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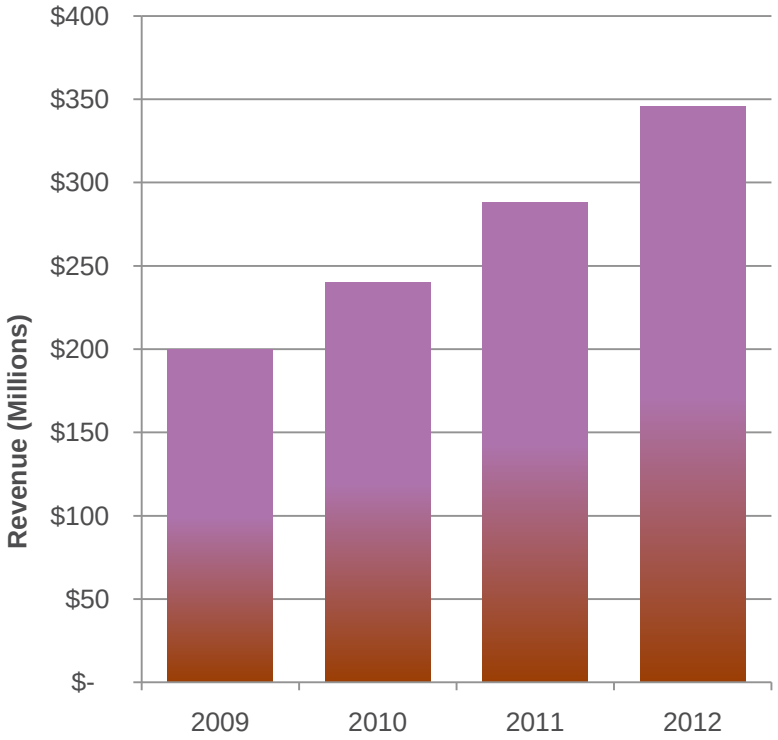
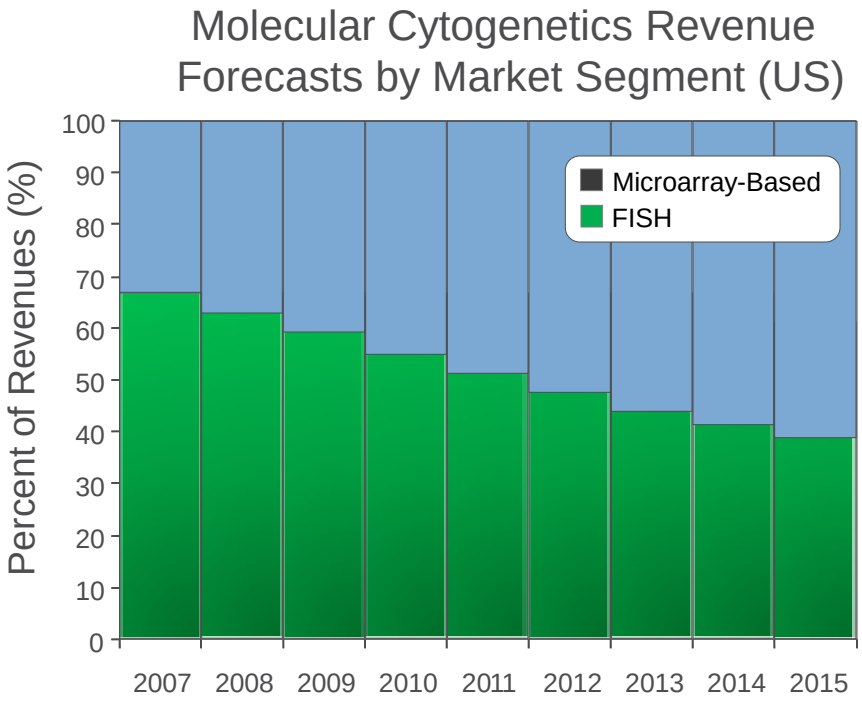
Identifying Chromosomal Abnormalities Using Infinium SNP BeadChips

Harper VanSteenhouse, PhD
Product Manager, Diagnostics
Molecular Cytogenetics

Large & Growing Opportunity in Array-Based Cytogenetics



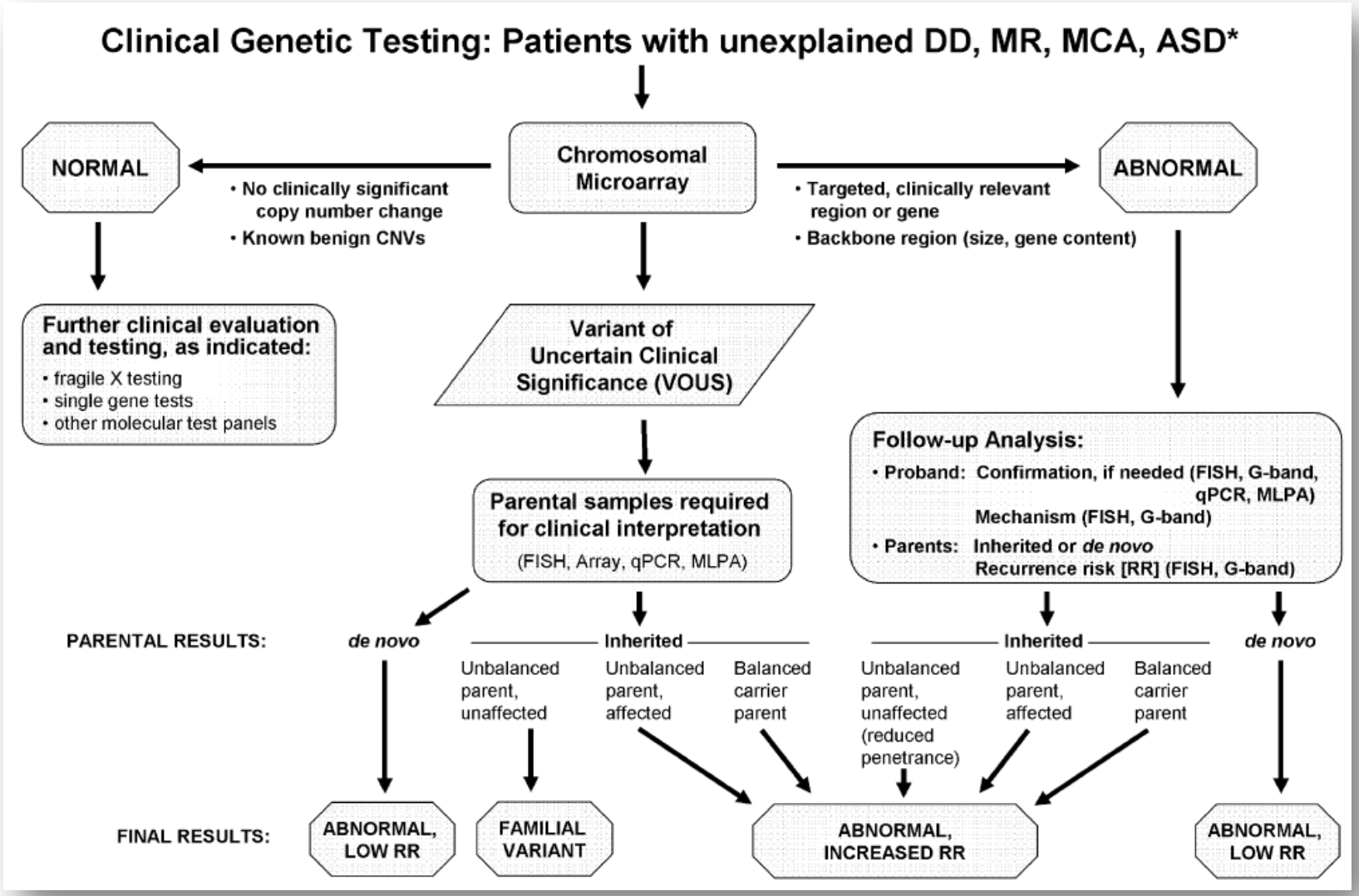
Array-based cytology market growing double digits (CAGR = 16–20%)



A technology substitution is underway

ILLUMINA IS POSITIONED FOR GROWTH

Building momentum: Arrays as first-line?
ISCA Consensus Statement: AJHG, May 2010



The World's Most Complete Cytogenetics Offering

Genome-Wide SNP BeadChips

- Multiple arrays for efficient identification and discovery of aberrant regions
- Flexible software options for any cytogeneticist (GenomeStudio or KaryoStudio)

Next-Gen Sequencing

- Sequencing to obtain the ultimate molecular karyotype and detection of any variant in any genome at single base resolution
- Ideal for discovering new aberrations across the genome with precise breakpoint identification

Plans for FDA Submission: iScan Platform

- Q4 ’09 Illumina submitted Pre-IDE for Cytogenetics, followed by additional discussions with FDA
- Package includes iScan platform, arrays, reagents and software
- Intended use
  - *Post-natal*
  - *DD/MR/MCA*
  - *Broad, genome-wide coverage*

iScan/KaryoStudio

HumanCytoSNP-12

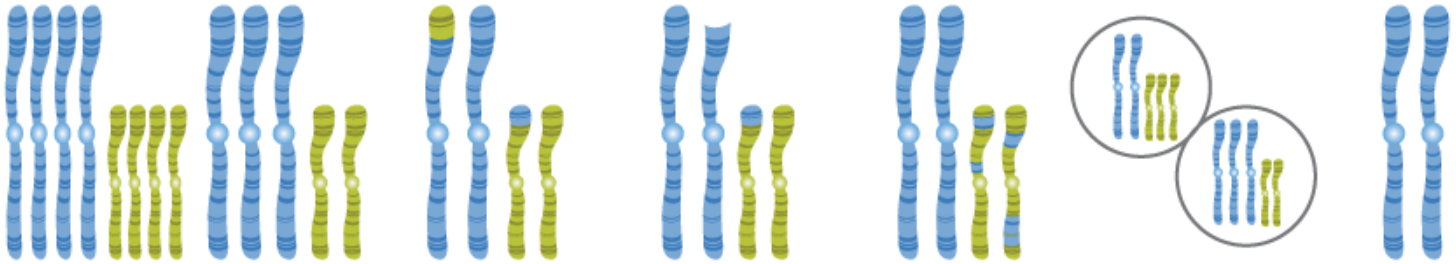
- Optimized for cytogenetics

HumanOmni1-Quad

- High resolution detection of micro-deletions
- Efficient four-sample format

Detection of a Wide Range of Aberrations

ILLUMINA DNA ANALYSIS AND SEQUENCING PRODUCTS IDENTIFY STRUCTURAL VARIATION

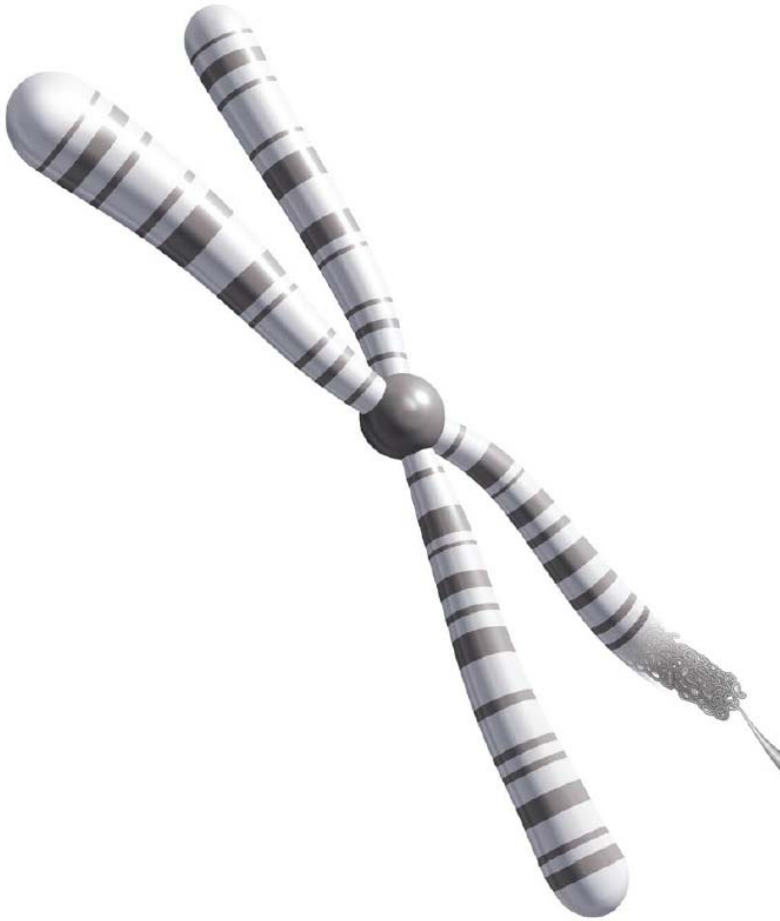


	POLYPLOIDY	ANEUPLOIDY	BALANCED ABERRATION	UNBALANCED ABERRATION	AMPLIFICATION	LOW-LEVEL MOSAICISM	COPY-NEUTRAL LOH
Infinium HD Assay BeadChips	+ (3n)	+	-	+	+	+	+
Illumina Sequencing	+ (1n–4n)	+	+	+	+	+	+
Banding	+	+	+	+	-	+	-
FISH/SKY	+	+	+	+	+	+	-
Array-CGH	-	+	-	+	+	-	-

Adapted from Speicher and Carter, 2005

Getting High-Quality Cytogenetic Data is a SNP

- ***Highest data quality***
  - Proven assay and analysis
  - High accuracy and signal-to-noise
  - Consistently high call rates
- ***Most intelligent content***
  - BeadChips target high-value regions
  - And provide genome-wide coverage
- ***Most informative marker panel***
  - High proportion of SNP markers
  - Optimized to identify the most relevant aberrations with high confidence
- ***Streamlined sample to answer workflow***
  - Fast, simple, and proven protocols
  - Automated processing
  - Results visualization

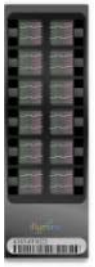


Infinium HD Portfolio of Cytogenetic Products

Arrays

HumanCytoSNP-12

- Meticulously selected SNPs for whole-genome coverage enhanced in cytogenetic regions and genes
- Efficient detection of gains, losses, copy-neutral LOH, aneuploidy, and mosaicism



HumanOmni1-Quad

- High-resolution identification of aberrations and breakpoints across the whole genome
- Cutting-edge content in high-value genes and functional regions

Software

KaryoStudio™ v1.0

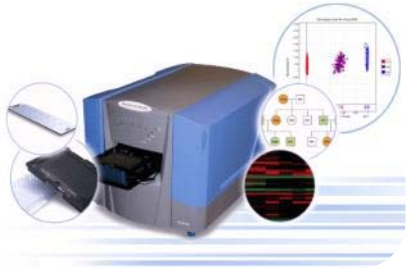
- Developed in collaboration with molecular cytogeneticists
- Automated cross-matching and reporting

GenomeStudio™ 2008

- Numerous options for copy number analysis with graphical, table, and data displays
- Links to 3rd party software offerings

Instruments

BeadArray Reader



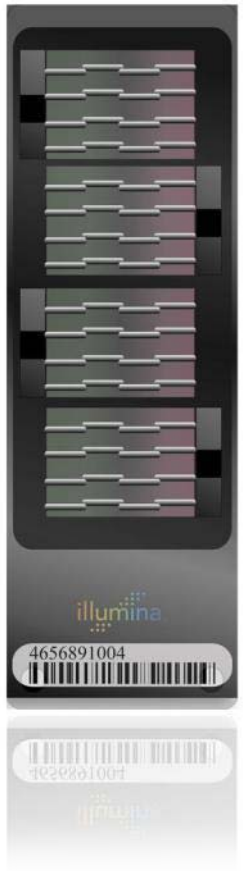
iScan System

HumanCytoSNP-12 BeadChip: Optimized for Efficient Cytogenetic Analysis

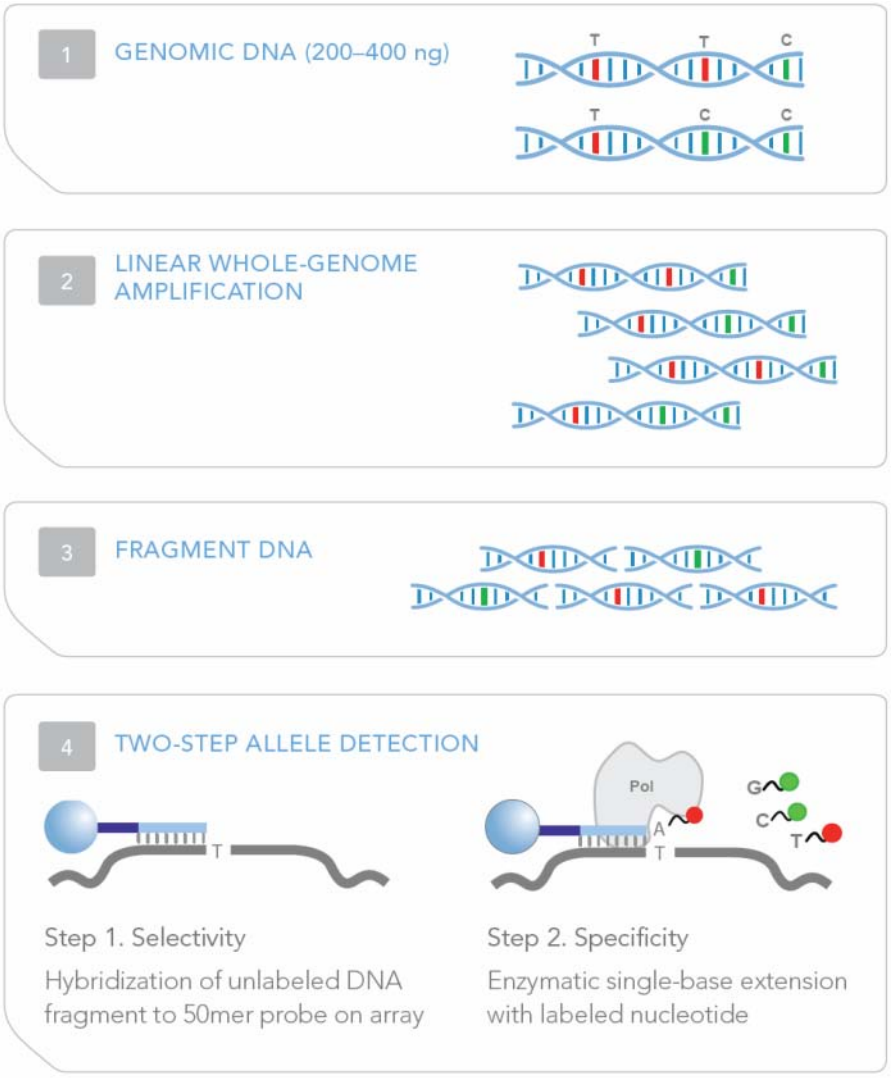
- Streamlined, most informative set of targeted and whole-genome SNP and non-polymorphic markers
- Uniform picket fence of entire genome (including ~92% of RefSeq genes)
  - 300,000 markers, mostly SNPs
  - 6.2kb median marker spacing yields ~30kb resolution
- Higher density in cyto high-value regions (~250 for ~40% of genome)
  - All pericentromeres and subtelomeres
  - Sex chromosomes
  - Common regions of interest (e.g., associated with known syndromes)
  - Regions contain ~9000 genes
- Higher density in ~400 “disease genes”

HumanOmni1-Quad BeadChip: High-Density Backbone Plus High Value Regions

- Dense, uniform whole-genome backbone
  - Intelligent SNP selection from all 3 phases of HapMap
  - 1.2kb median marker spacing yields ~6kb resolution
  - > 98% of RefSeq genes
- Most valuable, cutting-edge content
  - Gene-centric SNPs, indels
    - > 600,000 markers within 10kb of a gene
  - 1000+ disease-associated SNPs
  - Selected SNPs from the first phase of the 1000 Genomes Project
- Rare and Common CNVs
  - discovery and characterization



The Infinium HD Assay



- Unconstrained Marker Design
  - Freedom to select the best, most informative SNPs then fill-in with intensity-only probes
- Well-proven
  - High reproducibility (> 99.9%)
  - High call rates (> 99%)
- Streamlined, automatable
- No need to run a reference sample
- High locus selectivity and allele specificity
  - Two-step enzymatic discrimination

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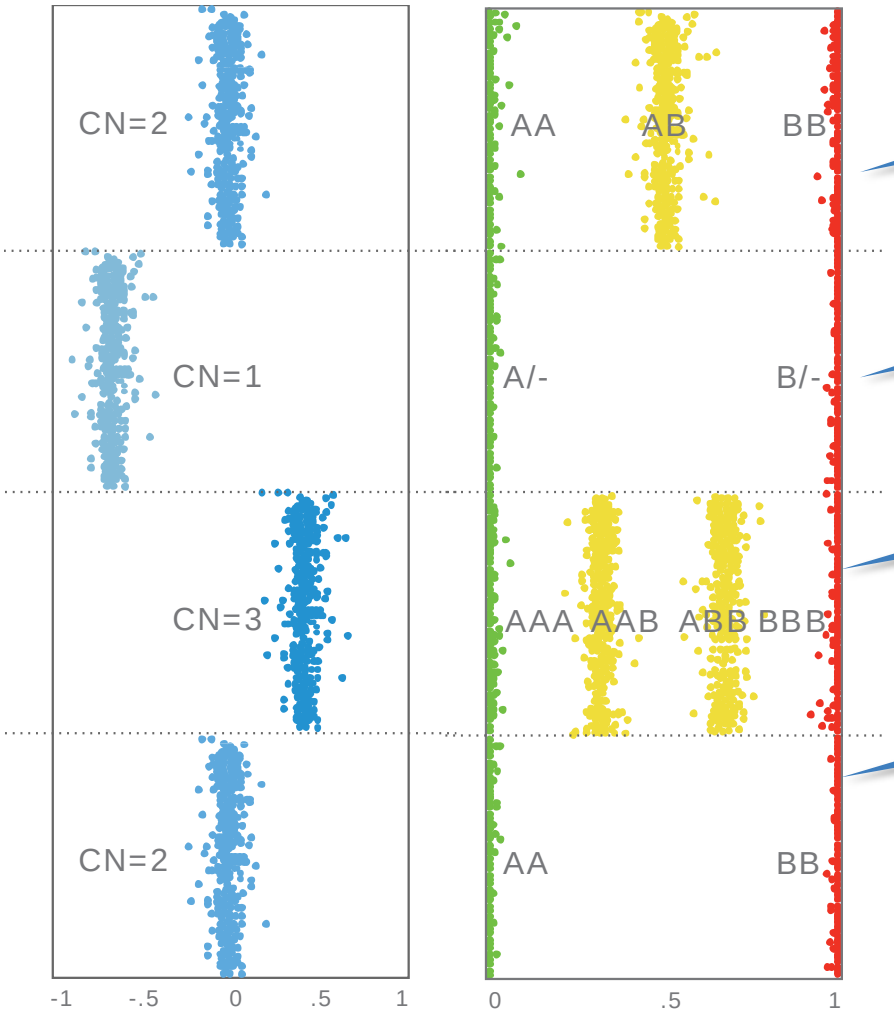
SNPs Provide More Information to Detect Copy Number



Normal (diploid)

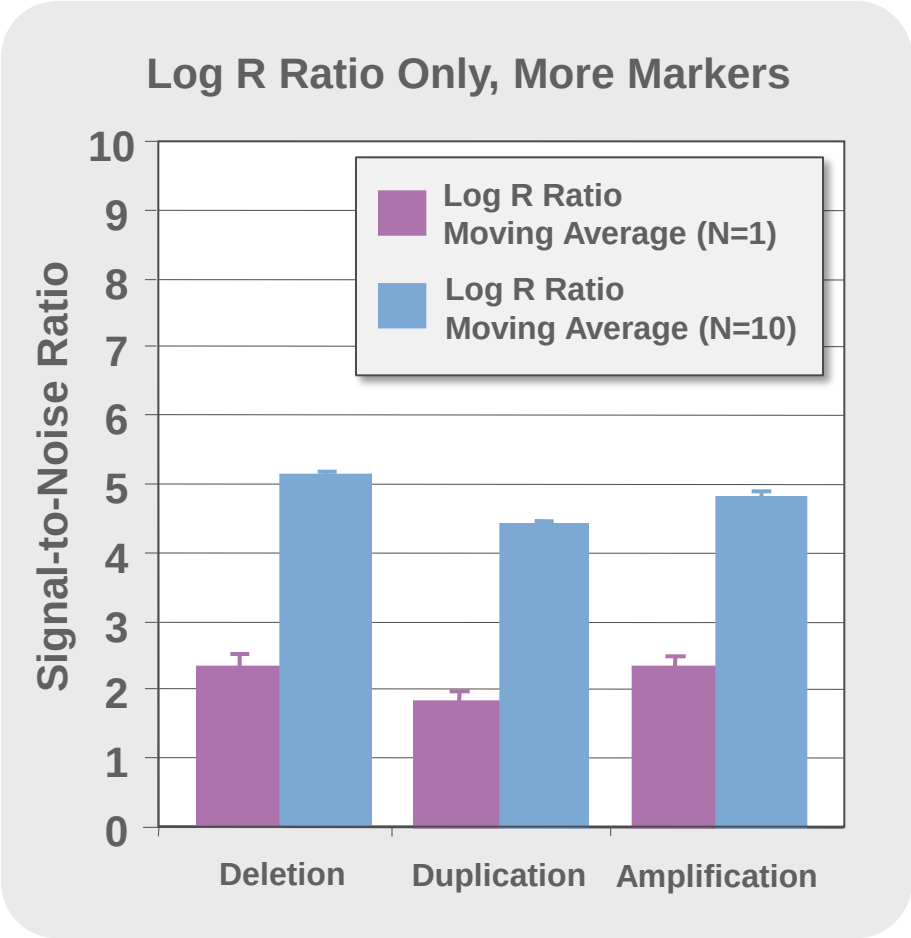
Deletion (loss of one copy)

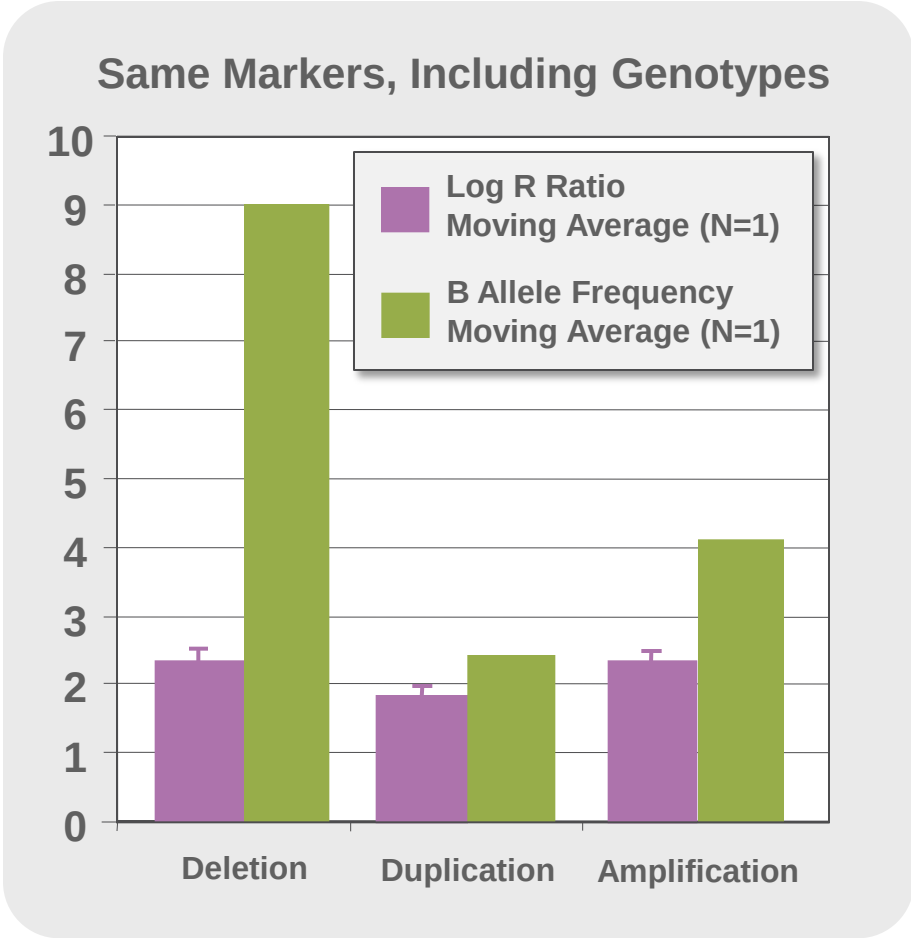
Duplication (gain of one copy)

Copy-Neutral LOH (UPD)

- Also can detect:
  - Amplification
  - Unbalanced aberration
  - Aneuploidy
  - Mosaicism

SNPs Provide Higher Signal-to-Noise Ratios





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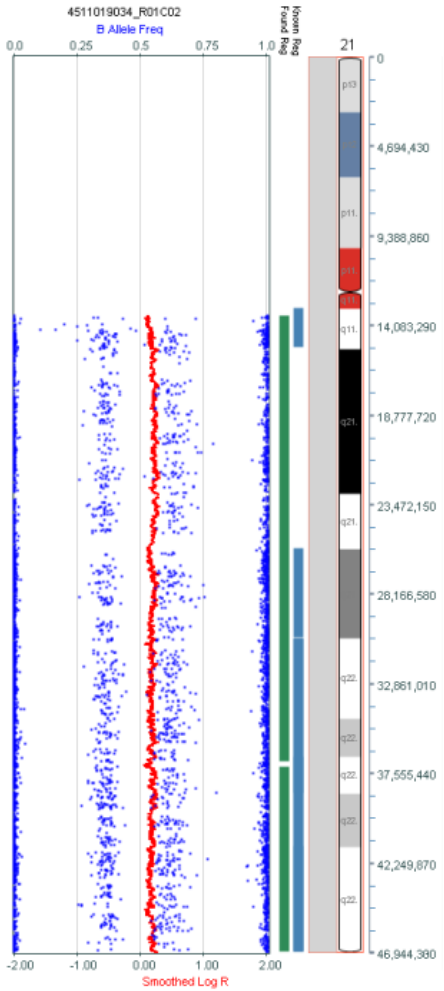
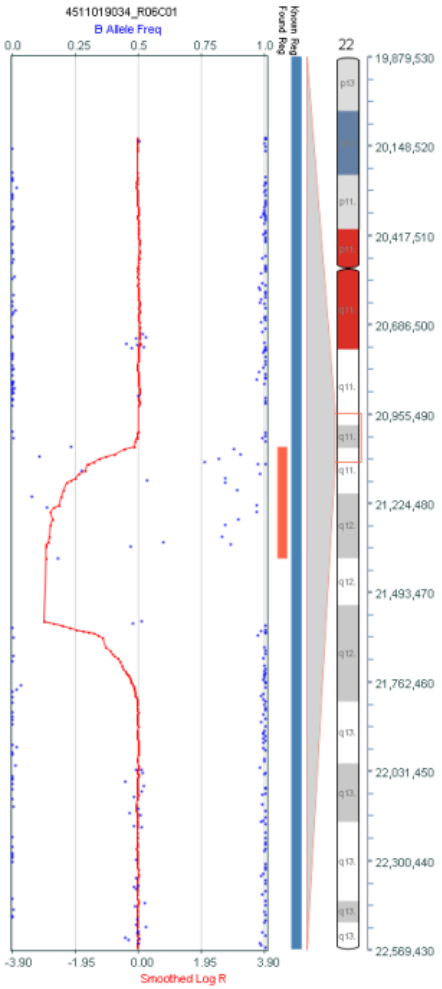
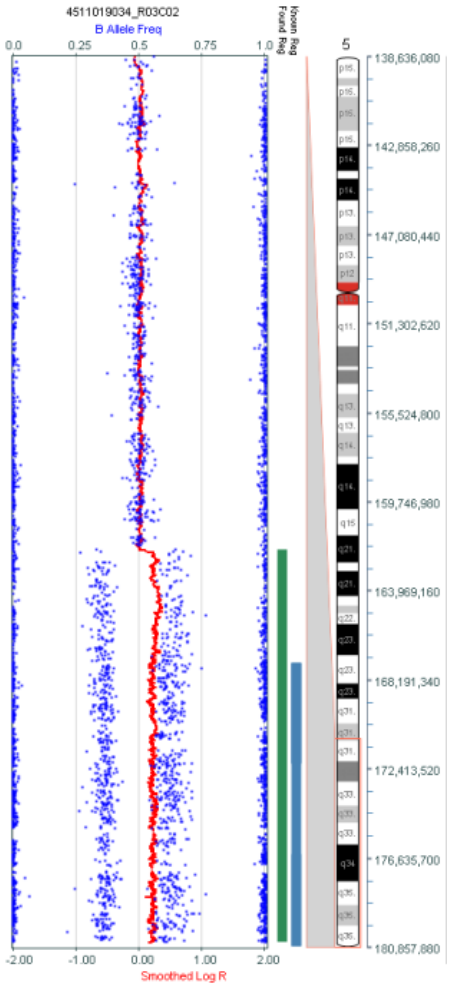
Abnormal NIGMS Cell Repository on HumanCytoSNP-12







Trisomy 21

22q11.2 Homozygous Deletion

5q34-qter Gain

Example: Detecting Copy-Neutral Abnormalities

- Platform comparison performed by independent cytogenetics lab
  - Human1M-Duo and Agilent 244A arrays
  - Looking for cytogenetic abnormalities with focus on copy-neutral events
- 24 pre-characterized samples plus single unrelated male and single unrelated female reference samples
  - 400ng of input DNA
  - Standard quality filters for each platform
    - Illumina: SNP genotype call rate >99% per sample, copy number measurements log R std dev < 0.2
    - Agilent: DLR spread < 0.3
  - Manufacturer recommended algorithms

Copy-Neutral Cytogenetic Aberrations

Forms of copy-neutral cytogenetic aberrations

- Uniparental disomy
  - Case in which individual receives two copies of a chromosomal region from one parent, none from the other
- Copy-neutral loss of heterozygosity (or “acquired uniparental disomy”)
  - Case in which one allele of a gene in a heterozygote is already inactivated and the second, “good” allele is lost without a net change in copy number. This can occur through a gene conversion event in which the chromosome region containing the inactivated allele is used as a template to repair a gap occurring in the corresponding region of the other chromosome

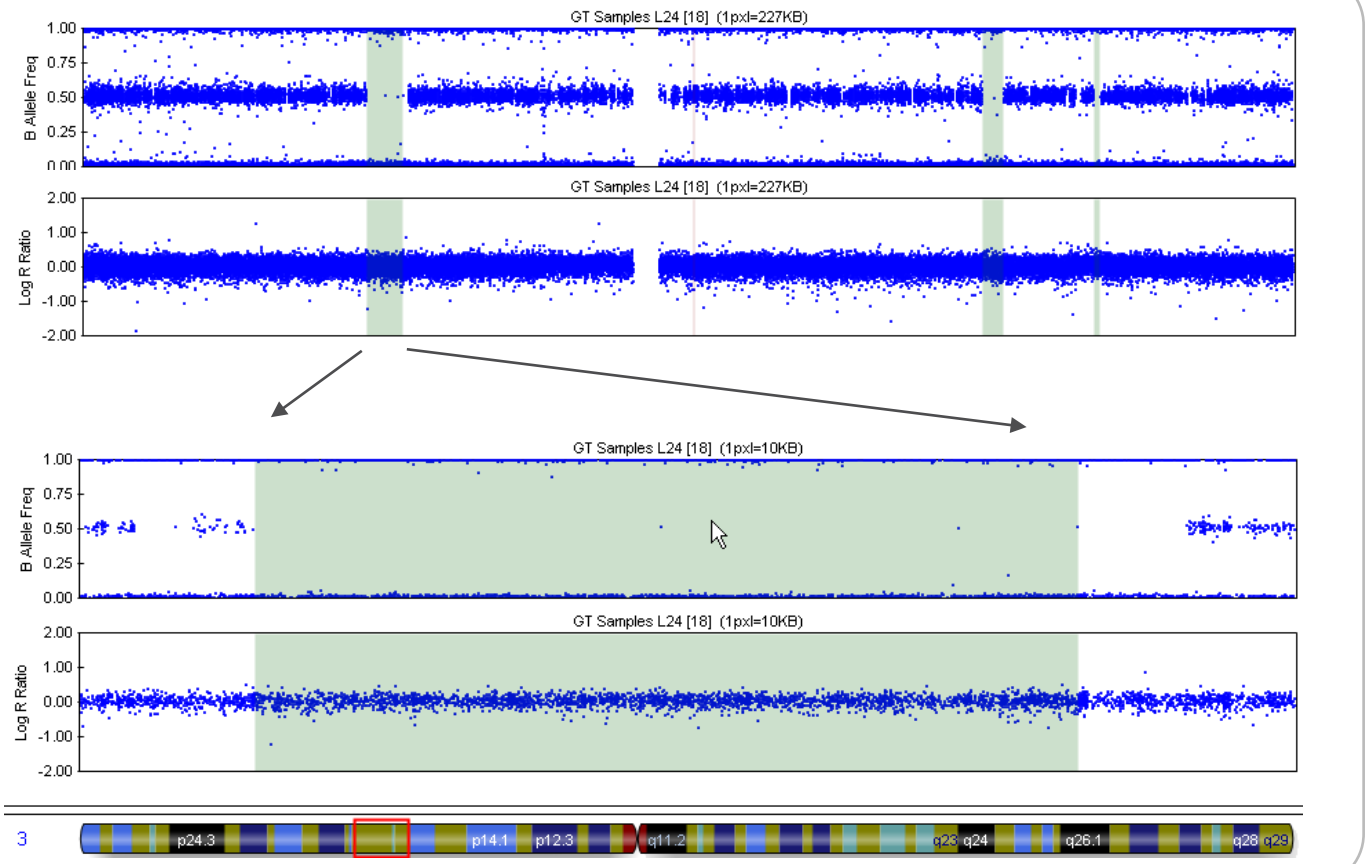
In either case, the absence a functional allele leaves the individual vulnerable to phenotypes that may be associated to the effected gene(s)

Copy-Neutral Cytogenetic Aberrations are Being Associated with a Rapidly Growing Number of Clinical Phenotypes

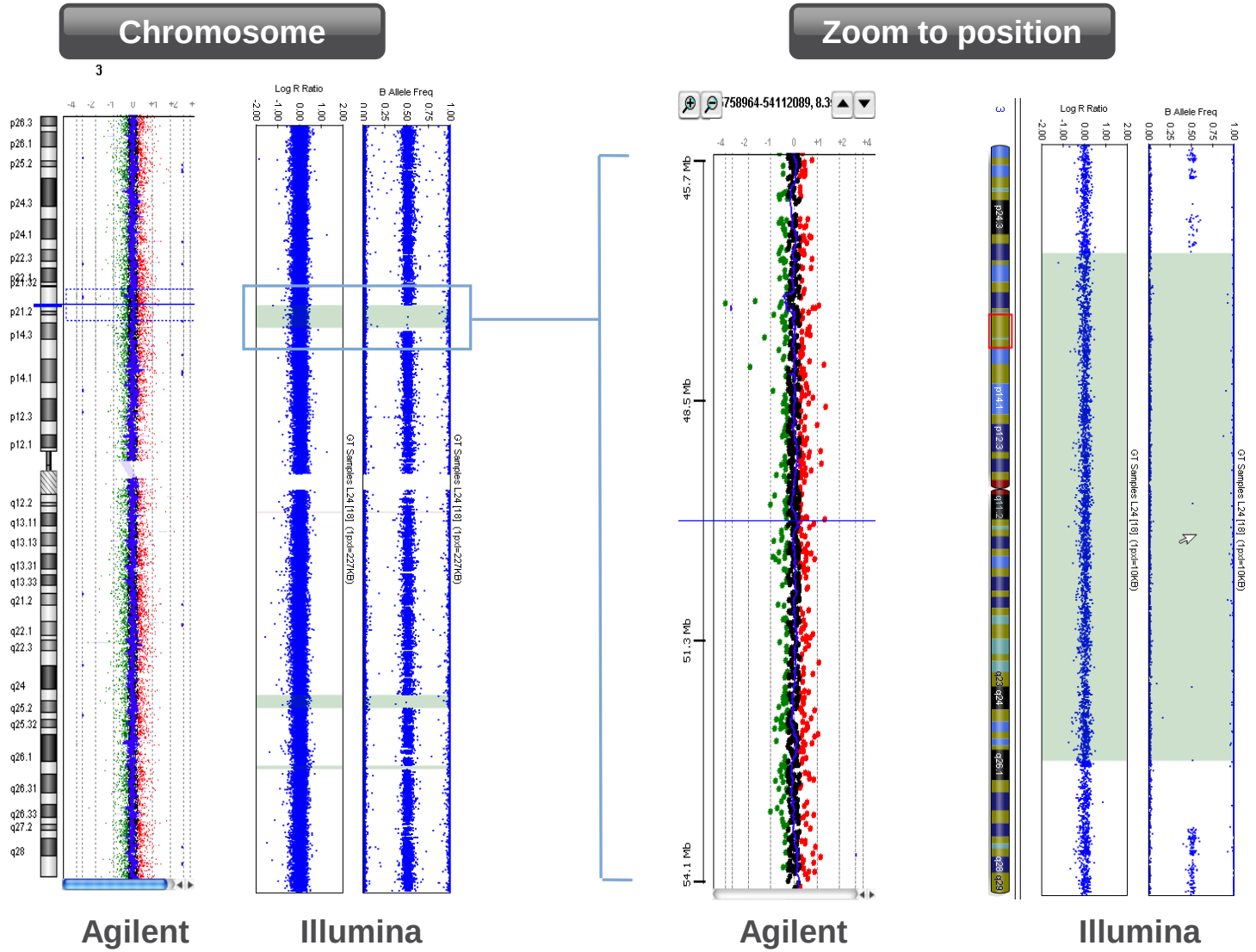
Pycnodysostosis	Am J Hum Genet. 1998 Apr;62(4):848-54
Herlitz junctional epidermolysis bullosa	J Invest Dermatol. 2000 Aug;115(2):307-11
Steroid 5alpha-reductase 2 deficiency	J Clin Endocrinol Metab. 2000 Sep;85(9):3147-50
Angelman syndrome	Hum Mol Genet. 1999 Jan;8(1):129-35.
Primary ciliary dyskinesia	Proc Natl Acad Sci U S A. 2002 Aug 6;99(16):10282-6. Epub 2002 Jul 25.
Late-infantile-onset neuronal ceroid lipofuscinosis	Hum Mutat. 2009 Mar 3
Pediatric acute lymphoblastic leukemia	Int J Oncol. 2009 Jun;34(6):1603-12
Prader-Willi syndrome	Endocrine. 2009 May 7
Myelodysplastic syndromes	Leukemia. 2009 Apr 23.
Silver-russell syndrome	Pediatrics. 2009 May;123(5):e929-31. Epub 2009 Apr 13
Angelman syndrome	Res Dev Disabil. 2009 Sep-Oct;30(5):1095-106.
Waldenstrom's macroglobulinemia.	Cancer Res. 2009 Apr 15;69(8):3579-88. Epub 2009 Apr 7
Autism Spectrum Disorders.	Biol Psychiatry. 2009 Mar 17.
Nephropathic cystinosis	Eur J Hum Genet. 2009 Mar 4
Silver-Russell syndrome (SRS)	J Med Genet. 2009 Mar;46(3):192-7. Epub 2008 Dec 9
Galactosaemia	J Inherit Metab Dis. 2008 Oct 29.
Myoclonus-dystonia	Arch Neurol. 2008 Oct;65(10):1380-5
Charcot-Marie-Tooth / Gaucher disease	Neurology. 2008 Mar 18;70(12):976-8
Glioblastomas	Neuro Oncol. 2008 Dec;10(6):995-1003. Epub 2008 Aug 12
Neuroblastoma	BMC Genomics. 2008 Jul 29;9:353
CLL	Blood. 2007 Feb 1;109(3):1202-10. Epub 2006 Oct 19
Head and neck squamous cell carcinoma	Head Neck. 2008 Oct;30(10):1361-83
Esophageal adenocarcinoma	Cancer Res. 2008 Jun 1;68(11):4163-72
MUTYH-associated polyposis carcinomas	J Pathol. 2008 Sep;216(1):25-31
Follicular lymphoma	Clin Cancer Res. 2007 Aug 15;13(16):4777-85

The Combination of B Allele Frequency and Log R Ratio Data Enables the Detection of CNLOH / UPD

- > 30 cases of CNLOH detected in sample cohort on Human1M-Duo BeadChip



Copy-Neutral Features Are Missed by Array CGH



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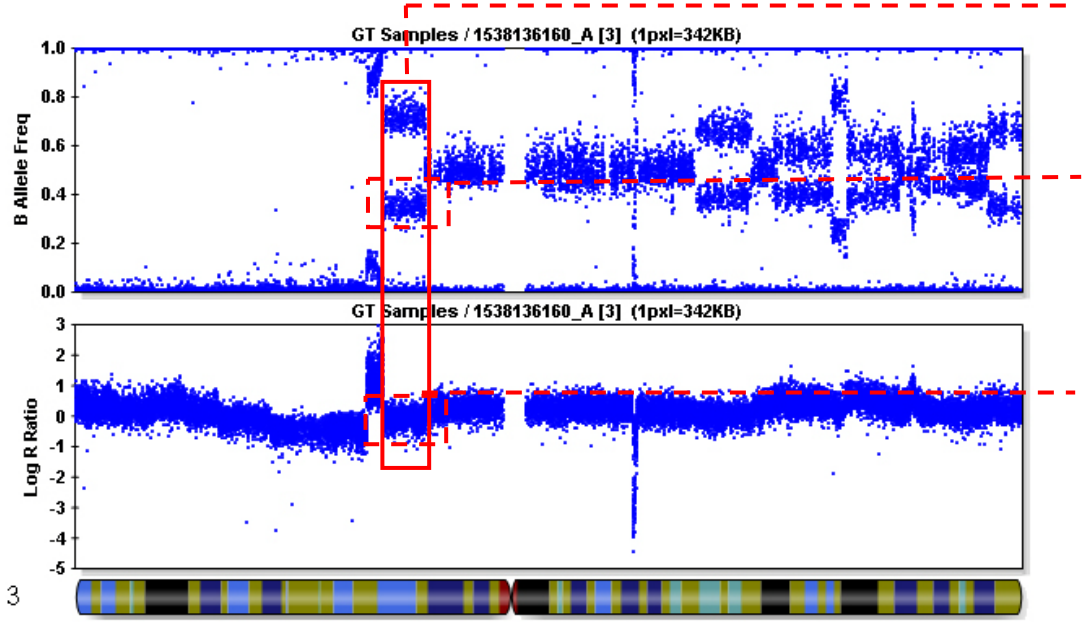
SNP-based Detection Provides More Information for Better Characterization of Chromosomal Aberrations



A profile of chromosome 3 of a cell line derived from a breast tumor.

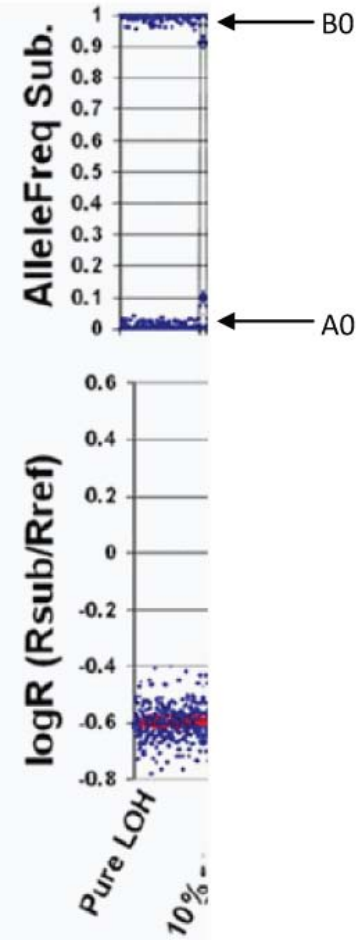
Detected duplication

B allele frequency data (AAB genotype)

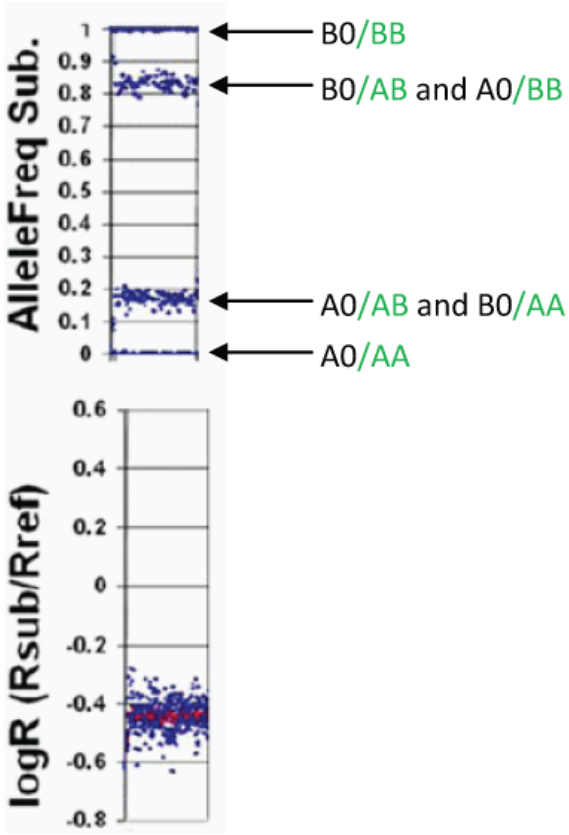
A shift in the LogR value is detectible, but the integration of B allele data improves the signal to noise ratio

Mosiacism Detection Benefits from SNP Markers

Pure LOH



Mosaic LOH



Green genotypes indicate the normal "contaminating" cells

- Adapted from Nancarrow DJ, Handoko HY, Stark MS, Whiteman DC, Hayward NK (2007) SiDCoN: a tool to aid scoring of DNA copy number changes in SNP chip data. PLoS ONE 2:e1093.

Human Molecular Genetics, 2010 **1–13**
doi:10.1093/hmg/ddq003

Mechanisms of mosaicism, chimerism and uniparental disomy identified by single nucleotide polymorphism array analysis

Laura K. Conlin[1,2], Brian D. Thiel[1], Carsten G. Bonnemann[2], Livija Medne[4], Linda M. Ernst[4], Elaine H. Zackai[2], Matthew A. Deardorff[2], Ian D. Krantz[2], Hakon Hakonarson[2,3] and Nancy B. Spinner[1,2,*]

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Main conclusions:

- Frequency of mosaicism higher than previously reported
- Successful detection of low-level mosaicism
- Increased risk of UPD with mosaicism
- Interesting chimeric case

Mechanisms of Mosaicism, Chimerism, and UPD

Laura Conlin, et al. 2010

- Frequency of mosaicism higher than previously reported
  - ~1% of their total of >2000 samples (~10% of "aberrant" samples)
  - Much higher than reported previously, likely owing to the new abilities to detect

Table 1. Patients with mosaic aneuploidy or chimerism

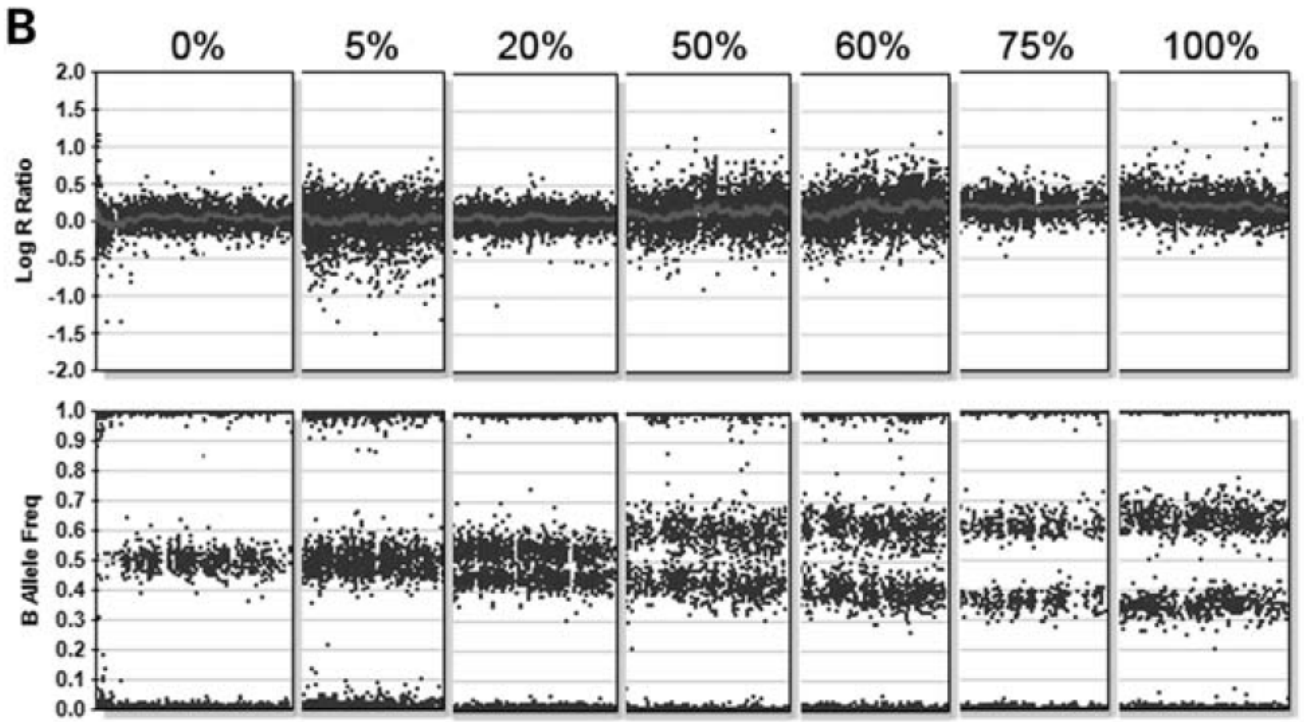
Patient number	Type of aneuploidy	Mosaic % by array	Mosaic % by G-banded chromosomes	Mosaic % by metaphase FISH	Mosaic % by interphase FISH	Parental chromosome gained or lost	Mitosis/Meiosis	UPD	Tissue	Cell lines
1	Monosomy X	5	6.67				Mitosis	Yes	Blood	45,X/46,XX
2	Monosomy X	25	16.00			Paternal	Mitosis	Yes	Skin	45,X/46,X,r(Y)
3	Monosomy X	30	25.00		22.00	Paternal	Mitosis	Yes	Blood	45,X/46,XY
4	Monosomy X	30	40.00			Maternal	Mitosis	Yes	Blood	45,X/47,XXX
	Trisomy X	70	60.00			Paternal	Mitosis	No	Blood	45,X/47,XXX
5	Monosomy 7	40	0.00		7.00	Paternal	Mitosis	Yes	Blood	45,XY,-7/46,XY
6	Monosomy X	50	42.11				Mitosis	Yes	Blood	45,X/46,X,r(X)
7	Monosomy X	75	6.67				Mitosis	Yes	Blood	45,X/46,X,r(X)
8	Monosomy X	80	75.00				Mitosis	Yes	Blood	45,X/46,X,r(X)
9	Monosomy X	80	76.67				Mitosis	Yes	Blood	45,X/46,X,r(X)
10	Monosomy X	90					Mitosis	Yes	Blood	45,X/46,XX
11	Trisomy 14	5	0.00			Maternal	MI	No	Skin	47,XX, + 14/46,XX
		50	2.56			Maternal	MI	No	Blood	47,XX, + 14/46,XX
12	Trisomy 8	40	100.00				MI	Yes	Blood	47,XY, + 8/46,XY
13	Trisomy 9	50	0.00	2.50	24.00		MI	Yes	Blood	47,XX, + 9/46,XX
14	Trisomy 18	10	15.15				MII	No	Blood	47,XX, + 18/46,XX
15	Trisomy 14	20	10.00			Paternal	MII	Yes	Blood	47,XX, + 14/46,XX
16	Trisomy 8	5		1.50			Mitosis	No	Blood	47,XY, + 8/46,XY
17	Trisomy 9	20	2.00			Paternal	Mitosis	No	Blood	47,XY, + 9/47,XY
18	Trisomy 9	20	35.00				Mitosis	No	Blood	47,XX, + 9/46,XX
19	Trisomy 8	20			12.62		Mitosis	No	Blood	48,XY, + 8, + 19/46,XY
	Trisomy 19	20			14.00		Mitosis	No	Blood	48,XY, + 8, + 19/46,XY
20	Trisomy 21	50	85.00				Mitosis	No	Skin (hypo)	48,XX, + 7, + 21/46,XX
	Trisomy 7	50	85.00				Mitosis	No	Skin (hypo)	48,XX, + 7, + 21/46,XX
	Trisomy 21	60	50.00				Mitosis	No	Skin (hyper)	48,XX, + 7, + 21/46,XX
	Trisomy 7	60	50.00				Mitosis	No	Skin (hyper)	48,XX, + 7, + 21/46,XX
21	Trisomy 17	50					Mitosis	No	Skin (left)	47,XY, + 17/46,XY
		75					Mitosis	No	Skin (right)	47,XY, + 17/46,XY
30	Chimera	20	45.00			Paternal	Fertilization	Yes	Skin (hyper)	46,XX/46,XY

MI, meiosis I; MII, meiosis II.

Mechanisms of Mosaicism, Chimerism, and UPD

Laura Conlin, et al. 2010

- Successful detection of low-level mosaicism
  - ~5% limit for mosaic detection. This is way better than anything reported before using aCGH (20% limit).
  - The result is that they find many more mosaics than have been reported in aCGH papers (1% rather than 0.2%).

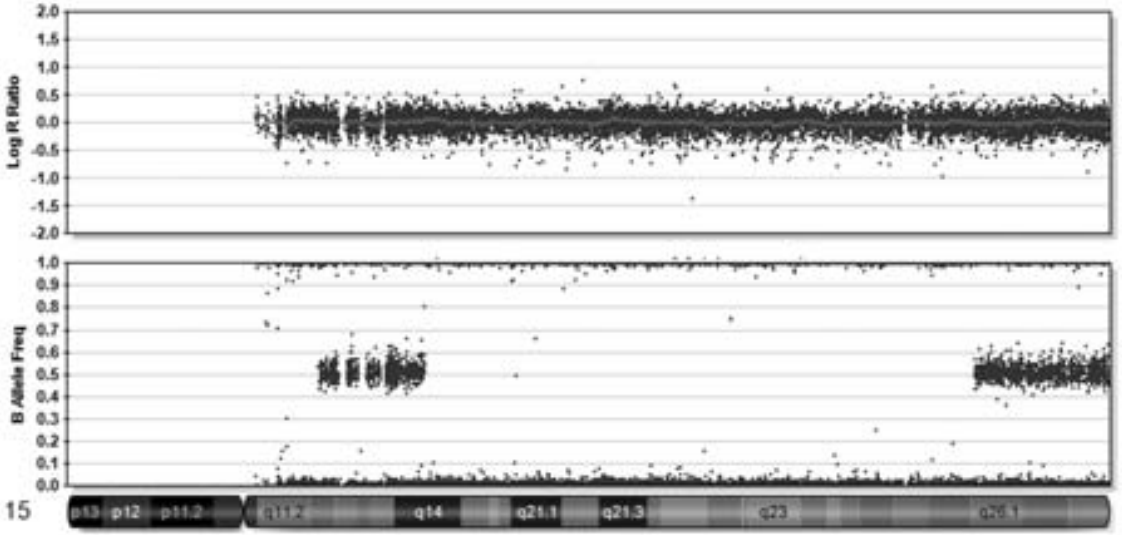


Mechanisms of Mosaicism, Chimerism, and UPD

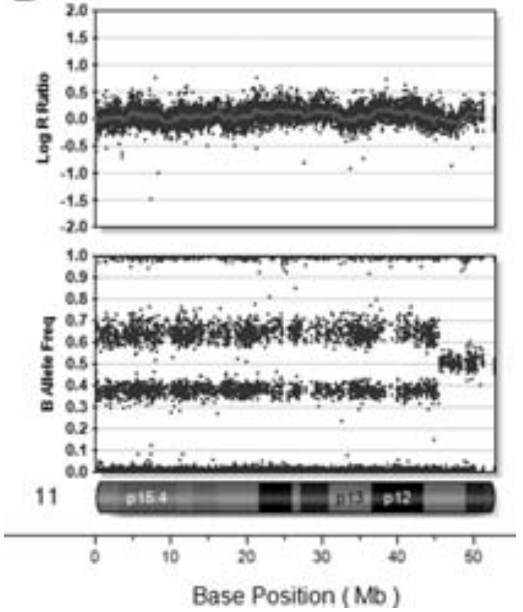
Laura Conlin, et al. 2010

- Increased risk of UPD with mosaicism
  - They found 6 cases of UPD, where there was no consanguinity and >20Mb of homozygosity. (see Table 2 below)
  - UPD was present in 60% of cases meiotic mosaic trisomies (trisomy rescue).

Segmental UPD



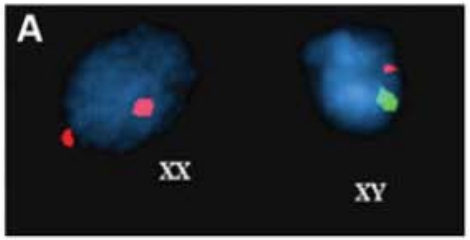
Mosaic UPD



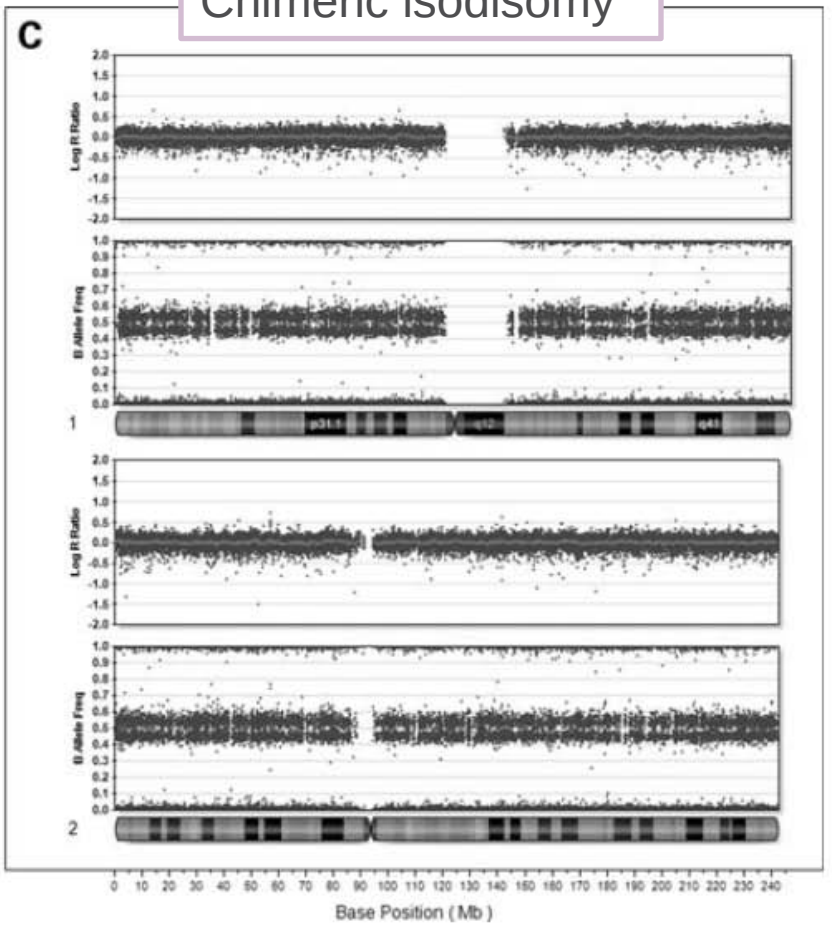
Mechanisms of Mosaicism, Chimerism, and UPD

Laura Conlin, et al. 2010

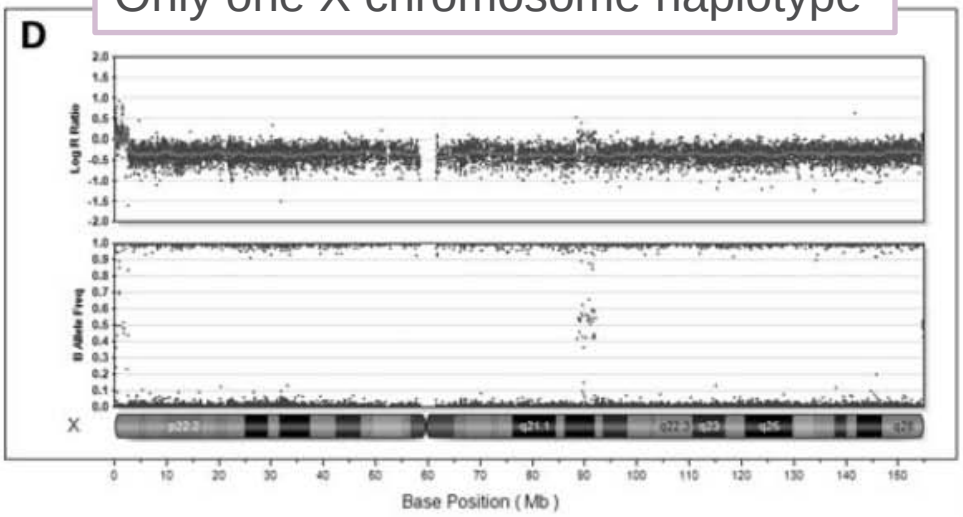
- Parthenogenic chimera case



Chimeric isodisomy



Only one X chromosome haplotype



The Industry's Best Cytogenetics SNP Arrays

	HumanCytoSNP -12	HumanOmni1 -Quad	300K Competitor	2.7M Competitor
Total Number of Markers for Copy Number Analysis	301,232	1,140,419	310,759	2,761,979
SNP Markers (% of Total)	294,748 (98%)	1,016,423 (90%)	16,666 (5%)	400,103 (14%)
Marker Spacing – Median	6.2kb	1.2kb	9.6kb	0.7kb
SNP Spacing – Mean	9.6kb	2.5kb	177kb	7.4kb
Feature Redundancy – Mean	12x		1x	
Hands on Time – Manual	< 2 hr per 48 samples (< 1hr Automated)		< 3hrs	
Protocol Duration	~40hr		~30hr	
Samples per Chip	12	4	1	1
Probe Length	50-mer		40-mer	
Hybridisation and Enzymatic Discrimination	Yes		No	
Assay Publications	>275		0	

iScan Reader

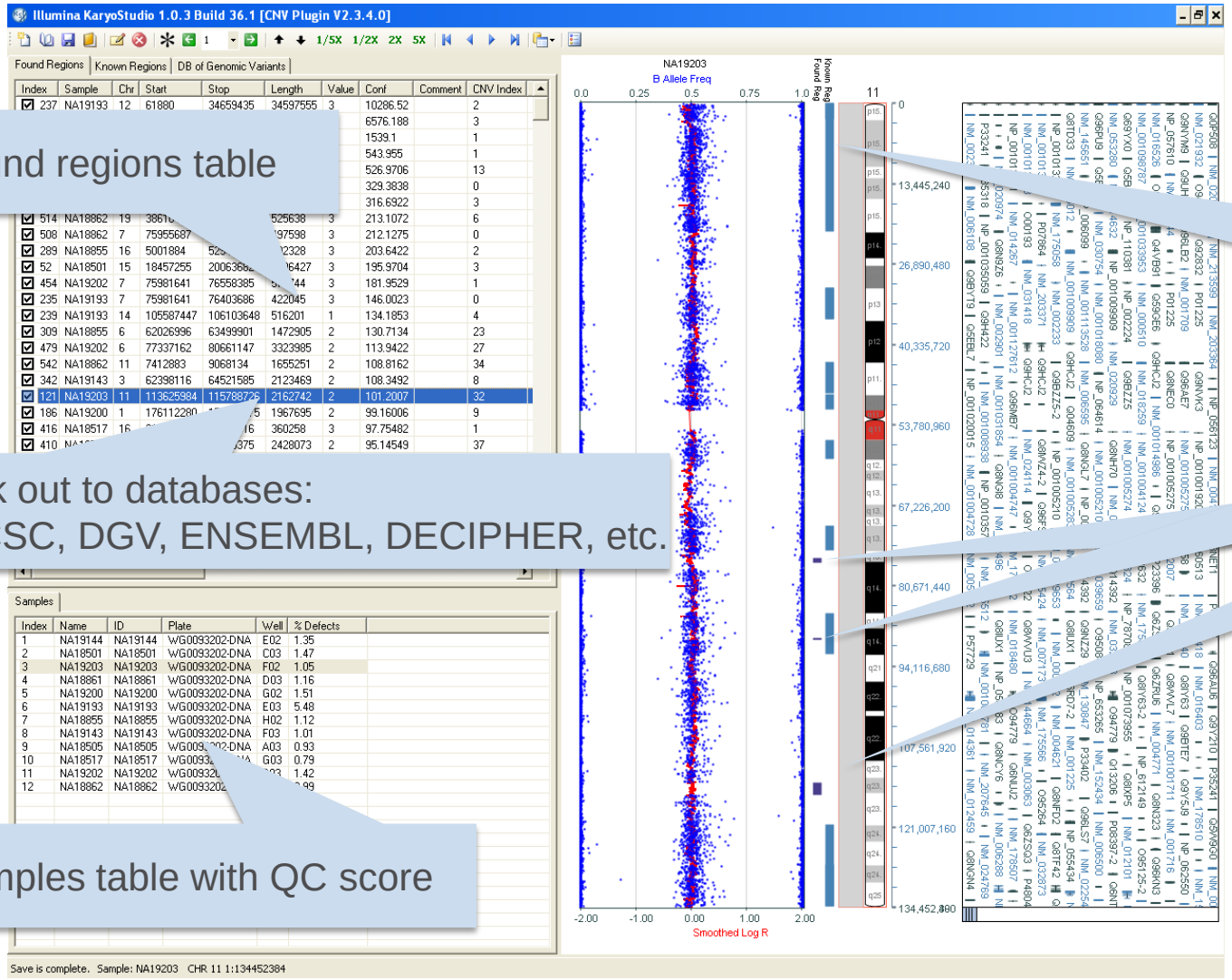
- Fast: 3–16 min per sample
- High resolution: 0.54 micron
- Dual line - lasers at 532 & 658nm
- Multi-application support
- Enhanced dynamic range and limit of detection
- Full integration with LIMS and Autoloader 2

KaryoStudio: Streamlined Cytogenetics Analysis

For Research Use Only

illuminaDx™

Found Regions Displayed in KaryoStudio



Found regions table

Link out to databases:
UCSC, DGV, ENSEMBL, DECIPHER, etc.

Samples table with QC score

User-defined
Known Regions track

Found Regions

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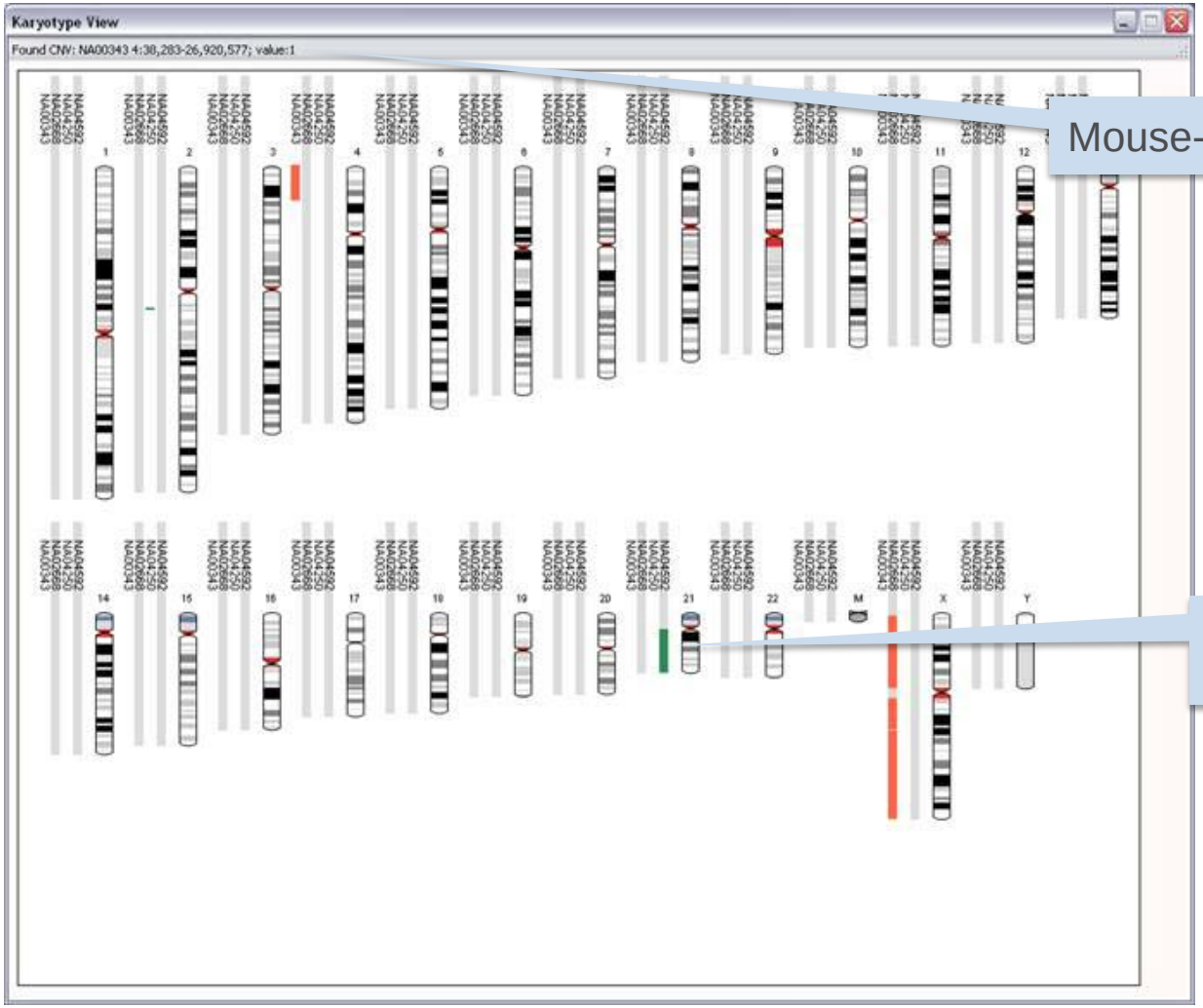
Karyotype View



Mouse-over region for details

Click identified region to zoom to in data plot

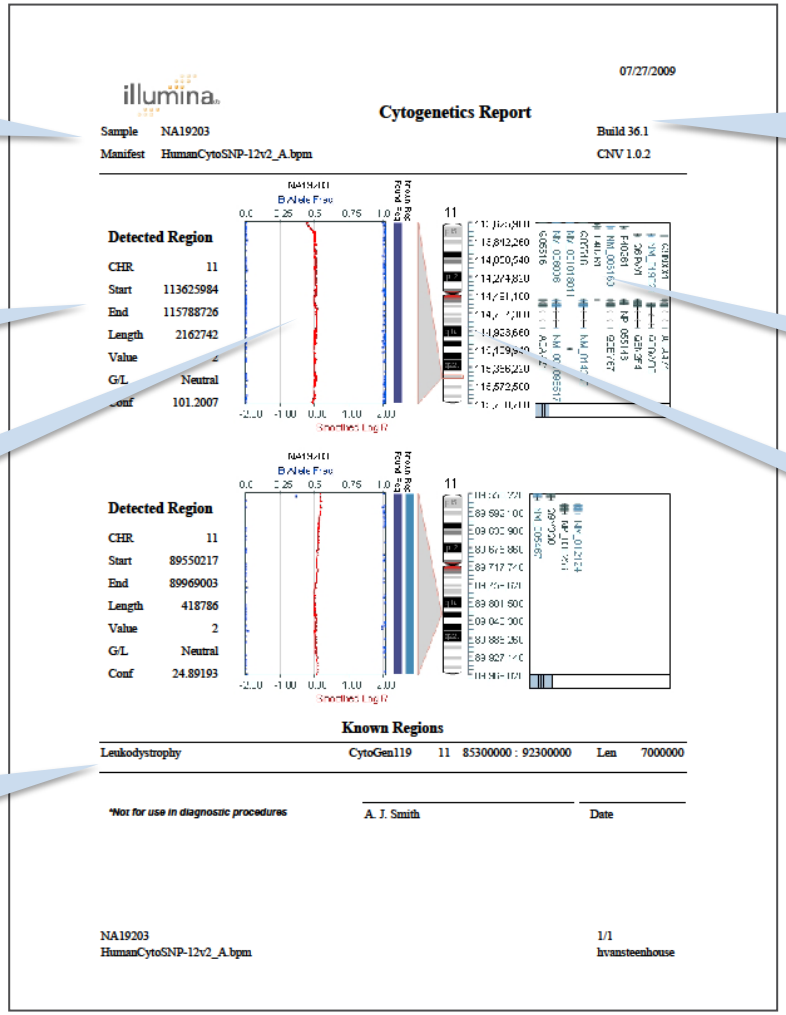
Report Generated From Aberrations

Sample and product information

Information on found region

Aberration display

Cross-matches to user-defined Known Regions list



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07/27/2009

Cytogenetics Report

Sample NA19203 — Build 36.1

Manifest HumanCytoSNP-12v2_A.bpm — CNV 1.0.2

Detected Region

CHR	11
Start	113625984
End	115788726
Length	2162742
Value	2
G/L	Neutral
Conf	101.2007

Detected Region

CHR	11
Start	89550217
End	89969003
Length	418786
Value	2
G/L	Neutral
Conf	24.89193

Known Regions

Leukodystrophy	CytoGen119	11	85300000 : 92300000	Len	7000000

*Not for use in diagnostic procedures

A. J. Smith — Date

NA19203
HumanCytoSNP-12v2_A.bpm

1/1
hvansteenhouse

Genome build and versions of software used

Gene information

Chromosome information

Recent Infinium Cytogenetics Publications
(www.illumina.com/publications)

- Conlin LK, Thiel BD, Bonnemann CG, et al. (2010) Mechanisms of mosaicism, chimerism and uniparental disomy identified by single nucleotide polymorphism array analysis. Hum Mol Genet 19: 1263-75
- Descipio C, Morrissette JD, Conlin LK, et al. (2010) Two siblings with alternate unbalanced recombinants derived from a large cryptic maternal pericentric inversion of chromosome 20. Am J Med Genet A 152A: 373-82
- Johnson DS, Gemelos G, Baner J, et al. (2010) Preclinical validation of a microarray method for full molecular karyotyping of blastomeres in a 24-h protocol. Hum Reprod 25: 1066-75
- Shaikh TH, Gai X, Perin JC, Glessner JT, Xie H, et al. (2009) High-resolution mapping and analysis of copy number variations in the human genome: A data resource for clinical and research applications. Genome Res Epub ahead of print.
- Beaujard MP, Chantot S, Dubois M, Keren B, Carpentier W, et al. (2009) Atypical deletion of 22q11.2: Detection using the FISH TBX1 probe and molecular characterization with high-density SNP arrays. Eur J Med Genet Epub ahead of print.
- Gijsbers AC, Lew JY, Bosch CA, Schuurs-Hoeijmakers JH, van Haeringen A, et al. (2009) A new diagnostic workflow for patients with mental retardation and/or multiple congenital abnormalities: test arrays first. Eur J Hum Genet Epub ahead of print.
- Jackson EM, Sievert AJ, Gai X, Hakonarson H, Judkins AR, et al. (2009) Genomic analysis using high-density single nucleotide polymorphism-based oligonucleotide arrays and multiplex ligation-dependent probe amplification provides a comprehensive analysis of INI1/SMARCB1 in malignant rhabdoid tumors. Clin Cancer Res 15:1923-30.
- Kamath BM, Thiel BD, Gai X, Conlin LK, Munoz PS, et al. (2009) SNP array mapping of chromosome 20p deletions: genotypes, phenotypes, and copy number variation. Hum Mutat 30:371-8.
- Fernández L, Nevado J, Santos F, Heine-Suñer D, Martinez-Glez V, et al. (2009) A deletion and a duplication in distal 22q11.2 deletion syndrome region. Clinical implications and review. BMC Med Genet 10:48.
- Sievert AJ, Jackson EM, Gai X, Hakonarson H, Judkins AR, et al. (2009) Duplication of 7q34 in pediatric low-grade [illegible]tomas detected by high-density single-nucleotide polymorphism-bas[illegible]ype arrays results in a novel BRAF [illegible]in Pathol 19:449-58.
- [illegible]r DA, Zhou L, Chan OT, et al. (2008) Geno[illegible]genes in archived [illegible]inoma tissues. Int J Cancer 122:2[illegible]
- [illegible]P, Bertherat J, Stratakis [illegible]: highly [illegible]matic gen[illegible]

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Cytogenetic Analysis Solutions from Illumina

- **Infinium HD Assay:** Unconstrained marker design to select the best, highest value SNPs
- **HumanCytoSNP-12:** Optimized for efficient cytogenetic analysis
- **HumanOmni1-Quad:** The most comprehensive and cost-efficient high-density tool to find chromosomal aberrations
- **KaryoStudio:** Streamlined analysis workflow for rapid identification of cytogenetic aberrations
- **Sequencing:** The ultimate digital karyotype