Copy Number Variation

Biostatistics 666

Outline

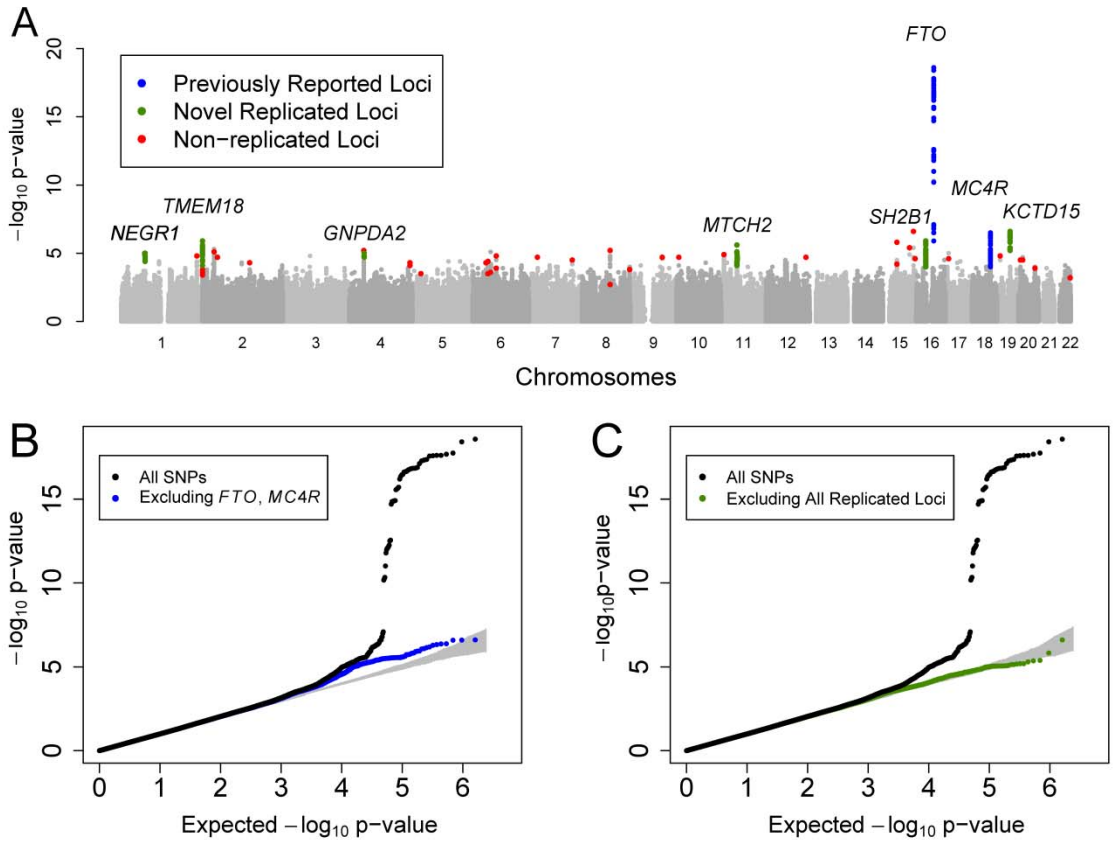
- Evidence that Copy Number Variation Contributes to Disease
- Signal of Copy Number Variation in Sequence Data
- A Method for Identifying Deletions Using Next-Generation Sequence Data

Evidence that Copy Number Variants Contribute to Disease

Some Background

- Copy number variation spans about 10% of the genome
- Copy number variation affects 5-10 Mb of sequence in an average individual
- Copy number variation is a major driver of disease in cancer
- A large fraction of copy number variants occur in and around regions of duplicated sequence
  - Due to “Non-Allelic Homologous Recombination” (NAHR)

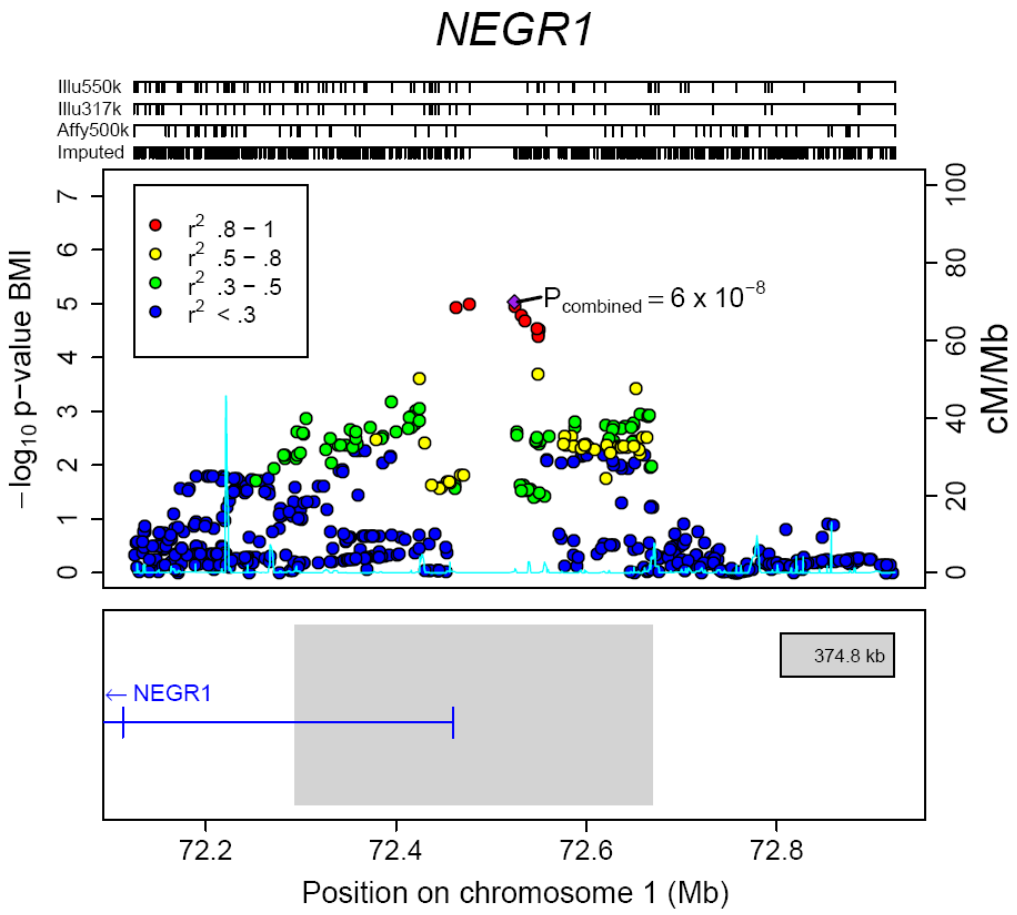
Evidence that Copy Number Variants Important Examples from Genetics of Obesity



Seven of eight confirmed BMI loci show strongest expression in the brain…

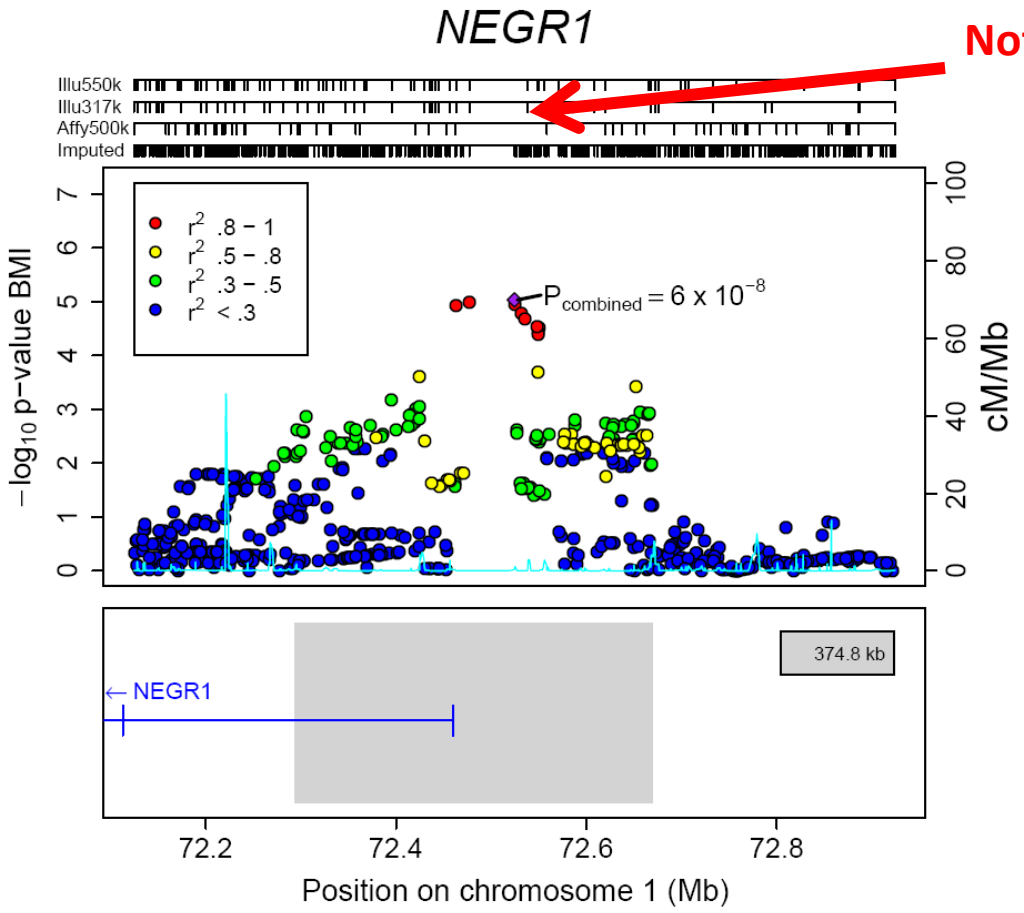
Willer et al, *Nature Genetics,* 2009

Some BMI Associated SNP Haplotypes …



Willer et al, *Nature Genetics,* 2009

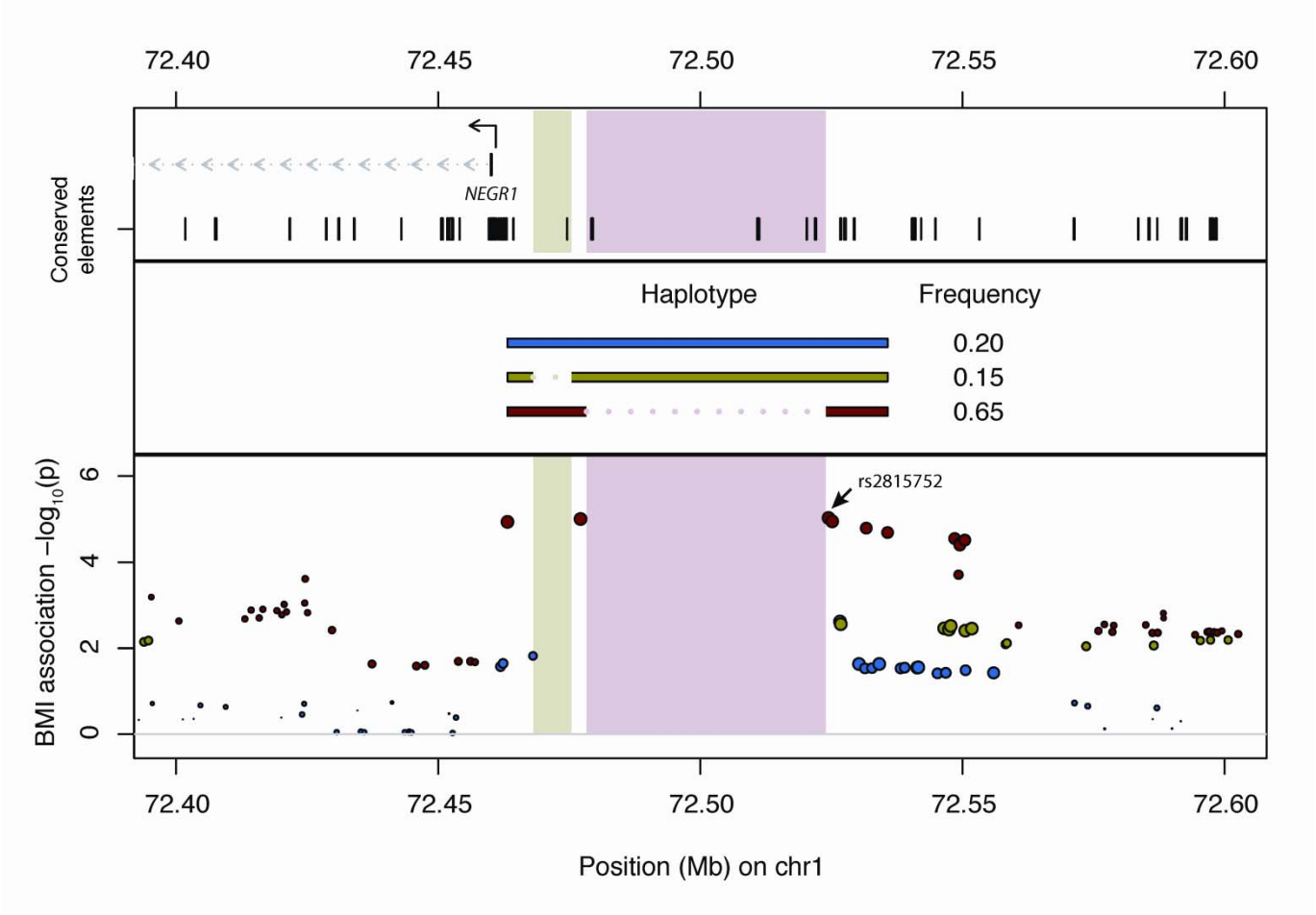
… Turn Out To Be Unusual …



Note hole in marker panels….

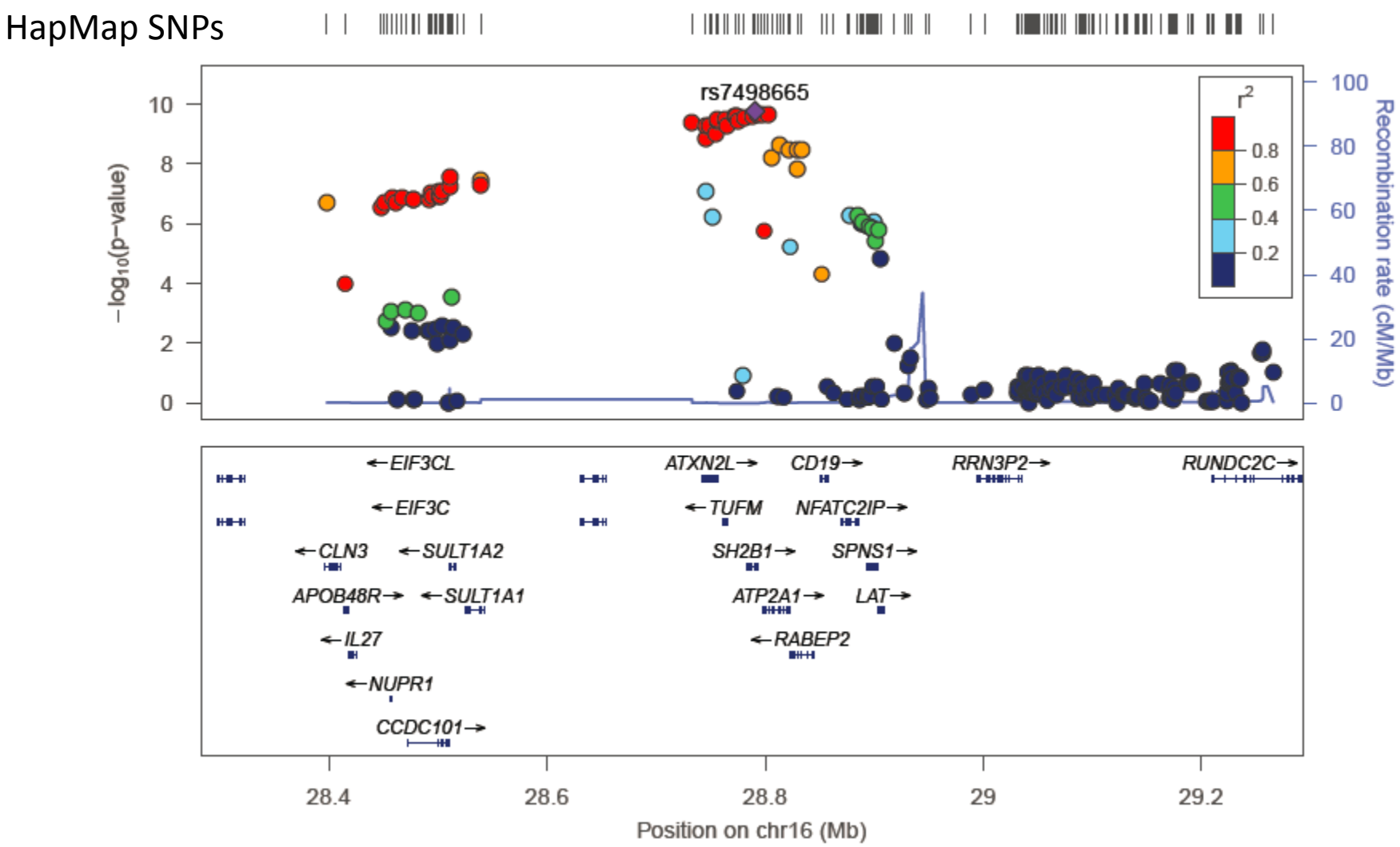
Willer et al, *Nature Genetics,* 2009

And Carry Unusual Deletion

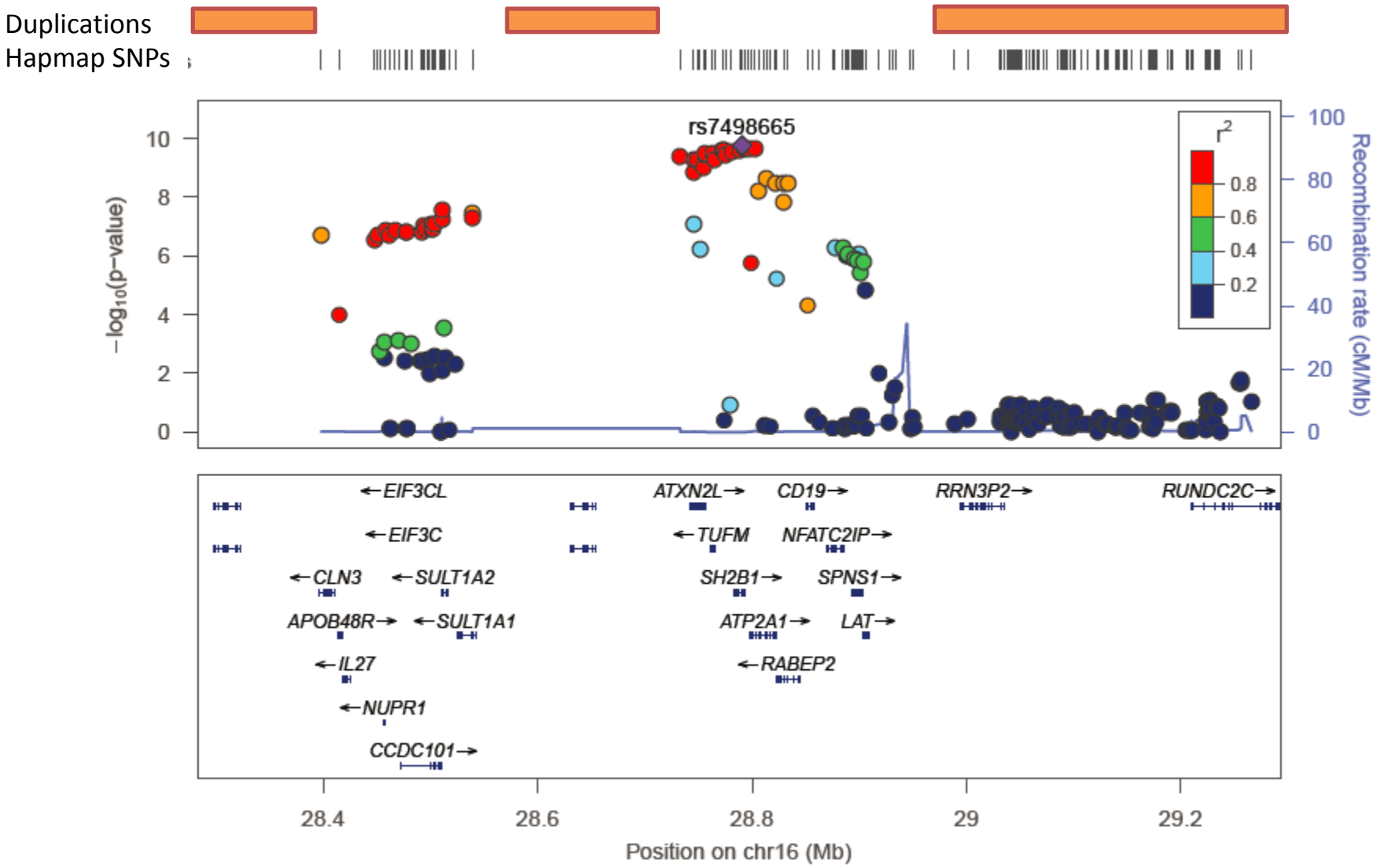


Willer et al, *Nature Genetics,* 2009

Other BMI Associated Haplotypes…



…Point To Complex Genomic Regions…



… With Large Obesity Associated Deletions

- Bochukova et al (Nature, 2009) showed that large deletions were present in 1.0 – 2.0% of children with severe obesity, 0.5% of controls
- The most common of these deletions was observed in 5 of 300 children with severe obesity but only 2 of 7,366 controls
- Similar patterns reported for other complex traits, including autism and schizophrenia

Next Generation Sequencing And Copy Number Variation

Massive Throughput Sequencing

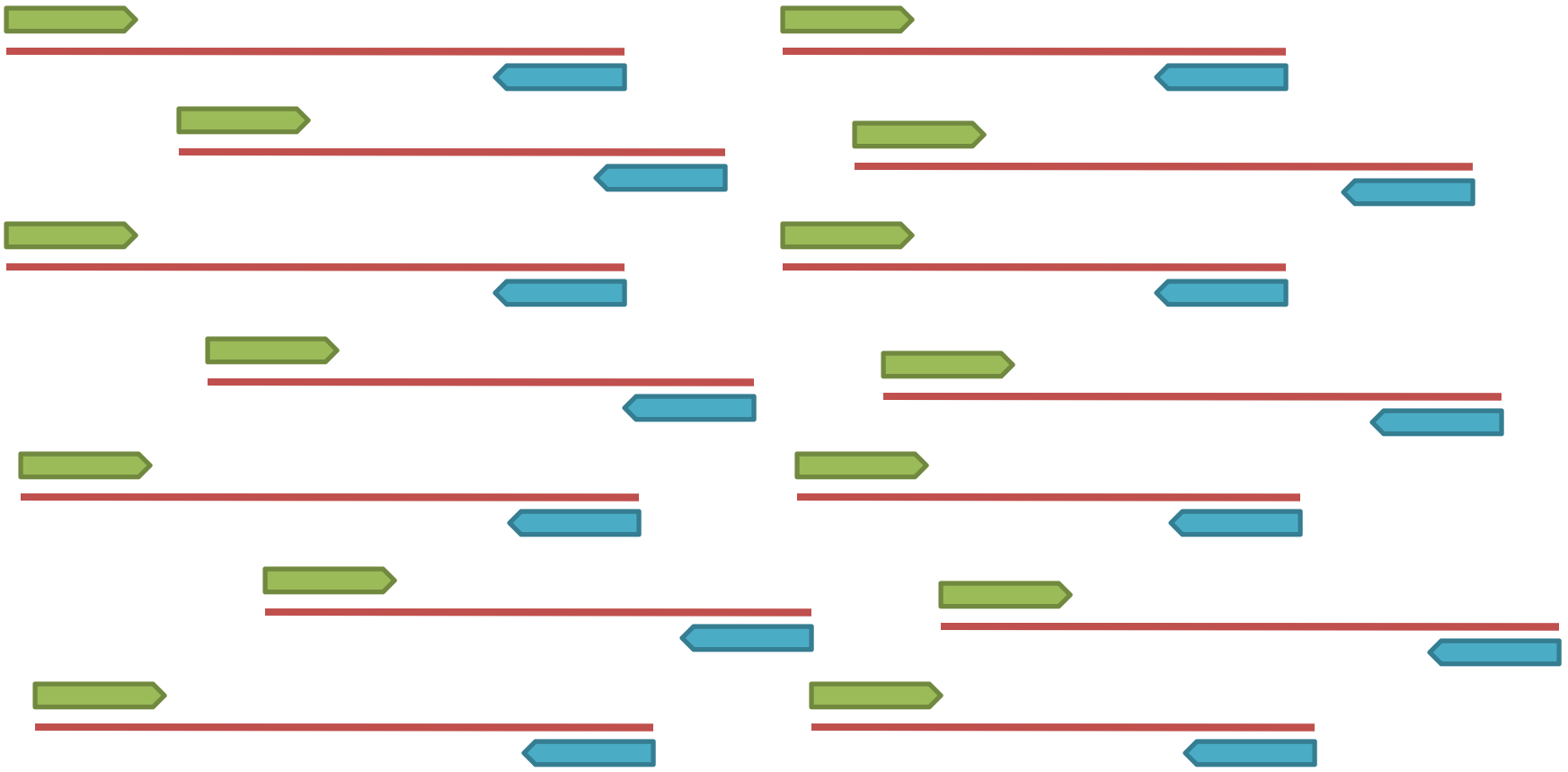
- Tools to generate sequence data evolving rapidly
- Commercial platforms produce gigabases of sequence rapidly and inexpensively
  - ABI SOLiD, Illumina Solexa, Roche 454, Complete Genomics, and others…
- Sequence data consist of thousands or millions of short sequence reads with moderate accuracy
  - 0.5 – 1.0% error rates per base may be typical

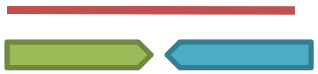
Shotgun Sequence Reads

- Typical short read might be <25-100 bp long and not very informative on its own

- Reads must be arranged (*aligned*) relative to each other to reconstruct longer sequences

Paired End Sequencing





Population of DNA fragments of known size (mean + stdev)
Paired end sequences

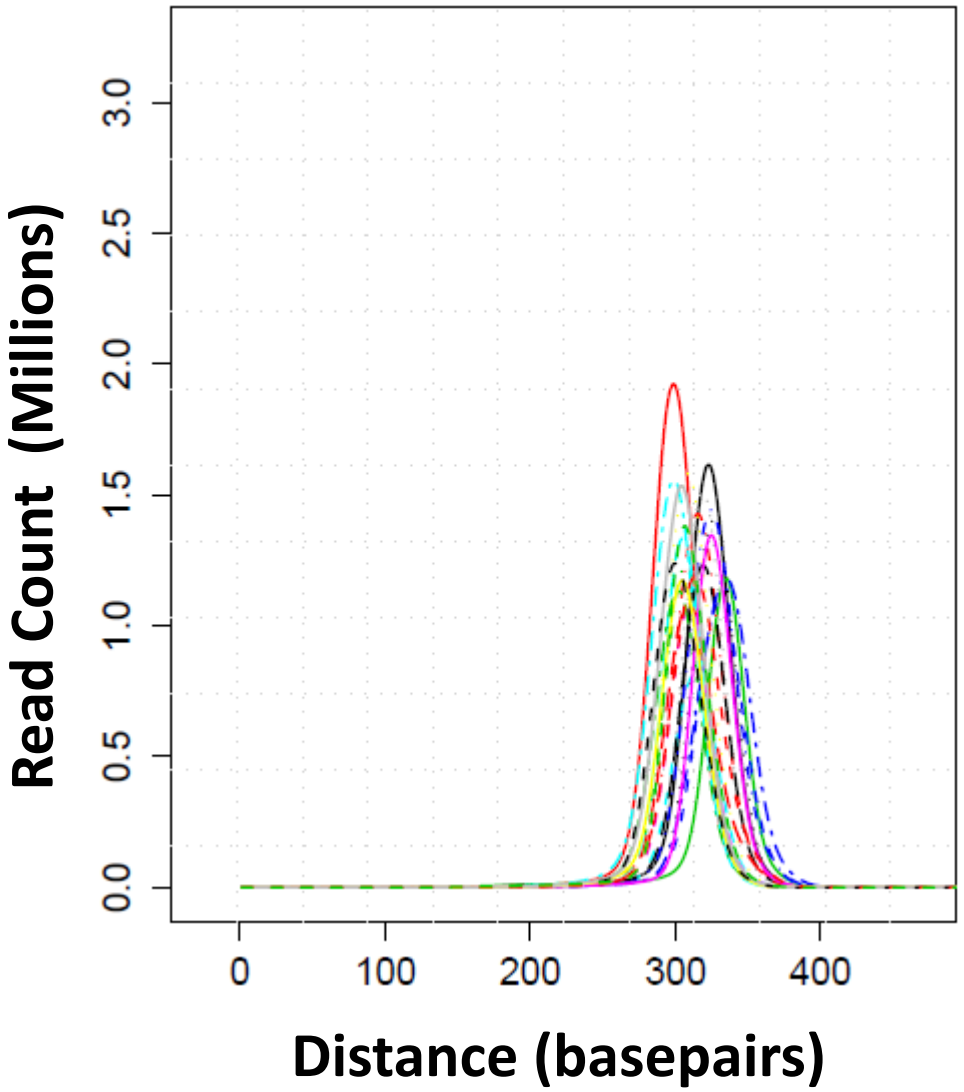
Paired End Sequencing

Paired Reads

Initial alignment to the reference genome

Paired end resolution

Distance Between Pair Ends



- The graph shows distance between paired end reads
- Data summarized across 24 samples
- Courtesy: Xiaowei Zhan, University of Michigan DNA Sequencing Core

Evidence For A Deletion Within A Single Individual

- Split Reads
- Read Pair Separation
- Read Depth

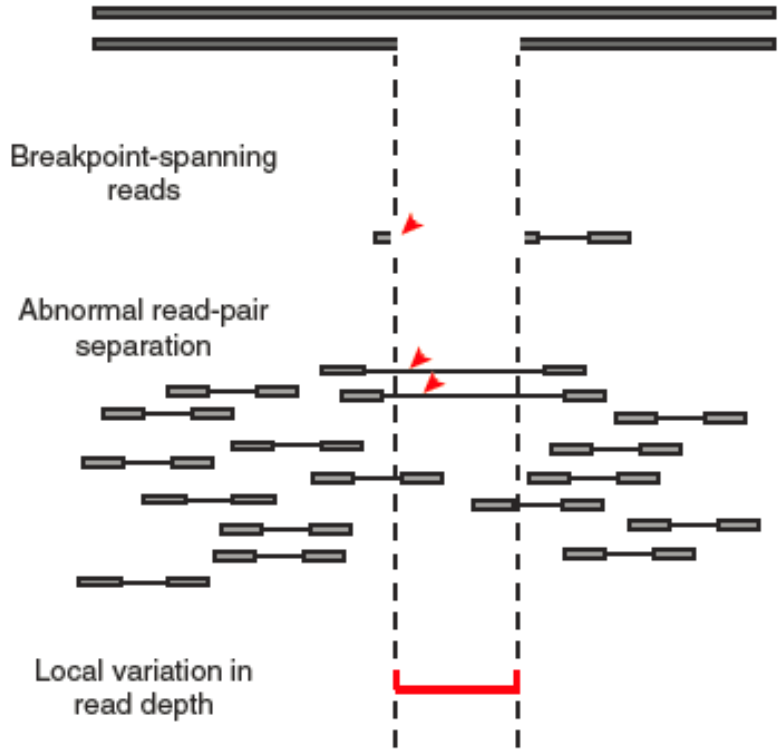


Figure from Handsaker et al (2011)

Detecting Copy Number Variation (Approach I)

- Focus on a particular feature of the data
  - e.g., read depth
- Normalize depth for each individual
  - e.g., adjust for total read count
  - e.g., adjust for GC content specific read count
- Model data as a mixture of distributions, characterized using maximum likelihood

Sara Rashkin

Detecting Copy Number Variation (Approach I)

$$d_i \sim p_0 N(\mu_0, \sigma_0^2) + p_1 N(\mu_1, \sigma_1^2) + p_2 N(\mu_2, \sigma_2^2)$$

Where

d_i is the depth for individual *i*

p_j is the frequency of individuals with *j* deletions (assuming Hardy Weinberg Equilibrium)

μ_j and σ_j^2 are the mean and variance of adjusted read depth distribution for deletion count *j*

Sara Rashkin

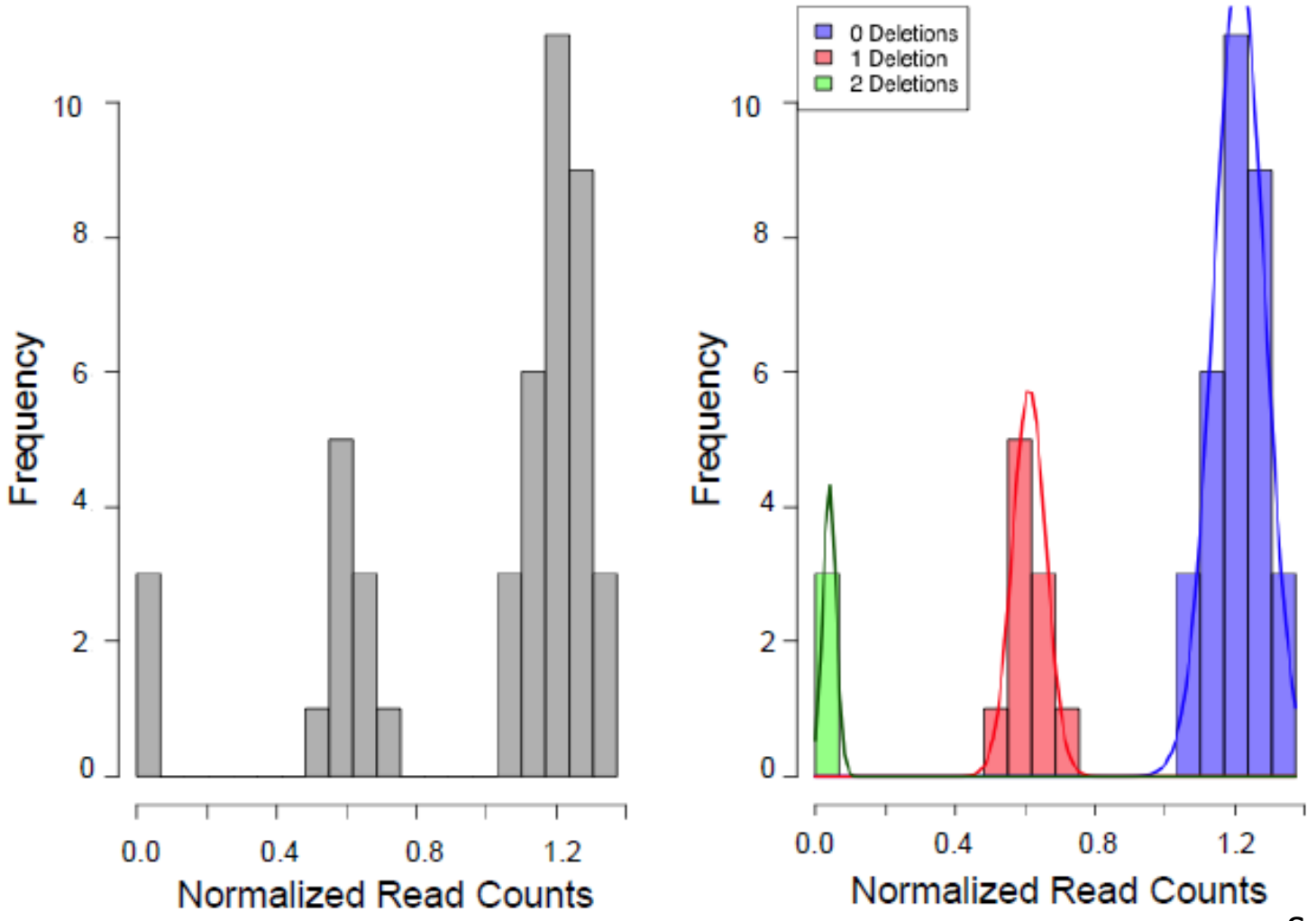
Detecting Copy Number Variation (Approach I)

- To estimate a deletion model, maximize

$$L(d_i) = \sum_j p_j (2\pi)^{\frac{1}{2}} \sigma_j^{-1} e^{-\frac{\frac{1}{2}(d_i - \mu_j)^2}{\sigma_j^2}}$$

- To keep number of parameters modest, we use HWE for modeling p_j (one parameter for three frequencies) and can impose additional structure on means and variances

Well Separated Region



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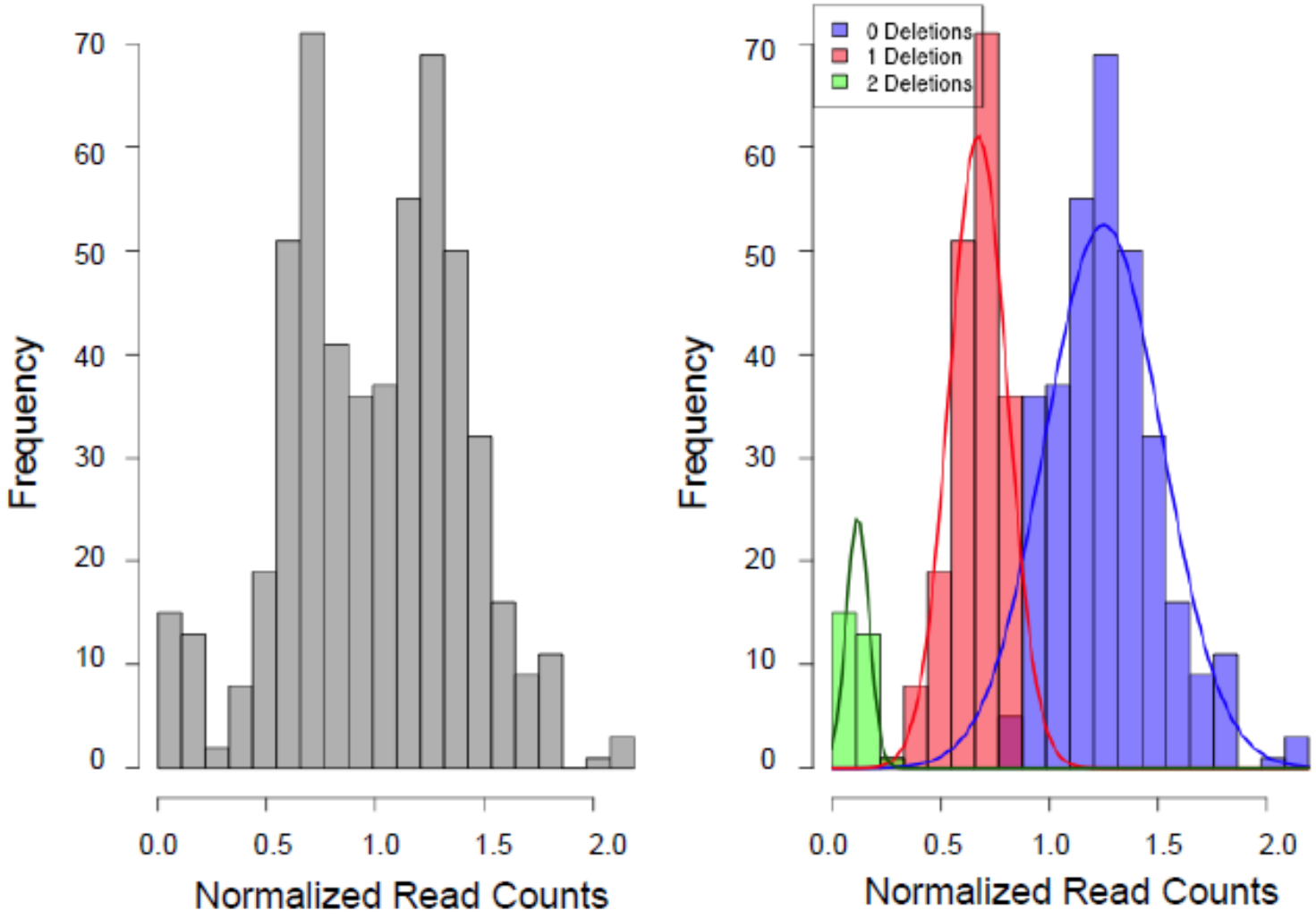
Moderately Separated Region



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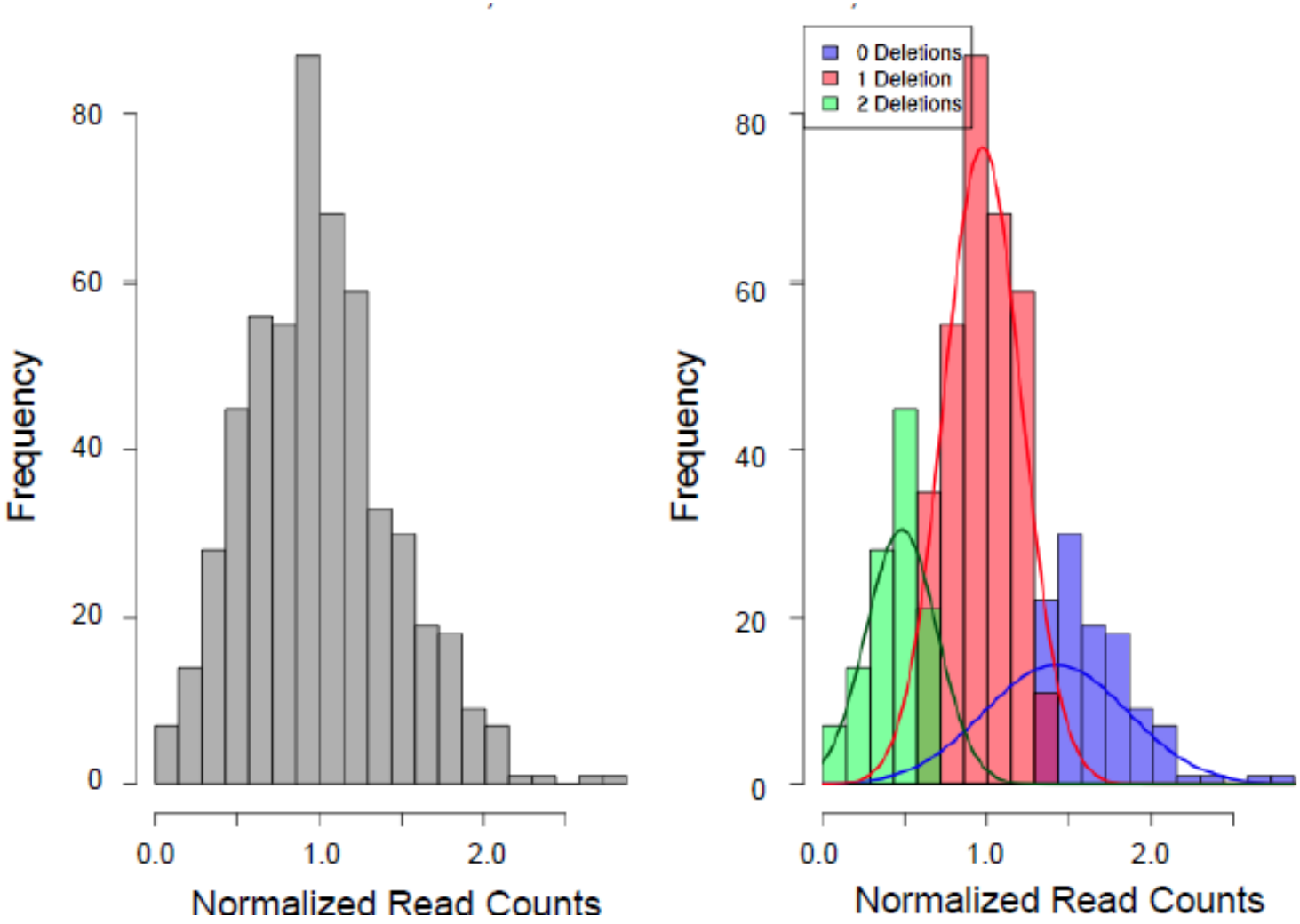
Hard to Call Region



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Challenges in Read Depth Based Calling

- Ideal if number of reads per region is large
- As technologies improve and reads get longer …
- … read depth based calling becomes harder
- Important to integrate different types of signal!

Evidence at the Population Level

- Allele Shared Between Multiple Individuals
  - Multiple individuals show cluster of reads with unusual separation in the same location
- Evidence for Deletion Recurs in the Same Individuals
  - Individuals with one unusually separated pair of reads, likely to show additional nearby read pairs with unusual separation
- Evidence for Reference Allele Decreases as Evidence for Deletion Increases
  - When the number of reads with unusual separation increases, the number of nearby reads with expected separation decreases
- Deletions Segregate on Specific Haplotypes

Refined Algorithm

- Build list of candidate variants by finding read pairs with abnormal separation
- Focus on regions supported by multiple pairs
- Check whether highly separated pairs are evenly distributed across individuals (*why?)*
- Evaluate read depth distribution
- Search for split reads spanning breakpoint
- Combine with haplotype based hidden Markov model analysis

Handsaker et al (2011)

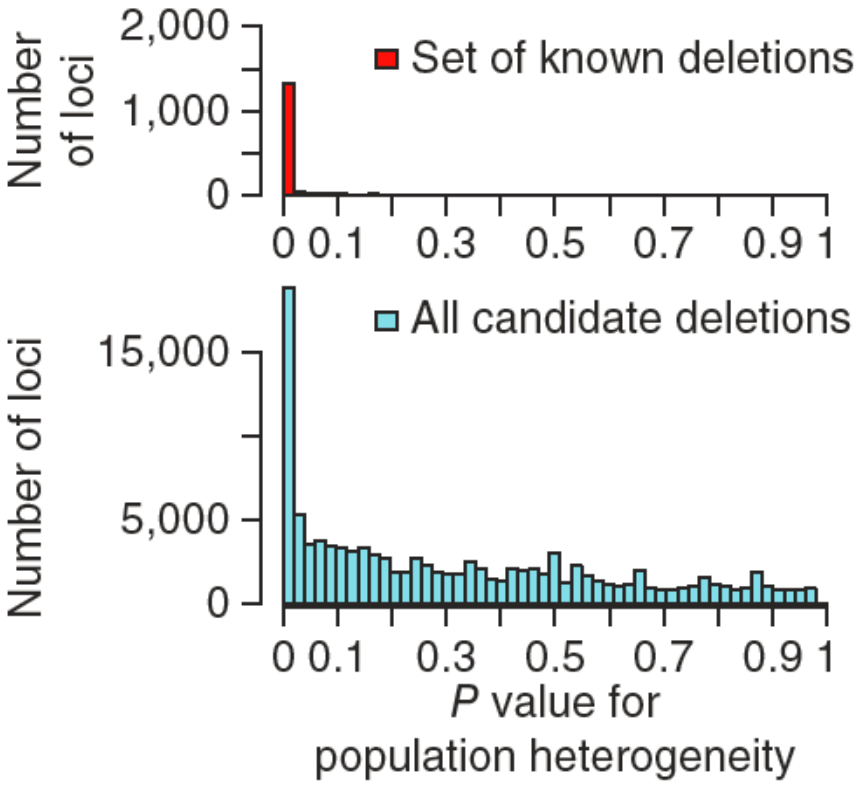
Search for Abnormal Read Pairs

- Search for read pairs where separation >10x the individual specific standard deviation
- Even if we require multiple supporting events, the number of potential copy number changes is ~10x larger than expected
- This is because of experimental limitation in preparing read pair libraries and of shortcomings in read mapping
- A major challenge is to reduce list of candidates

Handsaker et al (2011)

“Heterogeneity”

- Is rate at which widely separated read pairs occur constant among individuals?
- Calculated expected number of widely separated pairs using sequencing depth, average pair separation



Handsaker et al (2011)

Expected Number of Widely Separated Read Pairs

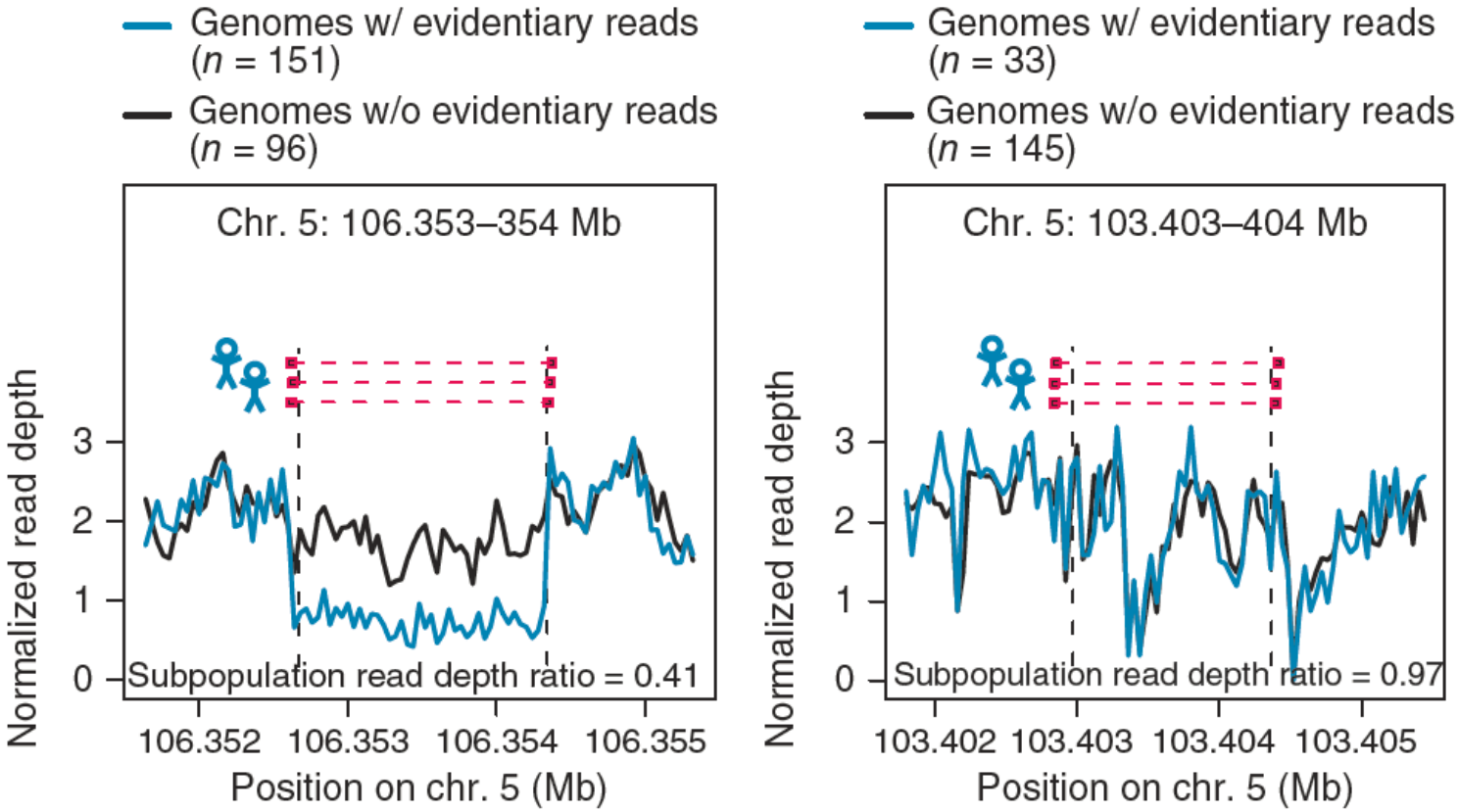
- The approach of Handsaker et al. requires that we calculate, for each individual, the expected number of widely separated read pairs

- To do this, Handsaker et al (2011) calculate the distance between every mapped pair of reads

- They then assume that the number of read pairs separated by *>x* bp is proportional to the number of reads (across the genome) for which this distance exceeds *x*

“Allelic Substitution”

- If we see evidence for deletion, based on read pair separation …
- Expect to see reduced evidence for reference based on read depth



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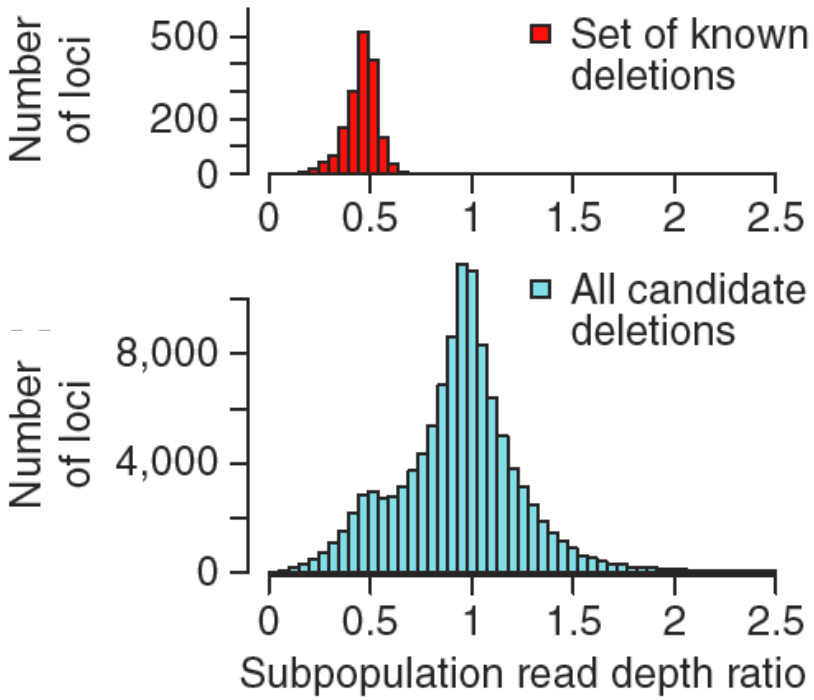
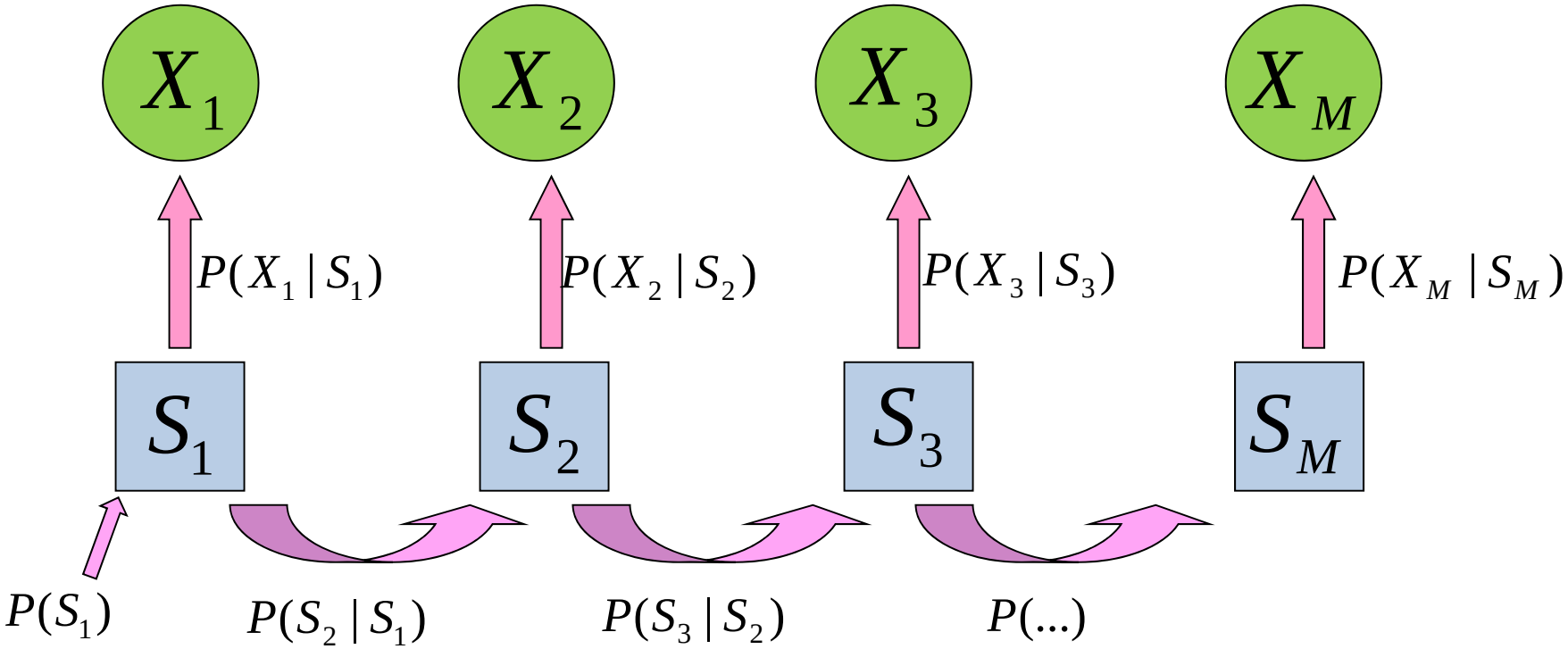


Handsaker et al (2011)

Integrate with Other Variant Types…

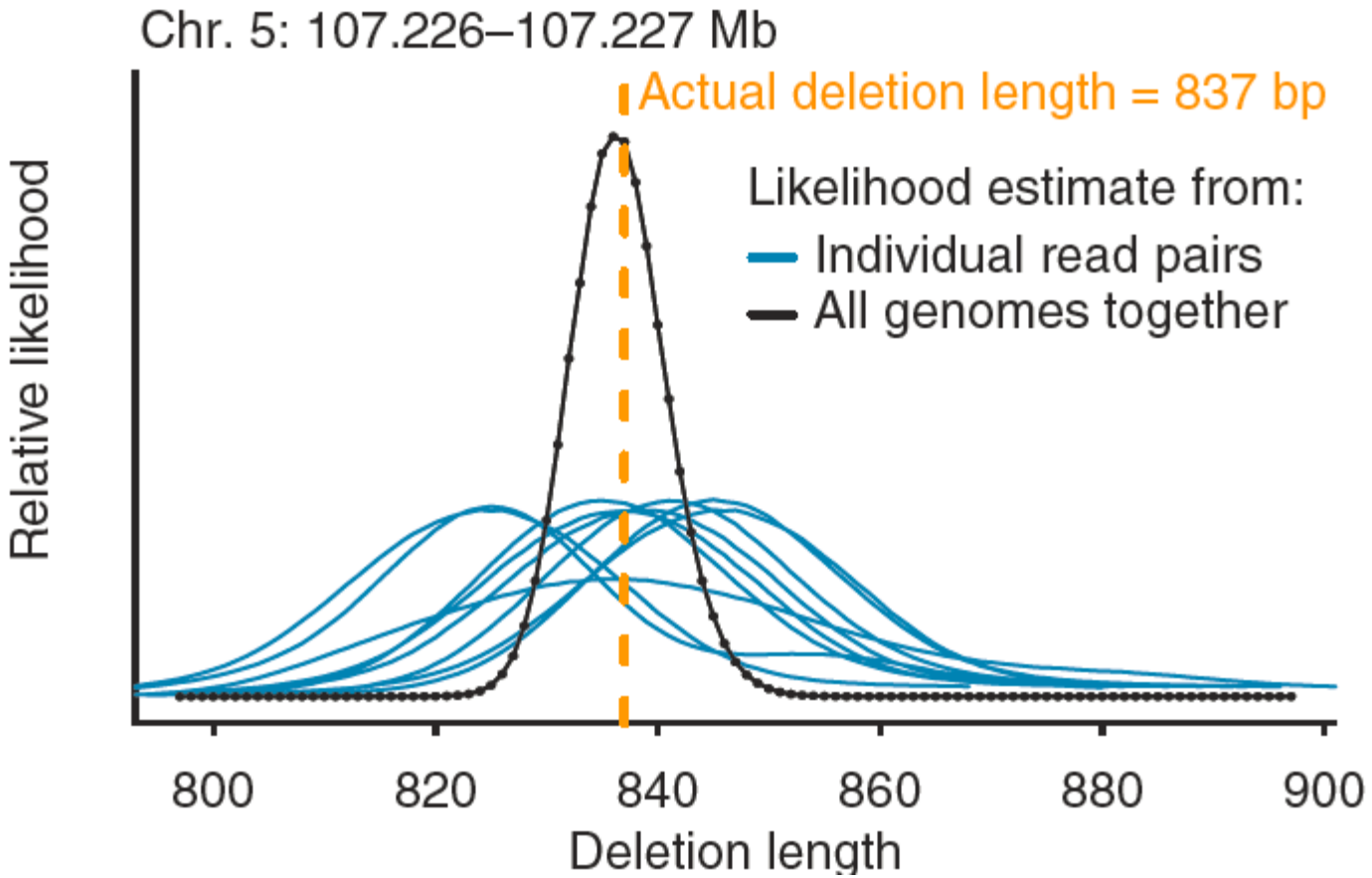


If we can calculated P(X|S) for a potential deletion, we can evaluate evidence for deletions jointly with other nearby variants

Sizing the Deletion

- If we know the distribution of read pair distances for one individual…
- Observing an abnormal read pair suggests a specific deletion size, but with low confidence
- Observing many abnormal read pairs gradually suggests more specific deletion sizes and locations

Combining Information Across Individuals is Key



Conclusions

- Combining information across individuals improves the power of deletion analyses
- Combining different sources of information within each individual also provides increased resolution
- Avoiding experimental artifacts is a major challenge in analysis of copy number

Recommended Reading

- Handsaker, Korn, Nemesh and McCarroll (2011) Discovery and genotyping of genome structural polymorphism by sequencing on a population scale.
  Nature Genetics **43:**269 - 276