Agilent CytoGenomics 1.5

Streamlining your cytogenetic workflow

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Take home message

Agilent CytoGenomics software is a workflow based easy to use analysis tool for CGH and CGH+SNP analysis to detect CNC, LOH and UPD

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Constitutional and cancer sample analysis

CGH analysis to detect CNCs:

- Constitutional samples
- Cancer samples: Liquid, Solid, FFPE, up to 10% mosaicism can be detected

SNP analysis to detect LOH/UPD and confirm CGH data:

- Constitutional samples only, no cancer samples and no mosaic detection

* Next version of CytoGenomics software will support SNP analysis for hematological cancer samples with ability to detect as low as 20% clonal fraction

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Positioning

- CytoGenomics is designed for the cytogenetics researcher who needs a simple streamlined software solution for cytogenetic analysis of human samples
- Agilent Genomic Workbench (AGW, previously DNA analytics) is designed for many aspects of DNA research of both human and non human samples and for the researcher who needs the ability of on the fly tweaking of all aspects of his (cytogenetics) workflow and who is not interested in a simple streamlined solution

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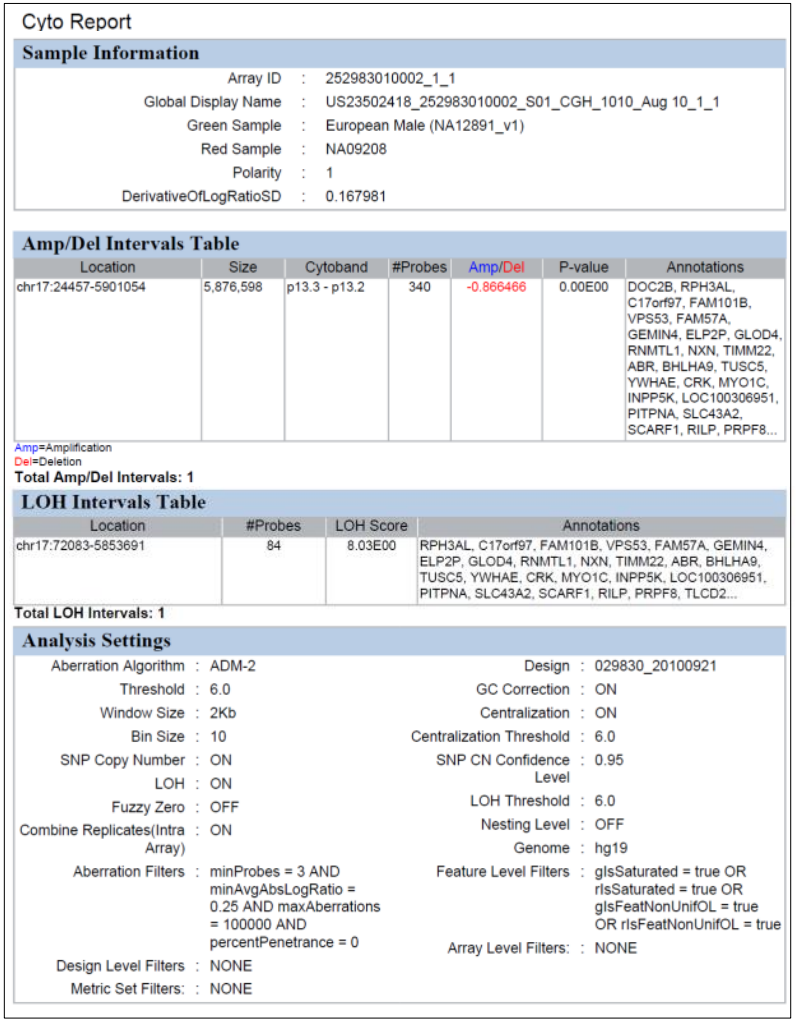
Result of the cytogenetic analysis: Cyto Report



Cyto Report

Sample Information

Array ID	:	252983010002_1_1
Global Display Name	:	US23502418_252983010002_S01_CGH_1010_Aug 10_1_1
Green Sample	:	European Male (NA12891_v1)
Red Sample	:	NA09208
Polarity	:	1
DerivativeOfLogRatioSD	:	0.167981

Amp/Del Intervals Table

Location	Size	Cytoband	#Probes	Amp/Del	P-value	Annotations
chr17:24457-5901054	5,876,598	p13.3 - p13.2	340	-0.866466	0.00E00	DOC2B, RPH3AL, C17orf97, FAM101B, VPS53, FAM57A, GEMIN4, ELP2P, GLOD4, RNMTL1, NXN, TIMM22, ABR, BHLHA9, TUSC5, YWHAE, CRK, MYO1C, INPP5K, LOC100306951, PITPNA, SLC43A2, SCARF1, RILP, PRPF8...

Amp=Amplification
Del=Deletion
Total Amp/Del Intervals: 1

LOH Intervals Table

Location	#Probes	LOH Score	Annotations
chr17:72083-5853691	84	8.03E00	RPH3AL, C17orf97, FAM101B, VPS53, FAM57A, GEMIN4, ELP2P, GLOD4, RNMTL1, NXN, TIMM22, ABR, BHLHA9, TUSC5, YWHAE, CRK, MYO1C, INPP5K, LOC100306951, PITPNA, SLC43A2, SCARF1, RILP, PRPF8, TLCD2...

Total LOH Intervals: 1

Analysis Settings

Aberration Algorithm	:	ADM-2	Design	:	029830_20100921
Threshold	:	6.0	GC Correction	:	ON
Window Size	:	2Kb	Centralization	:	ON
Bin Size	:	10	Centralization Threshold	:	6.0
SNP Copy Number	:	ON	SNP CN Confidence Level	:	0.95
LOH	:	ON	LOH Threshold	:	6.0
Fuzzy Zero	:	OFF	Nesting Level	:	OFF
Combine Replicates(Intra Array)	:	ON	Genome	:	hg19
Aberration Filters	:	minProbes = 3 AND minAvgAbsLogRatio = 0.25 AND maxAberrations = 100000 AND percentPenetrance = 0	Feature Level Filters	:	gIsSaturated = true OR rIsSaturated = true OR gIsFeatNonUnifOL = true OR rIsFeatNonUnifOL = true
Design Level Filters	:	NONE	Array Level Filters:	:	NONE
Metric Set Filters:	:	NONE			

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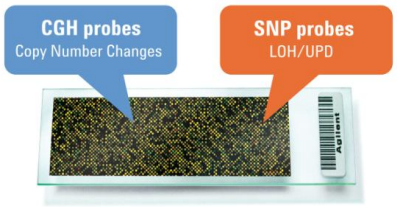
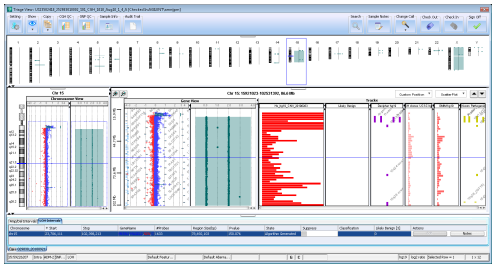
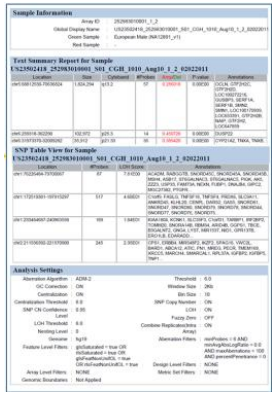
Value Proposition

- Streamlining the Cytogenetic data analysis workflow for CNC (copy number changes) and/or cnLOH / UPD detection of samples processed on Agilent SurePrint G3 CGH and CGH+SNP Microarrays.







To enable the Cytogenetic Researcher to analyze a normal CGH (or CGH+SNP) sample in less than 5 minutes, including report generation.

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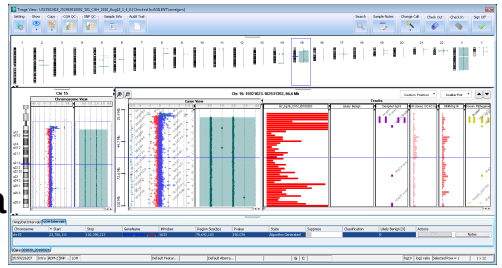
Streamlined data analysis for Cytogenetic research labs

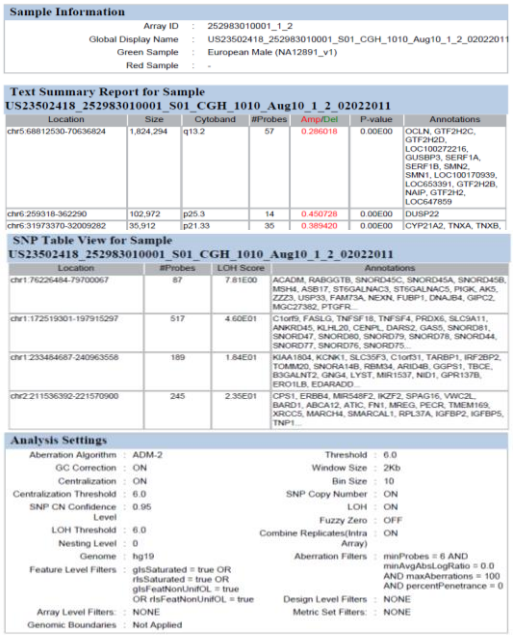
- Simple and quick workflow for data analysis
- Convenient input and output support allowing for integration with LIMS

Run data analysis



Triage & sign off

Cyto Report



Sample Information

Array ID	252983010001_1_2
Global Display Name	US23502418_252983010001_S01_CGH_1010_Aug10_1_2_02022011
Green Sample	European Male (NA12891_v1)
Red Sample	-

Text Summary Report for Sample US23502418_252983010001_S01_CGH_1010_Aug10_1_2_02022011

Location	Size	Cytoband	#Probes	Amp/Del	P-value	Annotations
chr5:68812530-70636824	1,824,294	q13.2	57	0.286018	0.00E00	OCLN, GTF2H2C, GTF2H2D, LOC100272216, GUSBP3, SERF1A, SERF1B, SMN2, SMN1, LOC100170939, LOC653391, GTF2H2B, NAIP, GTF2H2, LOC647859
chr6:259318-362290	102,972	p25.3	14	0.450728	0.00E00	DUSP22
chr6:31973370-32009282	35,912	p21.33	35	0.389420	0.00E00	CYP21A2, TNXA, TNXB,

SNP Table View for Sample US23502418_252983010001_S01_CGH_1010_Aug10_1_2_02022011

Location	#Probes	LOH Score	Annotations
chr1:76226484-79700067	87	7.81E00	ACADM, RABGGTB, SNORD45C, SNORD45A, SNORD45B, MSH4, ASB17, ST6GALNAC3, ST6GALNAC5, PIGK, AK5, ZZZ3, USP33, FAM73A, NEXN, FUBP1, DNAJB4, GIPC2, MGC27382, PTGFR...
chr1:172519301-197915297	517	4.60E01	C1orf9, FASLG, TNFSF18, TNFSF4, PRDX6, SLC9A11, ANKRD45, KLHL20, CENPL, DARS2, GAS5, SNORD81, SNORD47, SNORD80, SNORD79, SNORD78, SNORD44, SNORD77, SNORD76, SNORD75...
chr1:233484687-240963558	189	1.84E01	KIAA1804, KCNK1, SLC35F3, C1orf31, TARBP1, IRF2BP2, TCMBM20, SNORA14B, RBM34, ARID4B, GGPS1, TBCE, B3GALNT2, GNG4, LYST, MIR1537, NID1, GPR137B, ERO1LB, EDARADD...
chr2:211536392-221570900	245	2.35E01	CPS1, ERBB4, MIR548F2, IKZF2, SPAG16, VWC2L, BARD1, ABCA12, ATIC, FN1, MREG, PECR, TMEM169, XRCC5, MARCH4, SMARCAL1, RPL37A, IGFBP2, IGFBP5, TNP1...

Analysis Settings

Aberration Algorithm	ADM-2	Threshold	6.0
GC Correction	ON	Window Size	2Kb
Centralization	ON	Bin Size	10
Centralization Threshold	6.0	SNP Copy Number	ON
SNP CN Confidence Level	0.95	LOH	ON
		Fuzzy Zero	OFF
LOH Threshold	6.0	Combine Replicates(Intra Array)	ON
Nesting Level	0		
Genome	hg19	Aberration Filters	minProbes = 6 AND minAvgAbsLogRatio = 0.0 AND maxAberations = 100 AND percentPenetrance = 0
Feature Level Filters	gIsSaturated = true OR rIsSaturated = true OR gIsFeatNonUnifOL = true OR rIsFeatNonUnifOL = true	Design Level Filters	NONE
Array Level Filters:	NONE	Metric Set Filters:	NONE
Genomic Boundaries	Not Applied		

Sample info, export from LIMS

Laboratory LIMS

Cyto report, upload to LIMS

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Workflow setup and user roles

- Initial set up: Configuring the Workflow

1. Configure analysis method → **2. Configure CytoReport** → **3. Configure Workflow**

- Day to day usage:

  – **Technician (manual) / Auto-processing**

  1. Upload TIFF images (or FE files)
  2. Map array to sample attributes
  3. Engage **configured workflow** to run FE (TIFF only) and analysis

  – **Scientist**

  1. Triage the sample and sign off
  2. Generate the CytoReport

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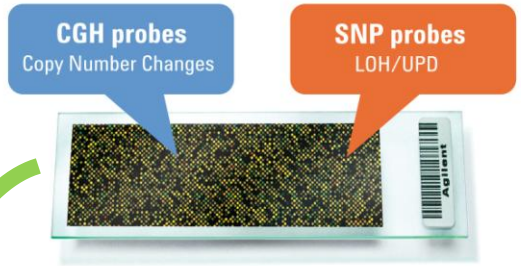
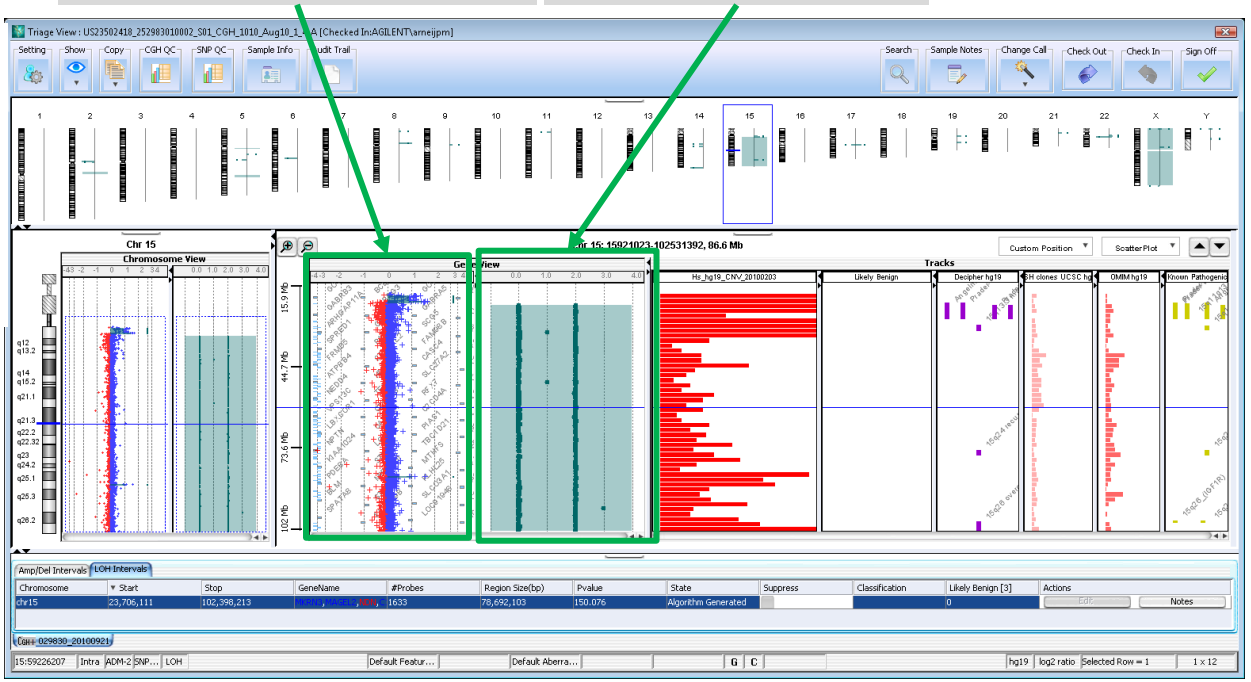
Powerful algorithms for calling CNC, LOH and UPD

Log_2 ratio of intensities to call CNCs

No. of uncut alleles to call LOH/UPD





Feature Extraction

ADM-1
ADM-2 — **Amp/Del calling**

SNP CN & LOH — **LOH calling**

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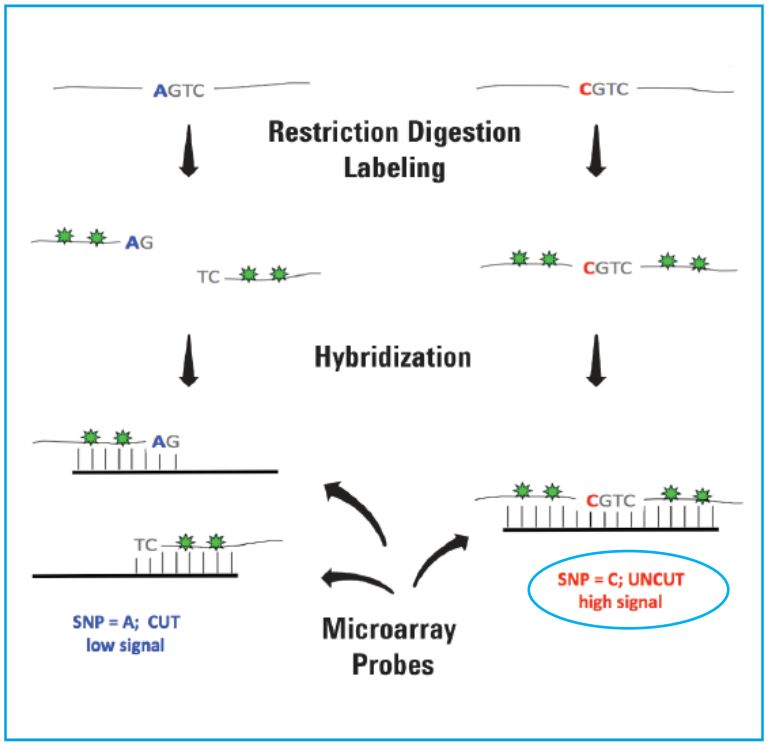
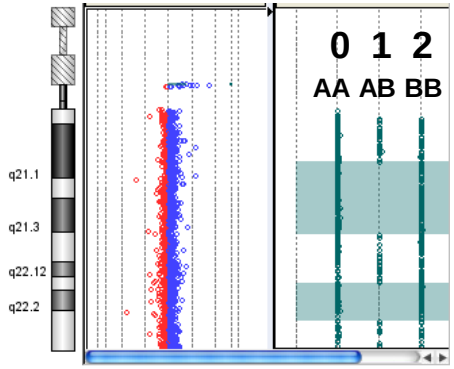
Using SNPs to detect LOH and UPD



- Genotype SNPs using **restriction digestion** (Alu/Rsa)
- 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 scarcity of heterozygous call**
- **~5-10 Mb LOH/UPD resolution** across the entire genome

Genomic status	Genotype	No. uncut allele
Normal diploid genome	AA, AB, BB	0, 1, 2
Diploid genome with copy-neutral LOH or UPD	AA, BB	0, 2
Hemizygous LOH	A, B	0,1
Amplification: e.g. trisomy	AAA, AAB, ABB, BBB	0, 1, 2, 3



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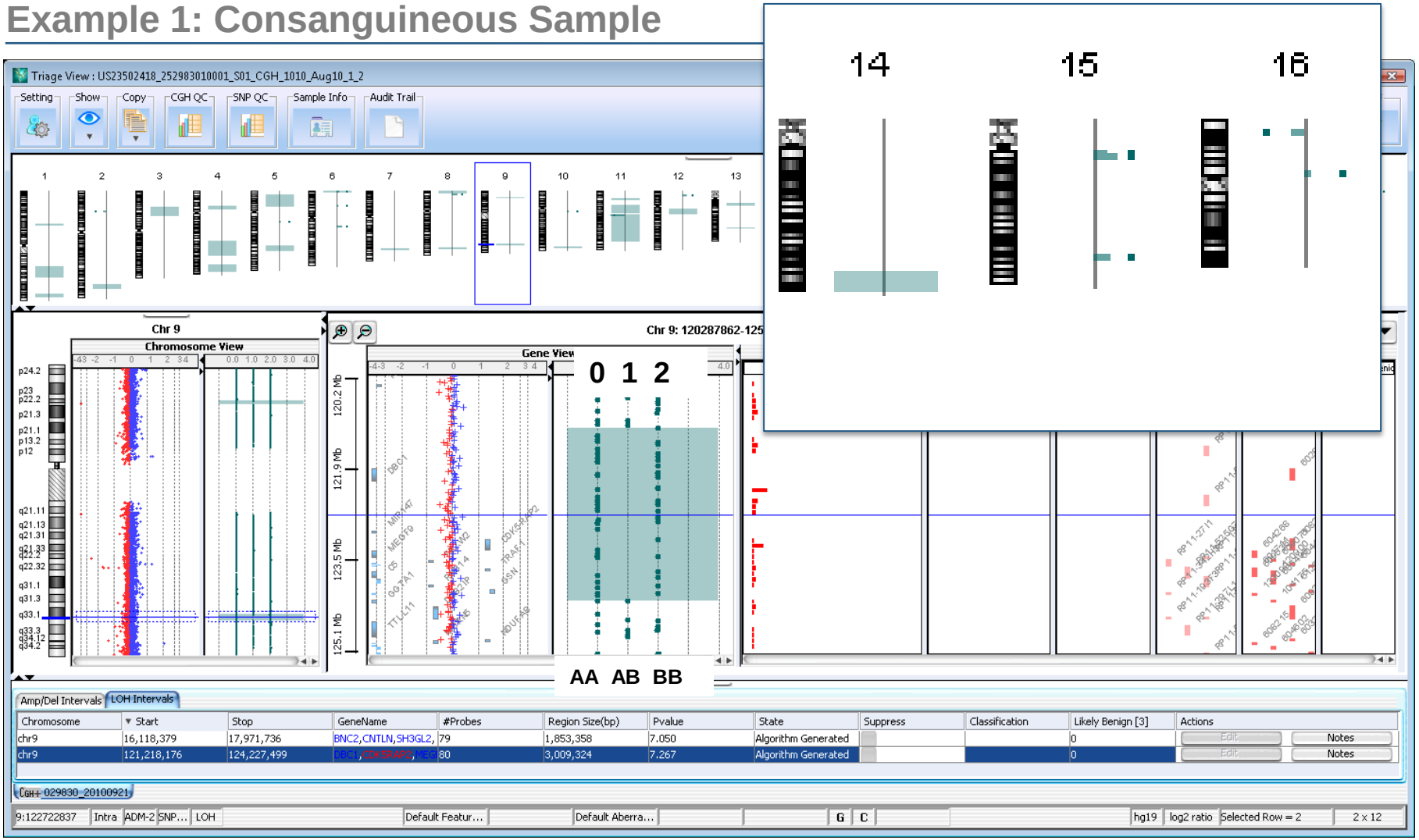
Example 1: Consanguineous Sample



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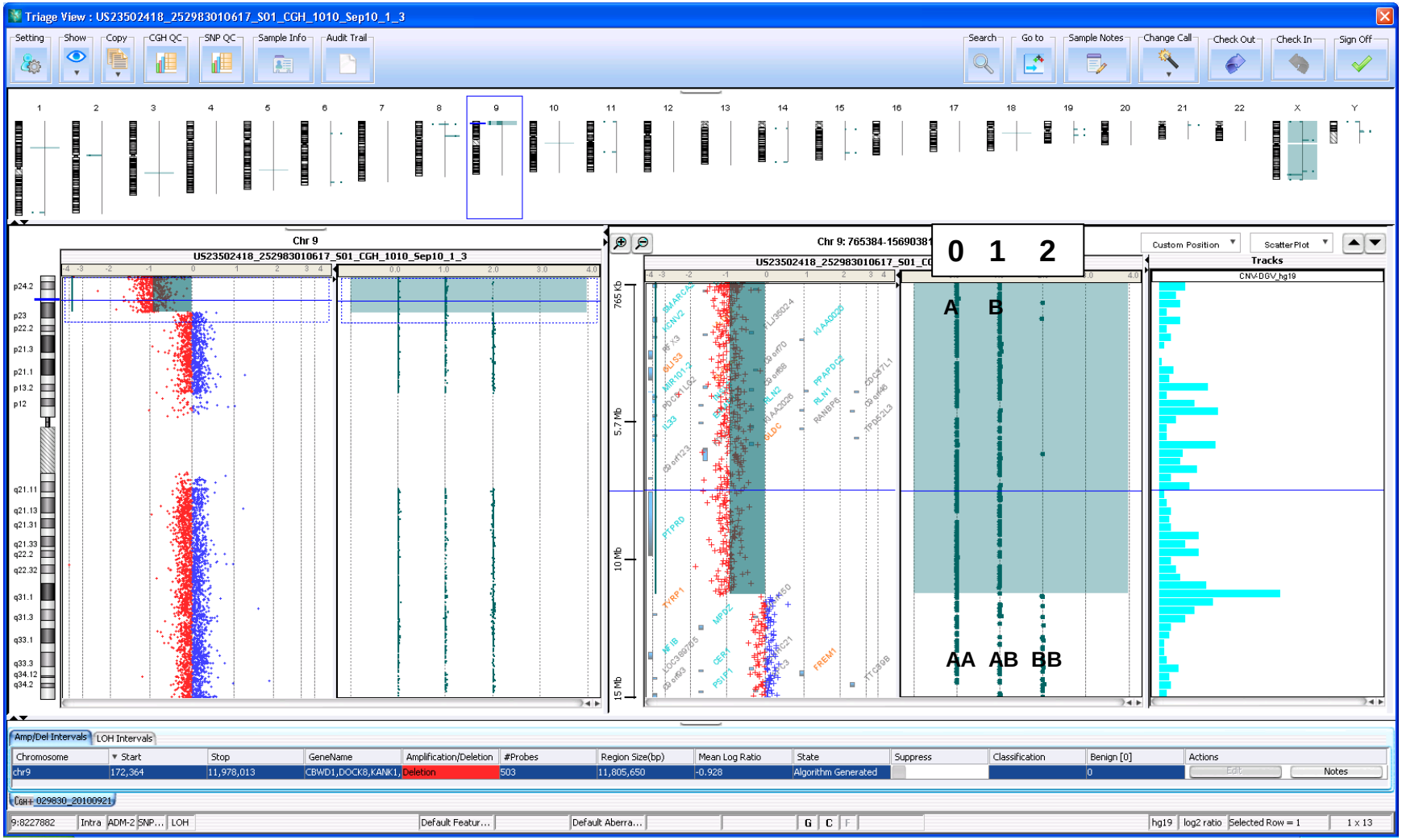
Example 2: Sample with one copy deletion on chr9 p-arm



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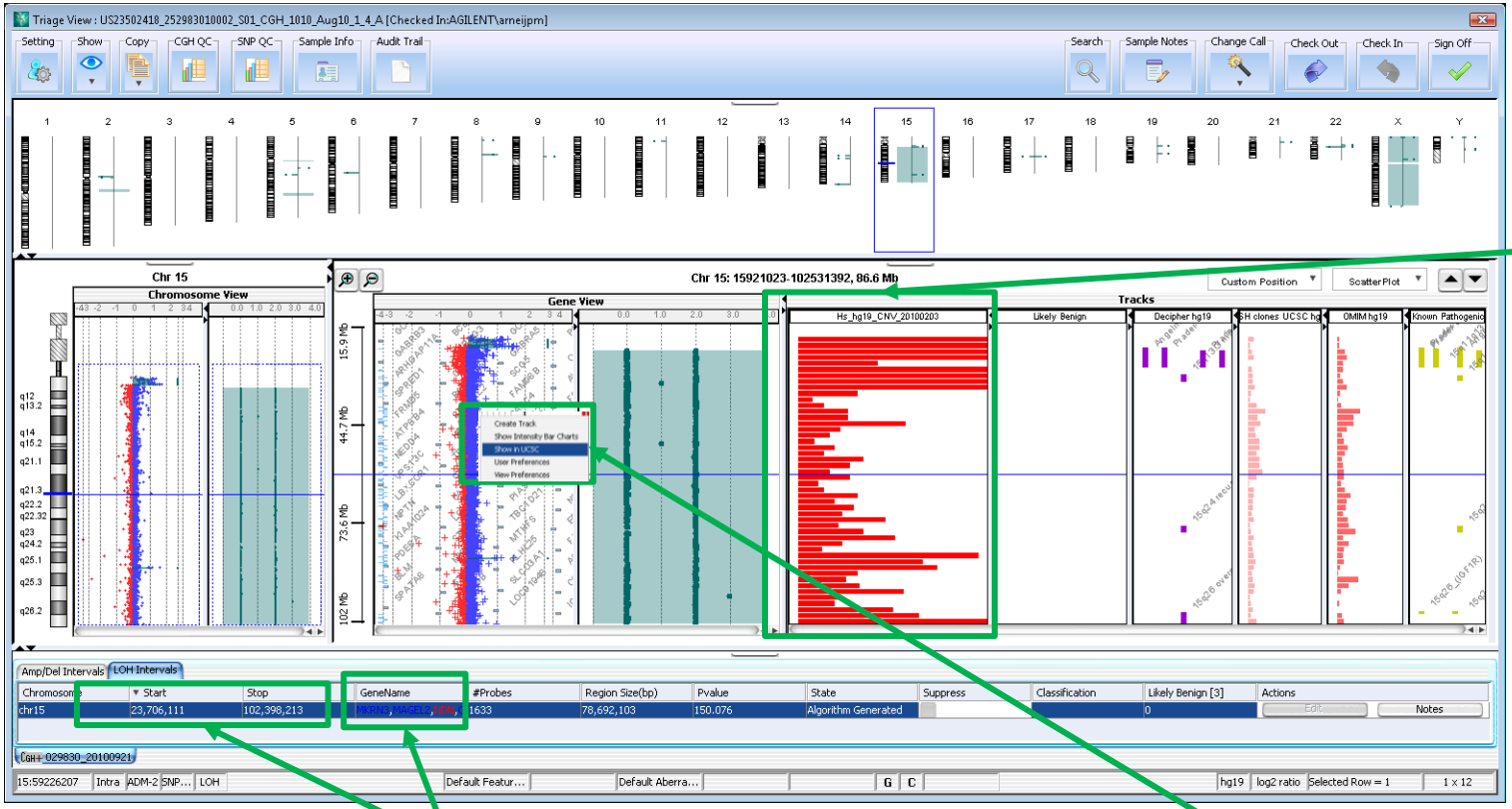
Sample Triage – Access to external databases



Preloaded track: DGV-CNV

Link out from aberrations and associated genes to external Databases: UCSC, DGV, OMIM, Entrez

View and compare aberrations in DGV and UCSC genome browser

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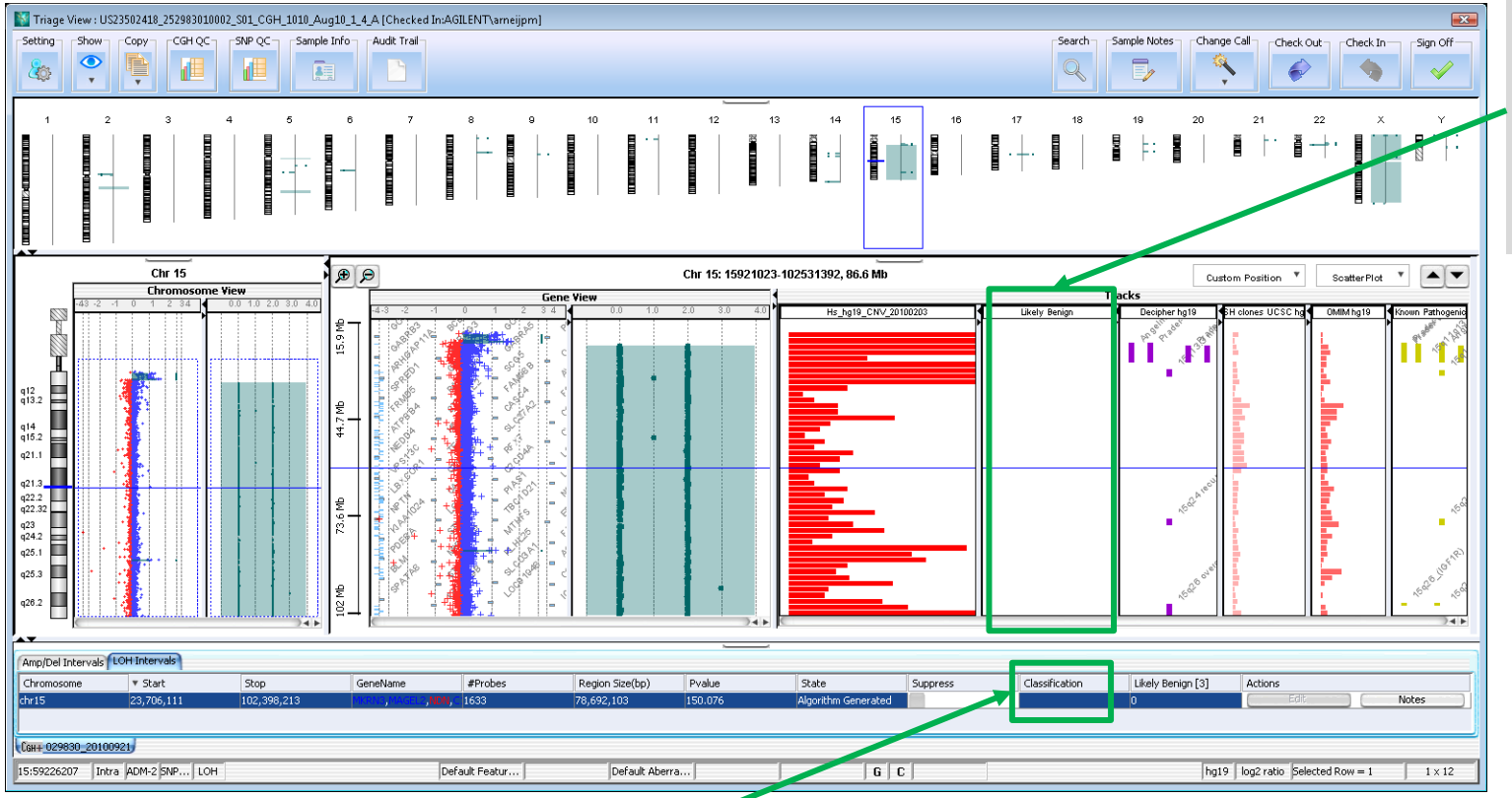
Sample Triage – Using CytoGenomics Database



Auto updated tracks based on aberration classification in CytoGenomics Database

Right click on 'Classification' allows query for overlapping aberrations in CytoGenomics database: results will be shown as new track along the gene view

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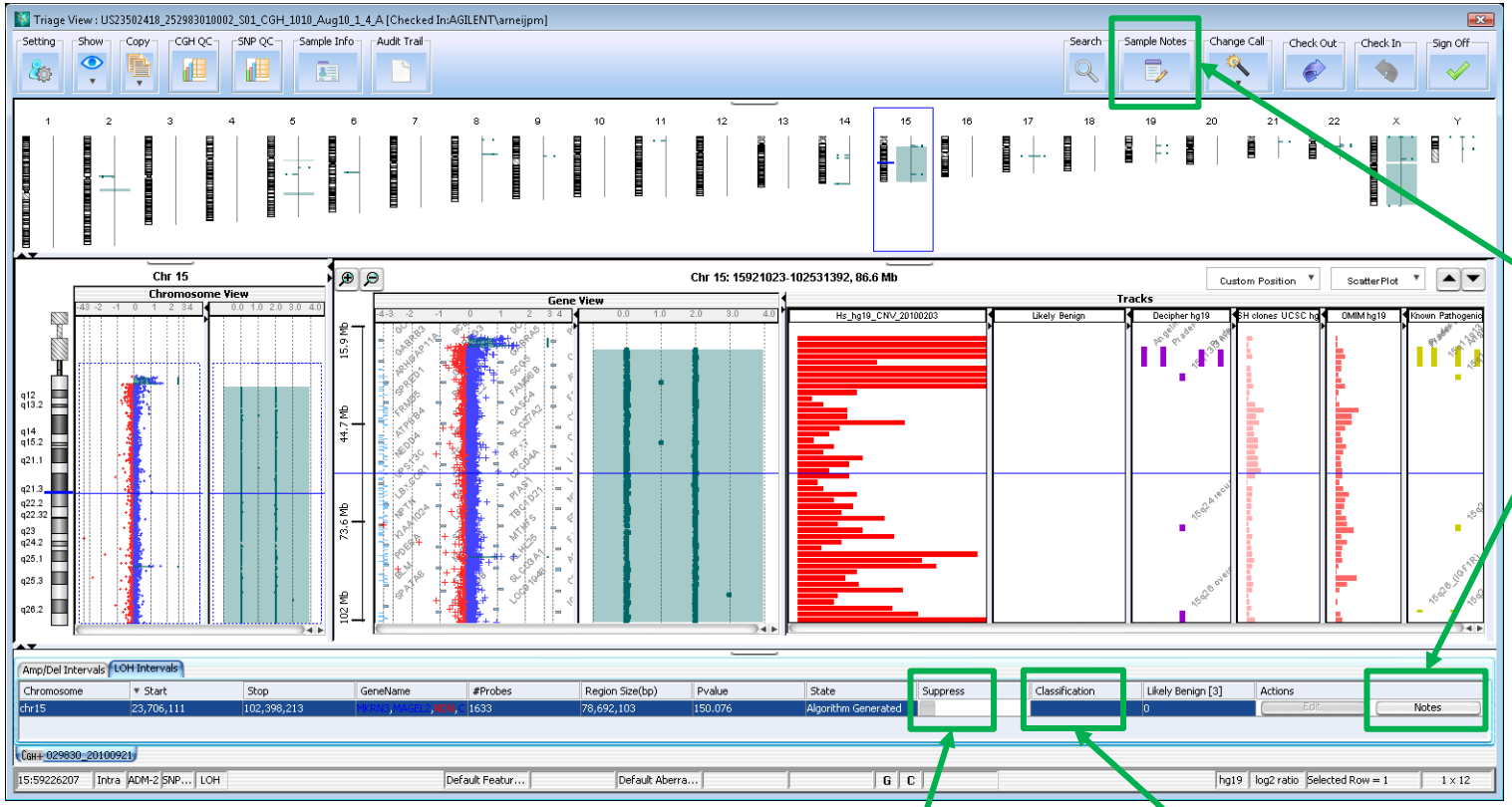
Sample Triage – Sample and Aberration annotation



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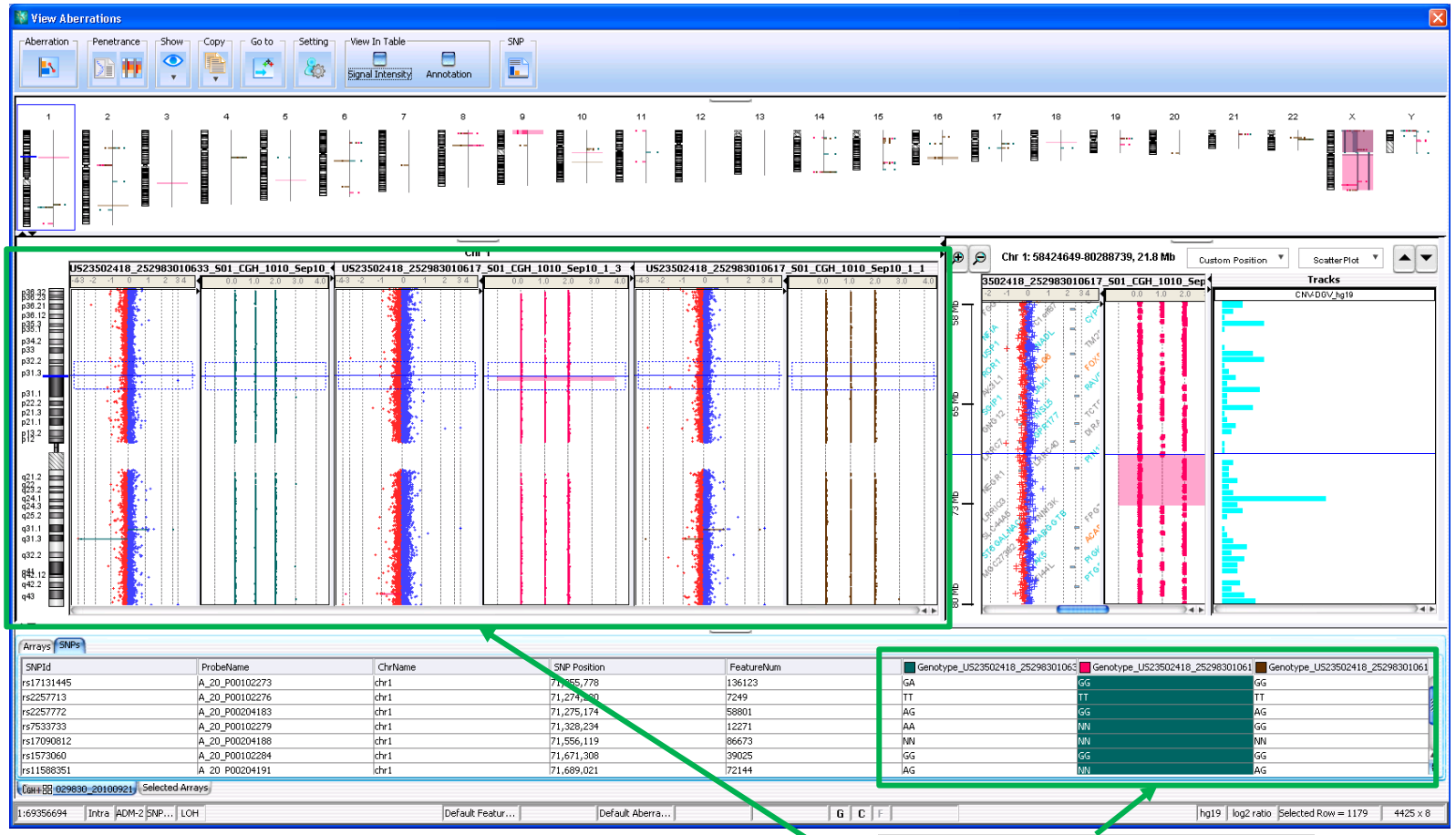
Multi Sample View



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Additional features

- Workflow Automation Mode for automatic processing of TIFF images and report generation.
- Data upload to ISCA DB via Cartagenia bench.

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System specs

- Client/server system with database (multiple users can access database at the same time).
- Runs on PC (XP, Windows 7).
- Runs on Mac (OS X Leopard and OS X Snow Leopard).
- Supported on both 32 and 64 bit machines.
- Minimum of 4GB of memory needed for 32 bit (8GB for 64 bit).
- Feature extraction is integrated component of CytoGenomics software.

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