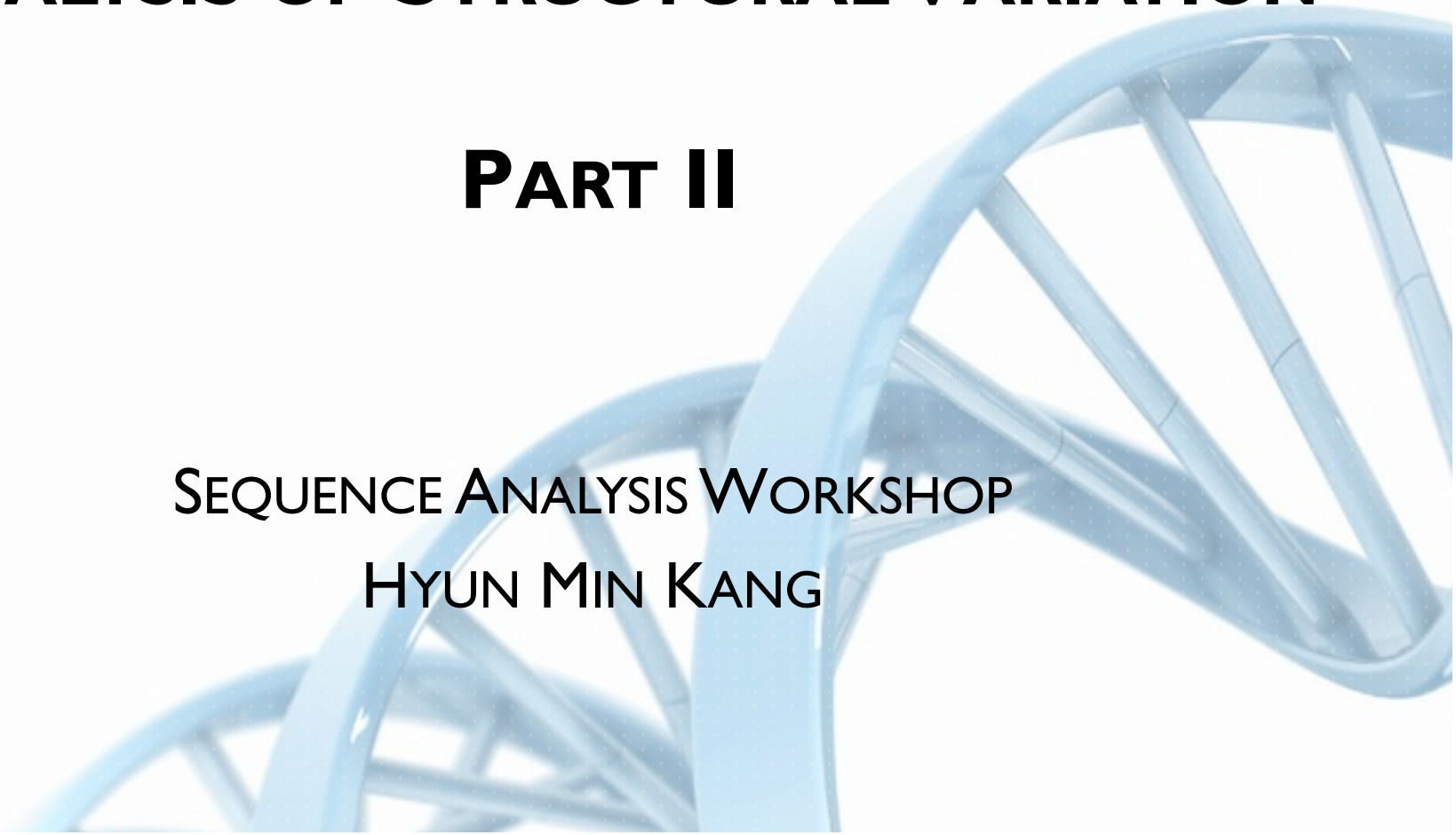
Analysis of Structural Variation

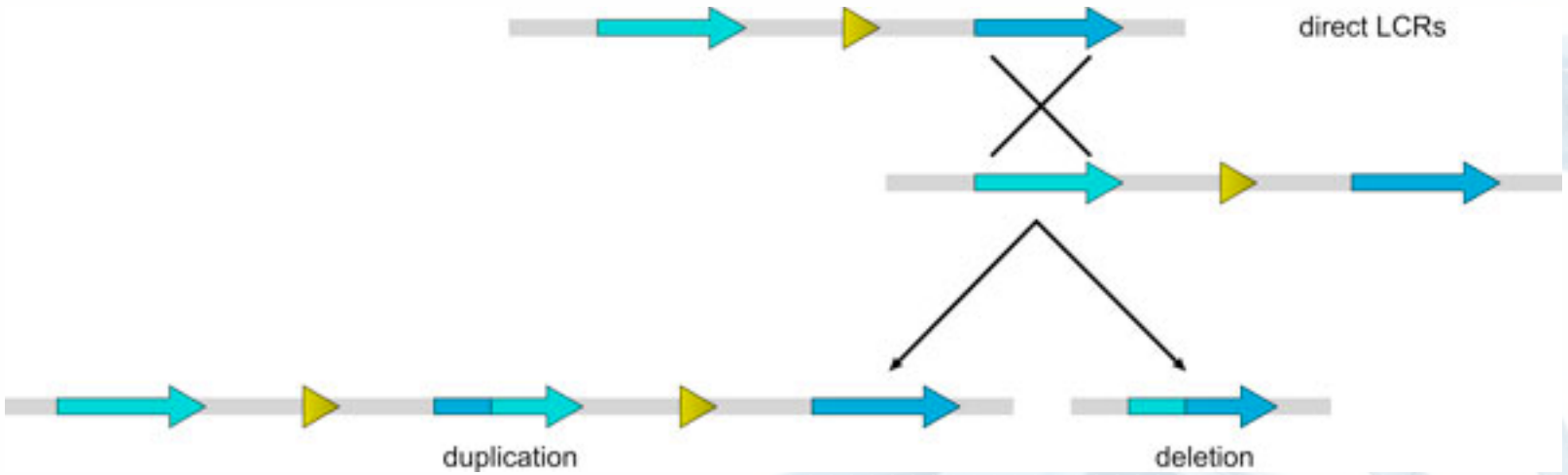
Part II



Sequence Analysis Workshop

Hyun Min Kang

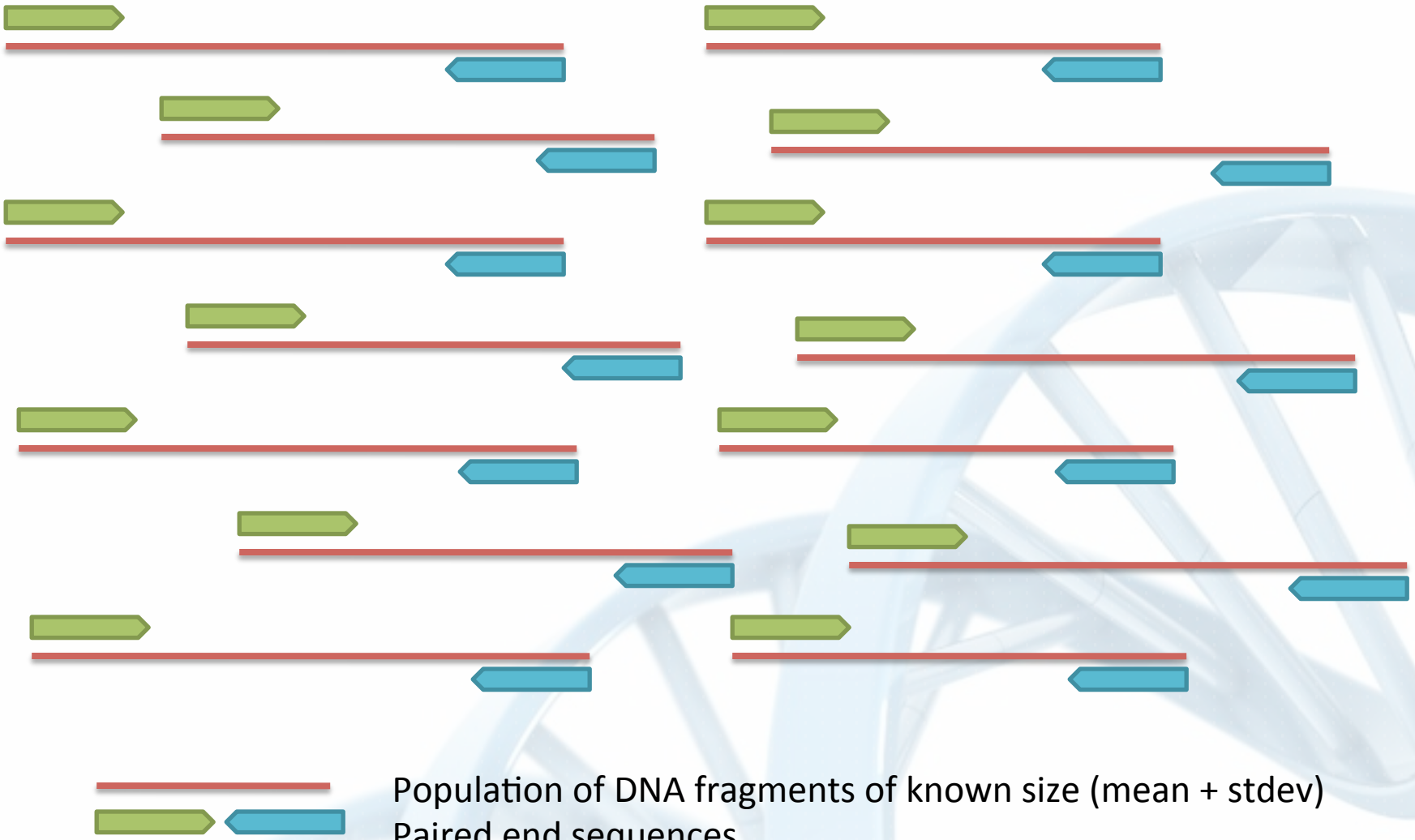
Many CNVs Are Contributed by Non-Allelic Homologous Recombination (NAHR)



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



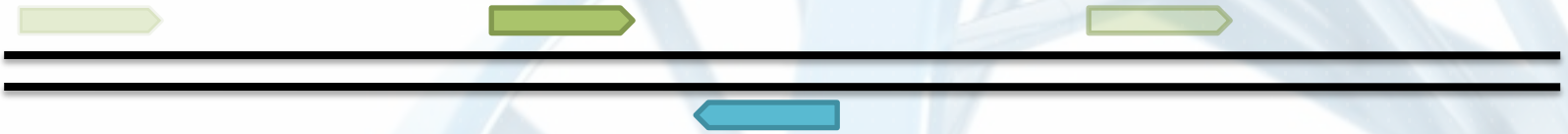
Population of DNA fragments of known size (mean + stdev)
Paired end sequences

PAIRED END SEQUENCING

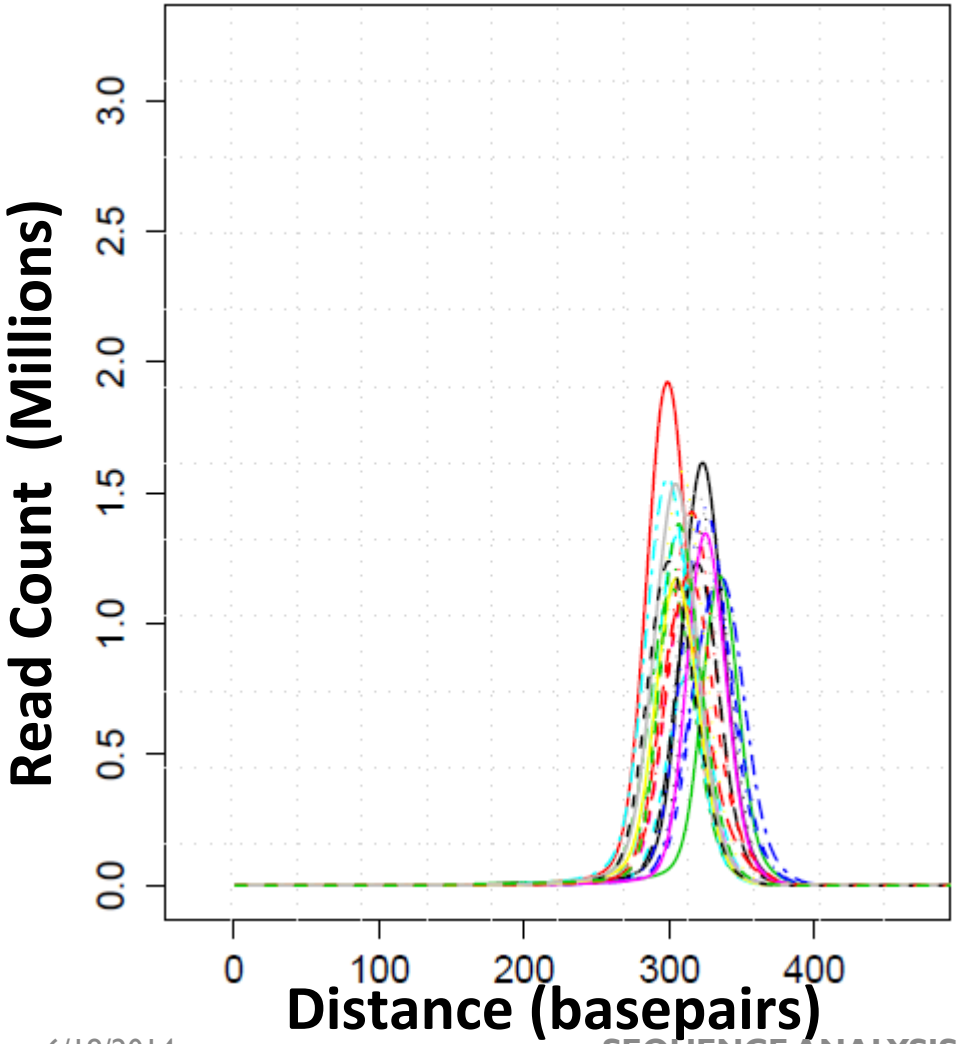
Paired Reads

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

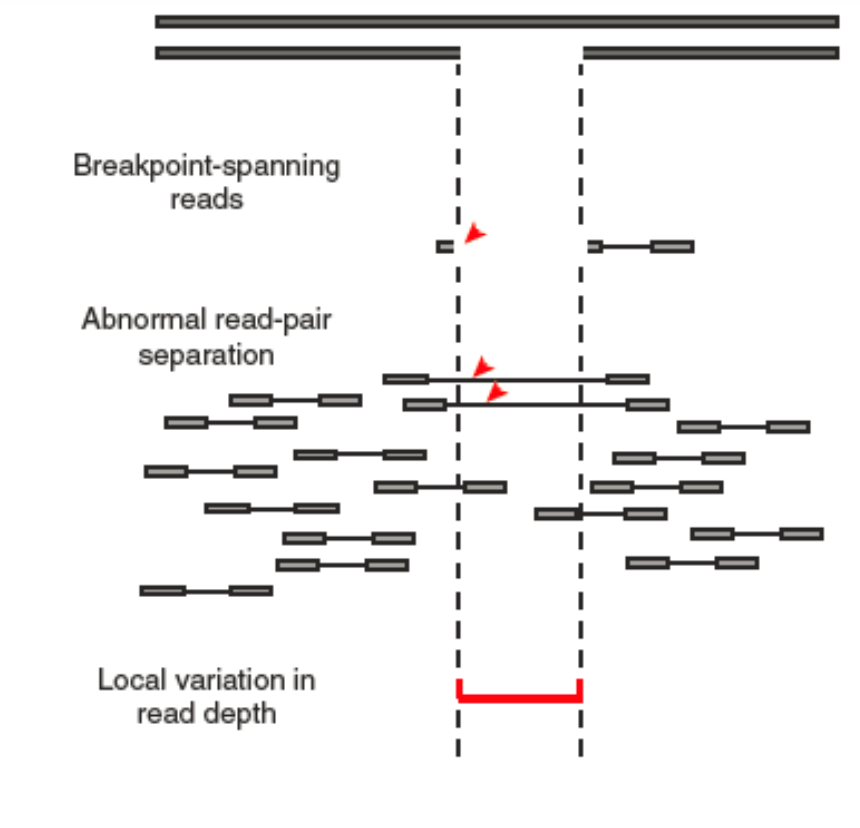
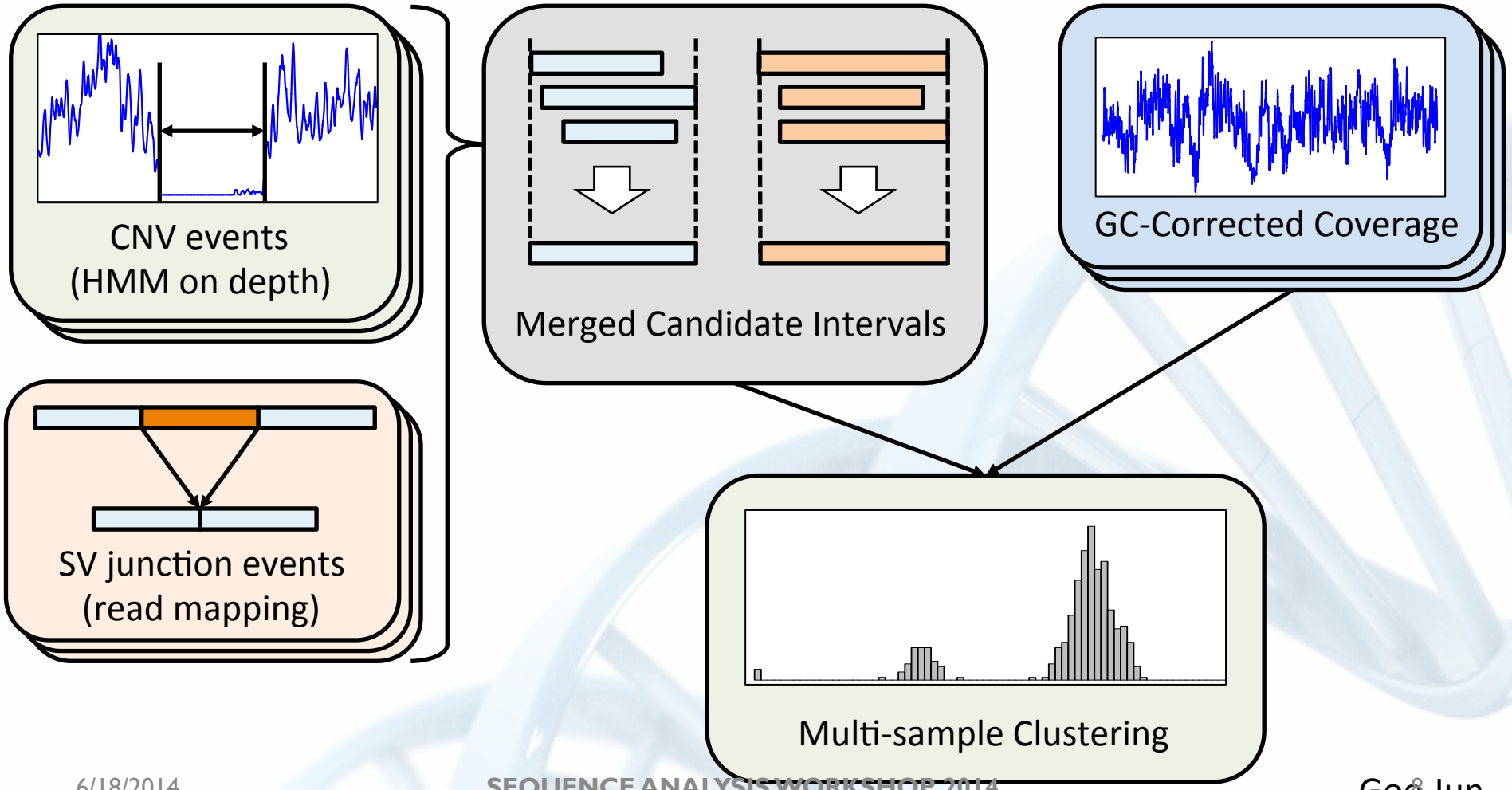


Figure from Handsaker et al (2011)

MULTI-SAMPLE CNV (DELETIONS) CALLING



Detecting Copy Number Variation Based on Read Depths

- 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 Based on Read Depths

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

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

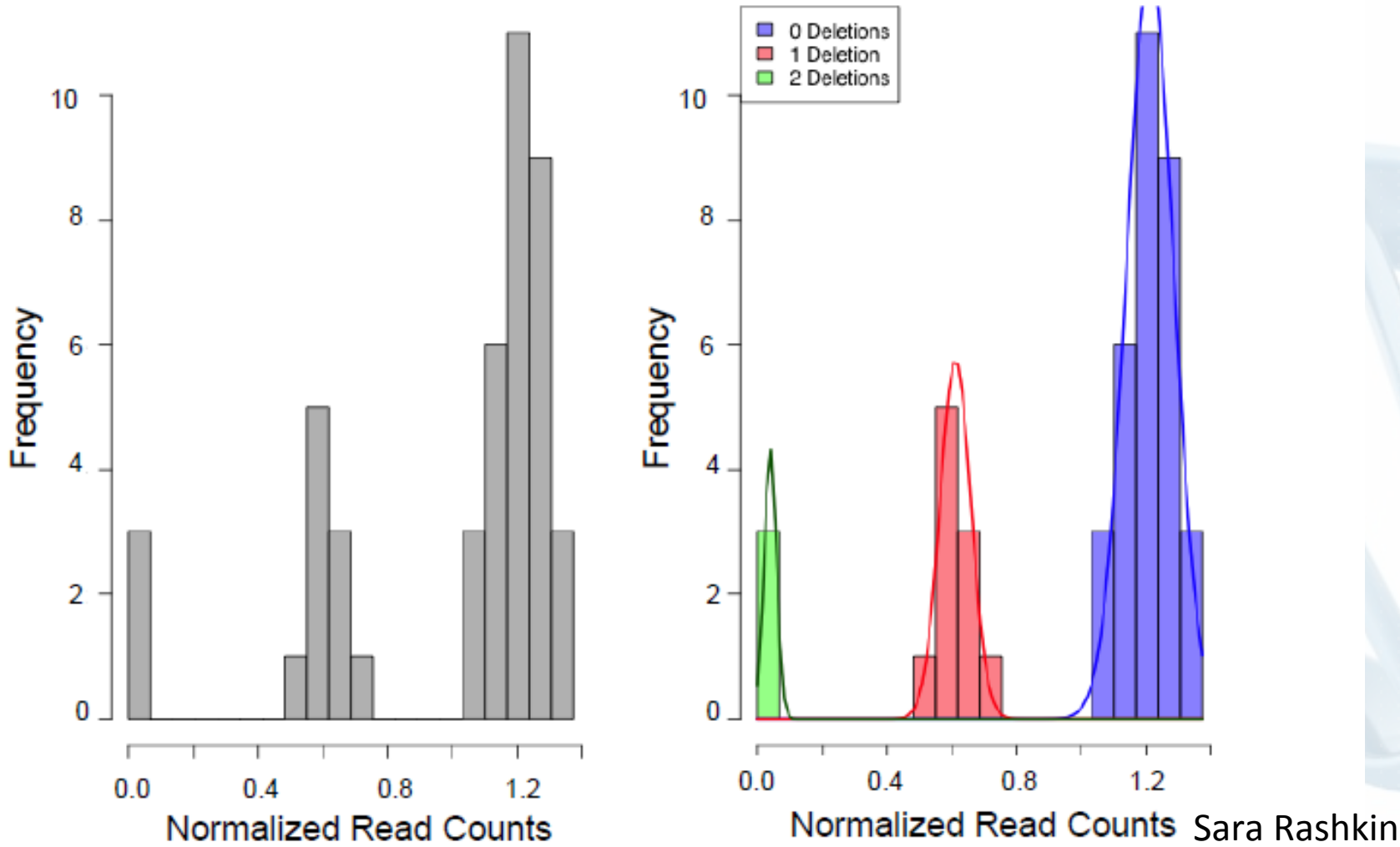
Detecting Copy Number Variation Based on Read Depths

- To estimate a deletion model, maximize

$$L(d_i) = \sum_j p_j (2\pi)^{\frac{1}{2}} \sigma_j^{-1} \exp\left[-\frac{(d_j - \mu_j)^2}{2\sigma_j^2} \right]$$

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

Well Separated Region



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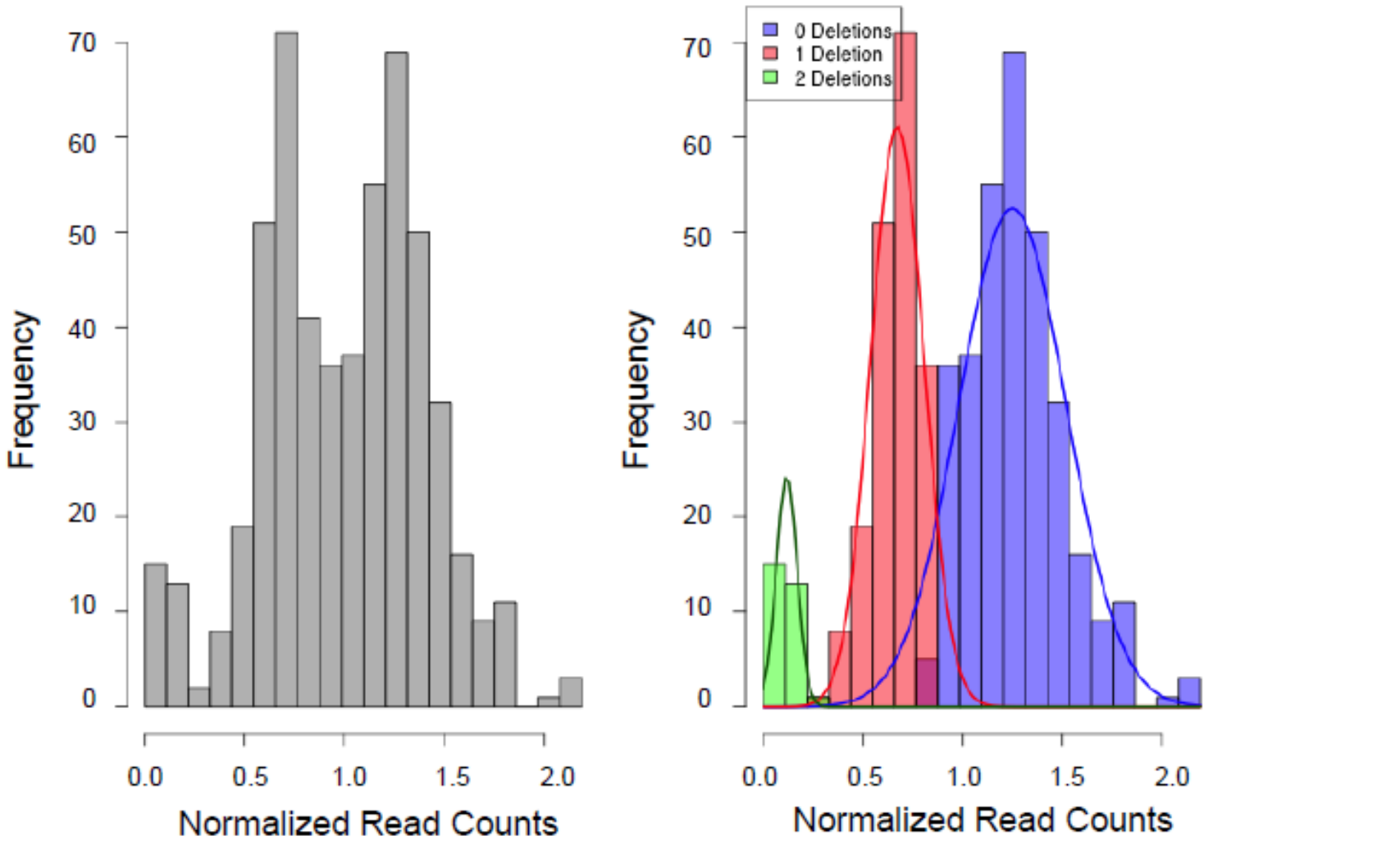
MODERATELY SEPARATED REGION



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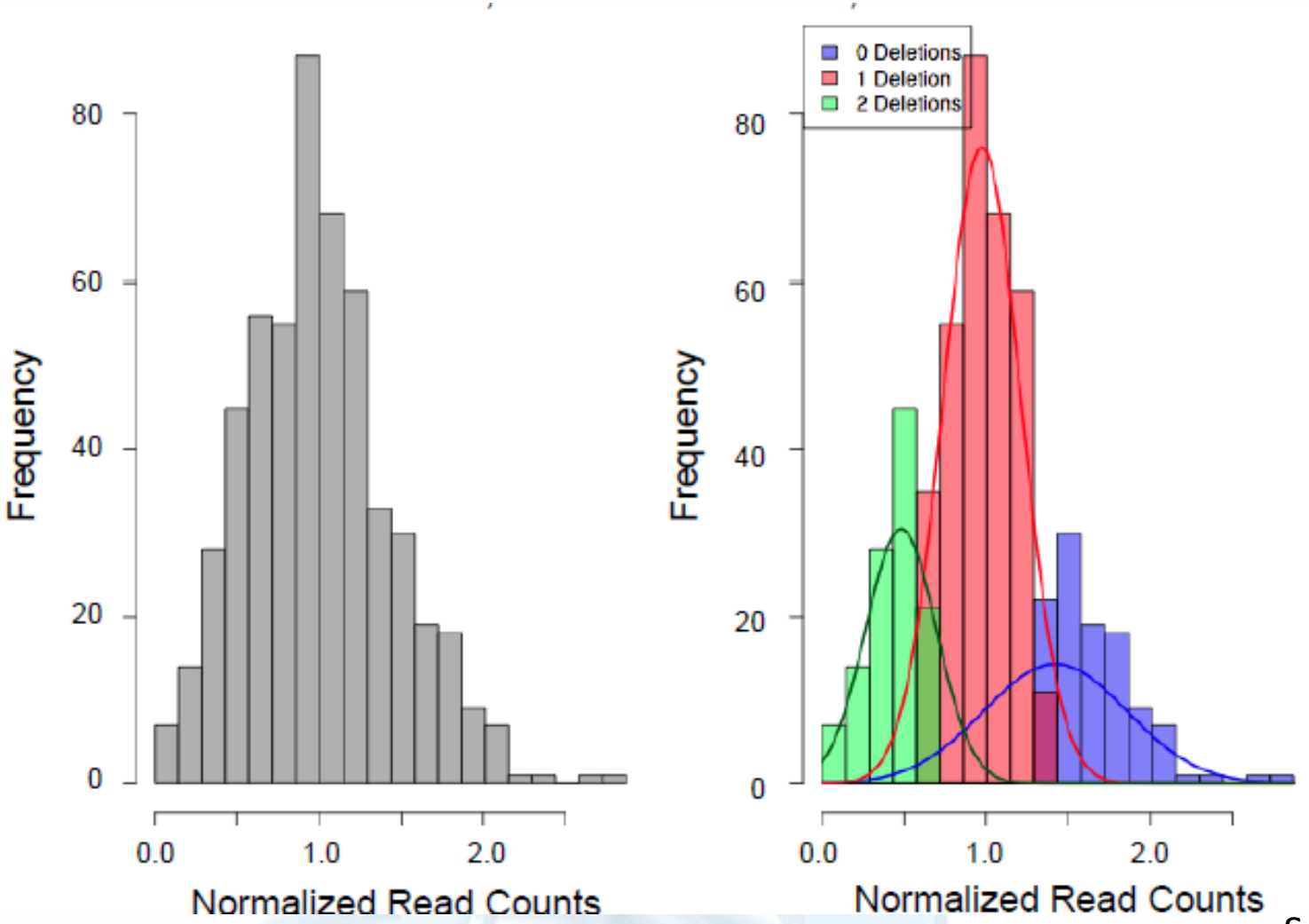
Hard to Call Region



Sara Rashkin

CLUSTER EVALUATION - # OF COMPONENTS

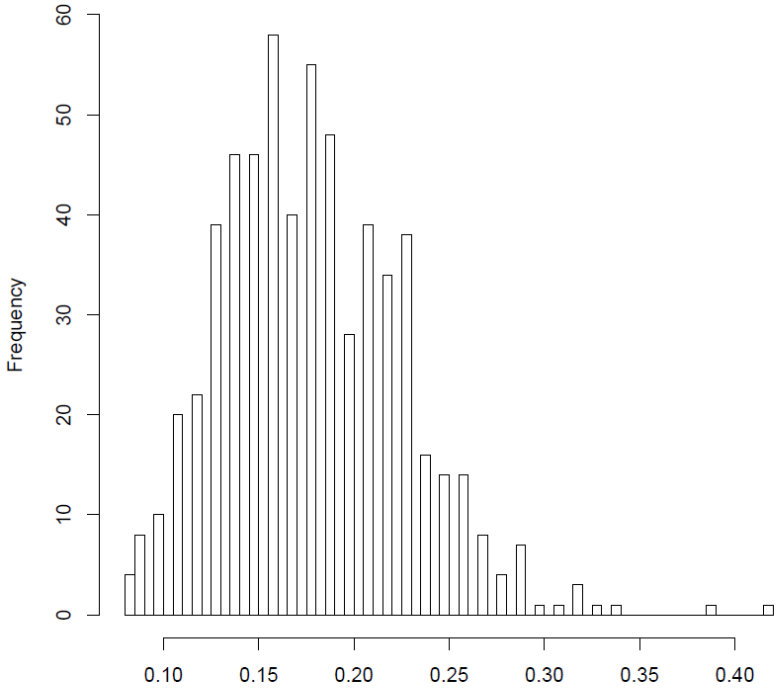
- Bayesian Information Criterion (BIC)

$$\mathrm{BIC} = -2\ \mathrm{LLK} + k \ln(n)$$

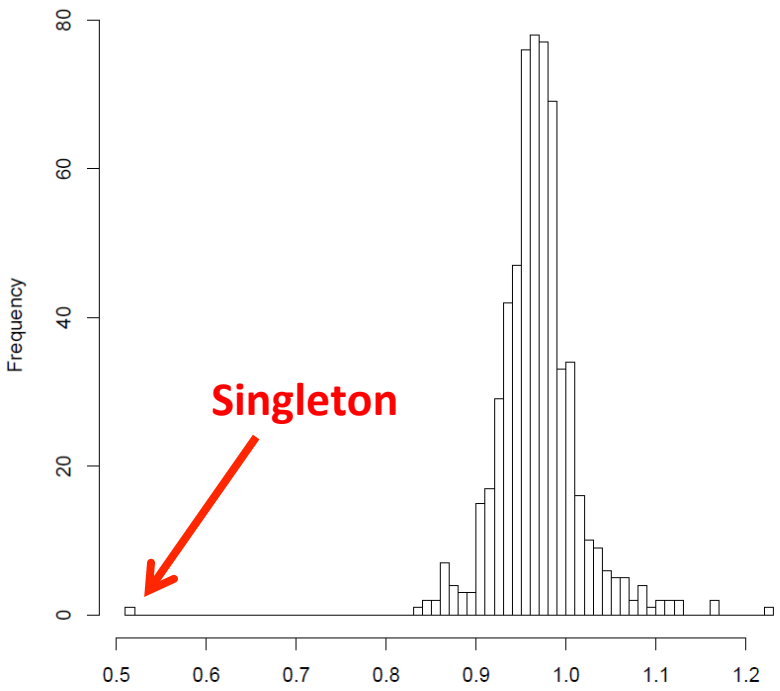
  - LLK : Log-likelihood
  - k : number of clusters
  - n : number of samples

- Fit Gaussian mixtures with 1, 2 and 3 components, and compare BIC of 1, 2, 3 component models to decide number of clusters.

BIC Example



BIC chooses 1 component



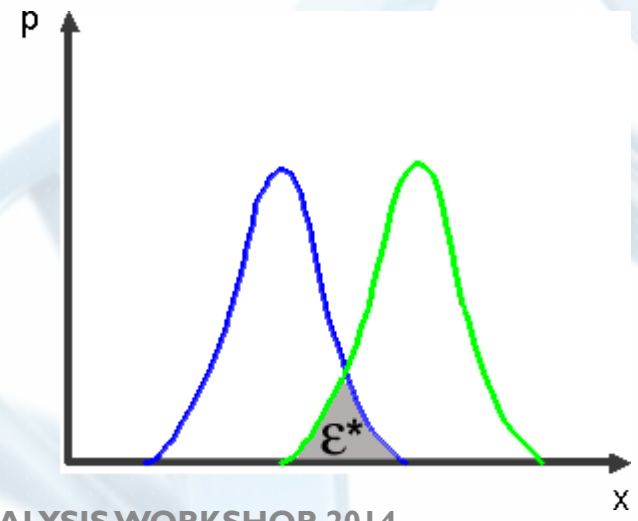
BIC chooses 2 components

CLUSTER EVALUATION – OVERLAP

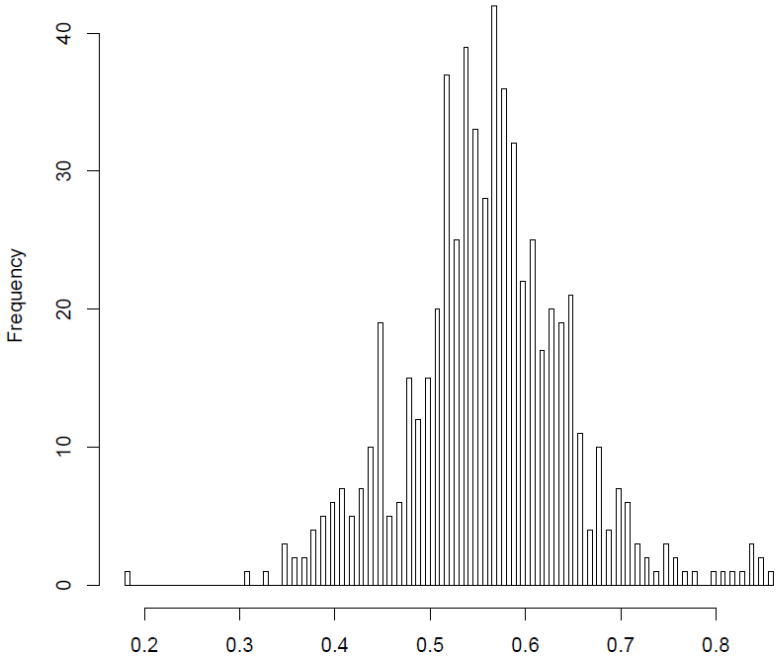
- Evaluation of cluster separation
  - Unavoidable error : overlap between two distributions
  - Bayes error rate ~ Bhattacharyya coefficient
  - For two Gaussian distributions,

    $D = (\mu_1 - \mu_2)^2 / (8\ \sigma_{avg}^2) + (1/2) \log [\sigma_{avg} / \mathrm{sqrt}(\sigma_a \sigma_a)]$

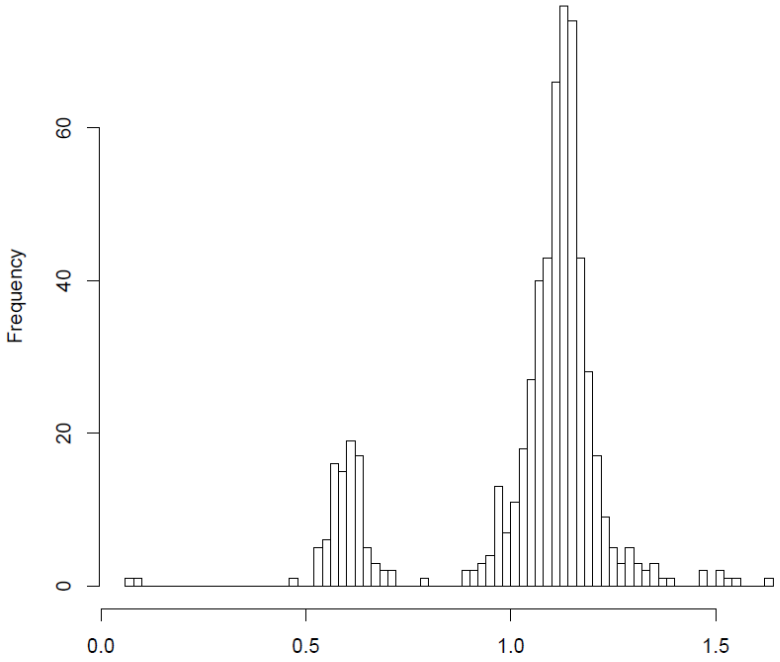
    $P(\mathrm{Overlap}) = \exp(-D)$



P(OVERLAP) EXAMPLE

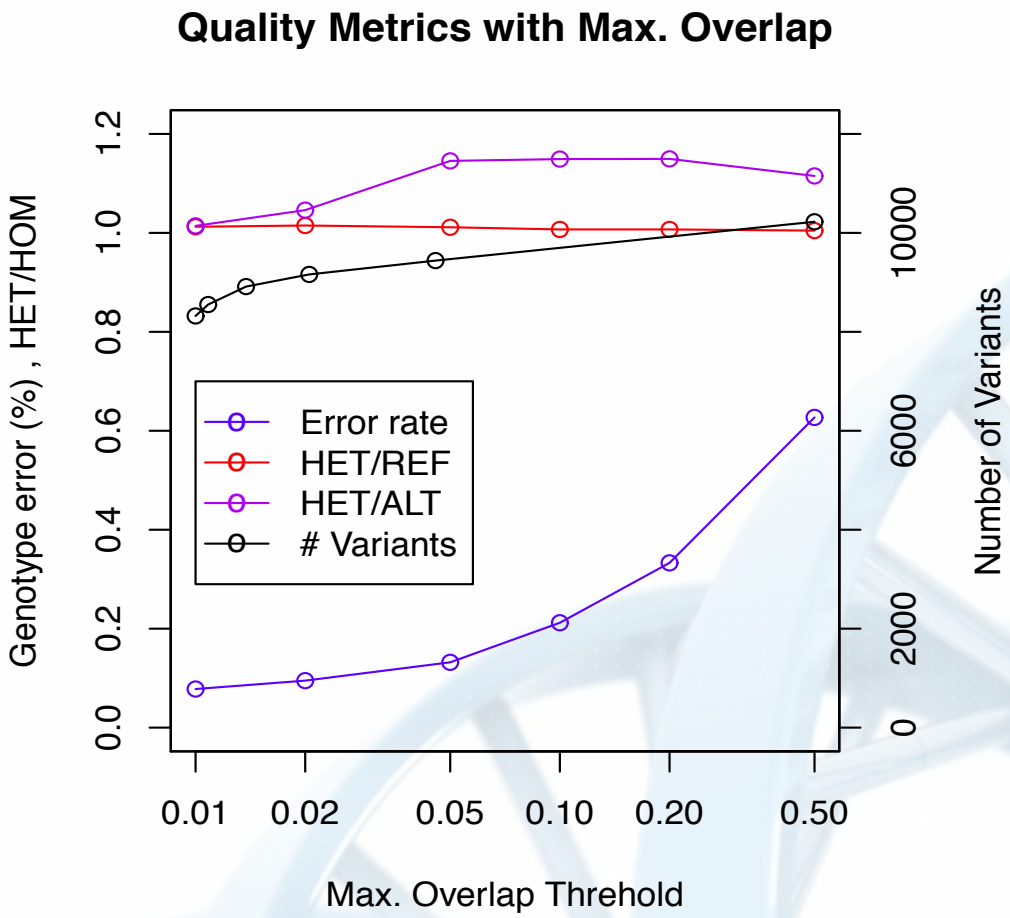


max exp(-D) = 0.933



max exp(-D) = 0.0032

SENSITIVITY/ERROR VS P(OVERLAP)



Goo Jun

1000G CNV Default CG vs Multi-sample

	CG Default	Multi-sample	SNPs
# Large Deletions	2,374	**8,321**	-
Call Rate (%)	95.2	**99.9**	96.73
Merlin-estimated Error Rate (%)	>10	**0.078**	0.494
Trio HET/HomREF	0.750	**1.012**	0.962
Trio HET/HomALT	1.055	**1.014**	1.001

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)

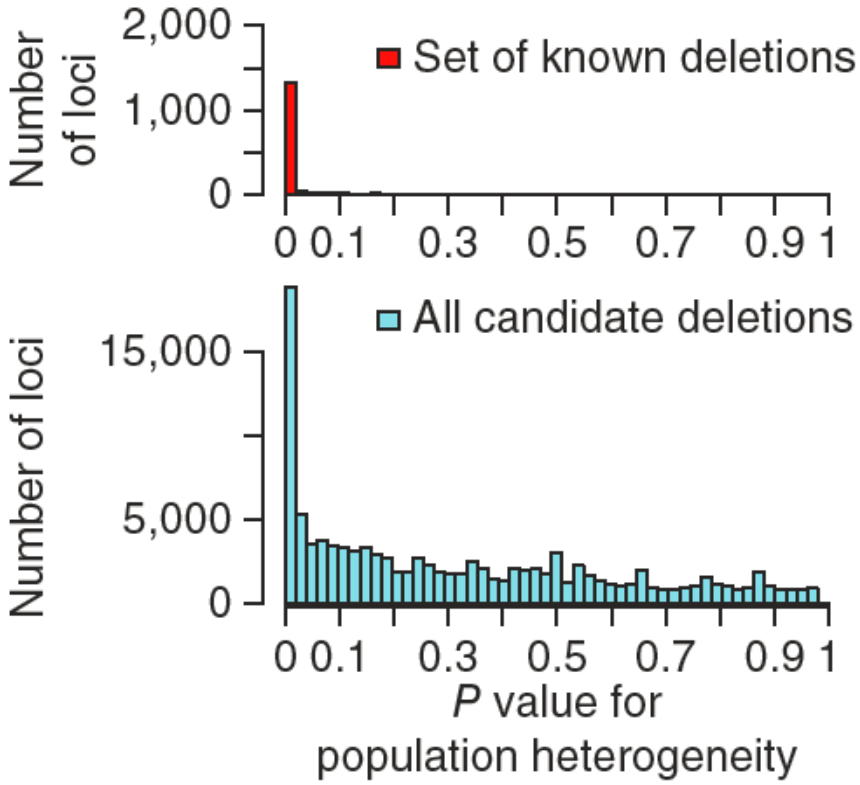
SEARCH FOR ABNORMAL READ PAIRS

- 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



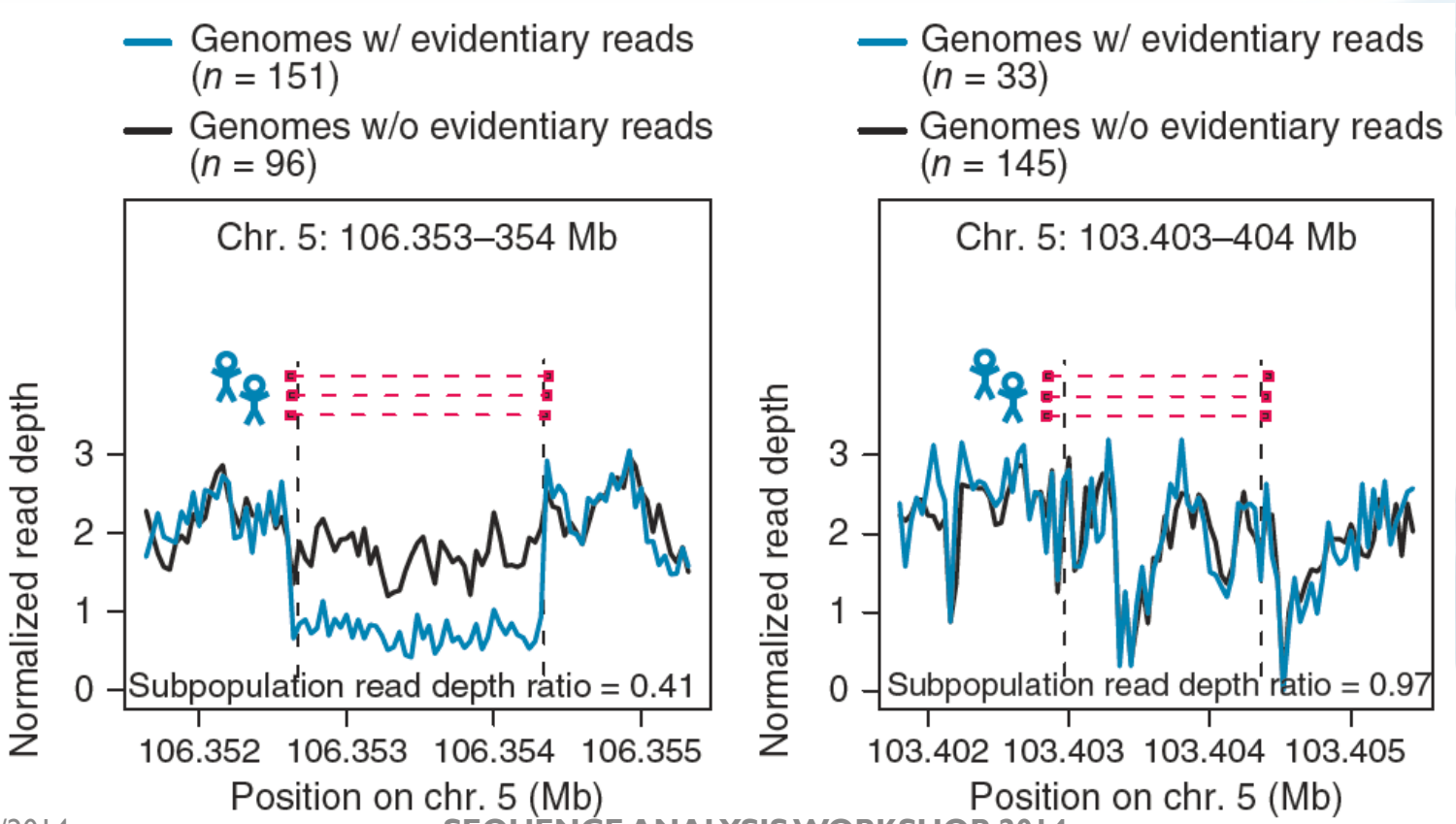
Handsaker et al (2011)

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



"Allelic Substitution"

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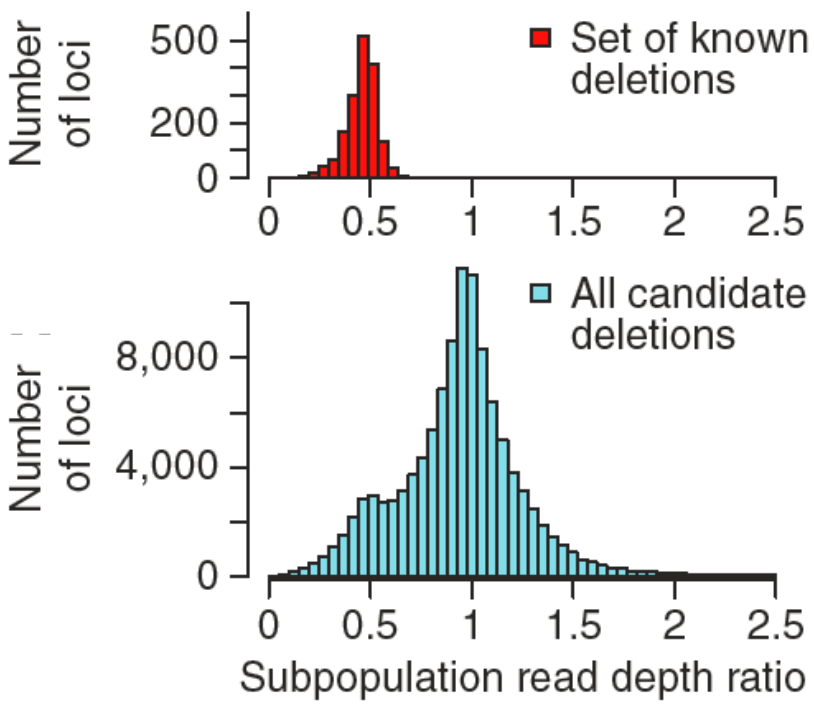
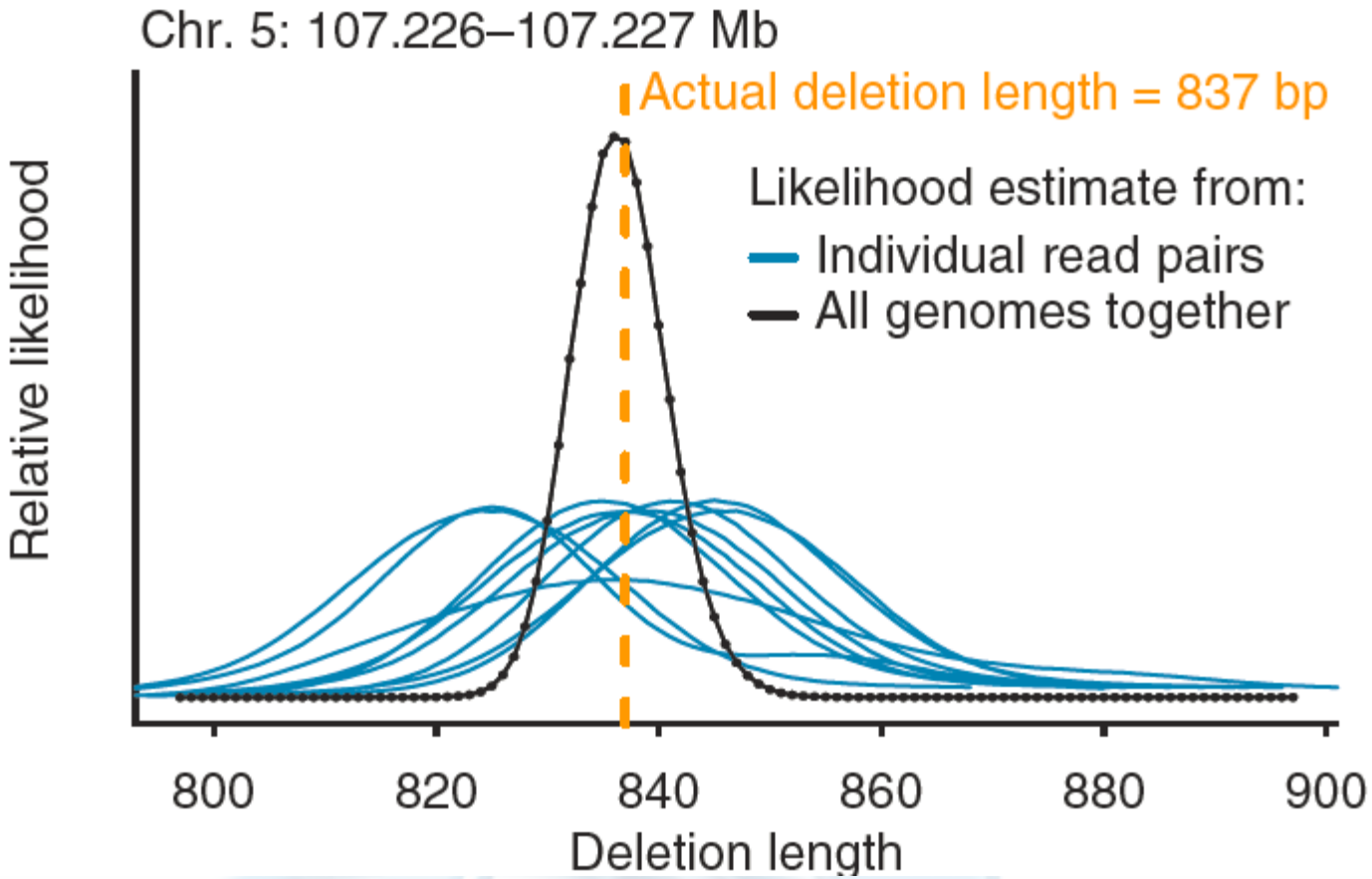


Handsaker et al (2011)

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