A Method for Rapid, Targeted CNV Genotyping Identifies Rare Variants Associated with Neurological Disease

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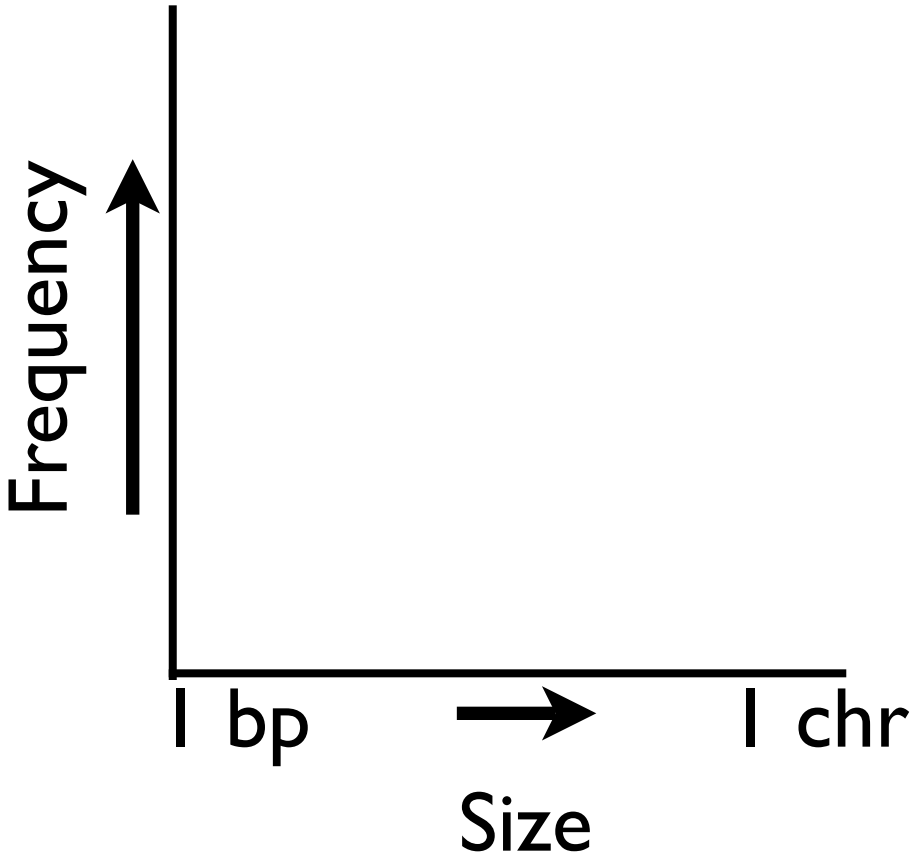
Genomic Structural Variation

Human Genetic Variation



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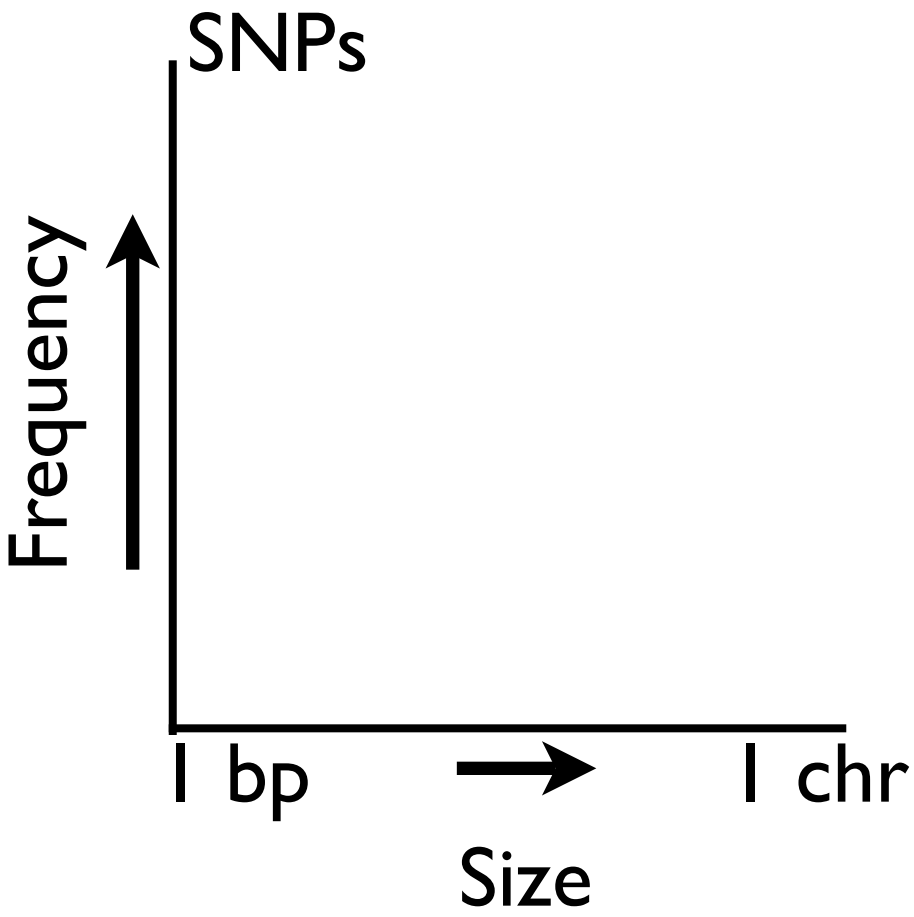

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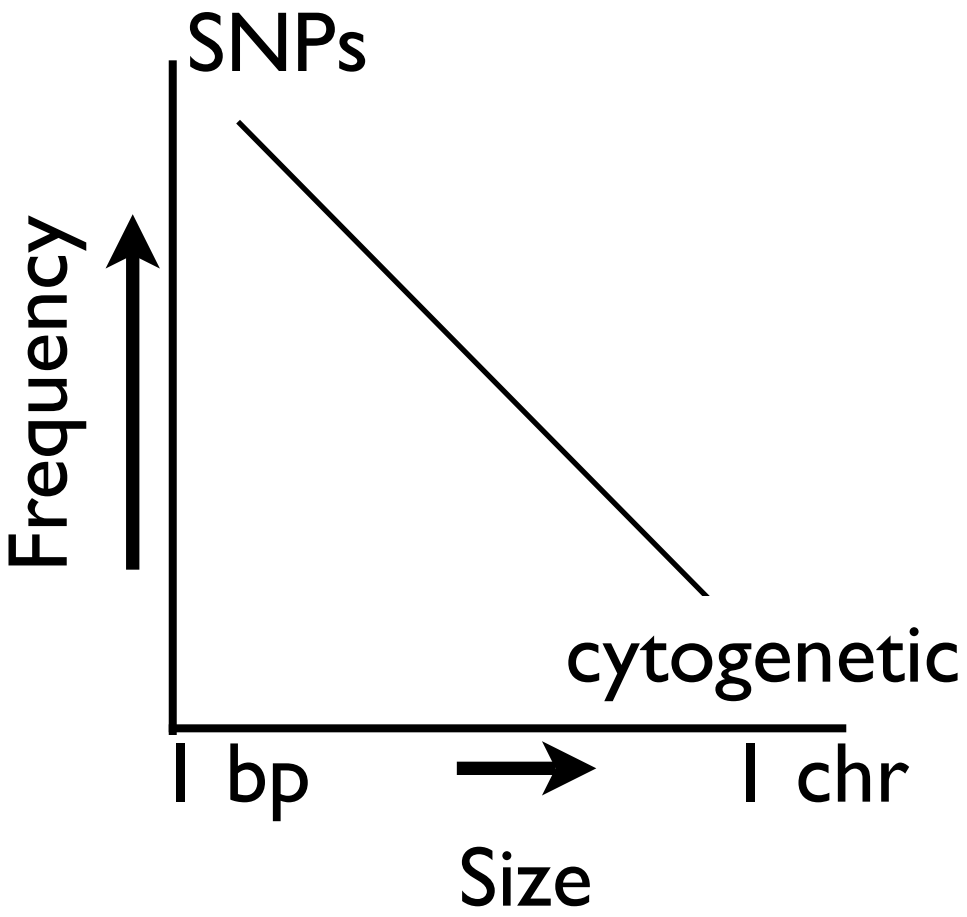

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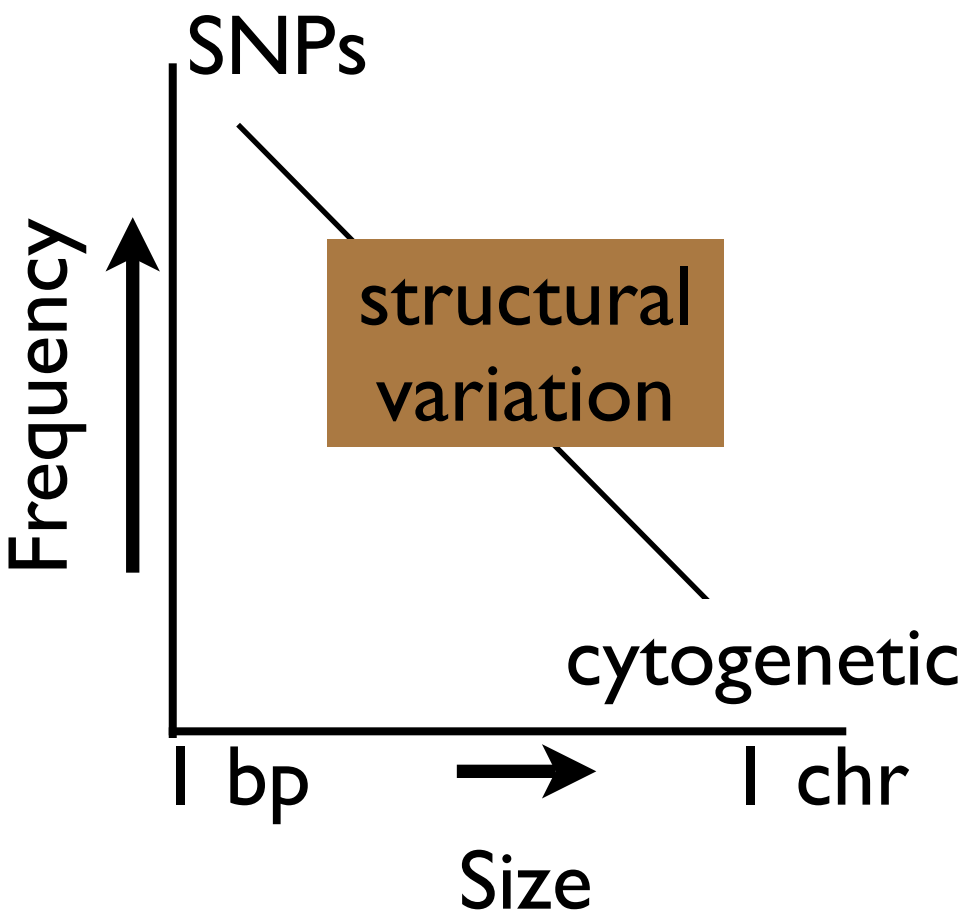

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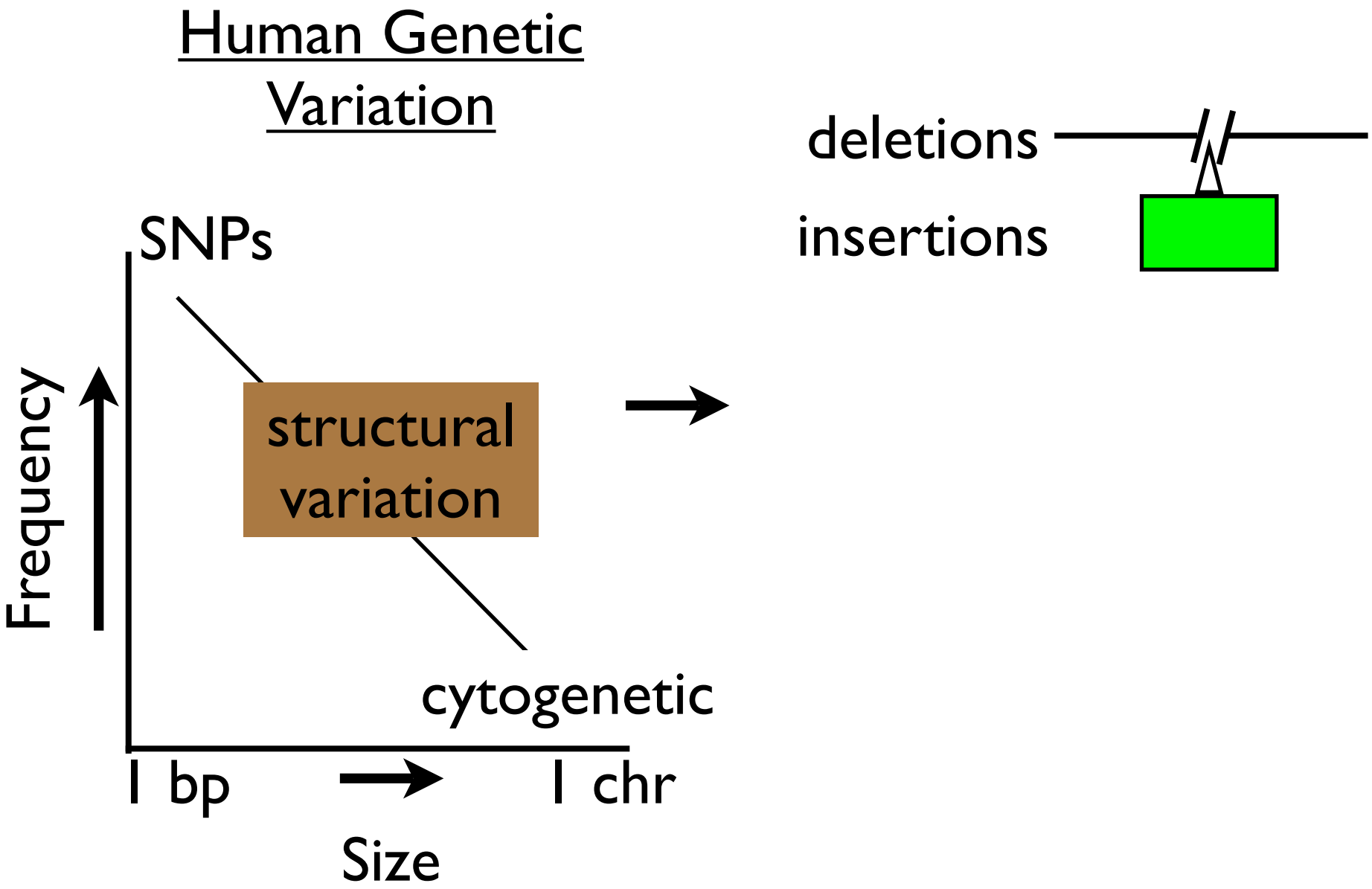

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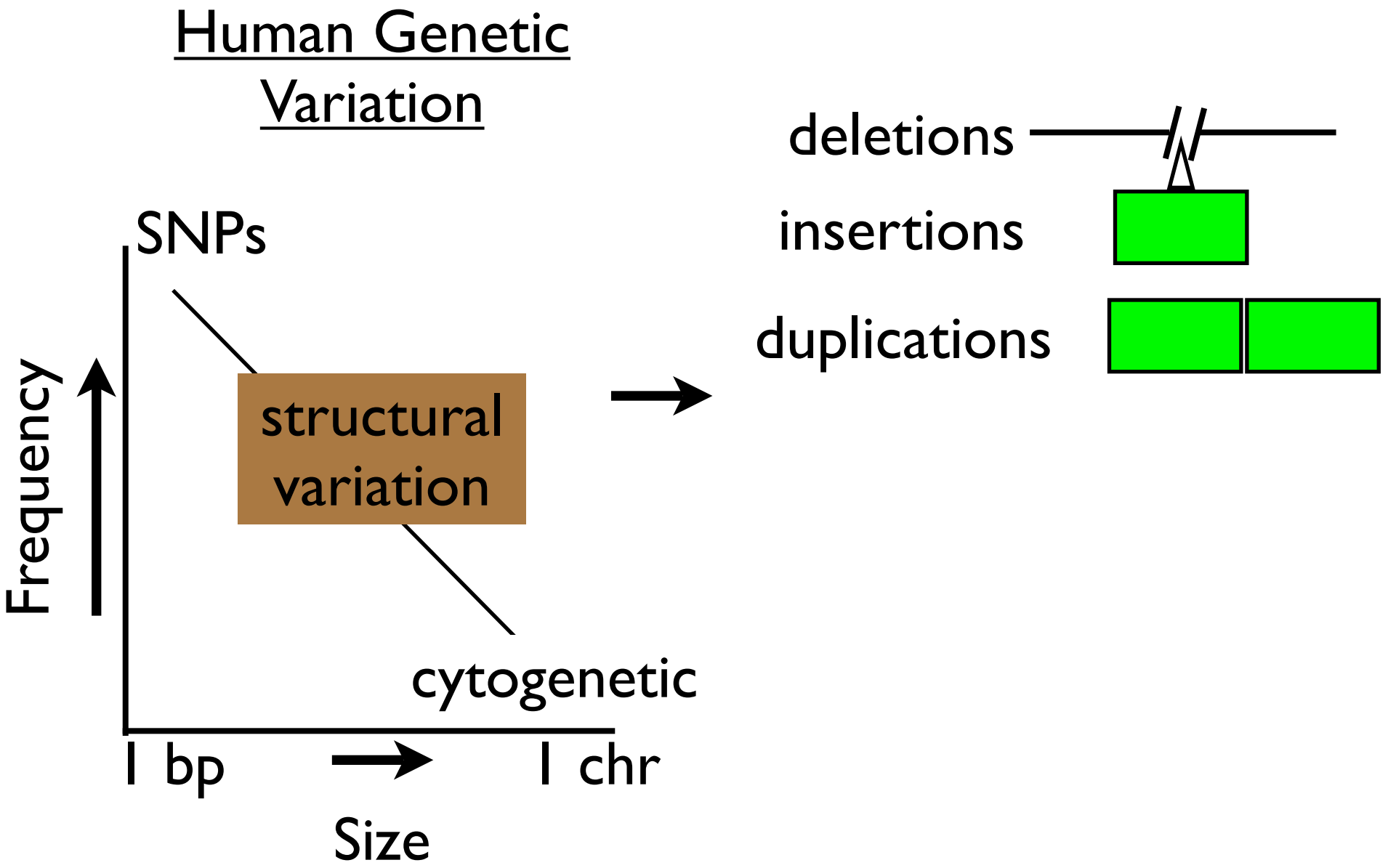

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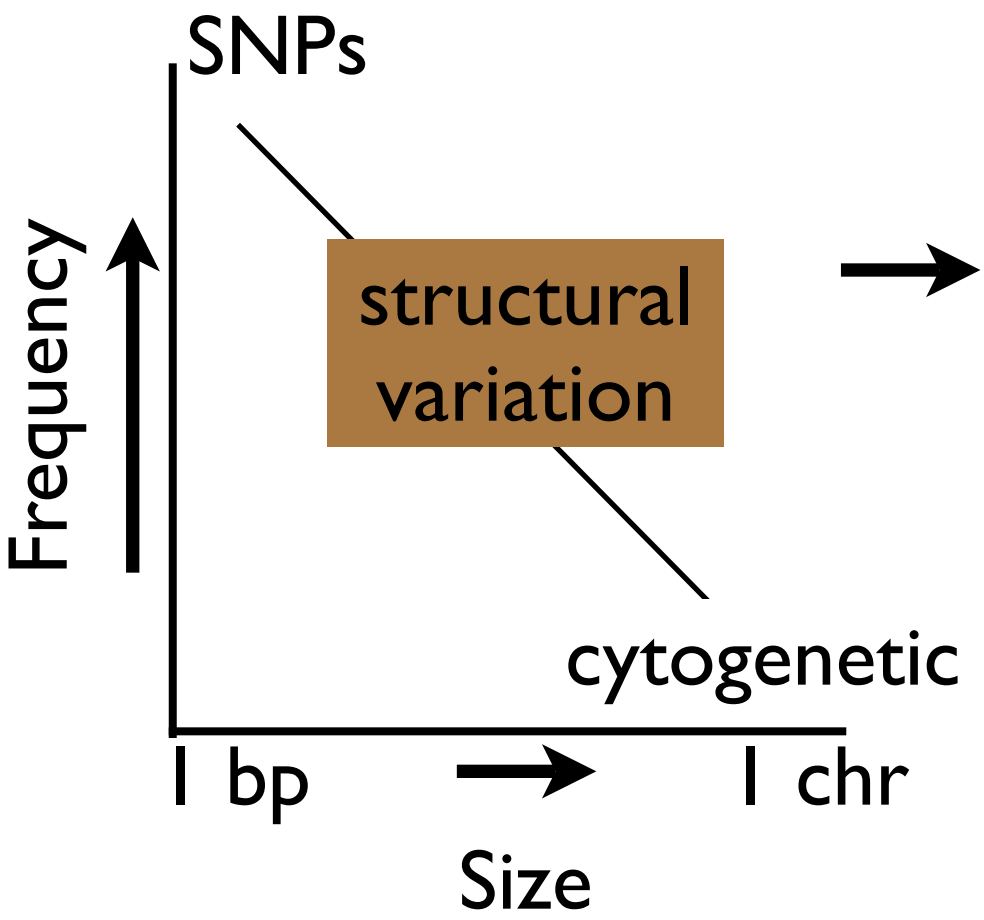
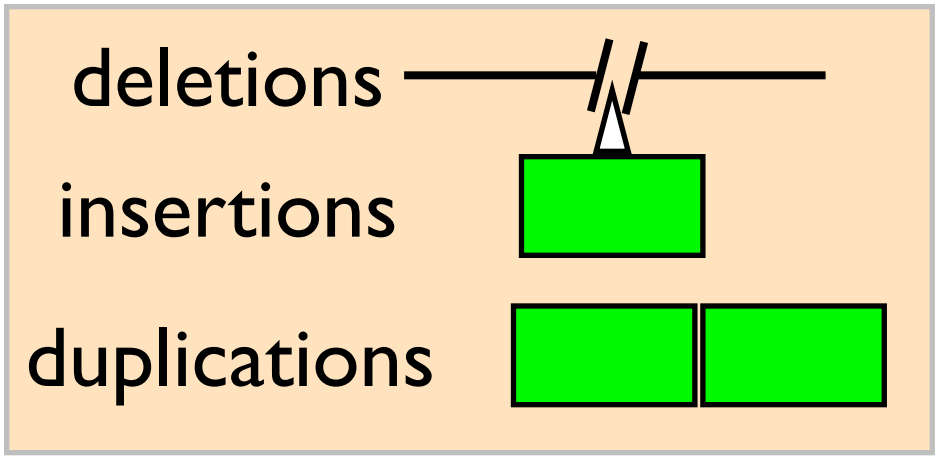


Copy-Number Variants



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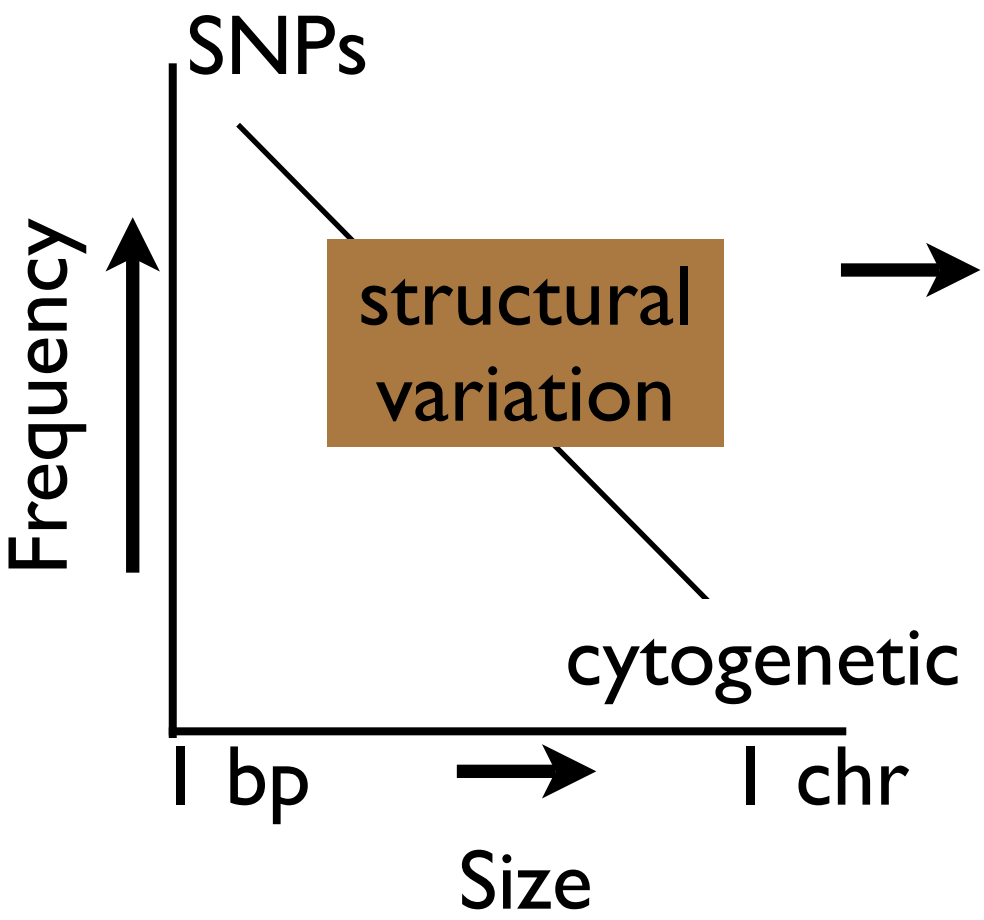

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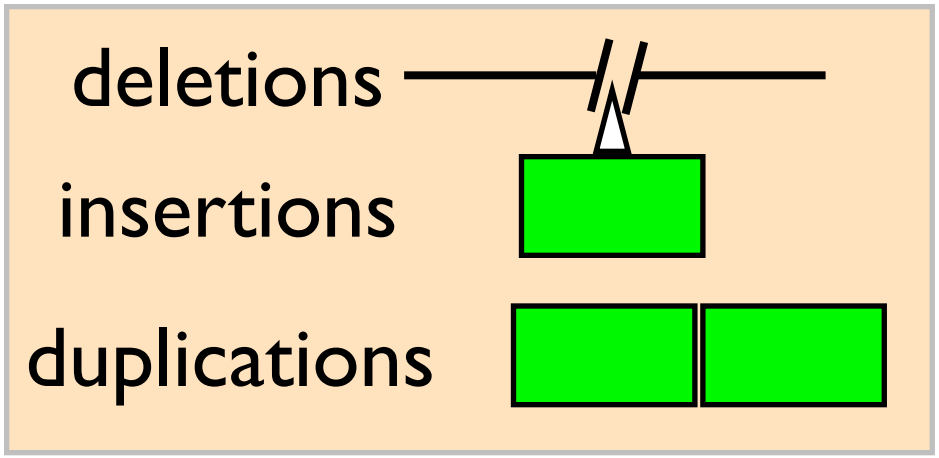
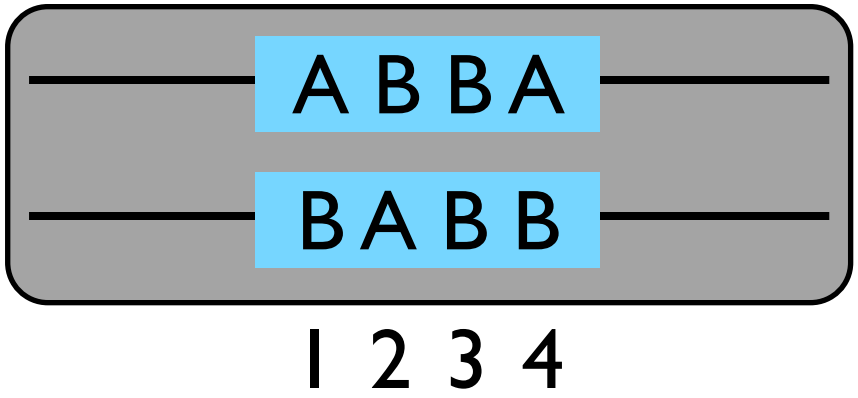


- Gene-rich, e.g. immune response, drug metabolism
- Abundant: majority of human heterozygosity
- Technological challenges have impeded large-scale analyses

SNP-based Deletion Discovery

SNP-based Deletion Discovery



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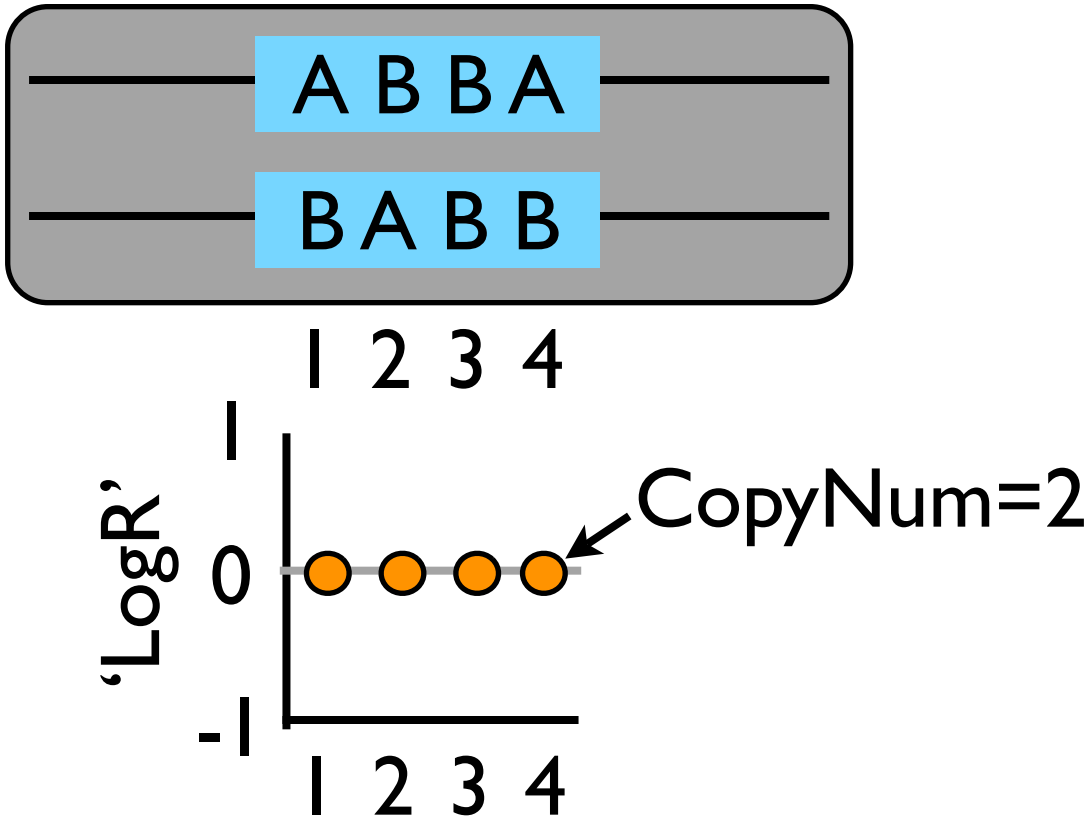

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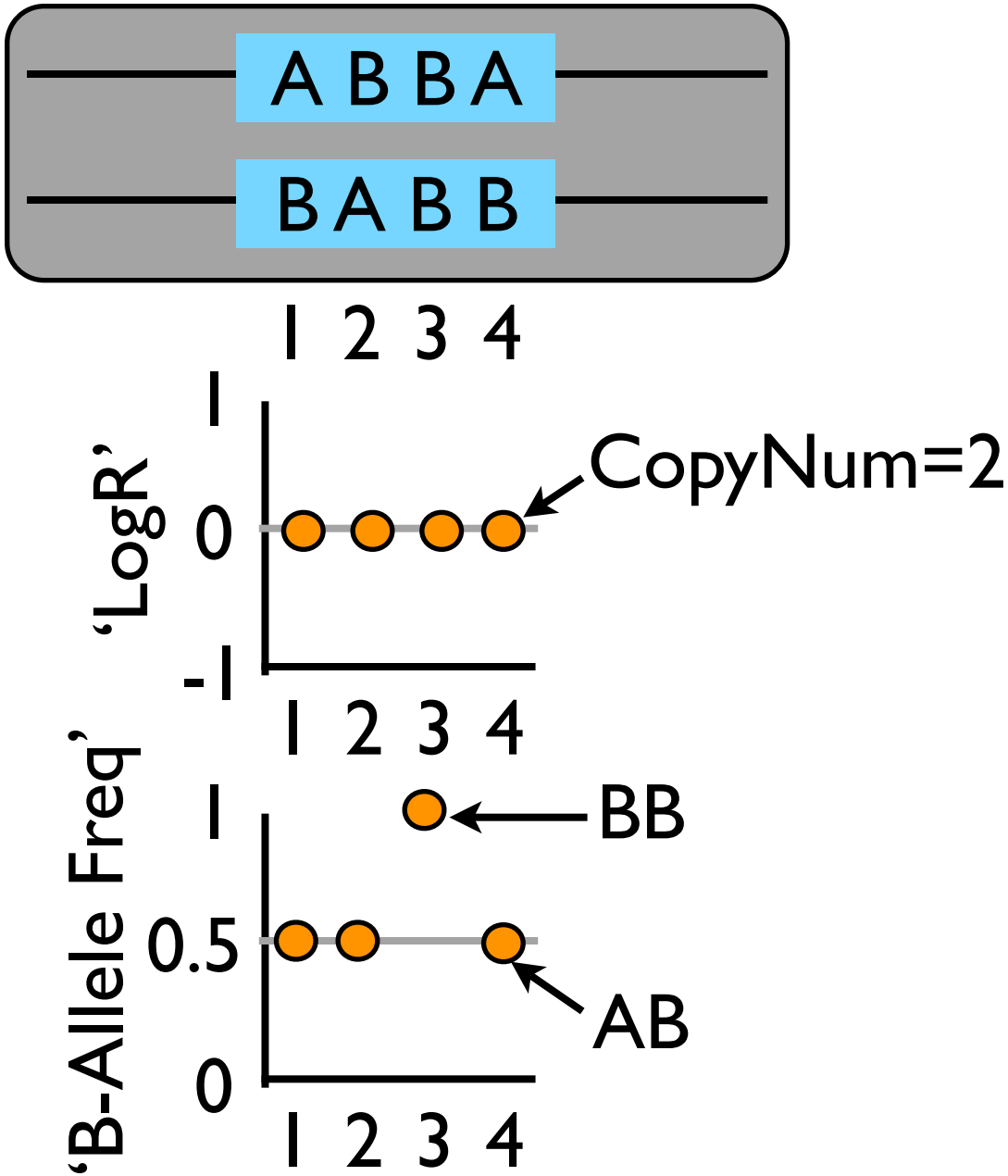

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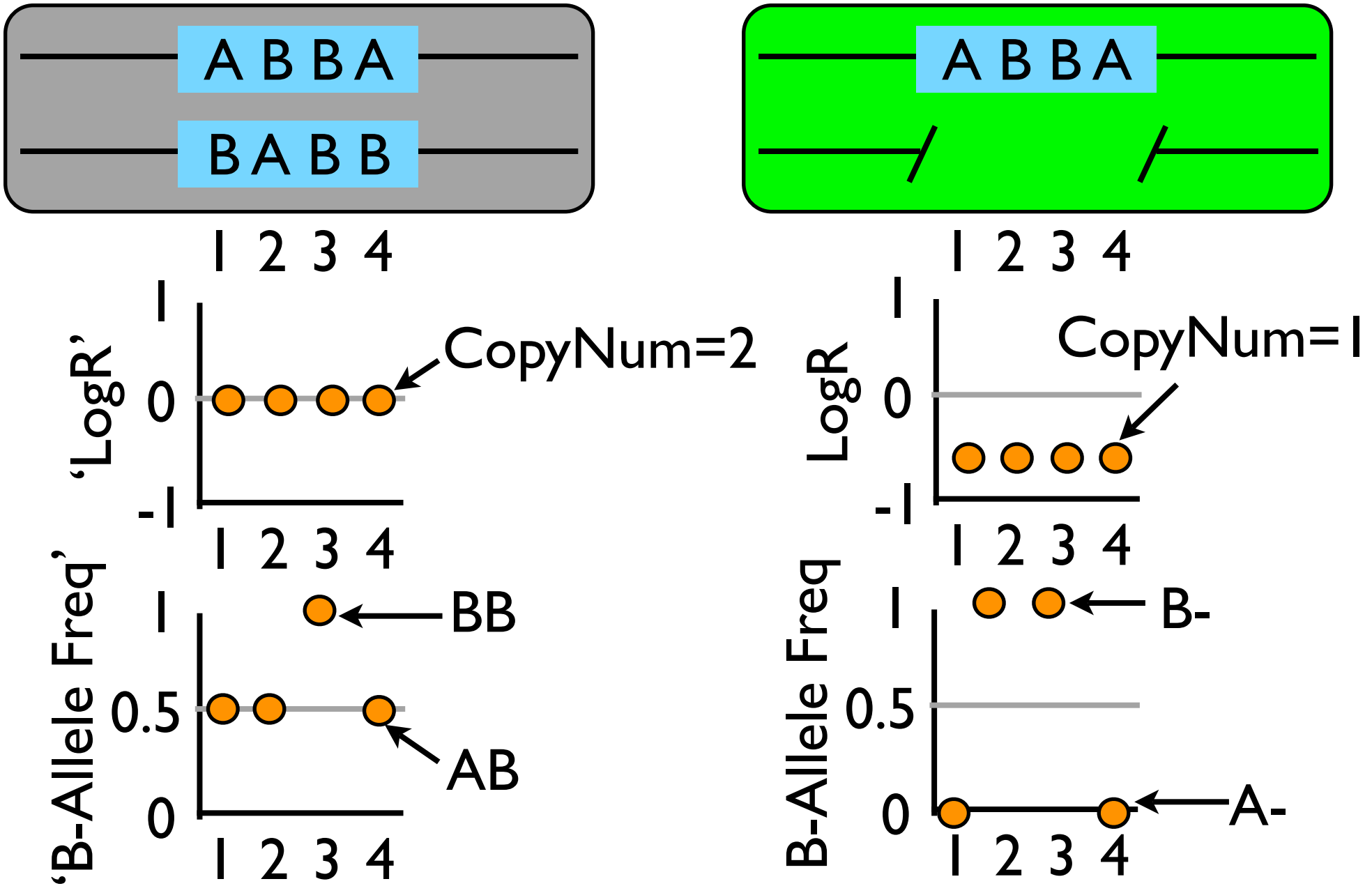
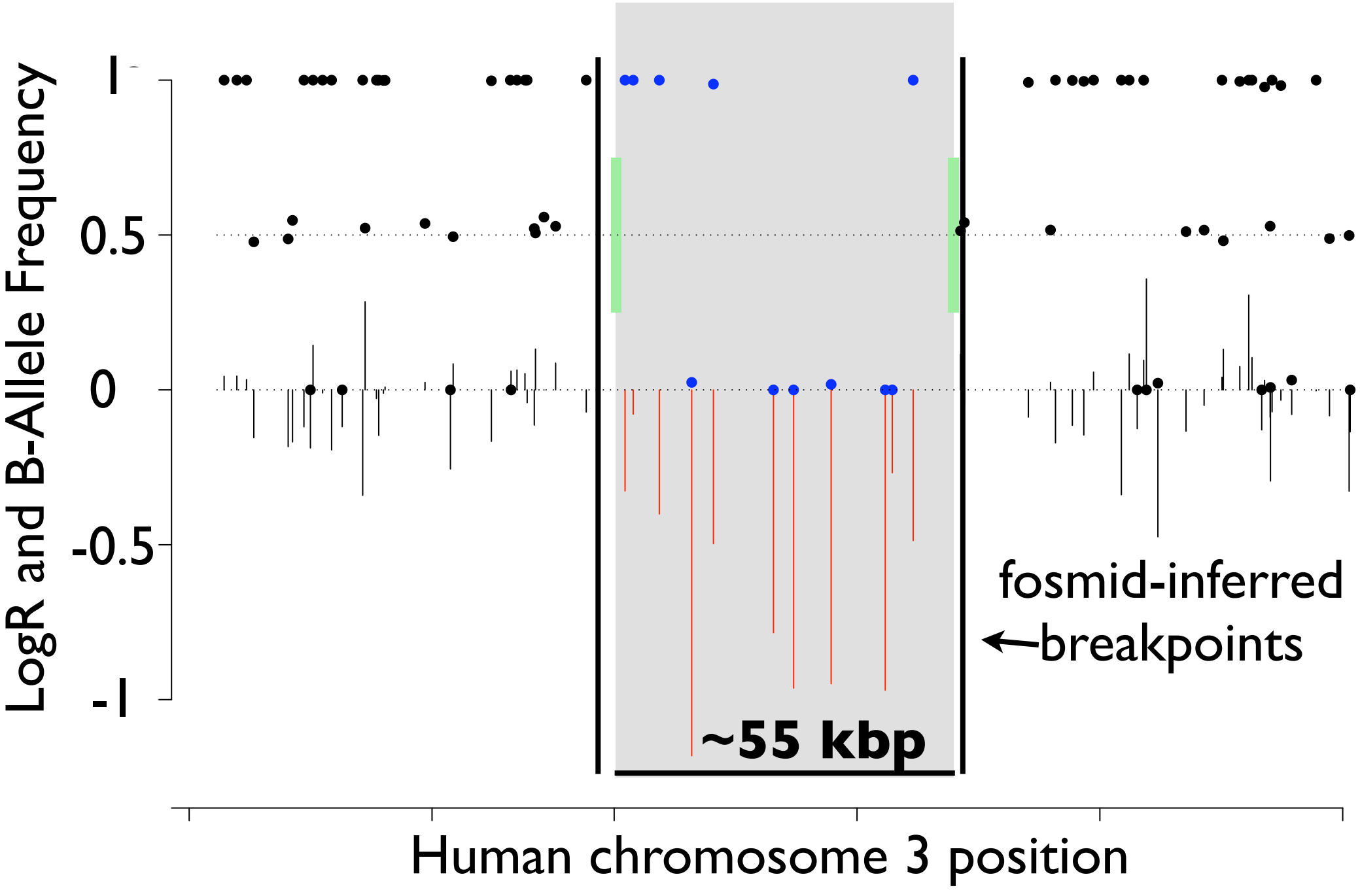


Illumina 1M Deletion Discovery



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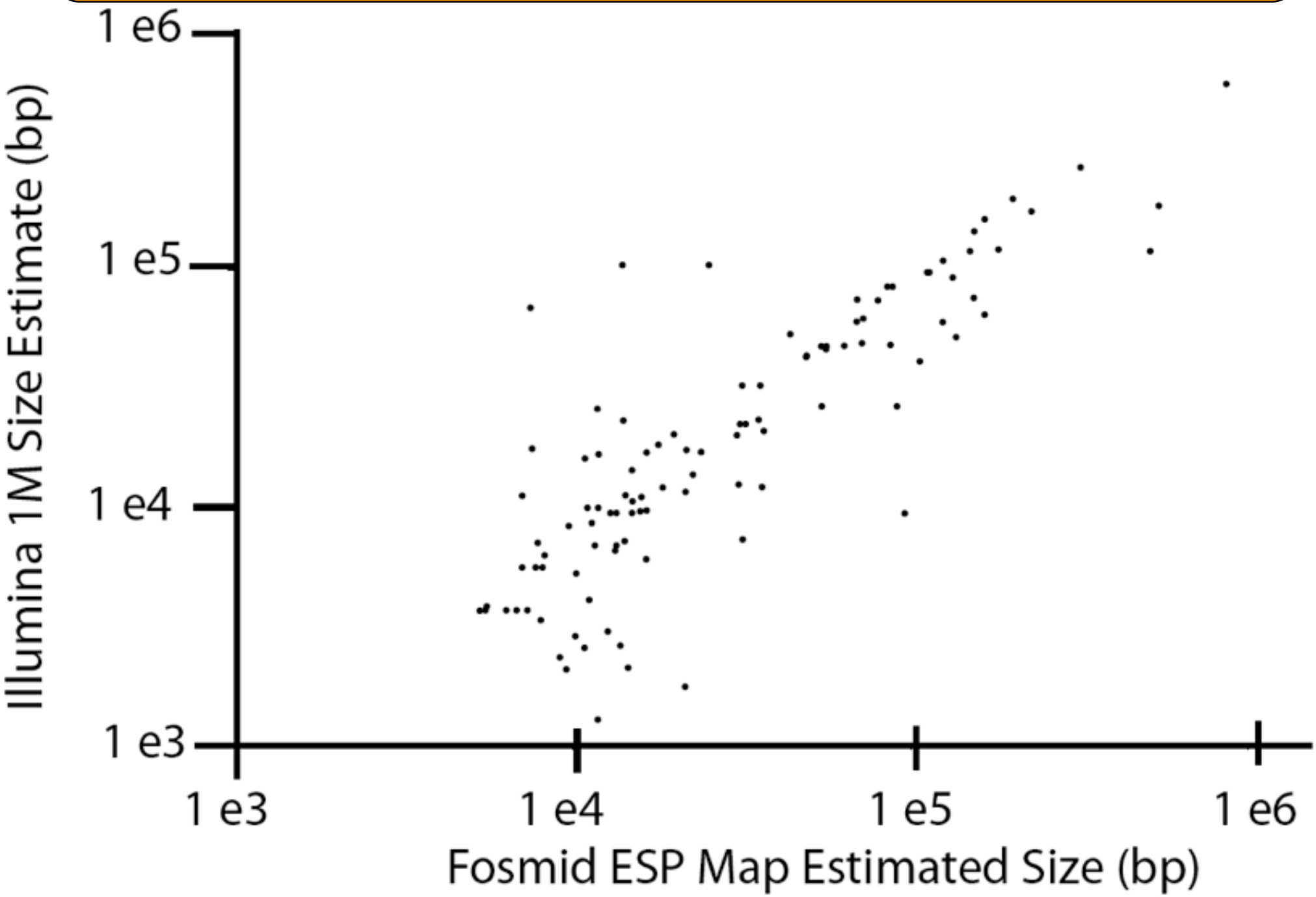
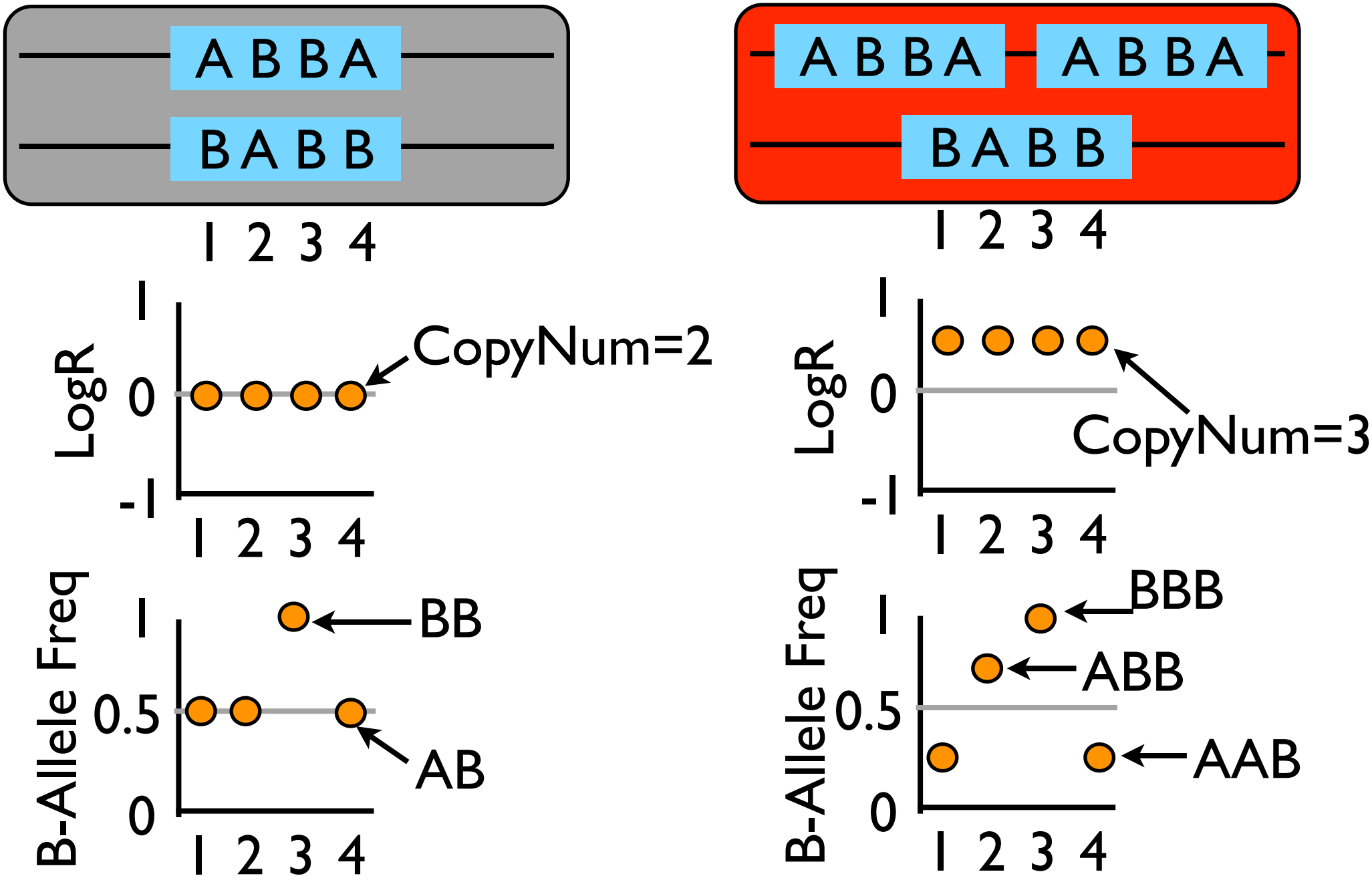
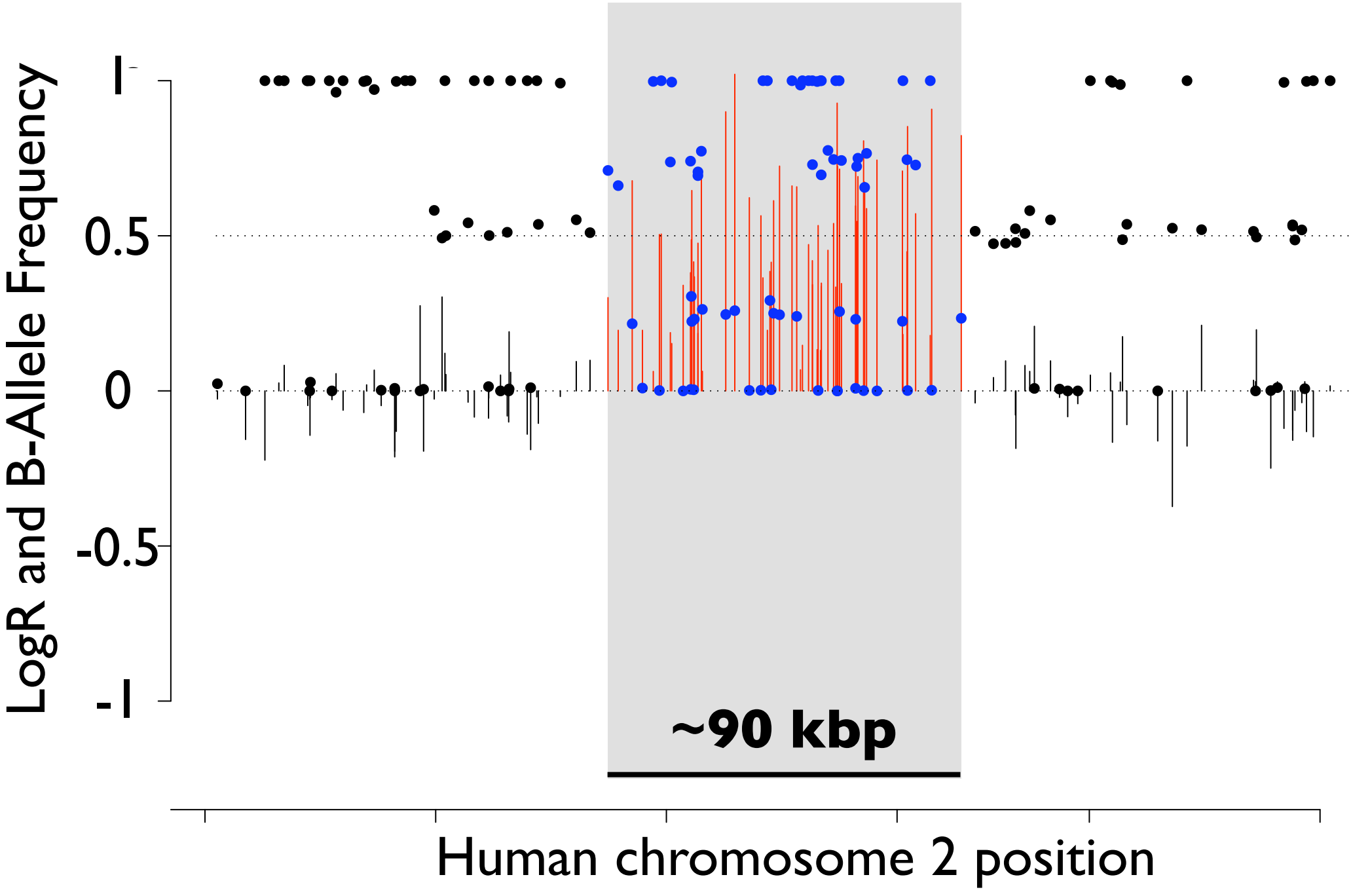


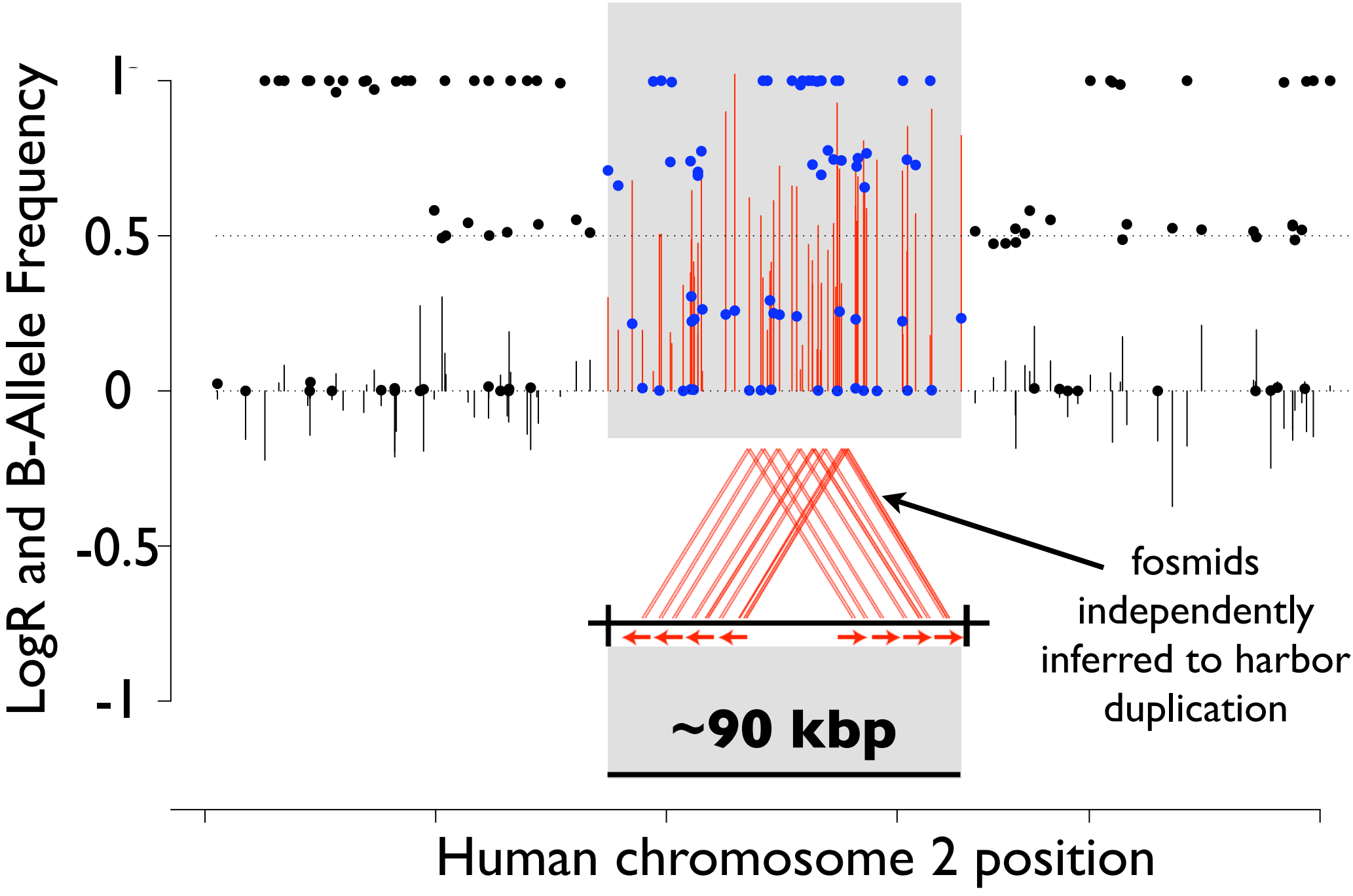
SNP-based Duplication Discovery



Illumina 1M Duplication Discovery



Illumina 1M Duplication Discovery



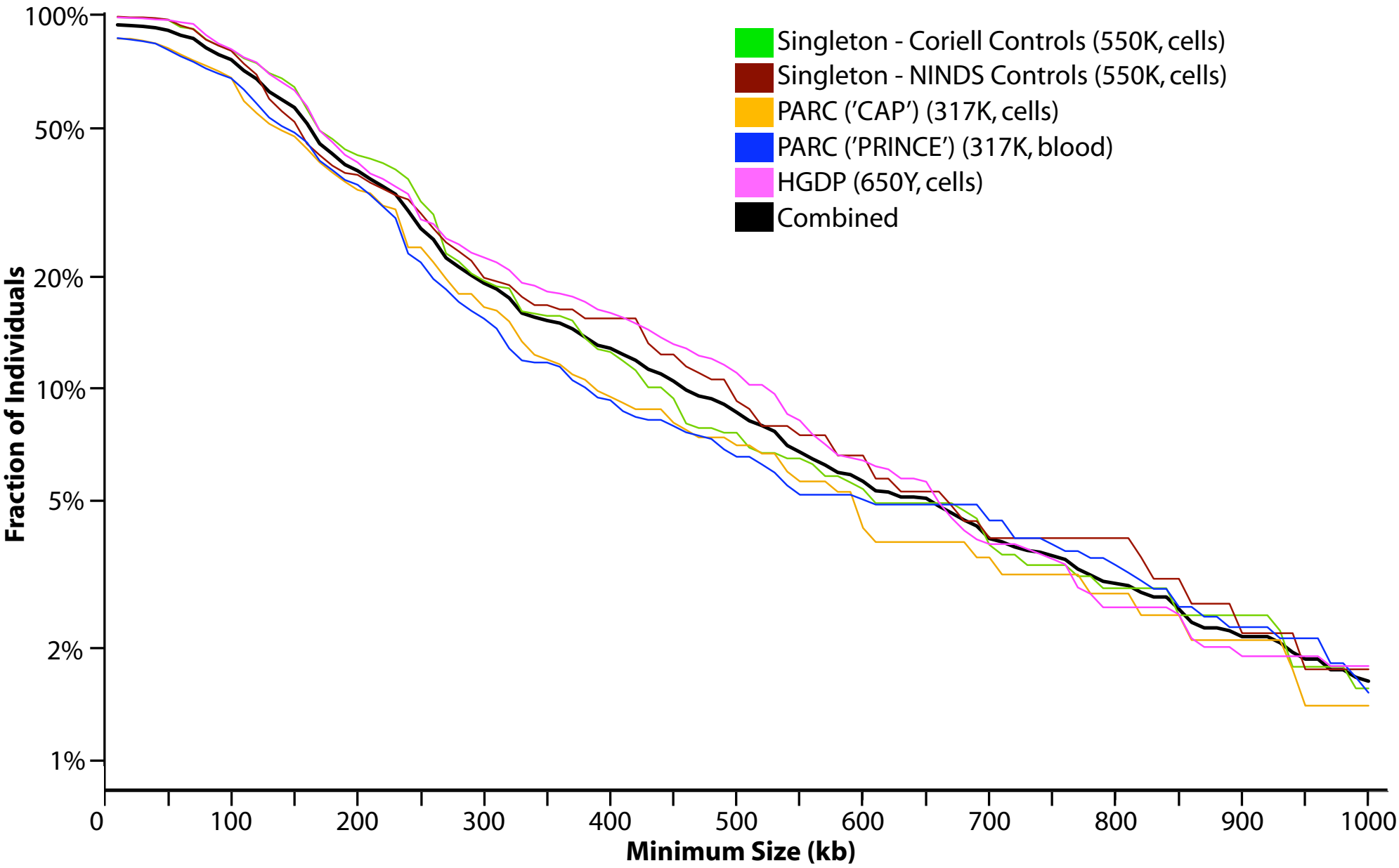
Discovering CNVs in Large Cohorts

- ~1,000 individuals from the PARC project (Illumina 317k SNP arrays)
  - Caucasian samples from a statin pharmacogenetics study
- ~1,000 samples from the Human Genome Diversity Panel (Illumina 650Y chips; Li, Absher, et al *Science* 2008):
  - samples collected in diverse regions of the world
- ~800 neurological disease controls (Illumina 550K chips; Andy Singleton; Walsh et al, *Science* 2008)
  - samples screened for symptoms of psychiatric disease
- n = 2,493 samples after QC (600 blood DNA)

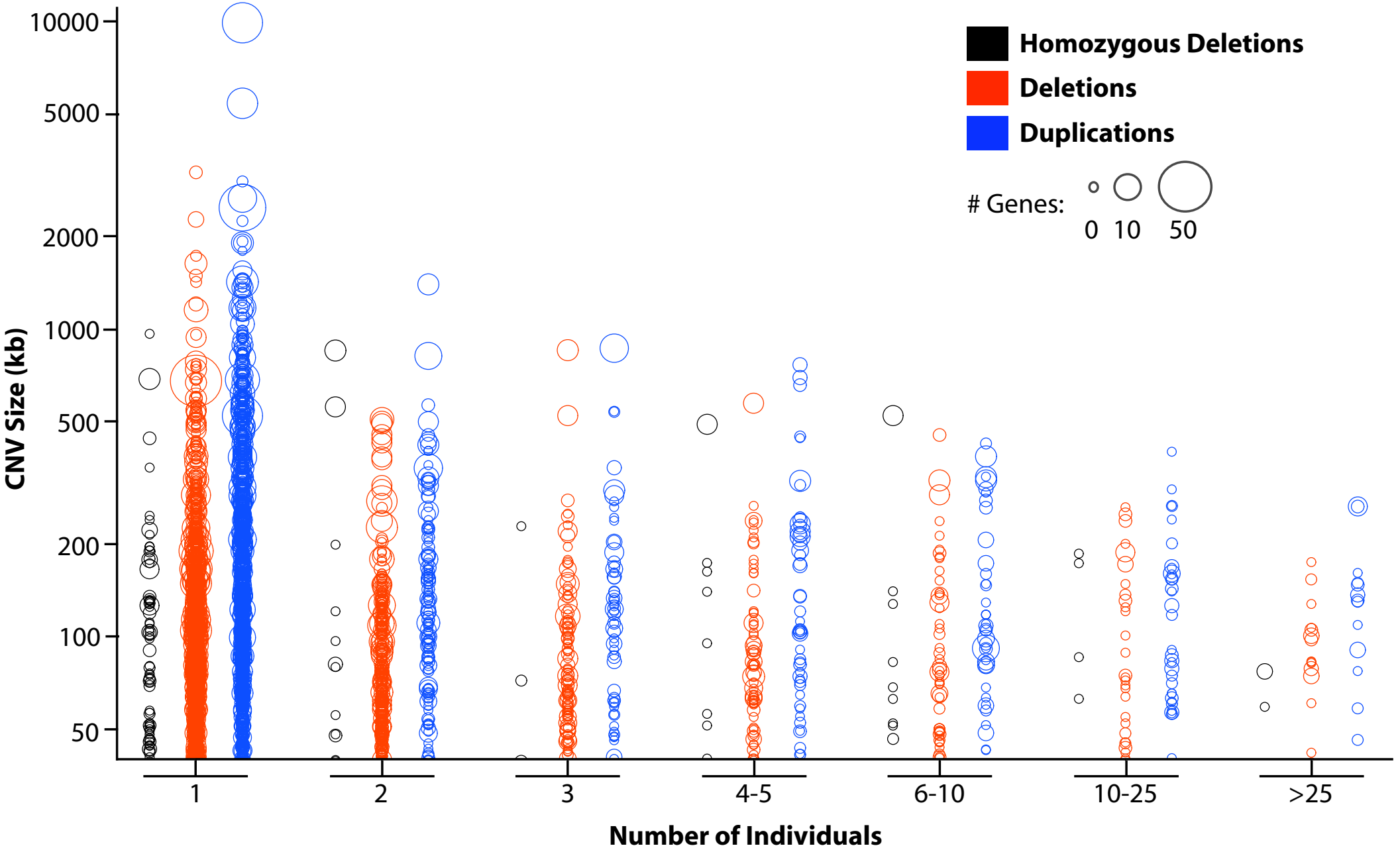
Large CNV Overview

Study	Platform	# Samples	CNVs	Calls/Sample
PARC	HH317K	936 (991)	2,664	2.85
Neurological Disease Controls	HH550K	671 (790)	4,641	6.92
HGDP	HH650Y	886 (941)	6,538	7.38
Total		**2,493 (2,722)**	**13,843**	**5.56**

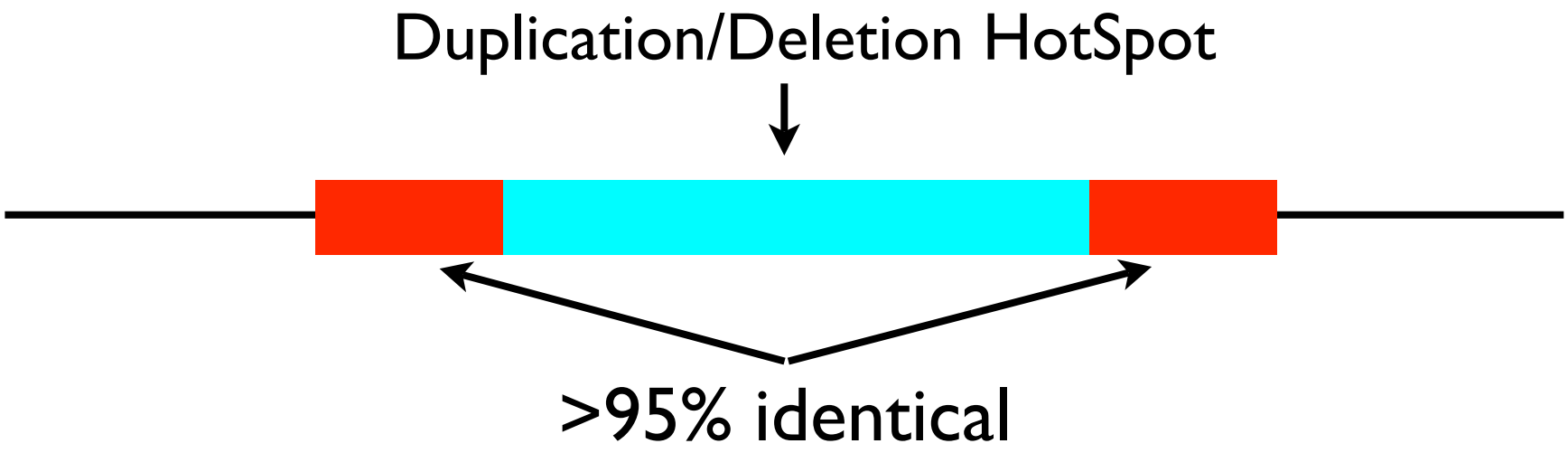
Many Individuals Carry Large Events



Collectively Frequent But Individually Rare

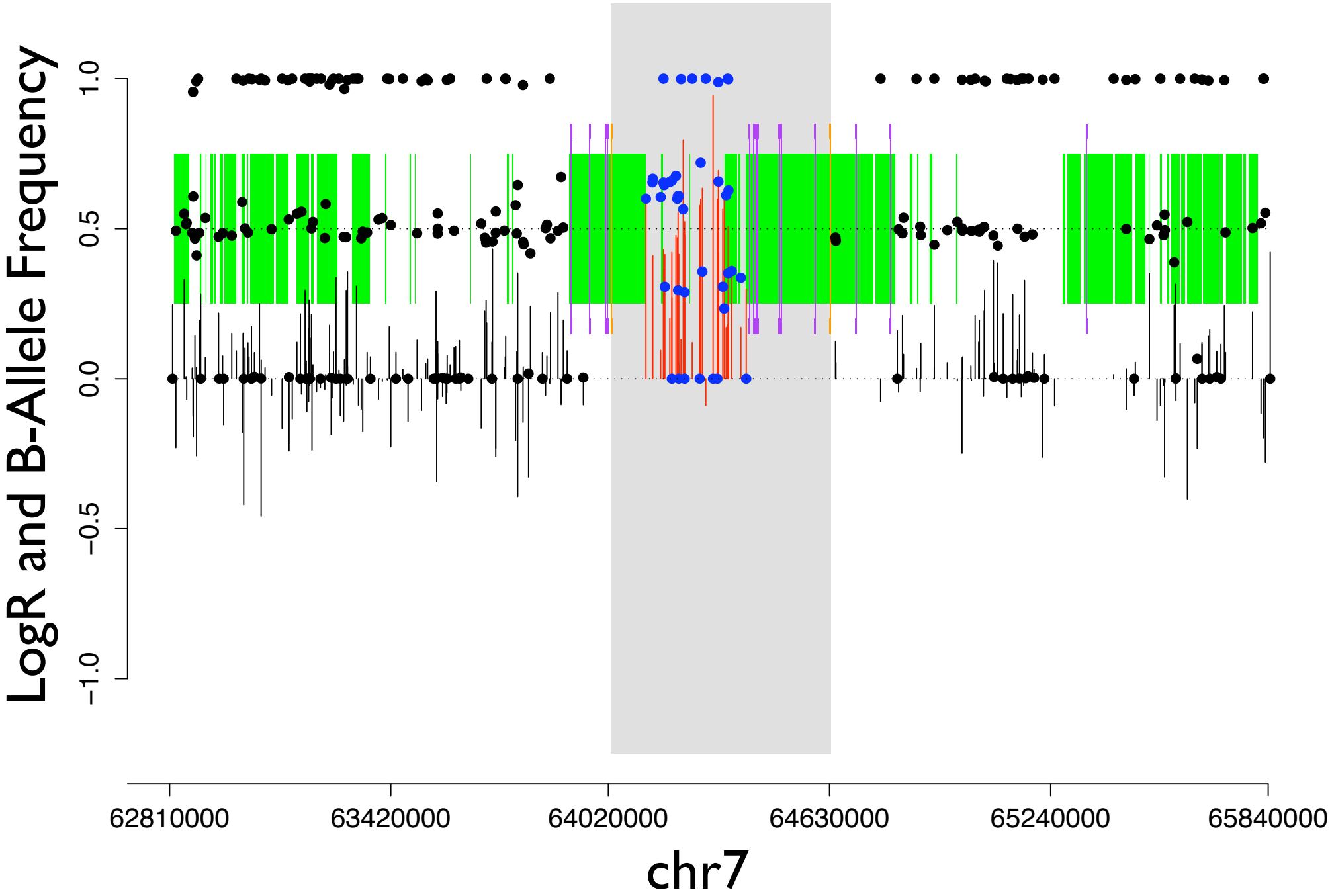


Genomic CNV 'HotSpots'



- Non-Allelic Homologous Recombination (NAHR) between duplicated sequences results in novel CNVs
- Thousands of potential hotspots in the reference assembly (1 kb to Mbps in size)
- *de novo* hotspot mutations have been implicated as causative for a number of diseases

CNVs Enriched Near Segmental Duplications



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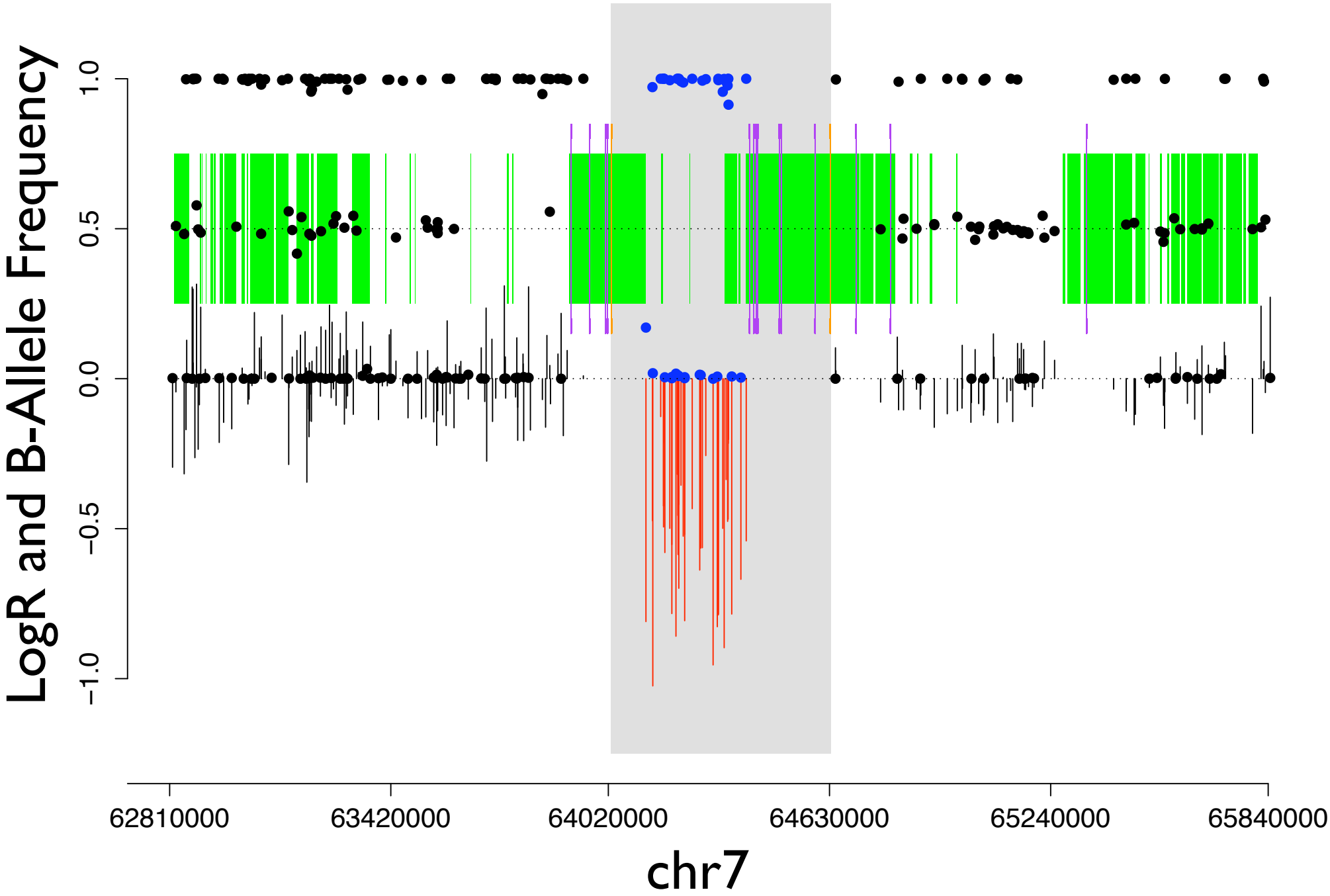

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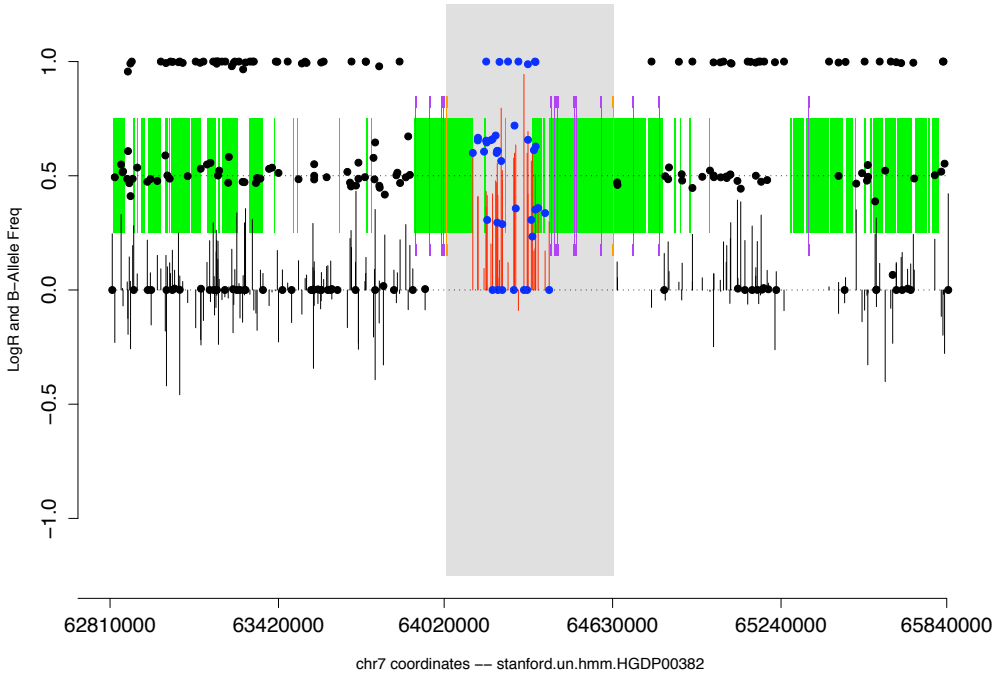
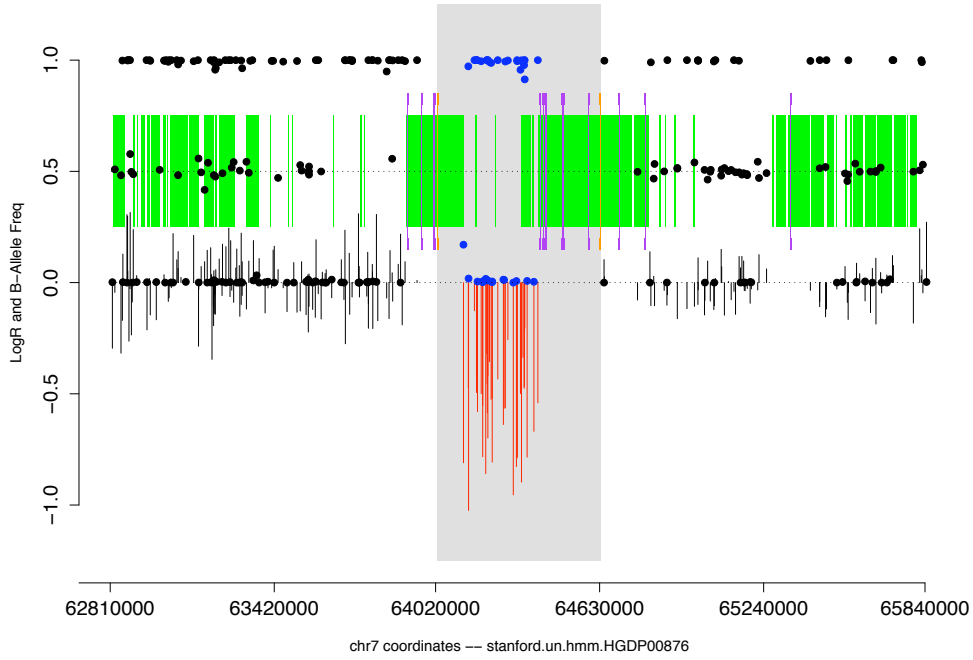
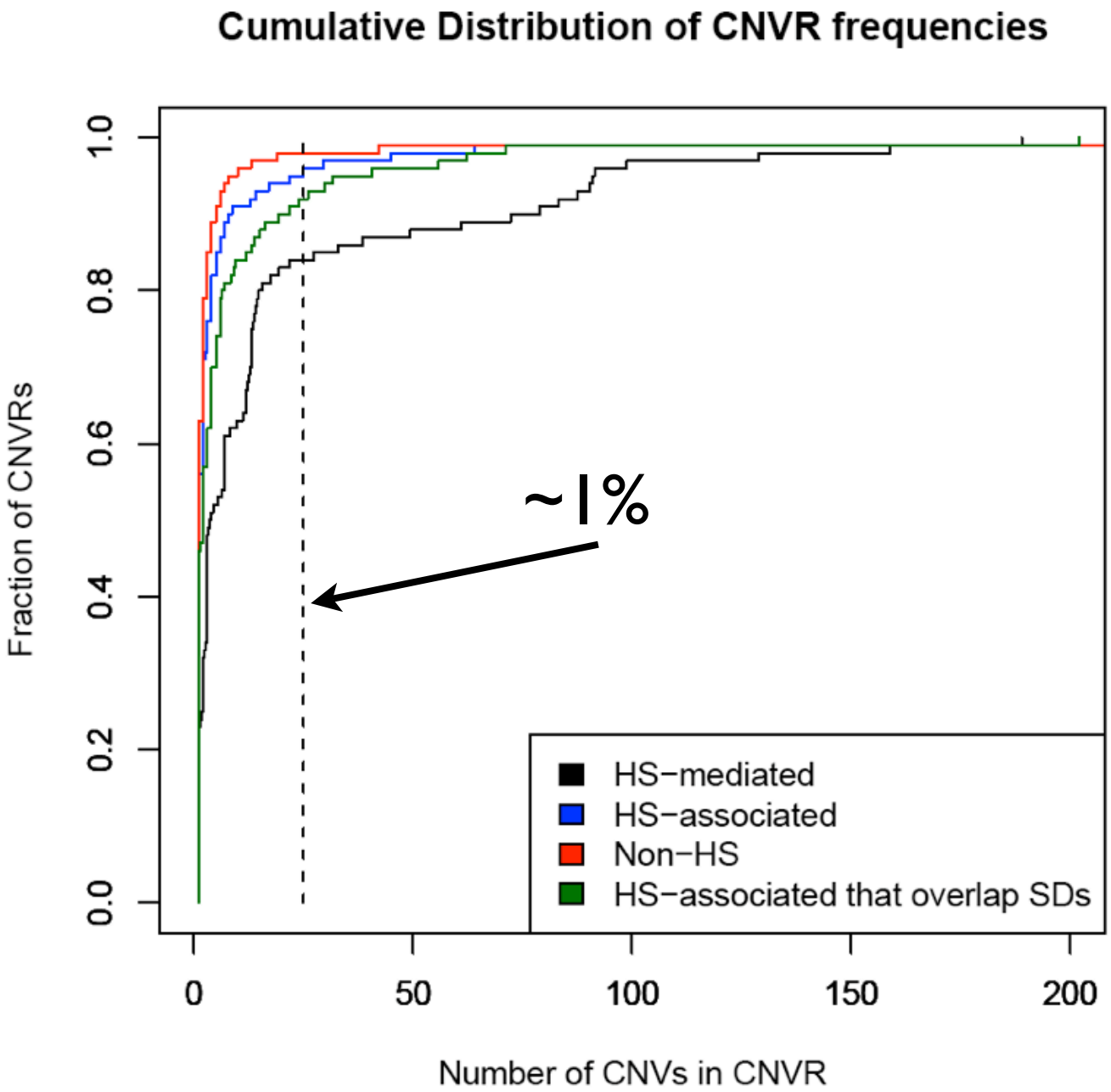




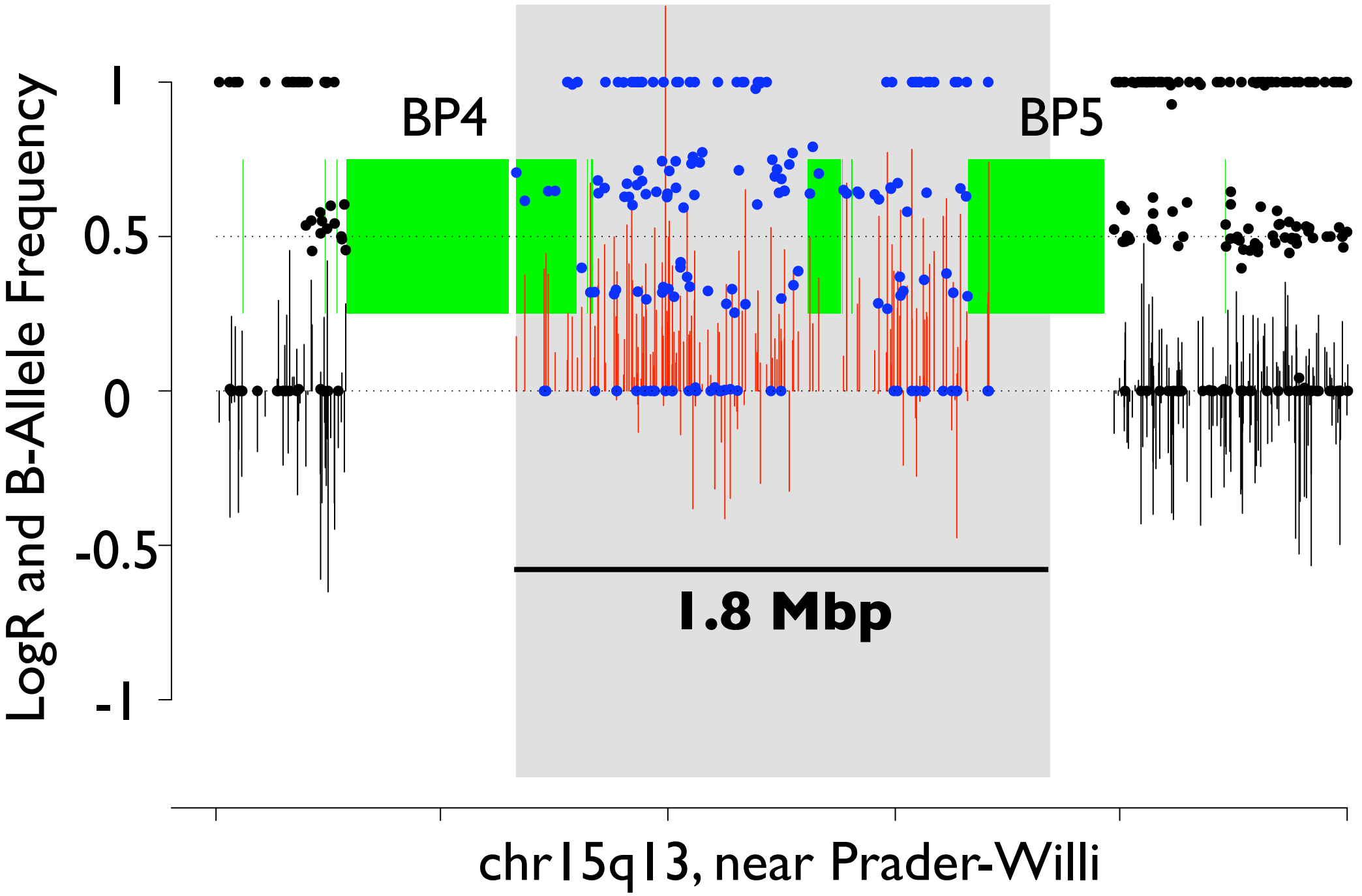
25X enrichment for CNVs between homologous duplications in the reference assembly

Hotspots Increase CNV Frequencies



Duplications increase CNV allele frequency

Large ‘HotSpot’ Duplication



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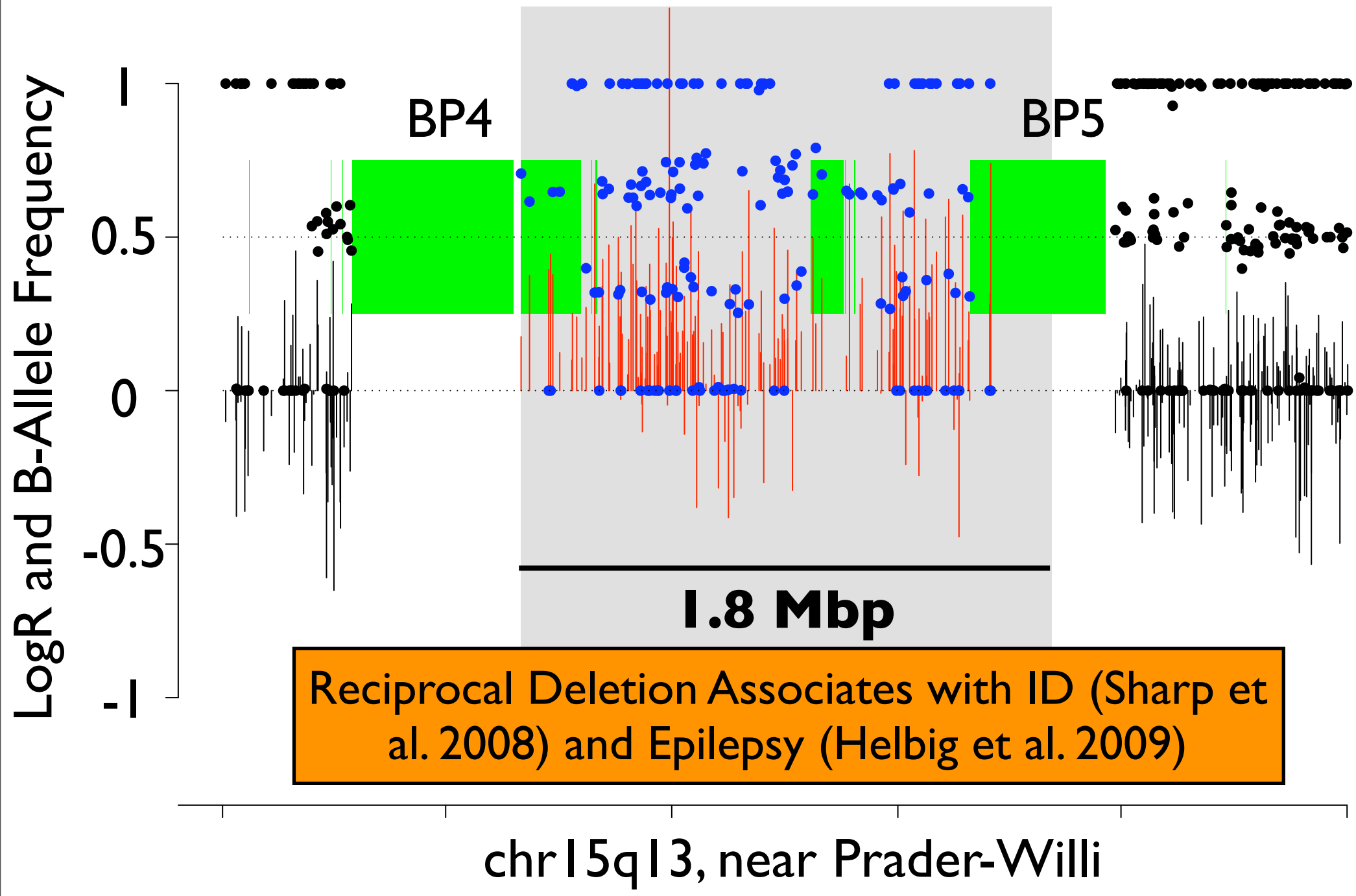
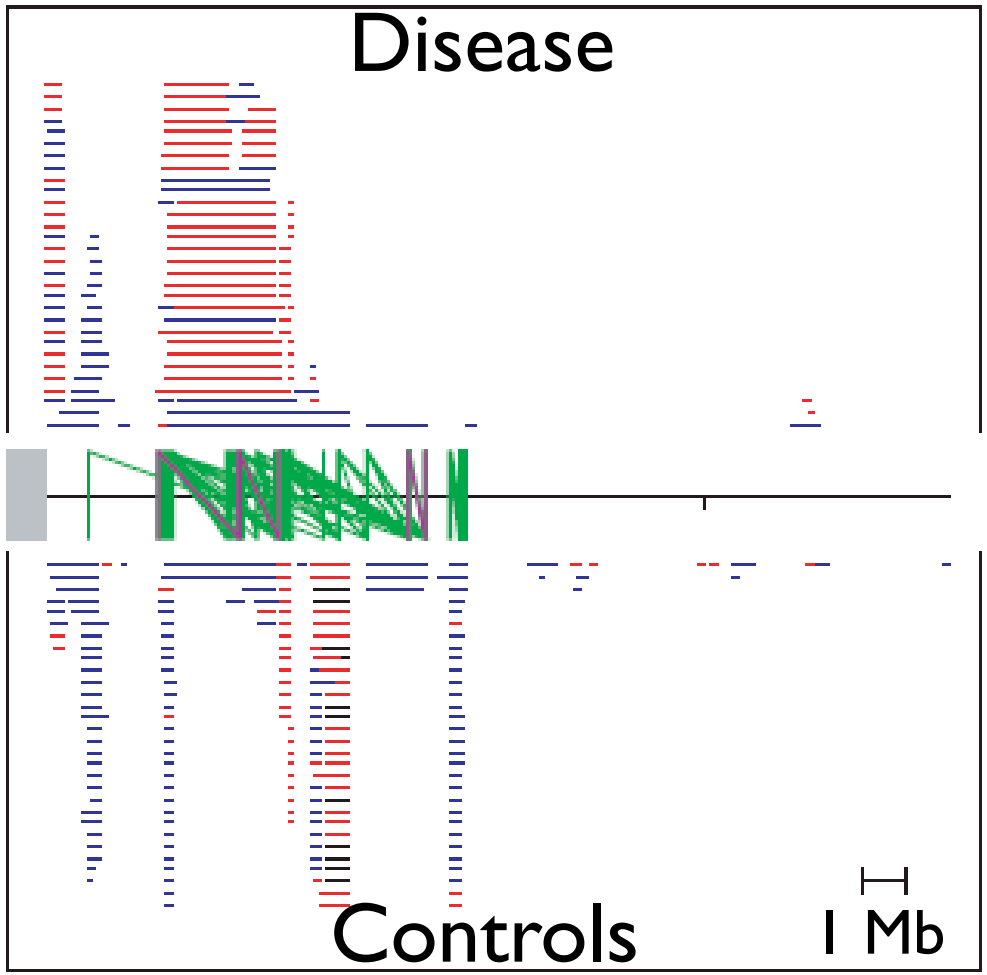


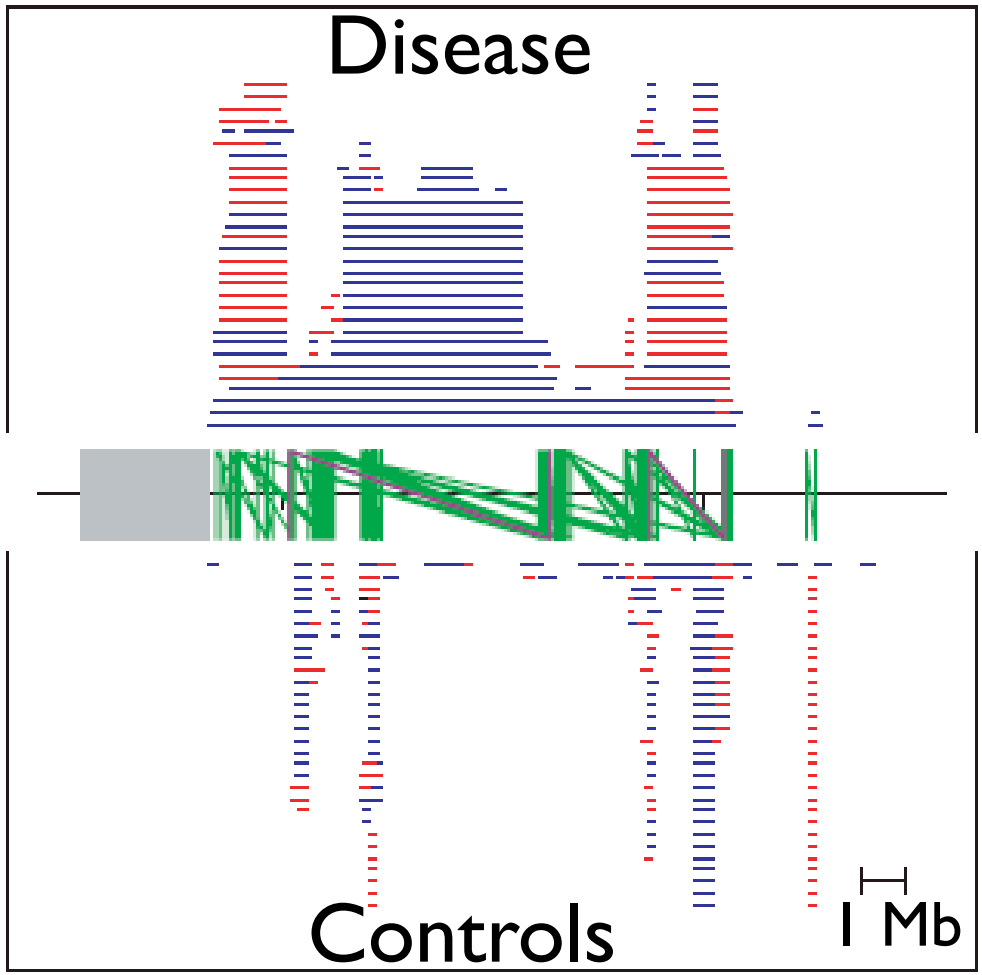
Reciprocal Deletion Associates with ID (Sharp et al. 2008) and Epilepsy (Helbig et al. 2009)

Neurological Disease Meta-Analysis

- Combined our ~2,500 samples with published CNV calls from ~3,000 controls analyzed using Affymetrix arrays (ISC *Nature* 2008)
- Combined genome-wide CNV annotations from 9 disease studies:
  - schizophrenia: ~3,500 individuals
  - autism: ~2,500 individuals
  - intellectual disability: ~500 individuals
  - mixture of Affy, Illumina, and CGH
  - only analyzed CNVs > 500 kb

Neurological Disease Meta-Analysis

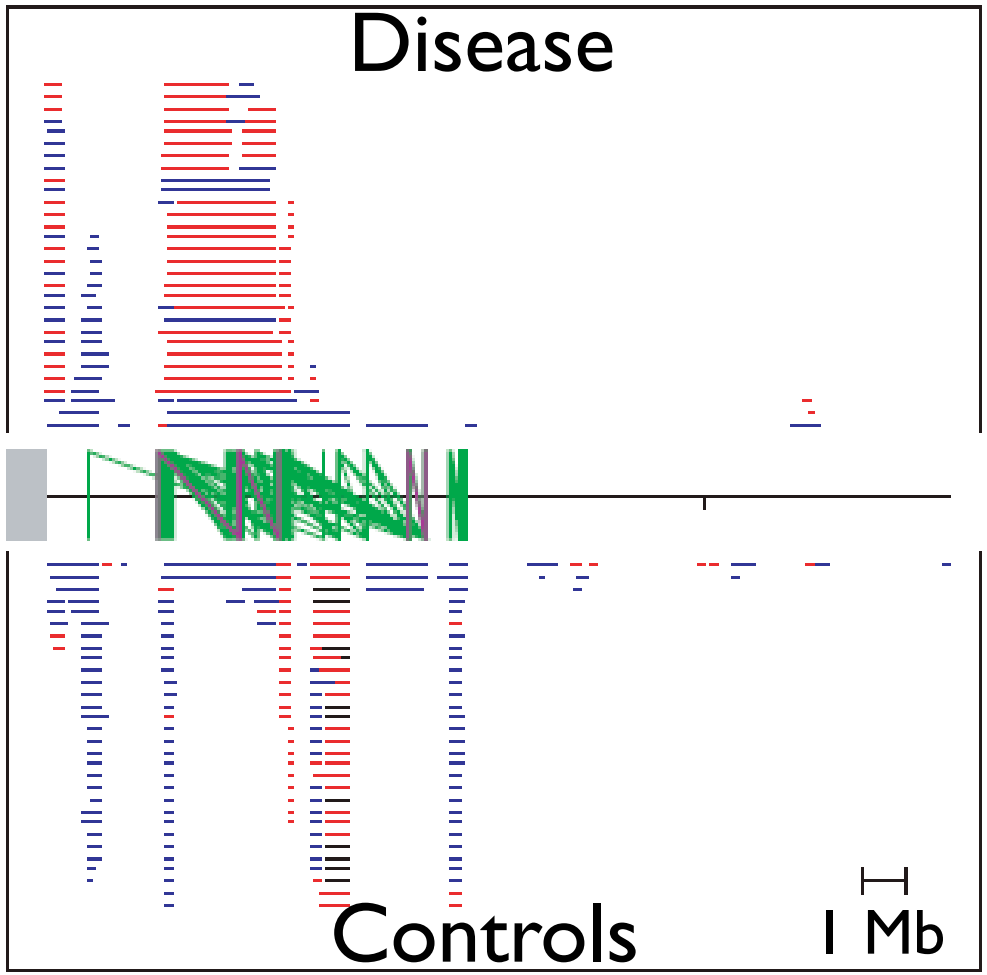




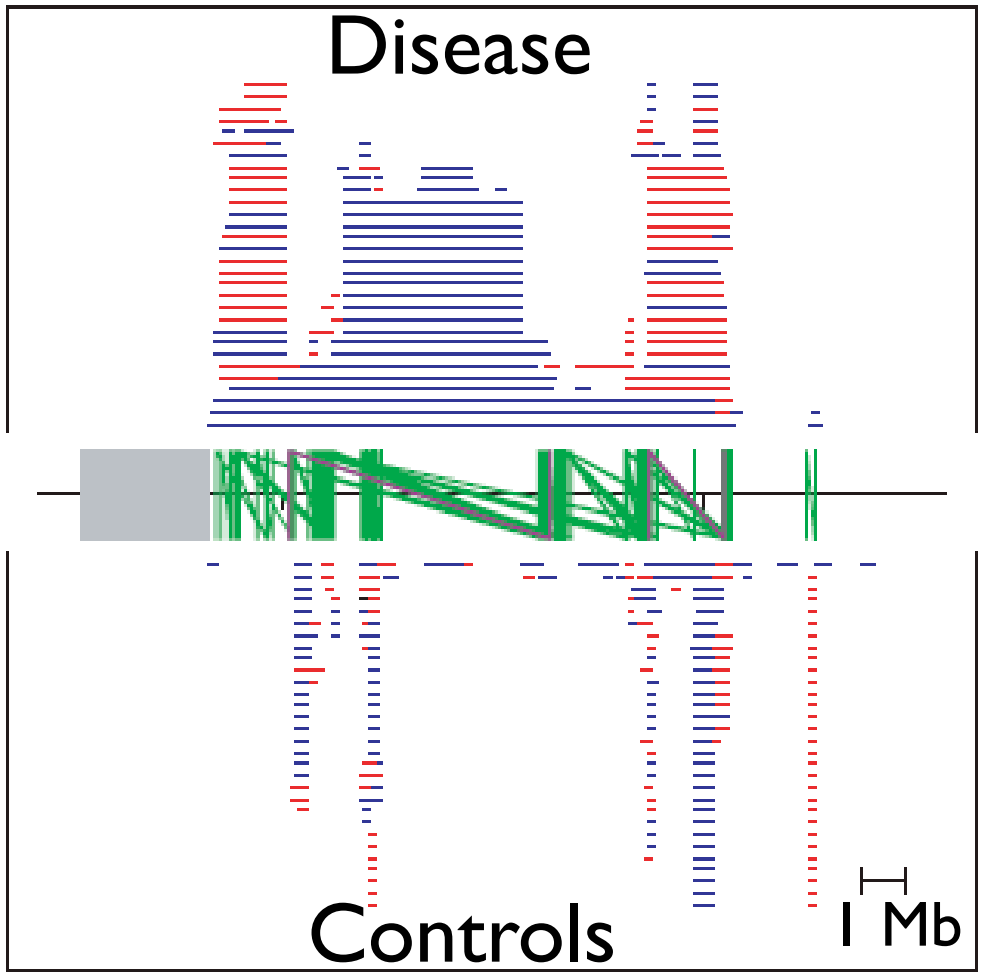
Deletions

Duplications

Neurological Disease Meta-Analysis



22q11-12 (VCFS)

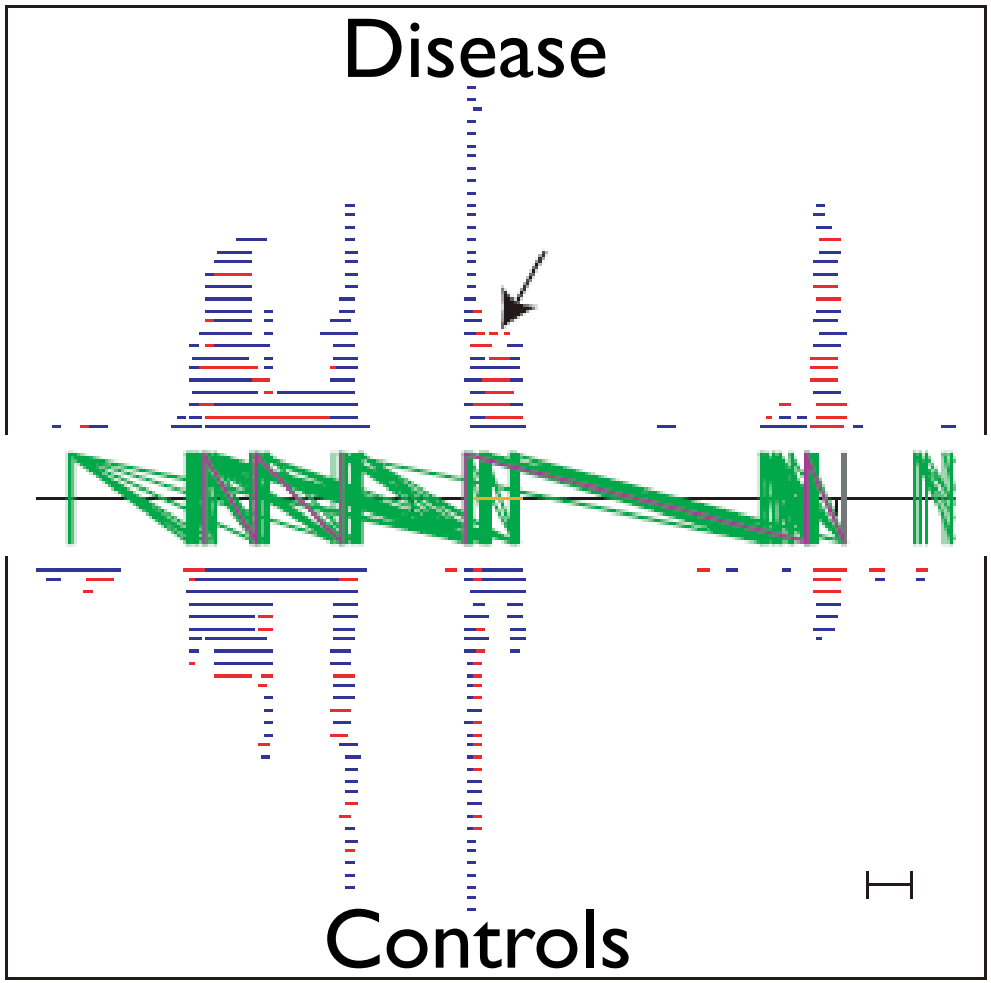


Prader Willi,15q13

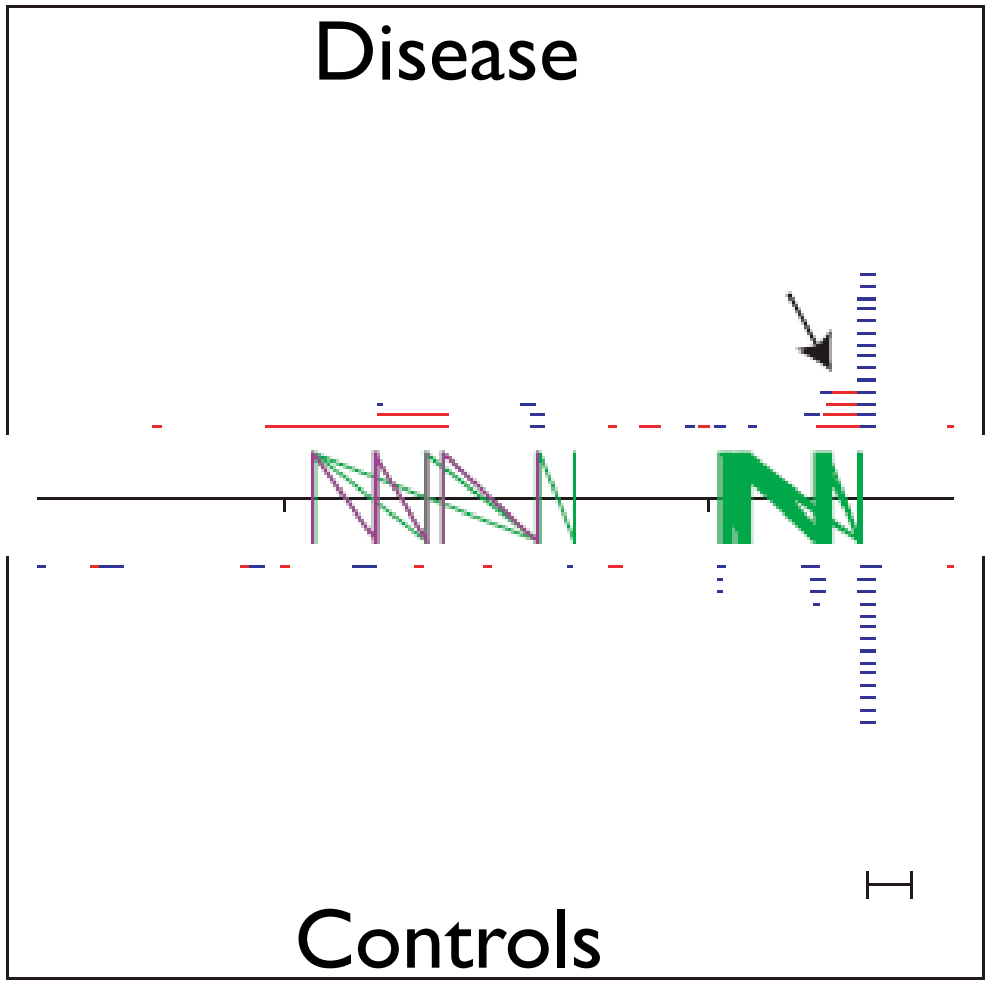
Deletions

Duplications

Neurological Disease Meta-Analysis



16p12 ?



15q25 ?

Deletions

Duplications

Neurological Disease CNVs

Chr	Start	Stop	Type	Note	NAHR?	Disease CNVs	Control CNVs	Diseases
chr22	17,014,900	19,993,127	loss	VCFS	yes	31	0	A,S,ID
chr15	27,015,263	30,650,000	loss	15q13	yes	19	0	S
chr1	142,540,000	146,059,433	loss	1q21.1	yes	24	3	A,S,C
chr15	18,376,200	30,756,771	gain	PW/15q13	yes	45	13	A,S,ID,C
chr1	142,800,580	146,009,436	gain	1q21.1	yes	12	3	A,S,ID,C
chr16	21,693,739	22,611,363	loss	16p12	yes	5	0	A,S
chr22	45,144,027	49,509,153	loss	Term 22	no	4	0	A
chr16	60,141,700	61,581,600	loss	16q21	no	4	0	A
chr15	82,573,421	83,631,697	loss	15q25	yes	4	0	A,S
chr16	80,737,839	82,208,451	gain	16q23.3	no	4	0	A,S
chr16	29,474,810	30,235,818	gain	16p11.2	yes	6	1	A,S,C
chr17	14,000,000	15,421,835	loss	HNPP	yes	6	1	A,S,C
chr22	47,572,875	48,323,417	gain	Term 22	no	5	1	A,S,C
chr11	78,120,000	85,610,000	loss	11q14	no	3	0	S,ID
chr2	184,270,000	186,892,000	gain	2q32	no	3	0	A
chr9	206456	1599250	gain	9p24	no	3	0	A,S
chr3	197,179,156	198,842,299	loss	3q29	yes	3	0	S
chr16	29,470,951	30,252,473	loss	16p11.2	yes	8	3	A,C
chr17	12,650,000	15,540,000	gain	CMT1A	yes	4	1	A,S,ID,C

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- 3q29 independently reported as an ID syndrome
- 16p12 deletion present in:
  - a schizophrenia/ID affected family (from Mary-Claire King)
  - 3 schizophrenic and 2 control samples (from Jonathan Sebat)
  - 12 out of ~10,000 children with various cognitive deficits (Lisa Shaffer and Signature Genomics)

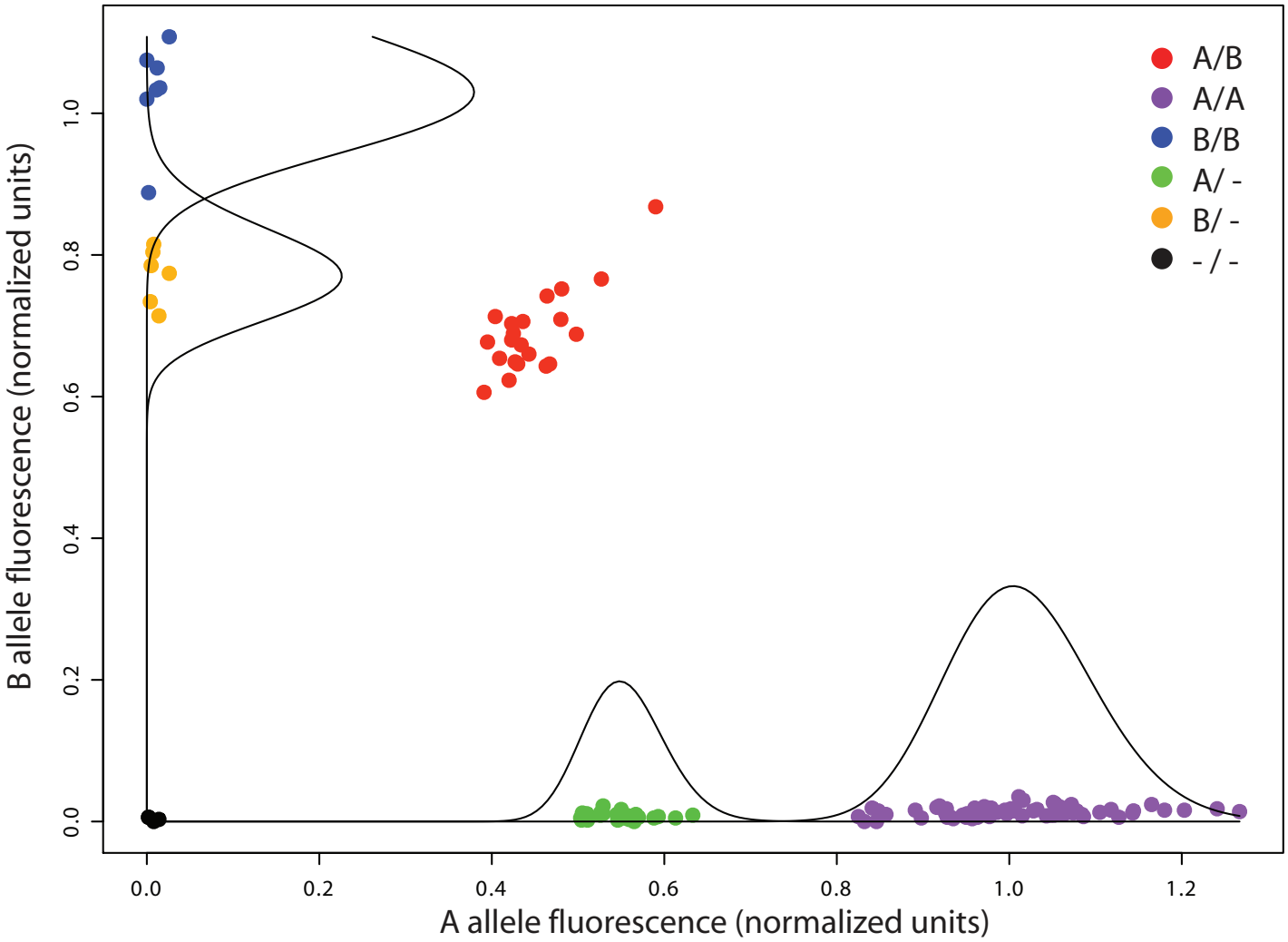
Large CNVs in Human Populations

- Large CNVs are collectively frequent (most individuals carry one or more large CNVs)
- Large CNVs are individually rare (usually <1% frequency)
- NAHR contributes to both common and rare variants:
  - many CNVs will not be 'taggable' via SNPs
  - ascertaining variation in/near duplications critical
- Highly penetrant CNVs, each explaining <1% of disease, may collectively make large disease contributions
  - Accurate genotyping of rare CNVs in large numbers will be required to identify these

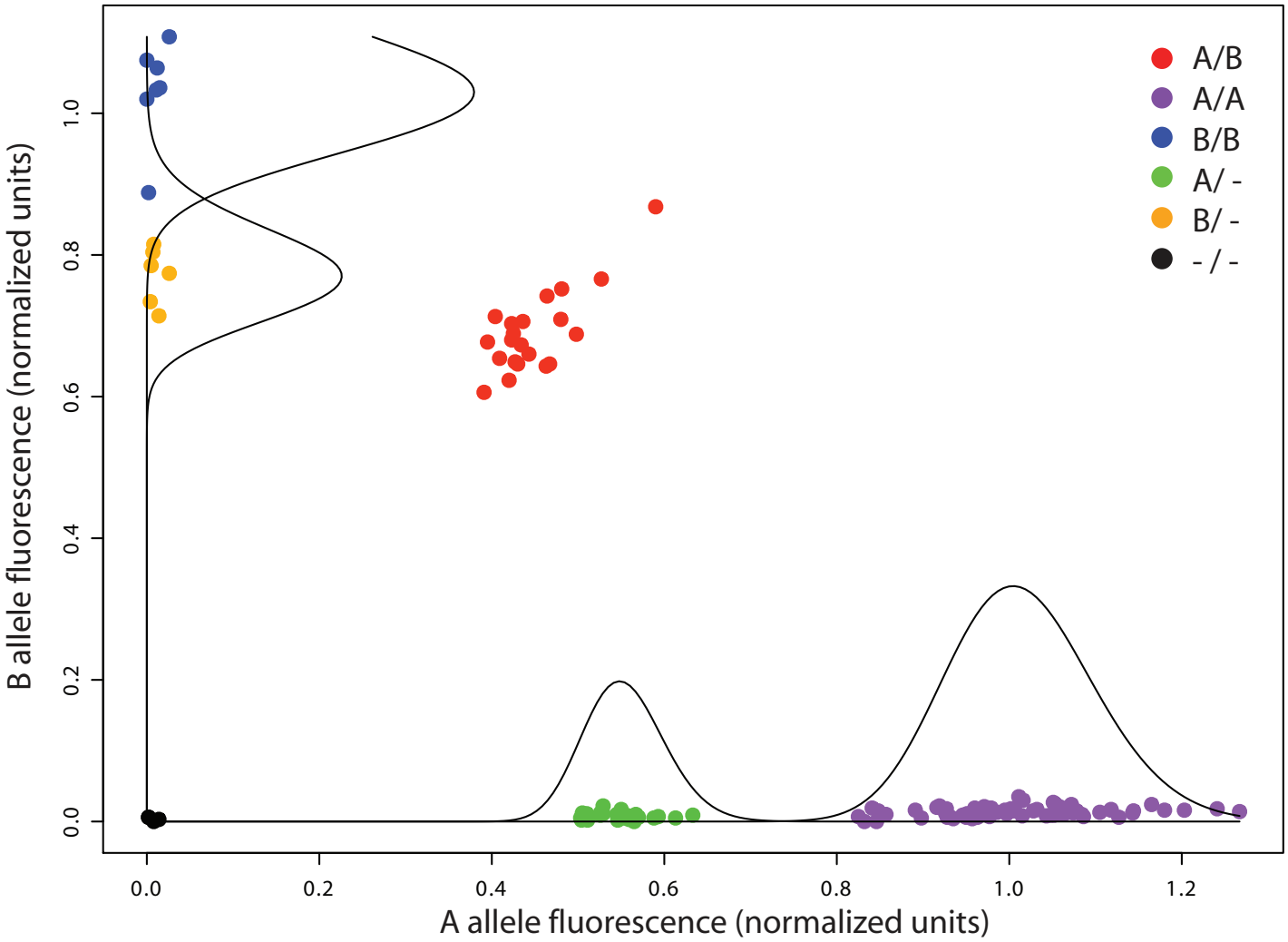
CNV Genotyping vs Discovery

- Discovery is done per-sample, genome-wide, and without assumptions about breakpoints
  - consequently, sensitivity is compromised to facilitate tolerable FDR
- Genotyping is targeted to known loci and applies to all samples simultaneously
  - good sensitivity **and** specificity are required
  - knowledge that a CNV is likely to exist and borrowing information across samples reduces the number of probes needed

SNP-based Common CNV Genotyping



SNP-based Common CNV Genotyping

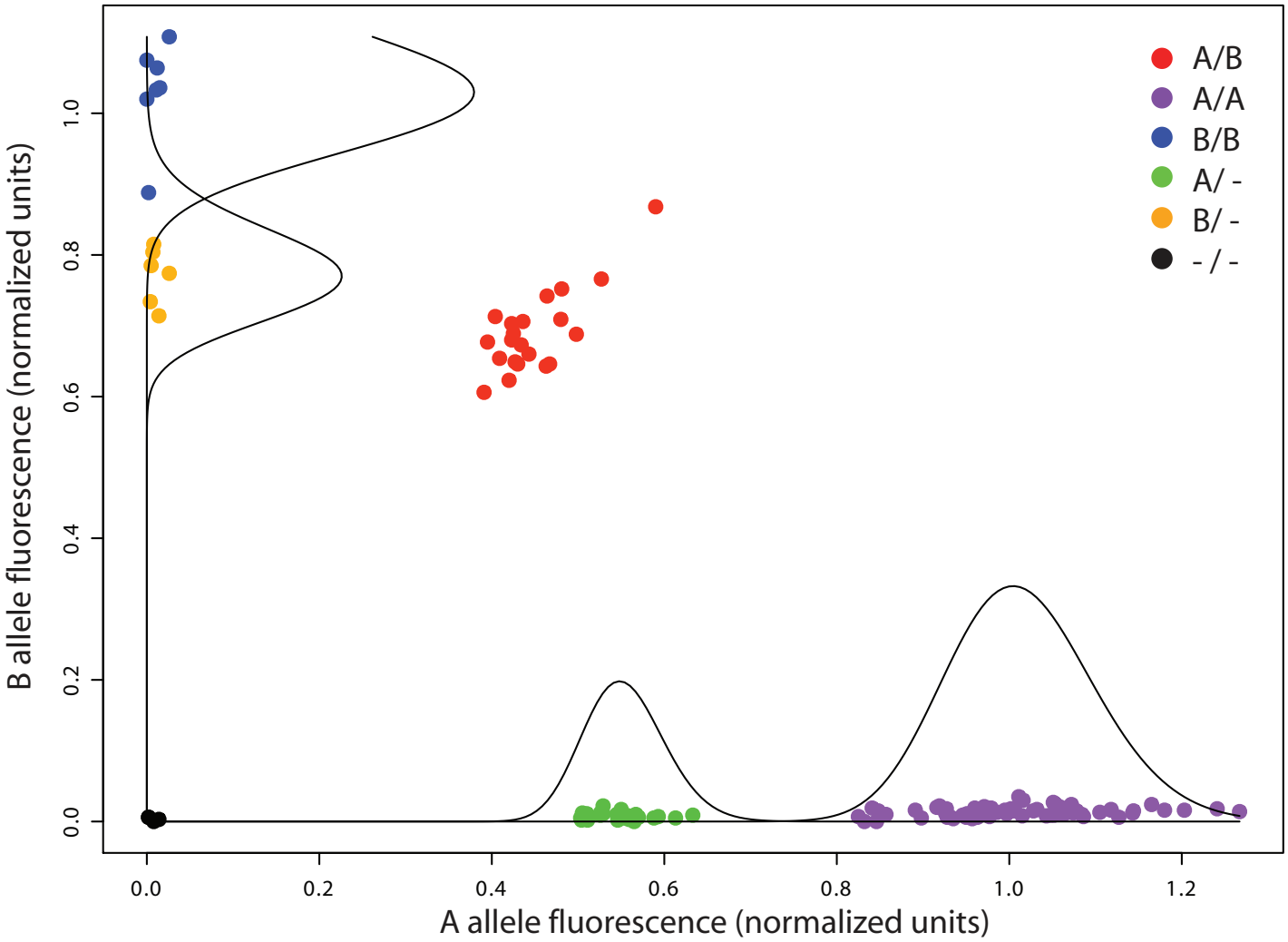


SNP-Conditional Mixture Modeling (SCIMM) for Deletion Genotyping

Input: SNP calls and quantitative data from multiple probes

Uses the EM algorithm to generate putative copy number calls (0, 1, 2) for each sample and a score for the probe set

SNP-based Common CNV Genotyping



SNP-Conditional Mixture Modeling (SCIMM) for Deletion Genotyping

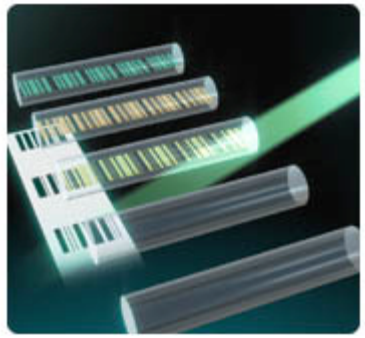
Input: SNP calls and quantitative data from multiple probes

Uses the EM algorithm to generate putative copy number calls (0, 1, 2) for each sample and a score for the probe set

SCIMM used to genotype hundreds of common deletions, replicate concordance and Mendelian consistency > 99%

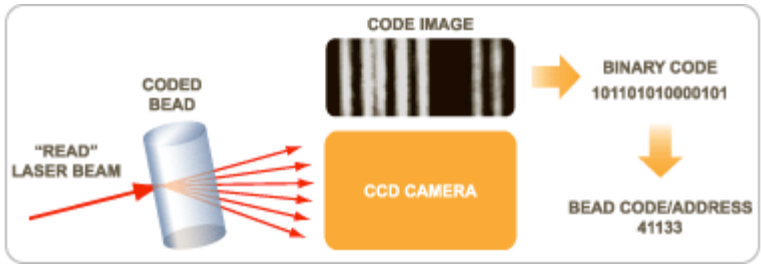
Scaling Up CNV Genotyping

- Identification of pathogenic variants seen in 0.1% - 1% of affected people requires sample sizes in excess of 10,000



- Genome-wide platforms are better for coverage, but expensive

- NAHR hotspots define breakpoints and enrich for copy-number variation



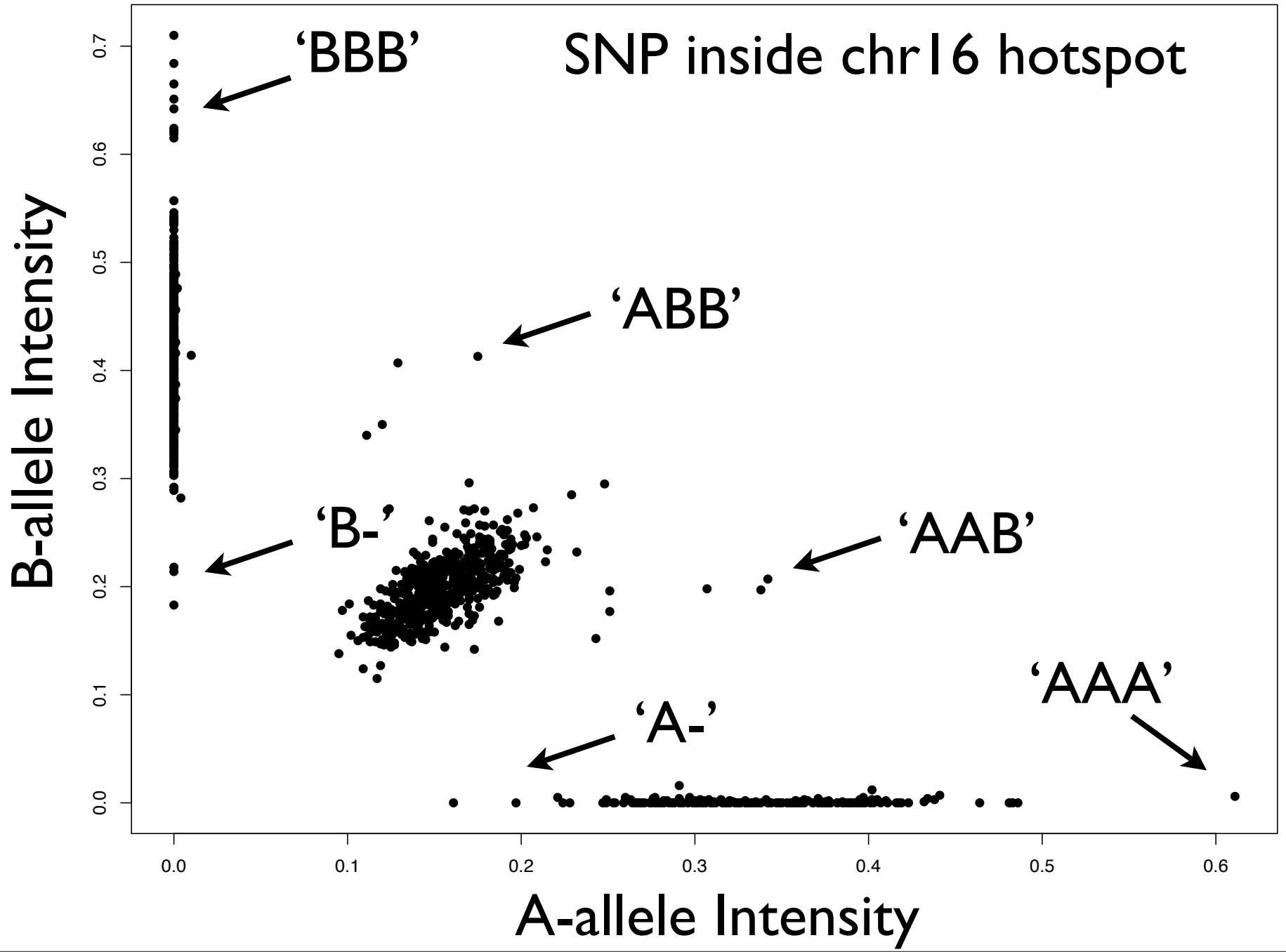
- We developed a custom 'BeadXpress' assay to genotype rare CNVs in children affected by idiopathic intellectual disability

BeadXpress CNV Detection

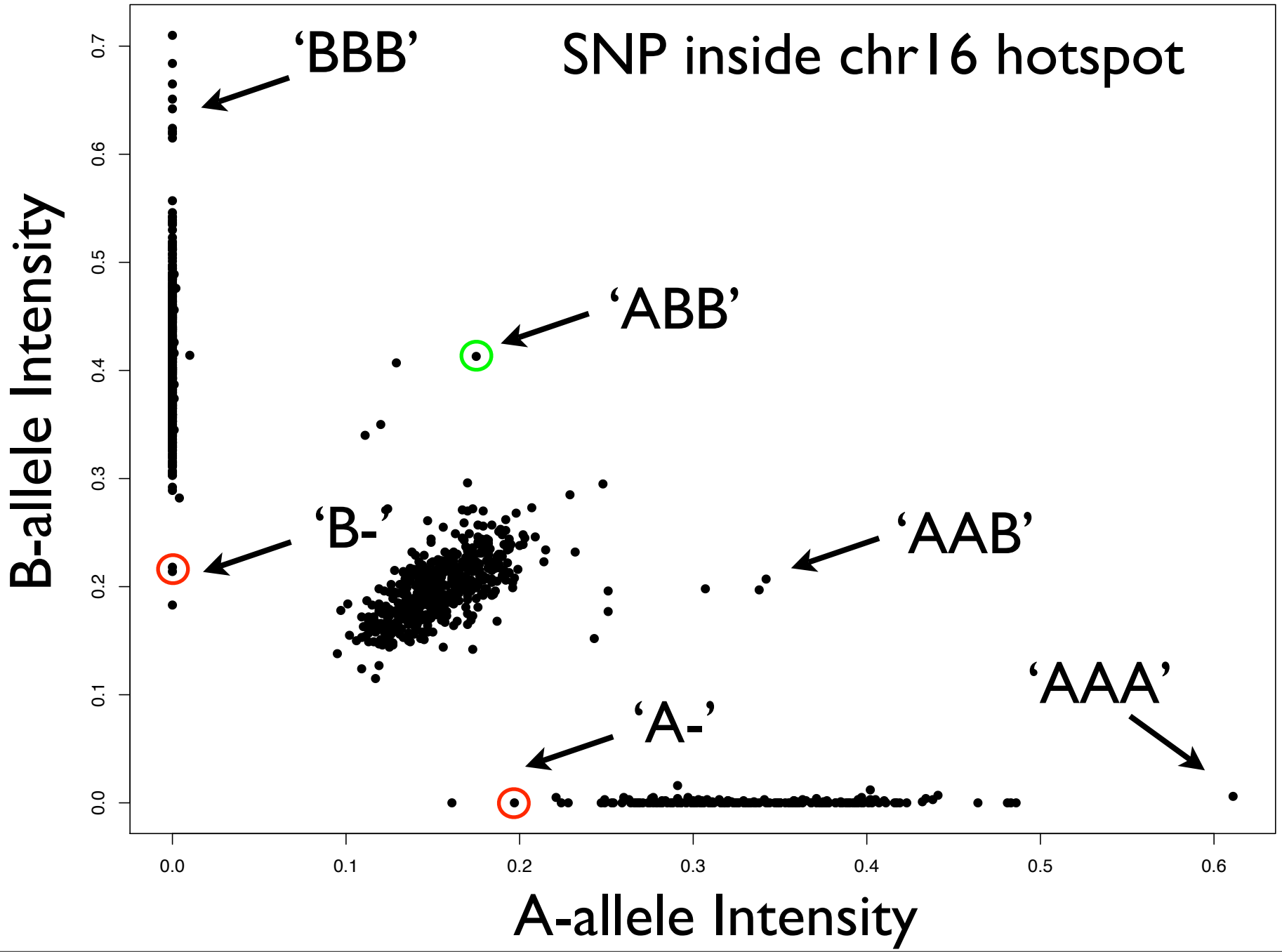
- 384-plex Illumina 'GoldenGate' SNP genotyping (PCR-based) assay performed in 96-well plates
- 69 known and putative disease hotspots targeted, ~5 probes each
- 2 common CNVs and 1 X-linked site
- Analyzed 1,105 affected children and 39 control samples
- Follow-up validation with targeted array-CGH

Snp-Conditional OUTlier (SCOUT) Detection

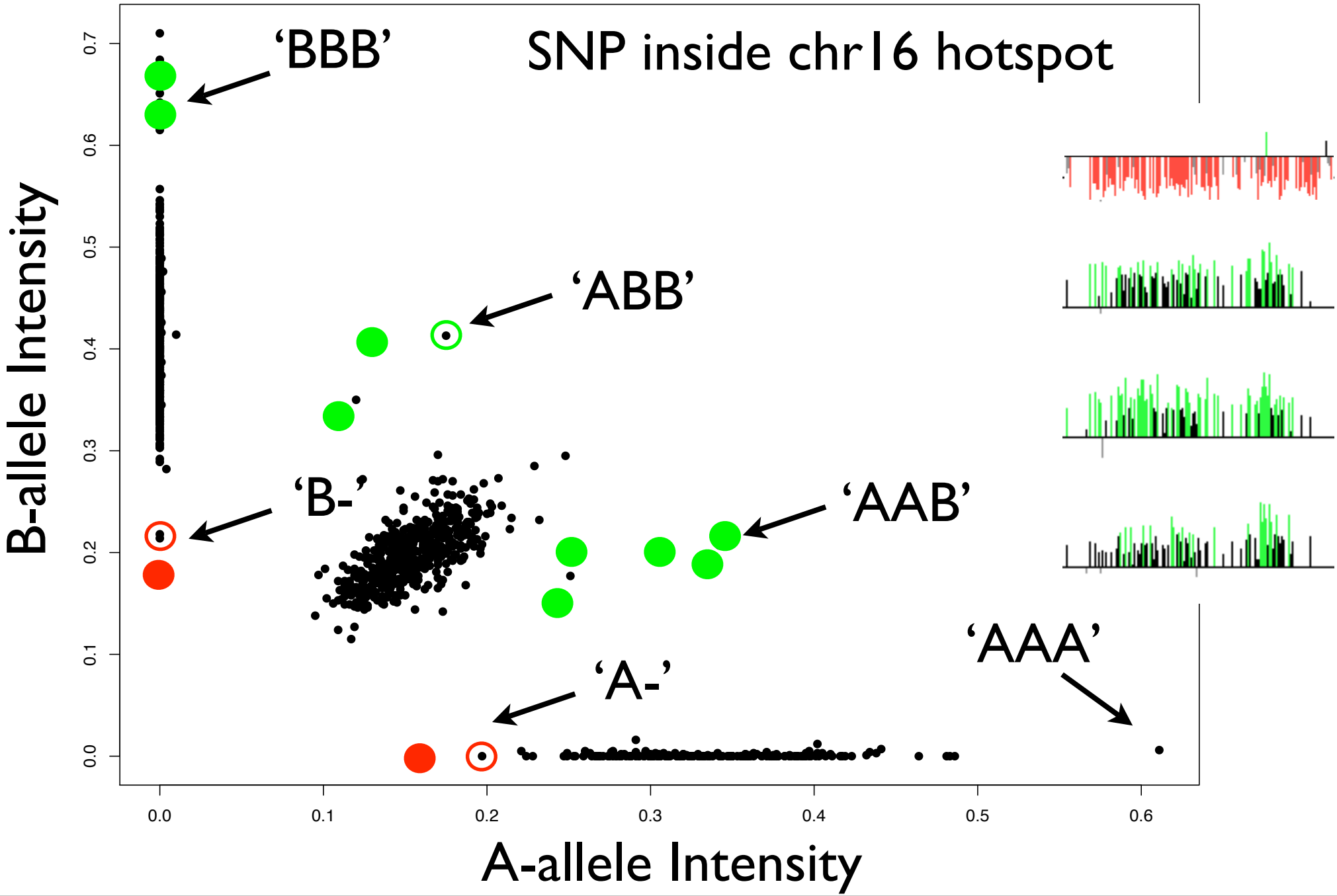
Snp-Conditional OUTlier (SCOUT) Detection



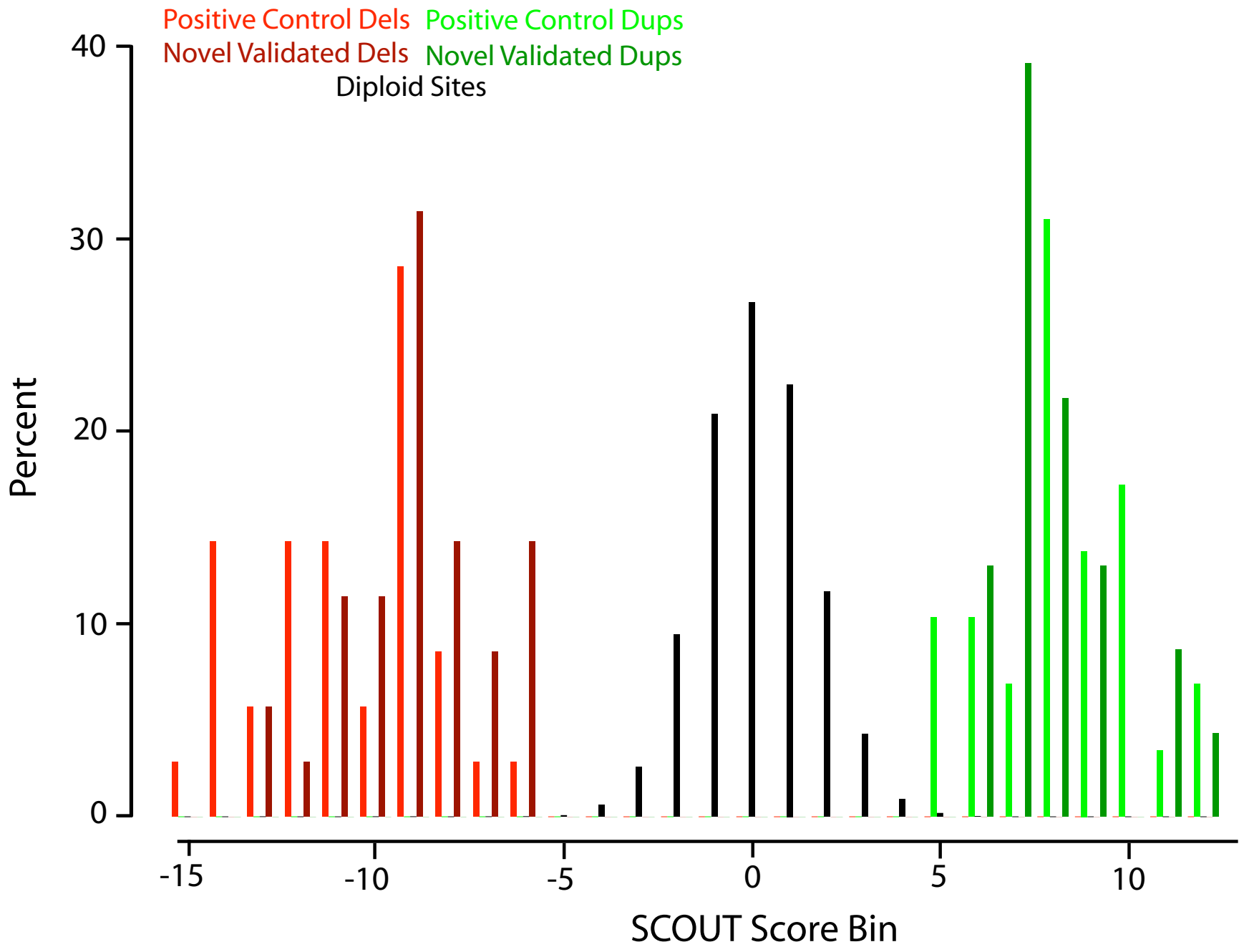
Snp-Conditional OUTlier (SCOUT) Detection



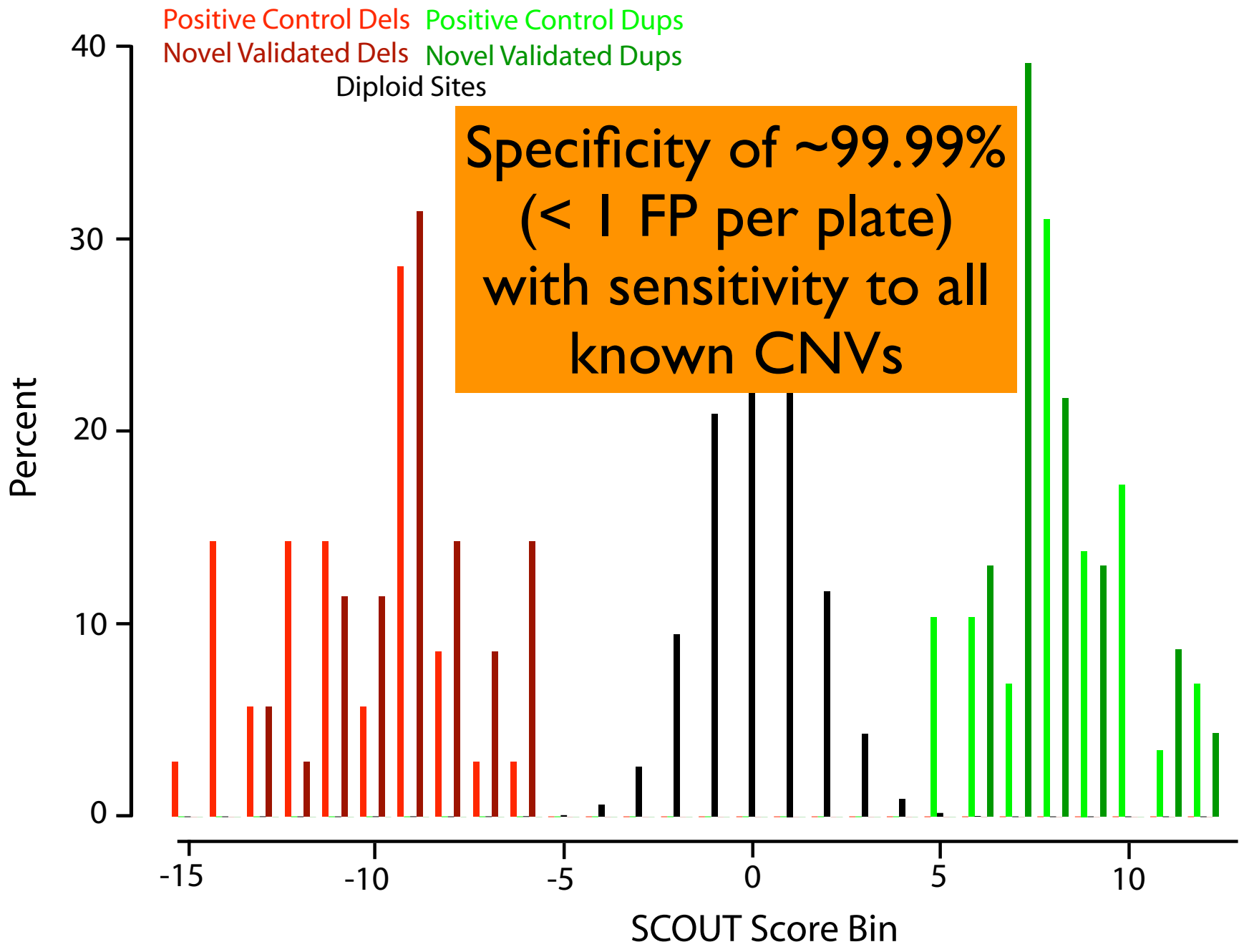
Snp-Conditional OUTlier (SCOUT) Detection



Accurate Detection of Rare Variants



Accurate Detection of Rare Variants



Disease-Relevant CNVs

Locus	Size (MB)	Confirmed by array-CGH	Predicted > |6|	Combined frequency (n = 1,010)
1q21.1 dup	1.4	1	2	0.30%
15q11 BP1-BP3 del	5.7	1	0	0.10%
15q11 BP2-BP3 del	5.3	1	0	0.10%
15q24 del	2.2	0	1	0.10%
16p11.2 del	0.9	6	1	0.69%
16p11.2 dup	0.9	1	0	0.10%
16p12.2-p11.2 del	8.1	0	1	0.10%
16p13 BP1-BP2 del	1.2	1	1	0.20%
16p13 BP1-BP3 del	3.2	1	0	0.10%
17p11.2 del (SMS)	3.5	2	0	0.20%
17p12 del (HNPP)	1.4	1	0	0.10%
17p12 dup (CMT1A)	1.4	0	1	0.10%
17q12 del	1.6	1	0	0.10%
22q11.21 3-Mb del (VCFS)	2.9	2	1	0.30%
22q11.21 3-Mb dup	2.9	2	0	0.20%
22q11.21-q11.22 distal del	1.4	2	1	0.30%
TOTAL		22	9	3.08%

Disease-Relevant CNVs

Locus	Size (MB)	Confirmed by array-CGH	Predicted > |6|	Combined frequency (n = 1,010)
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15q24 del	2.2	0	1	0.10%
16p11.2 del	0.9	6	1	0.69%

16p11.2 previously associated with autism, but most of the individuals here have intellectual disability without autism

17p11.2 del (SMS)	3.5	2	0	0.20%
17p12 del (HNPP)	1.4	1	0	0.10%
17p12 dup (CMT1A)	1.4	0	1	0.10%
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22q11.21-q11.22 distal del	1.4	2	1	0.30%
TOTAL		22	9	3.08%

CNVs of Uncertain Pathogenicity

- Duplications at 16p13 seen in 11 (1.1%) of samples
  - only seen in 2/2,493 controls ($p = 4.7 \times 10^{-5}$)
  - deletion is known to be pathogenic
  - duplication also enriched in schizophrenia
- Deletions at 15q11.2 seen in 8 (0.8%) of samples
  - only seen in 3/2,493 controls ($p = 0.003$)
  - near Prader-Willi critical region
  - also enriched in schizophrenia

BeadXpress Pilot Results

- High-confidence diagnosis of 31 (3.1%) children carrying pathogenic variants (e.g. 1q21.1, 16p11.2, VCFS, SMS, etc)
  - 16p11.2 associates with intellectual disability and is not an autism allele *per se*
- Evidence for disease relevance of 16p13 dups and 15q11.2 deletions collectively seen in 19 (1.9%) affected children
- 1,105 samples processed in < 1 month with costs 5-20X less than CGH or genome-wide SNP platforms
- New assay being designed for a larger batch of samples (5,000 - 10,000) and with probe selection optimizations

General Conclusions

- Copy-number variation is an influential source of genomic variation in human populations
- Duplication architecture is a major contributor to both common and rare variation via NAHR
- Rare variants are contributors to substantial amounts of disease heritability
- Tools are now available to dramatically scale the the size and accuracy of CNV-disease association studies

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