Microarrays

Eric Lee, Oct 2019

Microarrays detect changes in copy number

- Disease due to reduction in copy number (haploinsufficient) or gene disruption
- Disease due to increase in copy number (triplosensitive)

Microarray

Definition: Genomic targets spotted onto a solid support

Targets: Oligonucleotide or BAC targets with known positions within the genome and known location on array


illumina
9374160039 24x1-GR

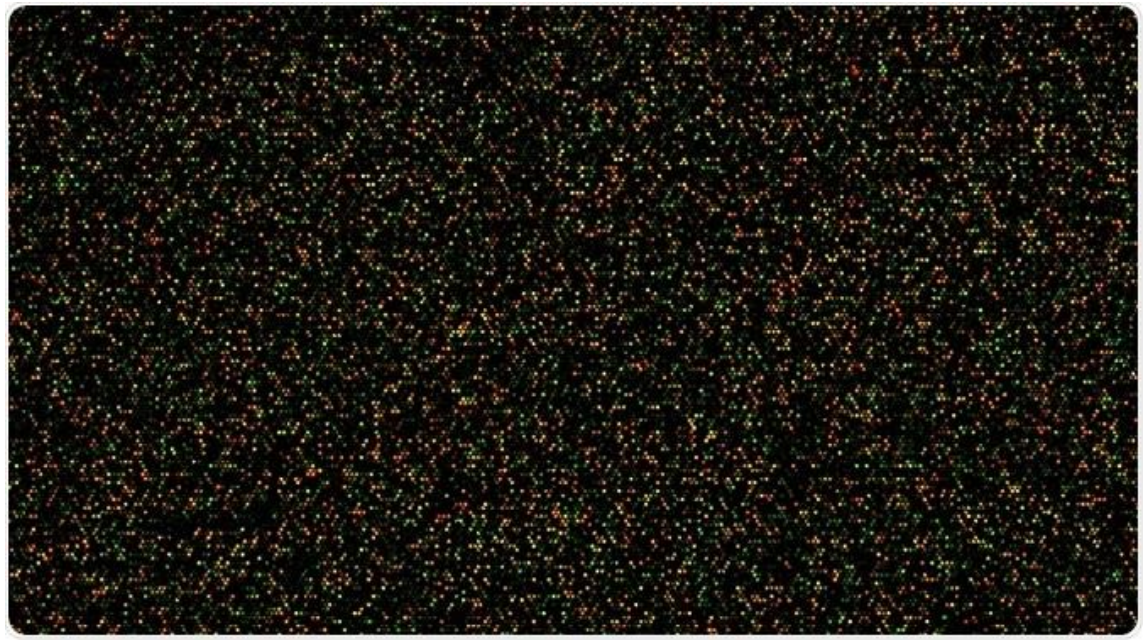
Microarray

1. Extract DNA
2. Amplify, digest, label with fluorophores
3. Hybridise to complementary targets on array
4. Scan array

Signal from patient’s genome is compared to signal from a reference genome

More fluorescence: sample has more copies of the target than reference

Less fluorescence: sample has fewer copies of the target than reference



Microarray

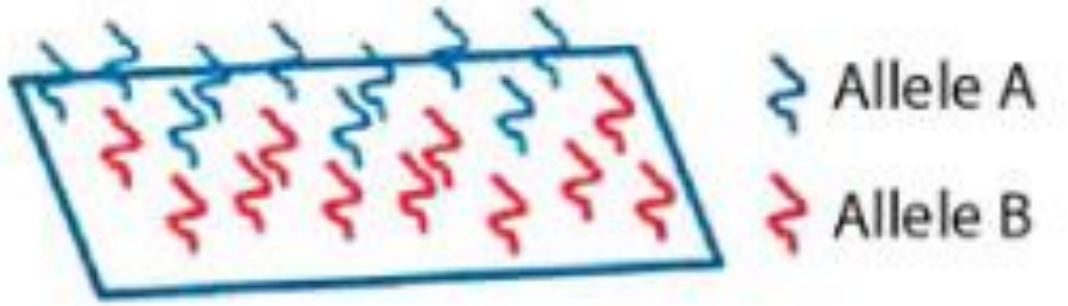
SNP (single nucleotide polymorphism)

- Compared to pool of normal individuals
- Targets any region of genome with multiple alleles

CGH (comparative genome hybridization)

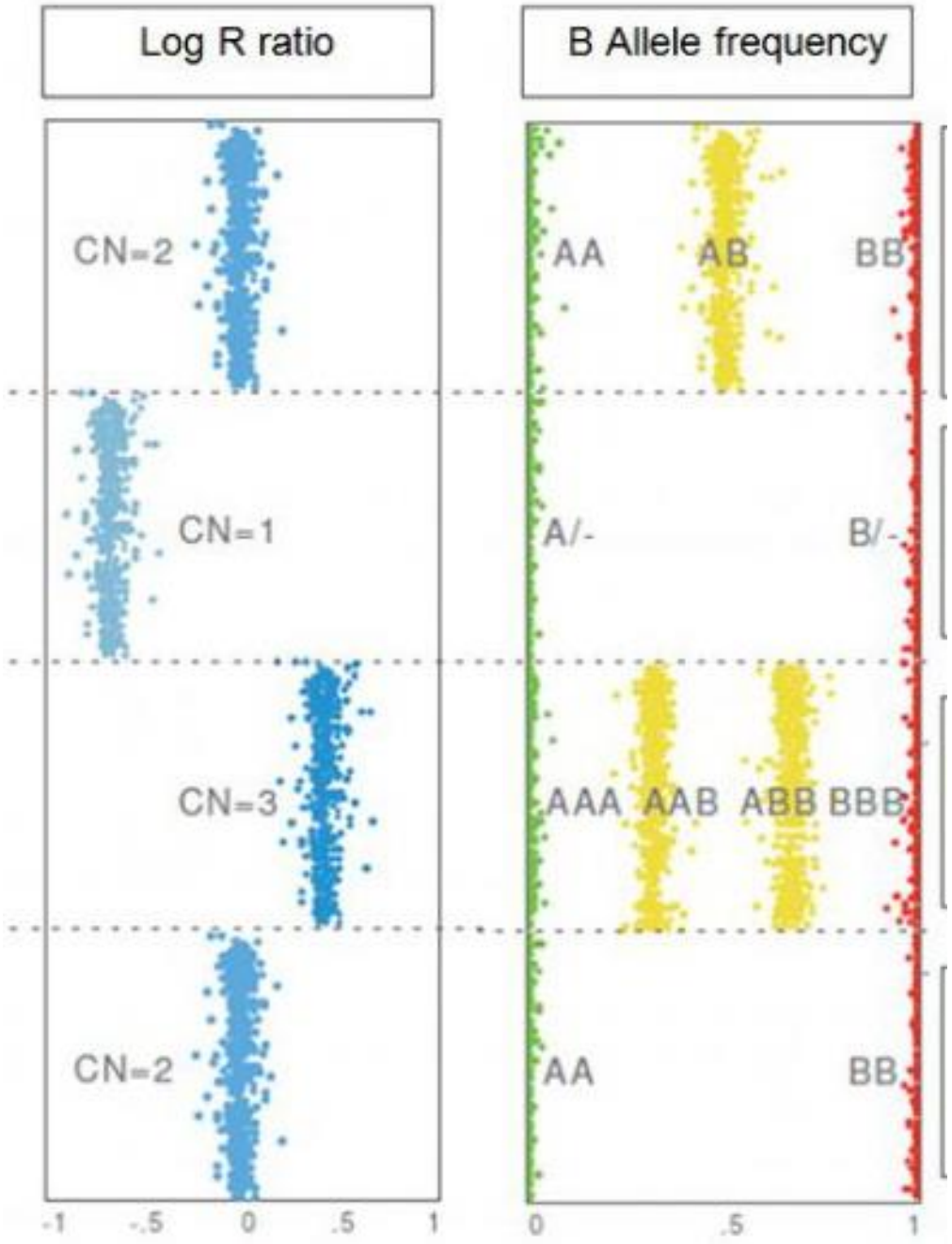
- Compared to reference DNA
- Targets any DNA sequence throughout genome

LogR ratio and BAF (B allele frequency)



LogR ratio = observed Allele A + Allele B signal intensity compared to expected (reference), at a particular locus

BAF = proportion of B allele signal at a particular locus



Normal copy number, heterozygous

Reduced copy number, hemizygous

Increased copy number, BAF split

Normal copy number, homozygous

Source: http://hmds.info/diagnostic-service/techniques/snp-arrays

…Different types of microarrays

…Relative quantification of sample DNA in comparison to a reference

…SNP arrays provide two data sets (LogR ratio and BAF) that must be interpreted concurrently

How are microarrays used in clinical laboratories?

Germline

- Child with autism/ ADHD/ intellectual disability
- Child with multiple congenital malformations
- Fetus with ultrasound abnormalities
- Patient with features of a disease primarily caused by large number variants (e.g. *PMP22*, *HNF1B*)

Category 6 - PATHOLOGY SERVICES

Group P7 - Genetics

73292

Analysis of chromosomes by genome-wide micro-array including targeted assessment of specific regions for constitutional genetic abnormalities in diagnostic studies of a person with developmental delay, intellectual disability, autism, or at least two congenital abnormalities (including a service in items 73287, 73289 or 73291, if performed)

- 1 or more tests.

Fee: $589.90 **Benefit:** 75% = $442.45 85% = $506.50

← Previous - Item 73291

Next - Item 73293 →

How are microarrays used in clinical laboratories?

Somatic

- Patient with CLL at diagnosis or relapse(including T12, 13q-, 11q-, 17p-)

What can we test?

- Peripheral blood
- Prenatal specimens (amniocentesis, CVS)
- Bone marrow
- Saliva or buccal swabs
- FFPE

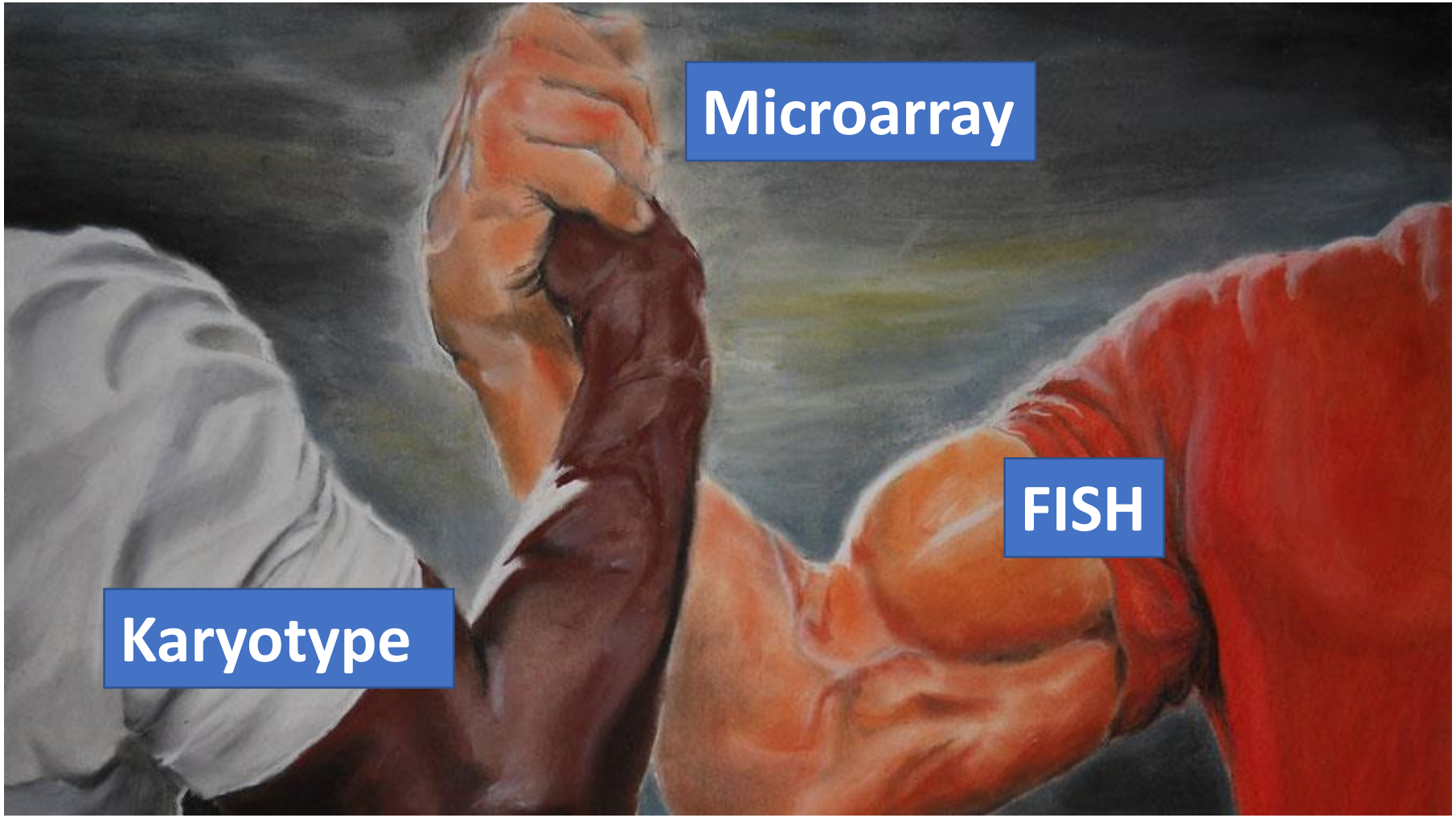
What does it detect?

- Copy number variants
- Regions of homozygosity
  - May indicate parental relatedness
  - May indicate presence of uniparental disomy

What does it NOT detect?

- Balanced chromosomal rearrangements
- Small copy number variants (<100kb)
- Low level mosaicism
- Uniparental heterodisomy
- Other: single nucleotide variants, oligonucleotide repeat expansions, mitochondrial genome variants, methylation abnormalities…

Why test by microarray?

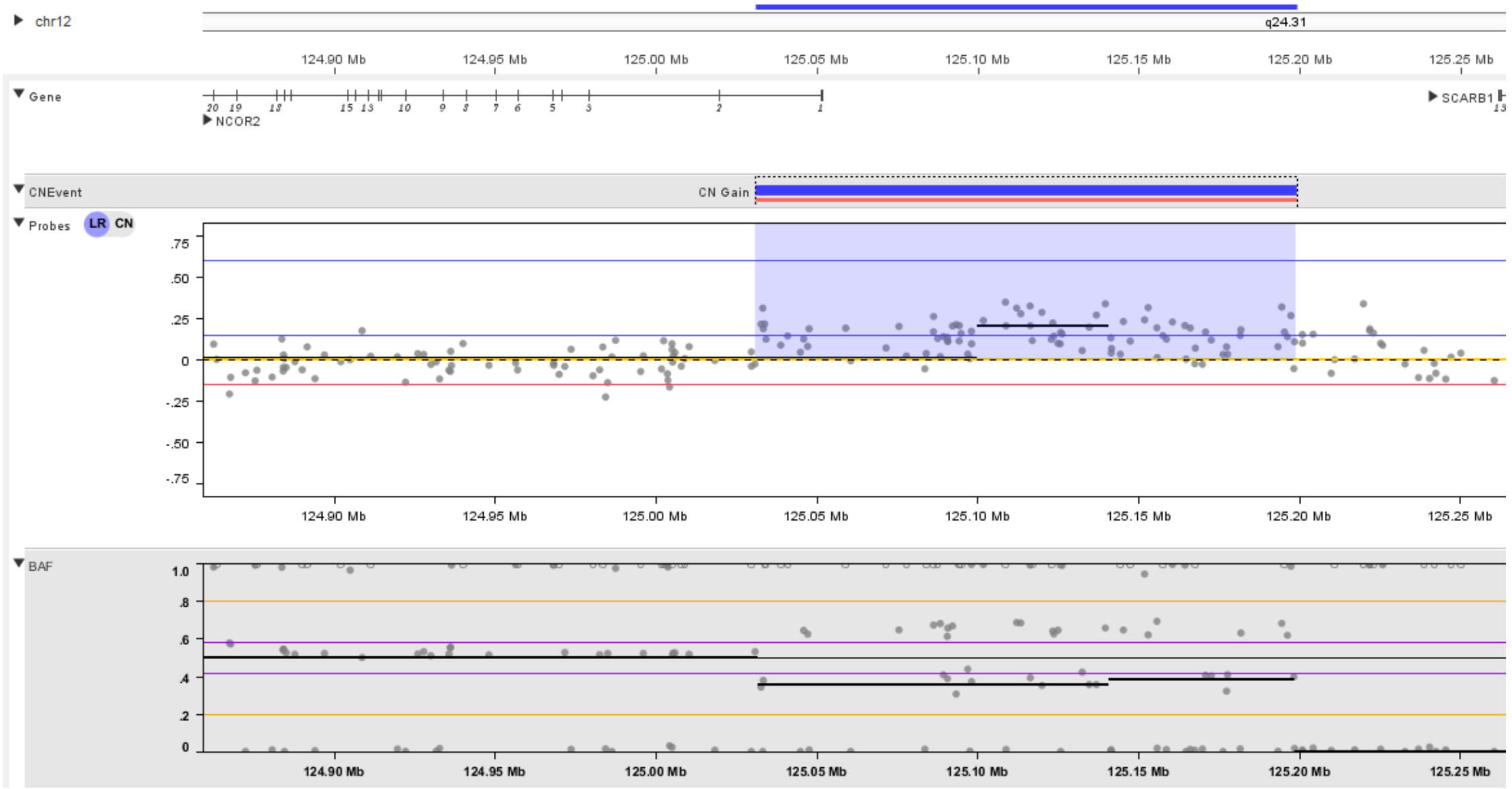


	Microarray	FISH	Karyotype
Targeted or screening	Screening	Targeted	Screening
Balanced or unbalanced	Unbalanced	Both	Both
Resolution	100-200kb	>300kb	5-10Mb
Mosaicism	20-30%	Better	Better
Regions of homozygosity	Yes	No	No
Sample preparation	DNA extraction	Direct or cultured	Cultured
Operator dependence	Lower	Higher	Higher

CNV interpretation

- Deletion/duplication; whole gene or partial
- Size
- Recurrent CNV regions
- Genomic content; gene-disease association(s); isoform
- Potential structural basis
- Population frequency
- Case studies; parental inheritance
- Case/control studies

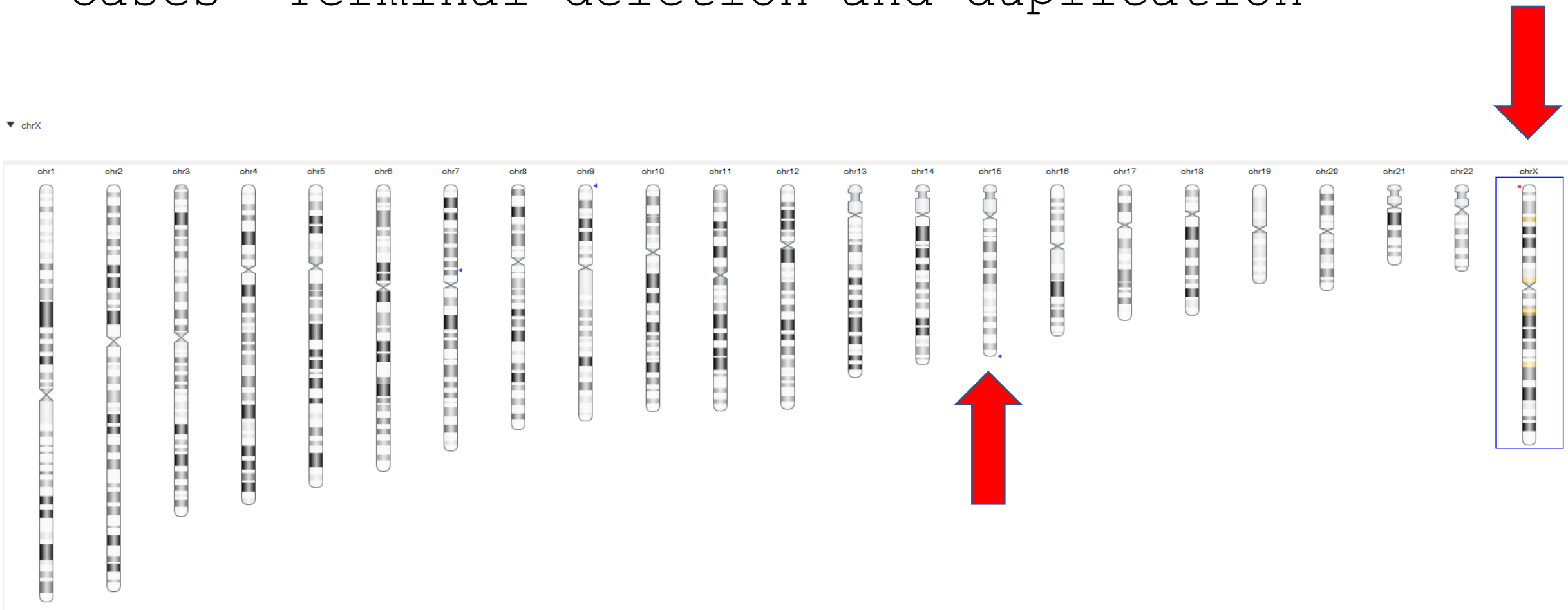
Cases – Extragenic partial gene duplication



Cases – Extragenic partial gene duplication

- Is the gene associated with disease?
- Is it known to be haploinsufficient?
- Location and orientation unknown
- Demonstrating presence in a parent would reduce likelihood of clinical significance
- Demonstrating it is de novo may increase likelihood

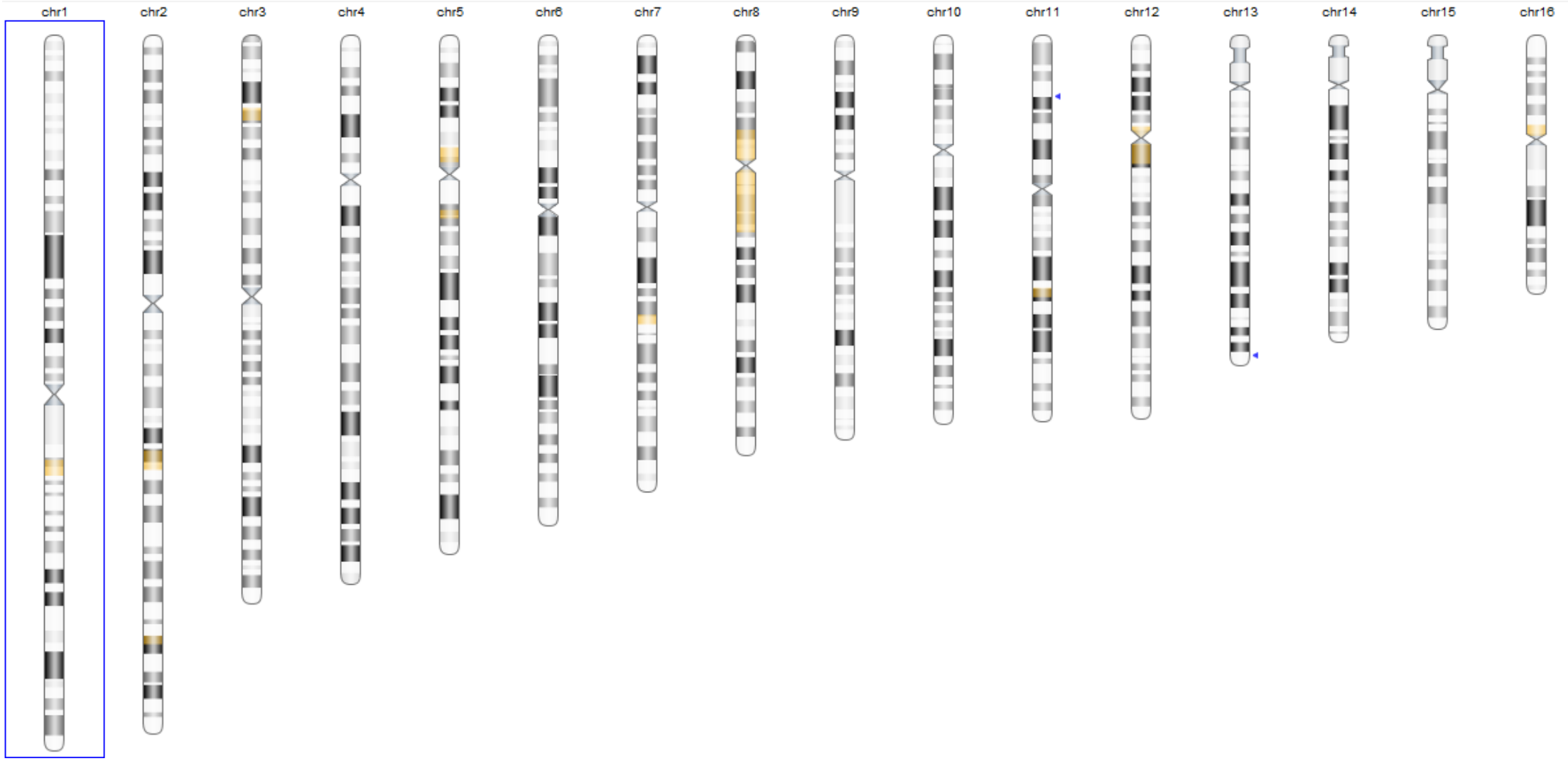
Cases –Terminal deletion and duplication



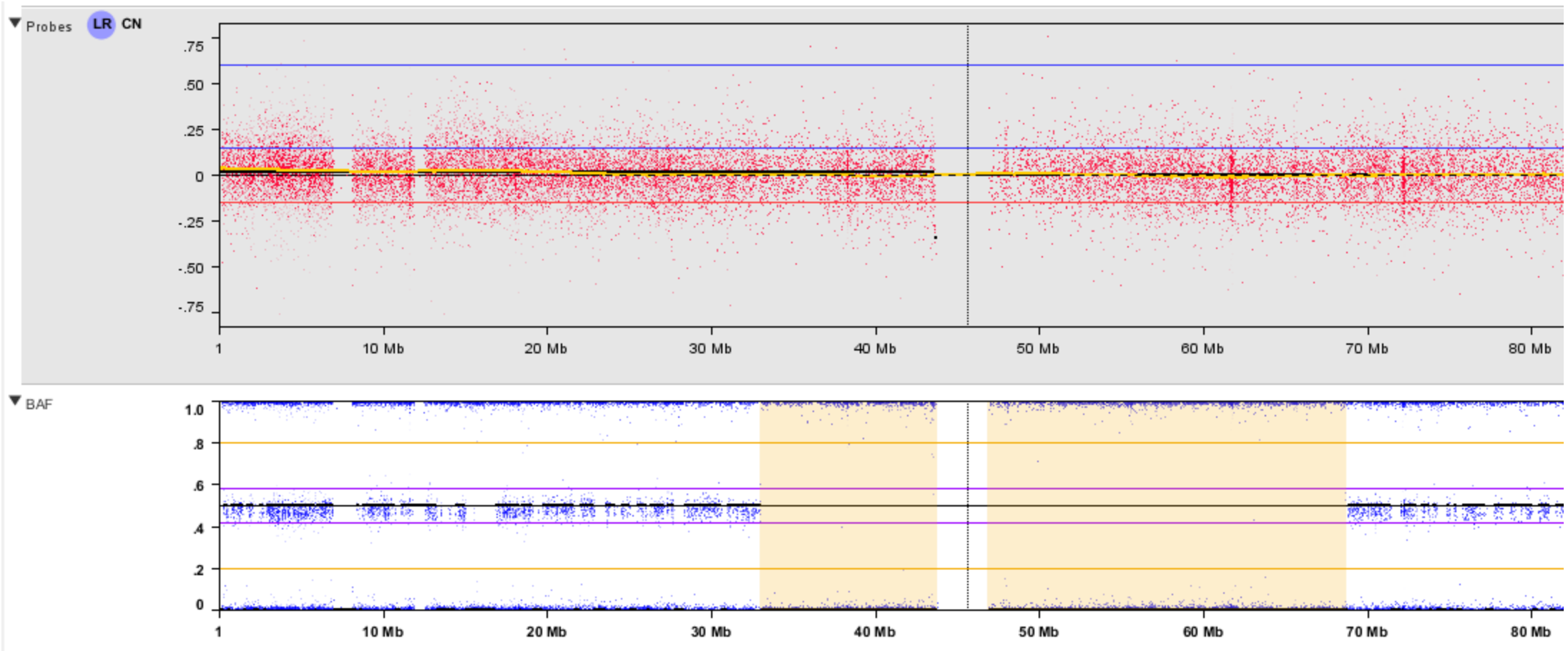
Cases – Terminal deletion and duplication

- Larger size, higher gene content, and deletion are more likely to be clinically significant
- Suggestive of unbalanced derivative chromosome resulting from parental reciprocal translocation
- Requires karyotype and/or FISH in proband and parents to confirm
- Recurrence risk

Cases – Elevated genome-wide ROH



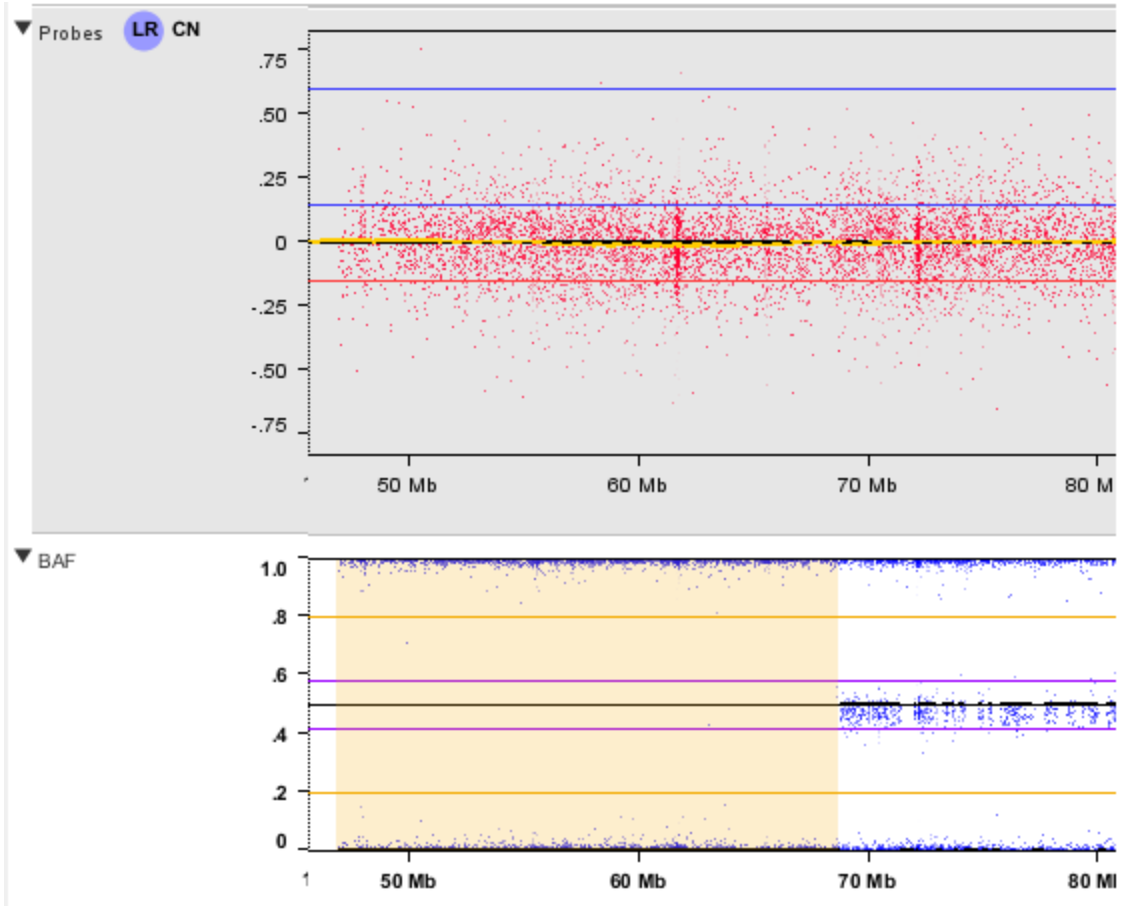
Cases- Elevated genome-wide ROH



Cases - Elevated genome-wide ROH

- Most suggestive of identity by descent
- May harbor a homozygous mutation in a recessive disease gene
- Very high levels of LOH (>10%) may be suggestive of a close parental relationship; liaison with clinician warranted

Cases – Large region of homozygosity on 15



Cases – Large region of homozygosity on 15

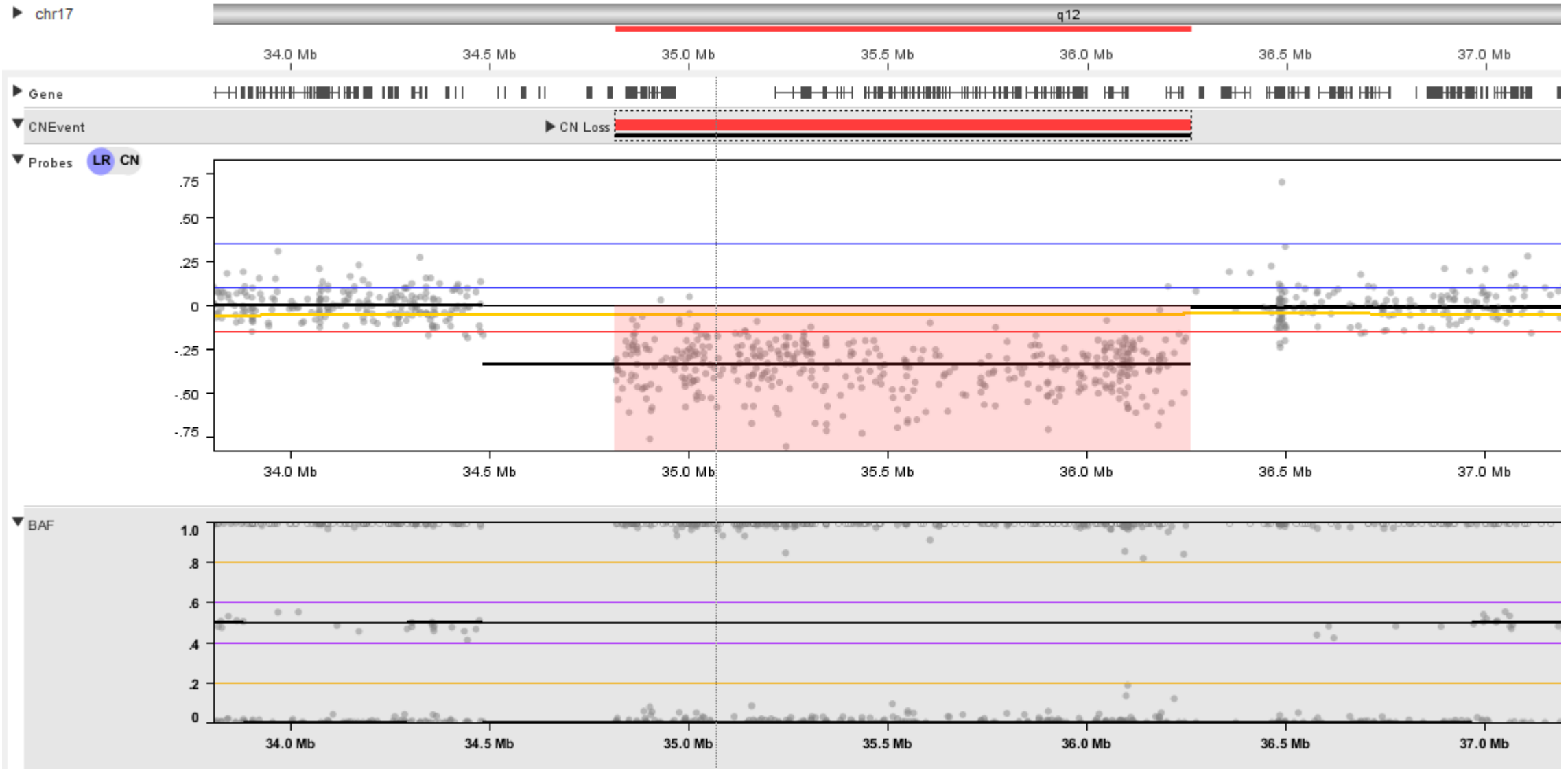
Uniparental disomy

- Associated with an abnormal phenotype when it affects imprinted regions on chromosomes 6,7,11,14,15,20
- Some genes expressed from only one parental allele
- Uniparental inheritance may lead to under- or over-expression of genes
- Iso-, hetero-, mixed heteroisodisomy

Cases – Large region of homozygosity on 15

- May suggest UPD; follow-up testing required to confirm
- UPD may be associated with residual trisomy below detection limit of array
- May harbor a homozygous mutation in a recessive disease gene

Cases – Incidental finding



Cases – Incidental finding

- 1.4Mb 17q12 deletion in a parent of a proband
- Includes HNF1B gene
- Associated with renal cysts, diabetes, and susceptibility to neurodevelopmental and psychiatric disorders
- Need for incidental findings policy

Cases – Susceptibility variant

15q11.2 BP1-BP2 deletion

- Incomplete penetrance – low
- Variable expressivity – DD, ASD, learning
- Can be inherited from unaffected or mildly affected parents
- Risk of passing onto future offspring
- Phenotype cannot be predicted in future offspring with the same variant

Nomenclature

International System for Human Cytogenetic Nomenclature (ISCN) 2016

arr[GRCh37] 6q21q25.1(113900413_149100458)x1

Nomenclature

arr[GRCh37] 6q21q25.1(113900413_149100458)x3

arr[GRCh37]
6q21q25.1(113900413_149100458)x1 mat

arr[GRCh37]
6q21q25.1(113898012x2,113900413_149100458x1,
149101546x2)

Nomenclature

arr[GRCh37]
6q21q25.1(113900413_149100458)x1~2

arr[GRCh37]
6q21q25.1(113900413_149100458)x2 hmz

46,XY.nuc ish(ABC1)x1.arr[GRCh37]
6q21q25.1(113900413_149100458)x1

Take-home messages

- Microarray, karyotype, and FISH each have their place
- Microarray only detects CNVs and ROH, but can be suggestive of other abnormalities requiring follow-up
- Interpretation of clinical significance requires consideration of many factors