New Agilent CGH microarrays focused on exons for clinical applications: constitutional, prenatal and cancer

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SANTIAGO DE COMPOSTELA
10 DE ABRIL 2014

Overview

- Introduction
  - Agilent Portfolio
  - Agilent Array CGH / CGH+SNP
- ISCA Consortium / CGH+SNP Arrays
- Baylor College of Medicine
  - Chromosomal Microarray Designs
  - Coverage Comparison
  - Exon-by-exon Performance
  - Cancer Research Case Studies

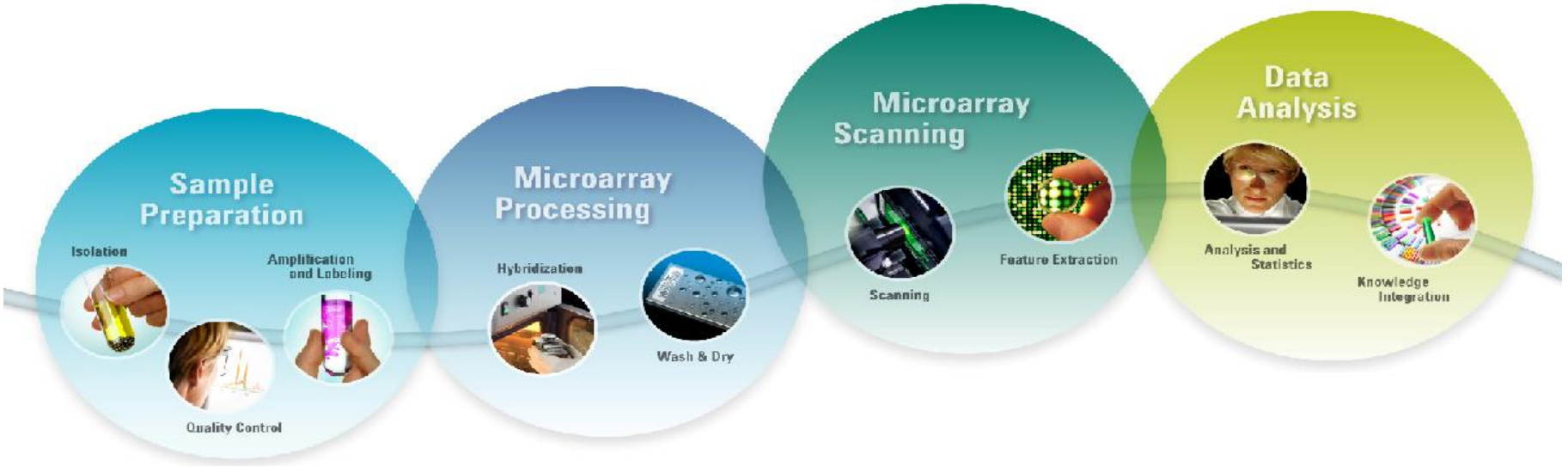
Application Areas

Target Enrichment

CGH+SNP Arrays

FISH Probes

Agilent Genomics Portfolio



2100 Bioanalyzer System

2200 Tape Station

Whole Genome labeling

FFPE labeling

Universal Reference RNA

LIQA Labeling Kits

Absolutely Nucleic Acid Purification

Target Enrichment

Arrays

FISH Probes

Hybridization kits

Wash buffer kit

Hybridization gaskets

eArray

qPCR QC

SureScan 2u Scanner

Feature Extraction Software

MX3000/3005 qPCR

8800 SureCycler

Brilliant III qPCR reagents

eArray

Cytogenomics 2.7

AGW 7

SureCall 2.0

GeneSpring 12.5

- **NGS**
- **Integrated Biology**
- **Mass Profiler Pro**

Clinical Research Conducted on Many Sample Types

Pre-implantation

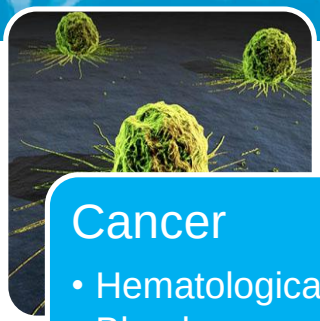
- One-two cells
- Parental samples

Pre-natal

- Amniotic Fluid
- Chorionic Villi
- Products of Conception

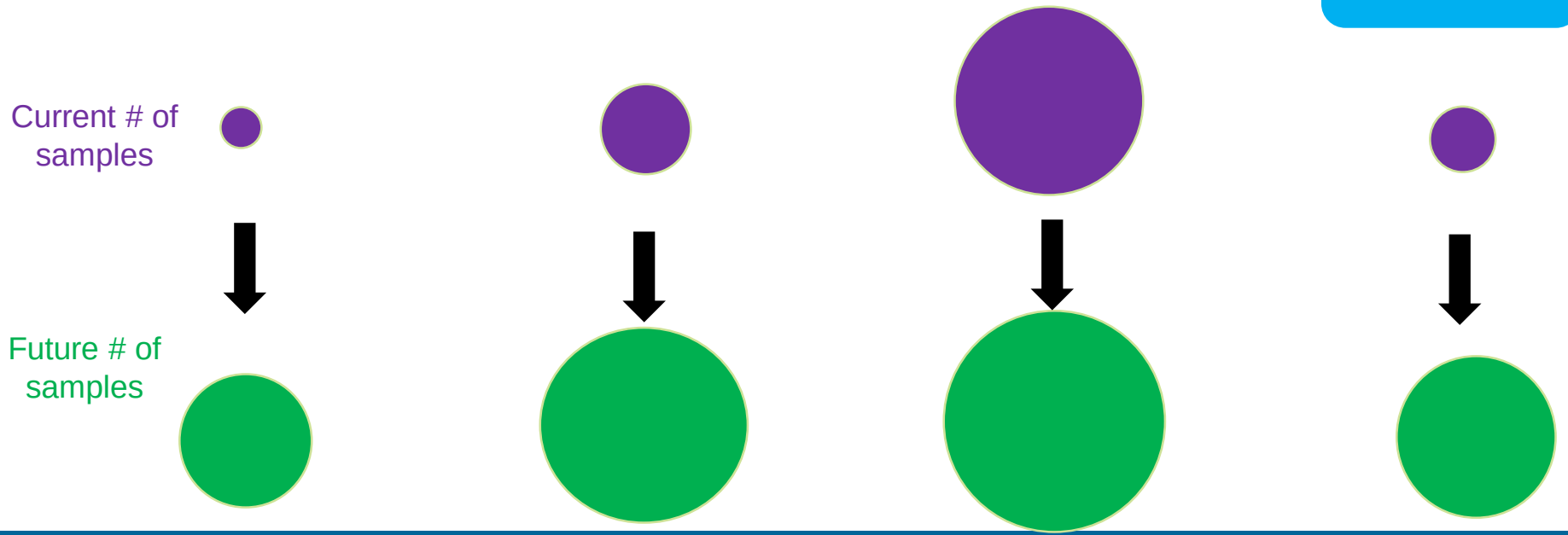
Post-natal

- Blood
- Saliva



Cancer

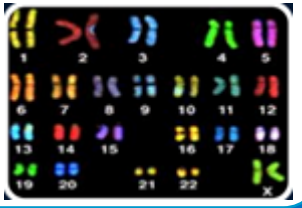
- Hematological
- Blood
- Bone marrow
- Solid tumor:
  - Frozen
  - FFPE



Molecular Analysis Made Easy With OLS
One Powerful Technology, Multiple Molecular Applications

Microarrays
- SurePrint CGH+SNP Microarrays
- Most trusted platform for cytogenetics research

FISH Probes
- SureFISH Probes
- Excellent resolution with high sensitivity and specificity

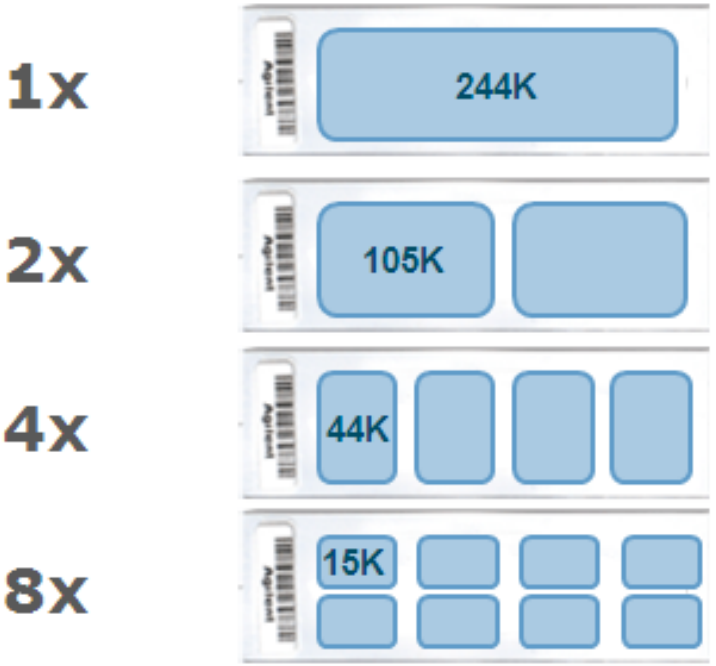


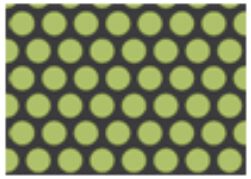
Targeted Sequencing
- HaloPlex Target Enrichment System
- Streamlined, single tube protocol for same day sequencing

Agilent Catalog Arrays

Product overview-Array formats

SurePrint HD Arrays



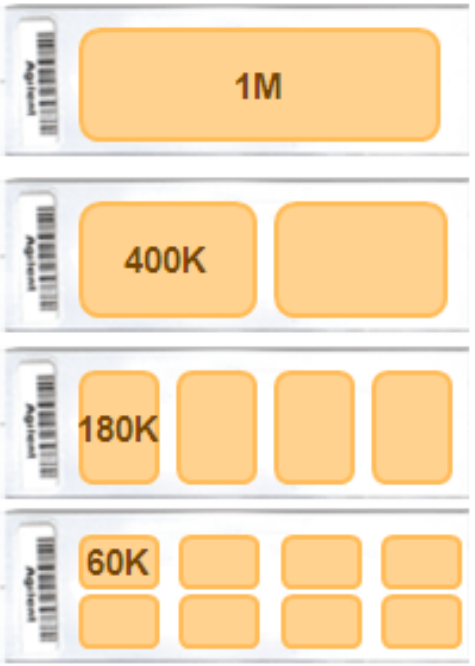


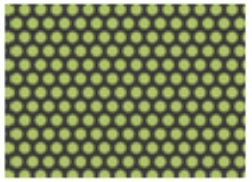
65 µm features

Compatible with:

➢Agilent B or C Scanner

SurePrint G3 Arrays



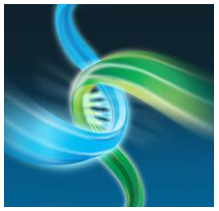


30 µm features

Compatible with:

➢Agilent C Scanner

Easily Create Your Custom Design

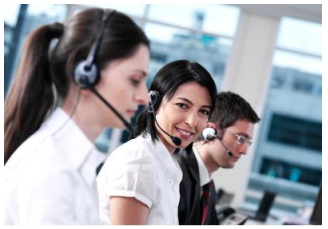


Any project

Streamlined
Design Software

eArray &
SureDesign
Free Web Portal

Genomic Coordinates
Genes names
Regions you want to target



Design Services
For Complex Designs

Your Own Custom
Agilent Genomic Kit
Specific of your project
Dedicated to your needs

Array Comparative Genomic Hybridization (CGH)

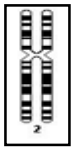
- Microarray-based comparative genomic hybridization (array CGH) is a powerful method for the genome wide detection of chromosome copy number changes at a higher resolution level than conventional chromosome-based CGH

- Based on the co-hybridisation of differentially labelled test and reference DNA onto arrays of oligonucleotide DNA

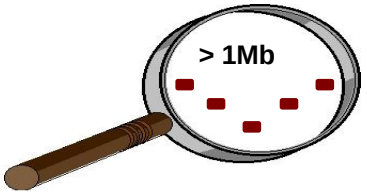
- Loss or gain in the test DNA can be indicated from spots showing aberrant signal intensity ratios

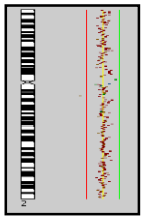
Platforms for genomic copy number analysis

Chromosome

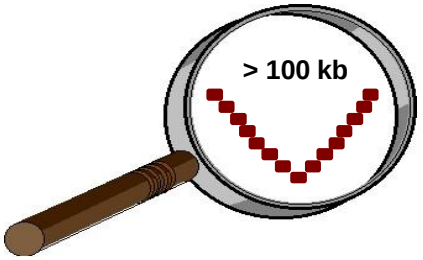


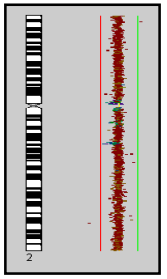
Conventional chromosome-based CGH



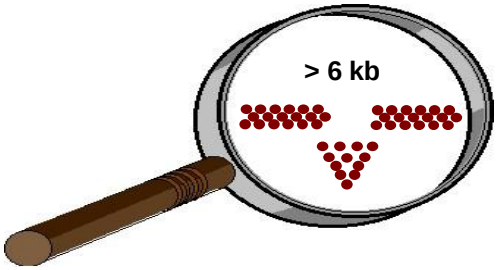


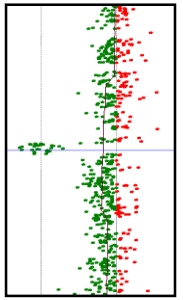
1 Mb BAC array





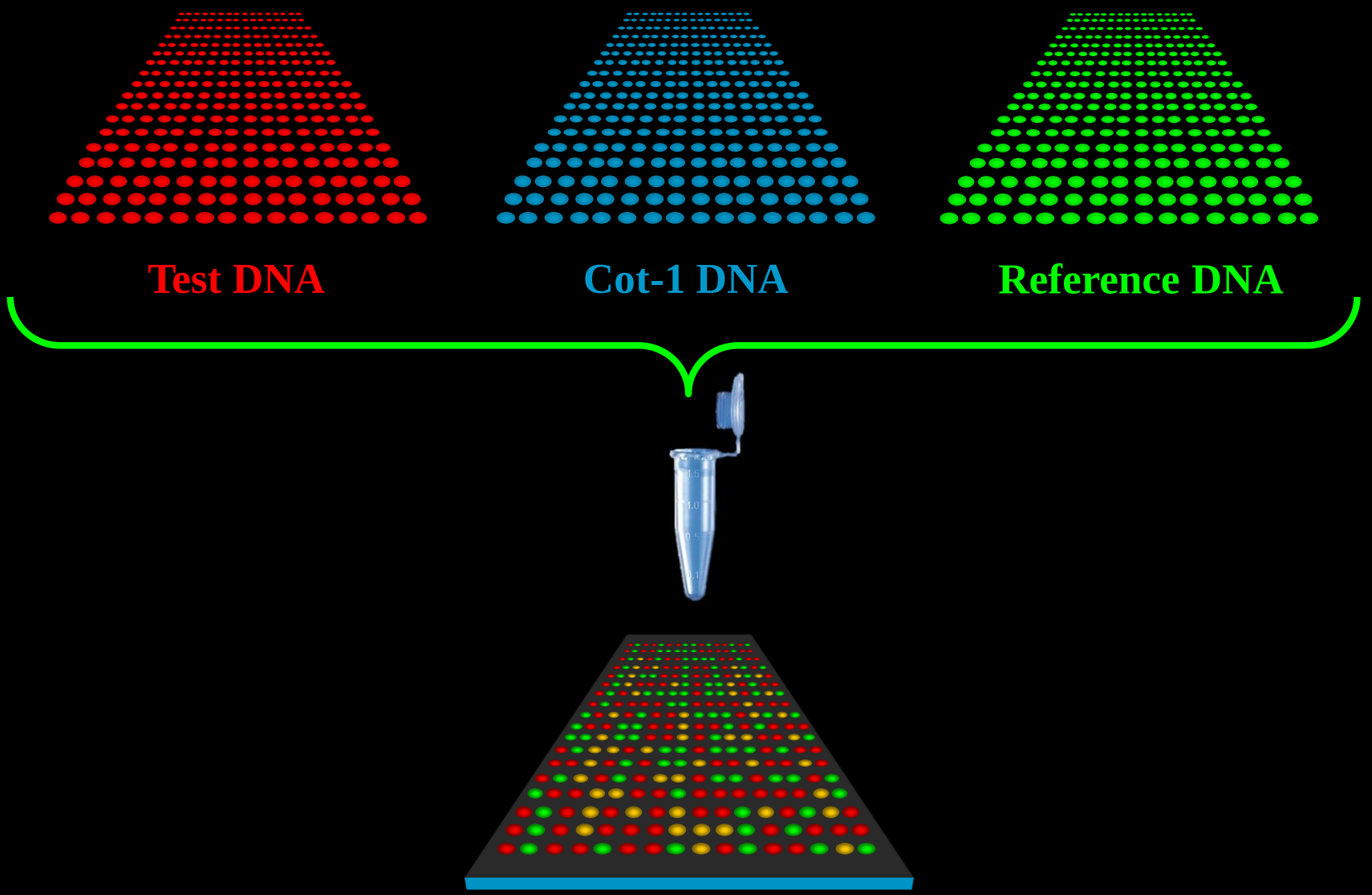
36K Tiling path BAC array



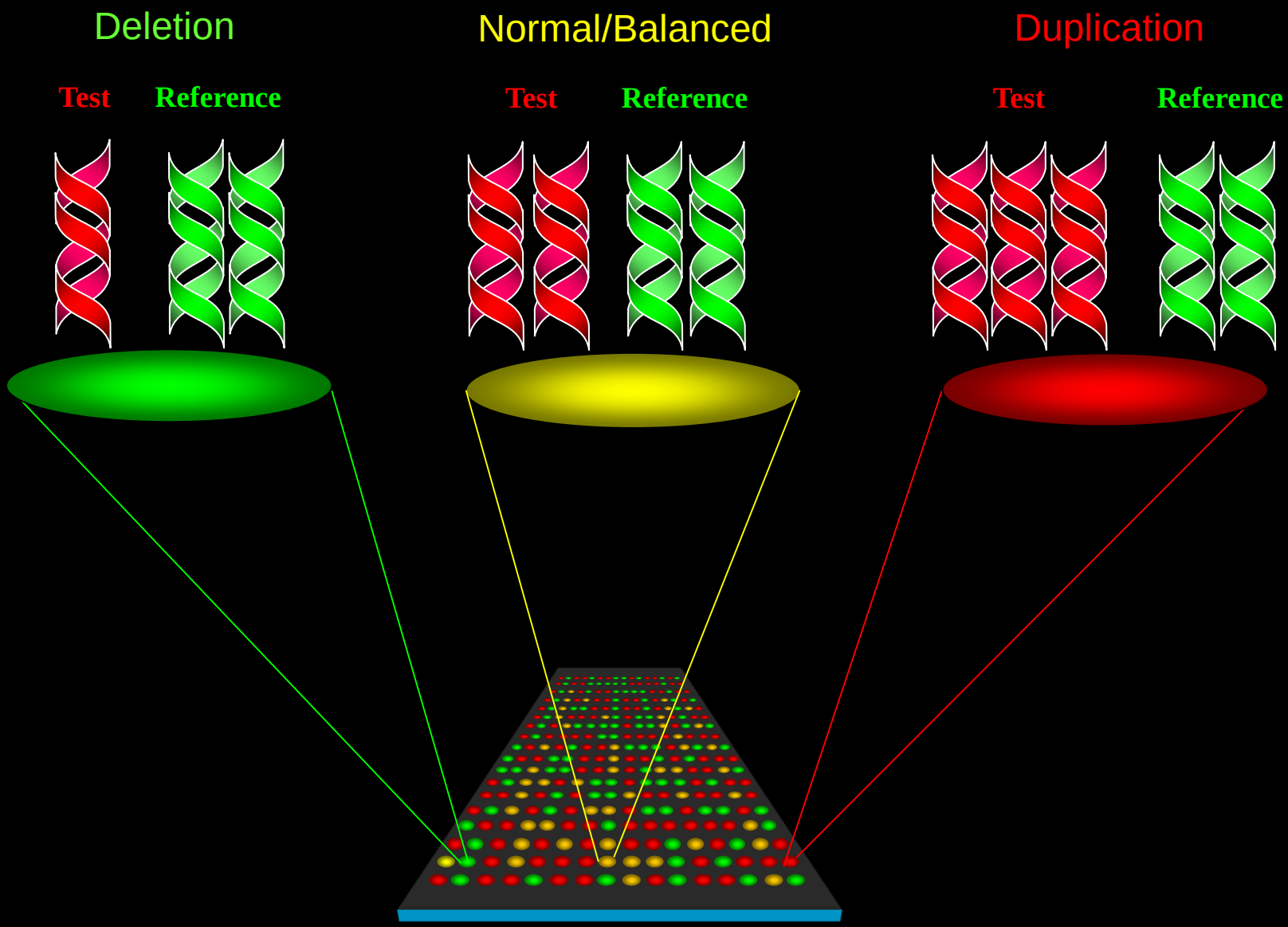


Agilent Oligonucleotide array

Probe generation and array CGH hybridization



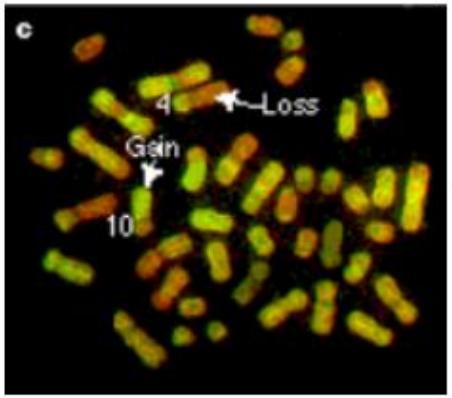
Microarray scanning

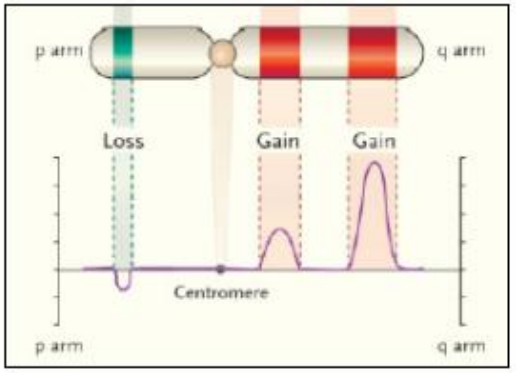


Measuring copy number changes

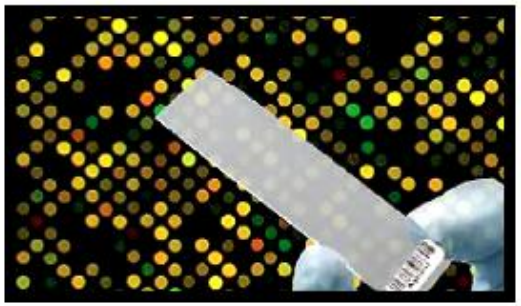
Virtual karyotypes have ~1000-fold greater resolution than conventional cytogenetics.

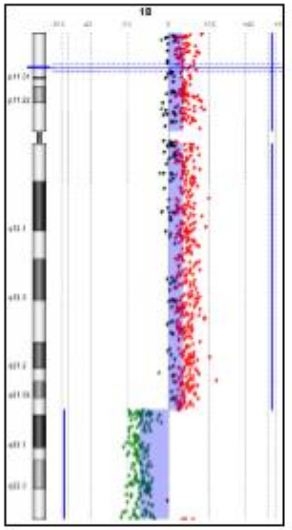
Conventional CGH: Metaphase Spreads



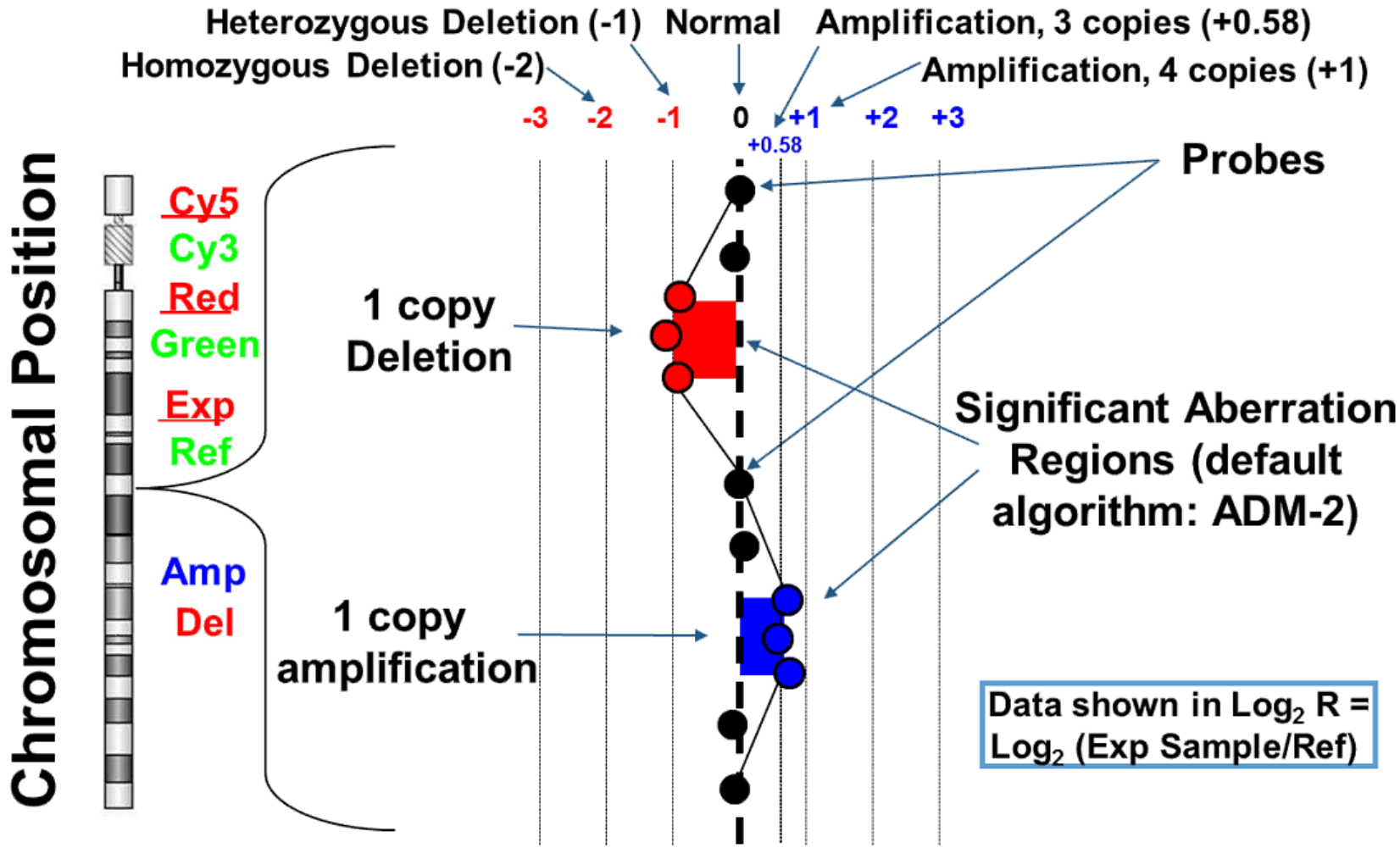


Array CGH: Agilent SurePrint G3 CGH+SNP microarrays



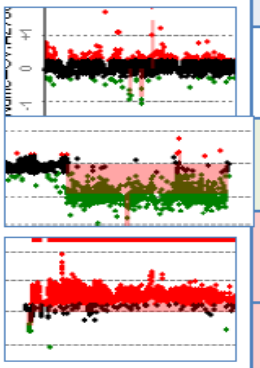


Schematic view of CGH Data Output



Overview of 2-color CGH "math"

We measure the difference between 1 sample and 1 reference DNA, usually expressed as log(2) (Sample/reference). Actual data are slightly compressed from ideal values.



Description	Average Sample CN	Ref CN	Ratio (S/R)	Ideal Log2(Ratio)	Actual data
Diploid	**2**	**2**	1	0	0
Deletion	**1**	**2**	0.5	-1	-0.9
Trisomy	**3**	**2**	1.5	+0.58	+0.53
Amplification	**4**	**2**	2	+1	+0.87
50% mosaic deletion	**1.5**	**2**	0.75	-0.41	-0.37
50% mosaic trisomy	**2.5**	**2**	1.25	+0.32	+0.29
20% mosaic deletion	**1.8**	**2**	0.9	-0.15	-0.13
20% mosaic trisomy	**2.2**	**2**	1.1	+0.14	-0.12

Agilent Catalog Arrays

CGH microarray portfolio (Human)

Array	Part Number	Format	Content	Information
Human CGH	G4447A G4448A G4449A G4450A	1M 400K 180K 60K	Comprehensive probe coverage with an empahsis on known genes, promoters, miRNA ad pseudoautosomal and telomreric regions	• SurePrint G3. • Also available in Bundle with SureTag Kit
	G4411B G4412A G4413A	244K 105K 44K	Old legacy designs	• HD
Catalog CNV	G4506A G4507A G4417A	1M 400K 105K	Designed to study the estimated 0.9-1.3% |normal| difference in copy number in the genomes of unrelated people	• Based on different Human projects
ISCA_v2	G4826A-031748 G4827A-031746 G4425B-031750 G4426B-031747	180K 60K 105K 44K	ISCA regions	• Made to order • Also available in Bundle with SureTag Kit

Agilent Catalog Arrays

CGH microarray portfolio (Other)

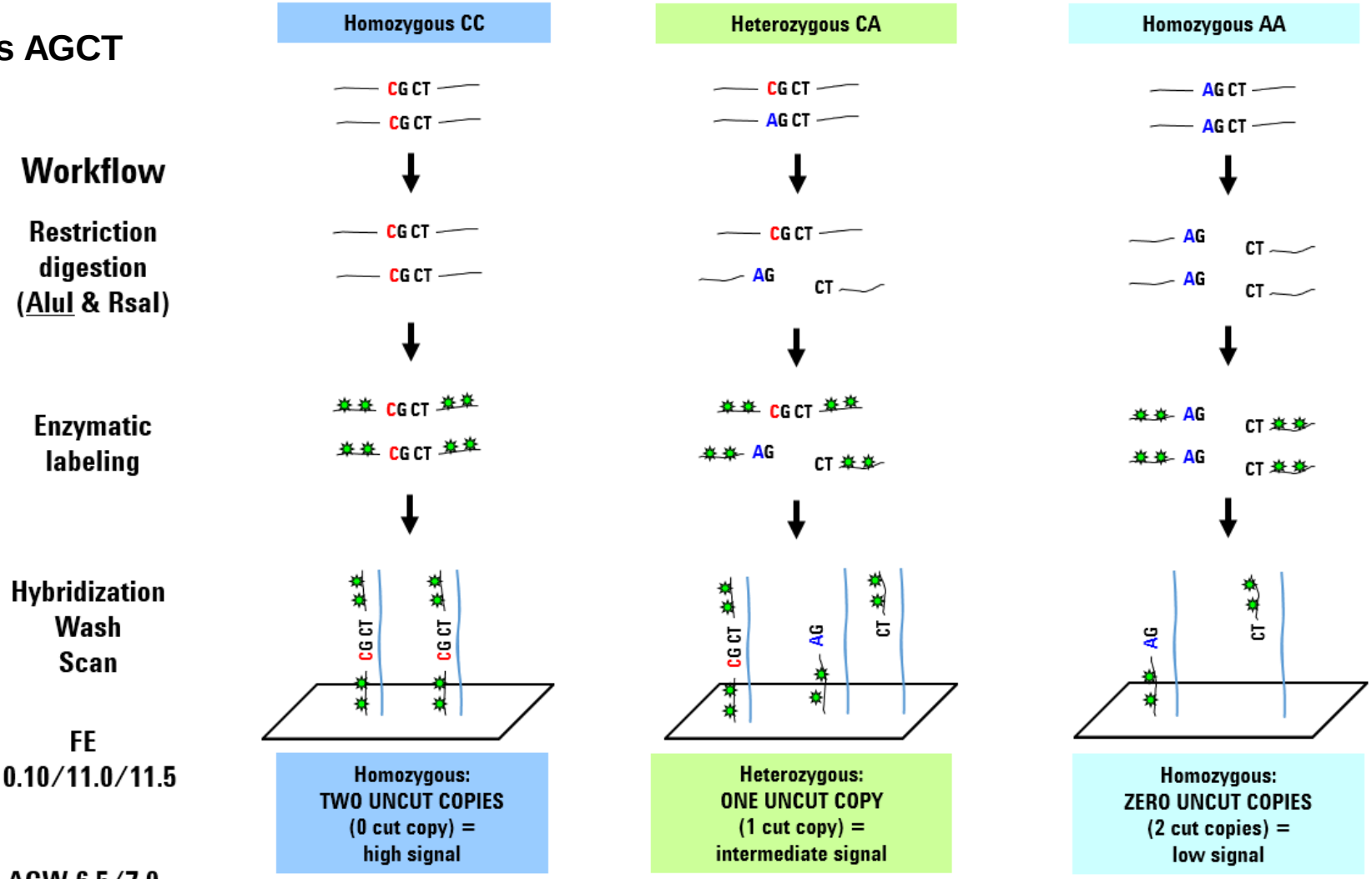
Array	Part Number	Format	Species	Content
Model Organism	G4838A G4938A G4415A G4416A	1M 180K 244K 105K	Mouse	High density coverage of coding and non-coding regions with emphasis on known genes
	G4840A G4841A G4435A G4436A	1M 180K 244K 105K	Rat	
	G4826A-025242 G4826A-025522 G4816A-024419 G4816A-024422 G4826A-025843 G4423B-019553	180K 180K 180K 180K 180K 244K	Bovine Canine Rhesus mcq Chimpanzee Rice Chicken	
Custom CGH	G4123A G4124A G4125A G4126A G4423A G4425A G4426A G4427A	1M 400K 180K 60K 244K 105K 44K 15K		

Principle of CGH+SNP
Copy Number and SNP Measurements

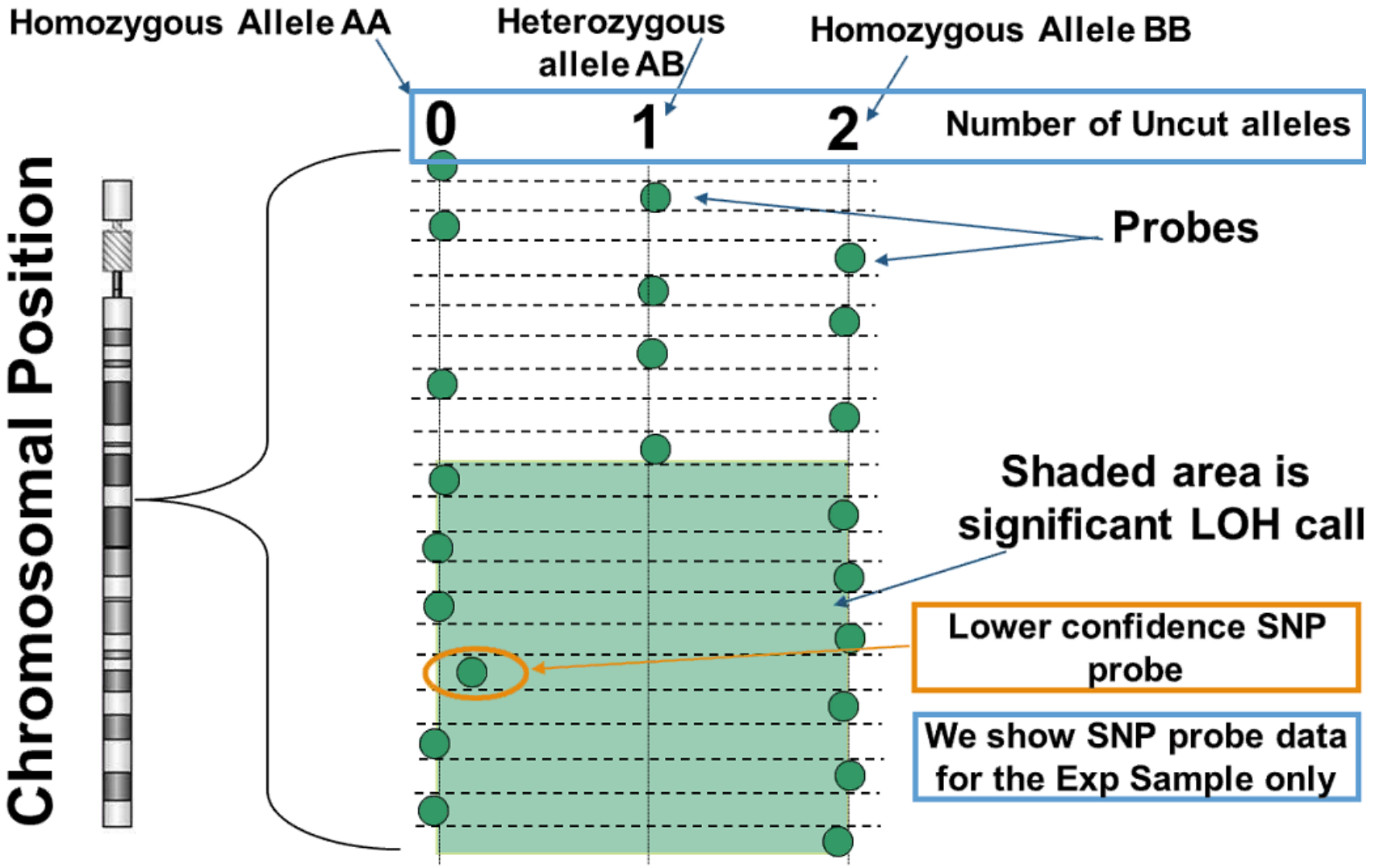
- A subset of probes on a CGH array measures SNPs, in parallel CGH probes measure copy number, **on a single array**
  - Detection of copy neutral aberrations such as LOH and UPD
  - ~5–10 Mb resolution for LOH/UPD detection across the entire genome
- Genotype SNPs using restriction digestion (Alu I/Rsa I)
- We measure the copy number of one allele at each SNP site relative to a known reference
- Regions of LOH are located by finding genomic regions with a statistically significant lack of heterozygous calls
- Scan on Agilent C-scanner/SureScan and analyze data in CytoGenomics 2.7

How it works!

Alu I & Rsa I cuts AGCT

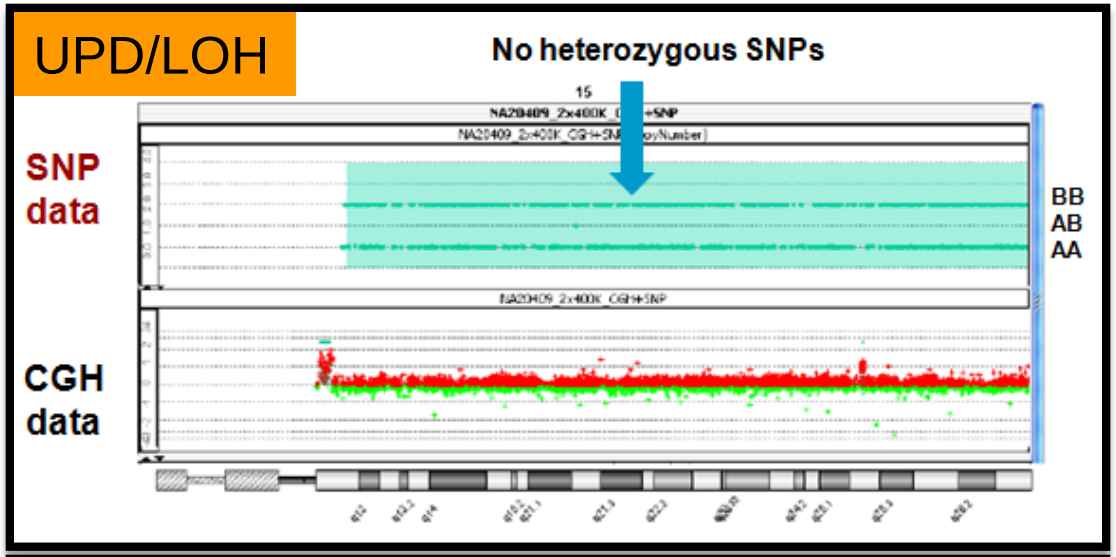


Principle of CGH+SNP and SNP Measurements

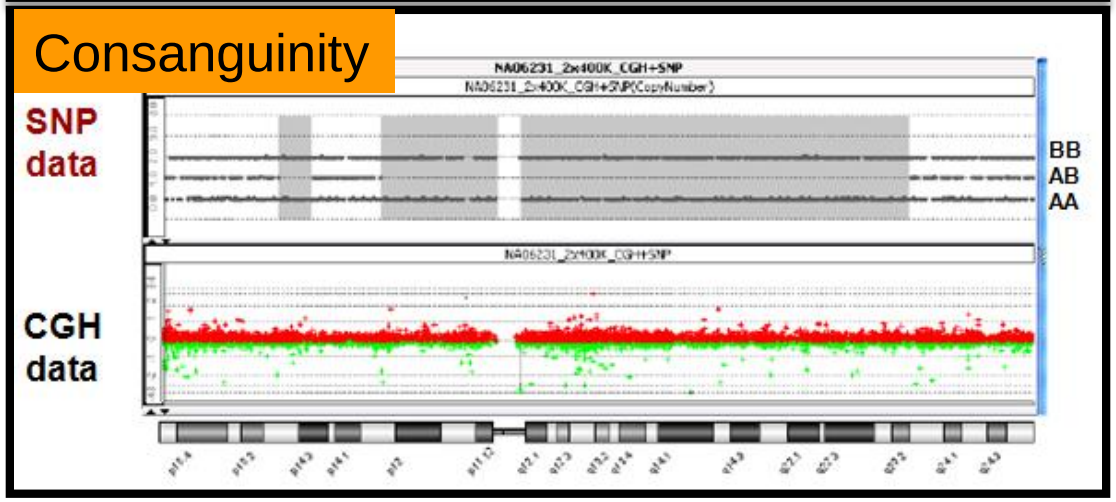


CGH+SNP: Copy Number and LOH/UPD in constitutional samples

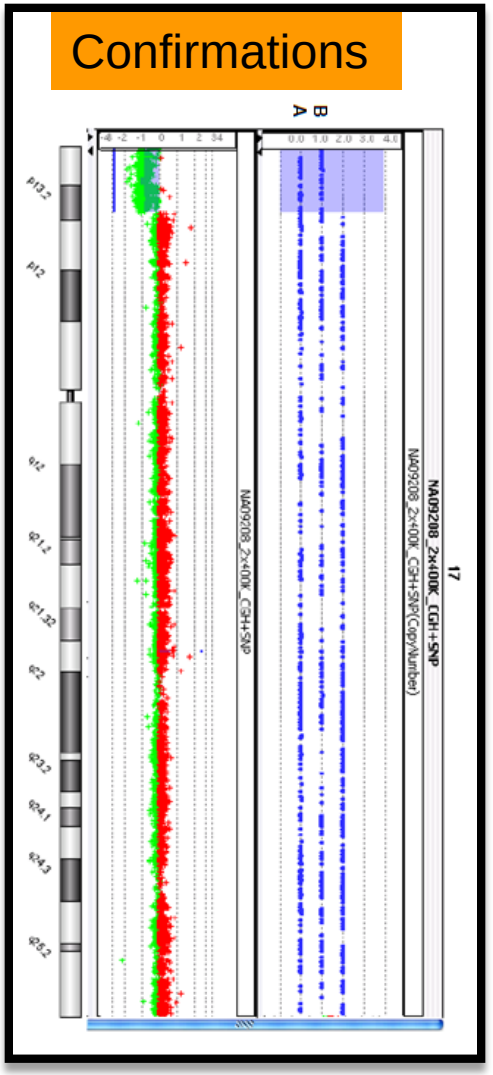
UPD/LOH



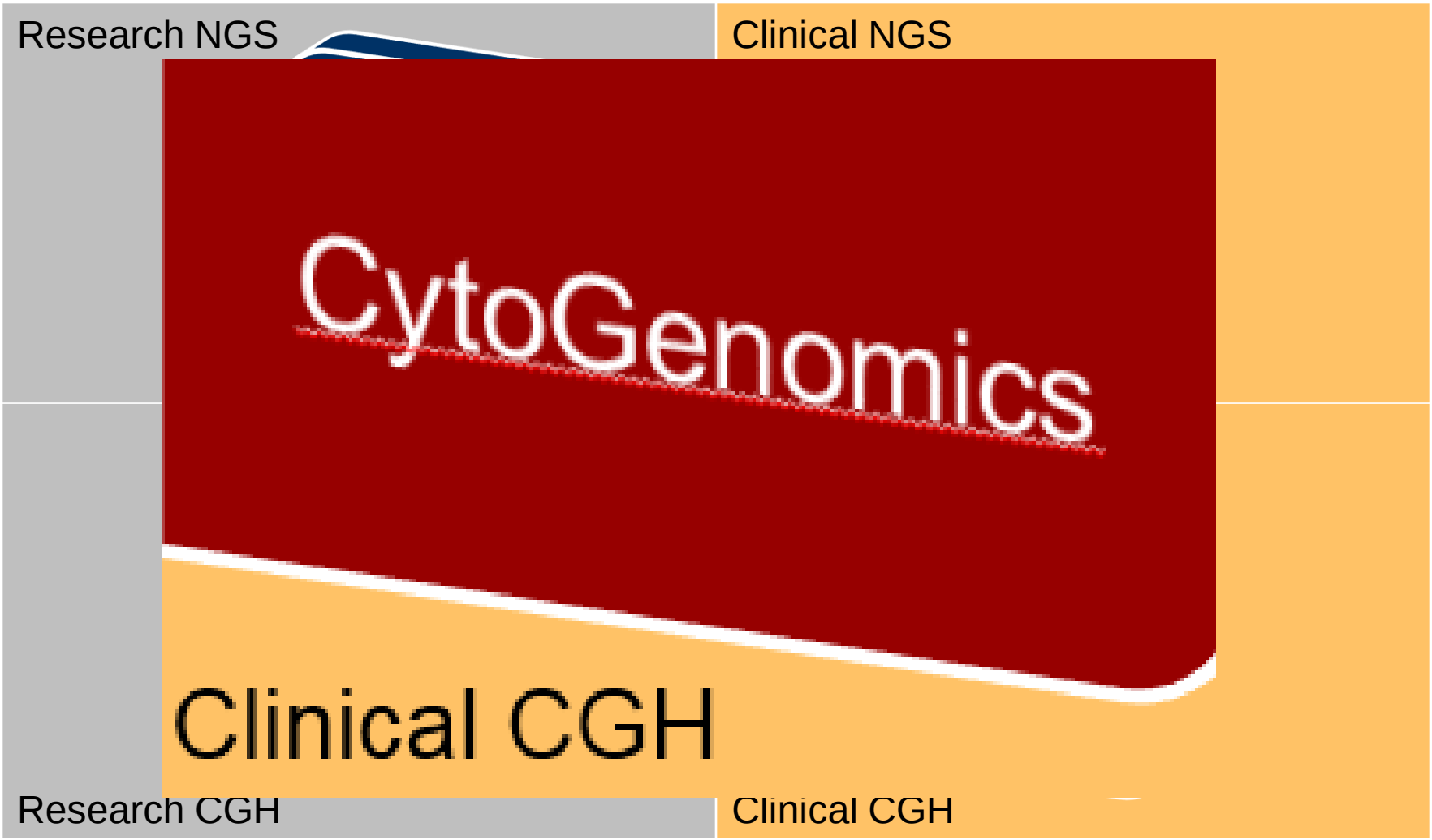
Consanguinity



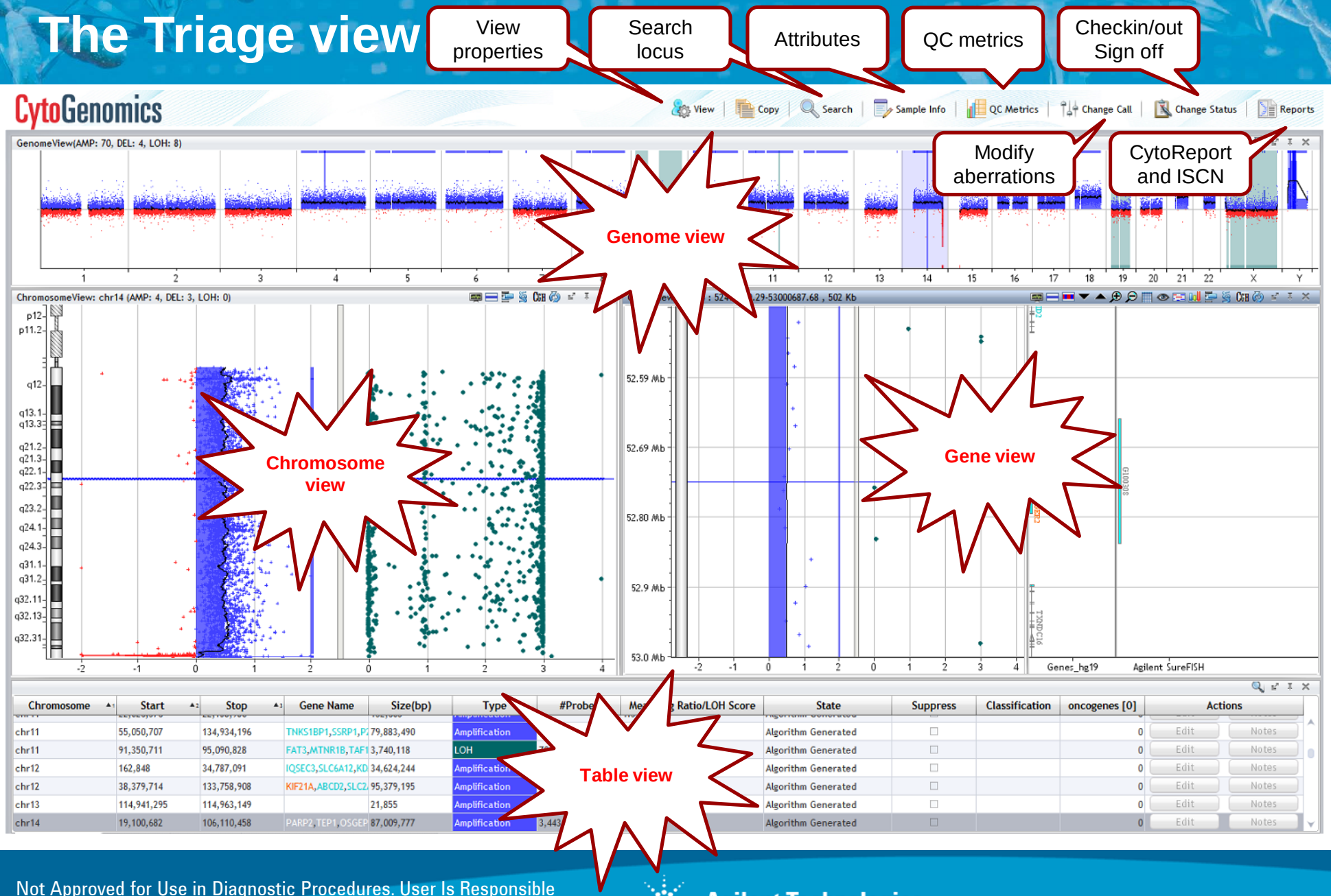
Confirmations



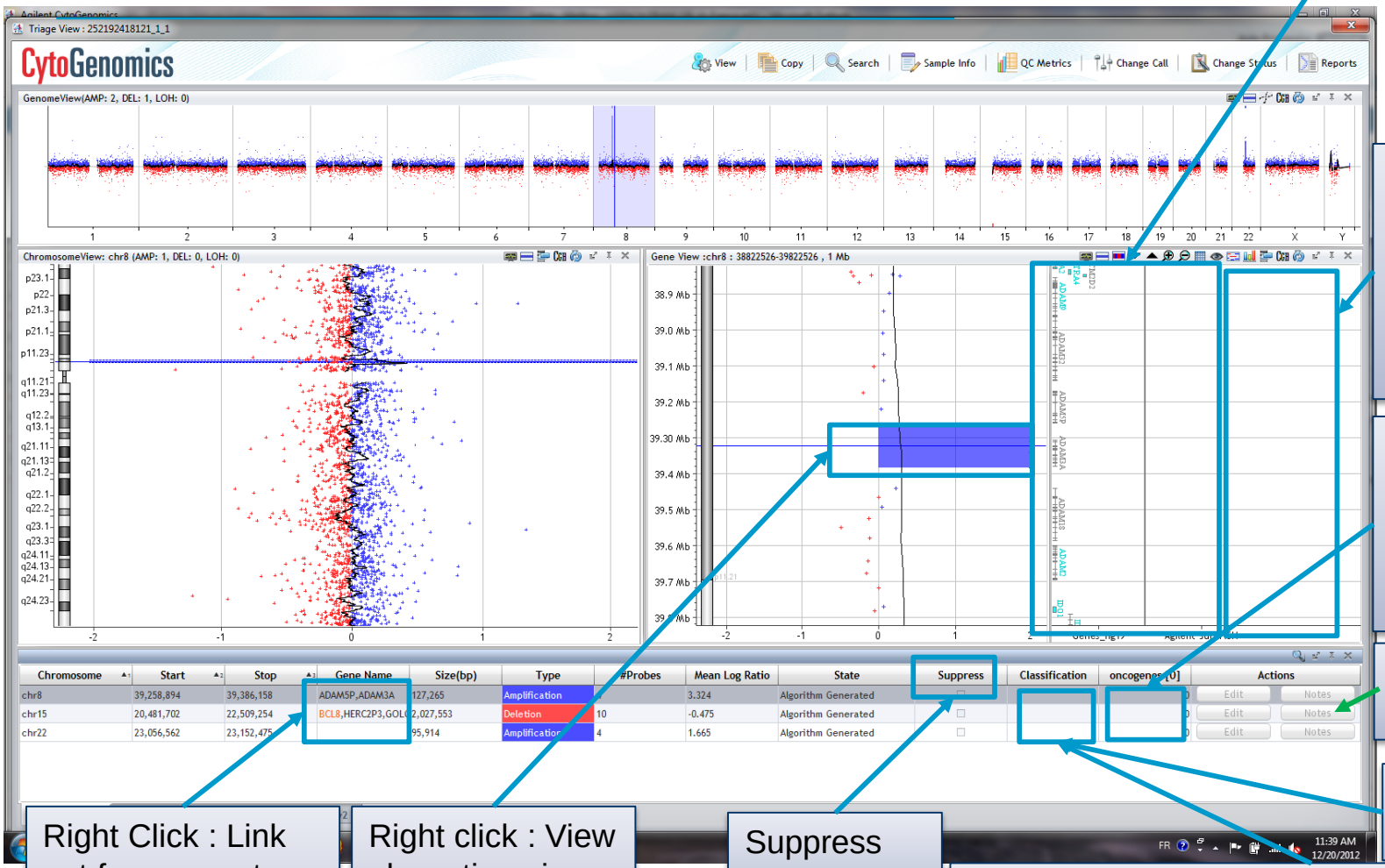
Agilent's software offering



The Triage view



The Triage view



Preloaded tracks: eg DGV-CNV

Auto updated tracks based on aberration classification in CytoGenomics Database

Showing number of samples with similar aberrations in database

Add notes to aberrations

Classify aberrations

Right Click : Link out from gene to external sources: DGV, OMIM, Entrez

Right click : View aberrations in UCSC genome browser, DGV

Suppress aberrations

Right click on Classification allows query for overlapping aberrations in database: results will be shown as new track

Technical Considerations:
Factors Influencing the Success of CGH

Aberration size:	**Large**	→		**Small**
Aberration Type:	**Large copy number change**	→		**Single copy change**
Composition:	**Homogeneous**	→		**Heterogeneous (normal cells)**
Material	**Lots**	→		**Small primary tumor**
Specimen:	**Blood/Cell Line**	→	**Fresh/Frozen tissue**	→ **FFPE**

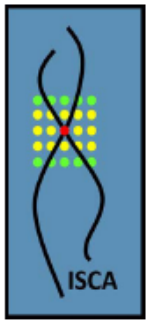
Adapted from Nat Genet. 2005 Jun; 37 Suppl:S11-7

ISCA Consortium
(International Standards for Cytogenomic Arrays)

- Established in 2007 and now includes >145 clinical laboratories worldwide
- The goals of the ISCA Consortium include:
  - standardization for genotype and phenotype data
  - guidelines for data interpretation
  - publicly available databases through NCBI
- Goal 200,000 cases in 2 years

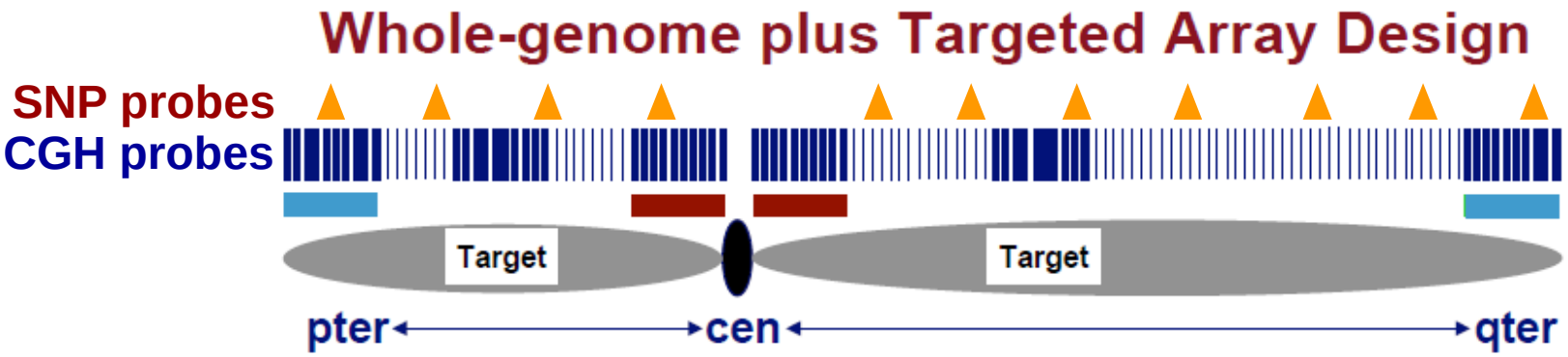
~100% Agilent data

http://iscaconsortium.org/



Slide content downloaded from ISCA website

Agilent Catalog ISCA CGH+SNP Microarray



	Resolution
Telomere FISH clone	
Unique centromere FISH clone	~20 kb
Known clinically relevant targets	
~25 kb interval backbone	~100 kb
60K SNP probes	~5-10 mb LOH

4x180K SurePrint G3 CGH+SNP kit P/N G4890A

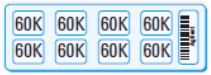
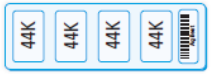
Choose Your Catalog ISCA Microarray

Use the ISCA microarray to discover copy number and copy-neutral genetic variations

CGH+SNP

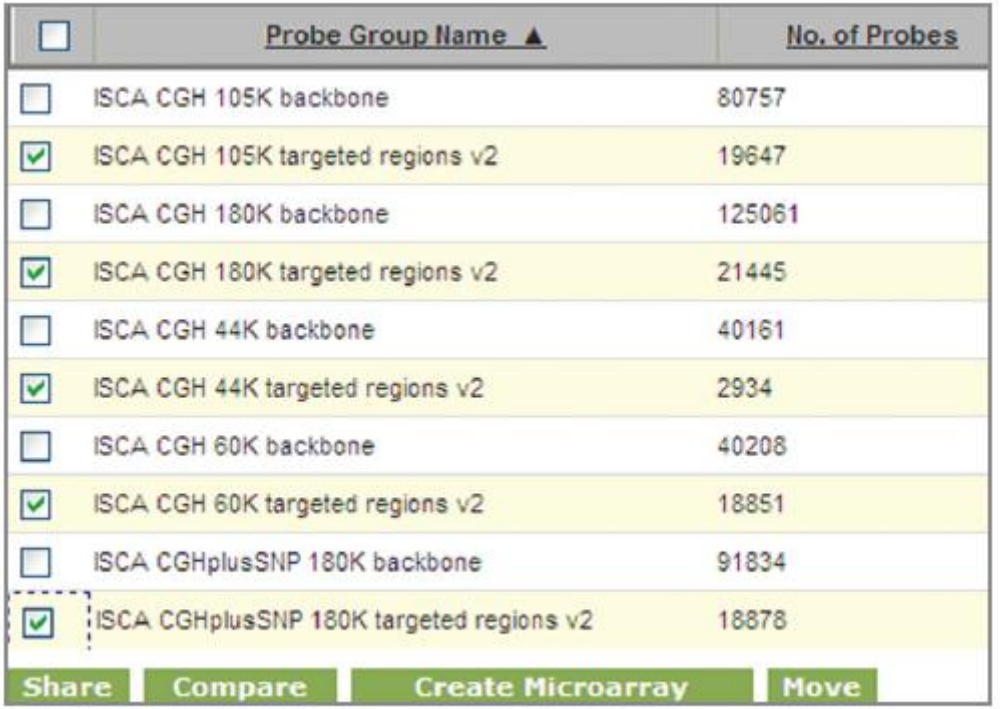
Description	Part Number	Number of targeted ISCA regions	Backbone probe density	LOH/UPD resolution	
CGH+SNP 4x180K	G4890A (kit of 3 slides)	~500	25 Kb	5-10 Mb	

CGH-only

Description	Part Number	Number of targeted ISCA regions	Backbone probe density	LOH/UPD resolution	
ISCA 4x180K v2	G4826A AMADID 031748	~500	25 Kb	NA	
ISCA 8x60K v2	G4827A AMADID 031746	~500	60 Kb	NA	
ISCA 2x105K v2	G4425B AMADID 031750	~500	35 Kb	NA	
ISCA 4x44K v2	G4426B AMADID 031747	~30	75 Kb	NA	

Design your Custom ISCA Microarray

Use the ISCA targeted regions probe groups and add your own content in eArray, Agilent's free, web-based tool to create custom array designs.



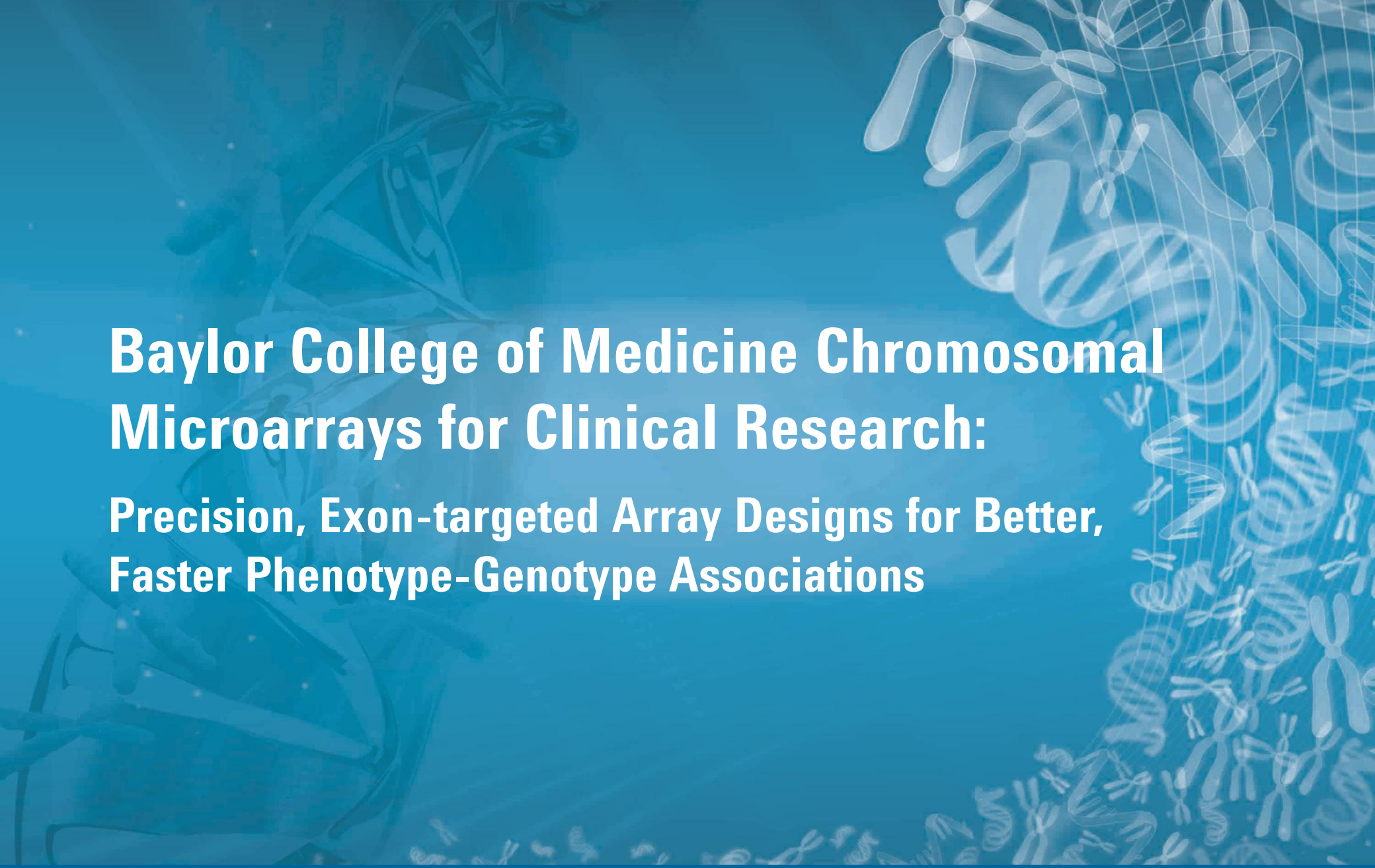
	Probe Group Name ▲	No. of Probes
☐	ISCA CGH 105K backbone	80757
☑	ISCA CGH 105K targeted regions v2	19647
☐	ISCA CGH 180K backbone	125061
☑	ISCA CGH 180K targeted regions v2	21445
☐	ISCA CGH 44K backbone	40161
☑	ISCA CGH 44K targeted regions v2	2934
☐	ISCA CGH 60K backbone	40208
☑	ISCA CGH 60K targeted regions v2	18851
☐	ISCA CGHplusSNP 180K backbone	91834
☑	ISCA CGHplusSNP 180K targeted regions v2	18878

Share | Compare | Create Microarray | Move

Visit: https://www.agilent.com/genomics/earray

Baylor College of Medicine Chromosomal Microarrays for Clinical Research:

Precision, Exon-targeted Array Designs for Better, Faster Phenotype-Genotype Associations



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Who is Baylor College of Medicine?

Baylor College of Medicine®

"The NIH named Baylor College of Medicine #1 in Clinical Genetics."

Source: Opening remark at Dr. Marilyn Li's talk in Baylor session at AMP 2013

- A medical school and center for biomedical research and clinical care
- Located in the Texas Medical Center in Houston, Texas - the largest medical center in the world
- Affiliations with eight teaching hospitals
- Extensive laboratory testing as a service

Baylor College of Medicine Chromosomal Microarray Designs

Baylor College of Medicine Chromosomal Microarrays

PN	Long Description	# slides per kit	Price per Array	Agilent Kit price
G5956A	SurePrint G3 CGH+SNP Cancer Research Microarray Kit, 2x400K	5 slides	$ 526	$ 5,261
G5957A	SurePrint G3 CGH+SNP Postnatal Research Microarray Kit, 2x400K	5 slides	$ 526	$ 5,261
G5958A	SurePrint G3 CGH Postnatal Research Microarray Kit, 8x60K	3 slides	$ 179	$ 4,292
G5959A	SurePrint HD CGH Prenatal Research Microarray Kit, 2x105K	5 slides	$ 448	$ 4,484
G5960A	SurePrint G3 CGH+SNP Prenatal Research Microarray Kit, 4x180K	3 slides	$ 309	$ 3,703
G5961A	SurePrint G3 CGH Postnatal Research Microarray Kit, 4x180K	3 slides	$ 308	$ 3,699

Premium Content and Design Differences

Part number	Agilent name	Hg18 or Hg19	# of genes	# SNPs	# Disease associated miRNA	Mitochondrial genome	Backbone Probe Spacing	Description
G5956A	SurePrint G3 CGH + SNP Cancer Research Array 2x400K	19	2,300 genes	60 K	235	no	12 KB	2,300 exon by exon, cancer focused with genes with known cancer association. Sanger consensus list included. Expansion of CCMC with Baylor exonic deletions and duplications 6 probes per exon.
G5957A	SurePrint G3 CGH + SNP Postnatal Research Microarray 2x400 K	19	1,700 genes, exon X exon	60 K, w/ 5 MB AOH resolution	755	yes	30 KB	Intense disease-region coverage with all exons and transcripts covered. Enriched with relevant peri-centromeric, subtelomeric and intragenic disease regions.1,700 selected genes with exon-by-exon coverage with 6 probes per exon to detect more disease relevant aberrations.
G5961A	SurePrint G3 CGH Postnatal Research Microarray 4x180K	19	1,700 genes, exon X exon	No	755	yes	30 KB	The same as 57A but no SNPs.
G5958A	SurePrint G3 CGH Postnatal Research Microarray 8x60K	18	no	no	no	no	60 KB	Tiling for enriched density in disease-associated microdeletion, microduplication and pericentrometric regions
G5959A	SurePrint HD CGH Prenatal Research Microarray 2x105K	18	no	no	no	no	30 KB	Targeted coverage for disease-associated microdeletions, microduplications, pericentromeric, subtelomeric regions. Basic prenatal research design with most experience and least VOUS.
G5960A	SurePrint G3 CGH + SNP Prenatal Research Microarray 4x180K	19	22 genes, exon x exon	60 k w 5 MB AOH resolution	no	no	30 KB	Targeted coverage for disease-associated microdeletions, microduplications, pericentromeric, subtelomeric. Exon-by-exon for 22 known disease genes and SNPs for AOH and UPD. Expanded coverage compared to 105 K.

How does this content relate to CCMC and ISCA content?

- CCMC content is a subset of the Cancer Research 2X400 K
- Baylor post and prenatal designs cover much of what is on ISCA and more
  - Baylor design has better exon coverage and picks up some aberrations missed by ISCA

Baylor College of Medicine tracks for CytoGenomics

- Research and investigate underlying genetics of phenotypes:
  - developmental delay
  - intellectual disability
  - congenital anomalies
  - neuropsychiatric disorders
  - cardiac malformations
- Accurately contextualize aberrations using tracks generated from a database of >60,000 samples
- Enables quick, easy analysis and interpretation in CytoGenomics

Postnatal Research Arrays: Comprehensive, exon-by-exon, disease-specific content enables efficient association of phenotype with genotype

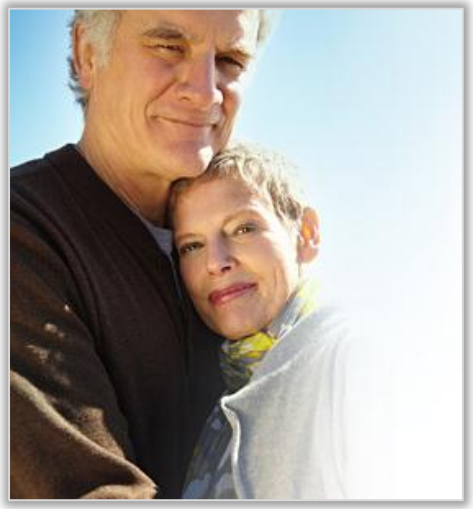
- Maximize detection of disease-specific aberrations
  - large number of aberrations, simultaneous
  - mosaicism down to 10%
  - sub-telomeric and pericentromeric deletions and duplications
- Minimize detection of variants of uncertain significance
  - i.e., systematic removal of probes in irrelevant regions
- Detect inherited and/or *de novo* CNVs and SNPs
- 3 formats for different detection sensitivity, throughput, and budgets

Prenatal Research Arrays: Content optimized for prenatal research

- Identify novel genomic imbalances that may correspond to birth defects or intellectual disability
- Basic design, thousands of cases run
  - maximize detection of disease-specific aberrations
  - detect disease-specific microdeletions and microduplications
  - not exon targeted, no SNPs to pick up fewer uncertain results
- Expanded version w/ SNPs and exonic coverage of 22 disease genes relevant for prenatal research detects:
  - absence of heterozygosity (AOH)
  - uniparental disomy (UPD)
  - consanguinity

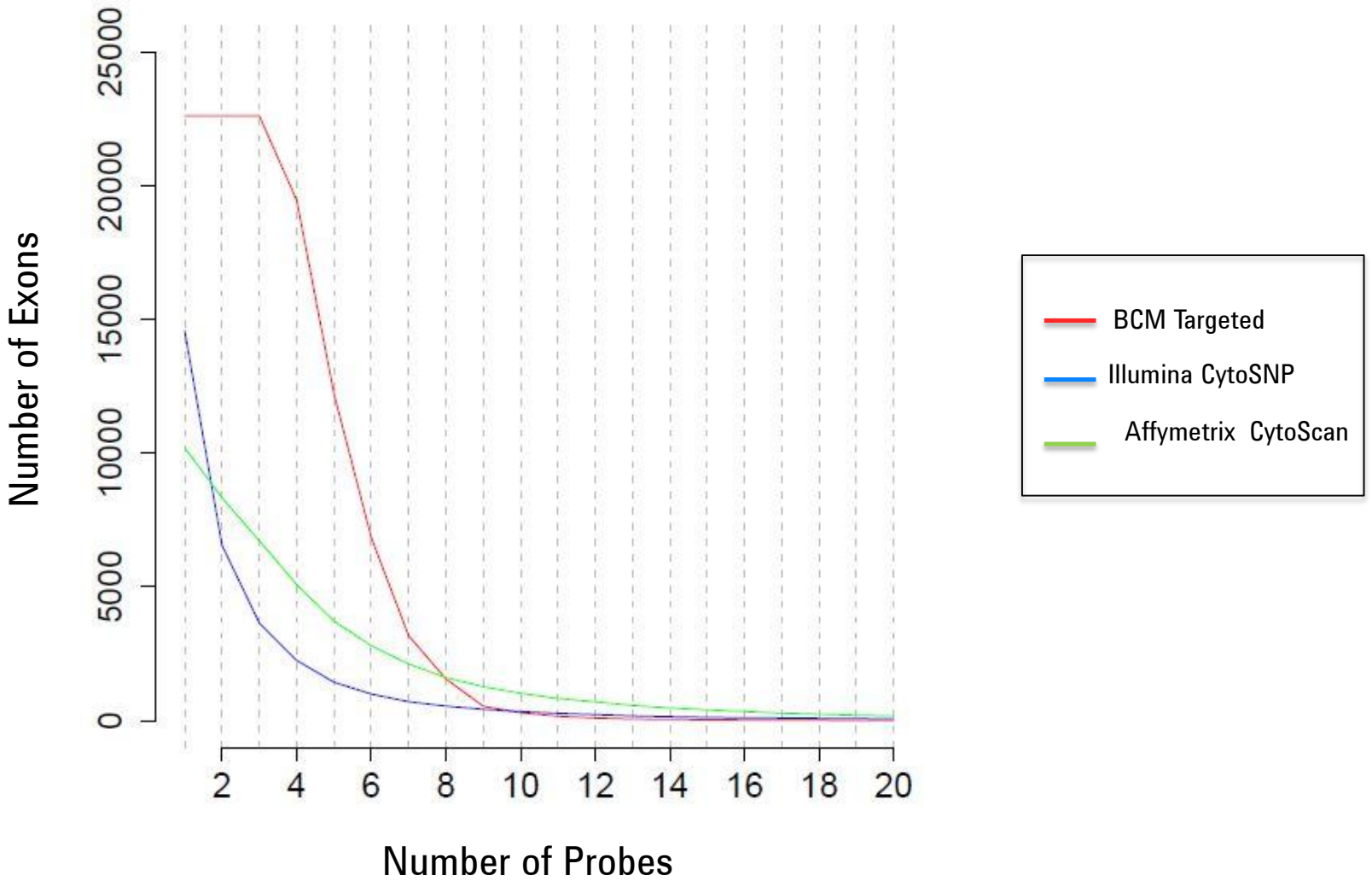
Cancer Research Array: Comprehensive, cancer-specific content

- Accurately pinpoint probable driver mutations and causal variants
- Compare tumor vs. normal samples in the same study subject (can't do with HD SNP technology)
- Characterize clonal heterogeneity and evolution
- Identify germline and somatic aberrations in solid tumor and hematological samples, including FFPE
- Combine with Agilent NGS TE, GEX and other technologies for deeper profiling and monitoring of heterogeneity

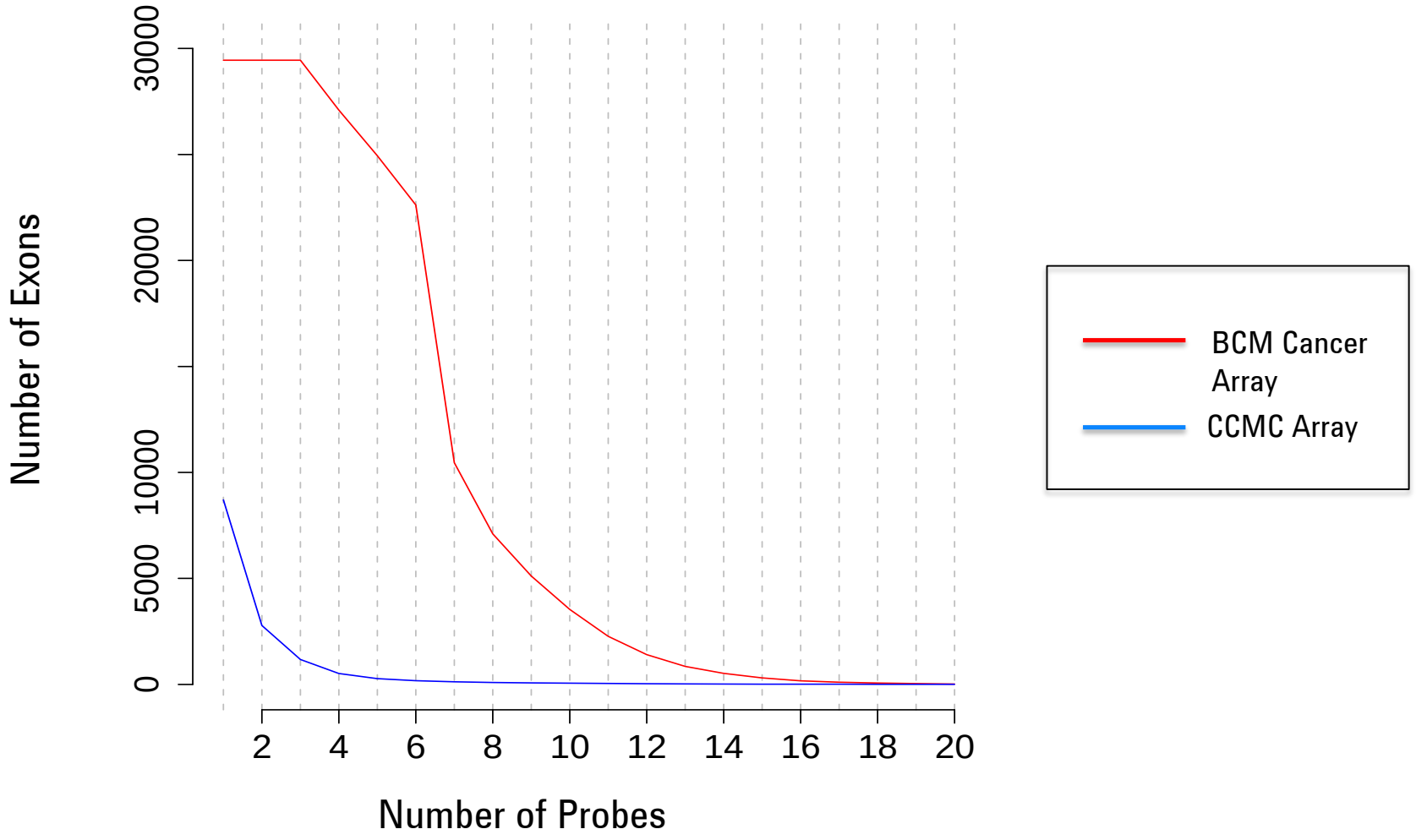


Coverage Comparisons

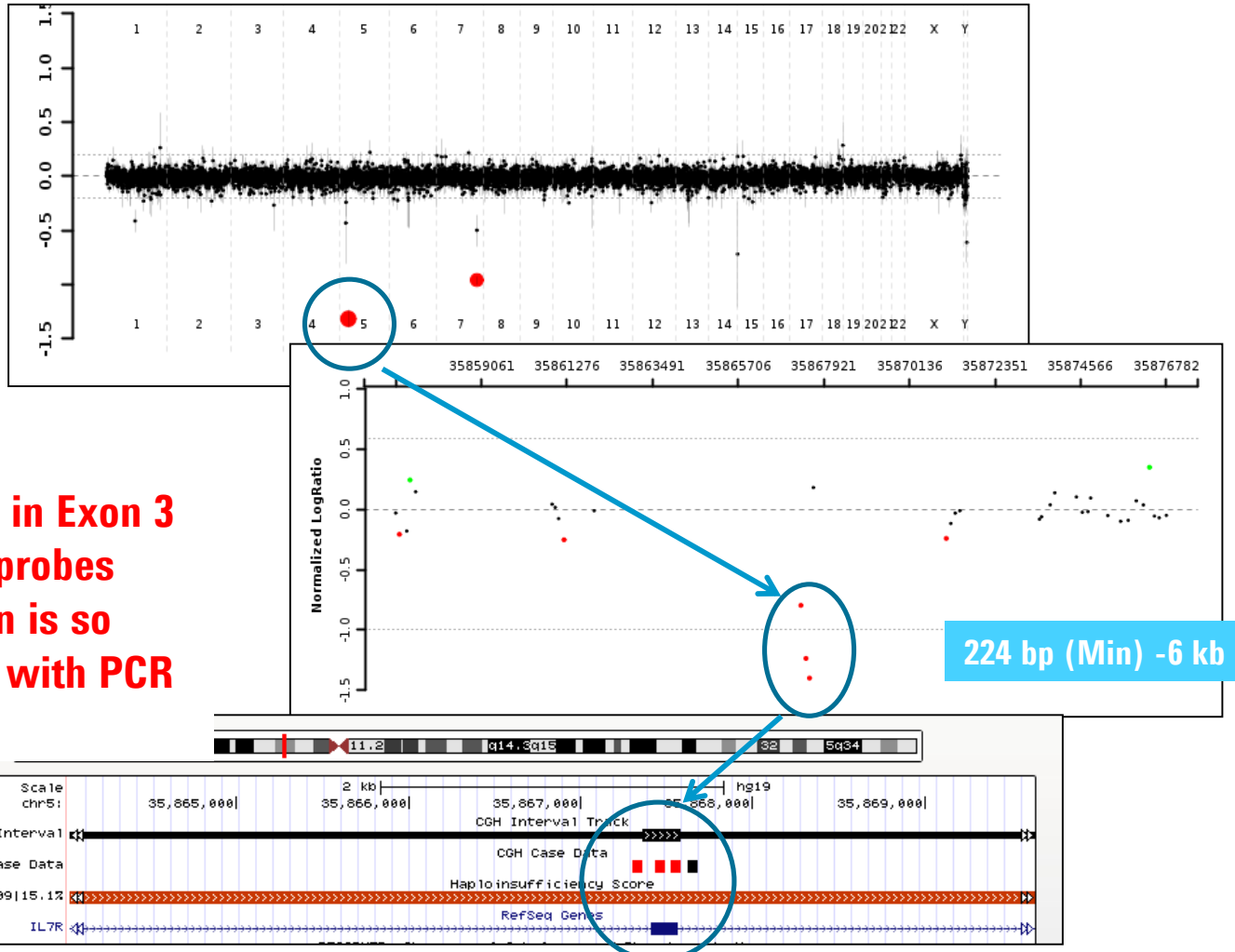
Coverage of Targeted Exons in Disease-associated Genes on Postnatal Research Array



Coverage of Cancer Research Array Targeted Exons



Disease Research Case Study: Severe Immunodeficiency is associated with small IL7R Exon 3 deletion



Very small deletion in Exon 3 found with 3 oligo probes (because the region is so small confirmation with PCR is recommended)

224 bp (Min) -6 kb (Max) loss

IL7R Exon 3

Take-Home Message: Use precision, exon-targeted array designs for better, faster phenotype-genotype associations

Baylor College of Medicine

Exon-targeted aCGH+SNP Arrays

- **Fit-for-purpose, genome-wide designs for specific applications-** precisely placed, dense oligo coverage in disease-relevant regions - highly conserved exons
- **Save time on interpretation** - Cytogenomics software with Baylor tracks (60 K cases) enable faster analysis and interpretation - fewer irrelevant results
- **Greater adaptability** – easy, inexpensive to remove irrelevant probes and add ones for new literature findings
- **Superior quality** - superior overall signal-to-noise ratio due to internal controls (2-color technology) and long probes

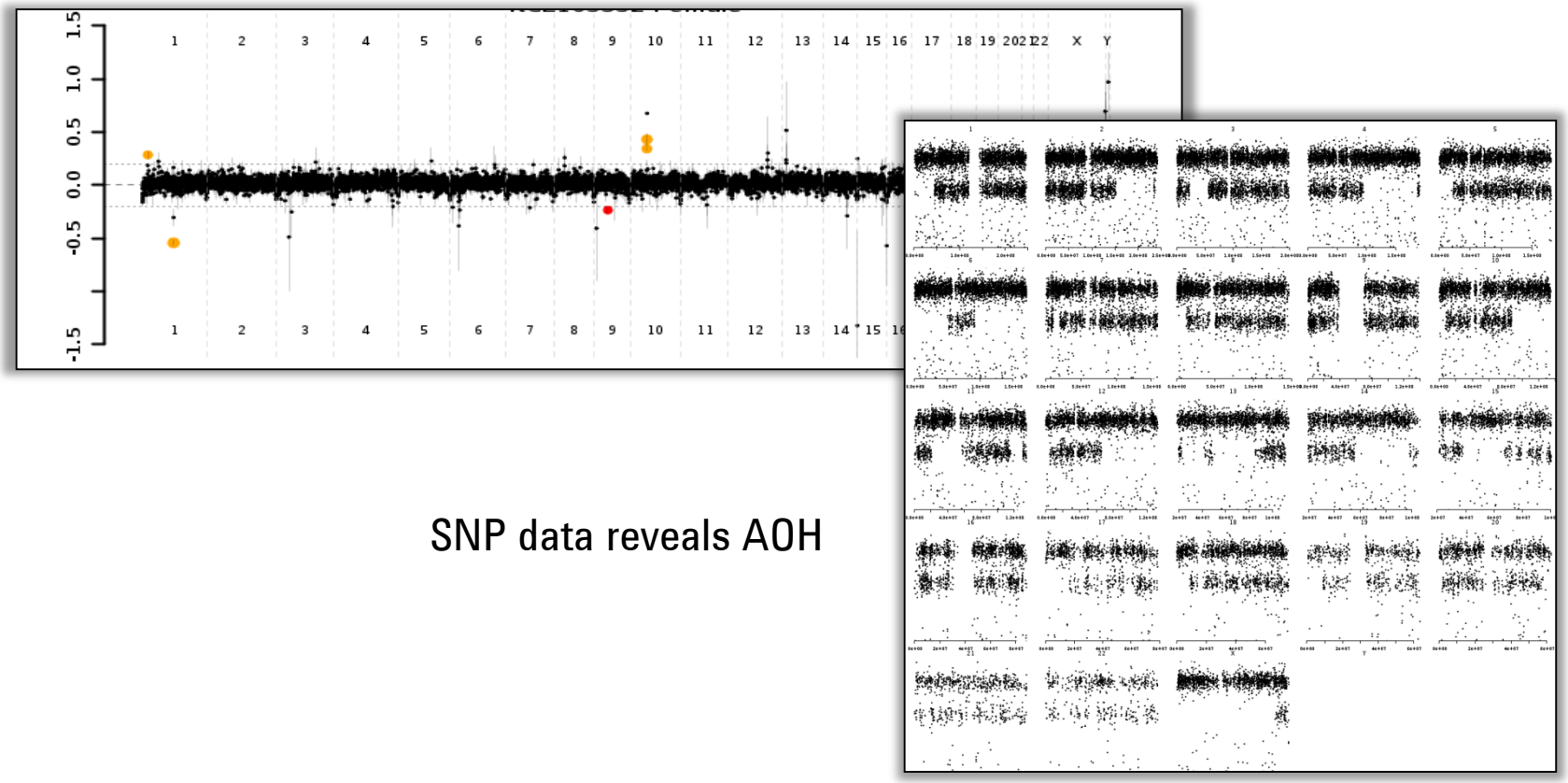
High-density SNP Arrays

- **One-size fits all -designed for genotyping, not copy number -** Detecting only naturally occurring SNPs limits coverage in conserved exons that matter most
- **More time to interpret -** more SNPs in irrelevant regions takes more time
- **Less flexibility -** Competitor arrays have less flexible, more costly manufacturing
- **More noise** - external control (one color), shorter oligos means more sources

Exon-by-exon Performance, Constitutional Research Case Studies

Consanguinity: Absence of heterozygosity (AOH) in close relative mating 180 K + SNP Microarray

Normal CMA profile



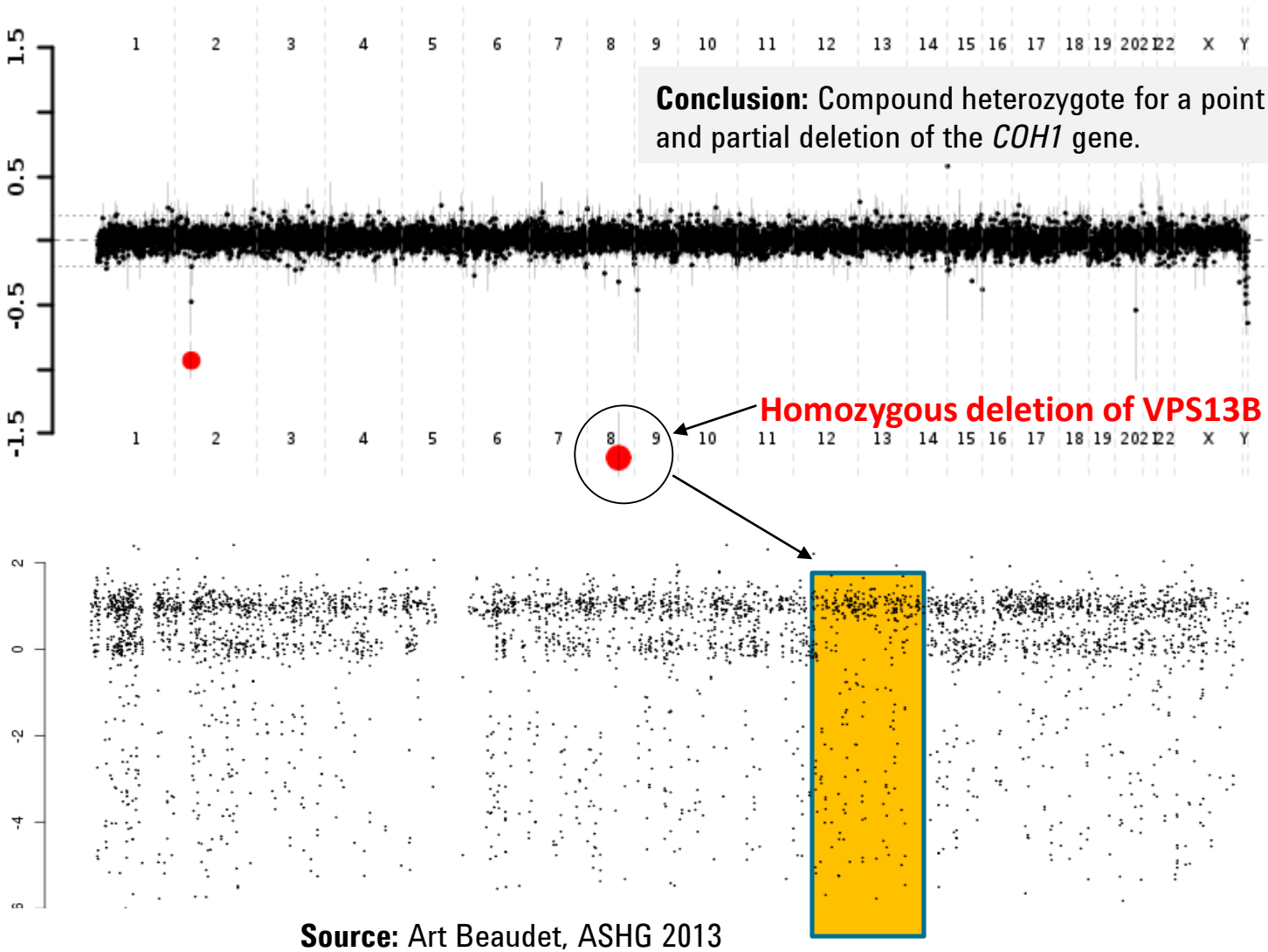
SNP data reveals AOH

Source: Art Beaudet, ASHG 2013

Autism Case Study 1: SNP data showing AOH region on chromosome 8 in *VSP13B* gene where phenotypically relevant homozygous deletion is also located

Genome-wide view

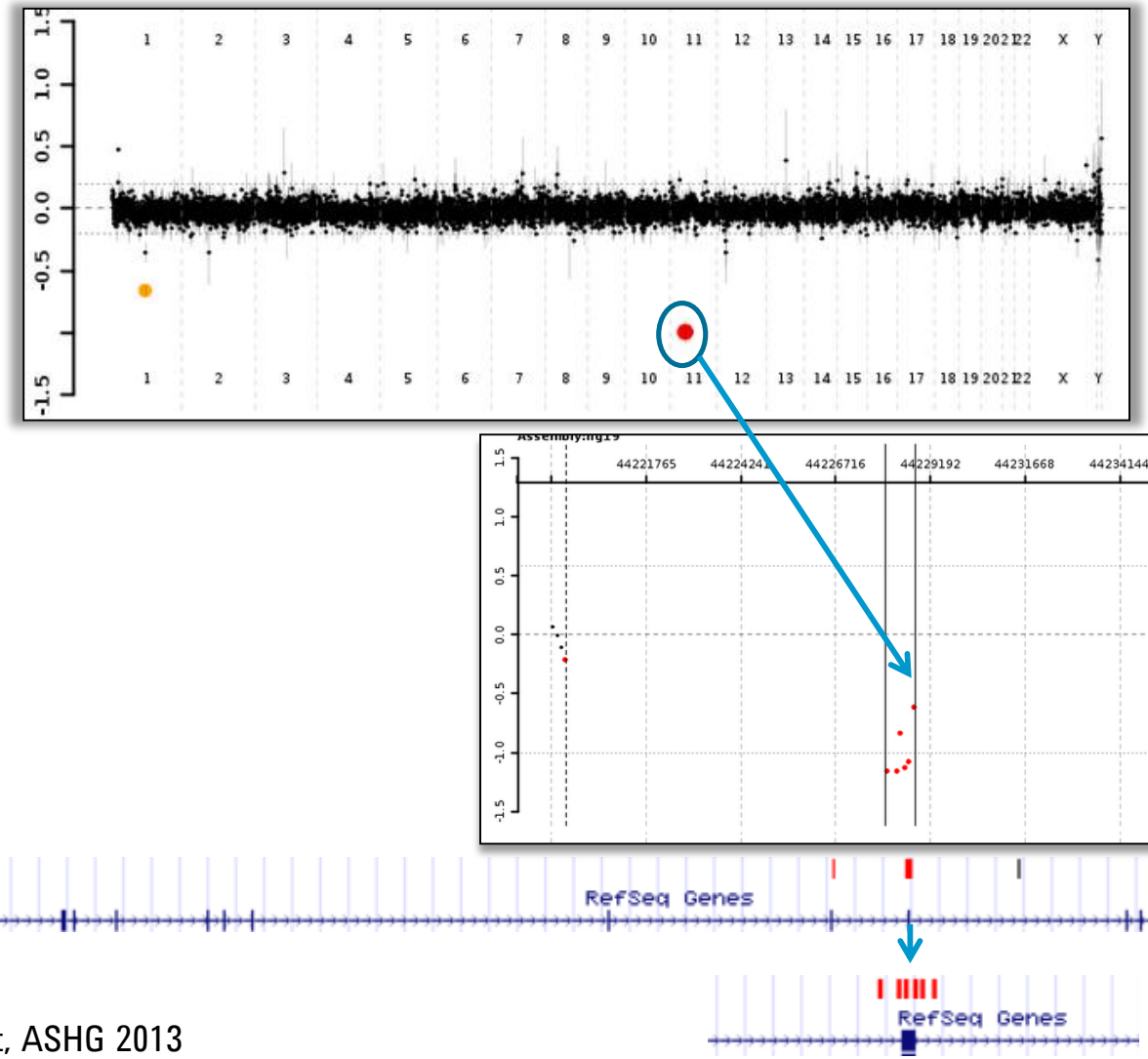
SNP Plot



Conclusion: Compound heterozygote for a point mutation and partial deletion of the *COH1* gene.

Source: Art Beaudet, ASHG 2013

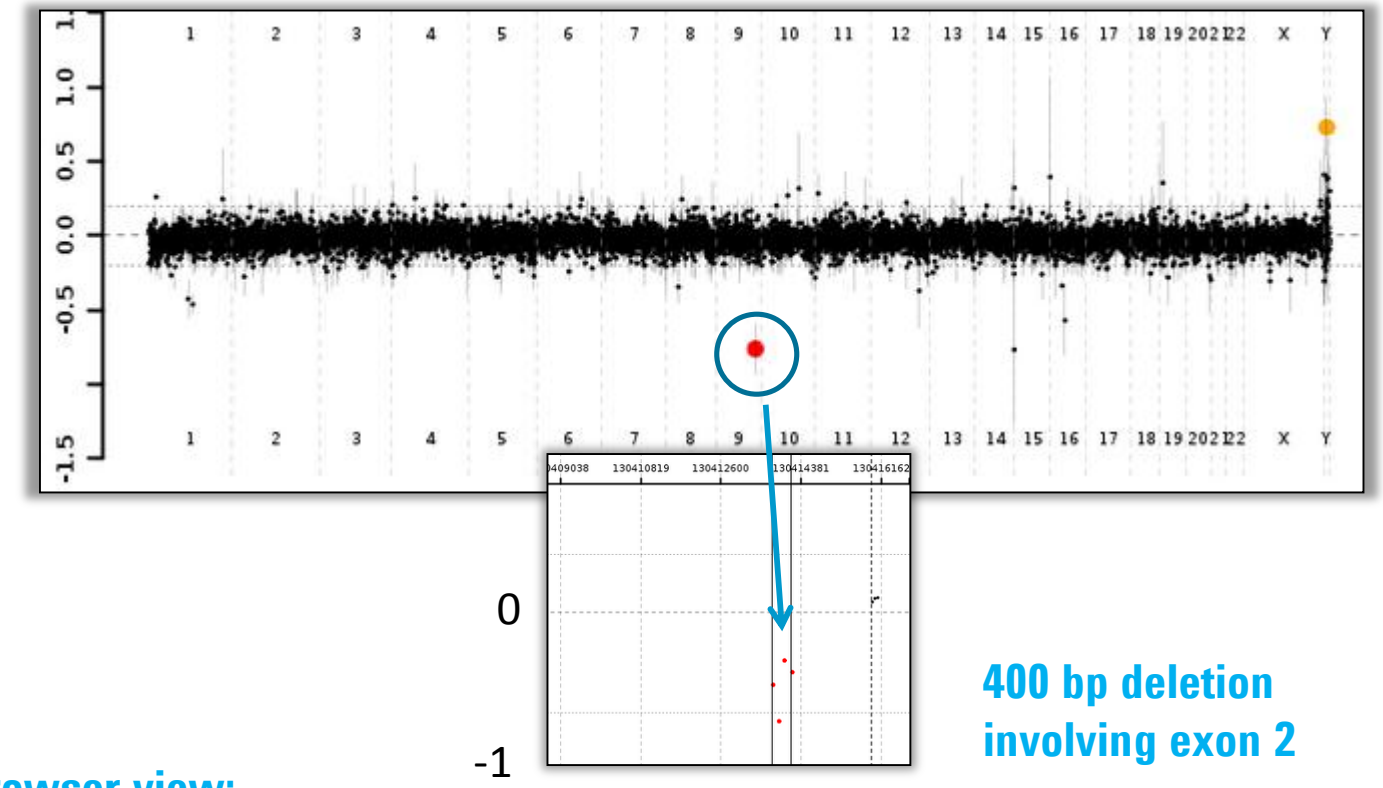
Disease Research Case Study: Deletion of exon 10 in the EXT2 gene demonstrates precise, exon-targeted coverage



Exon 10
800 bp deletion

Source: Art Beaudet, ASHG 2013

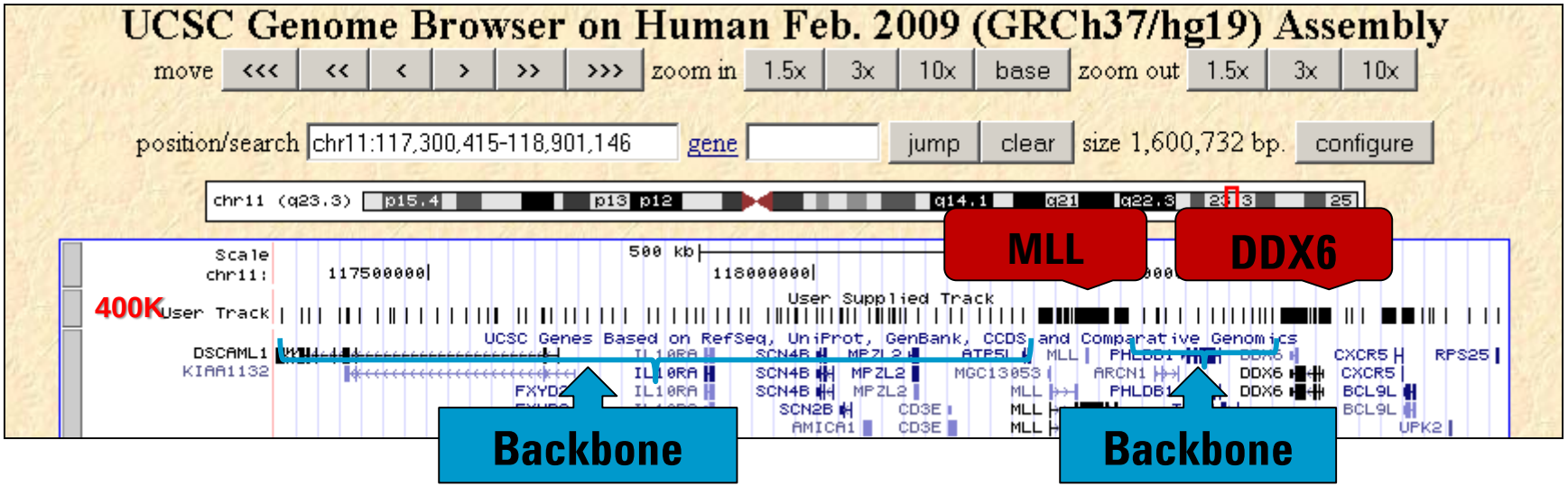
Disease Research Case Study: Detection of a small exon 2 deletion in the STXBP1 gene



Source: Art Beaudet, ASHG 2013

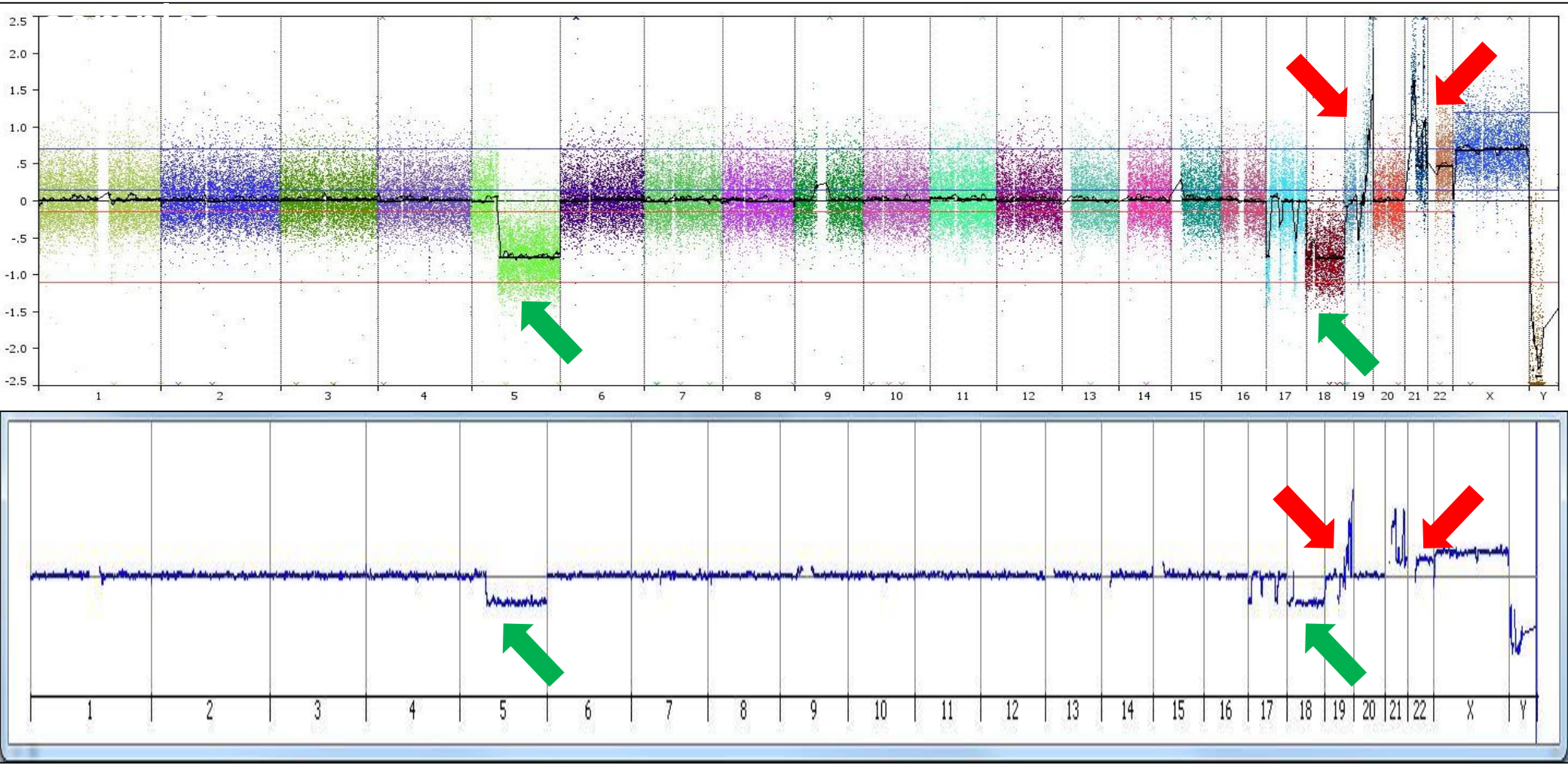
Cancer Research Case Studies

CGH + SNP Cancer Research Array (2X 400 K): Dense exonic coverage in cancer-related genes to maximize detection of causal CNVs and SNPs

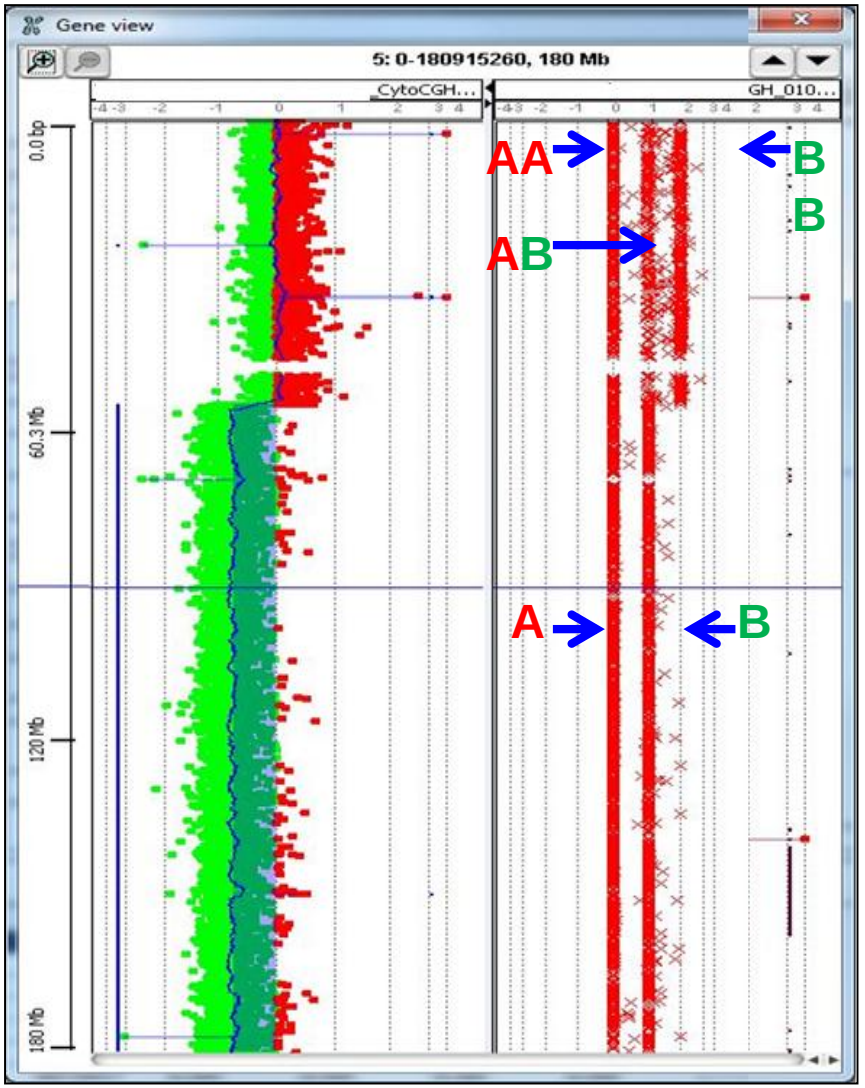


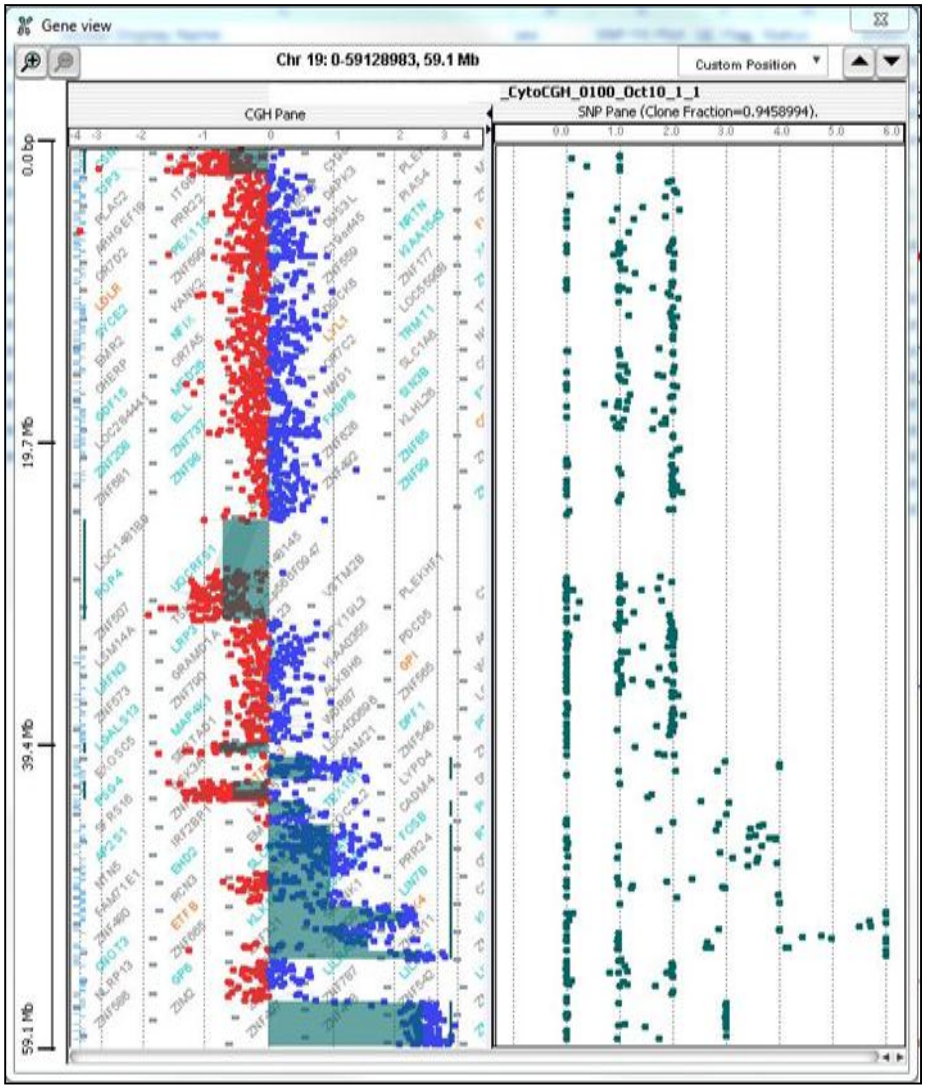
- Exon-by-exon coverage of 2,300 cancer genes or cancer-related genes
- Average of 6 probes per exon
- Average resolutions <1 Kb (large exons) to <10 Kb (cancer genomic targeted regions)
- ~12 Kb in backbone regions
- 60,000 SNPs
- 235 cancer-associated miRNAs

Hematological Research Case Study: aCGH reveals multiple copy number variations in Cytogenetically Normal AML (by karyotype)

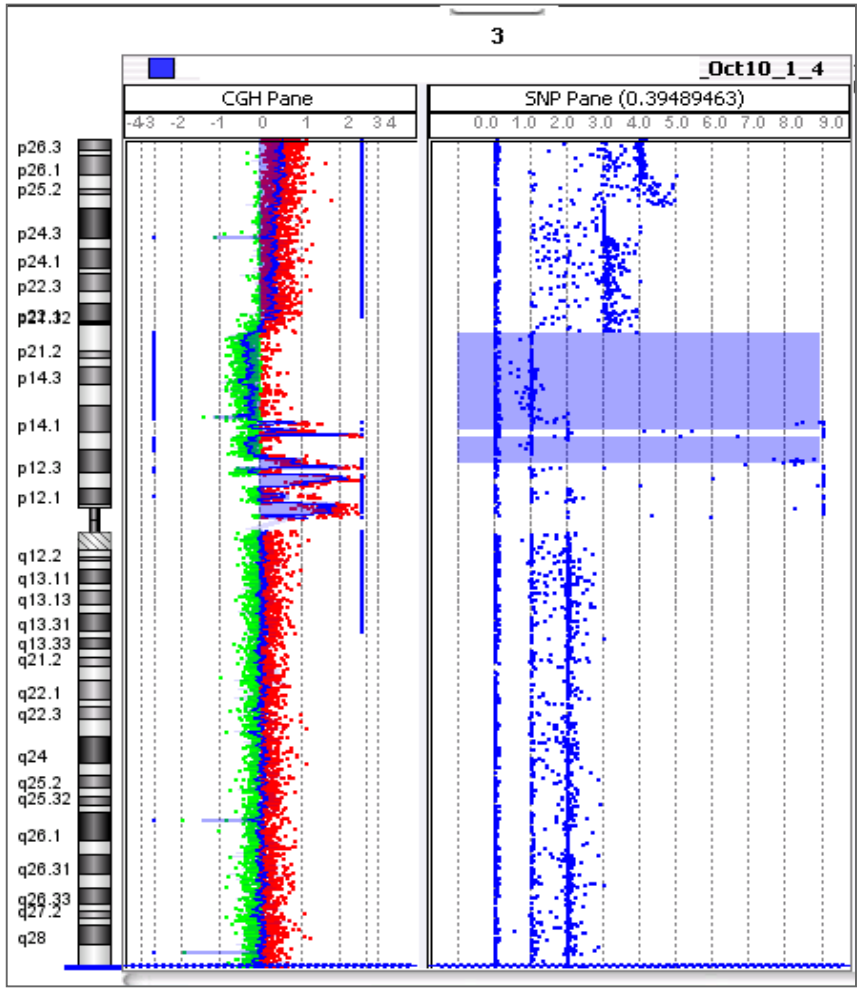


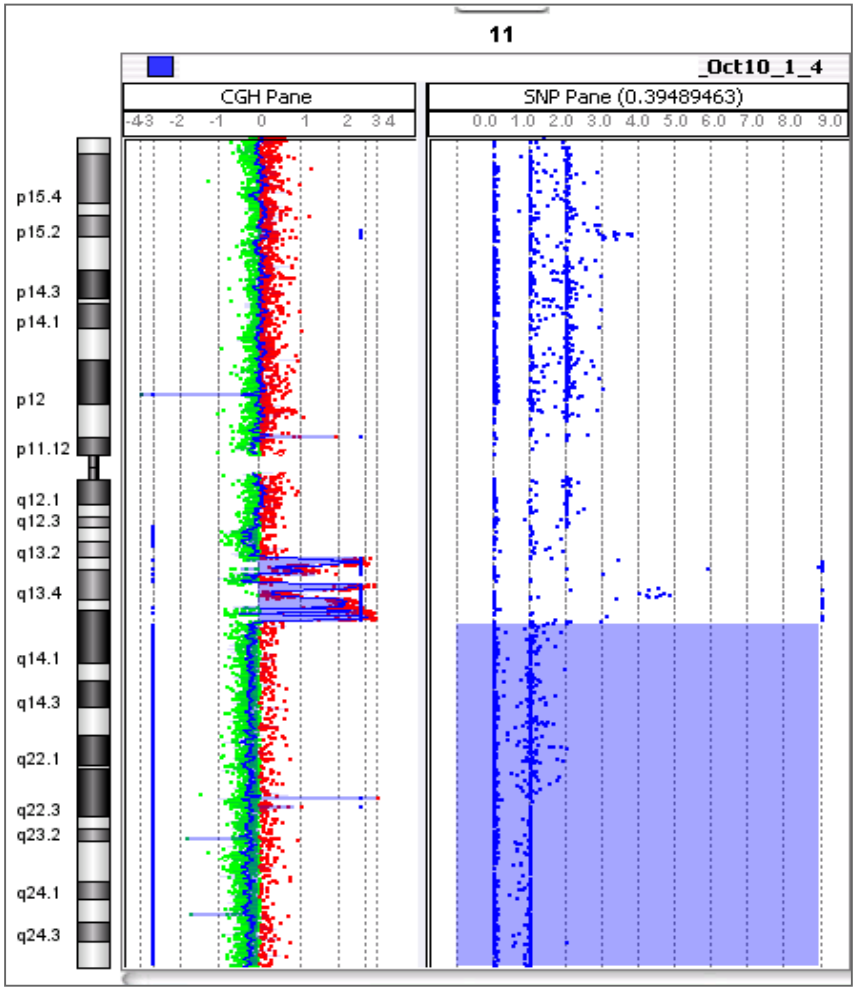
Novel finding with CMA: CN-AML: Del5q, Chromothripsis Chr19





Cancer Research Case Study: Metastatic malignant melanoma





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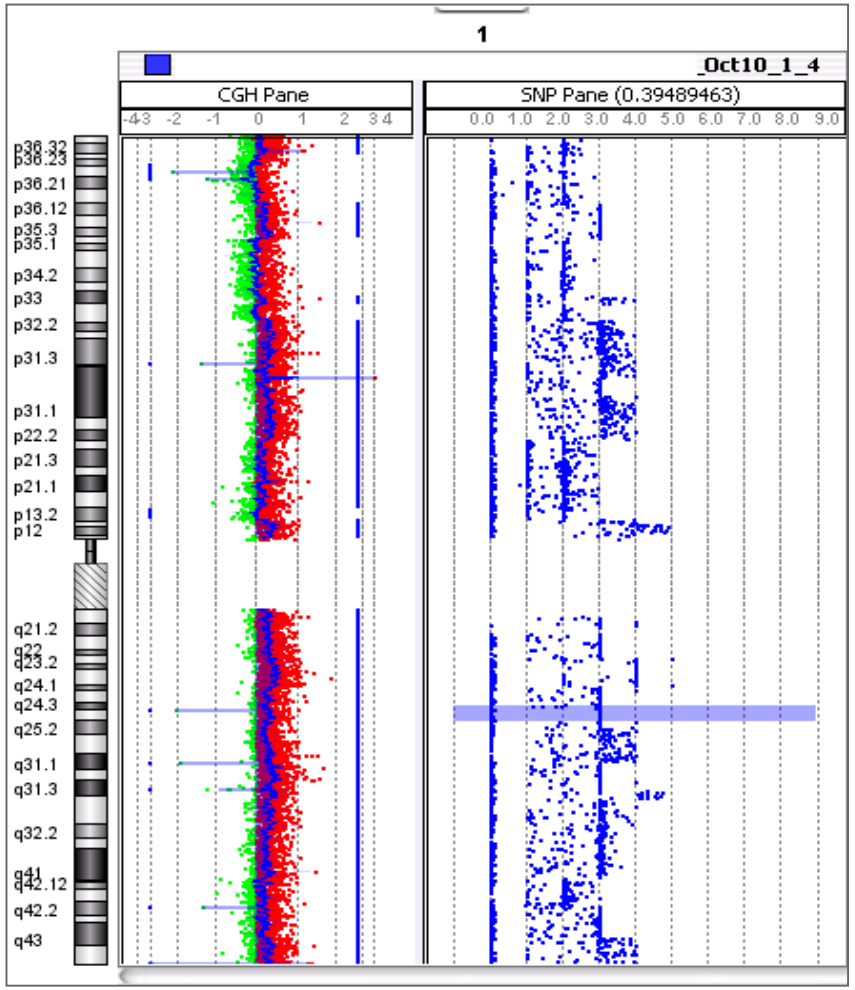
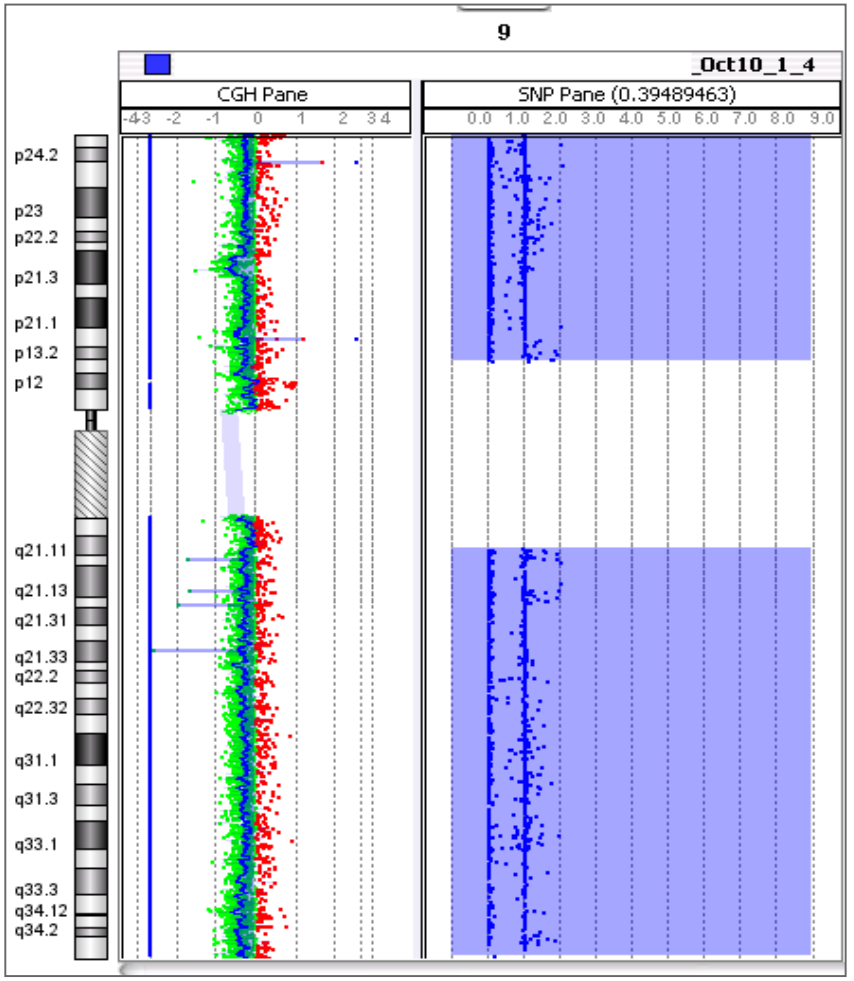
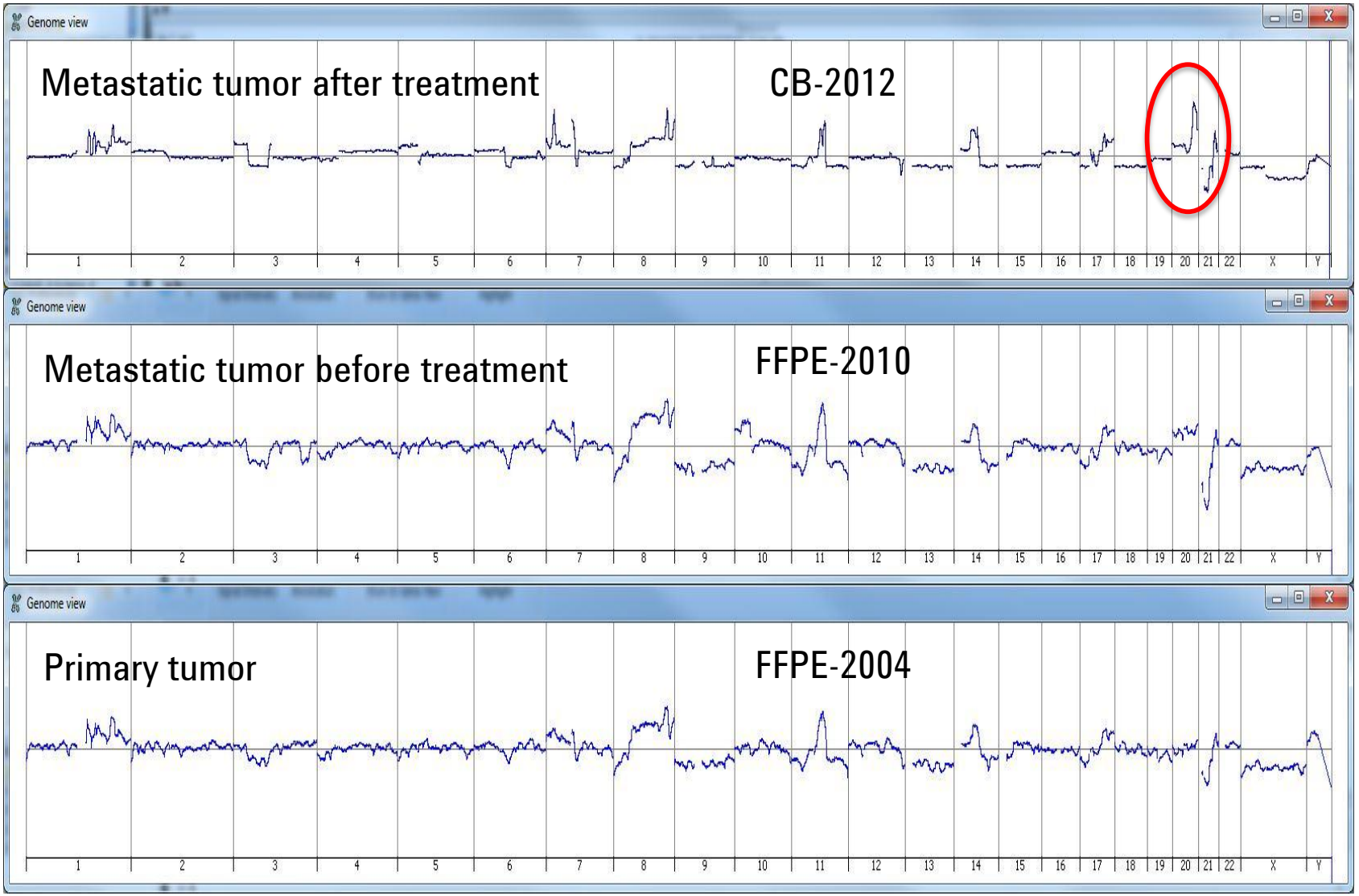
Cancer Research Case Study: Metastatic malignant melanoma





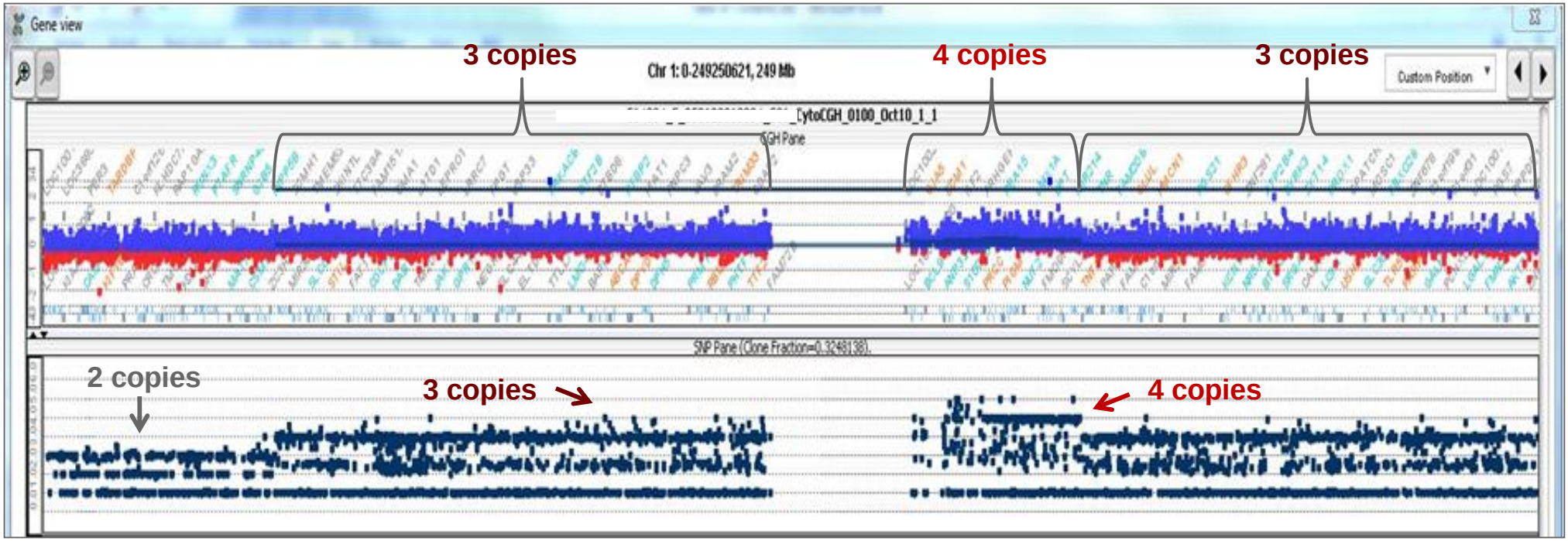
Cancer Research Case Study: CMA on CB & FFPE Samples - Genome View



MetastaticTreatment induced mutation?

NGS shows many changes at bp level from pre- to post-treatment.

Detection of Low-level mosaic aberrations: Chromosome 1 gain in Breast cancer sample



Thank you
for your attention