Axiom Technology and Genotype calling

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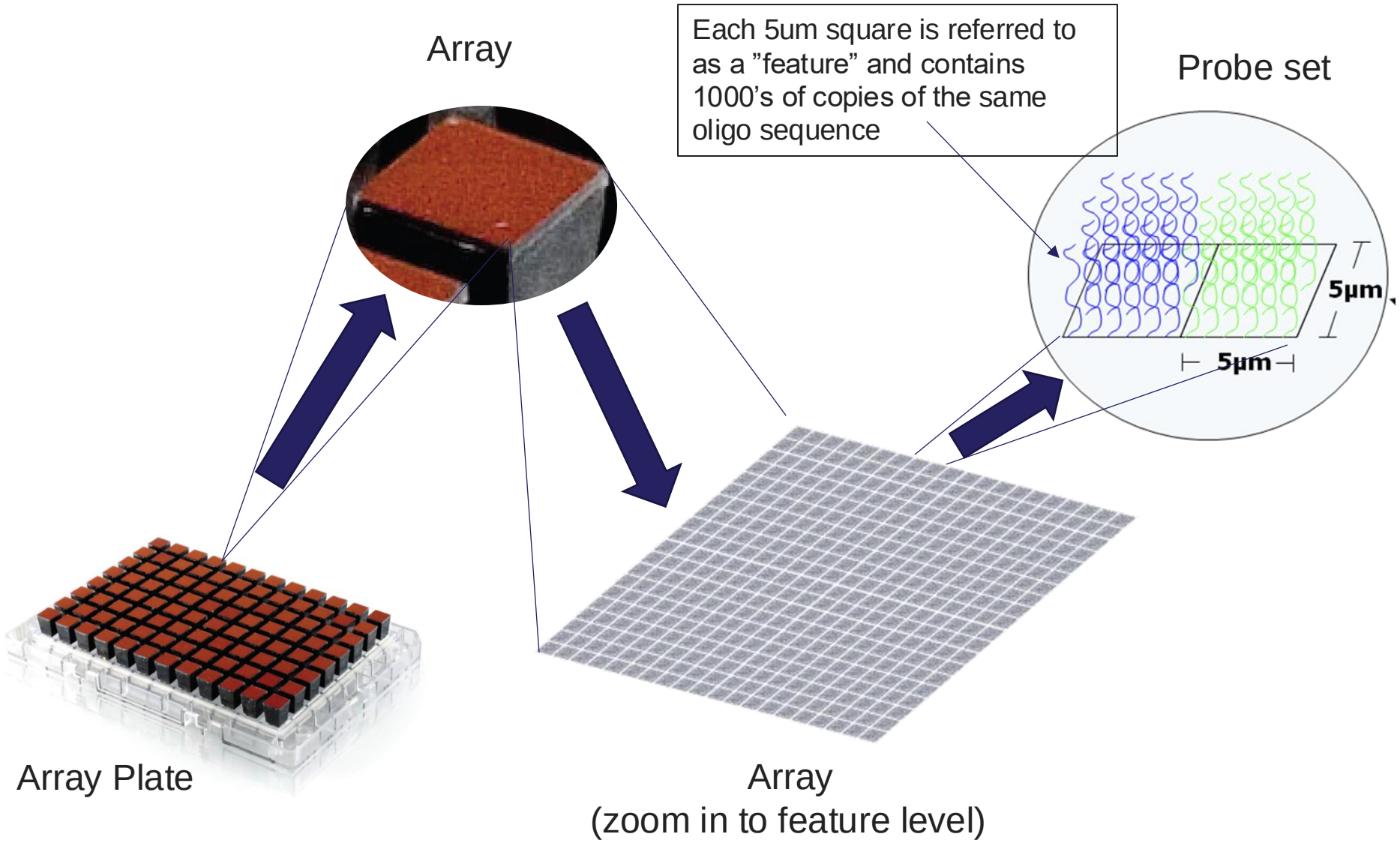
Outline

- Anatomy of a microarray
- Data files: DAT and CEL
- The AxiomGT1 genotype calling algorithm in five slides
- Probeset QC and classification

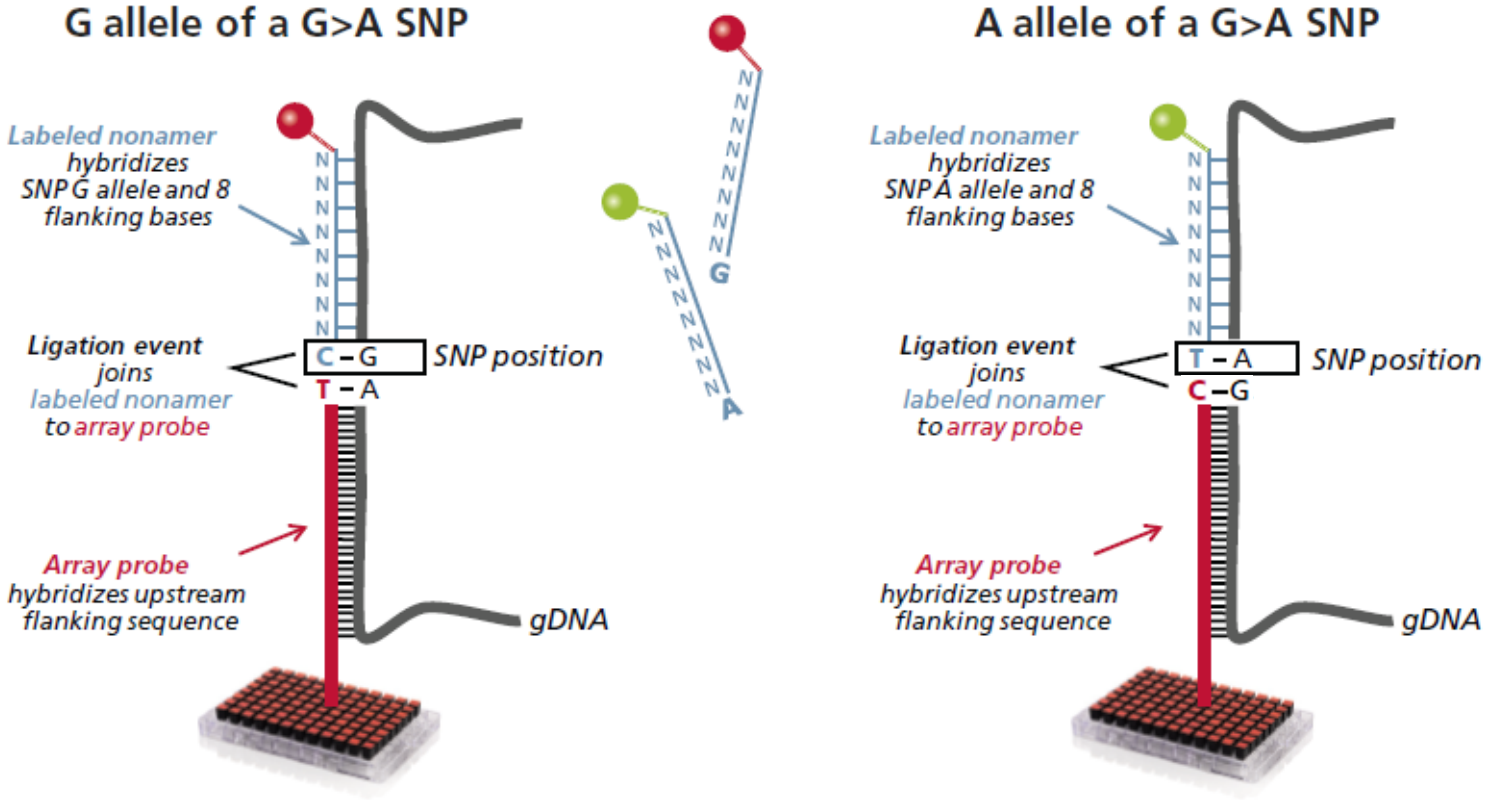
Anatomy of an Axiom Array



Allelic Discrimination (across channels) – non-AT/GC SNPs

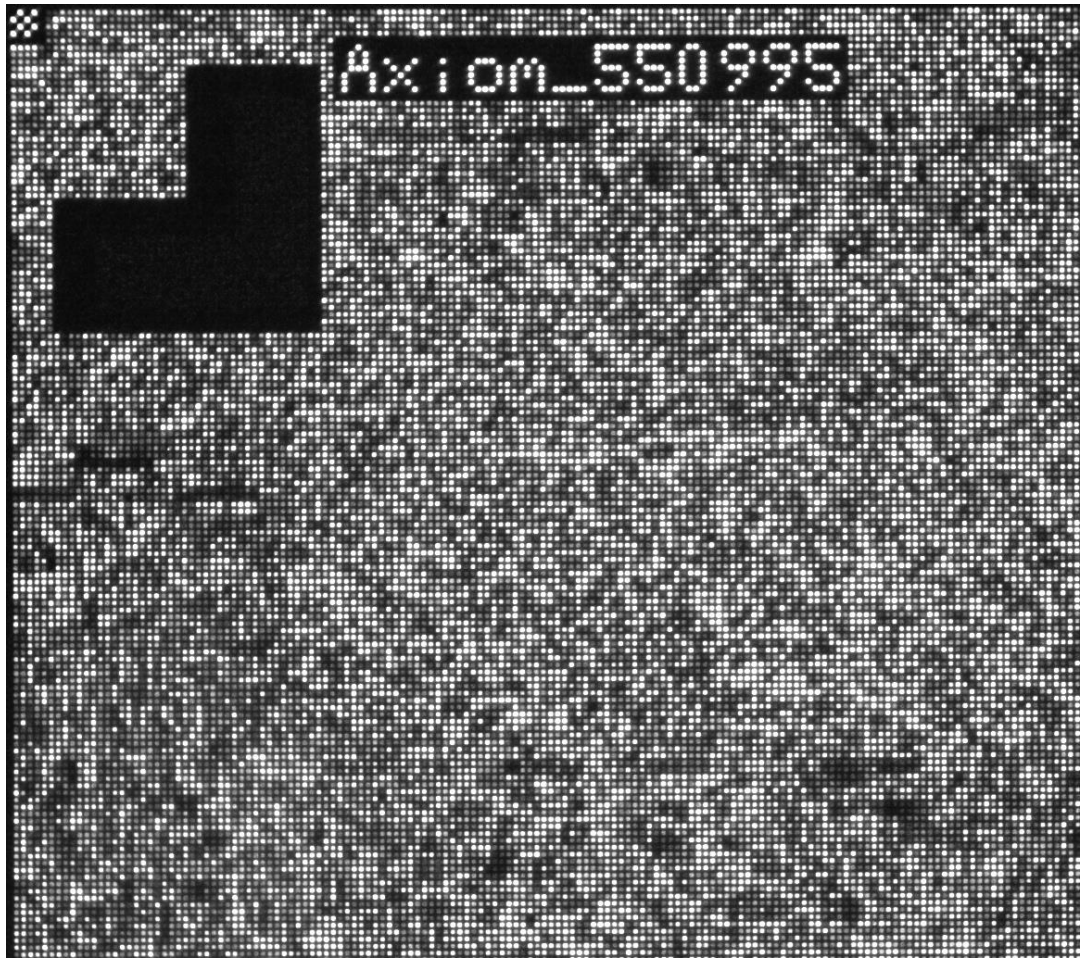
Axiom® standard probes

Used for [G/A], [G/T], [C/A] or [C/T] SNPs. Requires one array probe per SNP. Allelic discrimination is achieved by differentially labelled nonamers that hybridize each allele.



Raw Data: DAT and CEL Files

DAT File: Image from scanner



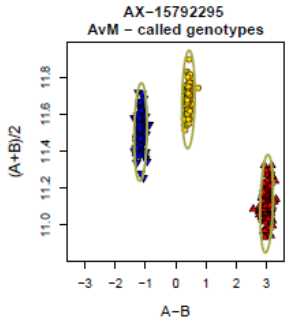
CEL File: Signal values extracted from DAT

X	Y	Channel	Signal
37	4	0	859
37	4	1	160
37	5	0	150
37	5	1	2261
…			

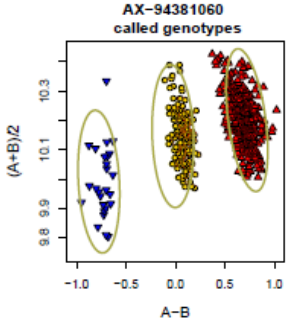
Binary XML – not this easy to read

Genotyping Algorithms and Capabilities

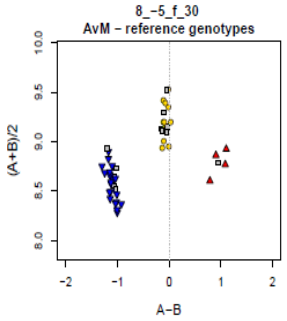
Biallelic
CYP3A5*3 6986A>G



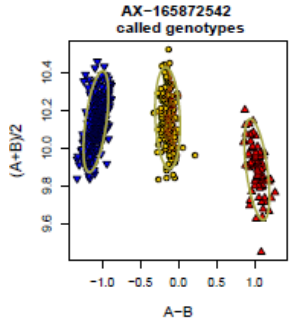
High GC content
ApoE-ε4 (rs429358)



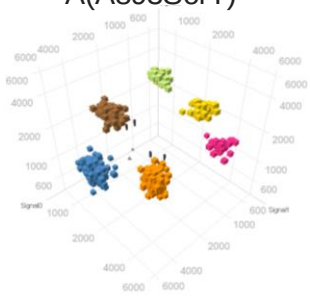
Homology/pseudogenes
CYP2D6*10 100C>T(P34S)



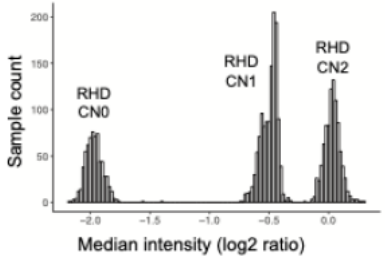
Nearby variation
SLCO1B1*1B



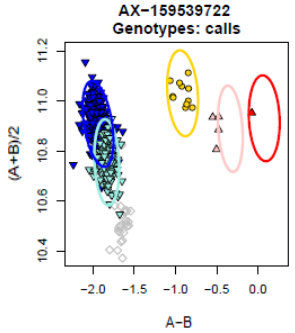
Multiallelic
ABCB1_c.2677G>T>
A(A893SorT)



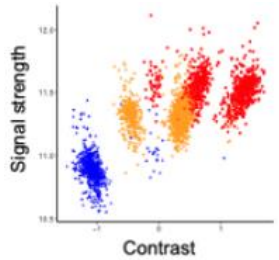
Copy number detection
(RHD)



Copy number aware
(RHDΨ)



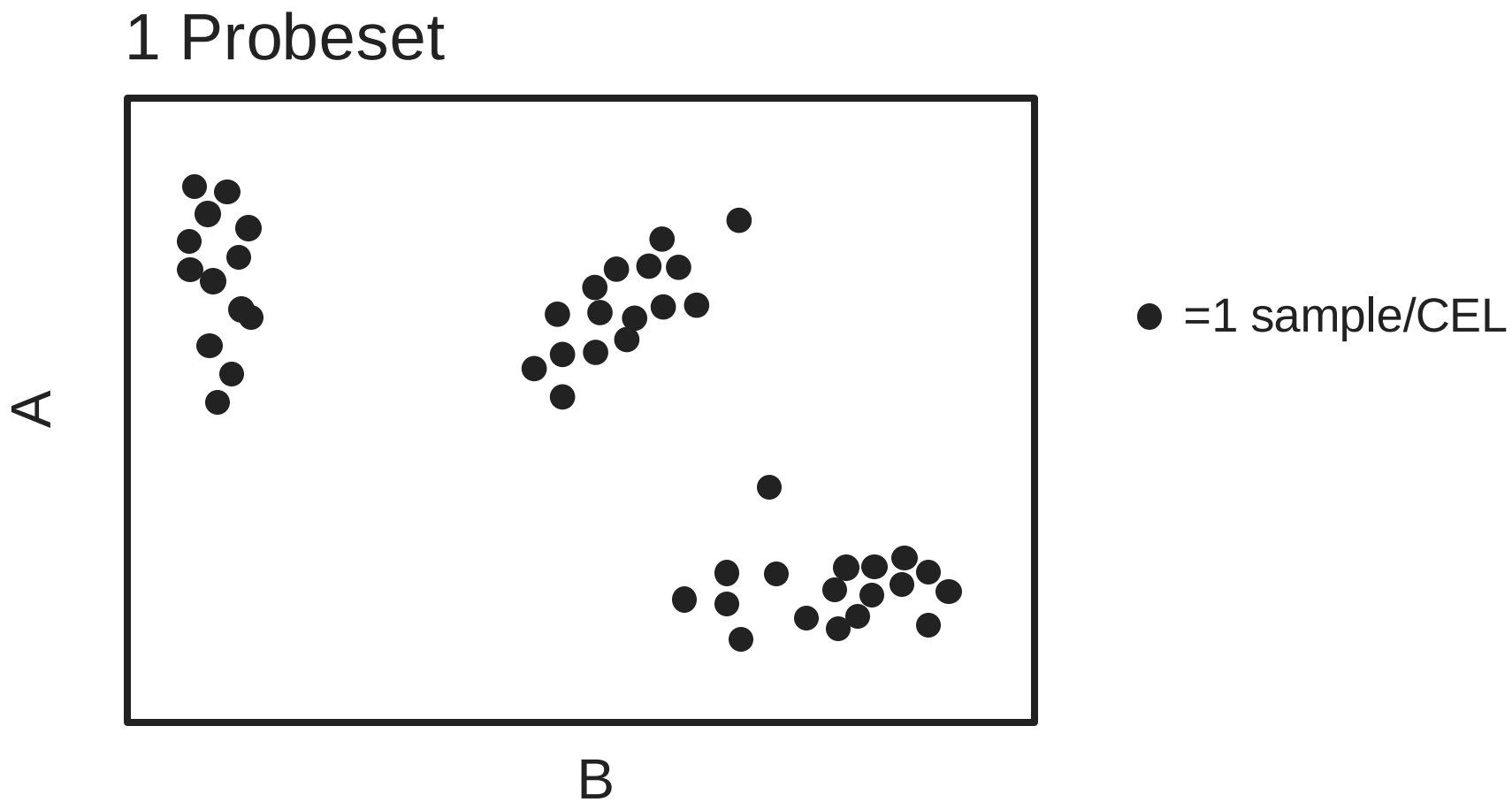
Remote CNV
(RHCE)
RHCE:c.307T>C



**All algorithms equally applicable to SNPs and indels*

AxiomGT1 Calling Algorithm

Signals from CEL files, plotted directly as A vs. B

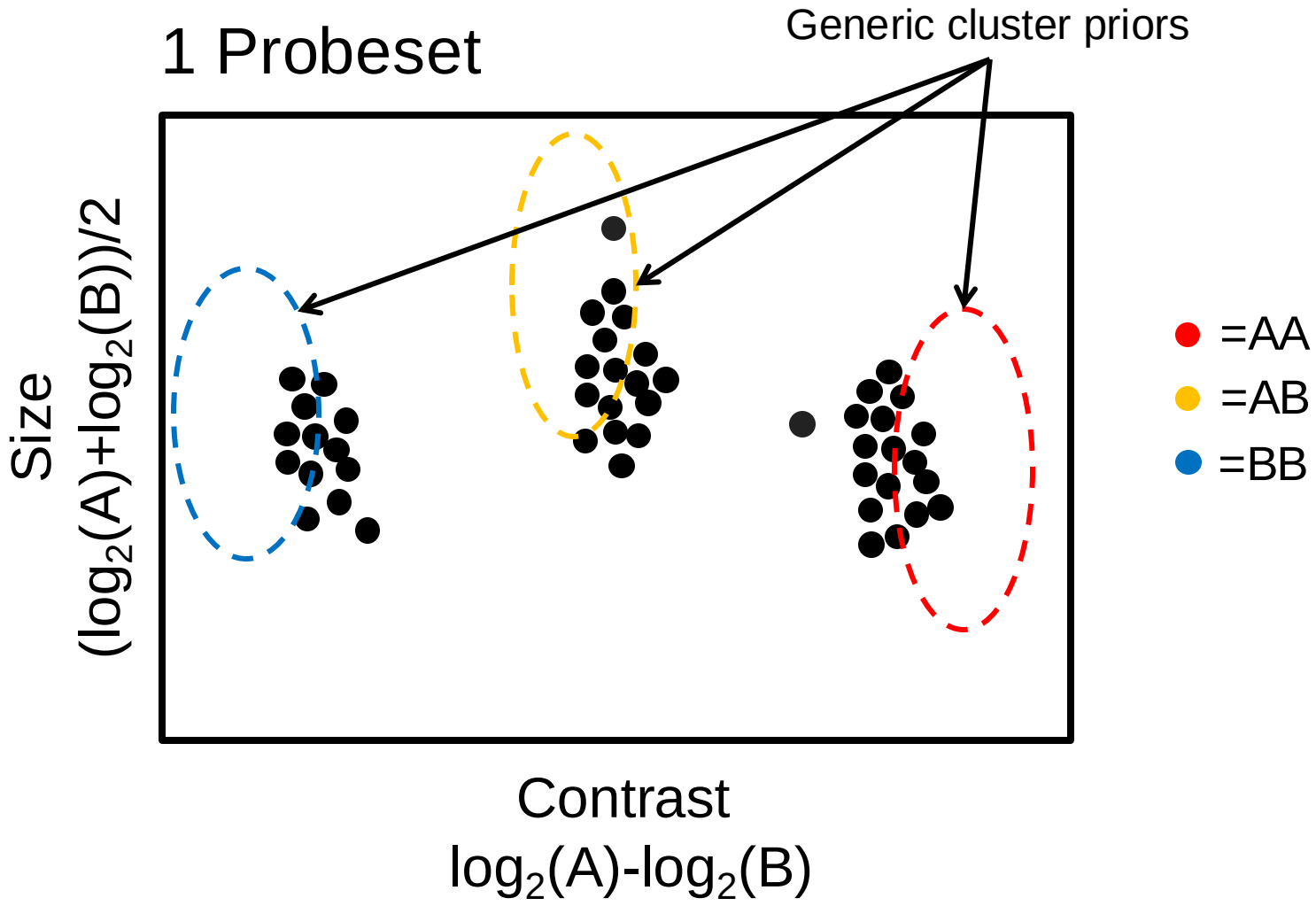


AxiomGT1 Calling Algorithm

Signals transformed into “clustering space” and plotted as size vs. contrast

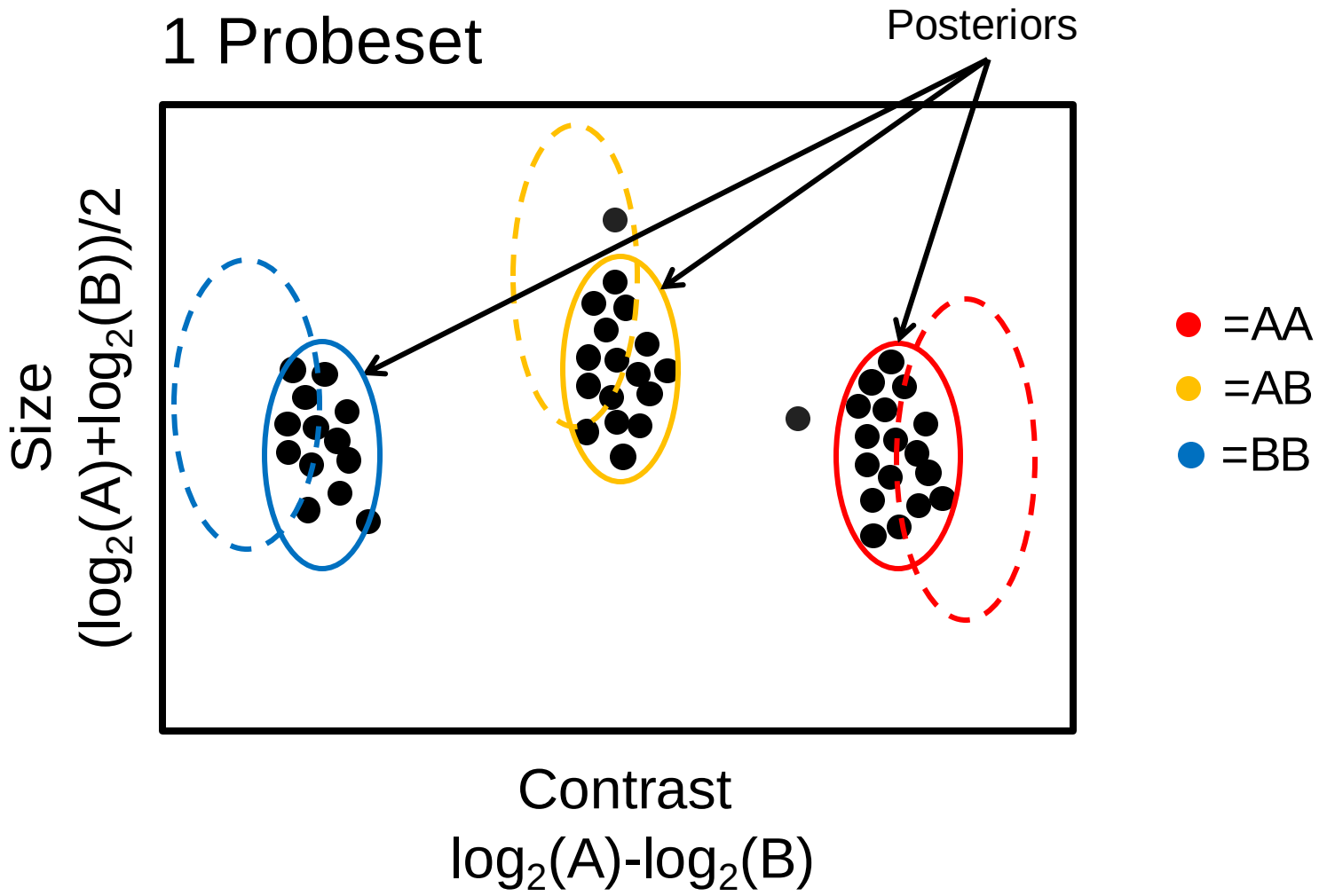
AxiomGT1 Calling Algorithm

Prior expectation of data location in clustering space



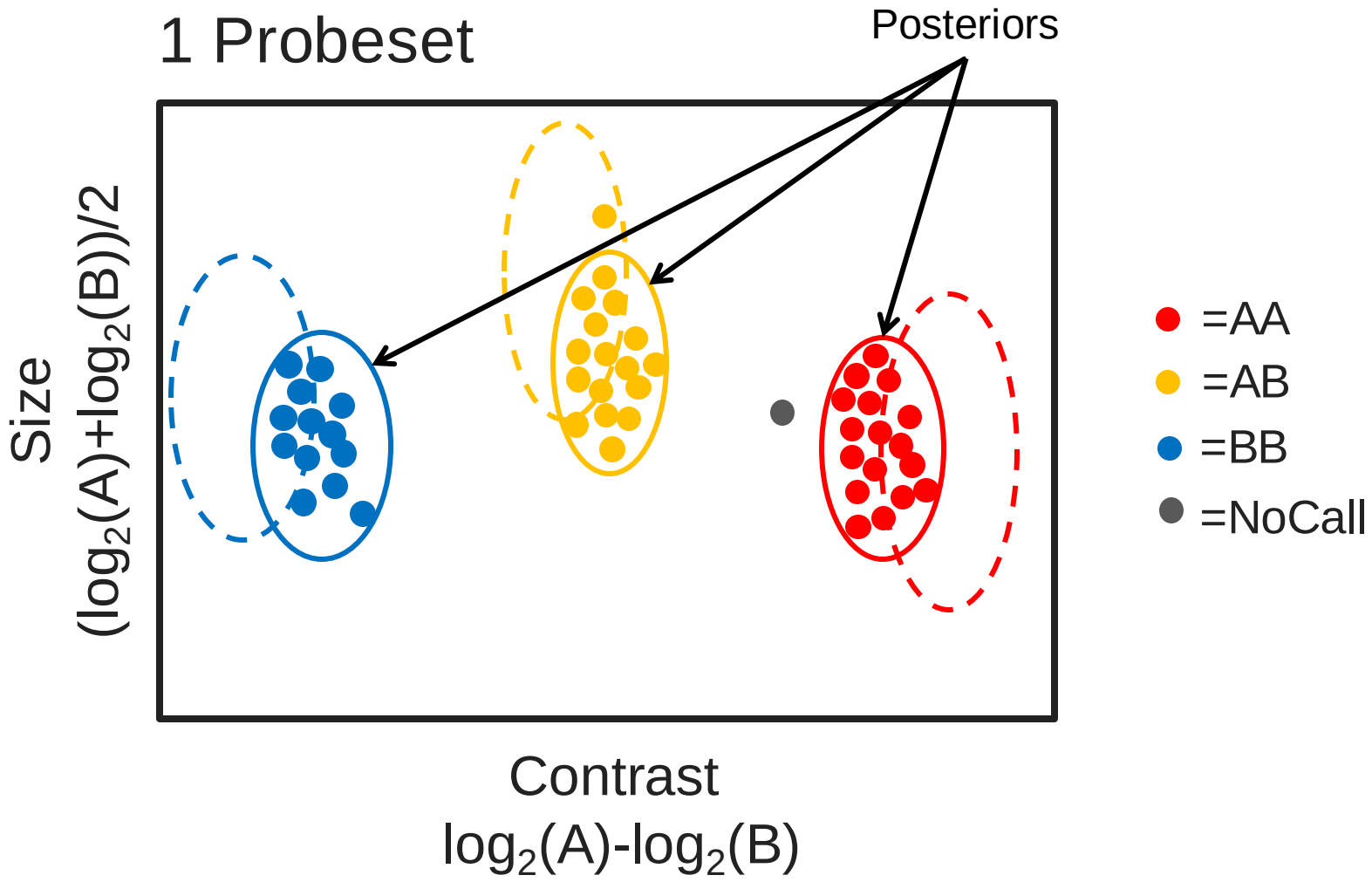
AxiomGT1 Calling Algorithm

Posteriors are the expected positions of the data after considering priors and data from the array.



AxiomGT1 Calling Algorithm

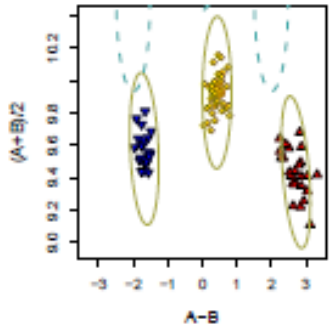
Samples close enough to the posteriors are called.



Probeset QC

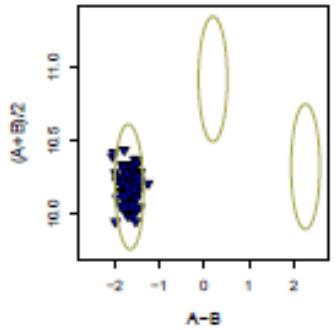
Software automatically classifies probesets “recommended” (top row) or non (bottom row).

Poly High Resolution



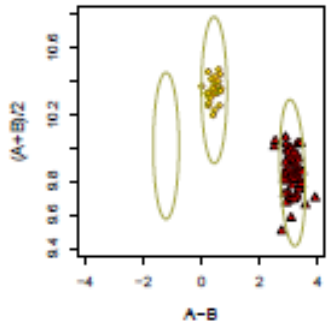
- Good cluster Resolution
- At least 2 examples of minor allele

Mono High Resolution



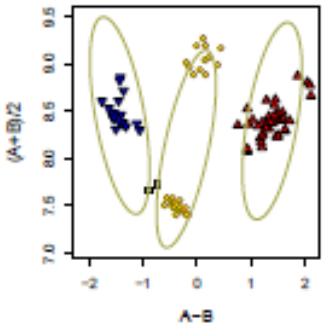
- All genotyped samples are monomorphic

No Minor Homozygote



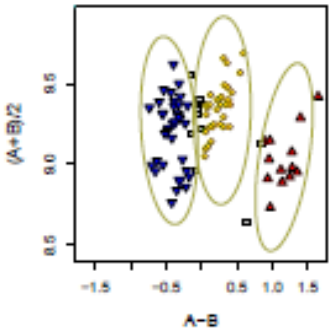
- Fewer than 2 examples of the minor allele

Off Target Variant



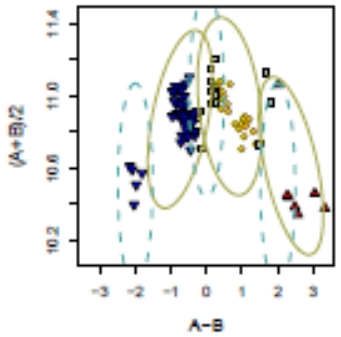
- Off Target Variants can be subjected to the off target variant calling algorithm

Call Rate Below Threshold



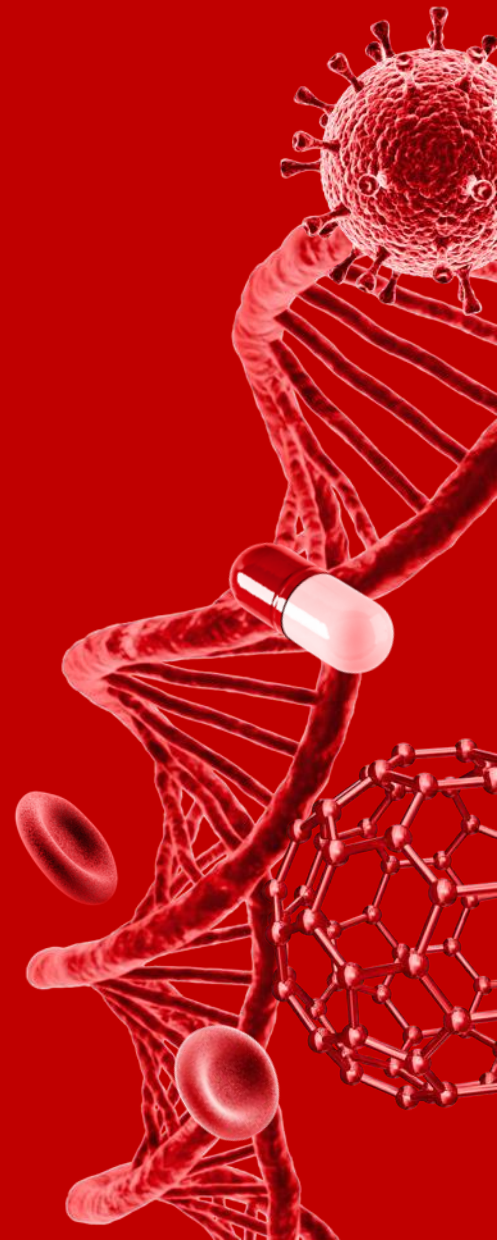
- Call Rate is below threshold, but all other cluster properties are normal

Other



- One or more cluster properties are below threshold values

Thank you



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