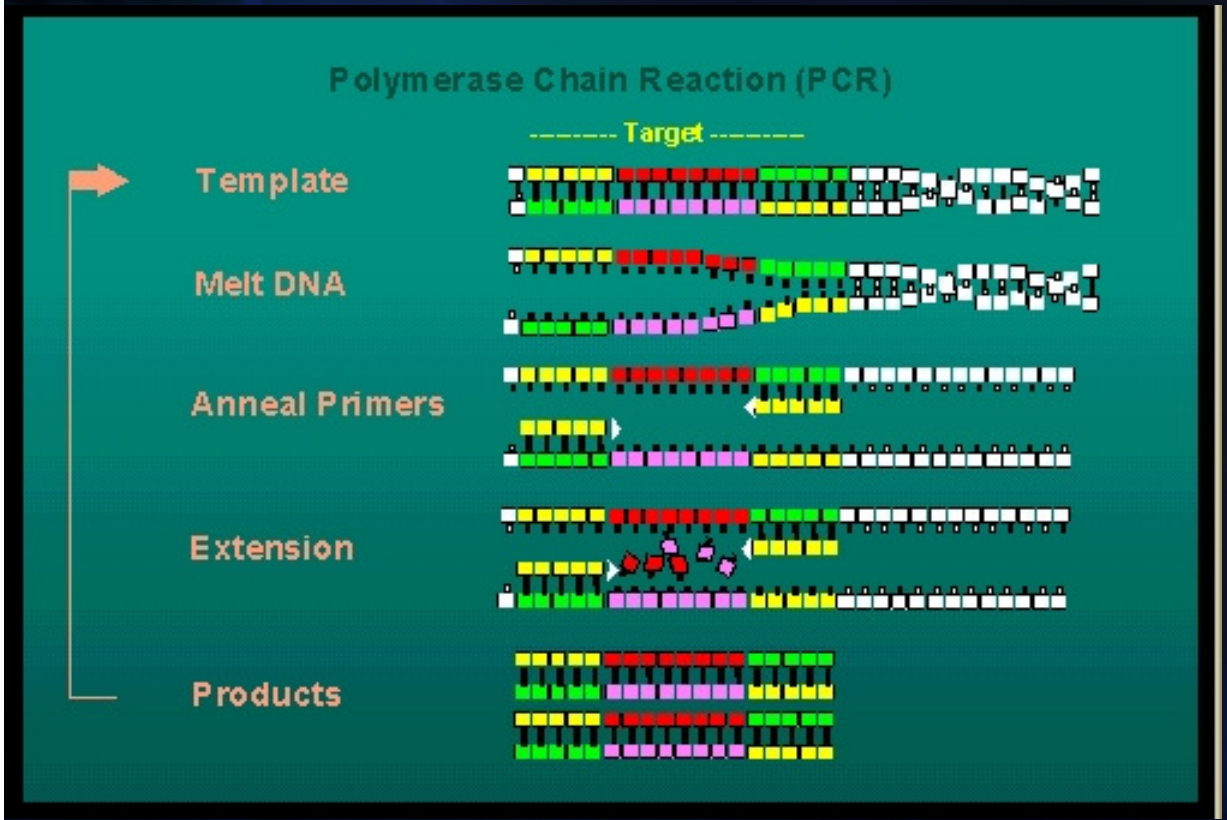
- FISH
  - Reported world-wide mis-dx is 6-11%
  - Some have suggested that FISH has a ~ 50% mis-dx rate !!! NO NO NO
    - Based upon rehybridizing completed PGD cases
    - Estimations
    - FISH experience



Materials and Methods

- n = 565 single cells (565 blastomeres (61 day-3 abnormal embryos) and 34 cell lines
- Embryo biopsy of a single cell – laser
  - Blastomere
- Modified whole genome amplification (WGA)

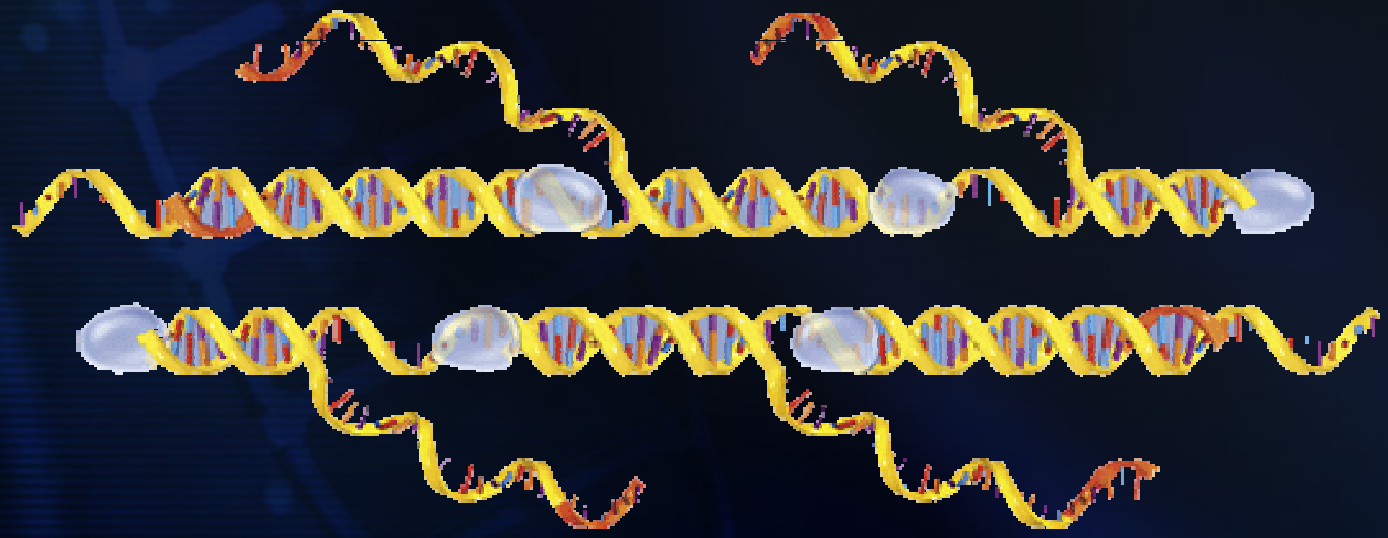
Experimental Problem to Overcome



- ~ 6 picograms DNA
- Need ~ 250,000 pg for microarray analysis
- PCR
  - Artifacts
  - Amplification
  - Allele dropout

Modified Whole Genome Amplification (WGA)

- Home Brew
- Parental DNA not required for PGS



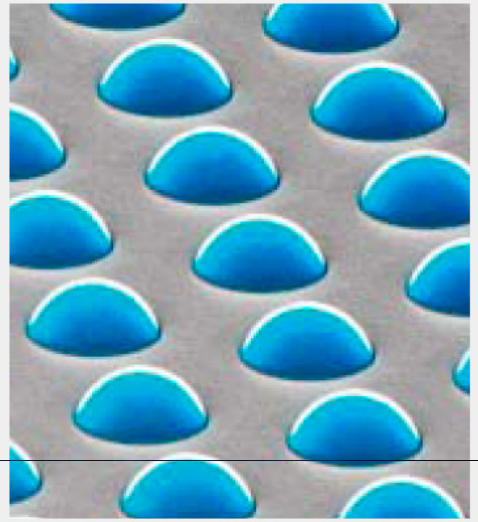
Materials and Methods

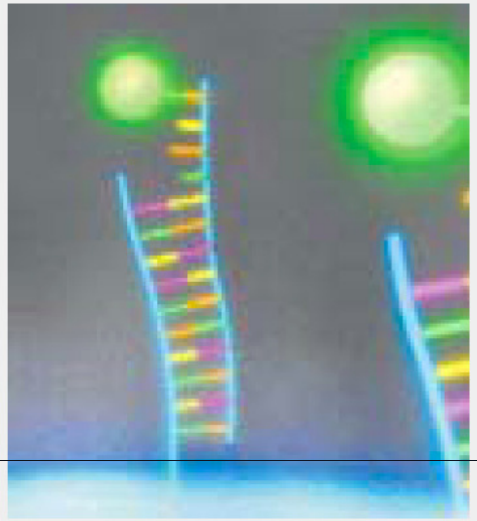
- Invariant DNA genomic loci to ensure the entire genome was amplified
- TaqMan PCR to ensure heterozygous allele amplification
- Illumina HumanHap370 ~370,000 SNPs

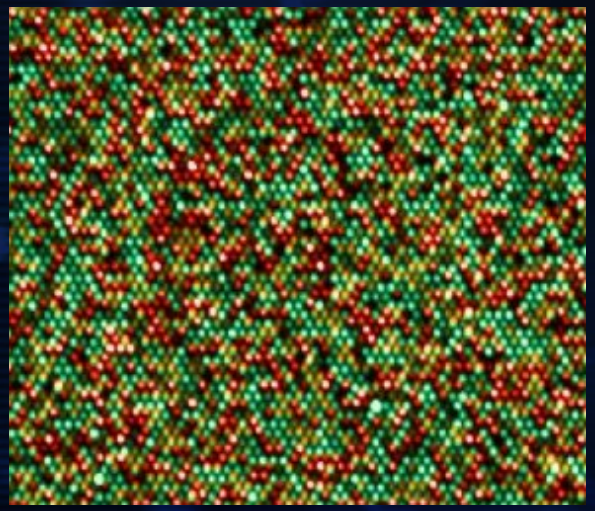
Materials and Methods

- Two-channel intensity values – high-resolution copy-number profile
  - Identify copy number variations (CNVs)
- Genome-wide scans
  - Modified DNA fingerprinting / genotyping
  - Embryo and parental DNA
    - Who provided the extra chromosome?
    - What embryo implanted?
- deCode genetics Disease Minor Professional, Illumina BeadStudio, GenomeStudio and KaryoStudio software

Microarray

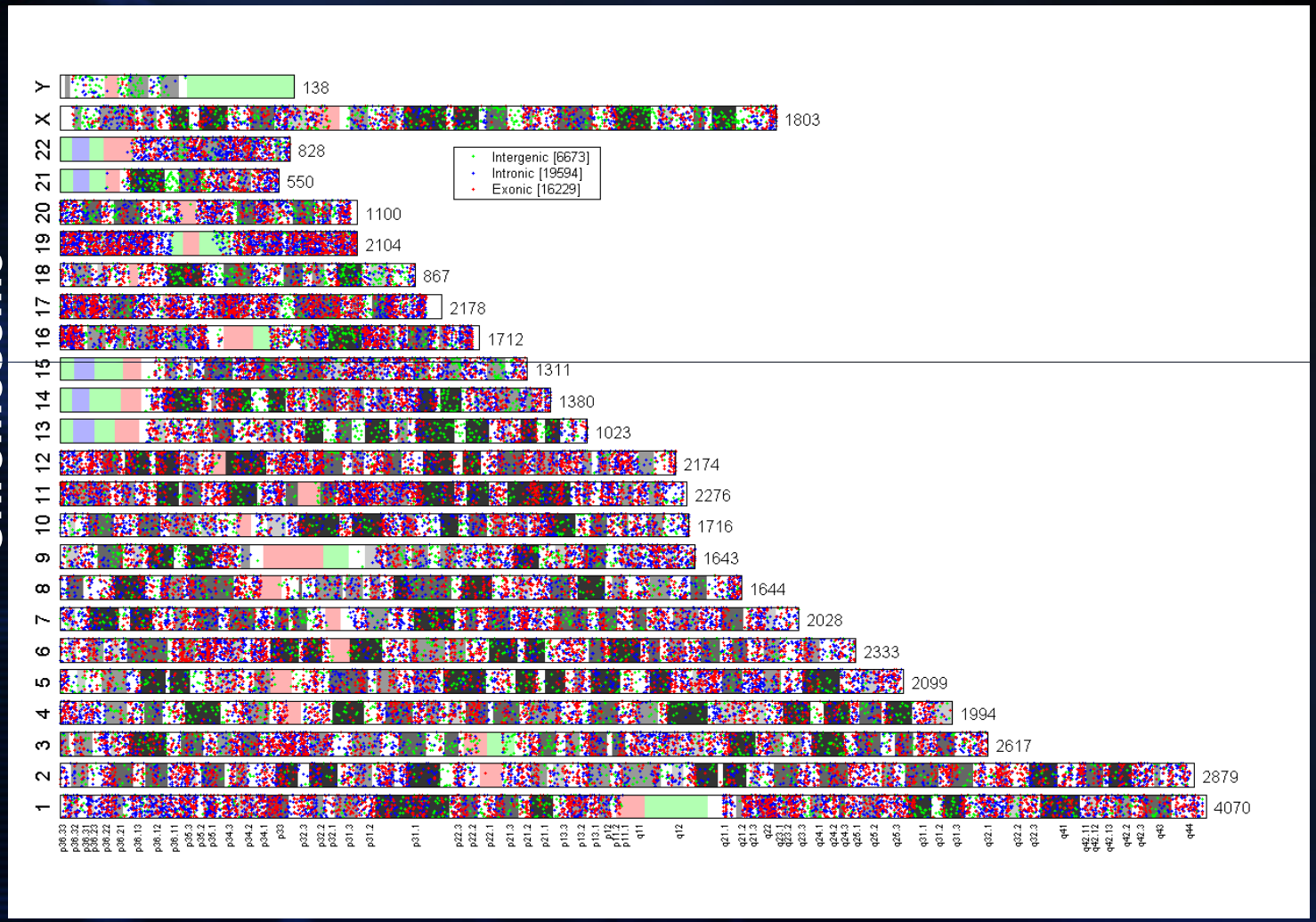






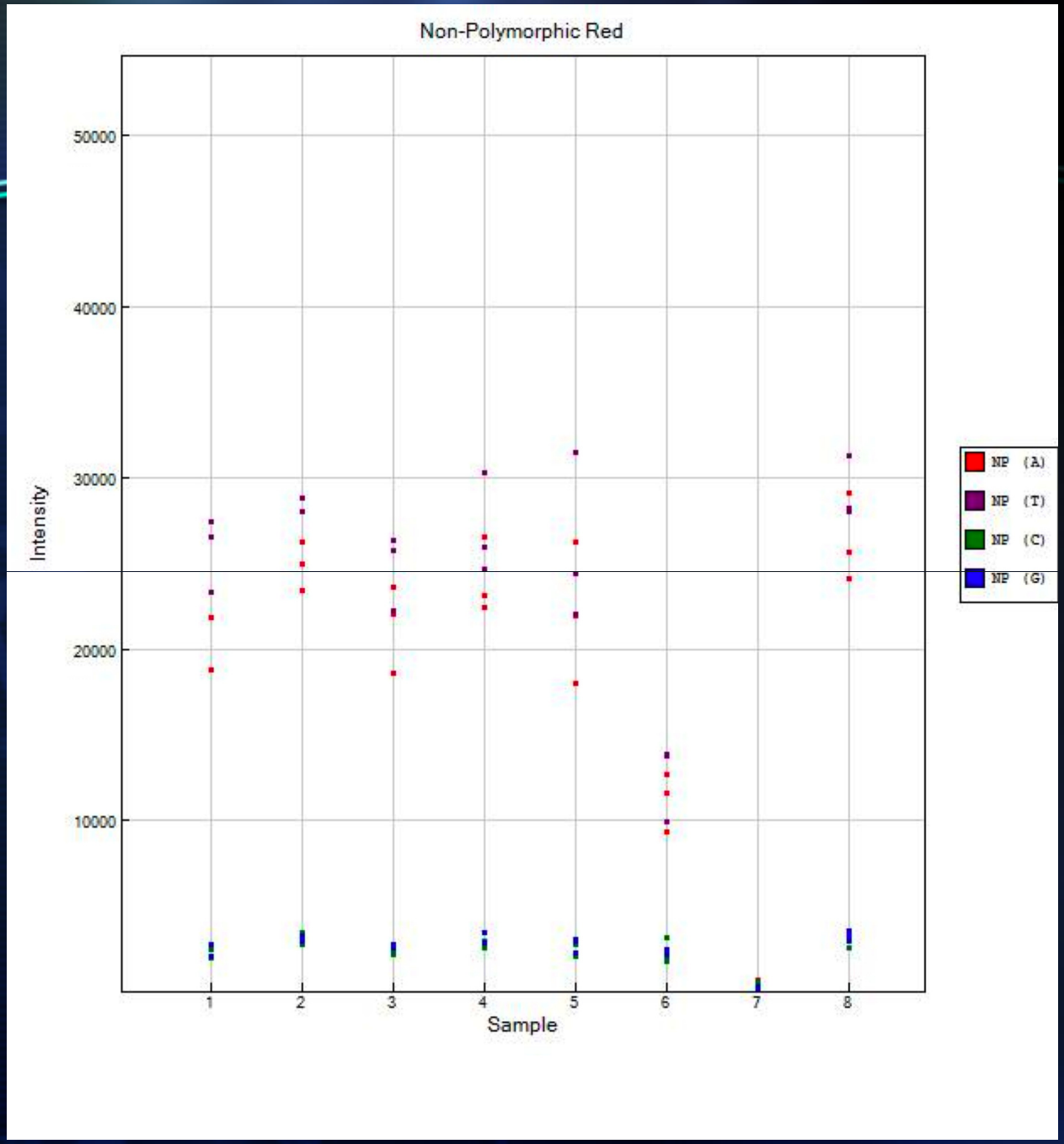
Illumina Human HapMap370 ~370,000

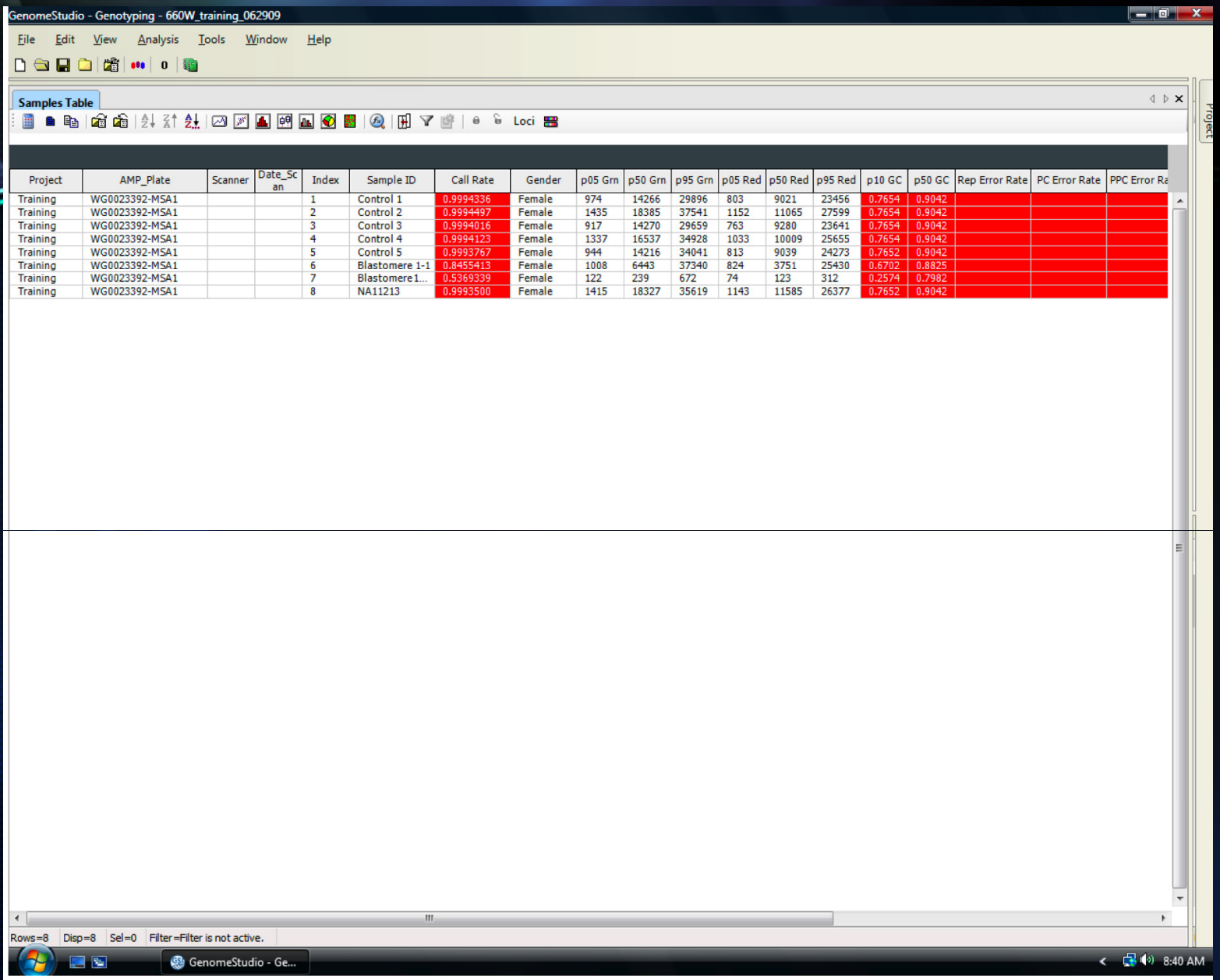
Genomic Coverage on CGH Microarray



Results

- Preimplantation blastomeres (n=565 cells from 61 embryos) and cell lines (n=34)
- DNA yields of 700-800 ng / 4-16 hrs reaction
- In many cases, a genomic coverage > 98% (Range 30-98%)
  - Correlates with day-3 embryo quality
- Heterozygous allele detection rate > 90%


Non-Polymorphic Red
Intensity
50000
40000
30000
20000
10000
1
2
3
4
5
6
7
8
Sample
NP (A)
NP (T)
NP (C)
NP (G)



Project	AMP_Plate	Scanner	Date_Scan	Index	Sample ID	Call Rate	Gender	p05 Grn	p50 Grn	p95 Grn	p05 Red	p50 Red	p95 Red	p10 GC	p50 GC	Rep Error Rate	PC Error Rate	PPC Error Ra
Training	WG0023392-MSA1			1	Control 1	0.9994336	Female	974	14266	29896	803	9021	23456	0.7654	0.9042			
Training	WG0023392-MSA1			2	Control 2	0.9994497	Female	1435	18385	37541	1152	11065	27599	0.7654	0.9042			
Training	WG0023392-MSA1			3	Control 3	0.9994016	Female	917	14270	29659	763	9280	23641	0.7654	0.9042			
Training	WG0023392-MSA1			4	Control 4	0.9994123	Female	1337	16537	34928	1033	10009	25655	0.7654	0.9042			
Training	WG0023392-MSA1			5	Control 5	0.9993767	Female	944	14216	34041	813	9039	24273	0.7652	0.9042			
Training	WG0023392-MSA1			6	Blastomere 1-1	0.8455413	Female	1008	6443	37340	824	3751	25430	0.6702	0.8825			
Training	WG0023392-MSA1			7	Blastomere 1...	0.5369339	Female	122	239	672	74	123	312	0.2574	0.7982			
Training	WG0023392-MSA1			8	NA11213	0.9993500	Female	1415	18327	35619	1143	11585	26377	0.7652	0.9042			

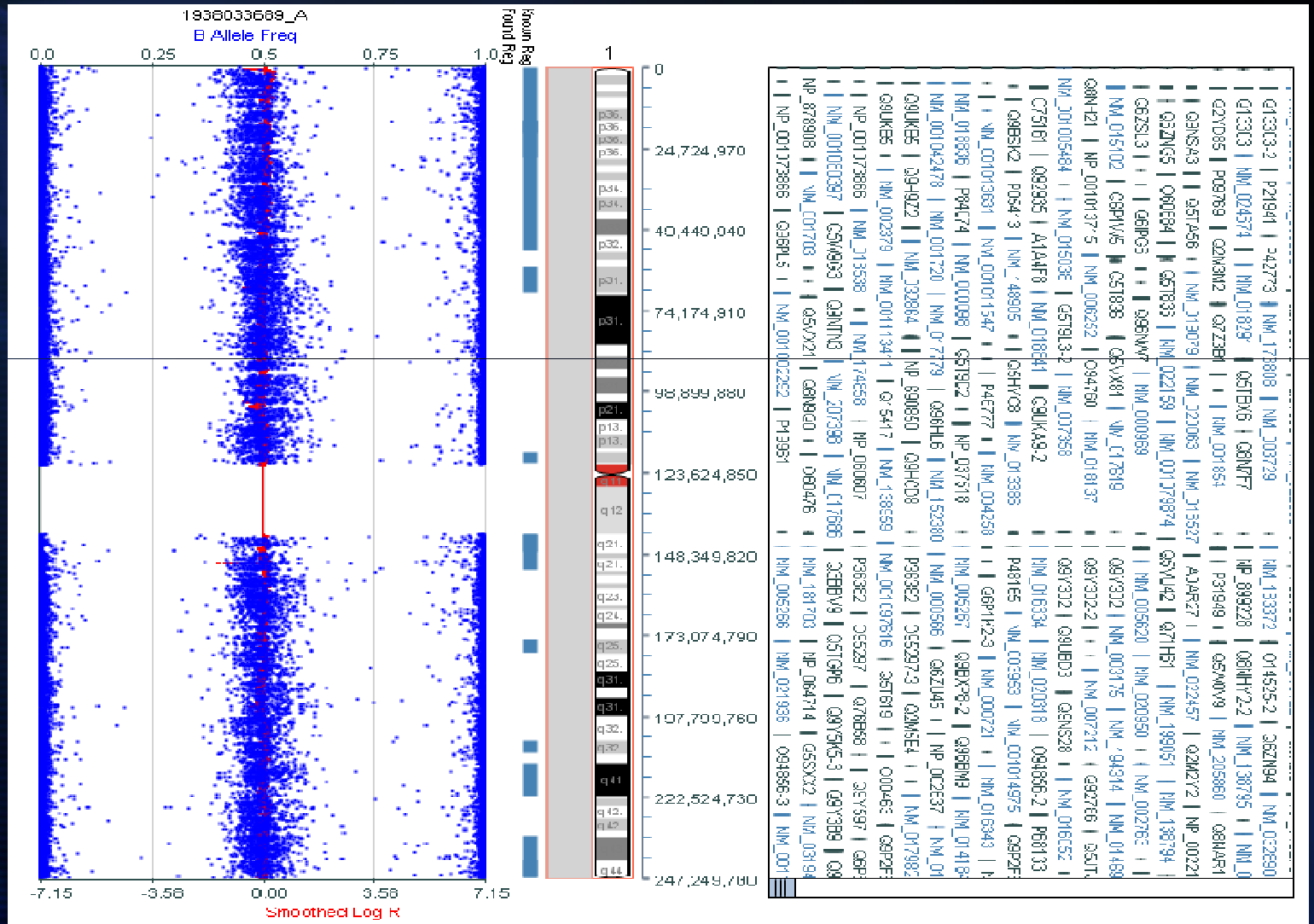
Results

- 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

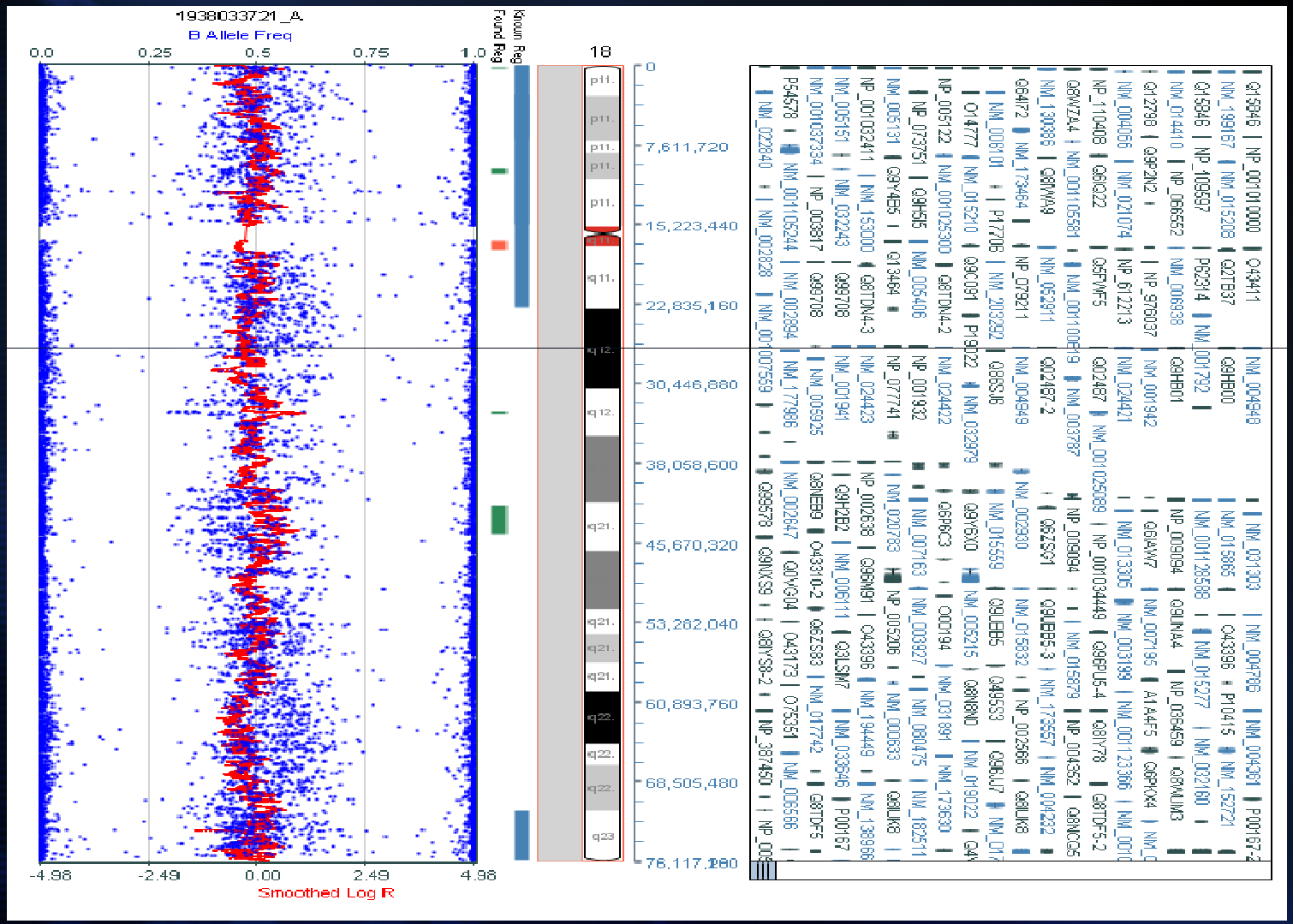
Aneuploidy Results

- 23-chromosome molecular karyotype was obtained from all 565 blastomeres and 34 cell lines
  - Blastomeres >25,000 individual chromosomes
  - Cell lines >1564 individual chromosomes

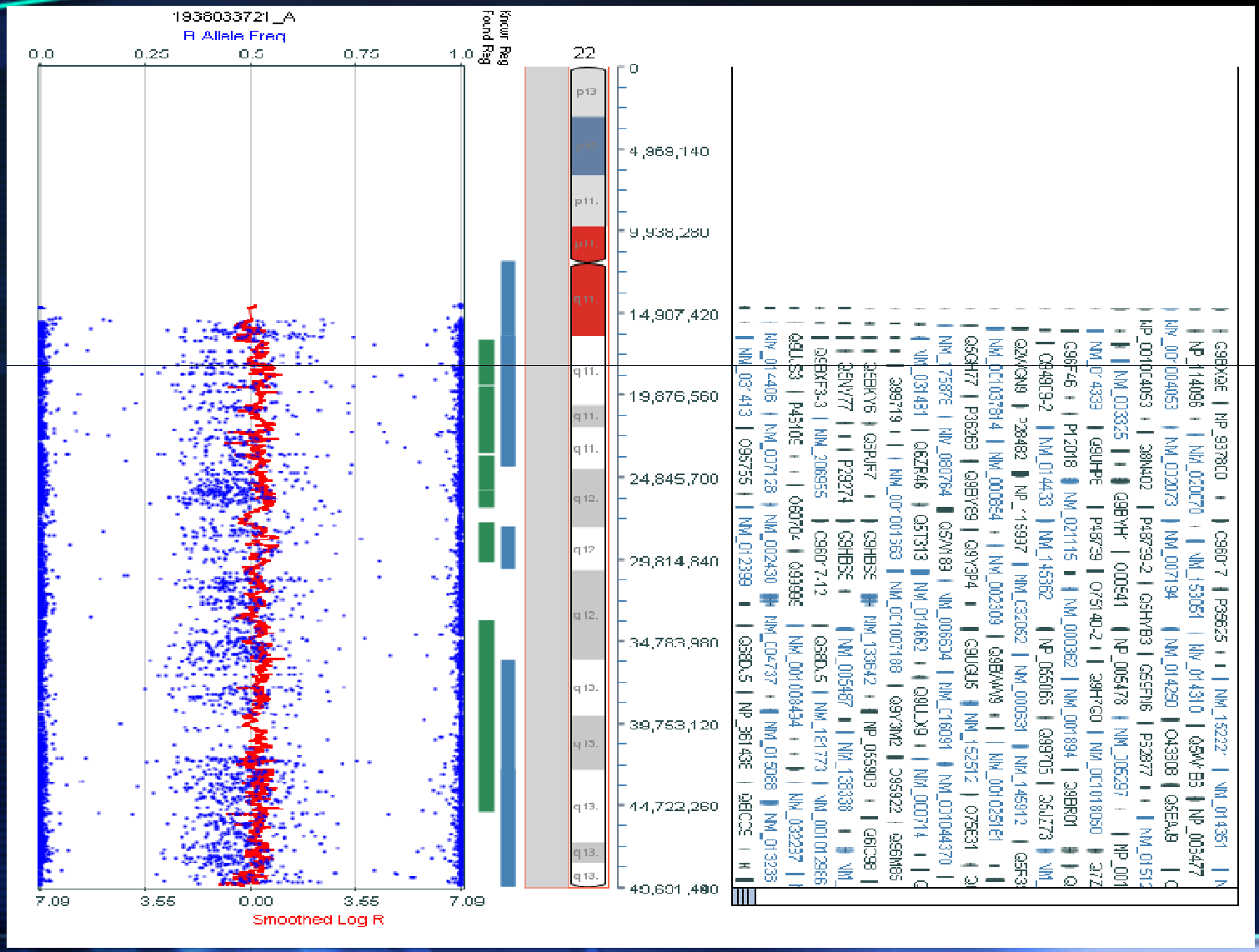
Two Copies of Chromosome 1



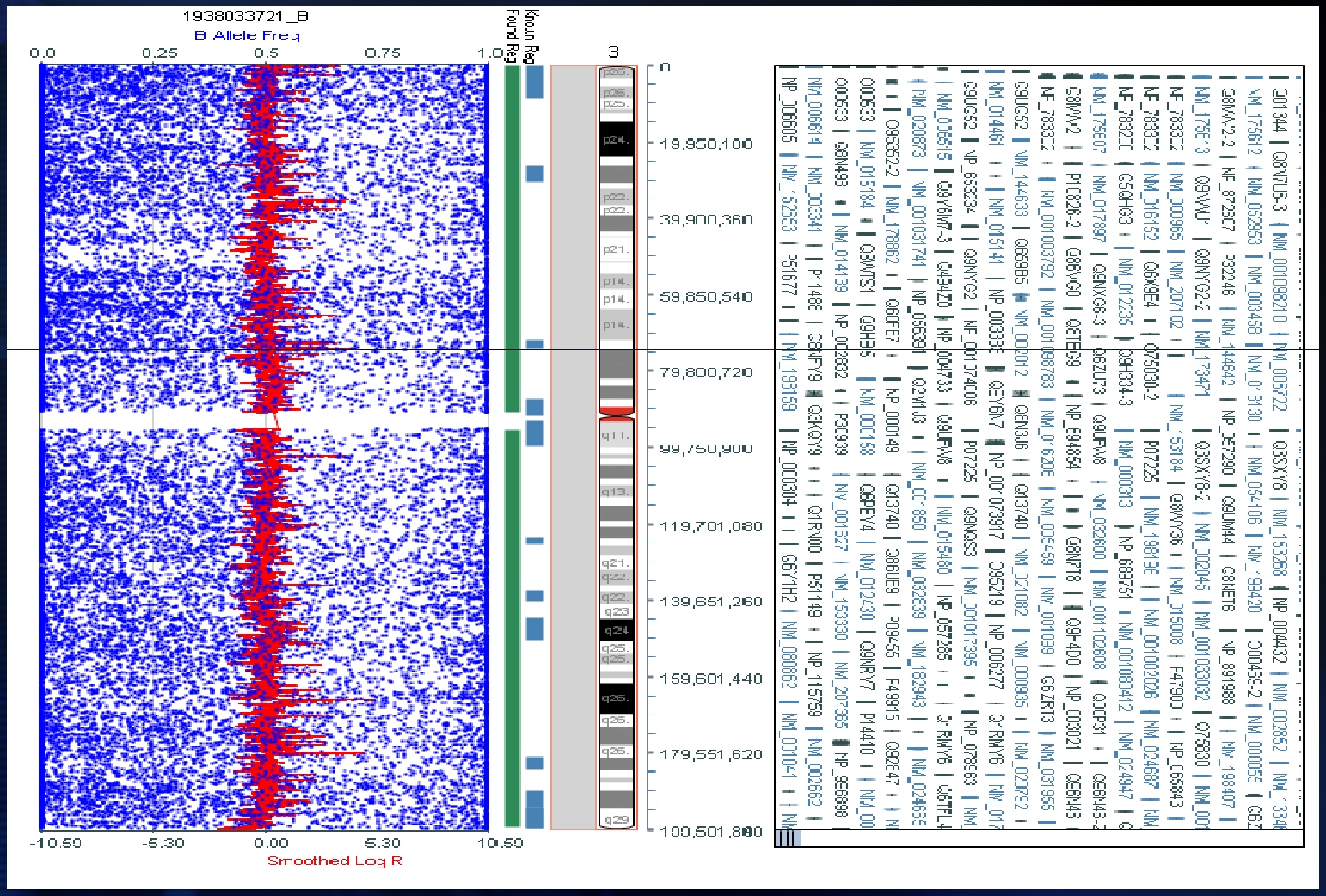
Two Copies of Chromosome 18



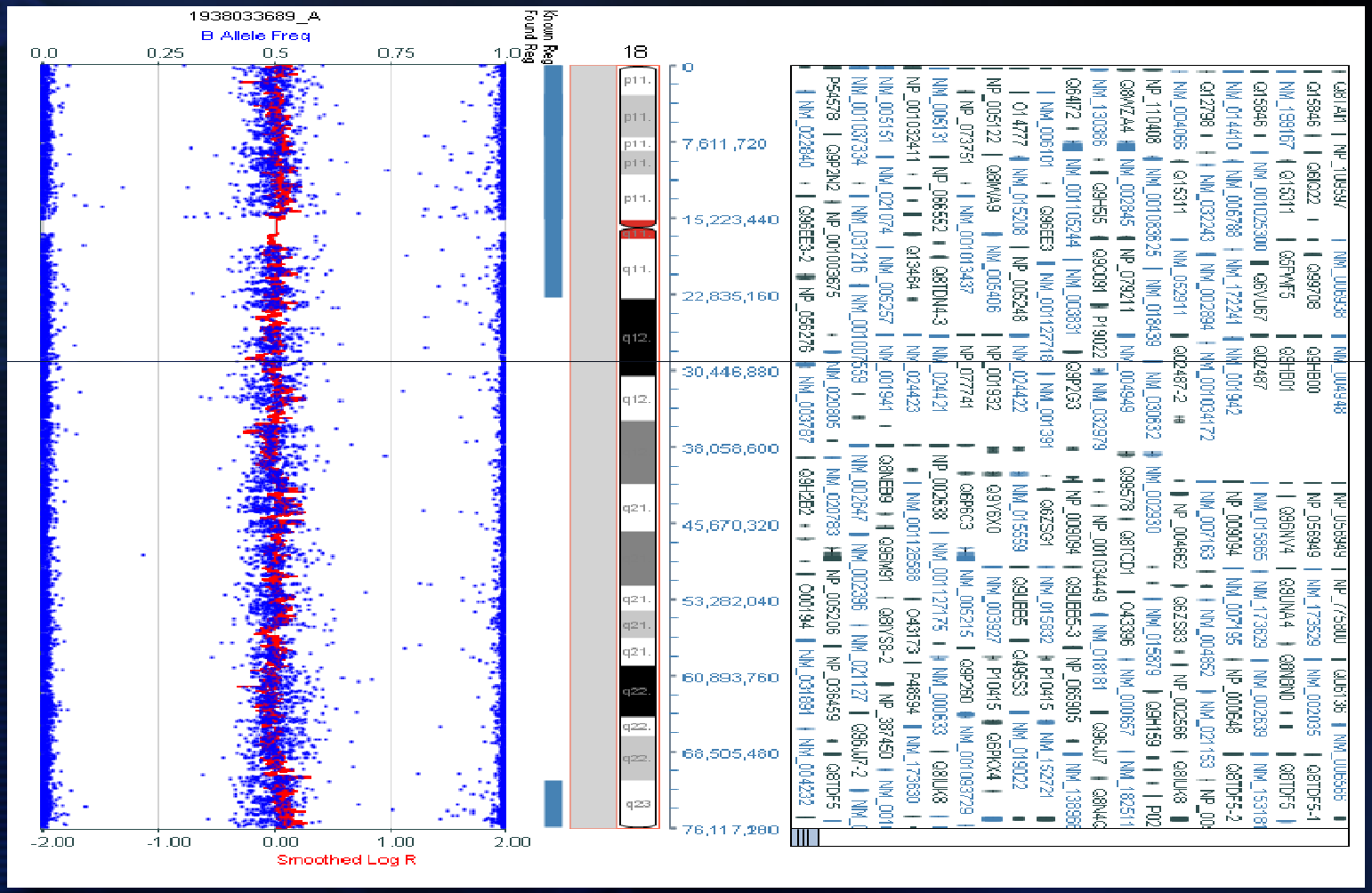
Three Copies of Chromosome 22



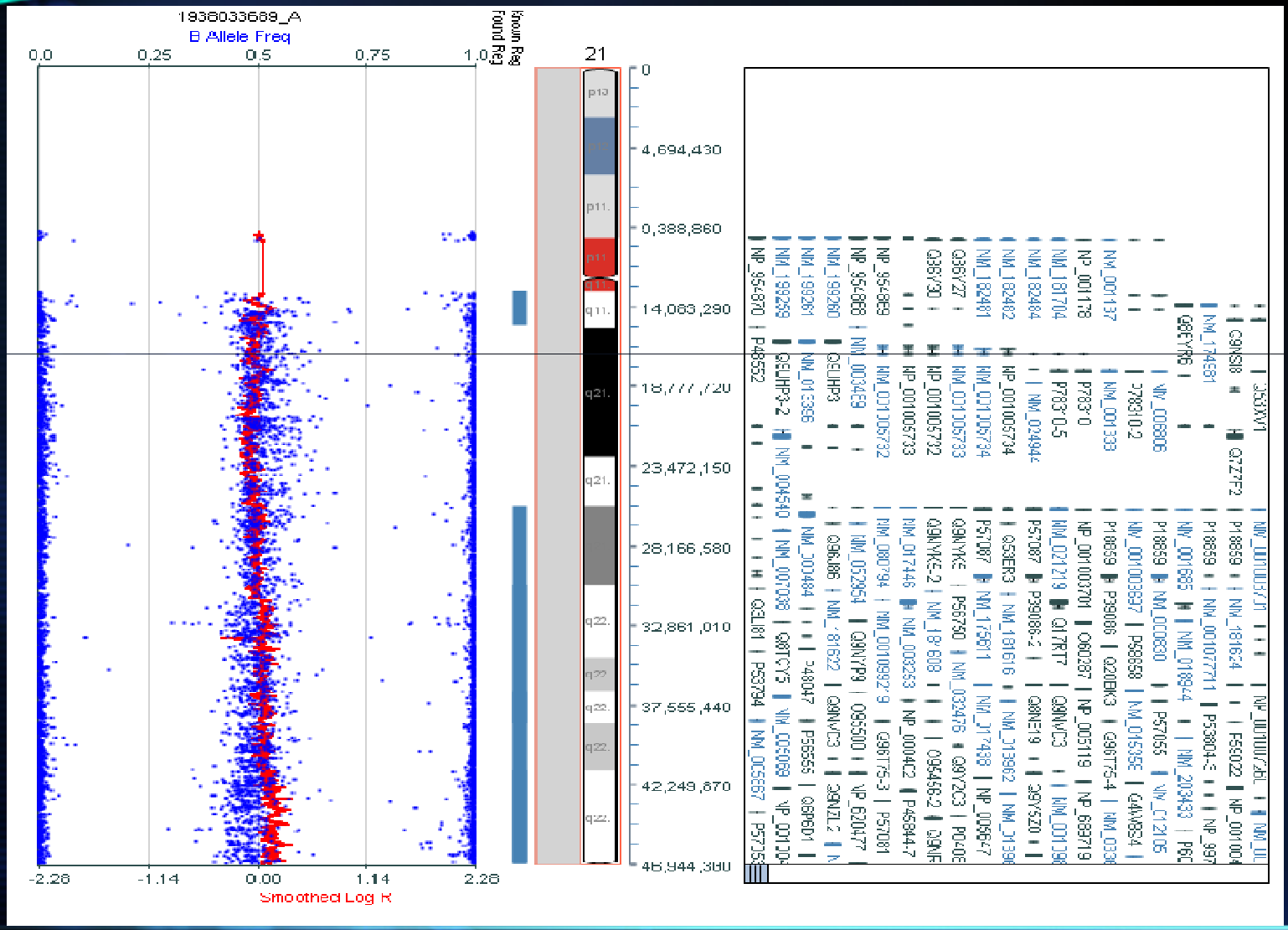
Three Copies of Chromosome 3 (Noise Due to Poor Embryo Quality)



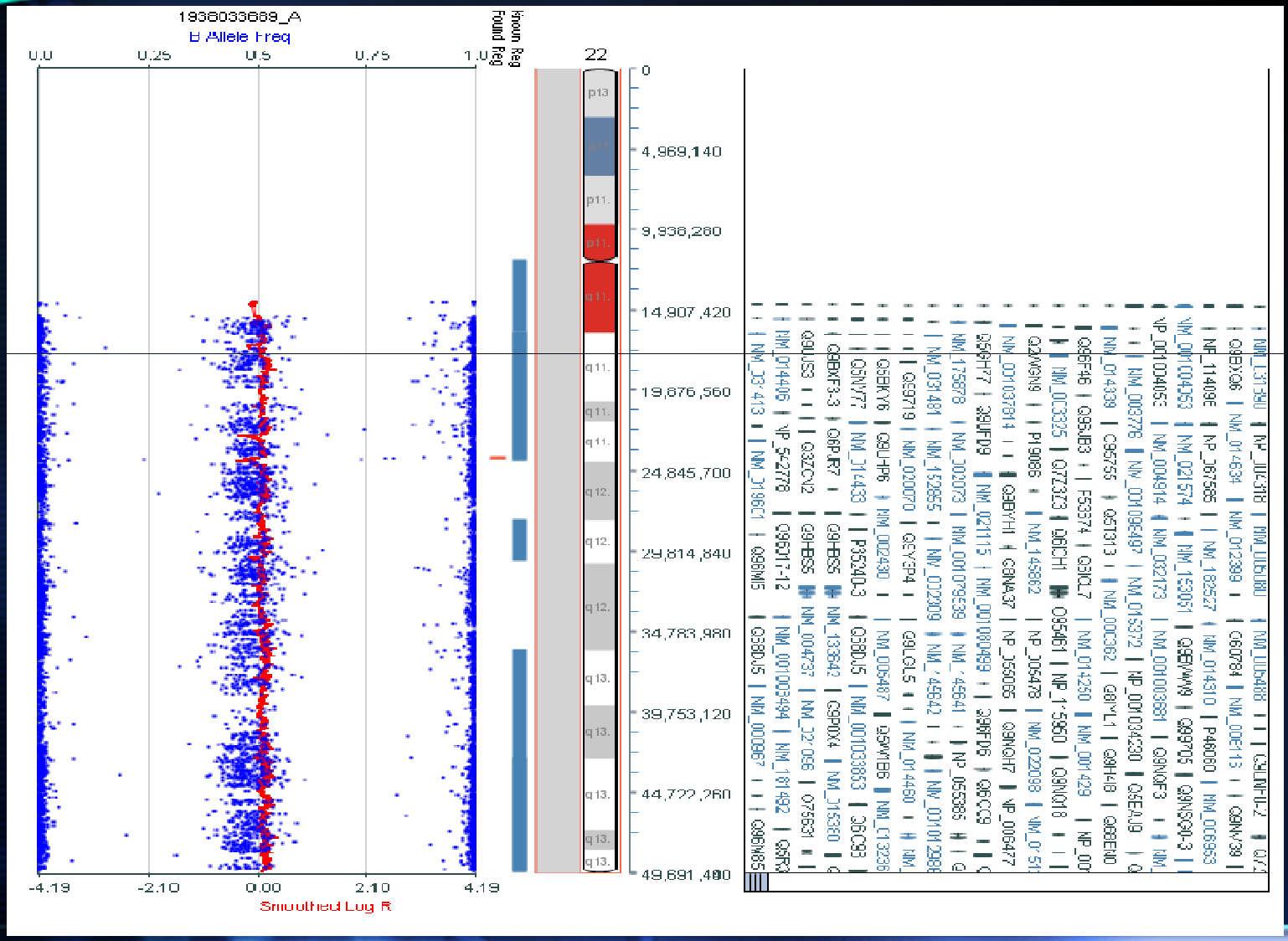
Two Copies of Chromosome 18



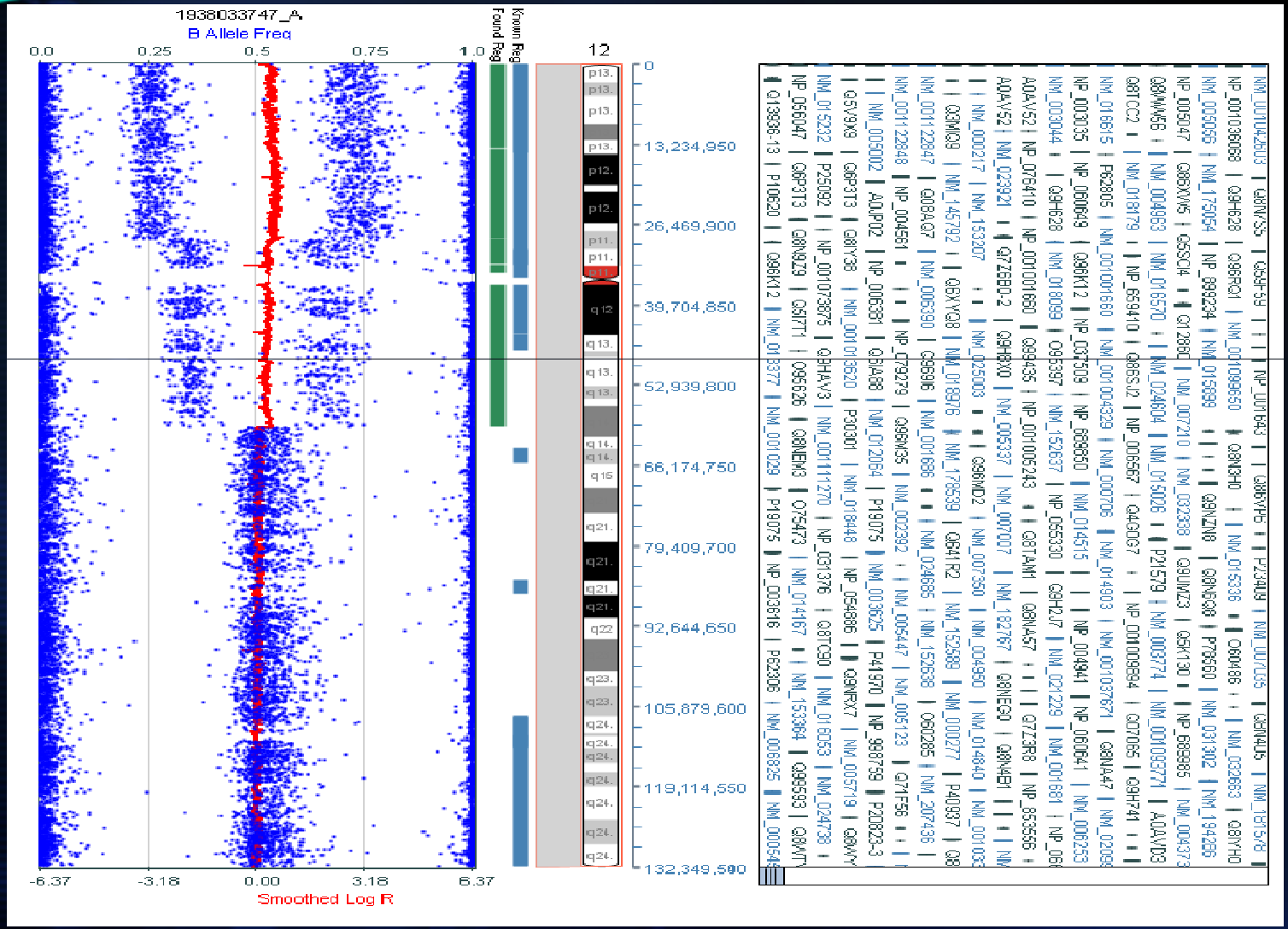
Two Copies of Chromosome 21



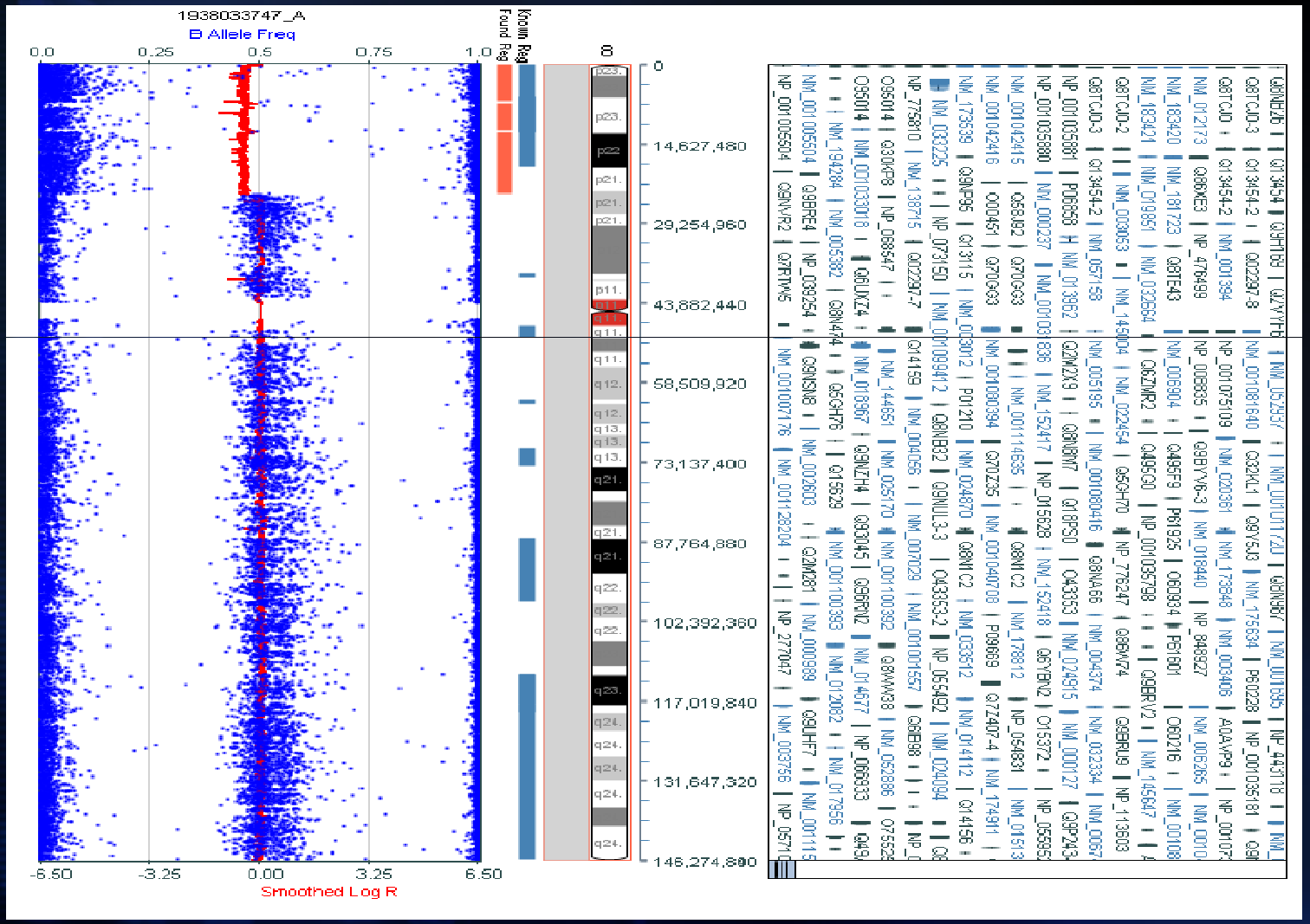
Two Copies of Chromosome 22



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



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

23-Chromosome Aneuploidy Results

- 61 embryos (day-3 PGS abnormal for 10-chromosome FISH)
  - 69% (42/61) = mosaic diploid / aneuploid
    - 2-7 Chromosomes
  - 25% (15/61) = mosaic aneuploid
    - 3-9 Chromosomes
  - 7% (4/61) = complex mosaic
    - 3-13 Chromosomes

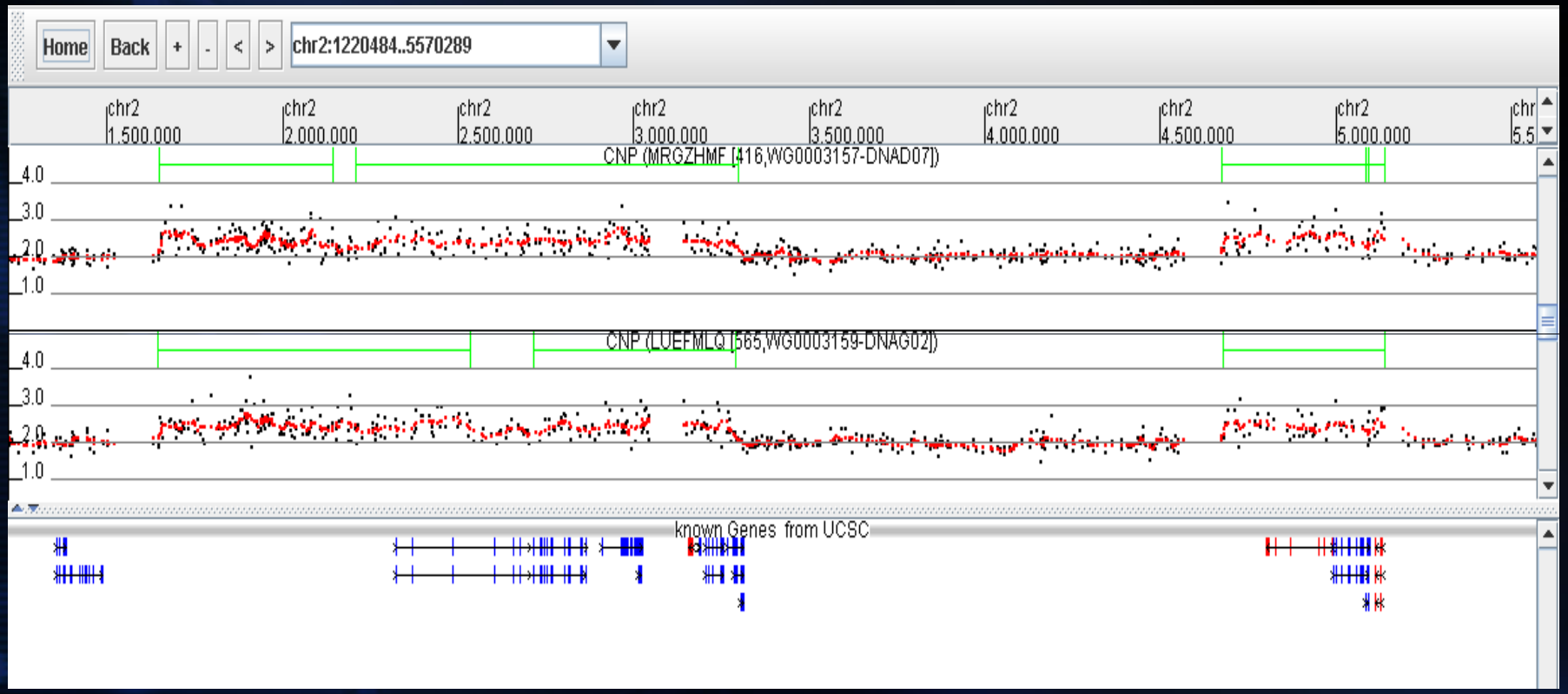
Results

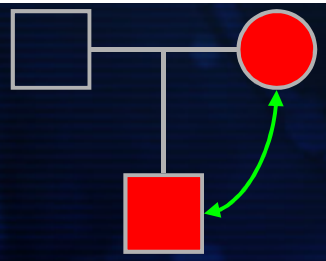
- Structural chromosome imbalances were identified from all 9 cytogenetically abnormal cell lines
  - del(8q), add(17p), del(17p), add(4q), add(9p), add(14q), dup(18p), dic(5), del(12p) and del(9p)
    - Based upon the density of the SNP microarray
    - CGH array couldn't identify genetic imbalances

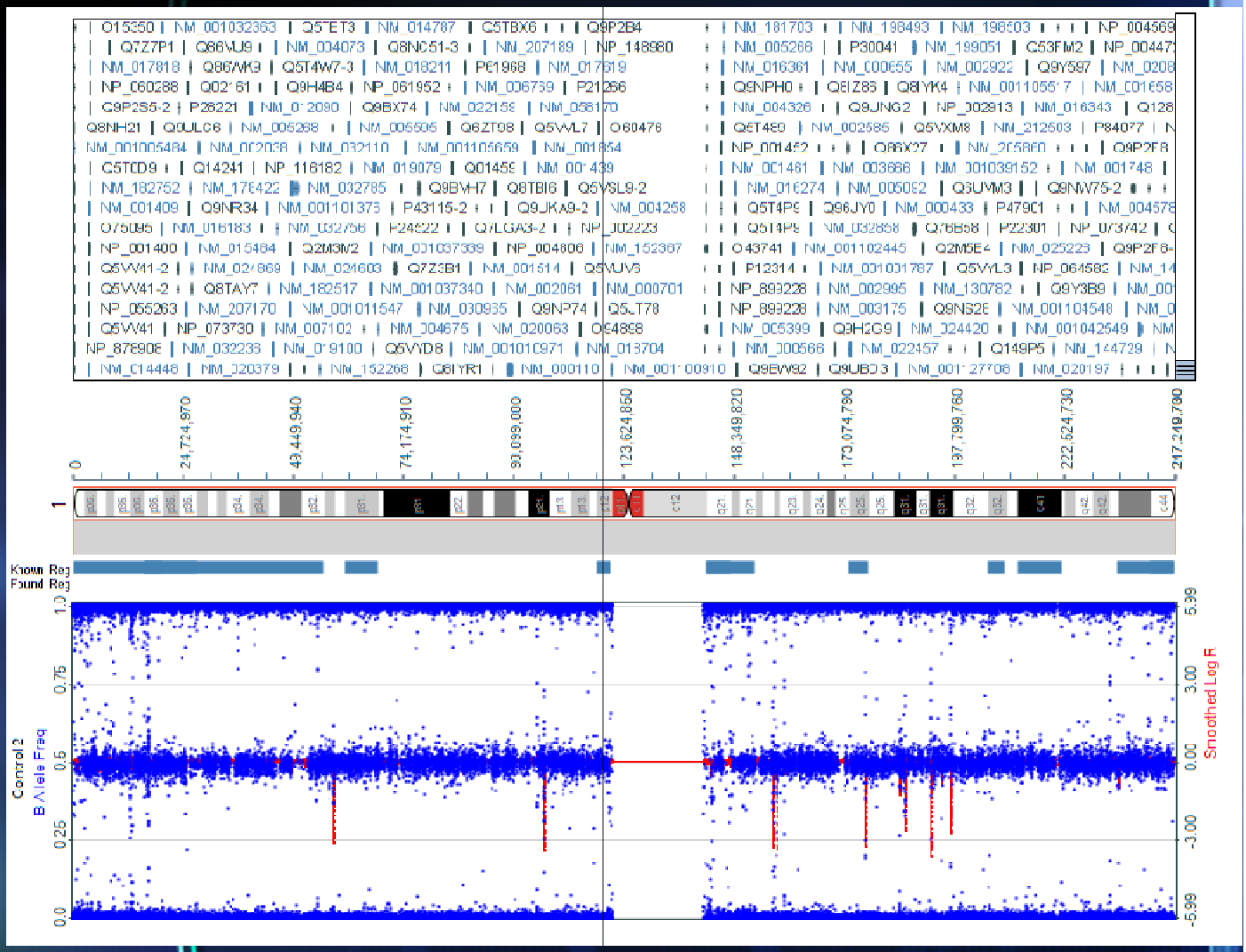
Copy Number Variations

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

Copy Number Variations



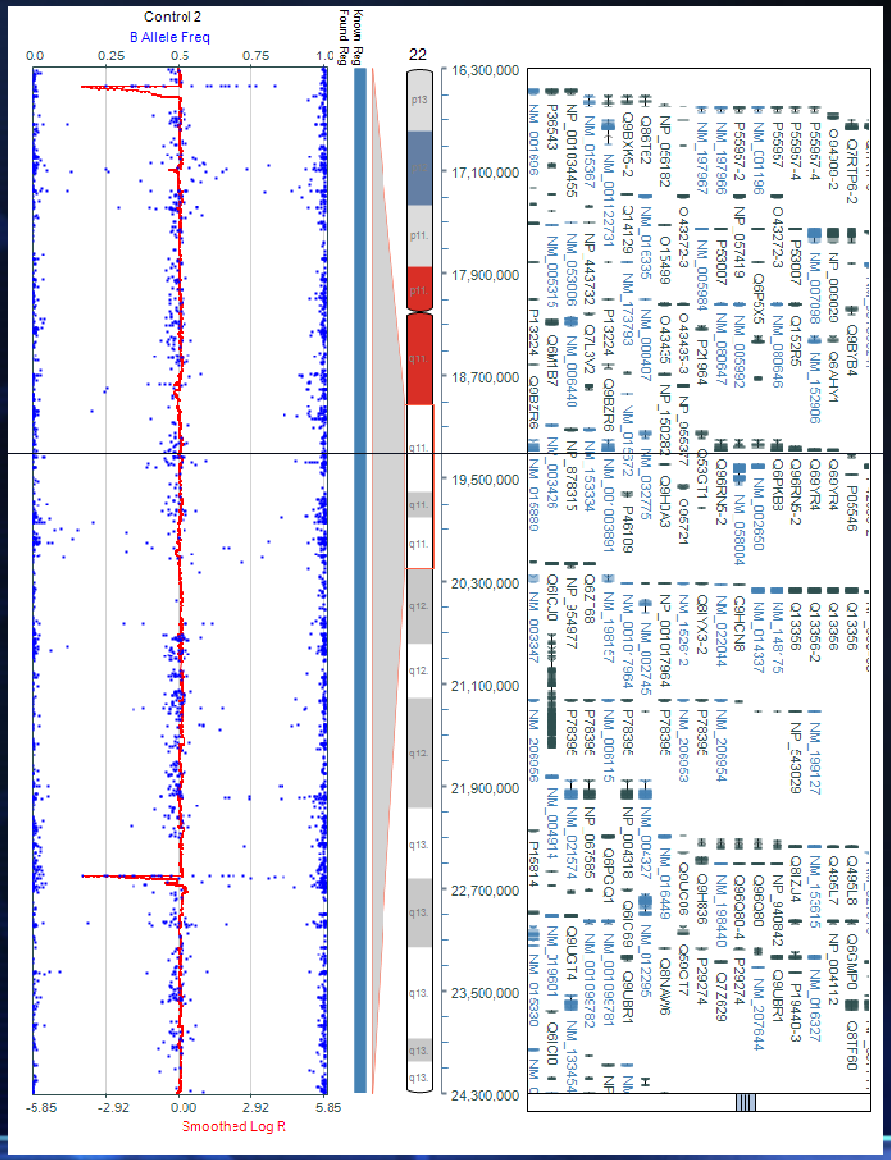



0
24,724,970
49,449,940
74,174,910
98,899,880
123,624,850
148,349,820
173,074,790
197,799,760
222,524,730
247,249,700
1
Known Reg
Found Reg
Control 2
B Allele Freq
1.0
0.75
0.5
0.25
0.0
5.99
3.00
0.00
-3.00
-5.99
Smoothed Log R

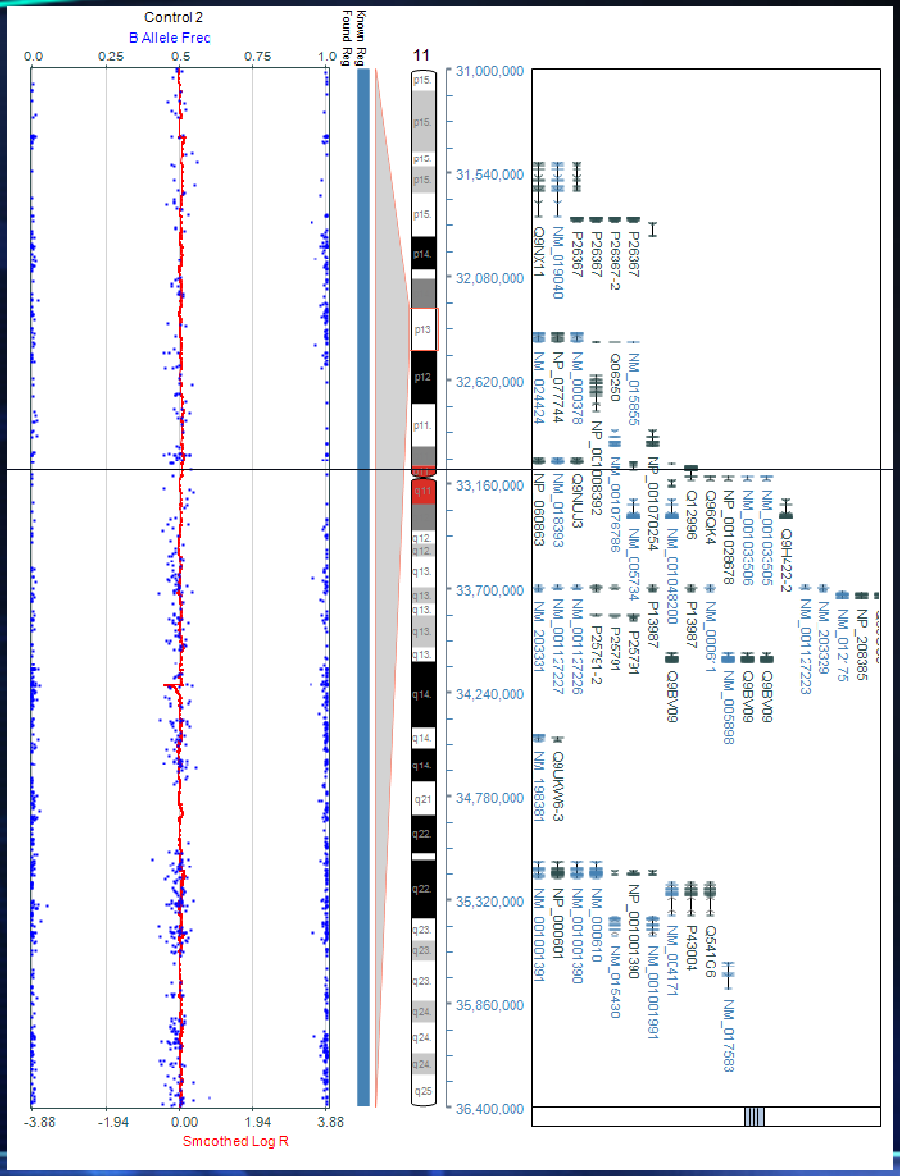
Genomewide Scans / Molecular Genetic Sequences

- Beckwith-Wiedmann Syndrome
- Some forms of Prader Willi / Angelman Syndrome
- DiGeorge Syndrome
- Some forms of Autism
- Uniparental Disomy
- Single Gene Disorders
- etc

DiGeorge Syndrome



Wilm's Tumor


Control 2
B Allele Frec
0.0
0.25
0.5
0.75
1.0
Known Reg
Found Reg
11
31,000,000
31,540,000
32,080,000
32,620,000
33,160,000
33,700,000
34,240,000
34,780,000
35,320,000
35,860,000
36,400,000
-3.88
-1.94
0.00
1.94
3.88
Smoothed Log R

Results

- Modified DNA fingerprinting / genotyping
  - What embryo implanted?
  - Who provided the extra chromosome?

Conclusions

Chromosomes

- Complete molecular karyotype for all 23/24-pairs of chromosomes
- Genetic imbalances due to reciprocal or Robertsonian translocations, pericentric and / or paracentric inversions
- Duplications (i.e Charcot-Marie-Tooth, type 1A)
- Microdeletion syndromes (i.e. DiGeorge syndrome)
- Using modified microarray and FISH, identify cryptic sub-telomeric rearrangements

Conclusions

- Using Genome-wide scans and modified DNA fingerprinting / genotyping
  - What partner provided the extra chromosome?
  - What embryo implanted?
    - Select best embryo for elective single embryo transfer (eSET)

Conclusions

- Genome wide scans / Molecular Genetic Sequences
  - Complex genetic disorders
  - Single gene disorders

Conclusions – SNP Microarrays

- Analyze polar bodies, blastomeres or trophectoderm cells

Laboratory and Clinical Collaborators

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- Andrew Siegel
- Eric Widra
- Richard Leach
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