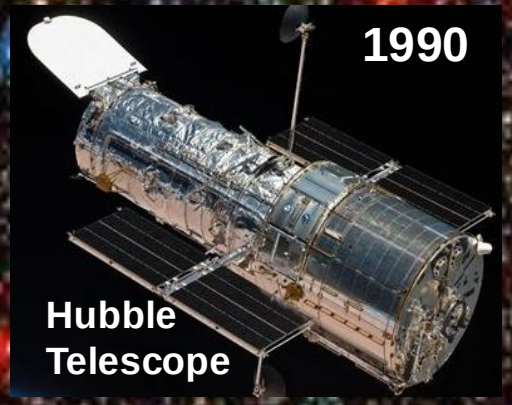
The Evolution of PGT Methods
PGT Back to the Future

PGDIS 21st Meeting
Kuala Lumpur, Malaysia
May 6, 2021

Mark Hughes, MD, PhD

The Symphony of Life…..The Genetic Cacophony of Starting It

Starry Southern Sky.
Kit Peak Nat’l Observatory – Arizona, USA



Same exact sky.
Nothing has changed.
Except the detection technology.

The Beginnings of PGT

1968 — Edwards/Gardner sexed whole rabbit embryos at the blastocyst stage (bar body, visual)

1987 — Penketh & McLaren
Proposed human embryo gene tests

1989 — Handyside & Winston
First human embryo sexing

1989 — Verlinsky & Kuliev
First Polar Body PGD attempts

1992 — Handyside, Winston & Hughes
First human PGD for specific genetic disease (Cystic Fibrosis)

The Beginnings ("science fiction") of PGT -M ("PGD)

The NEW ENGLAND JOURNAL of MEDICINE

Volume 327: 905-909 September 24, 1992 Number 13

Birth of a normal girl after in vitro fertilization and preimplantation diagnostic testing for cystic fibrosis

AH Handyside, JG Lesko, JJ Tarin, RM Winston, and MR Hughes

- Between 2014 and 2016, 94,935 PGT cases performed in USA
- Producing 26,822 babies.

Cooper Genetics is performing PGT-M on 400+ embryos/month

Nomenclature Mess

- PGD – for Diagnosis
- PGS – for Screening of Chromosomes
- CCS – for Comprehensive Chromosome Screening
- PIGD – ….

In 2016 PGDIS attempted to consolidate all the confusion:

- PGD – M - for monogenetic disorders; Mendelian conditions)
- PGD – A - for aneuploidy
- PGD – SR - for structural rearrangements

- World has settled on PGT

PGT-M Technologies

- Biggest problem was TIME.
  - In 1990, no technology existed for a 12-hour turn-around of molecular data. (shipment)
  - PCR and quick electrophoresis.
    - Cleverly designed, nested PCR oligo-primers to control what would amplify.

- Real Time, Quantitative PCR. (qPCR)
  - 3 or 4 locus-specific amplicons along any/each chromosome. Quick.
  - Good for very common gene mutations but otherwise, expensive.

- Multiplex-PCR. Mutation of interest AND flanking STRs for haploblock confirmation.
    - A single blastomere was analyzed for many different alleles in one reaction

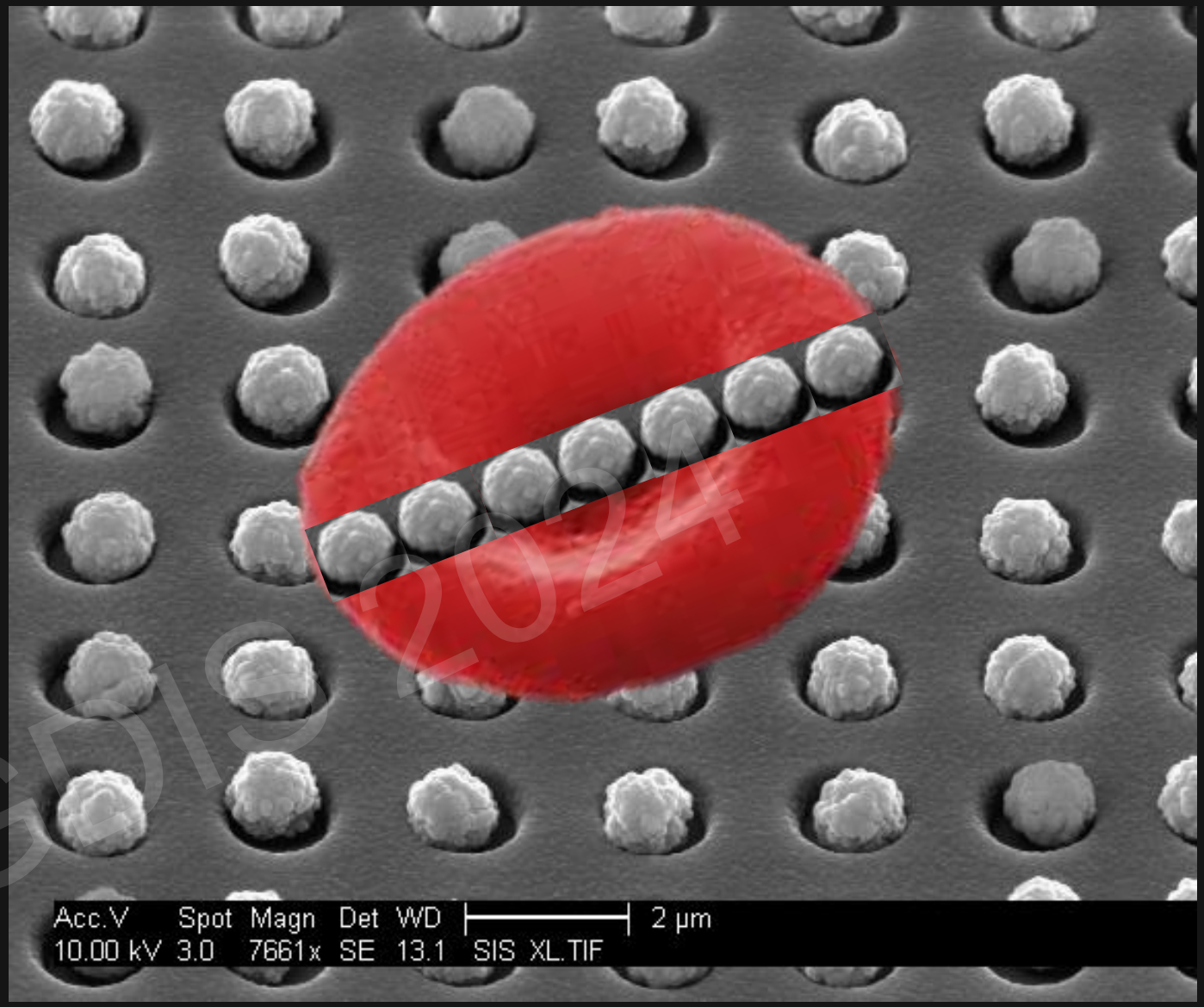
Whole Genome Amplification (WGA)

- Many different amplification techniques have been developed & tested (1 cell)
  - DOP-PCR (2002) used Degenerative Oligonucleotide Primer)
  - PEP-PCR (primer extension pre-amplification, with Klenow or T4 polymerase)
  - MDA (2006) used isothermic multiple displacement amplification)
    - Random hexamers and phi29 polymerase
    - Strand displacement technology
    - Produced chimeric DNA in high abundance, complication downstream analysis
  - Pico-plex PCR (2008) Proprietary quaisi-random primers bind to selective sites
    - Currently the most widely used WGA in single-cell testing.
  - PTA (2020) – Primary Template-directed Amplification (DNA photocopier)

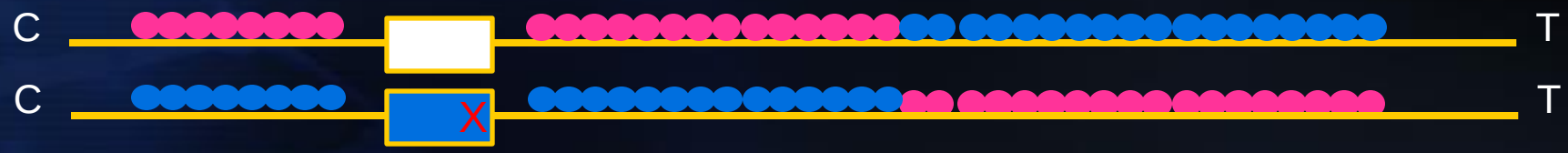
PGT-M Technologies

- SNP Arrays – a determination of “mutant” and “normal” haploblocks

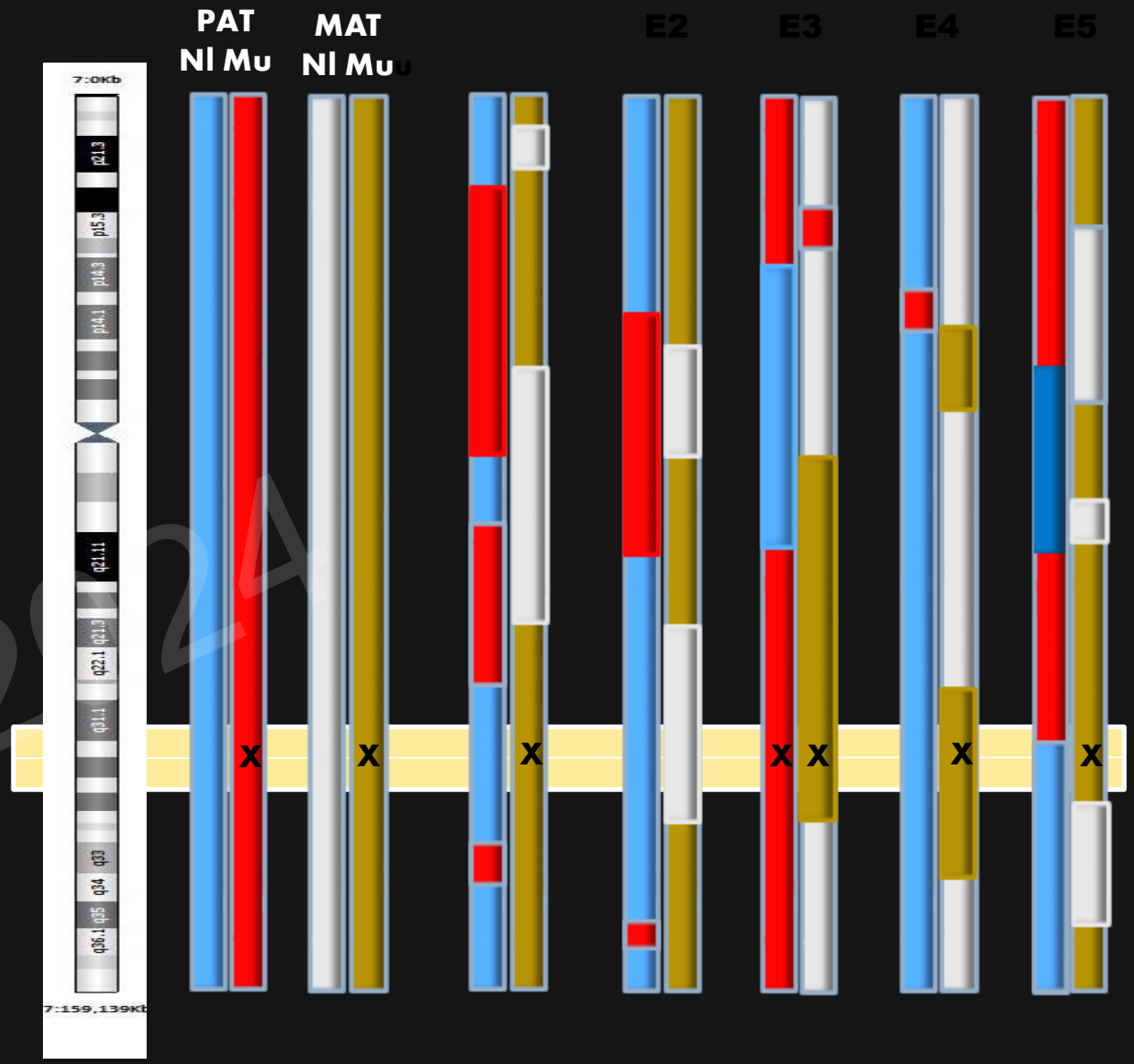
Oligonucleotides on a bead – millions of beads



Follow haploblocks highly validated SNP arrays



Follow the haplo-block shuffle



Cystic Fibrosis
Chromosome 7 ⇨

Massively Parallel Next-Gen Sequencing

Biopsy DNA

Fragmentation

300,000,000+ frags

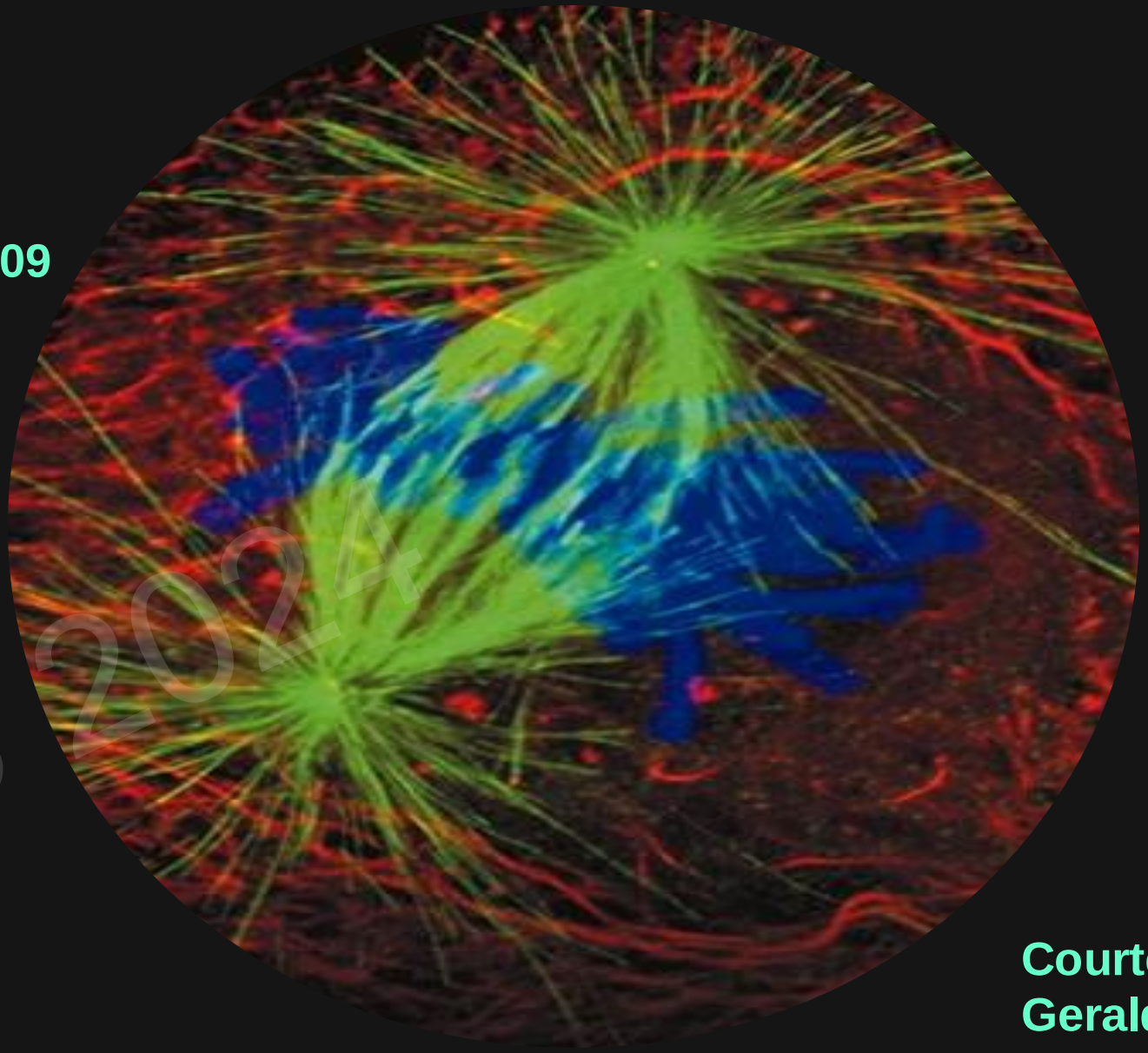
Ligate adaptors onto ends

Amplify fragments on flow cell

clonal clusters of each fragment

Human Fertilized Egg – Metaphase Plate Forming

Human Embryo - 2009

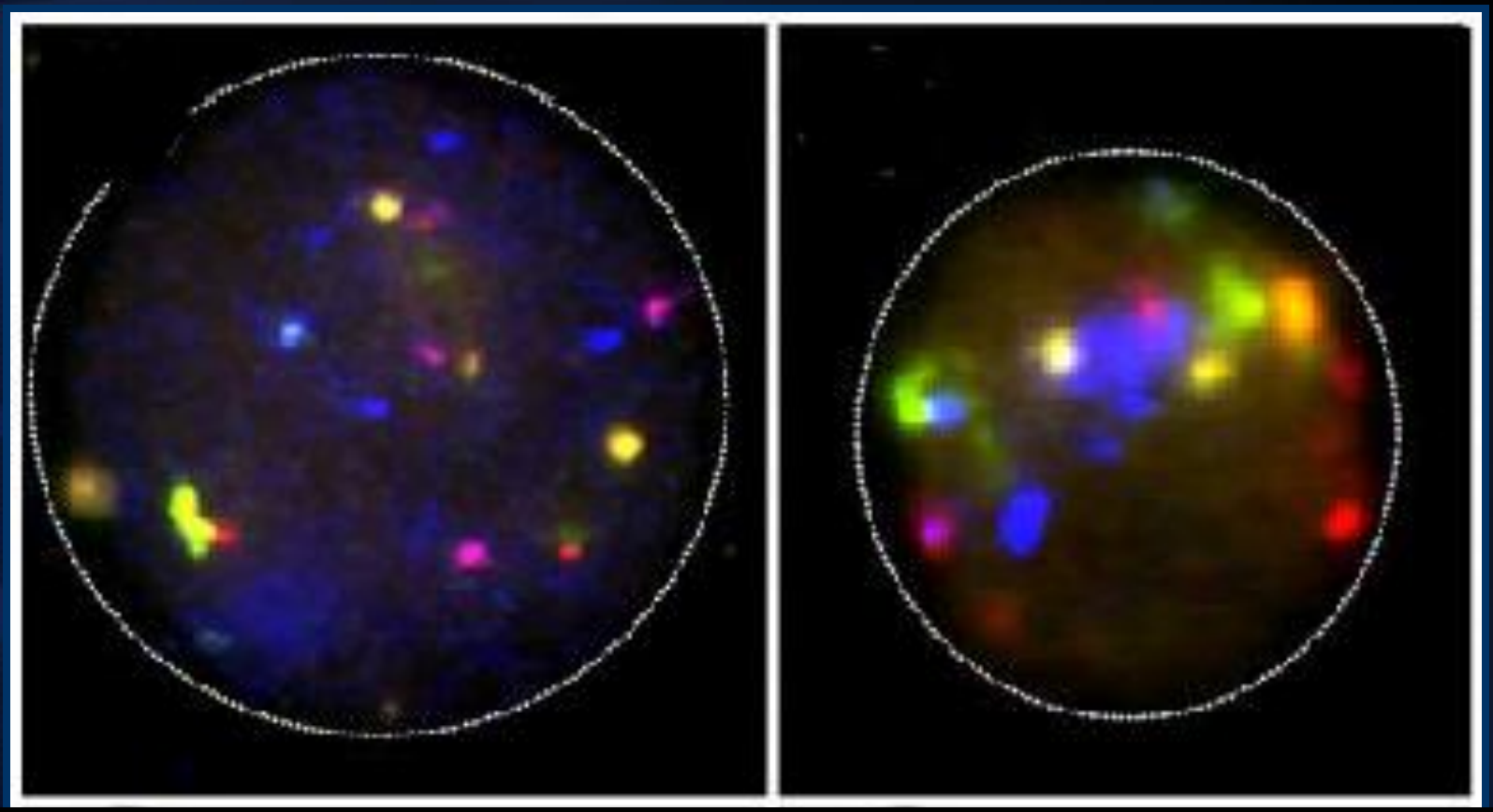


Courtesy
Gerald Schatten - 2009

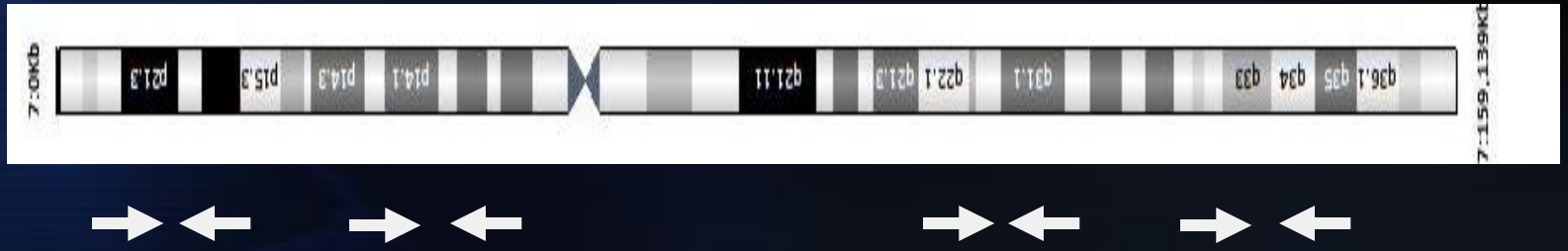
The Birth of PGT-A: FISH of Blastomere

Trisomy 21

11 Probe FISH



FISH on Steroids ("quantative PCR")



24 chromosome pairs x 4 markers = 96 rxns

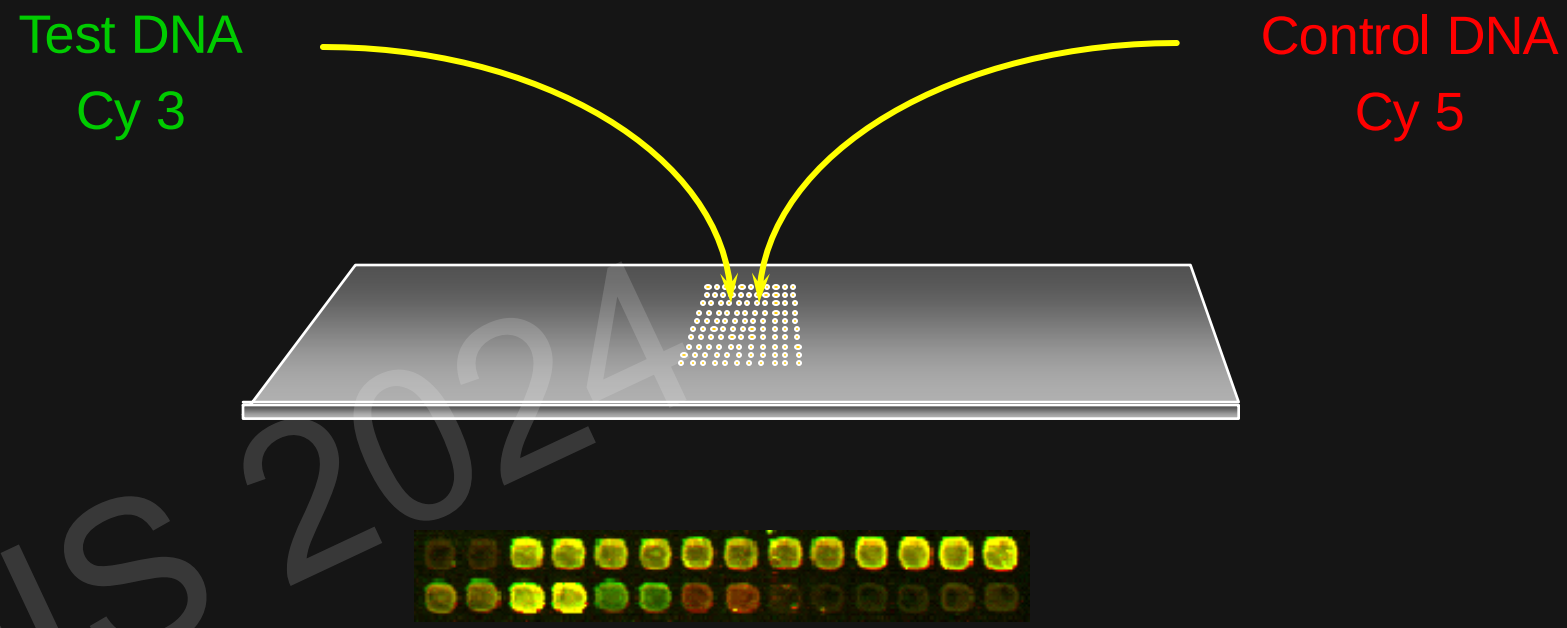
Misses Deletions, Duplications, Inversions

2007: Affymetrix (“original Grand-Daddy”) Platform

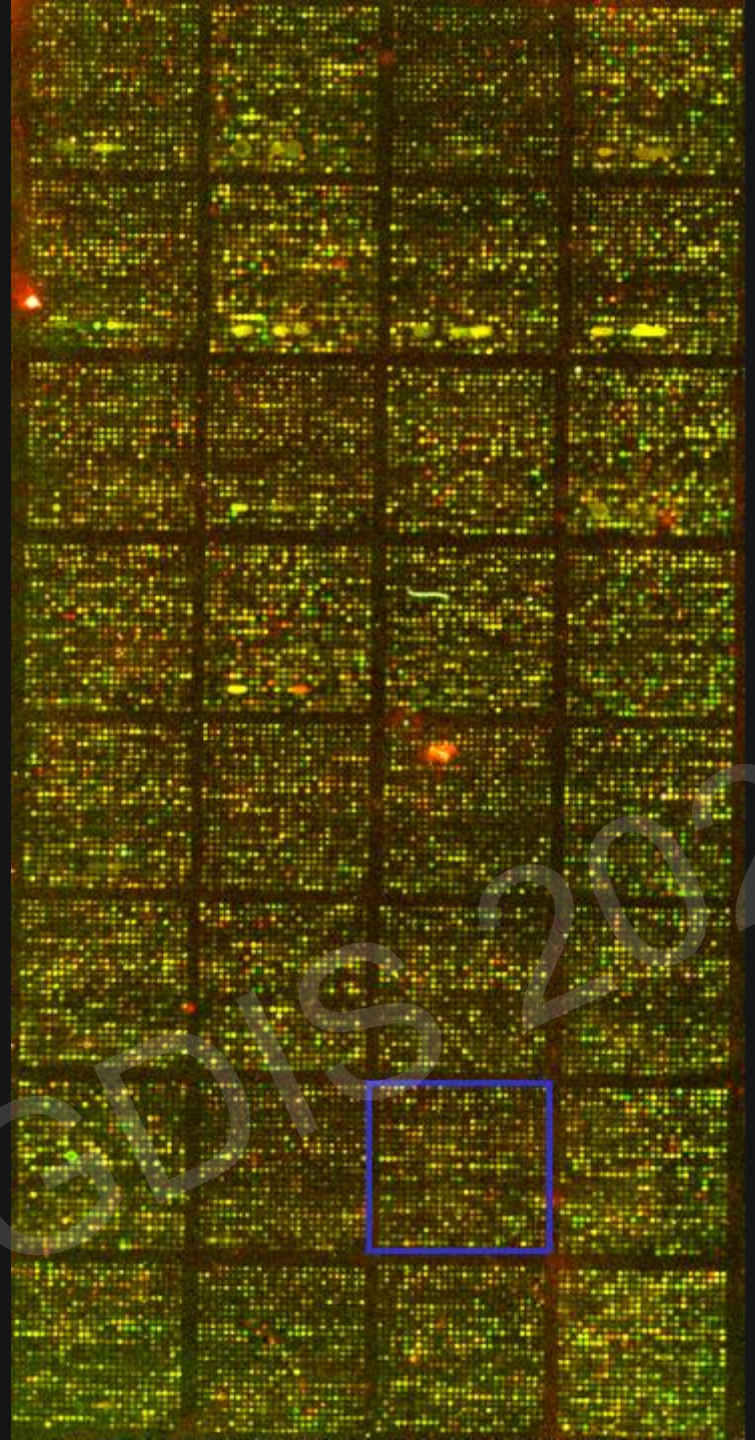
PGS One Blastomere – Multiple Genotypes (2007)

Chrom 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 15, 16, 18, 21, 22, X, Y

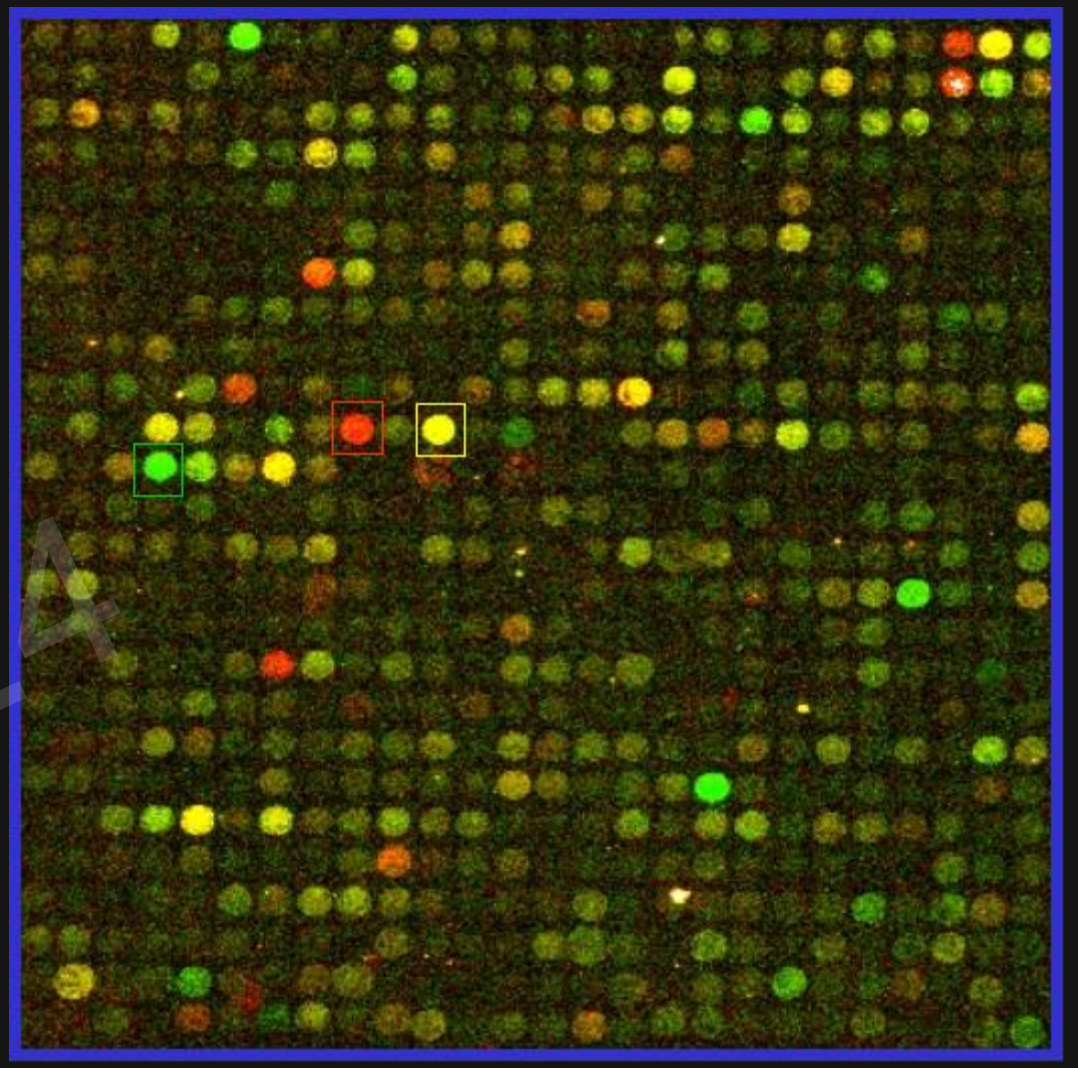
Microarrays: Comparative Genomic Hybridization (aCGH)



BlueGnome - London

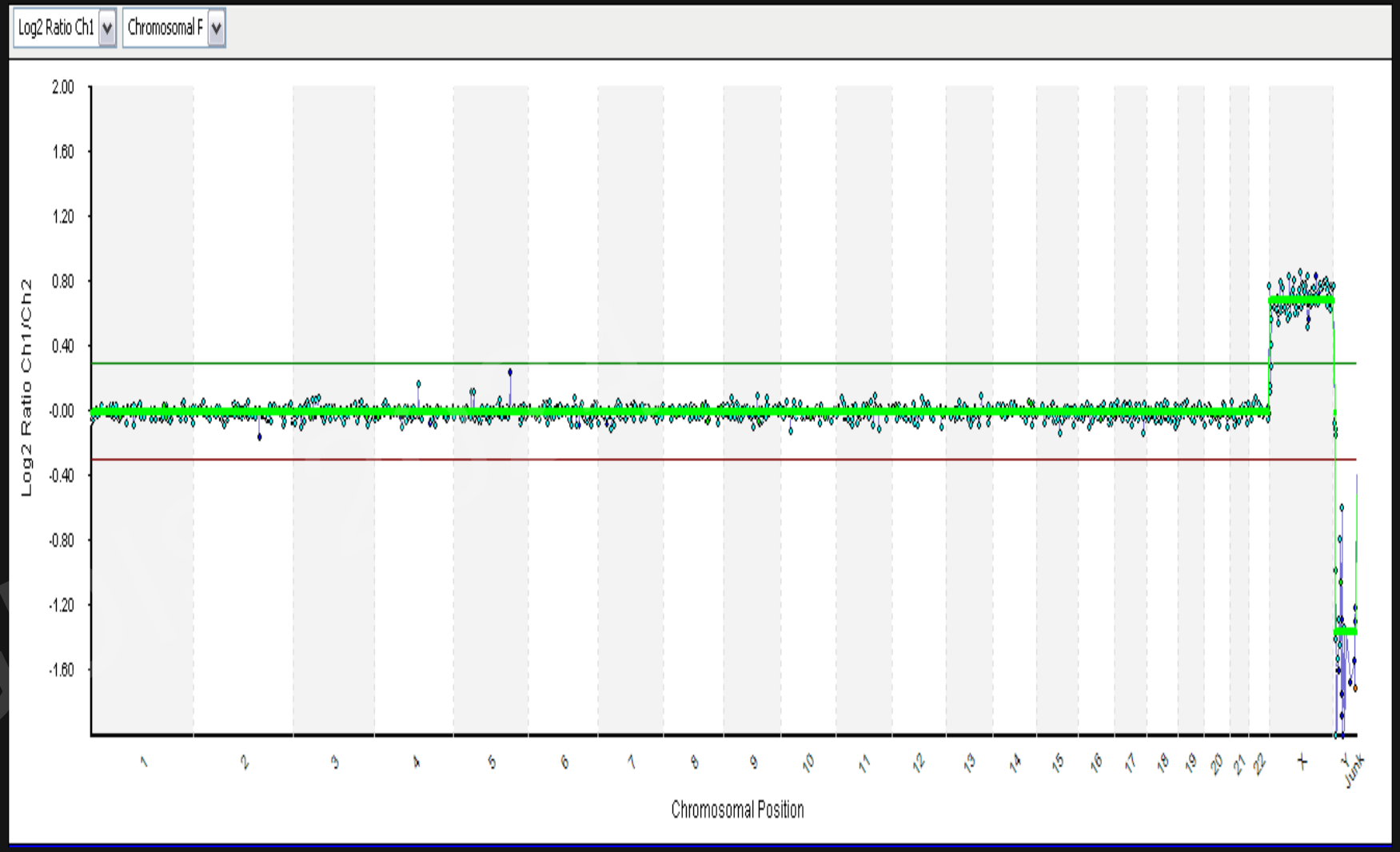


Human Cleavage-Stage Embryo
One Blastomere

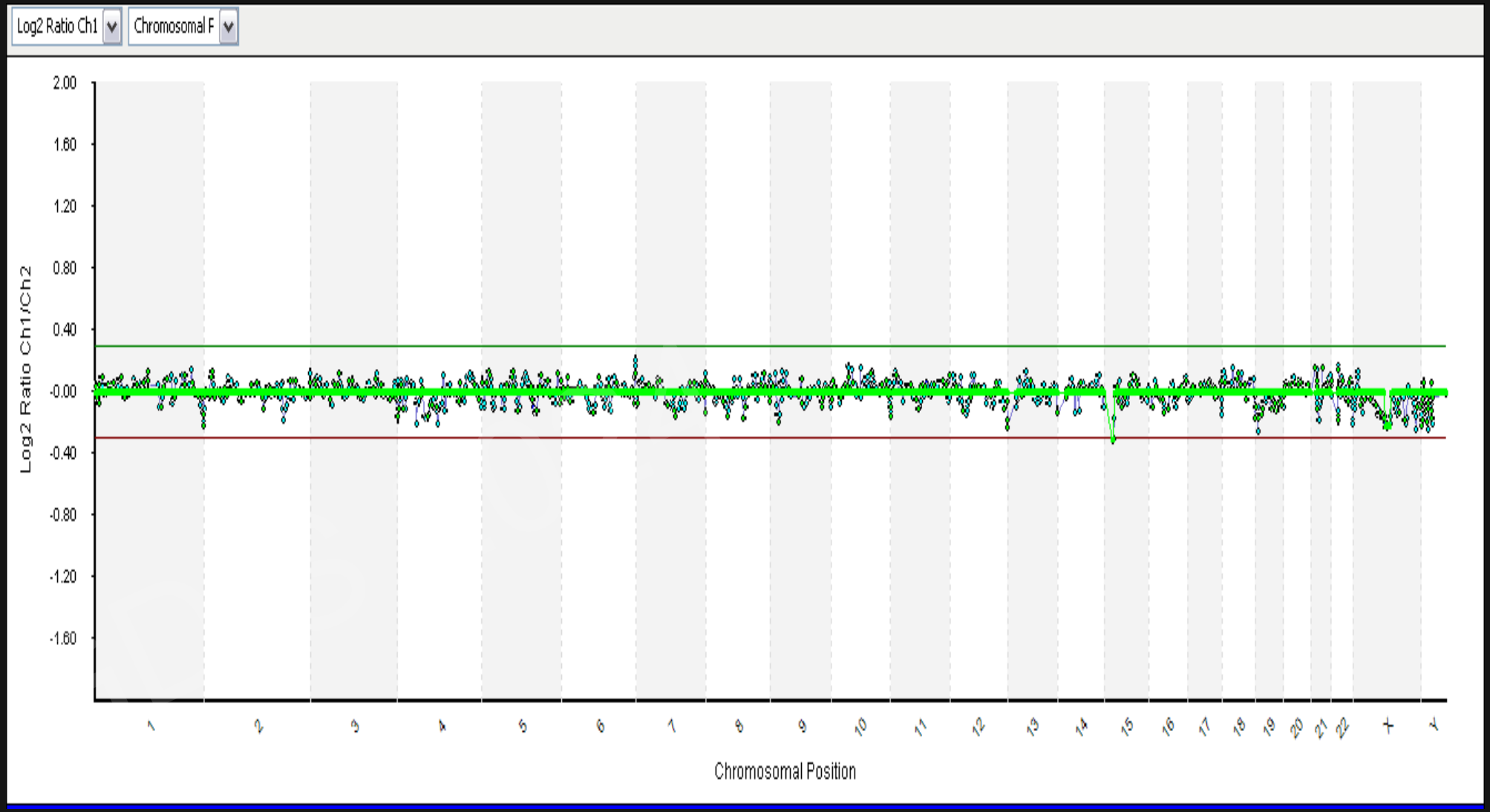


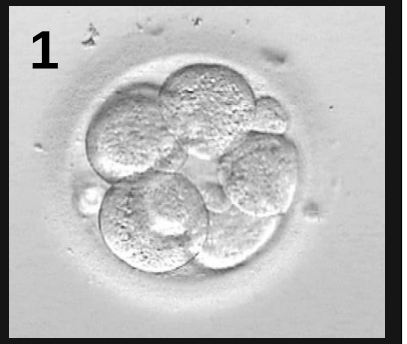
Hughes Lab

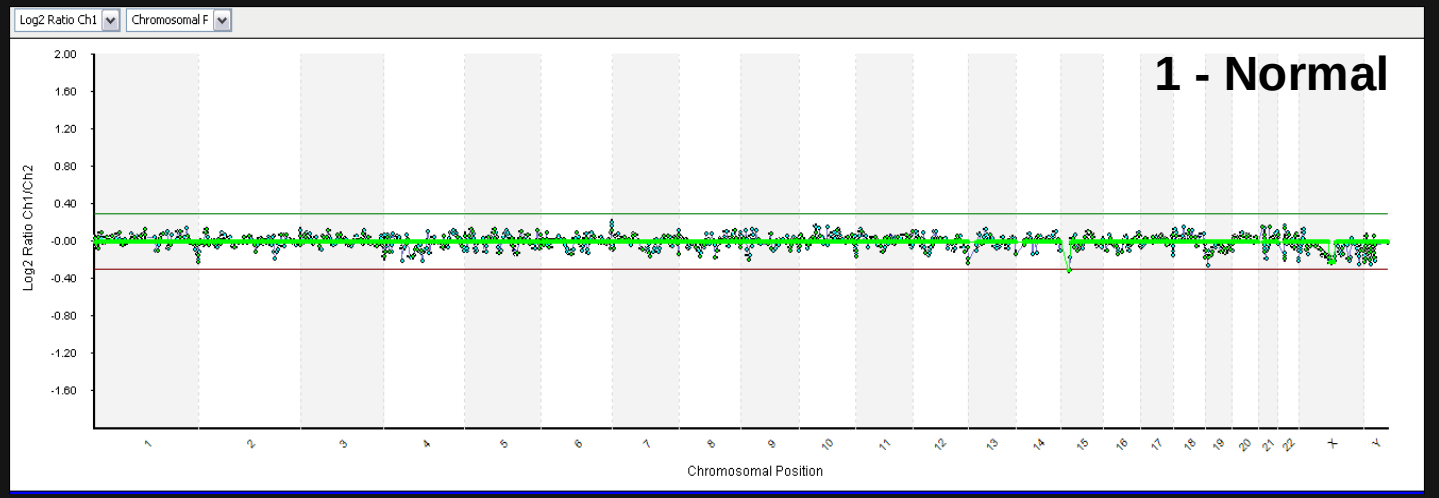
Normal Female Embryo

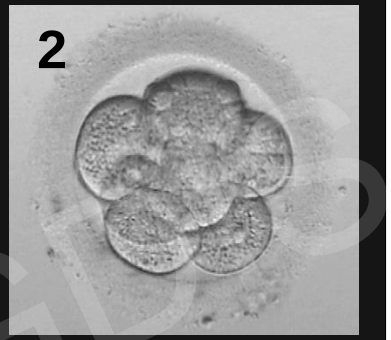


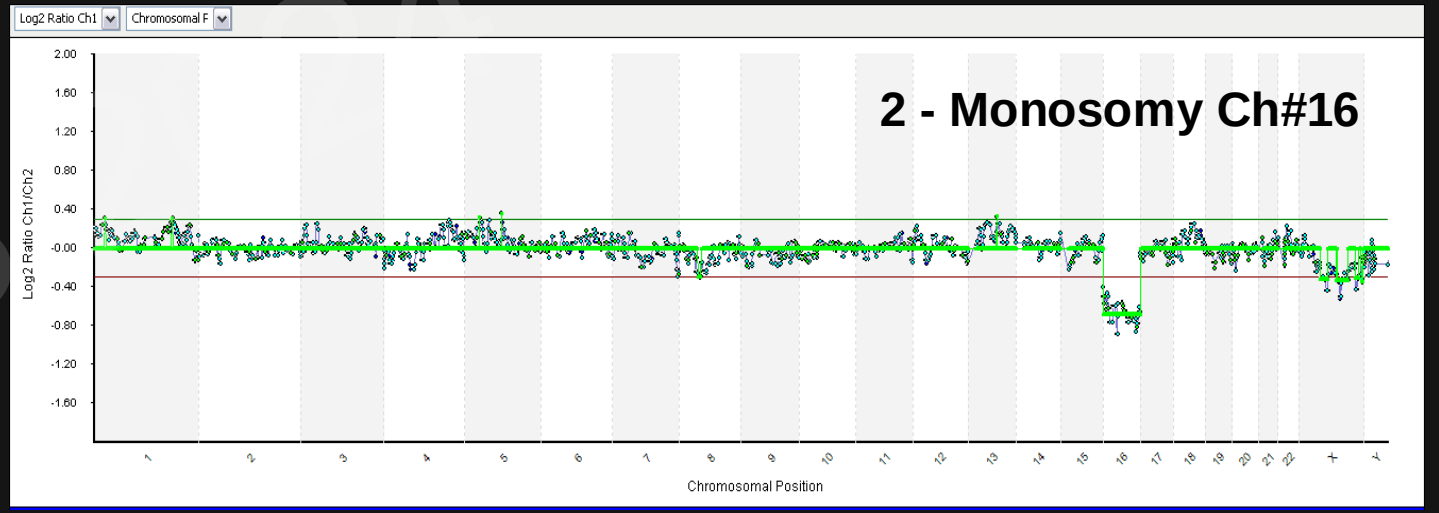
Normal Male Embryo




1

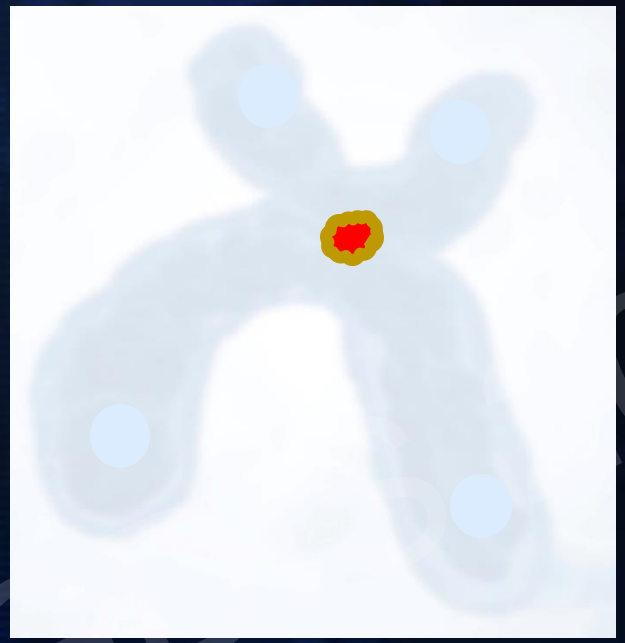

Log2 Ratio Ch1
Chromosomal F
1 - Normal
Log2 Ratio Ch1/Ch2
2.00
1.60
1.20
0.80
0.40
-0.00
-0.40
-0.80
-1.20
-1.60
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 X Y
Chromosomal Position


2

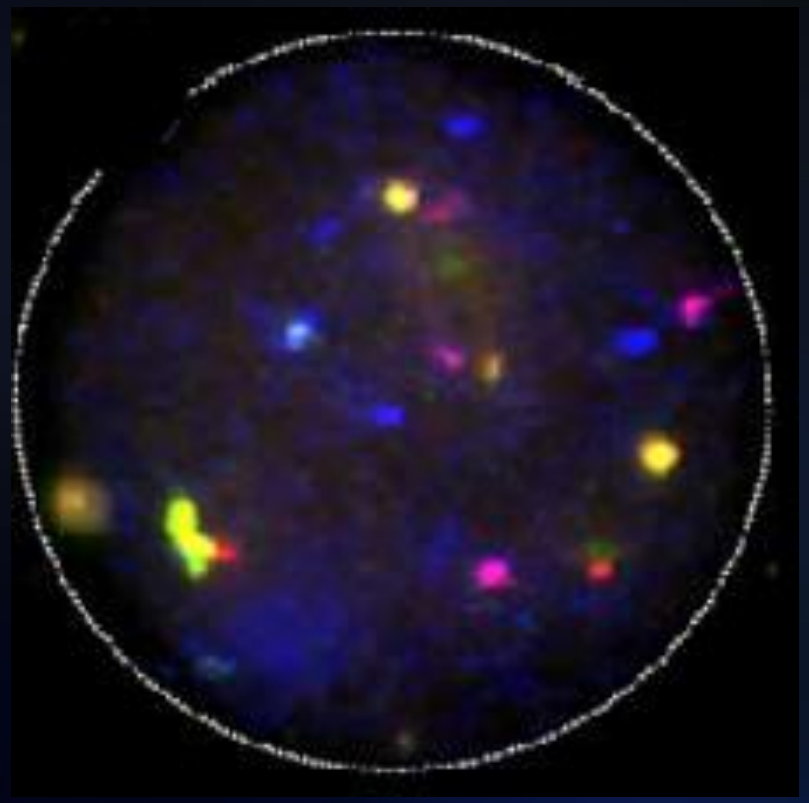

Log2 Ratio Ch1
Chromosomal F
2 - Monosomy Ch#16
Log2 Ratio Ch1/Ch2
2.00
1.60
1.20
0.80
0.40
-0.00
-0.40
-0.80
-1.20
-1.60
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 X Y
Chromosomal Position

Score Card: PGS Chromosome Technologies

	FISH	QPCR	aCGH	FAST-A	SNPs	hr-NGS
Total Data Signals	11	96	2,700	50,000	75,000	1.2 M



One Fluorescent data point per chromosome



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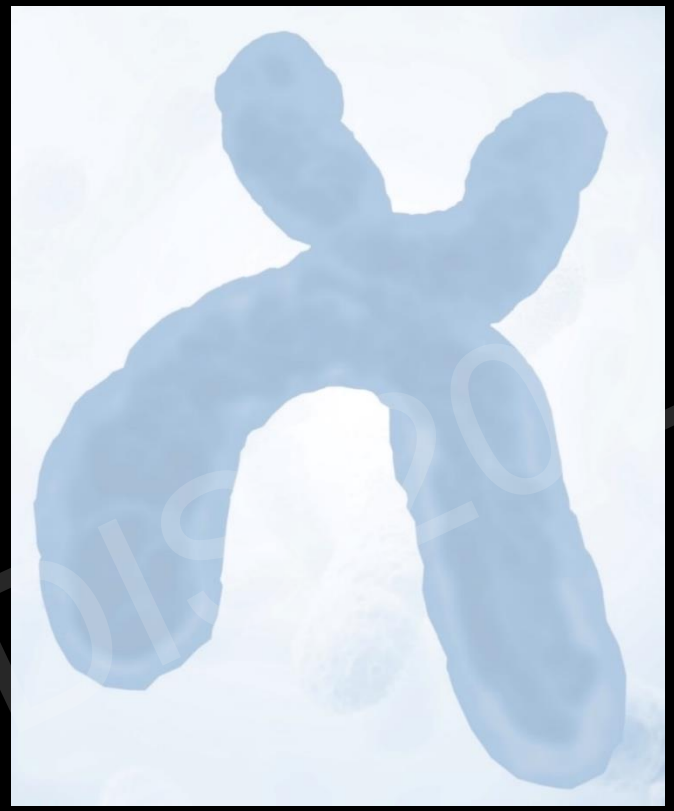
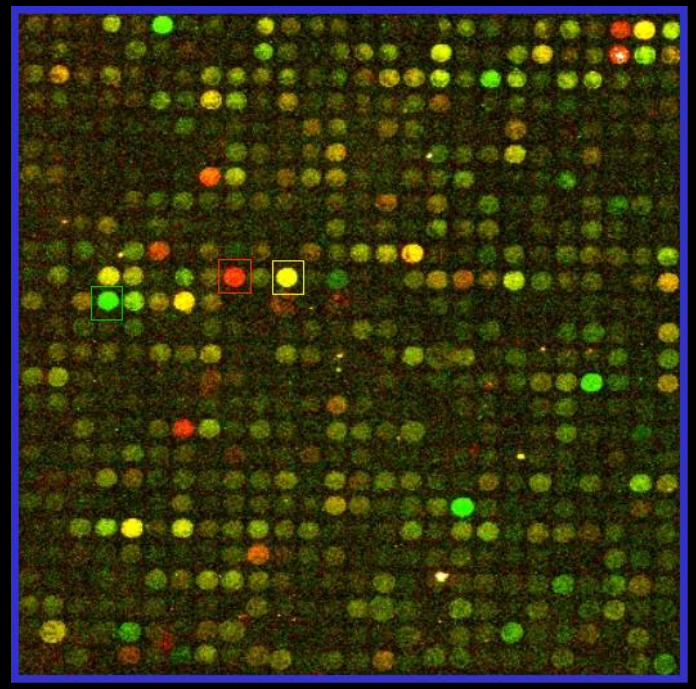


125 Data Signals per chromosome



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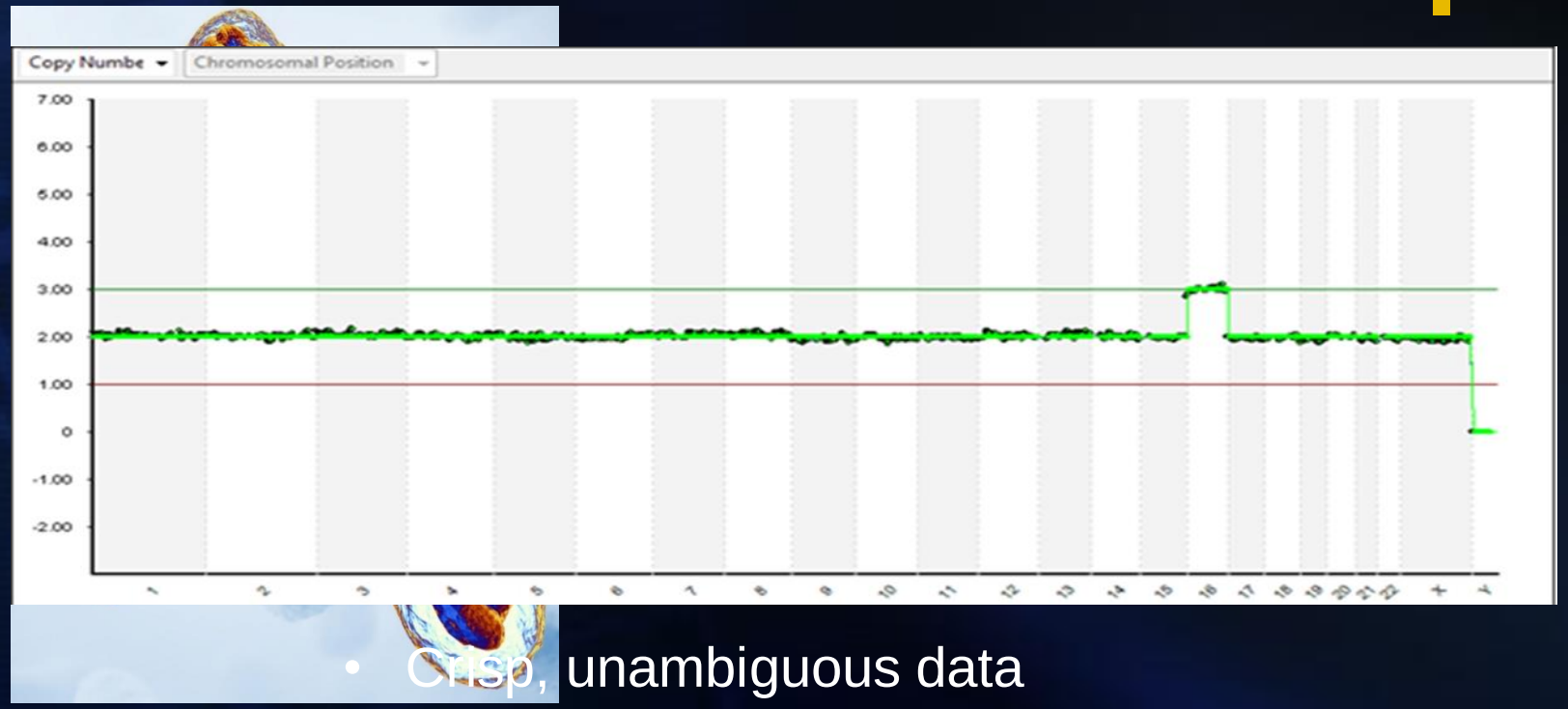

- Crisp, unambiguous data
- Detects meiotic aneuploidy and …

~130,000 data points / chromosome


PGDIS 2024
Genesis Genetics Institute

Next Generation DNA Sequencing

- Next Gen Sequencing
  - The $50,000,000 (March 2003)
  - $13,000 (February 2009)
  - $2,500 (Sept 2012)
  - $2,000 (July 2014)
  - $1,250 (Dec 2021)

Think about:

- Technology is the Fuel.
- The most profound discoveries come from collaborative interactions & serendipitous events
- Is more always better?
- Have we learned lessons from Mosaicism?
- How do we balance our Discovery Science with the money?
- Numbers drive everything. We must not compromise integrity.