

# Karyostat™ + Service : An Efficient and High Throughput Alternative to Karyotyping Stem Cells using Chromosomal Microarray

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## Abstract

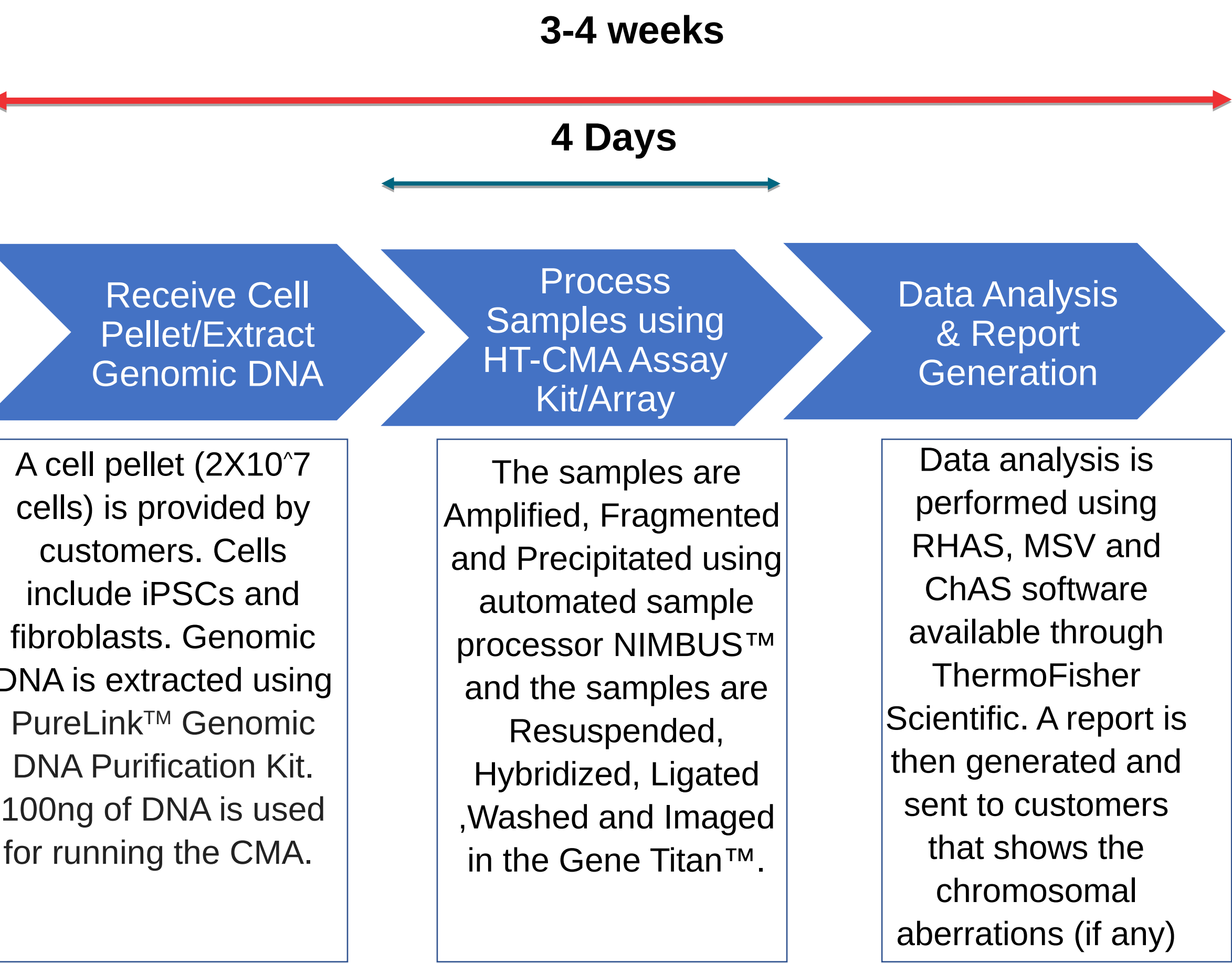
Genetic abnormalities, such as copy number and single nucleotide variants (SNVs), are common in several disease states. Population screening and cytogenetics studies are performed to identify these structural changes and point modifications in DNA. It is even more important in the context of hiPSC cells to confirm that the clonal population derived from the donors have the same genetic background and has not acquired any novel duplications or deletions in the derived hiPSCs. Chromosomal microarray analysis (CMA), also known as comparative genomic hybridization (CGH), and SNP arrays are clinical tools that are currently used to evaluate these stem cells. The Applied Biosystems™ CytoScan™ HT-CMA Arrays are optimized solutions for the detection of chromosomal abnormalities, such as deletions and duplications, in a high throughput automated format. We offer a high throughput Karyotyping service that provides much better resolution than the traditional G-Banding and provides customers with a fast turn around of data, which includes any chromosomal aberrations. The HT-CMA array provides a genome wide copy number analysis using an easy, interpretable Chromosome Analysis Suite software. In this poster, we describe in detail the Karyostat+™ service for cell characterization that produces reliable, low-noise image data with a high signal to noise ratio that is fast, efficient, high in throughput, and is offered at competitive pricing.

## CytoScan™ HT –CMA Arrays

CytoScan HT-CMA Array specifications	
Markers for copy number analysis	
Total number of copy number markers	1,162,042
Number of nonpolymorphic markers	133,823
Number of SNP markers	1,028,219
Genome build	hg19, hg38
Median marker spacing (base pairs)	
Intragenic	953
Intergenic	4,114
Overall	1,337
Percentage of genes covered (50 markers/100 kb)	
DECIPHER (1,778)	100%
Morbid OMIM (2,220)	99%
Percentage of genes covered (50 markers/400 kb)	
RefSeq (37,580)	96%

**Table 1.** Provides an overview of the CytoScan HT-CMA array attributes. The array is in a 96 format with a 5-micron feature size that has 400 Kb resolution of Whole genome backbone and Refseq genes; 100Kb resolution of Morbid OMIM and decipher genes with 178 sequence and targeted variants.

## WorkFlow of Karyostat+ Service



**Figure 1.** Shows the overview of the workflow of Karyostat + services. The boxes underneath shows more detailed breakdown of each step in the workflow.

## Comparison of Various Commercial Microarrays

Feature	Agilent aCGH (60K)	Illumina microarray	CytoScan 750K	CytoScan Optima	CytoScan HT-CMA	CNV-Seq	Karyotyping
Copy number resolution (genome-wide)	Variable, Mb	Variable (100–500 kb)	100–500 kb	500 kb–2 Mb	50–400 kb	2–5 Mb	>5 Mb
LOH detection	No	5 Mb	3 Mb	5 Mb	3 Mb	No	No
Triploidy detection	No	Yes	Yes	Yes	Yes	No	Yes
Maternal contamination detection	No	Yes	Yes	Yes	Yes	No	No
Input gDNA	100–200 ng	100 ng	50–250 ng	50–250 ng	50–100 ng	Low	NA
Sequence variant detection capability (e.g., hearing loss)	No	?	No	No	Yes	No	No
Throughput	Low	Flexible	Low	Low	High	High	Low

Table 2. Comparison of CytoScan HT-CMA Arrays to other microarrays and Karyotyping

## Principle Behind CytoScan HT CMA

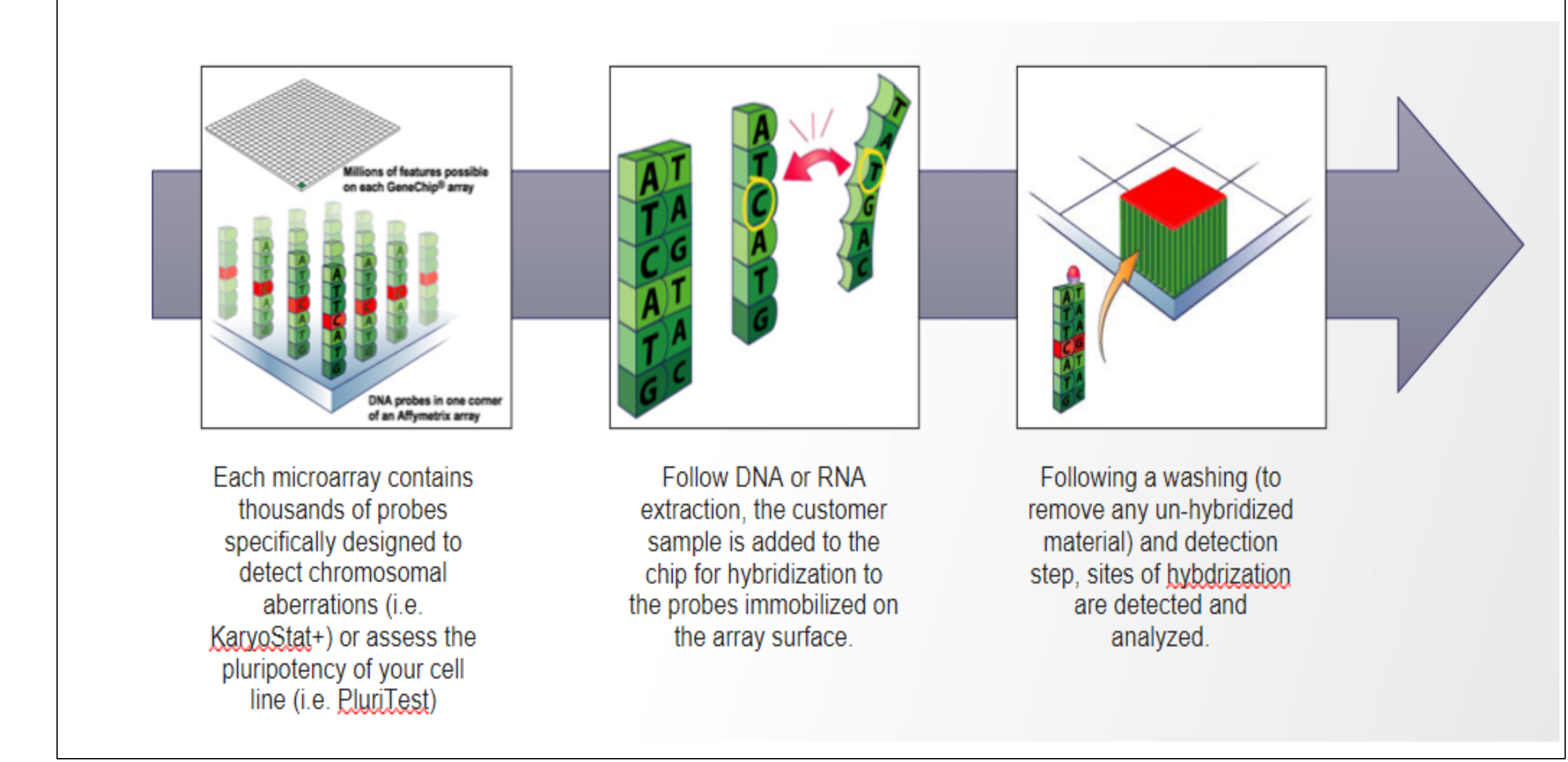


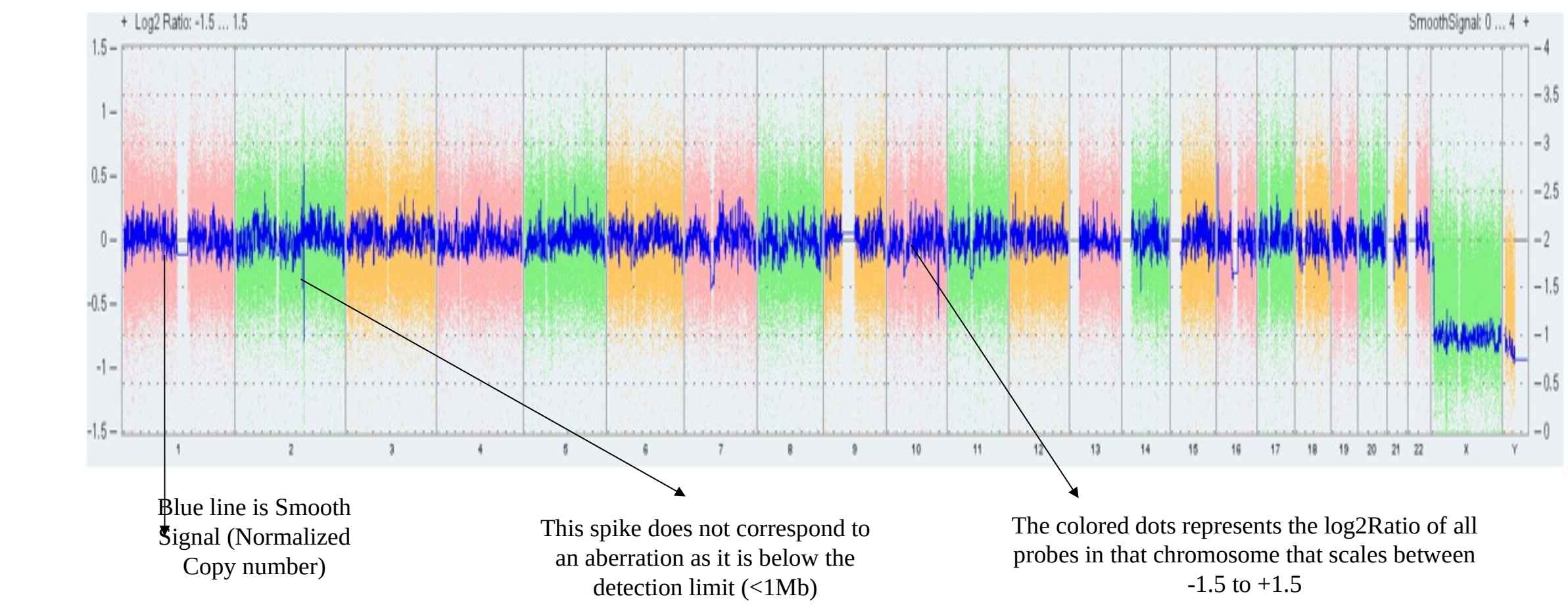
Figure 2. Shows the principle of chromosomal microarray in CytoScan HT assay.

## Statistical parameters for Sample QC

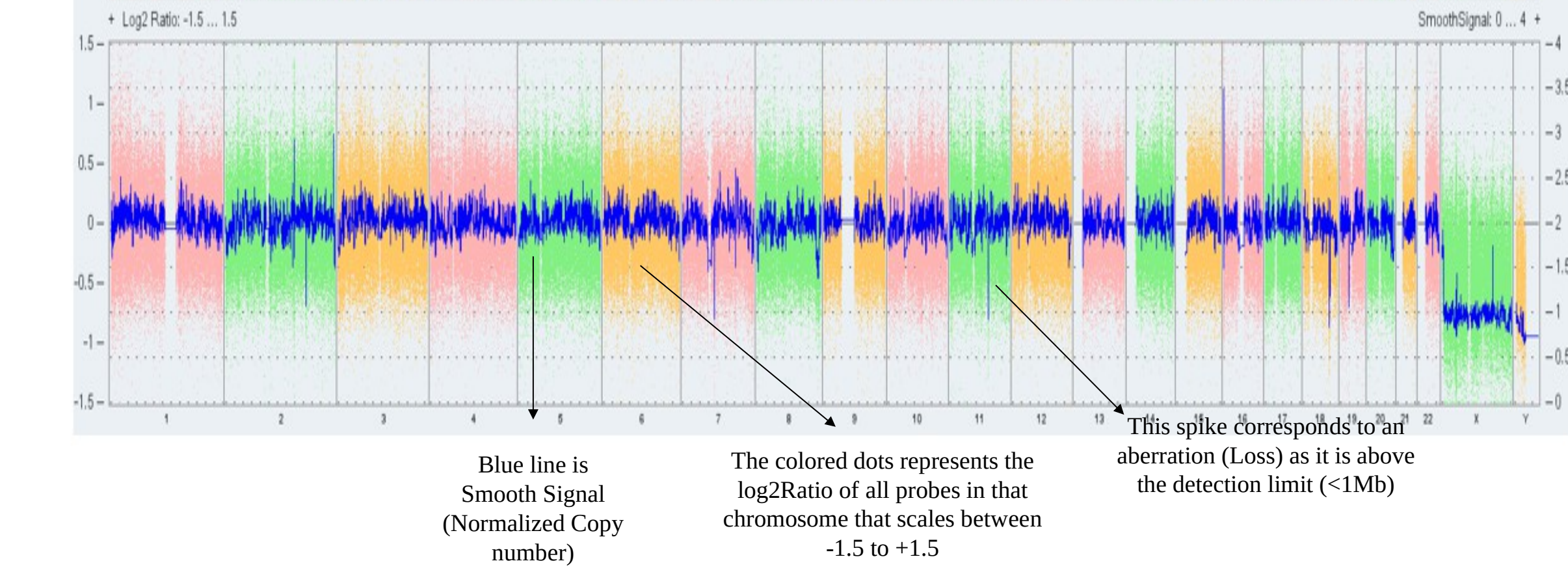
In Process QC	Passing Criteria
Genomic DNA quantitation/purity	260/280:1.8 to 2.0; 260/230:>1.5
DNA conc after resuspension	>1000 ng
Fragmentation gel QC	25-125bp
Data Analysis QC	
MAPD	≤0.29
SNPQC	≥ 10.0

Table 3. Provides the parameters for a sample to be considered passed. If these stringent conditions are not met, we fail the sample and reprocess the sample one more time as per our terms and conditions. If the sample fails a second time, we notify our customers.

## Whole Genome View of Normal sample

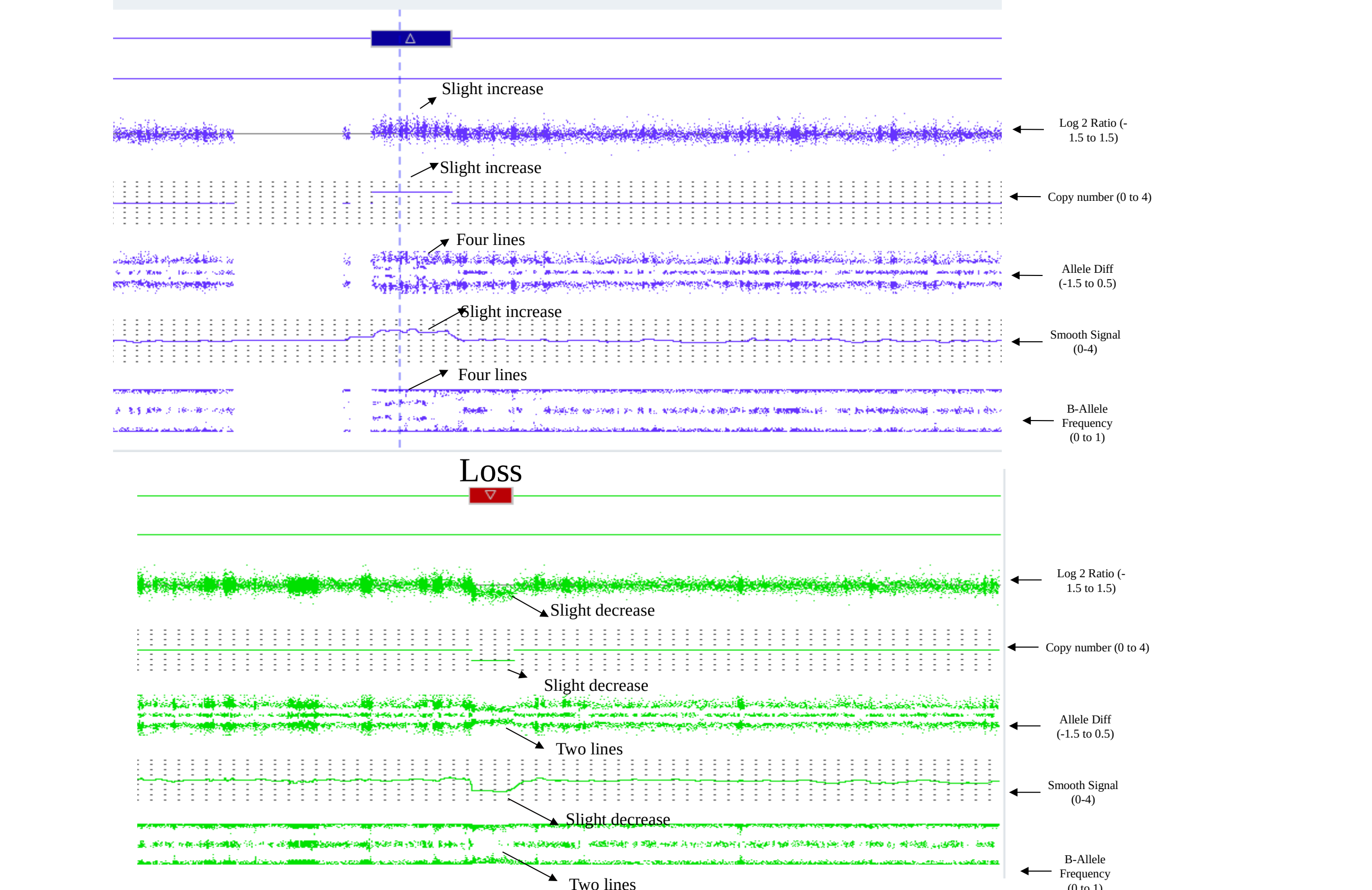


## Whole Genome View of sample showing deletion



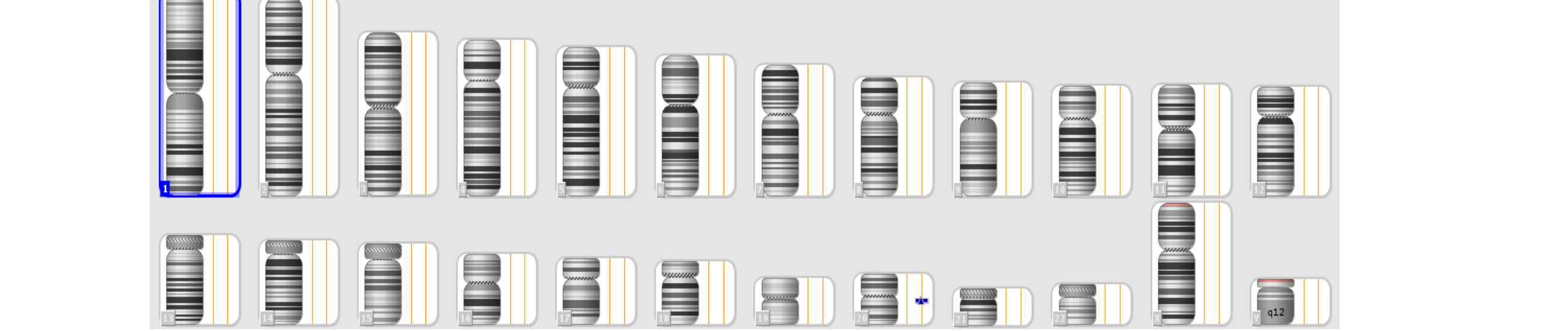
**Figure 3.** Shows the whole genome view showing log2 ratios and copy numbers for normal and aberrated sample.

## Side by side comparison of copy numbers, smooth signal, BAF, and AD in samples with aberrations



**Figure 4.** To decide if a spike is “True” aberration versus “False”, we look at copy number and log2 Ratios in the context of allele difference and B-allele frequency. If we see duplication, we see a trace of four lines in AD and BAF data, and in cases of deletion, we observe a tracer of two lines in BAF and AD data.

## Karyoview of samples



**Figure 5.** A Karyoview of sample is provided with the report only when samples have aberrations. In the above example chromosome 20 has a gain with a copy number =3

## Segment table showing the summary of aberrations

File: KS-11789_1_KSR-10051-real.rchcp CN State: 3.00 Type: Gain Chromosome: 20 Materially Modified By: Cytoband Start: q11.21 Cytoband End: q11.22 Size (bp): 4,561 Marker Count: 2,315 Gene Count: 166 Genes: DEFB118, DEFB118, DEFB118, DEFB121, DEFB122, DEFB123, DEFB124, REM1, LINC00028, HM13, HM13-AS1, ID1, MIR3193, COX4I2, BCL2L1, ABALON, TPX2, MYLK2, FOXS1, DUSP15, TTL9, PORGI1, XKR7, CCM2L, HCK, TM6SF4, TSPY2P, PLAGL2, POFU11, MIR1825, KIF3B, ASXL1, NOL4L, LOC101926968, NOL4L-DT, C20orf203, COMMD7, DNMT3B, MAPRE1, EPCAB2, SUN5, BPIFB2, BPIFB3, BPIFB4, BPIFB5, BPIFB6, BPIFB7, BPIFB8, BPIFB9, BPIFB10, BPIFB11, BPIFB12, BPIFB13, BPIFB14, BPIFB15, BPIFB16, BPIFB17, BPIFB18, BPIFB19, BPIFB20, BPIFB21, BPIFB22, BPIFB23, BPIFB24, BPIFB25, BPIFB26, BPIFB27, BPIFB28, BPIFB29, BPIFB30, BPIFB31, BPIFB32, BPIFB33, BPIFB34, BPIFB35, BPIFB36, BPIFB37, BPIFB38, BPIFB39, BPIFB40, BPIFB41, BPIFB42, BPIFB43, BPIFB44, BPIFB45, BPIFB46, BPIFB47, BPIFB48, BPIFB49, BPIFB50, BPIFB51, BPIFB52, BPIFB53, 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