SNP 6.0 GeneChip arrays: Strengths and limitations for copy number analysis

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Major applications of SNP arrays

- GW Linkage analysis : *Hum Mol Genet 2006, 15, 2903*
- GW Association studies : *Nature, 2007, 447, 661*
  Nature, 2007, 447, 1087
- Copy number and LOH estimation : *Genes Chr. & Cancer, 2008, May 27 [Epub]*
  PNAS 2008,105,6708
  Nature 2007, 446, 758

Major suppliers for SNP arrays:

- Affymetrix and Illumina

Affymetrix GeneChip® arrays

Hybridization based

- Mapping 10K 2.0 :10,000 SNPs
- Mapping 100K (2 x 50K) : 2 x 50,000 SNPs
- Mapping 500K (2 x 250K) : 2 x 250,000 SNPs

- GW Human SNP arrays 6.0 : 900,000 SNP
  + 900,000 CN probes

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ARTICLES

Genome-wide analysis of genetic alterations in acute lymphoblastic leukaemia

Charles G. Mullighan[1]*, Salil Goorha[1]*, Ina Radtke[1], Christopher B. Miller[1], Elaine Coustan-Smith[2], James D. Dalton[1], Kevin Girtman[1], Susan Mathew[1]†, Jing Ma[5], Stanley B. Pounds[3], Xiaoping Su[5], Ching-Hon Pui[2], Mary V. Relling[4], William E. Evans[4], Sheila A. Shurtleff[1] & James R. Downing[1]

Chromosomal aberrations are a hallmark of acute lymphoblastic leukaemia (ALL) but alone fail to induce leukaemia. To identify cooperating oncogenic lesions, we performed a genome-wide analysis of leukaemic cells from 242 paediatric ALL patients using high-resolution, single-nucleotide polymorphism arrays and genomic DNA sequencing. Our analyses revealed deletion, amplification, point mutation and structural rearrangement in genes encoding principal regulators of B lymphocyte development and differentiation in 40% of B-progenitor ALL cases. The *PAX5* gene was the most frequent target of somatic mutation, being altered in 31.7% of cases. The identified *PAX5* mutations resulted in reduced levels of PAX5 protein or the generation of hypomorphic alleles. Deletions were also detected in *TCF3* (also known as *E2A*), *EBF1*, *LEF1*, *IKZF1* (*IKAROS*) and *IKZF3* (*AIOLOS*). These findings suggest that direct disruption of pathways controlling B-cell development and differentiation contributes to B-progenitor ALL pathogenesis. Moreover, these data demonstrate the power of high-resolution, genome-wide approaches to identify new molecular lesions in cancer.

Mullighan et al, Nature, 2007

Microdeletions are a general feature of adult and adolescent acute lymphoblastic leukemia: Unexpected similarities with pediatric disease

Kajsa Paulsson*†‡, Jean-Baptiste Cazier*§, Finlay MacDougall*, Jane Stevens*, Irina Stasevich*, Nikoletta Vrcelj*, Tracy Chaplin*, Debra M. Lillington*, T. Andrew Lister*, and Bryan D. Young*

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Paulsson et al, PNAS, 2008

Map of UPDs in 450 AML samples

GENES, CHROMOSOMES & CANCER 00:000–000 (2008)

RESEARCH ARTICLES

Novel Regions of Acquired Uniparental Disomy Discovered in Acute Myeloid Leukemia

Manu Gupta,[1] Manoj Raghavan,[1] Rosemary E. Gale,[2] Claude Chelala,[3] Christopher Allen,[2] Gael Molloy,[1] Tracy Chaplin,[1] David C. Linch,[2] Jean-Baptiste Cazier,[1,4] and Bryan D. Young[1]*

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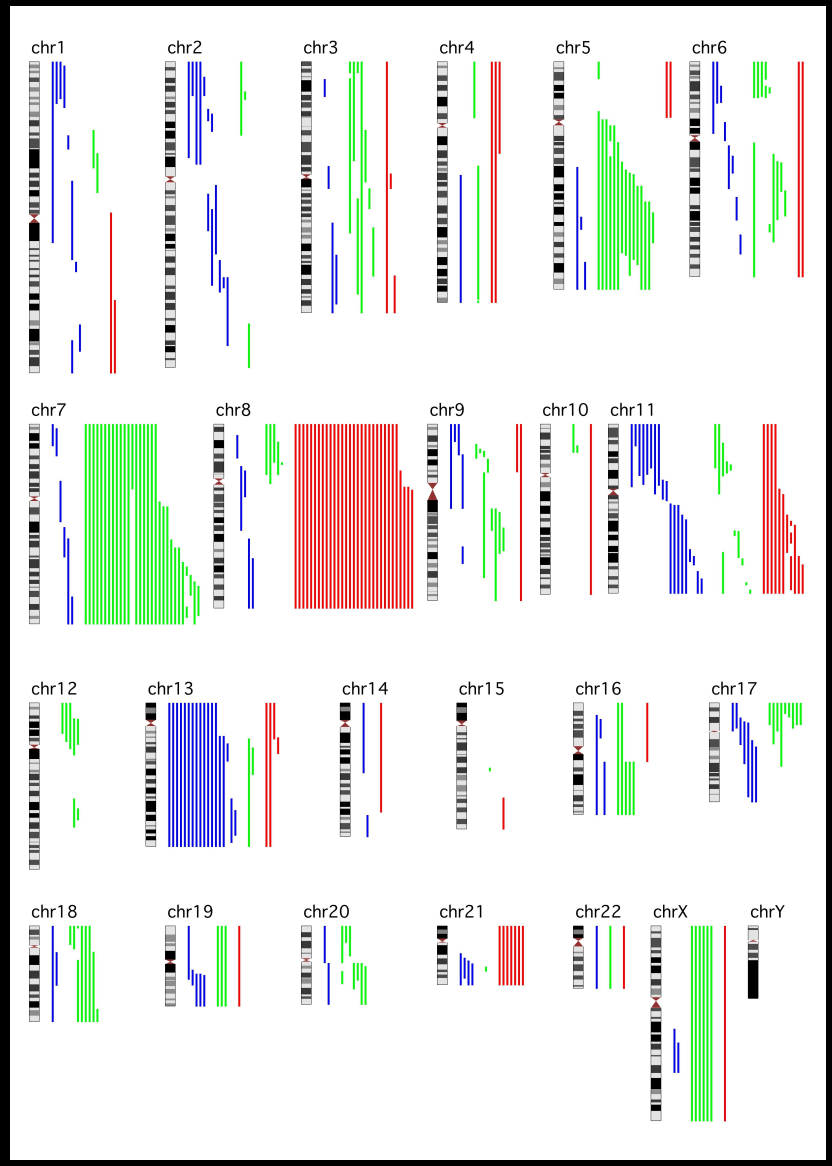
[4]Cancer Research UK Bioinformatics & Biostatistics Service, London WC2A 3PX, UK

Gupta et al,
Genes Chromosomes and Cancer, 2008

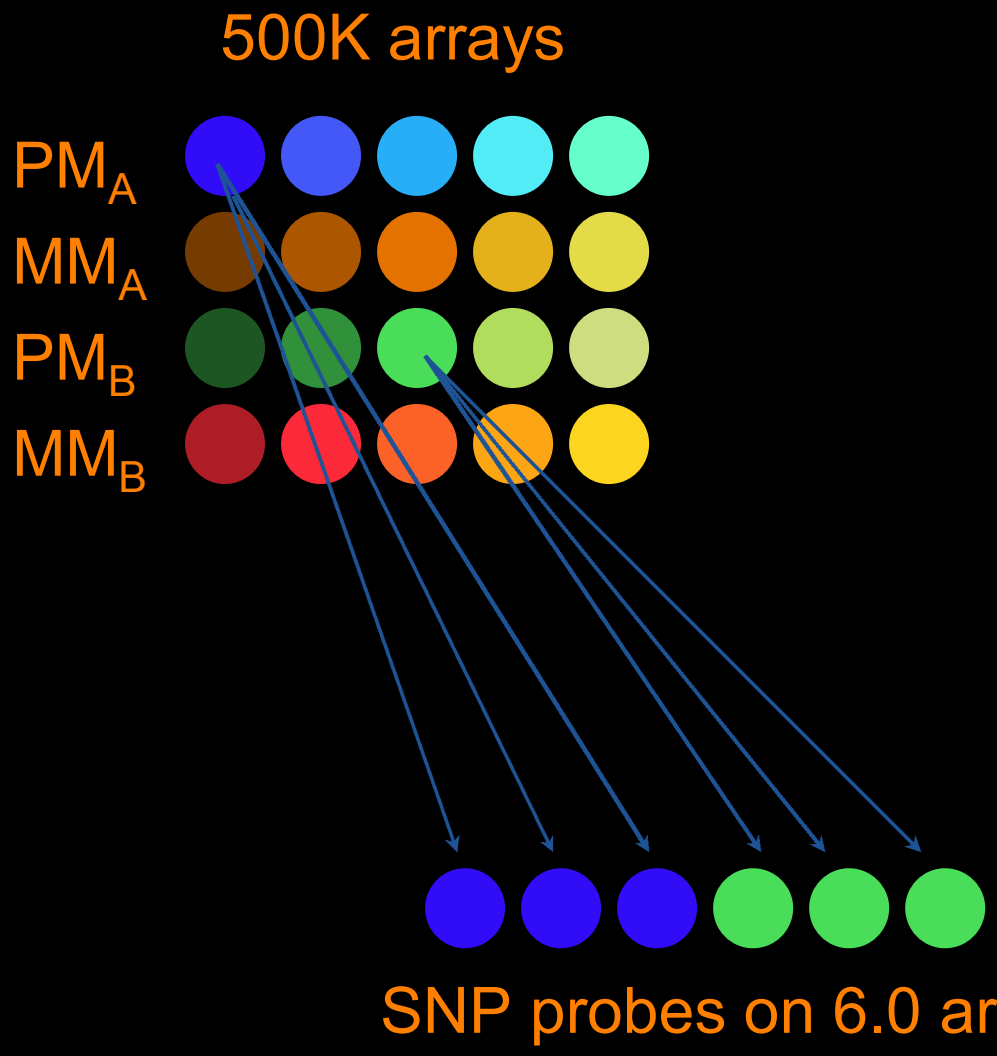


UPD Loss Gain

The 6.0 array - Design

- Over 1.8 million markers of genetic variation
  - 906,600 SNPs
    - 482,000 from the 500K arrays
    - Another 424,000 SNPs (HapMap, mitochondrial, recombination hotspots, X and Y chr)
  - Over 946,000 non-polymorphic probes for Copy number detection
    - 202K probes in known CNV regions
    - 744K probes “tiled evenly” along the genome

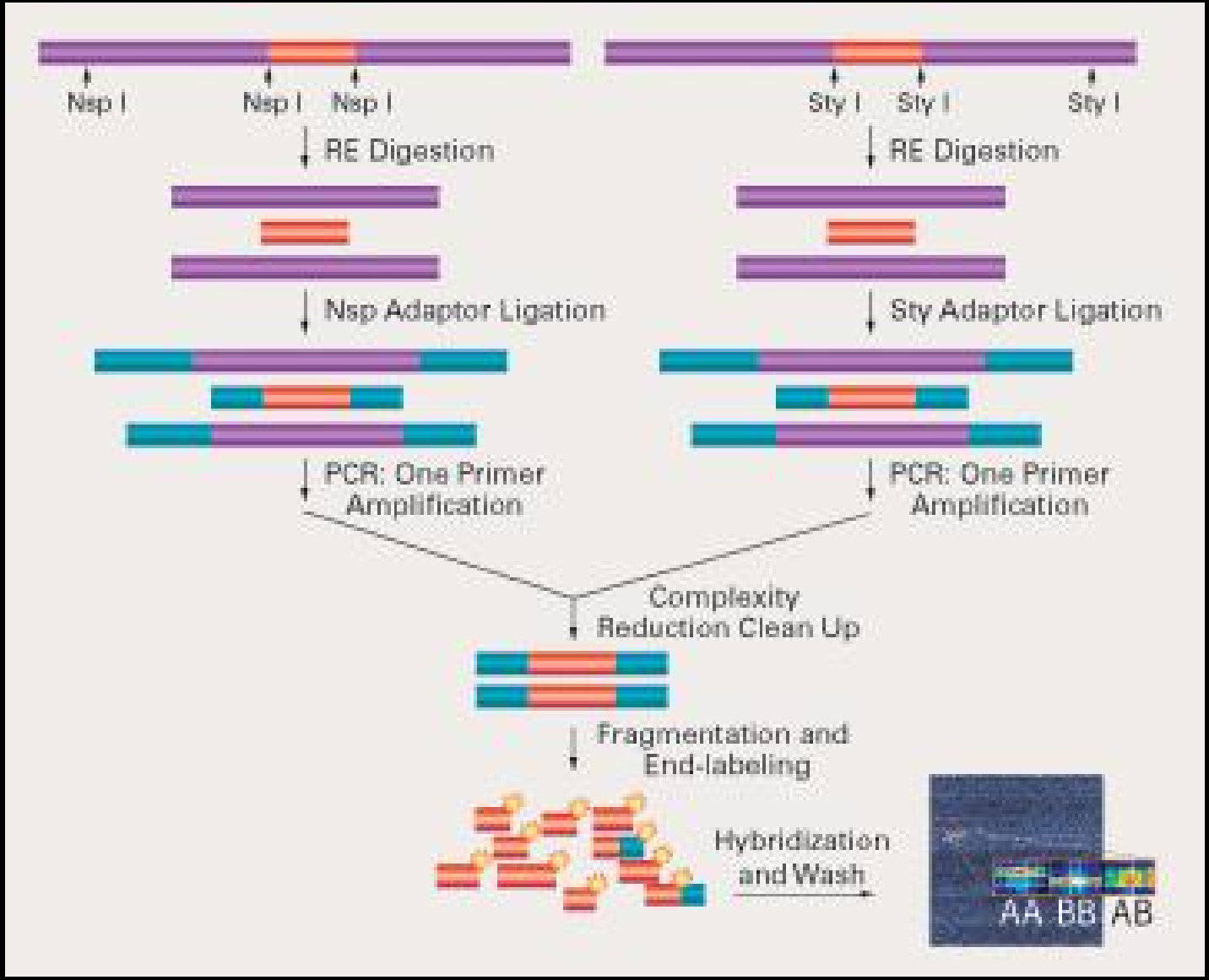
The 6.0 array - Design



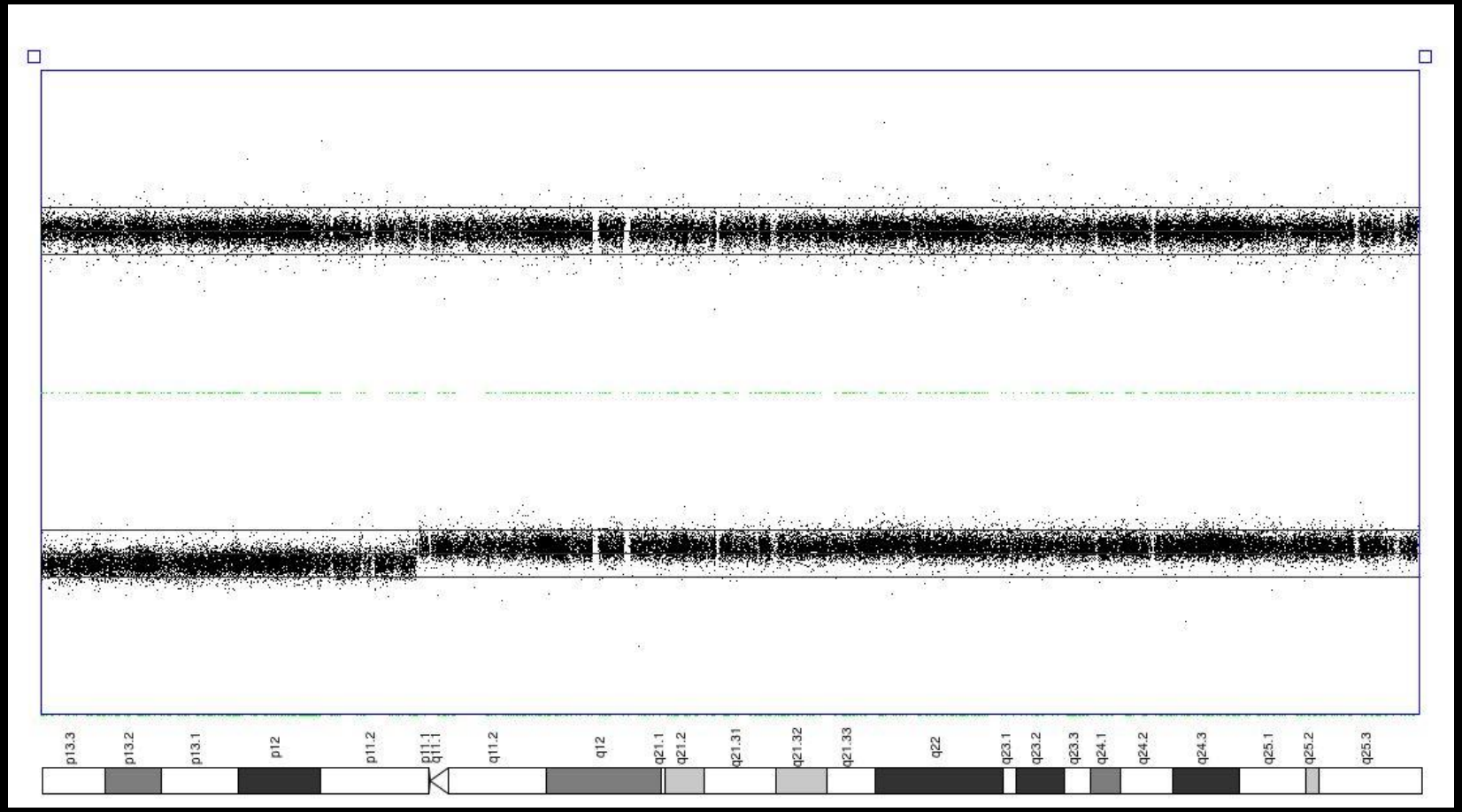
Resolution:
1 marker every
700 bp on average

CN probe

Experimental protocol for 6.0 arrays



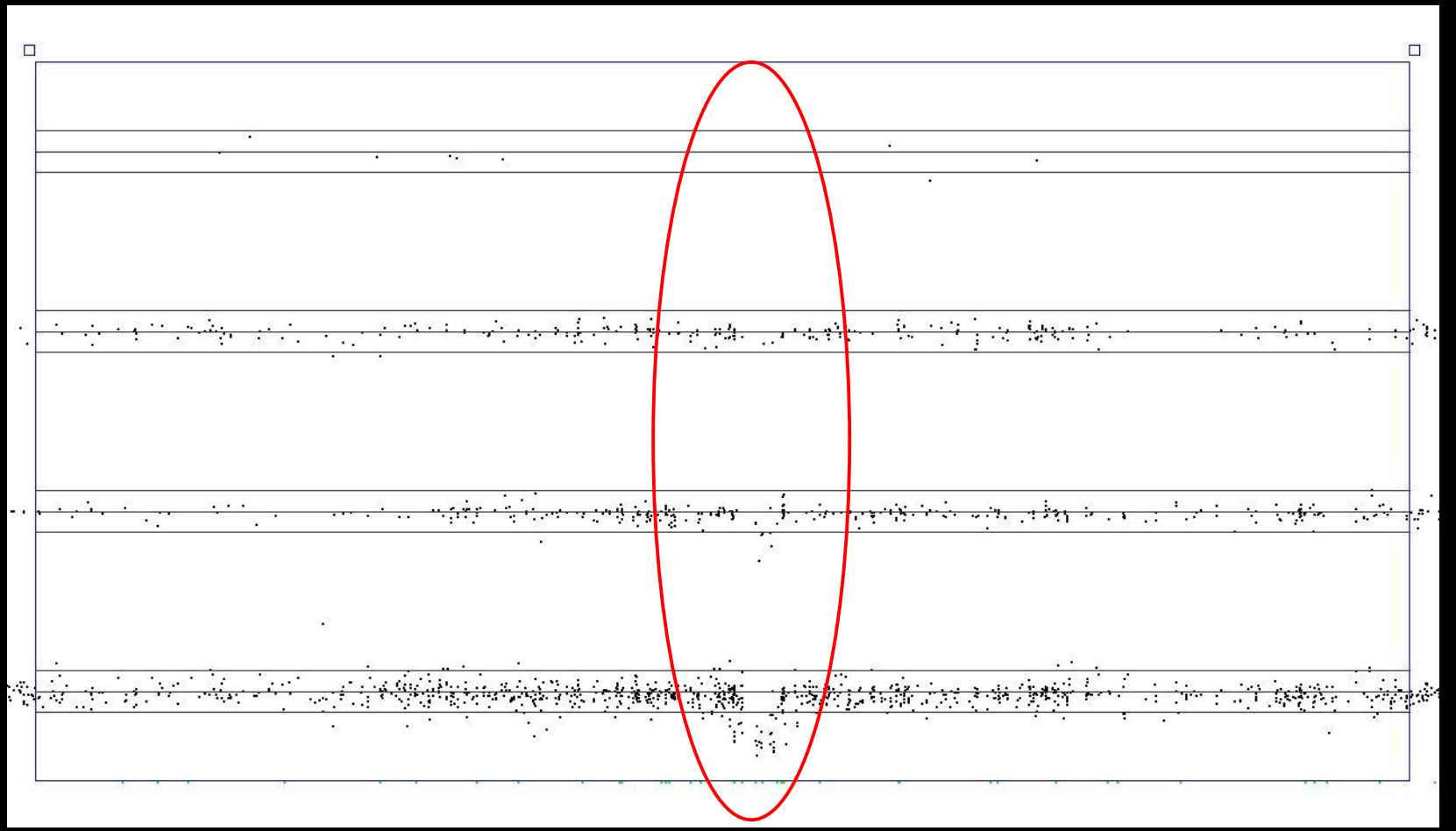
6.0 SNP array results: Chr17



Sample 0067

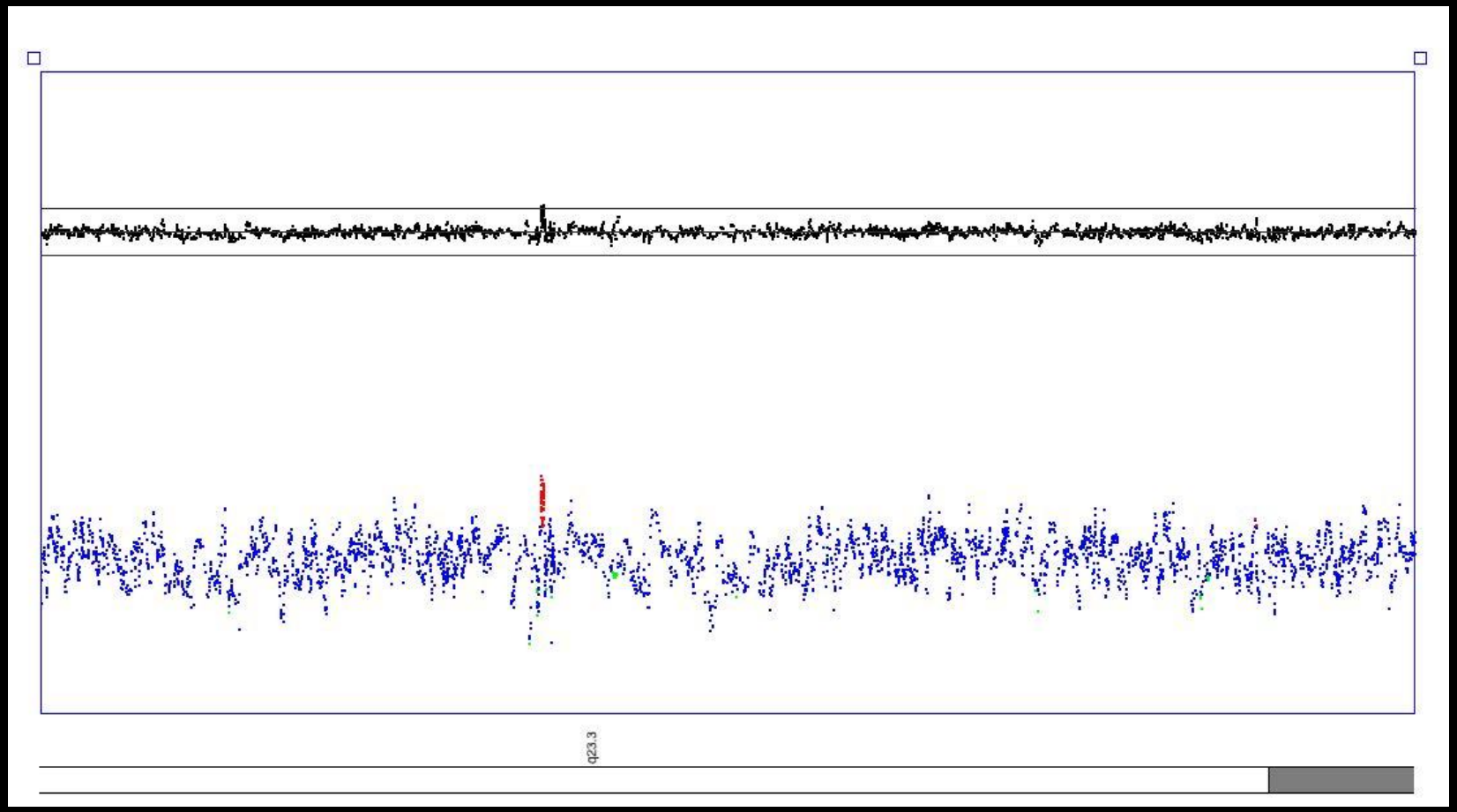
Sample 0065

Increased resolution is the strength of 6.0 arrays: Sample 69_D: UPD6p



Loss of ~65 Kb

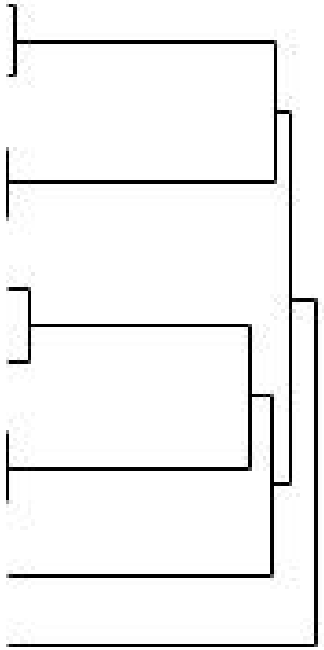
Detection of MLL-PTD in an AML sample by 6.0 arrays



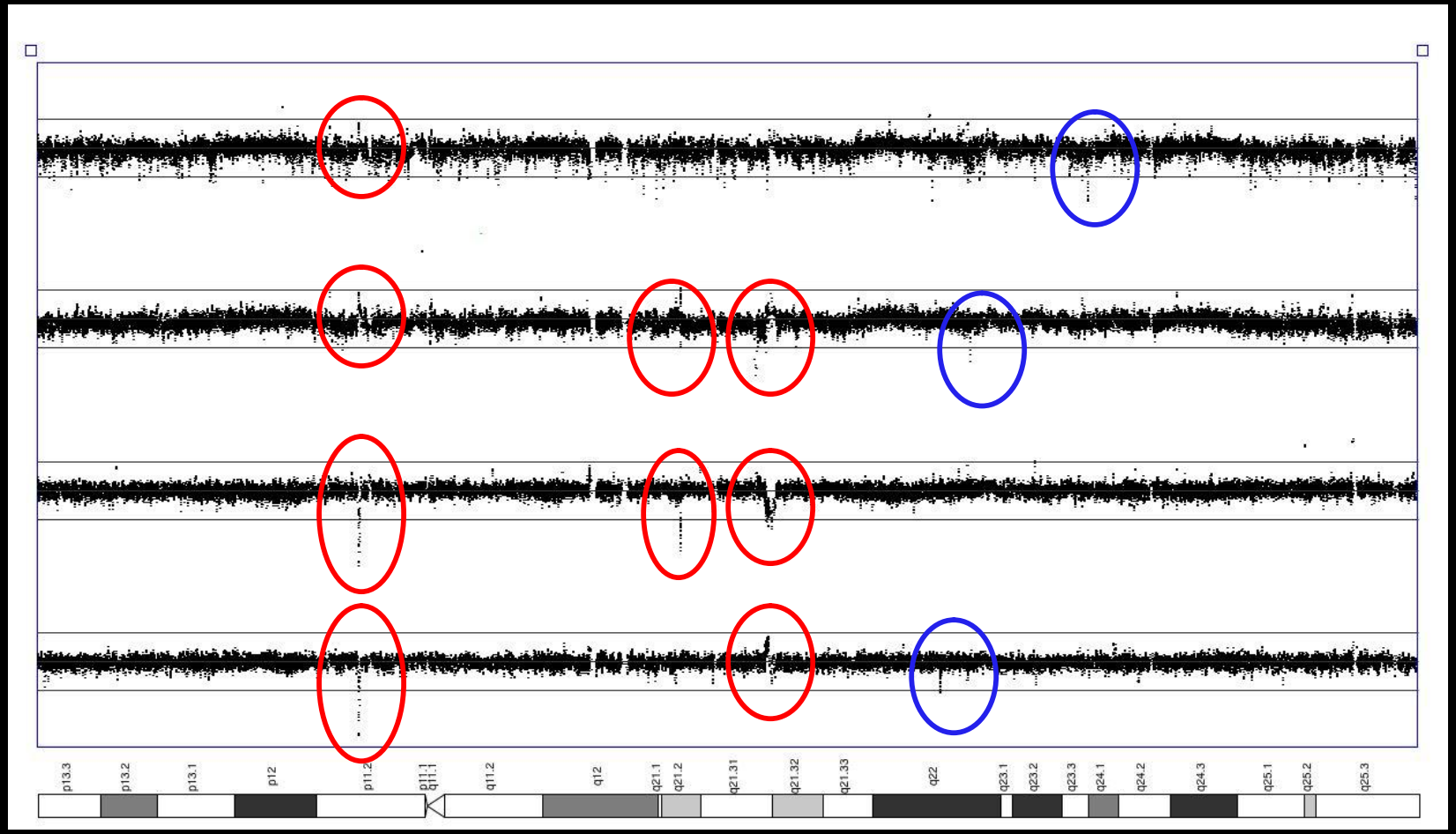
Size: 5Kb-17Kb 43 CN probes, 3 SNP probes

Genotype information allows QC of patient samples

AML26_R_14_SNP6.birdseed.txt
GSM173401_SNP6.birdseed.txt
AML132_R_09_SNP6.birdseed.txt
GSM173397_SNP6.birdseed.txt
AML94_R_04_SNP6.birdseed.txt
AML69_D_04_SNP6.birdseed.txt
AML92_R_22_SNP6.birdseed.txt
AML59_D_22_SNP6.birdseed.txt
S0275FL0031.birdseed.txt
S0275FL0032.birdseed.txt



CNVs detected in human genome (Fig: Chr 17)

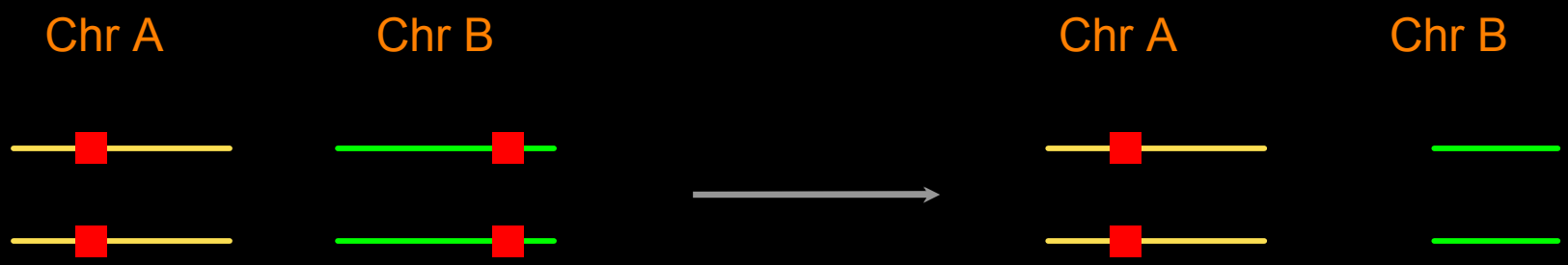


4 normal samples compared against a pooled control

Analysis can further be made complex by Constitutional CNVs/ segmental duplications

Germ Line

Tumor sample

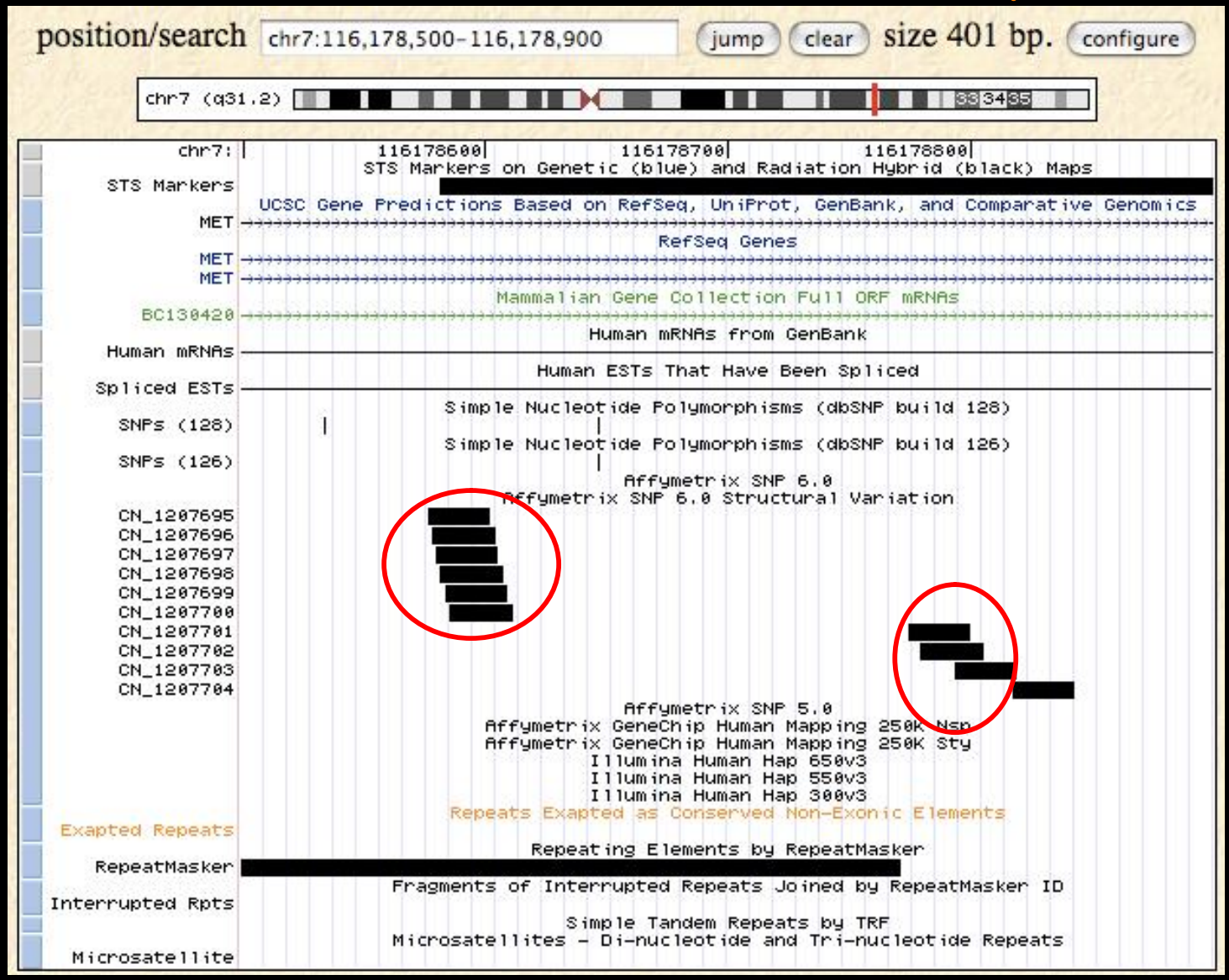


4 copies of ■ marker

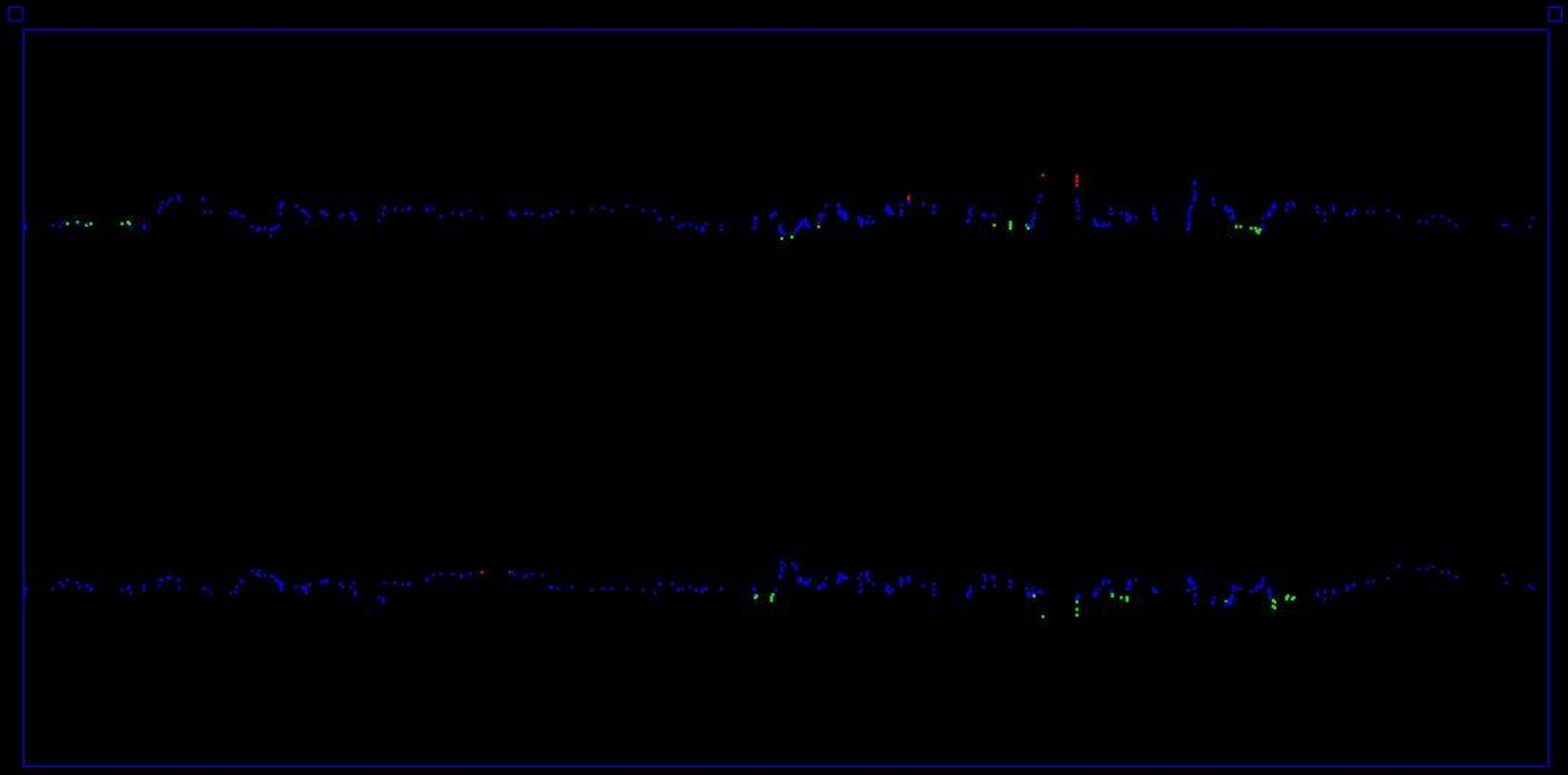
2 copies of ■ marker

A deletion on Chr B would also reflect a heterozygous deletion on Chr A, even though Chr A is normal

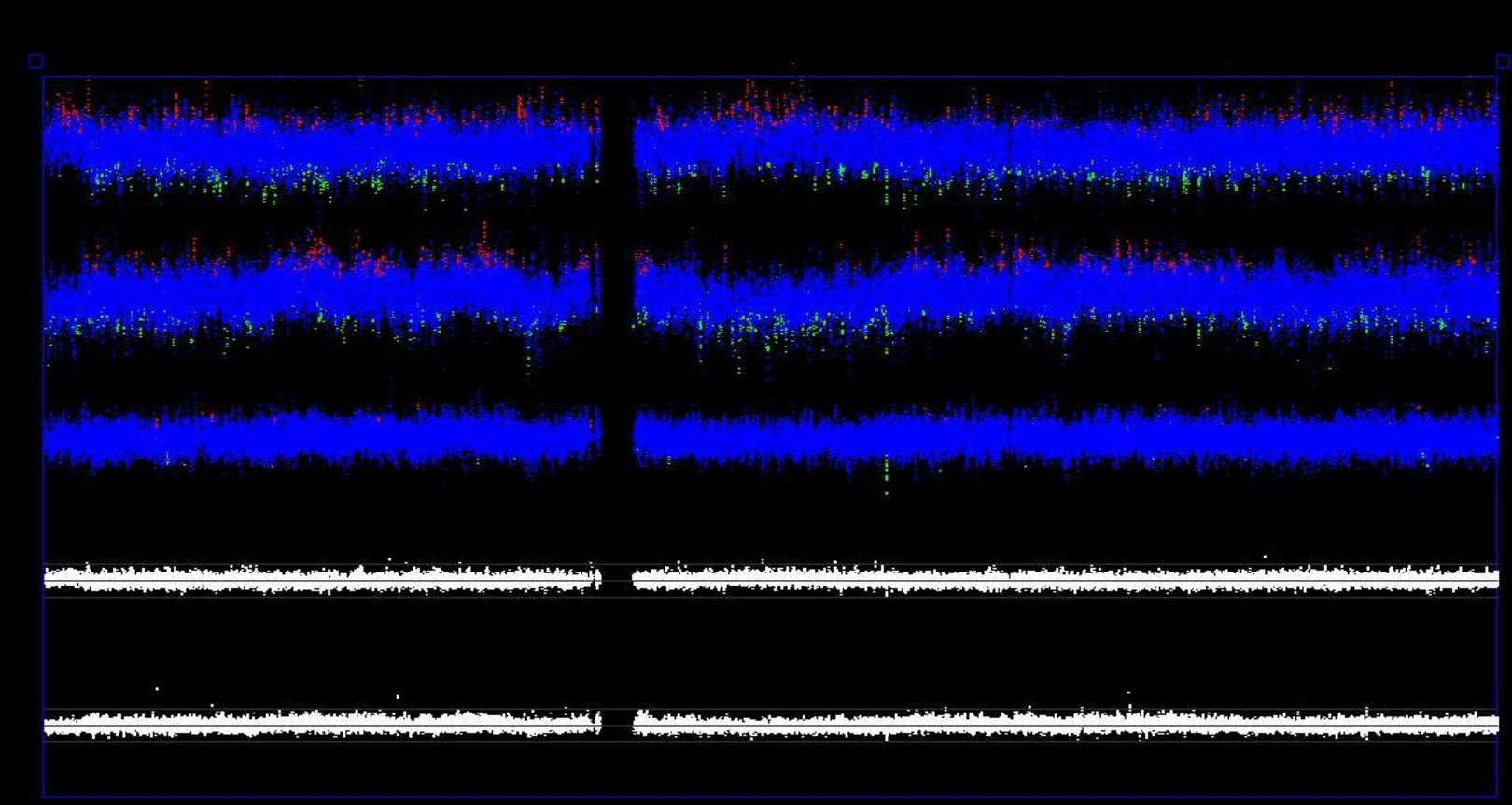
Limitation I: Overlapping probes considered as independent



Limitation I: Overlapping probes considered as independent



Replicates increase signal to noise ratio: avoid false positives and true negatives

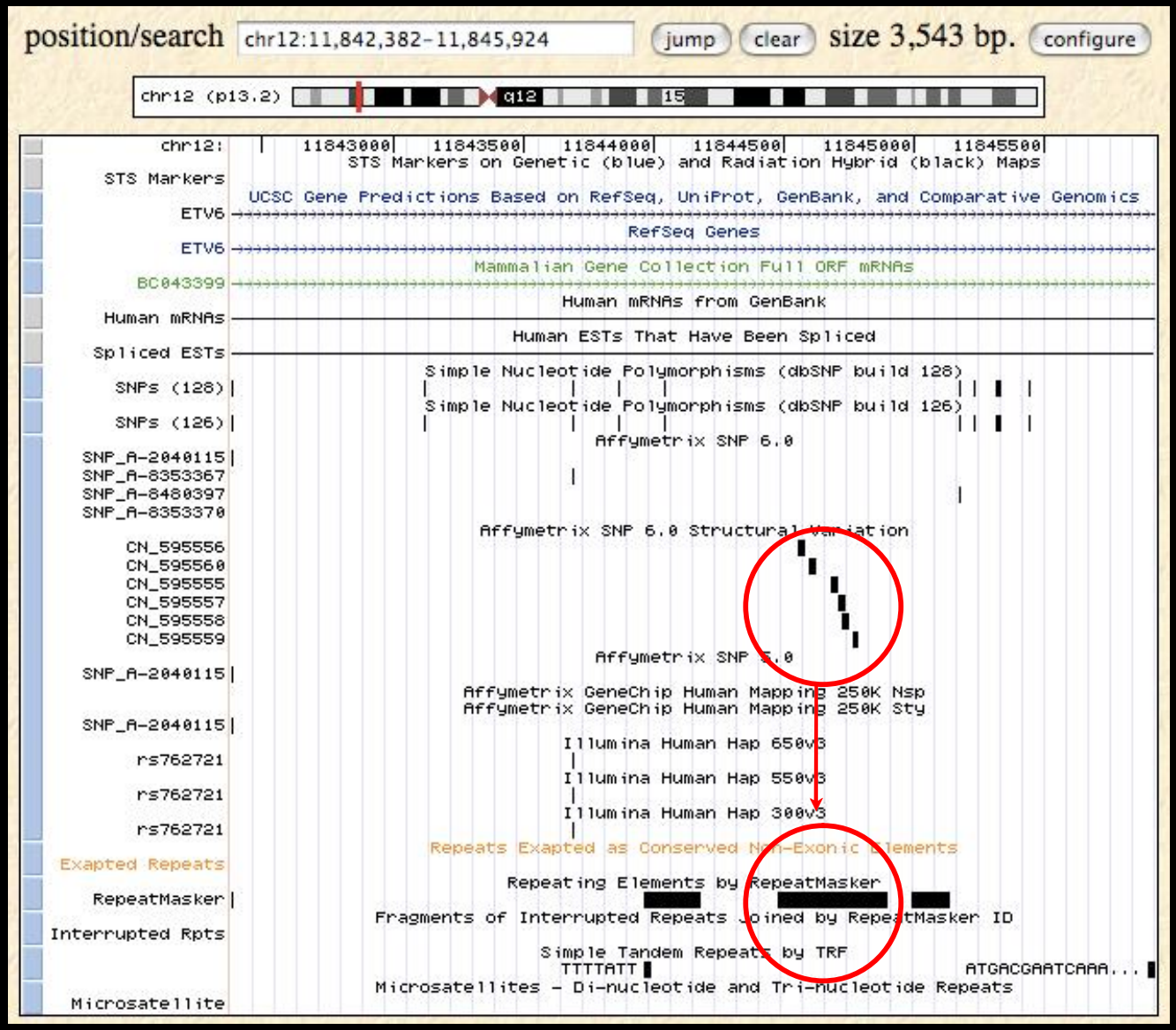


029/030
Run 1

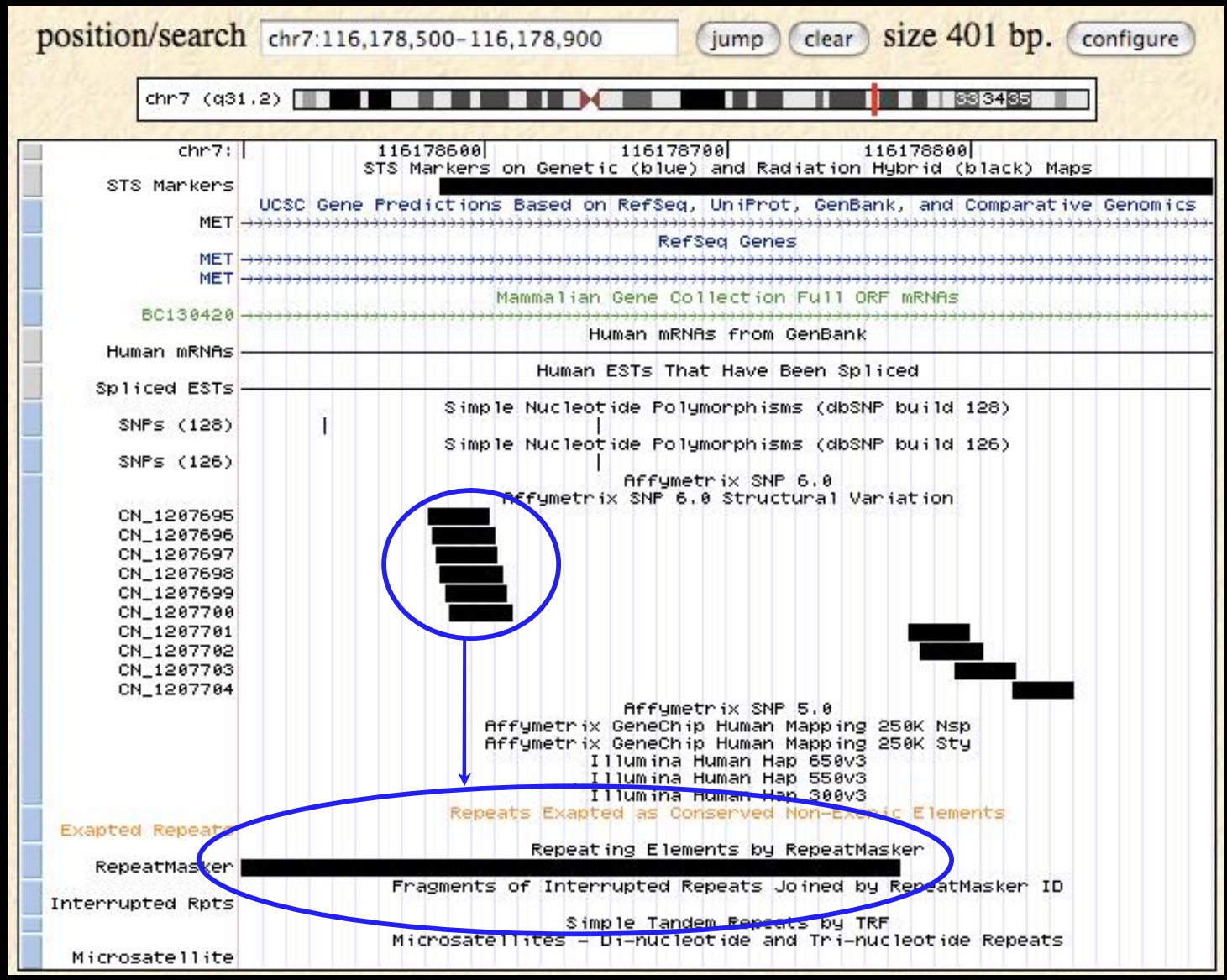
029/030
Run 2

Summation
of above
two

Limitation II: Probes in repeat elements



Limitation II: Probes in repeat elements



Take home message

- SNP 6.0 are very good arrays to detect “micro events”, if used carefully
- Control for overlapping probes and probes in repeat regions should improve analysis
- Running experiments in duplicate increases power of analysis; but is expensive!

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