Detecting INDELs and CNVs with High Throughput Sequencing

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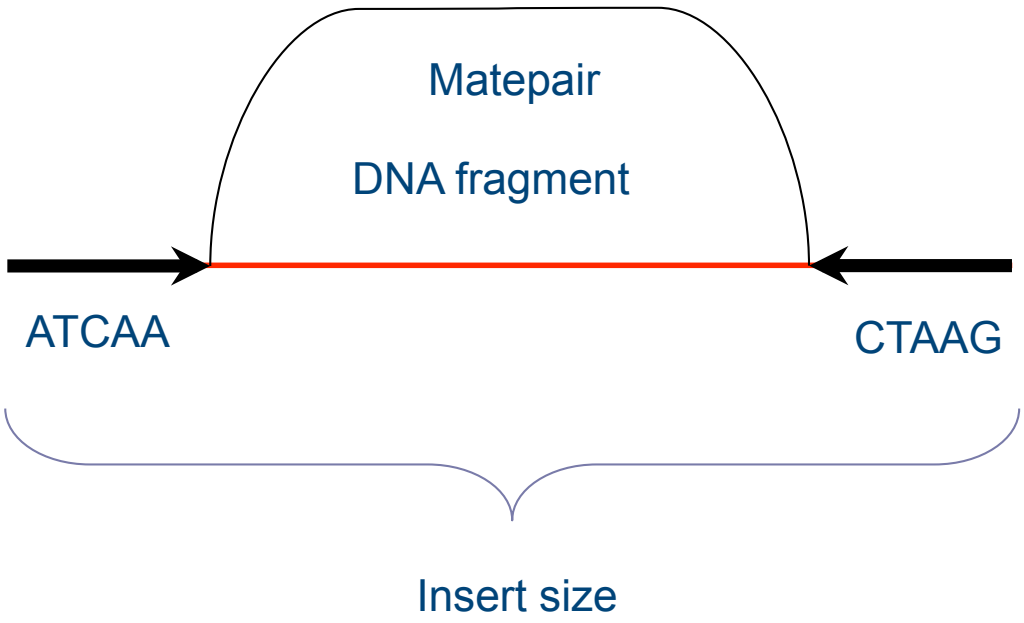
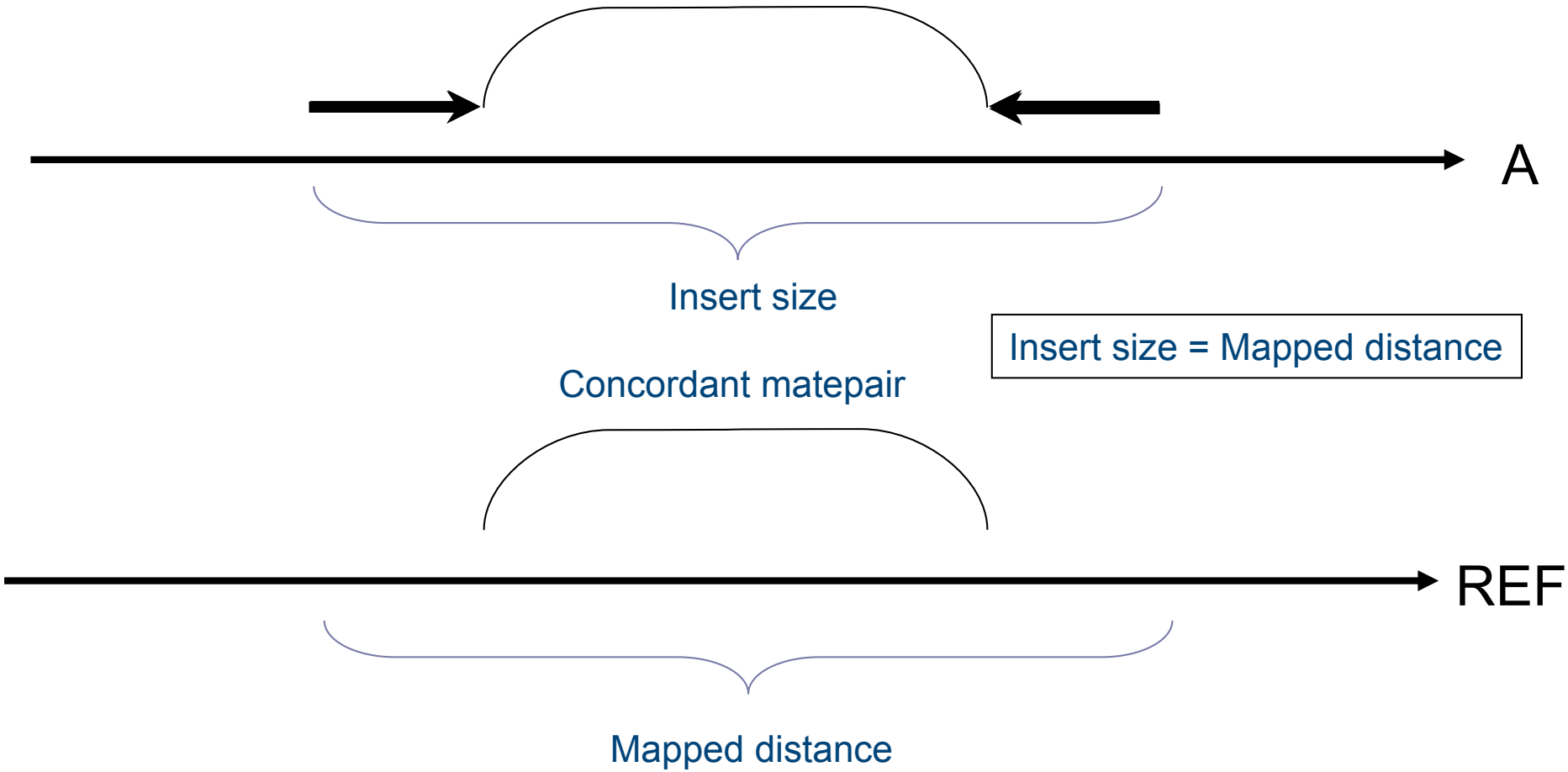
What are Matepairs?



For now, assume insert size is perfect

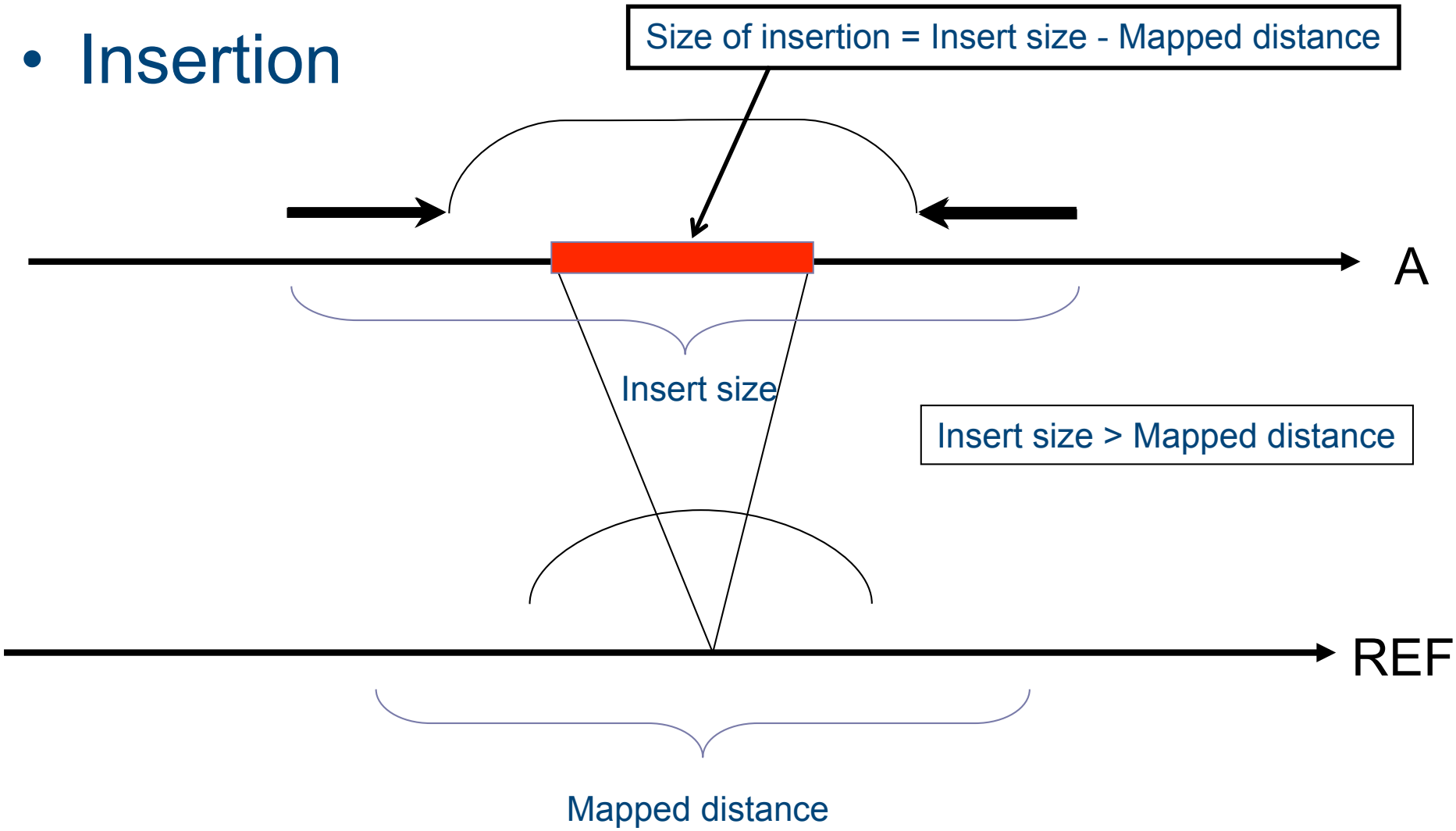
Detecting Structural Variants

- No structural variants



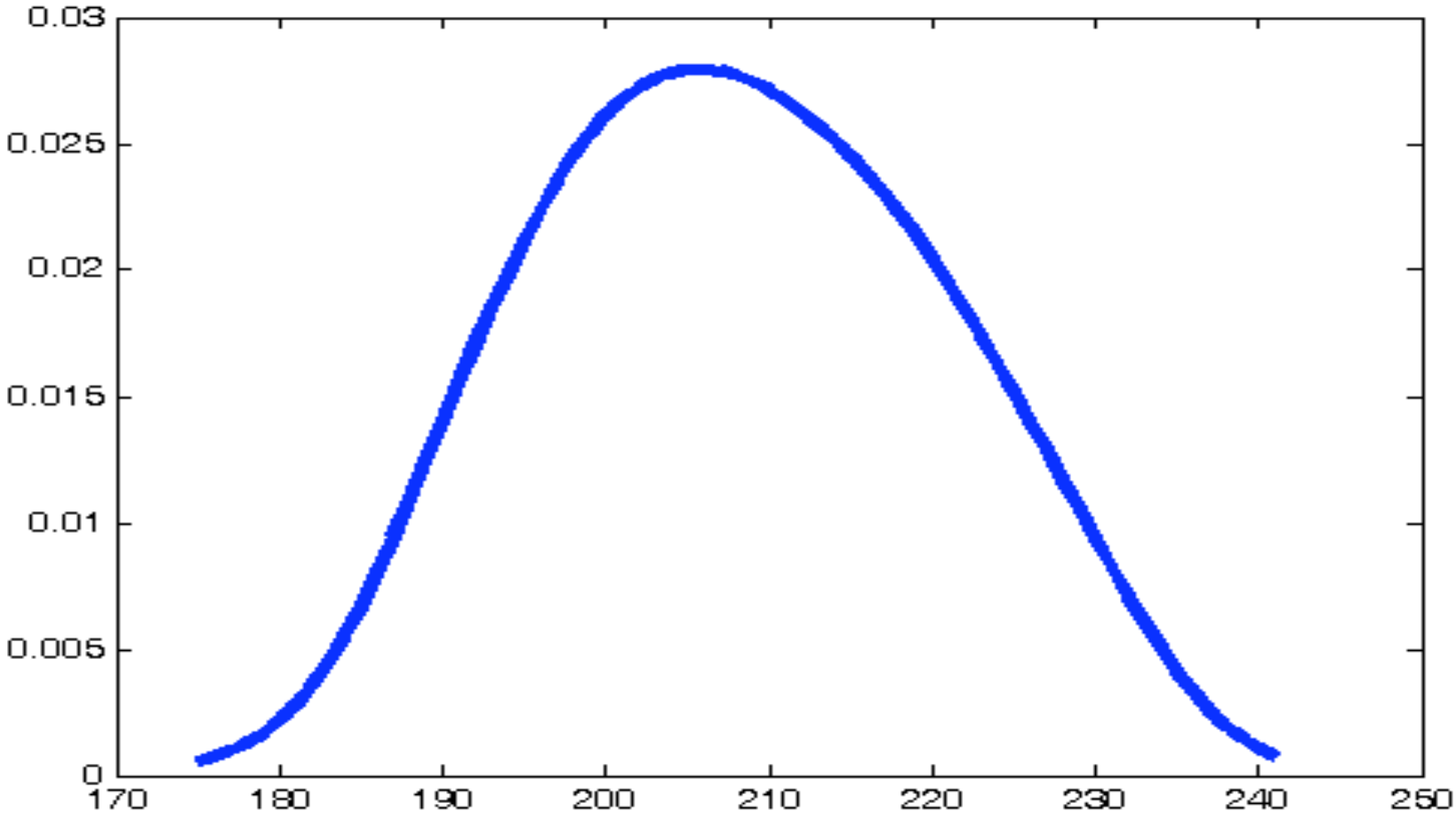
Detecting Structural Variants

- Insertion

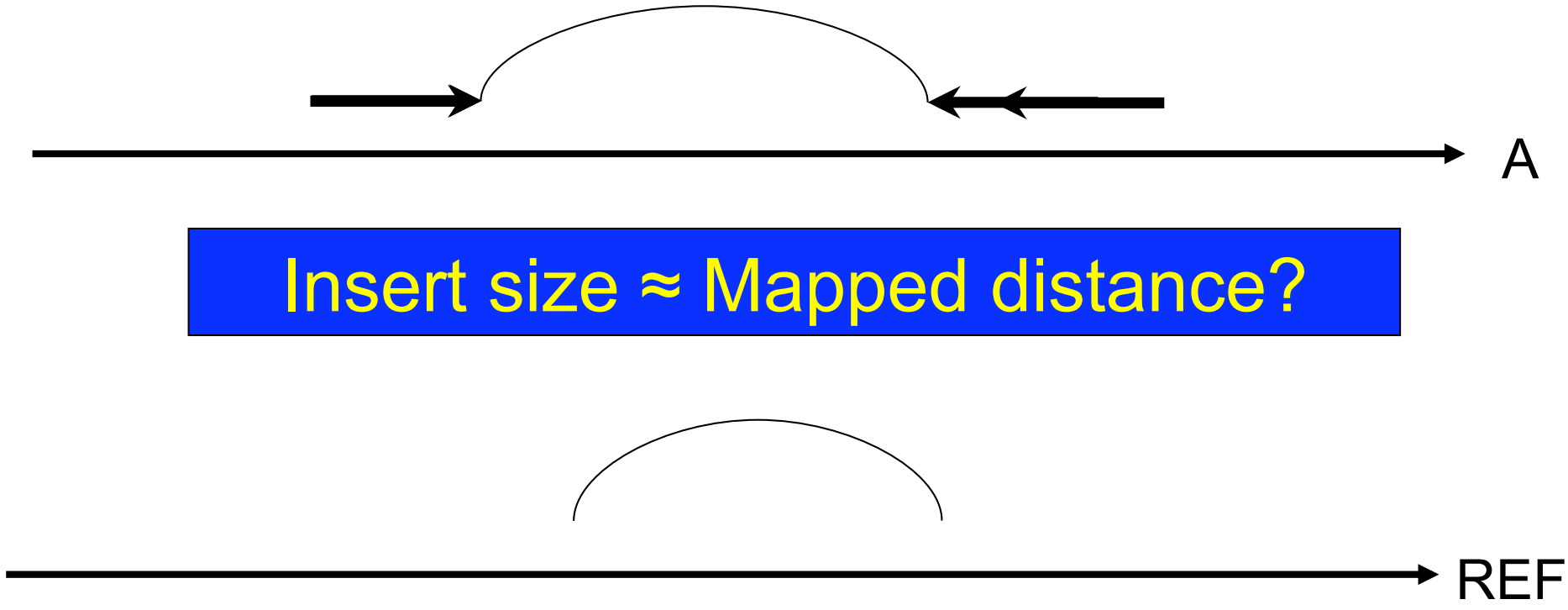


Distribution of Insert Sizes

- In reality, insert sizes of matepairs are not perfect

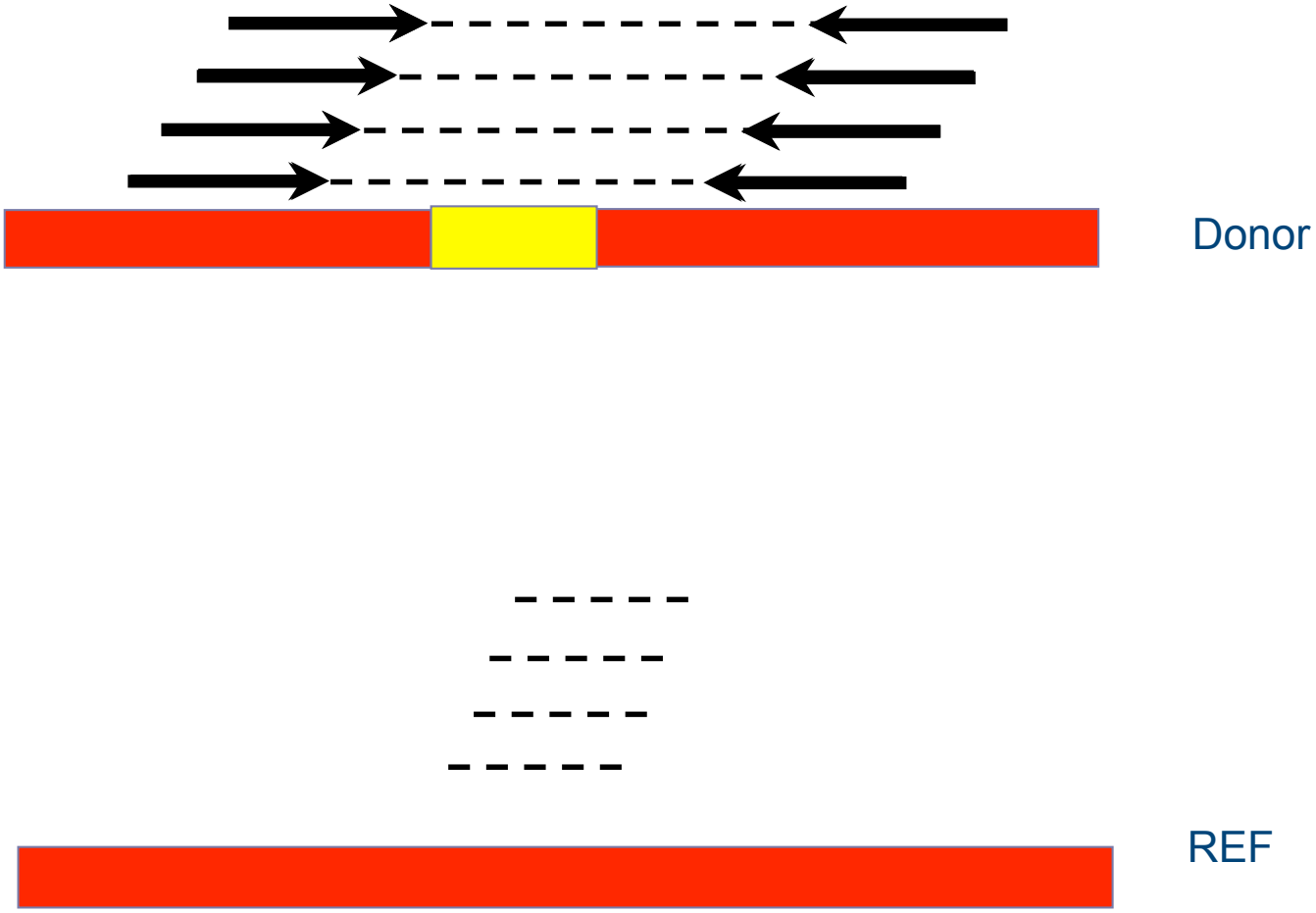


Detecting Smaller INDELS



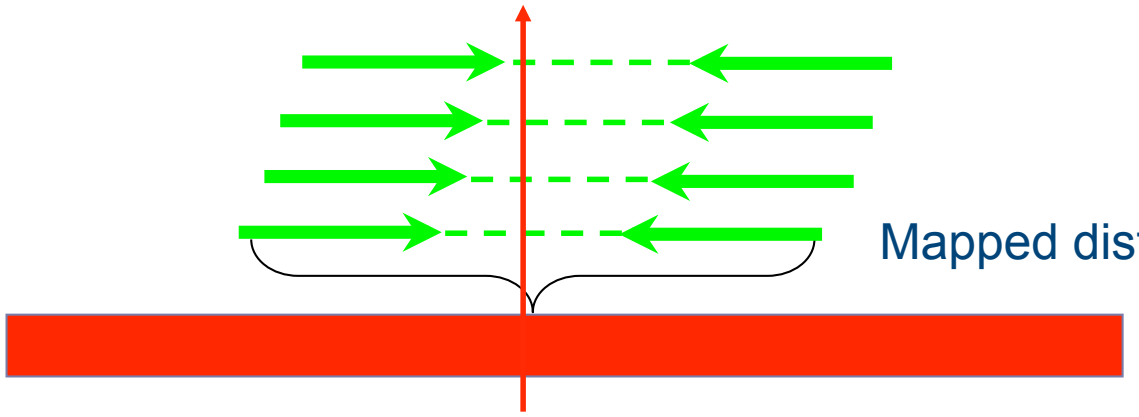
Small Insertion… or noise

Haploid Case – Alignment



Haploid Case – Alignment

Donor



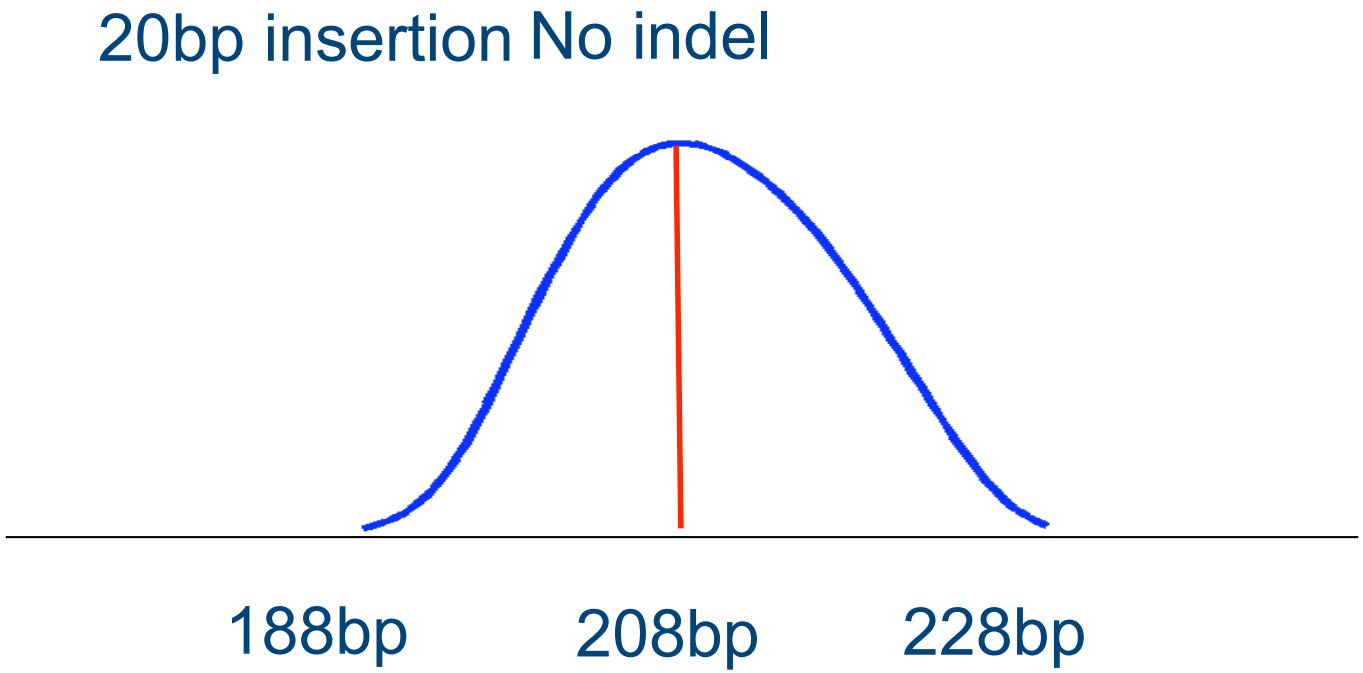
Mapped distance

REF

Cluster

Haploid Case – Distribution

Make a distribution of mapped distances in each cluster
=> The distribution shifts if there is an INDEL

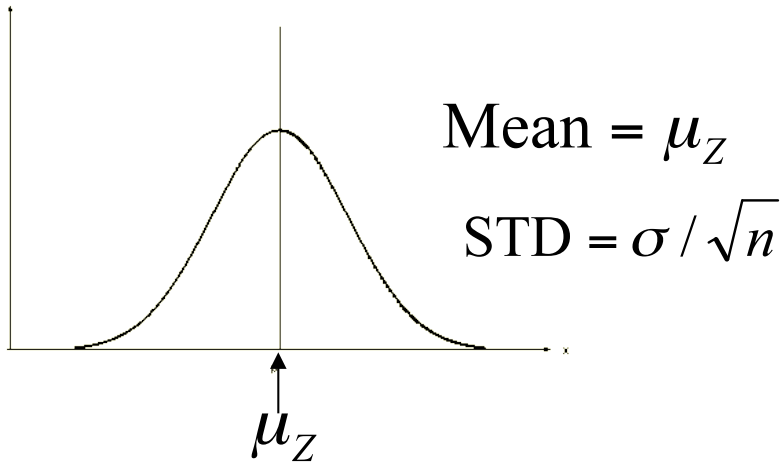


Accuracy of INDEL Estimation

Central limit theorem

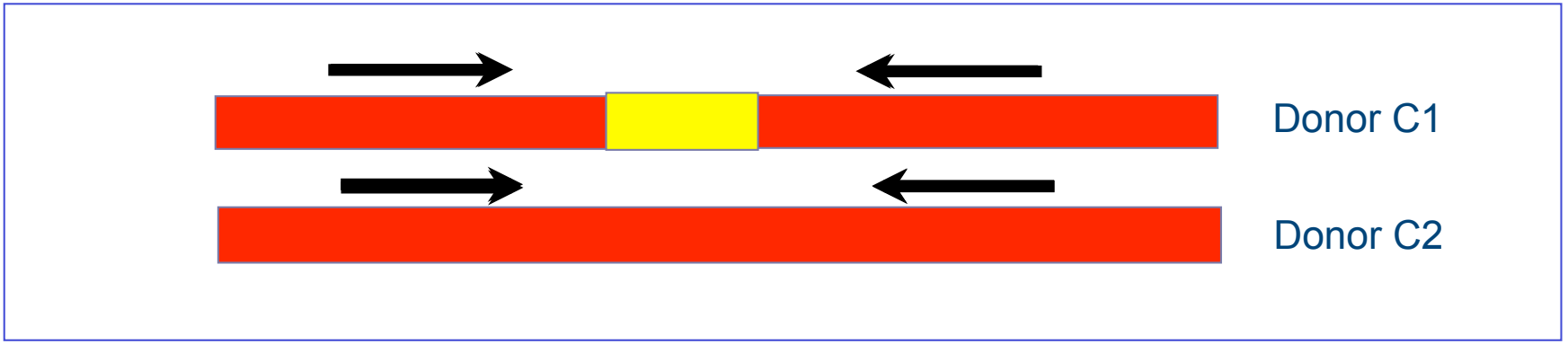
Mean of N independent random variables with finite mean μ and variance σ^2 follows Gaussian with mean μ and standard deviation $\sigma / \sqrt{N}$

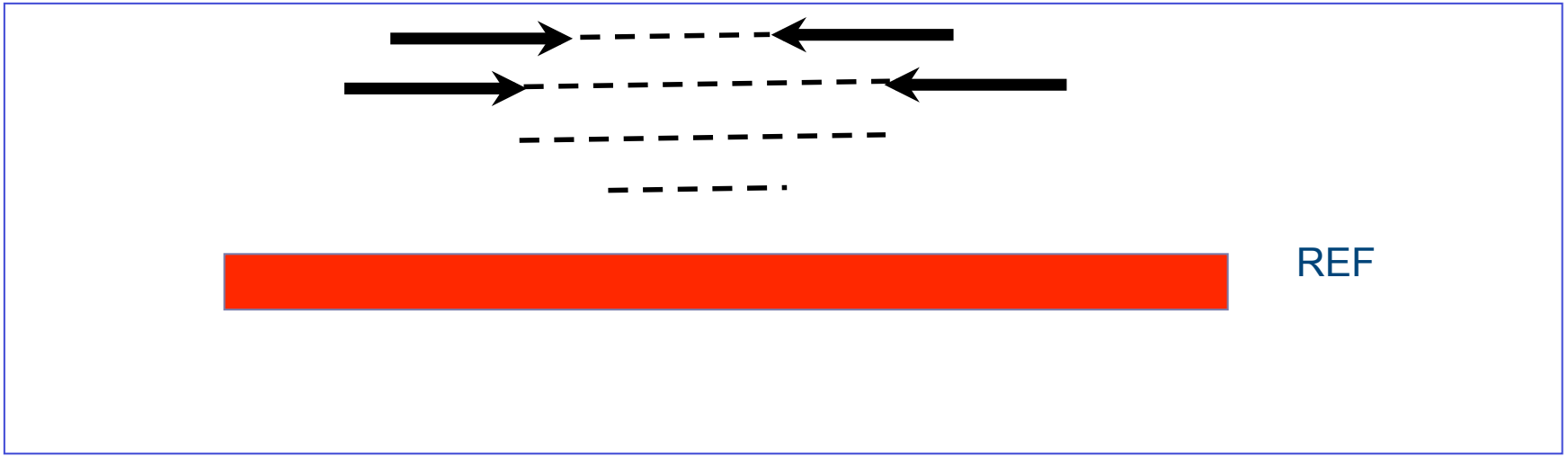
$\mathbf{Z} = \{Z_1 \ldots Z_n\}$: random vars for size of indels from each pair



Diploid Case – Alignment & Clustering

Heterozygous insertion





Diploid Case – Alignment & Clustering

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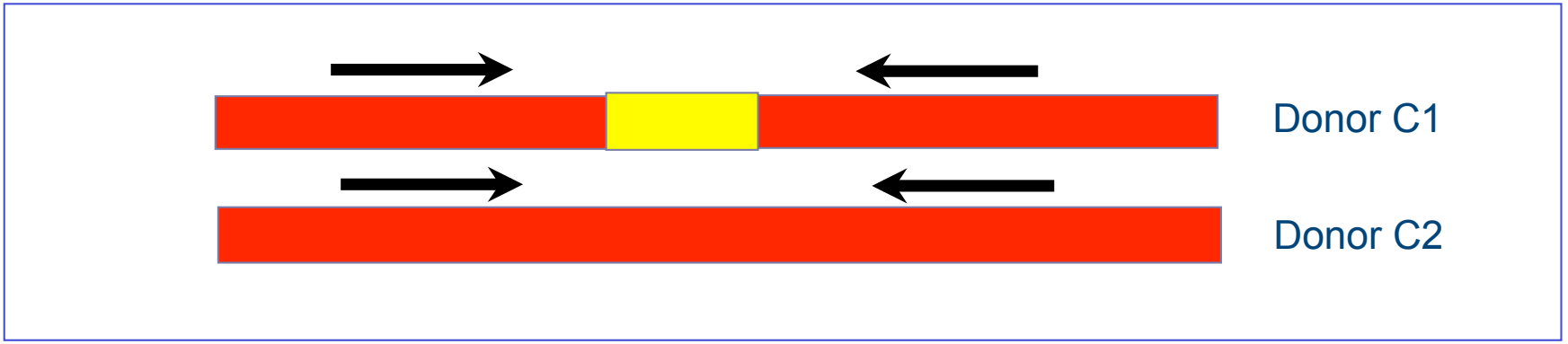
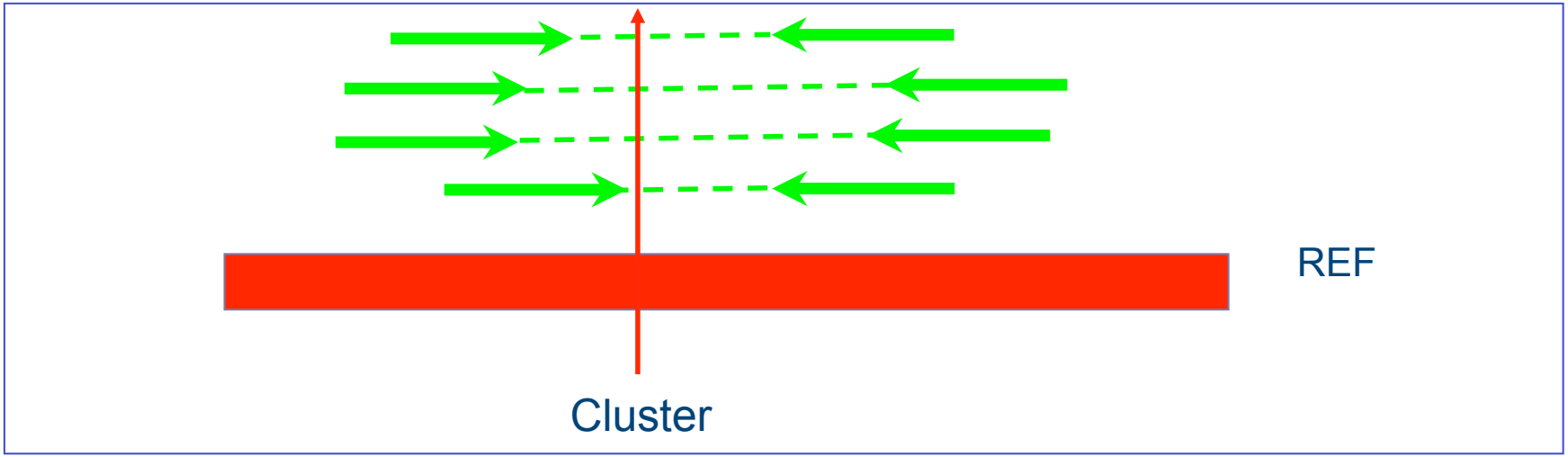
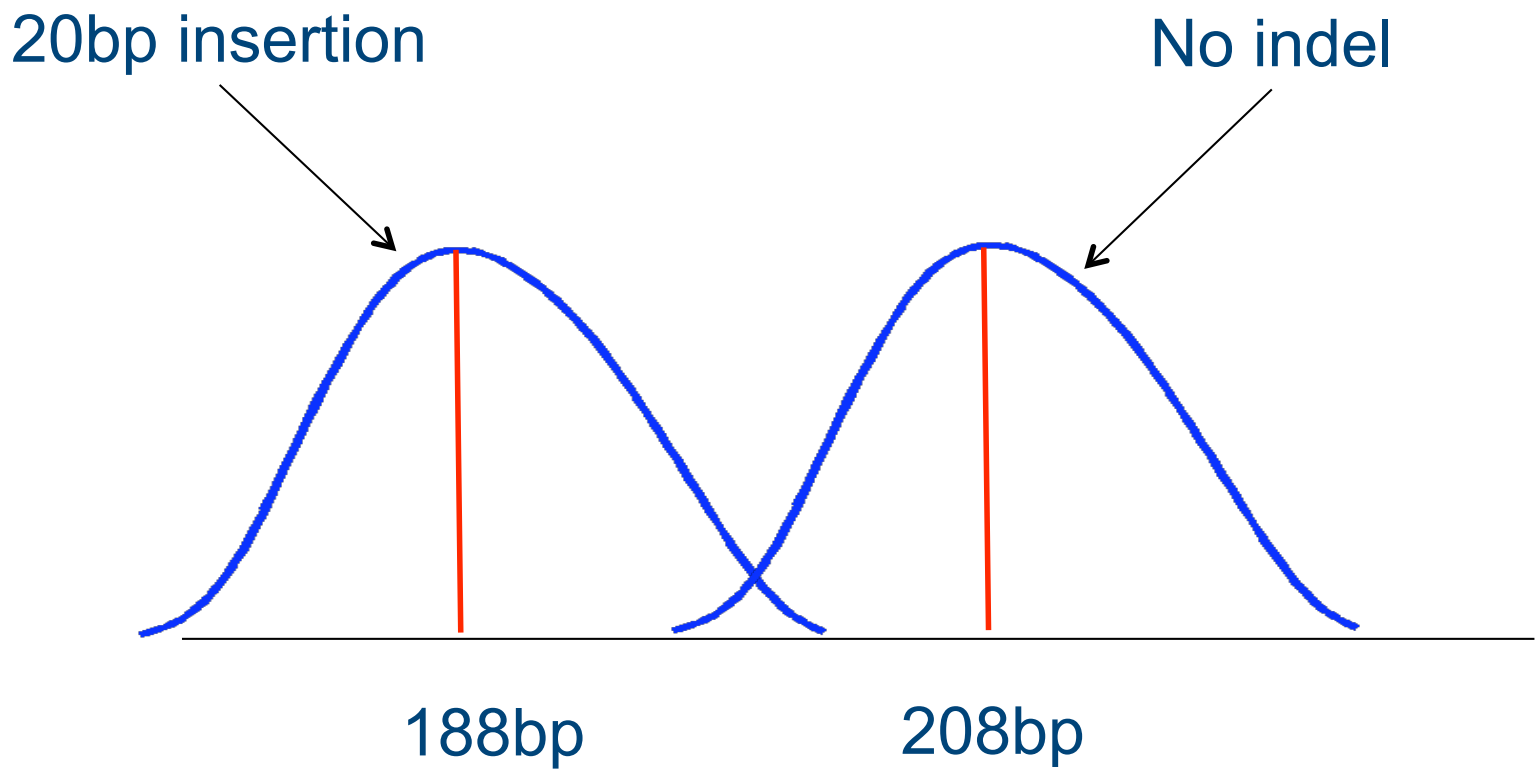




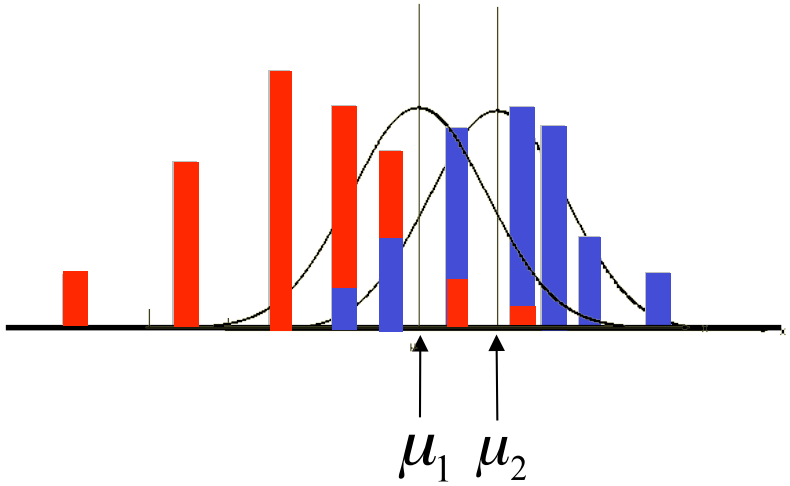
Diploid Case – Distributions

You expect to see matepairs from two distributions.



MoDIL EM Algorithm

1. Randomly initialize μ_1 and μ_2



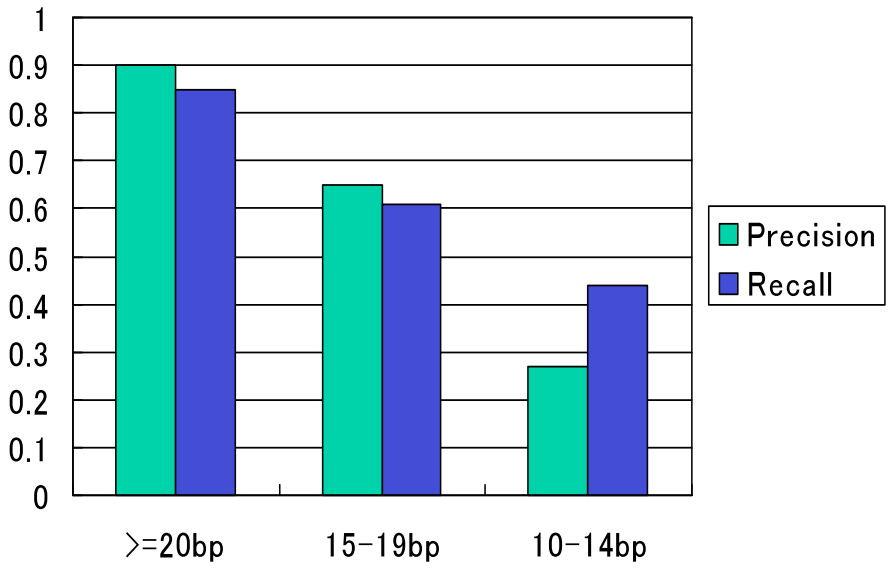
2. E step: Assign each matepair, M_i, to one of two distributions

Assign M_i to p_1 *with* probability $\frac{p_1(M_i)}{p_1(M_i)+p_2(M_i)}$, p_2 with $1-\frac{p_1(M_i)}{p_1(M_i)+p_2(M_i)}$

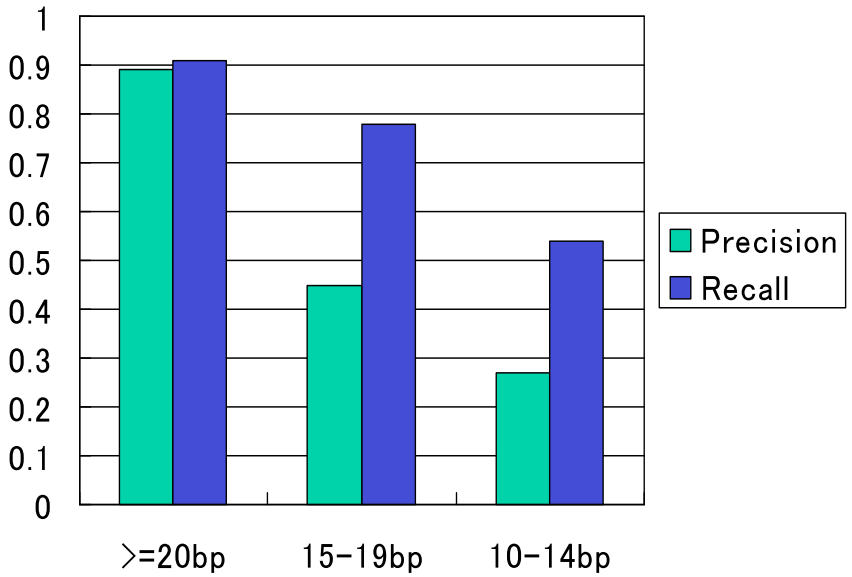
3. M step: Update μ_1 and μ_2 by searching the optimal μ_1 and μ_2 which minimizes Kolmogorov–Smirnov statistic $D = \sum_{t=1}^{2} l_t \sup\left|F_t^o(z) - F_t(z)\right|$

Simulation Results

- Implanted all indels from Mills et al. into chromosome 1 and generated ~51 million matepairs
- Run MoDIL on simulated data and compute precision & recall.



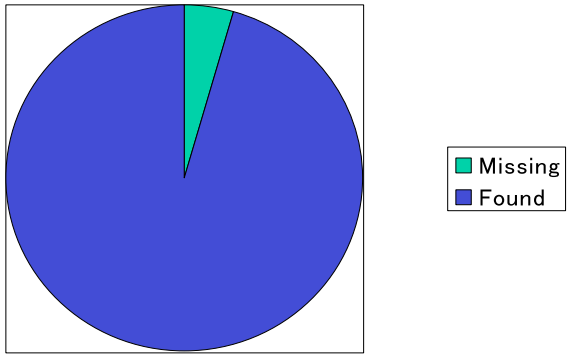
Insertion



Deletion

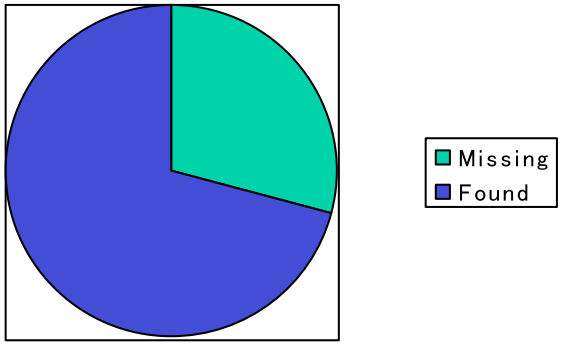
Analysis of NA18507

- NA 18507 (40x Illumina coverage, 208±13bp pairs)
- Kidd et al. found small fraction of INDELs using Sanger style reads (0.3x coverage)
- Computed False Negative Rate (FNR) by taking into account Kidd et al. indels covered by >=20 matepairs



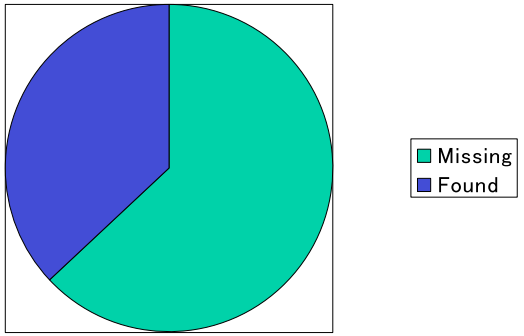
>=20bp INDELs

FNR=0.05



15-19bp INDELs

FNR=0.3

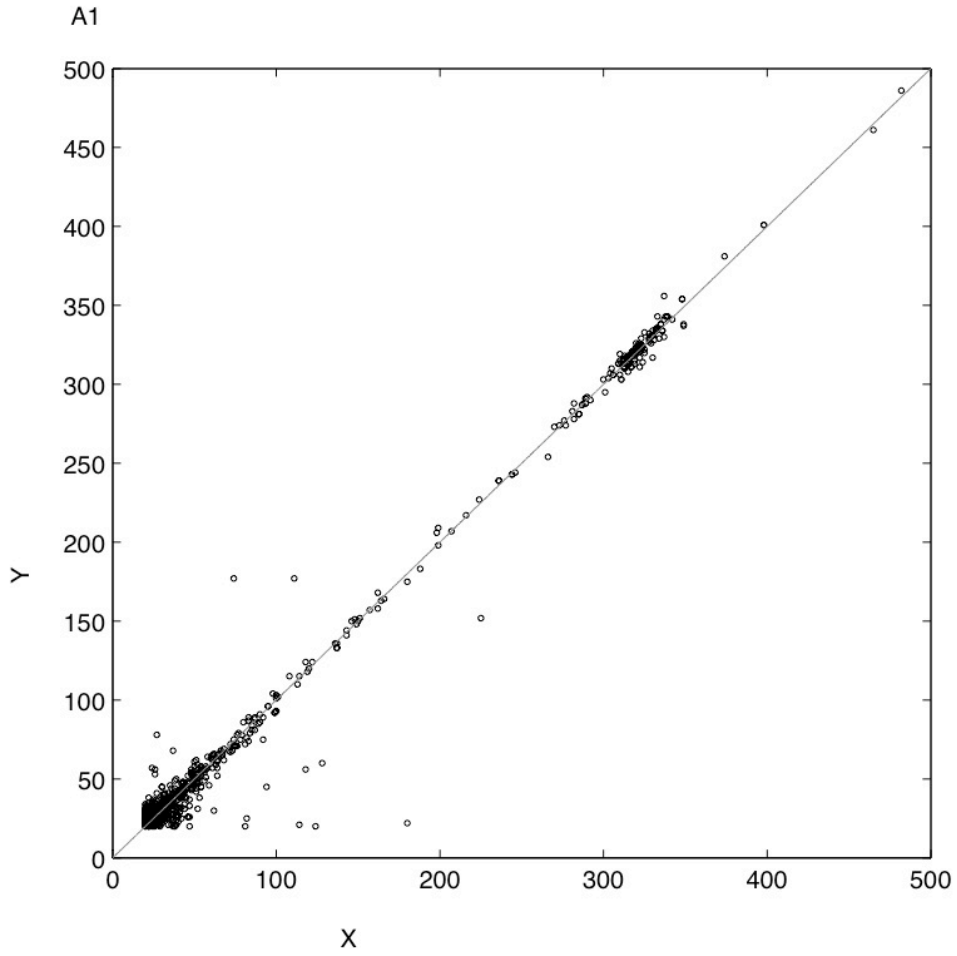


10-14bp INDELs

FNR=0.65

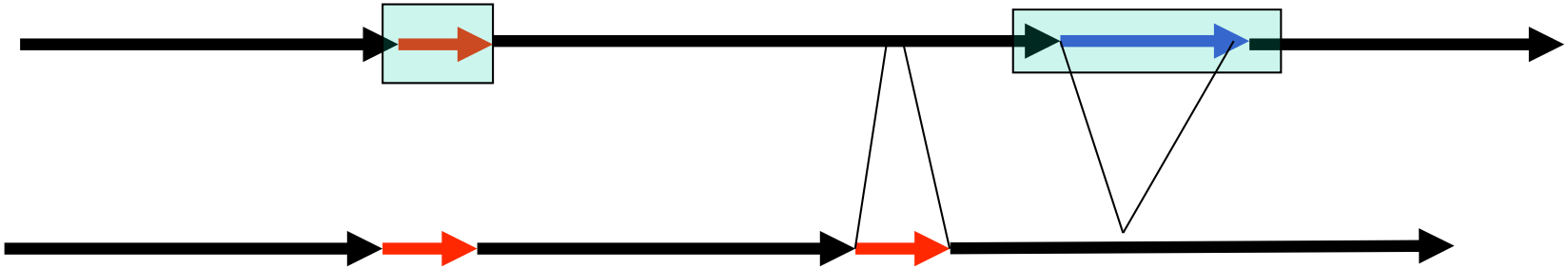
Analysis of NA18507

- NA 18507 (40x Illumina coverage, 208±13bp pairs)

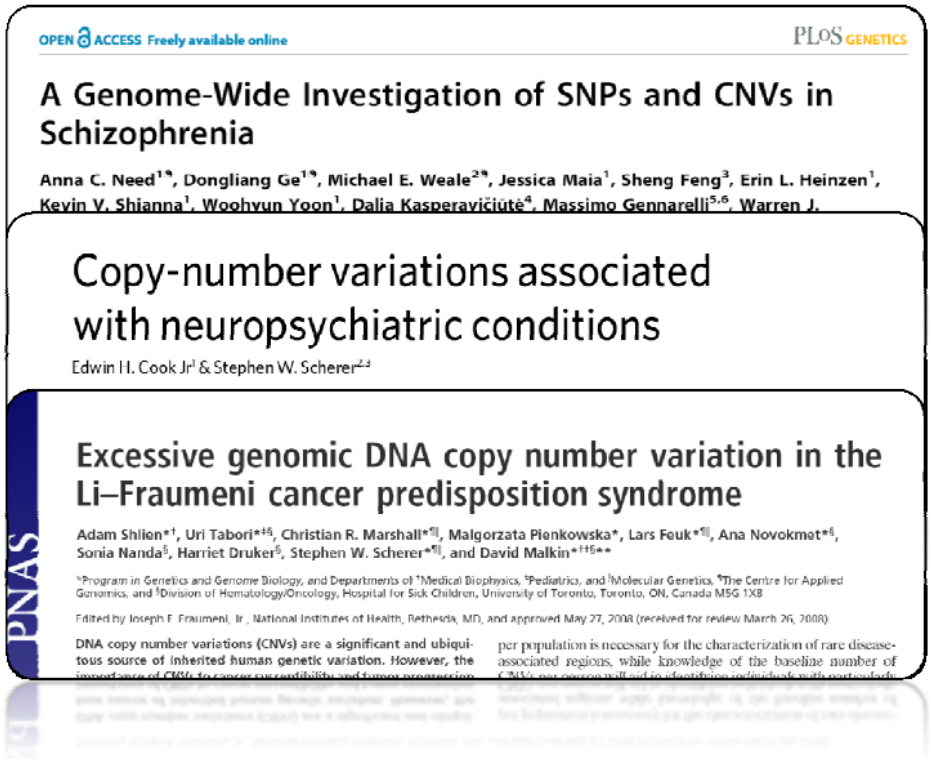


Copy Number Variants (CNVs)

- Large regions that appear a different number of times within different indiv.



- CNVs are associated with a number of diseases
- Input
  - reference human genome
  - sequenced donor genome
- Output
  - CNV annotations in ref



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PLoS GENETICS

A Genome-Wide Investigation of SNPs and CNVs in Schizophrenia

Anna C. Need[1¶], Dongliang Ge[1¶], Michael E. Weale[2¶], Jessica Maia[1], Sheng Feng[3], Erin L. Heinzen[1], Kevin V. Shianna[1], Woohyun Yoon[1], Dalia Kasperaviciute[4], Massimo Gennarelli[5,6], Warren J.

Copy-number variations associated with neuropsychiatric conditions

Edwin H. Cook Jr[1] & Stephen W. Scherer[2,3]

PNAS

Excessive genomic DNA copy number variation in the Li–Fraumeni cancer predisposition syndrome

Adam Shlien*†, Uri Tabori*‡§, Christian R. Marshall*¶‖, Malgorzata Pienkowska*, Lars Feuk*¶‖, Ana Novokmet*§, Sonia Nanda§, Harriet Druker§, Stephen W. Scherer*¶‖, and David Malkin*†‡§**

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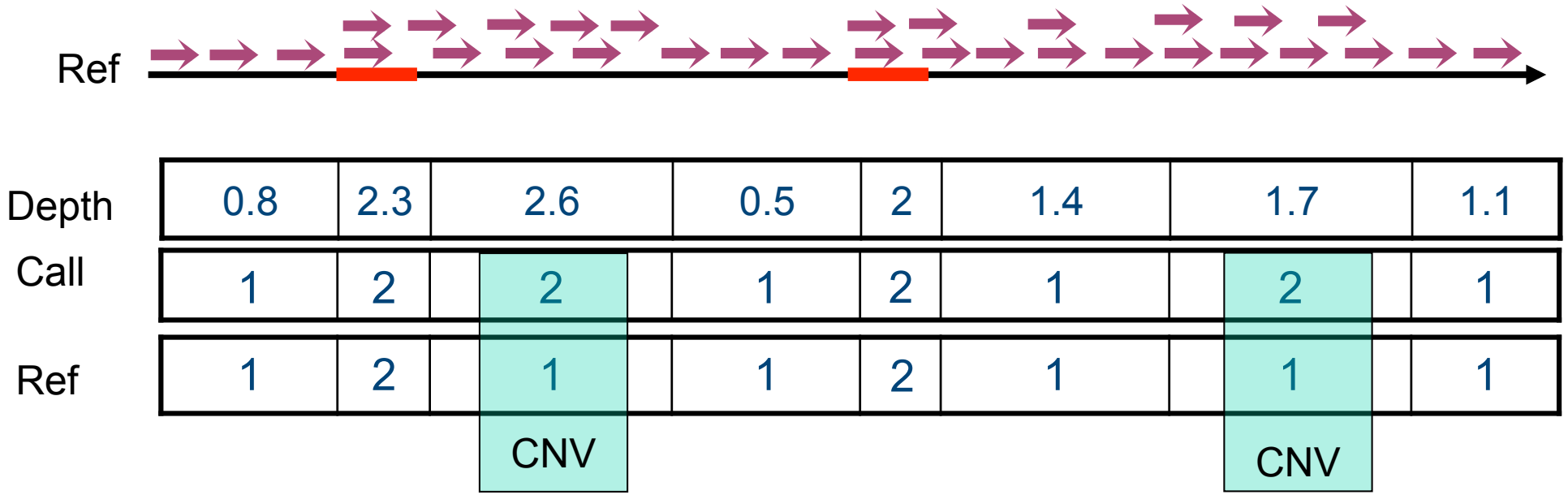
Edited by Joseph F. Fraumeni, Jr., National Institutes of Health, Bethesda, MD, and approved May 27, 2008 (received for review March 26, 2008)

DNA copy number variations (CNVs) are a significant and ubiquitous source of inherited human genetic variation. However, the

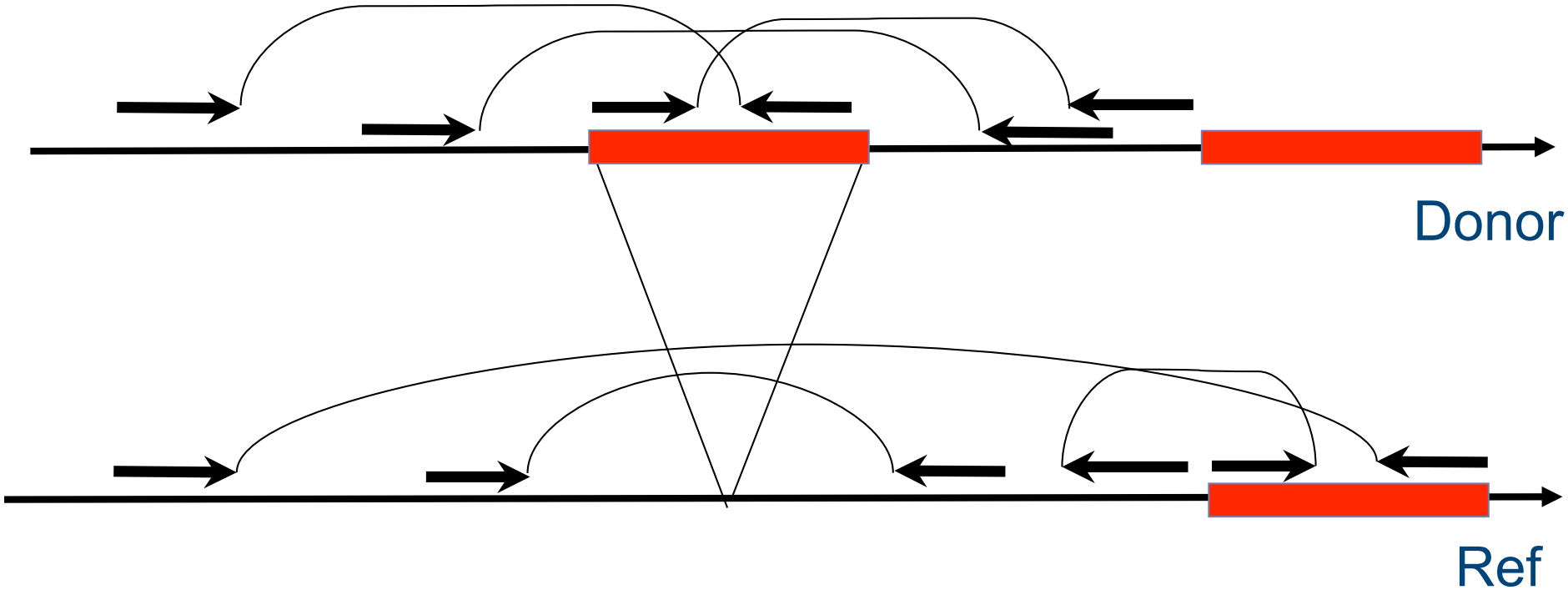
per population is necessary for the characterization of rare disease-associated regions, while knowledge of the baseline number of

Calling CNVs

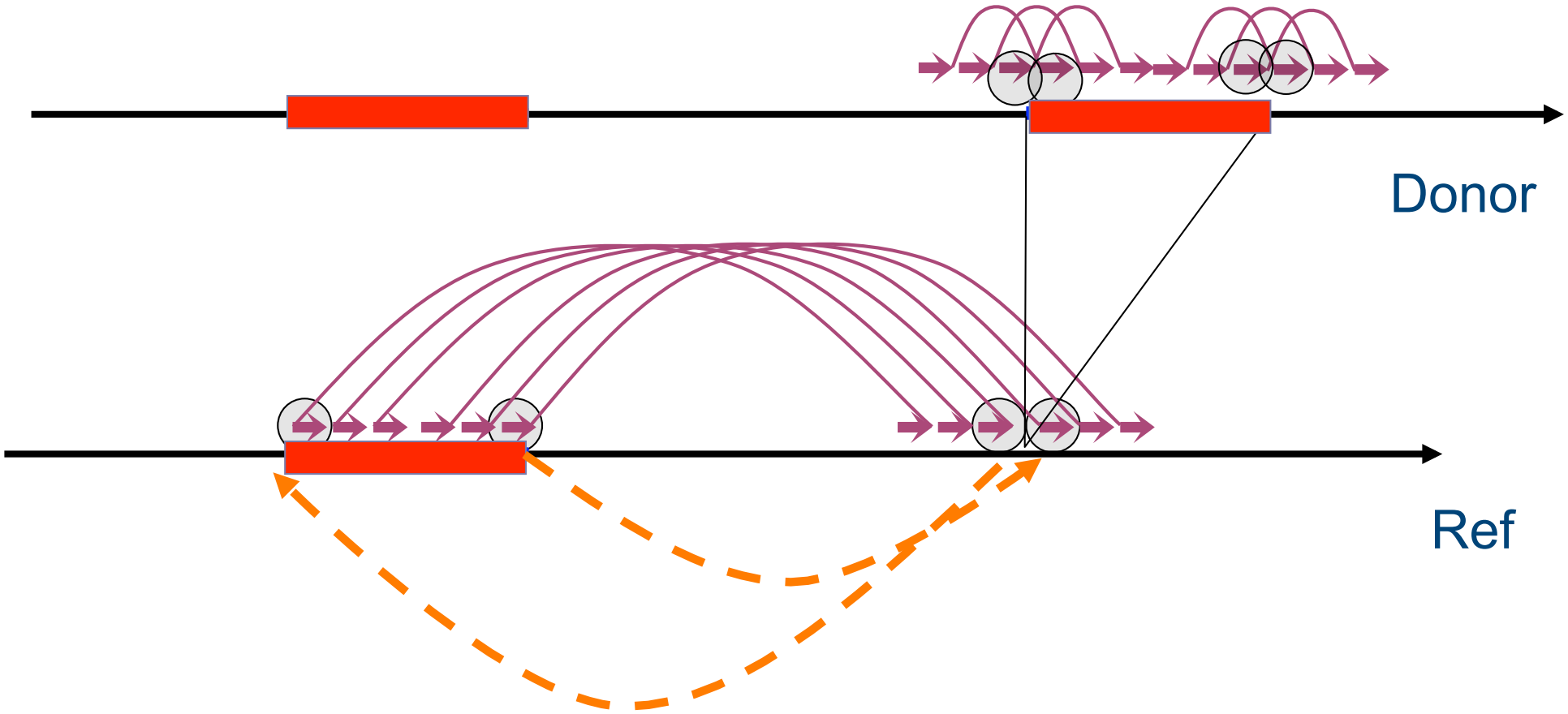


Back to… Structural Variants

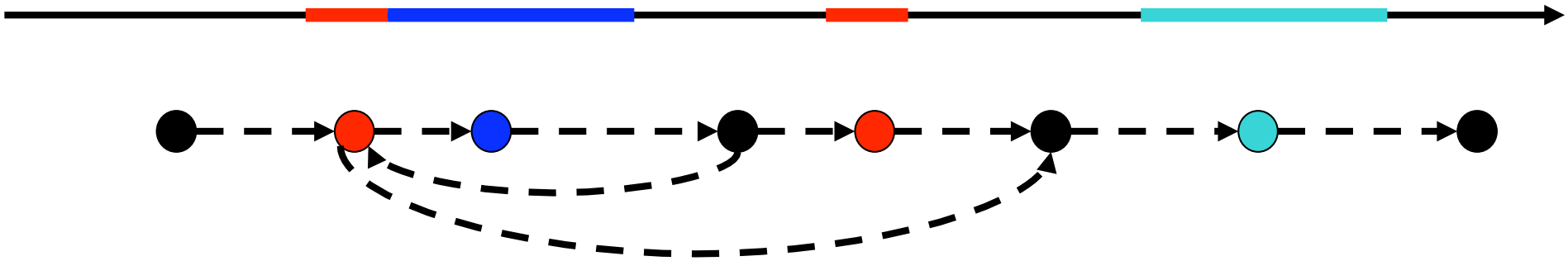
- What if the inserted segment is present elsewhere?



The Linking Signature



Step 1 – Build Repeat Graph



Pevzner, Tang, Tesler (2004)

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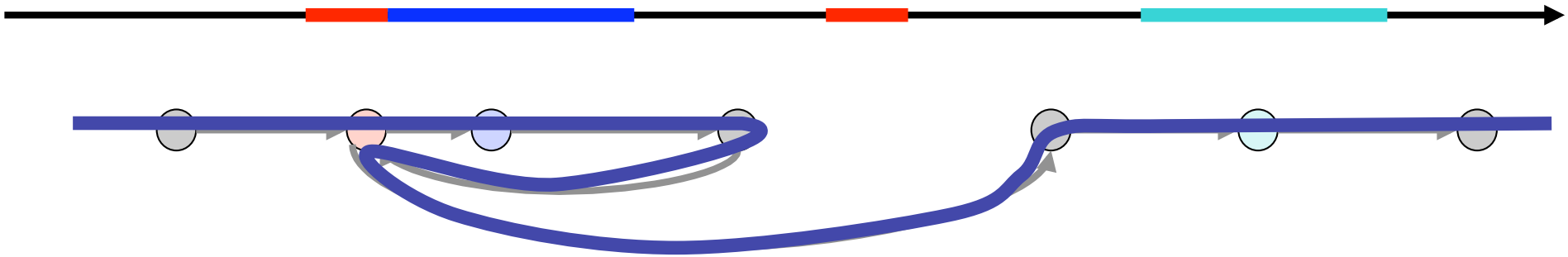
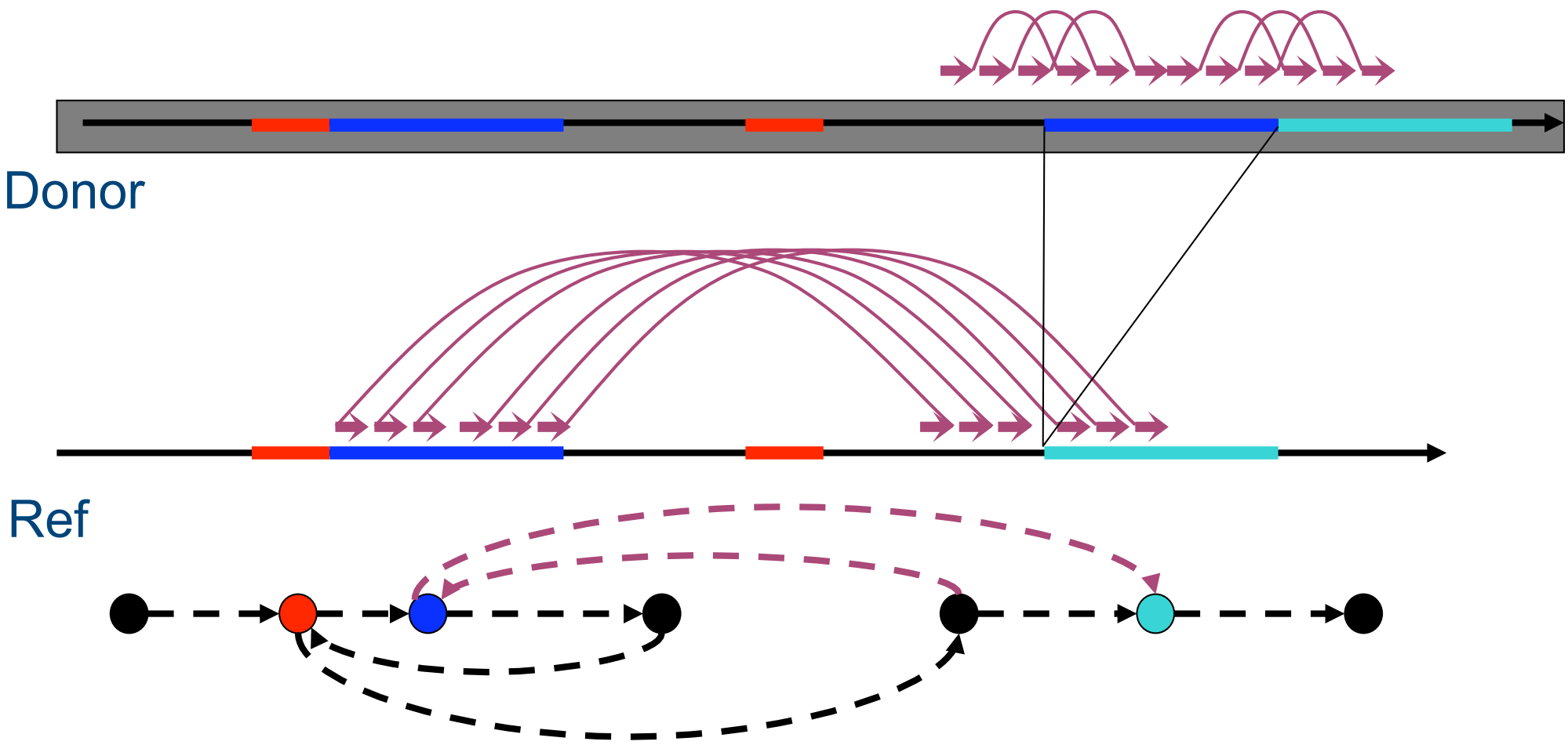


Pevzner, Tang, Tesler (2004)

Step 2 – Capture Donor Adjacencies



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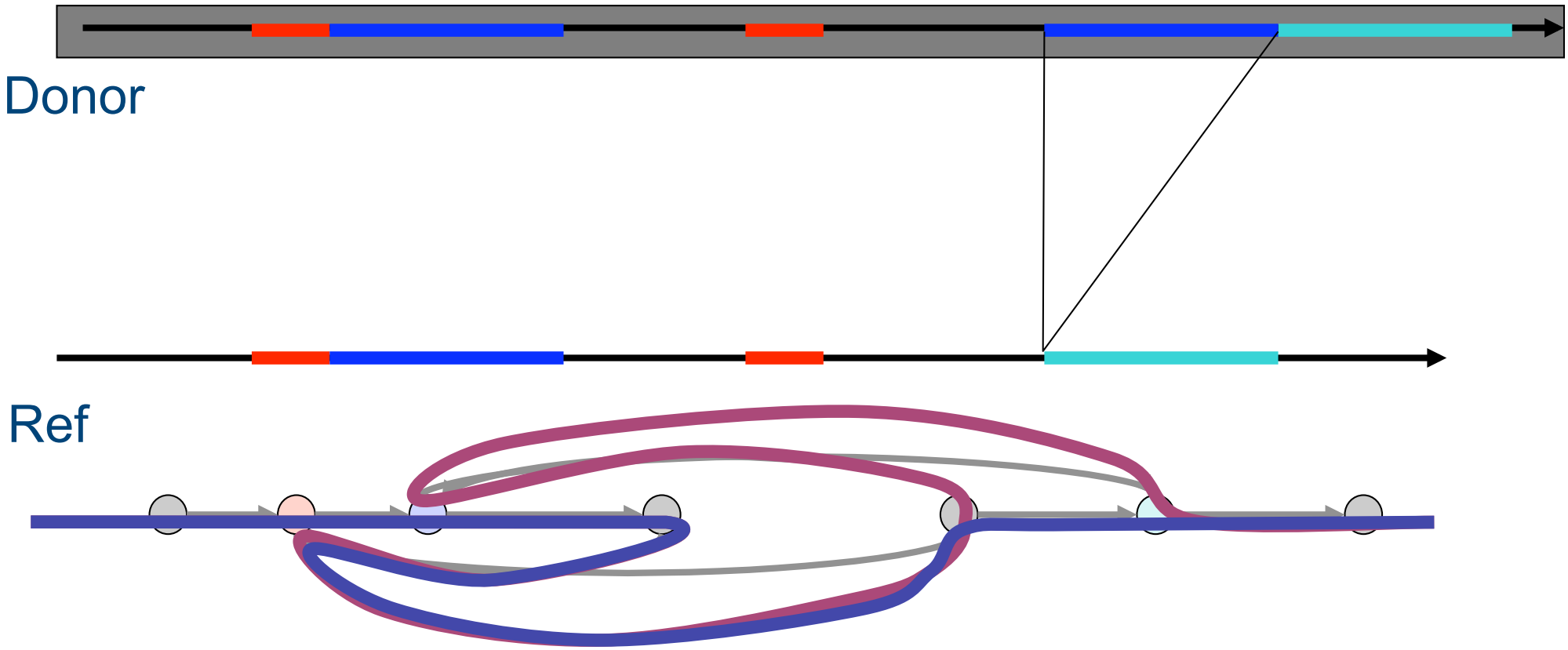
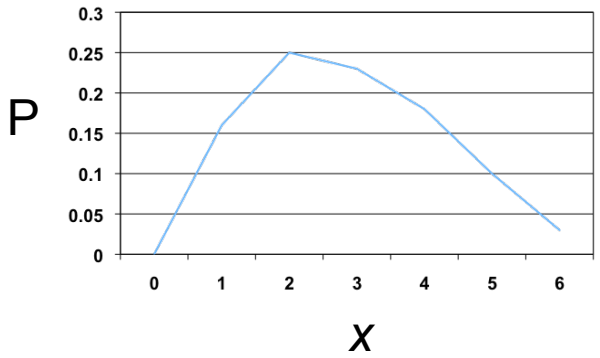
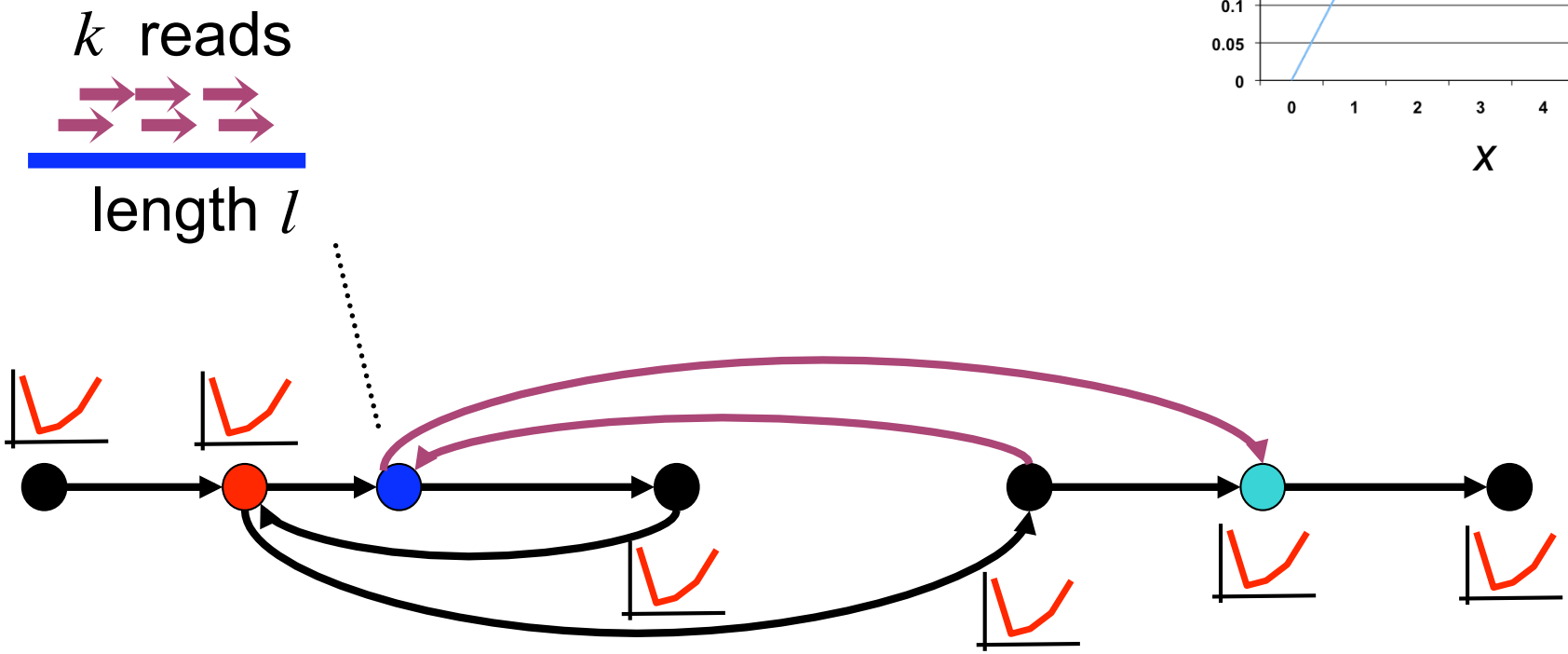


Step 3– Defining Walk Costs

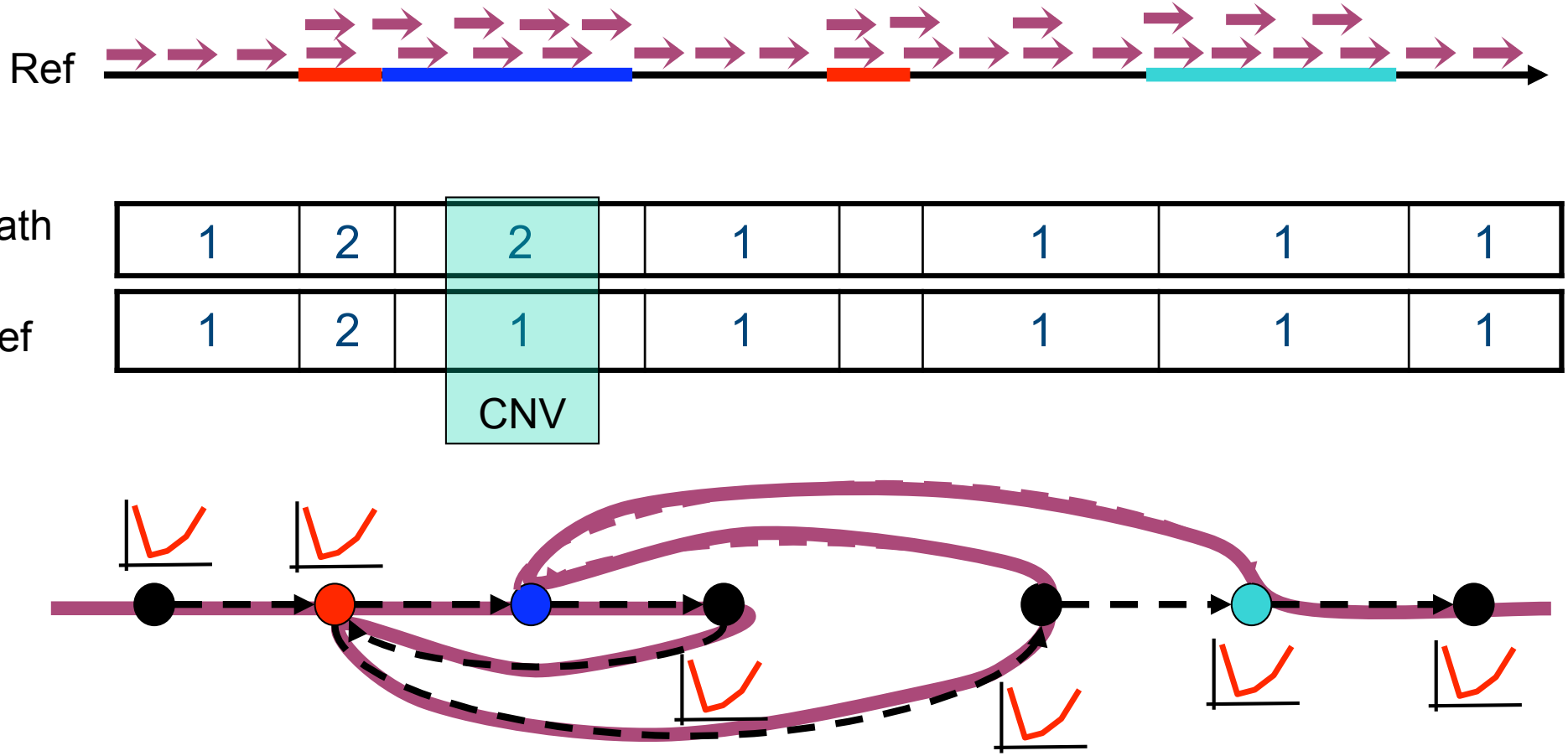
Each function represents the probability that the segment of length *l* appears *x* times in the donor given that there are *k* reads mapped to that segment

$$P(k \mid xl) = \frac{(x\lambda)^k e^{-(x\lambda)}}{k!} \quad \text{where} \quad \lambda = N * l / G$$





Calling CNVs



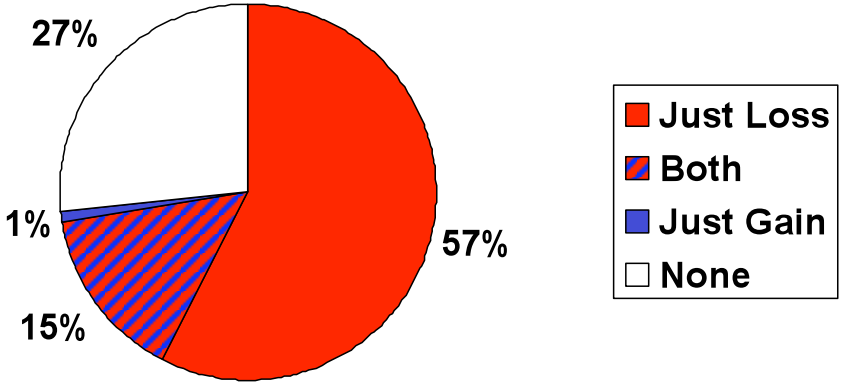
Finds the path “most faithful” to the DOC (Network Flow)

- Probabilistic model to score “faithfulness”

Preliminary Results

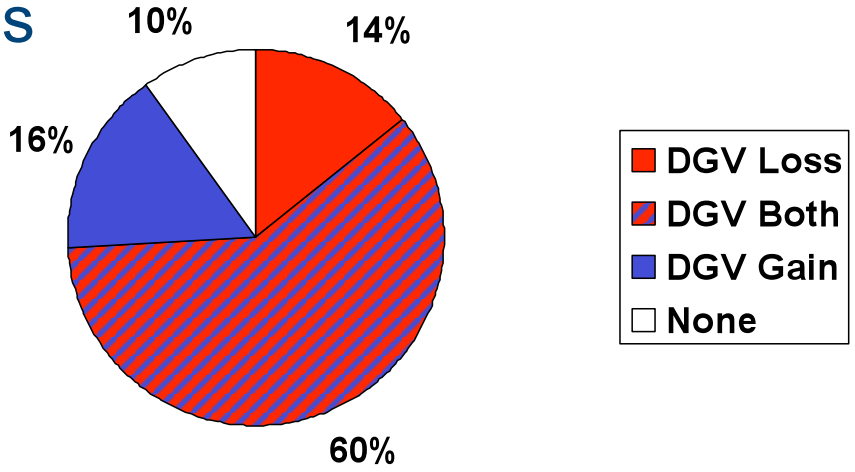
- Total 9909 CNV calls (>1k; 2.5%) – 5795 losses, 4114 gains

Kidd et al's variants detected (out of 146; Sensitivity)

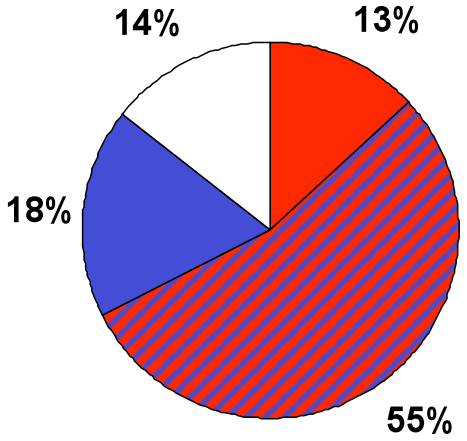


DGV Overlap (Specificity)

Gains



Losses



Take-home points

- MoDIL
  - Take advantage of high clone coverage to find smaller INDELs with high accuracy
  - ~90% accuracy and recall for INDELs ≥ 20bp.
- CNVs
  - Combine pair-end and arrival information to find CNVs
  - Good Concordance with previous results
- Matepairs are key
  - Length & distribution of insert sizes key
  - Read length (sometimes) less so

Acknowledgments

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Can Alkan (UW)
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Marc Fiume
Misko Dzamba
Tim Smith

http://compbio.cs.toronto.edu/modil

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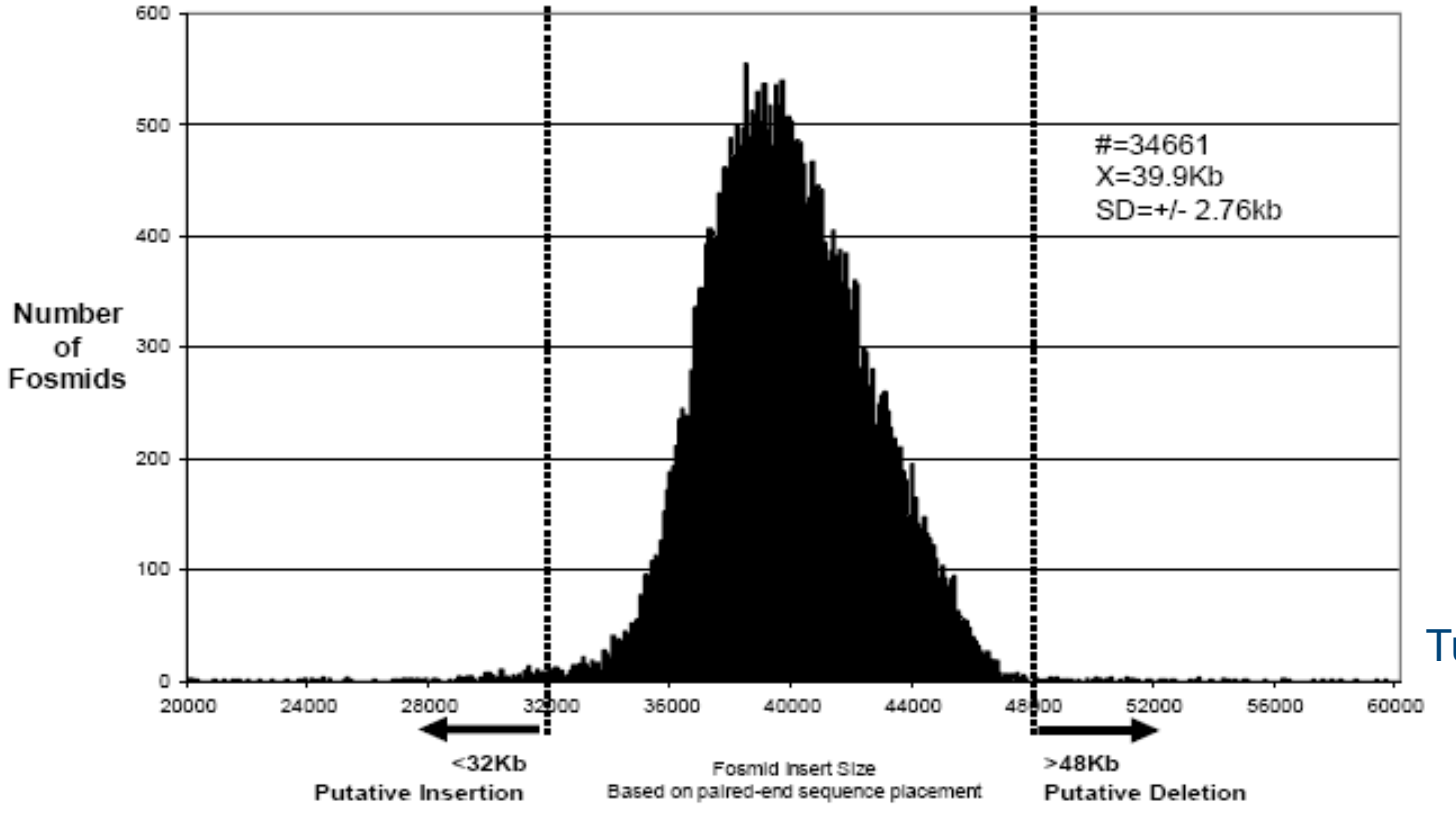
Outline

1. MoDIL: Detecting INDELs with Mixtures of Distributions
   - Haploid case - Detecting INDELs with a distribution
   - Diploid case – Detecting INDELs with mixtures of distributions
   - Results

2. Finding CNVs with Matepairs and Depth-of-coverage

Difficulties in Small INDEL Detection

- In reality, insert sizes of matepairs are not perfect
  - Unable to detect small indels (e.g. < 3STD)



Tuzun et al. 2005

Outline: MoDIL

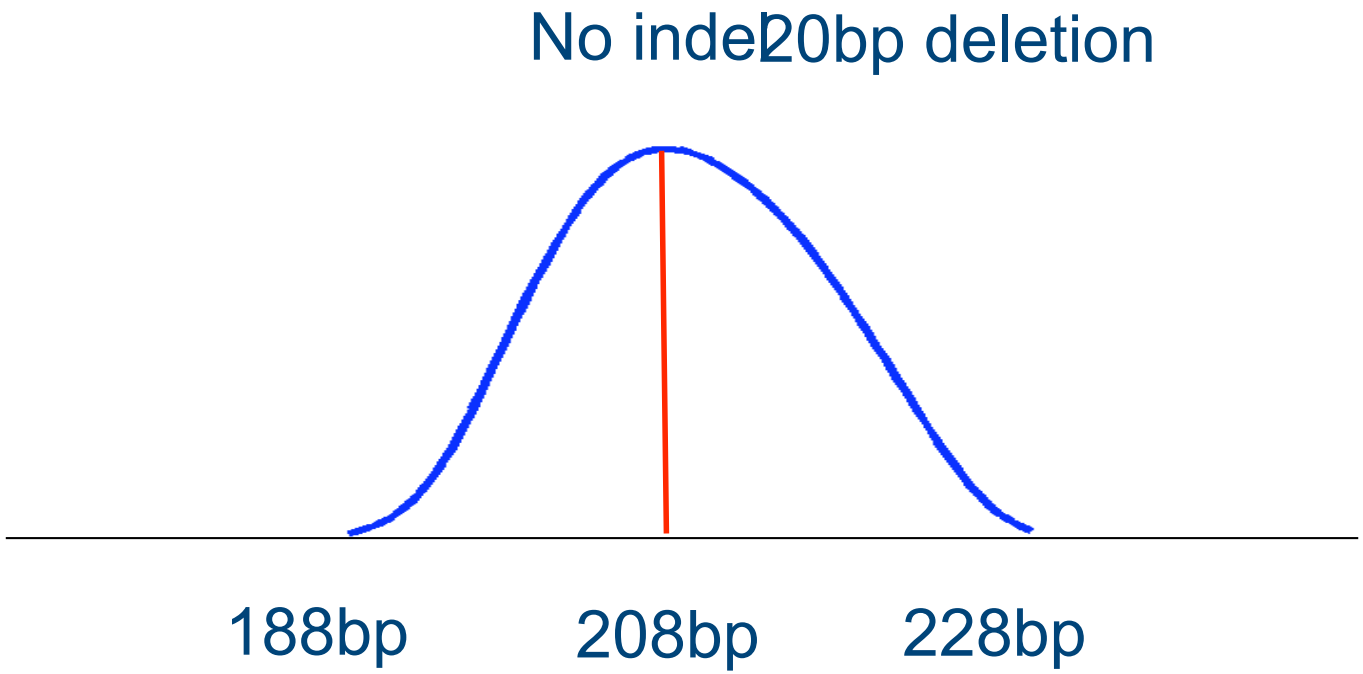
1. Haploid case - Detecting INDELs with a distribution

2. Diploid case – Detecting INDELs with mixtures of distributions

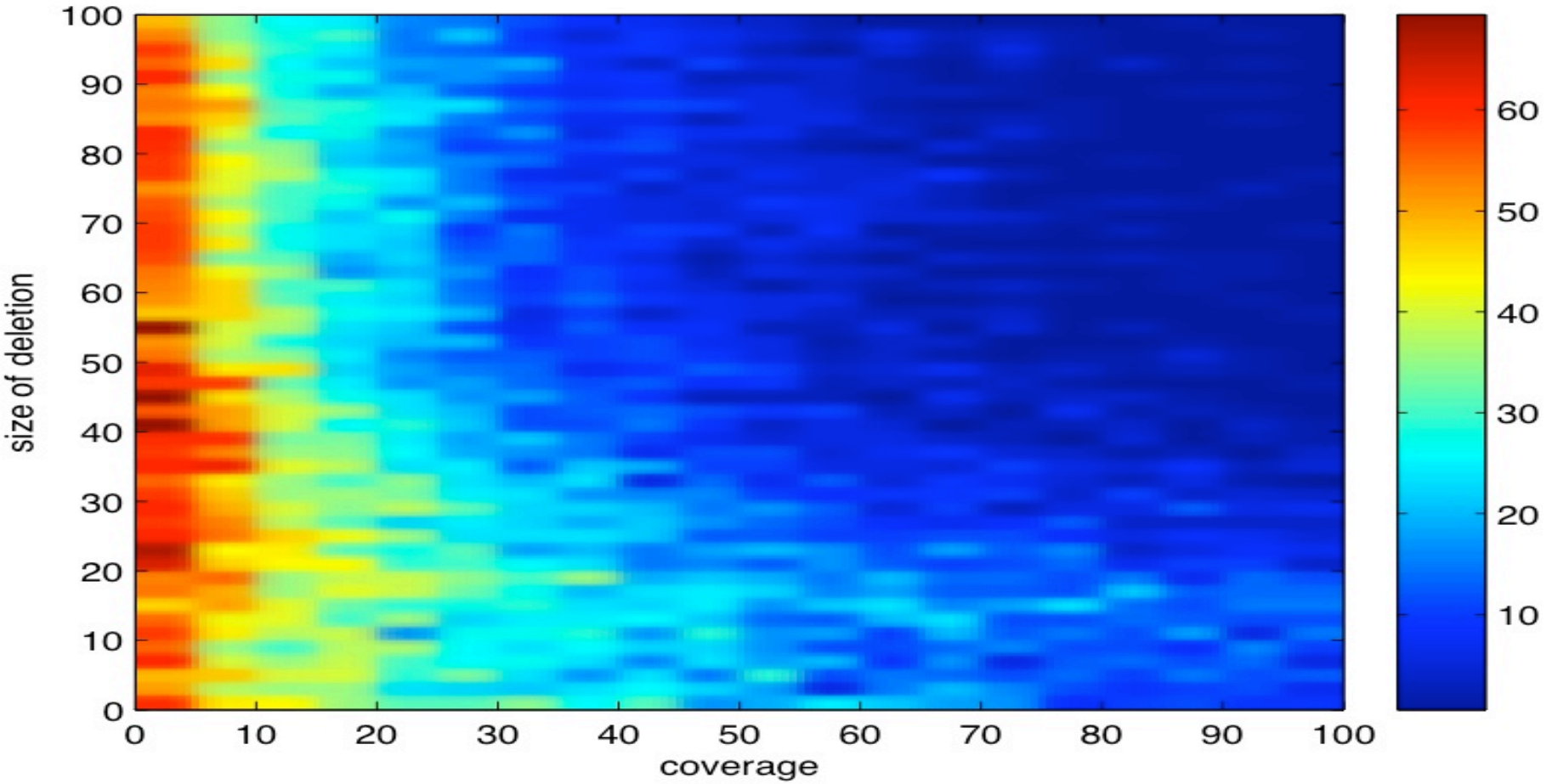
3. Results

Haploid Case – Distribution

Make a distribution of mapped distances in each cluster
=> The distribution shifts from distribution of insert size
if there is an INDEL



EM Algorithm Sensitivity



Percent error (> 5bp off)

Probabilistic Framework

- S_{ACAT} = 2
- S_{GGCA} = 1

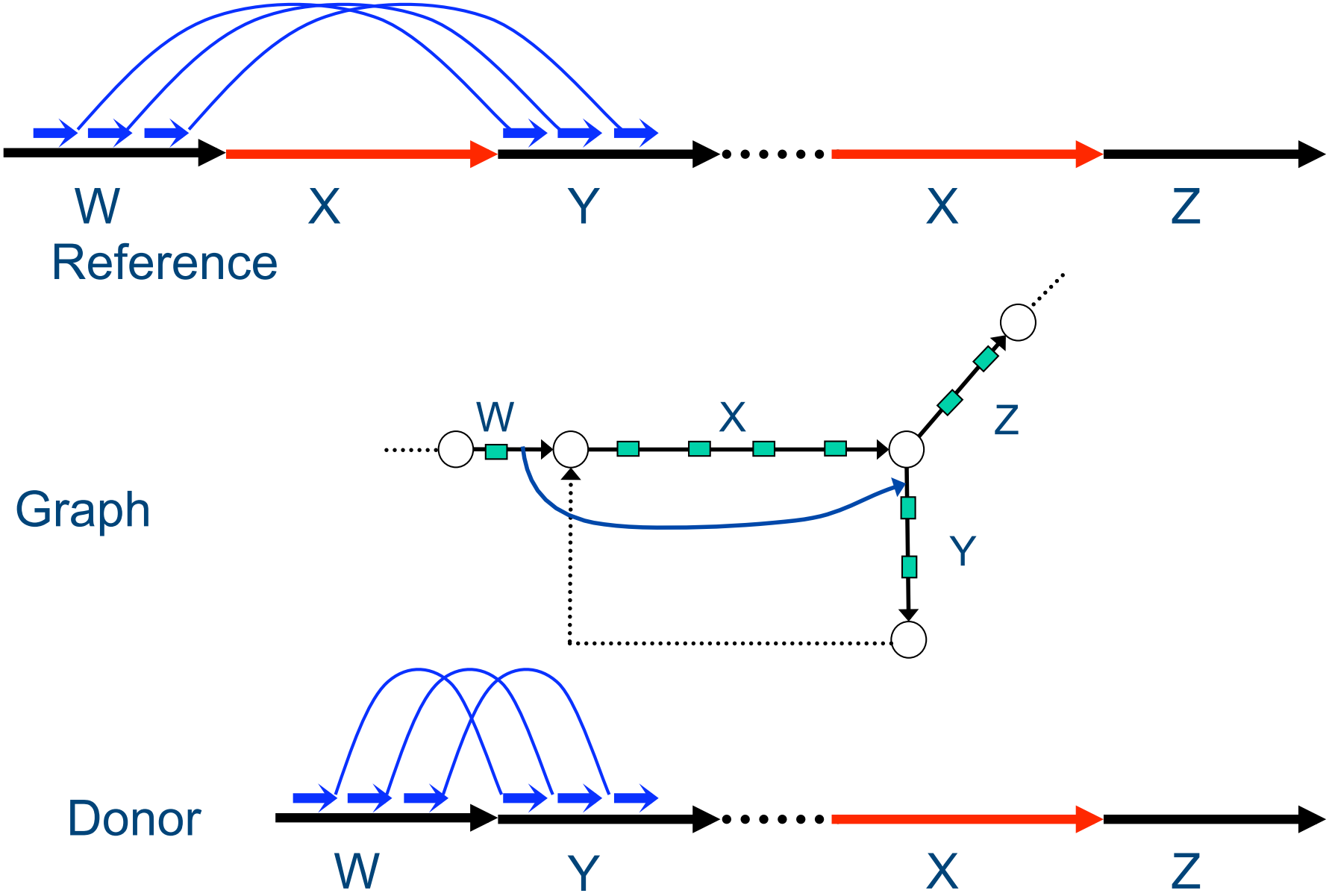
- Example:
  - 50x coverage
  - 25-long reads

- Every 25-long window of the genome is sampled 2 times, on average.

- Let s_i=4
- $g_i = s_i / 2$, so $g_i \approx 2$

ACAT	ACAT	GGCA

Discordant Matepairs



MoDIL: Detecting INDEL Variation with Clone-end Sequencing

Seunghak Lee,Fereydoun Hormozdiari,
Can Alkan, Michael Brudno

This paper is in pressat Nature Methods

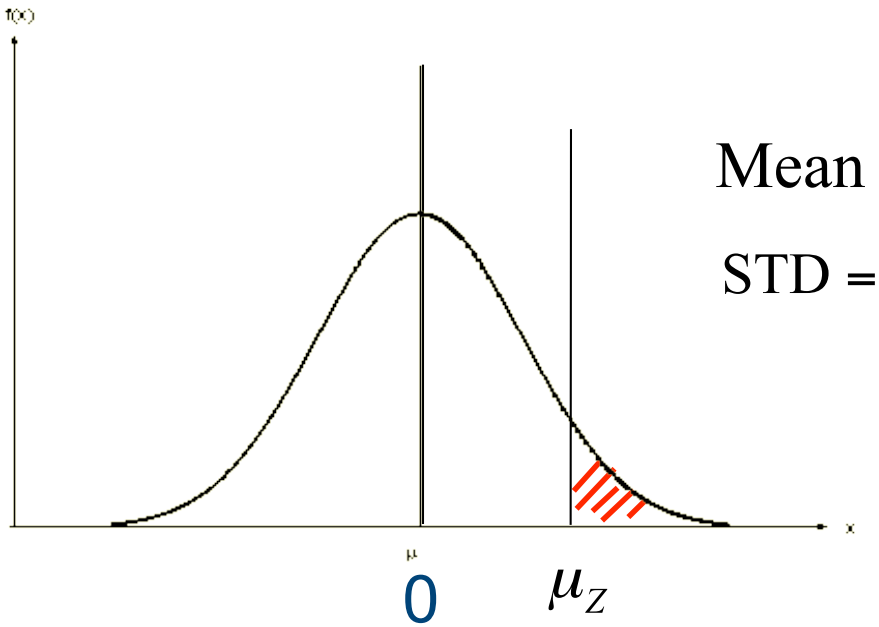
http://compbio.cs.toronto.edu/modil/

P-value (assigning a confidence)

P-value

Probability that a cluster is generated from a region without an indel

$$\text{P - value} = \sum_{\mu_Z}^{\infty} p(Z' = z \mid 0)$$



P-heterozygosity

P-heterozygosity

posterior probability that an indel is hetozygous

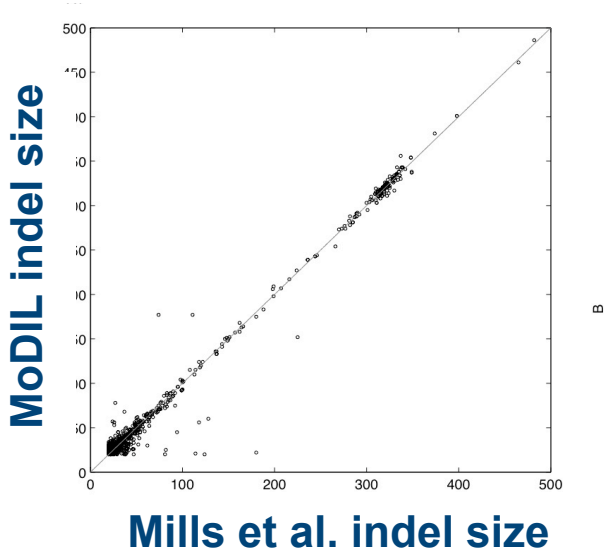
$$\mathrm{P-het} = P(hetero \mid X_1, \ldots X_N) = \frac{P(X_1, \ldots X_N \mid hetero)}{P(X_1, \ldots X_N \mid herero) + P(X_1, \ldots X_N \mid \mathrm{hom})}$$

$$P(X_1, \ldots X_N \mid hetero) = \prod_{i=1}^{N} P(X_i \mid hetero)$$

$$= \prod_{i=1}^{N} \left\{ 0.5P(X_i \mid \mu) + 0.5P(X_i \mid 0) \right\}$$

Accuracy of Size Estimation of MoDIL

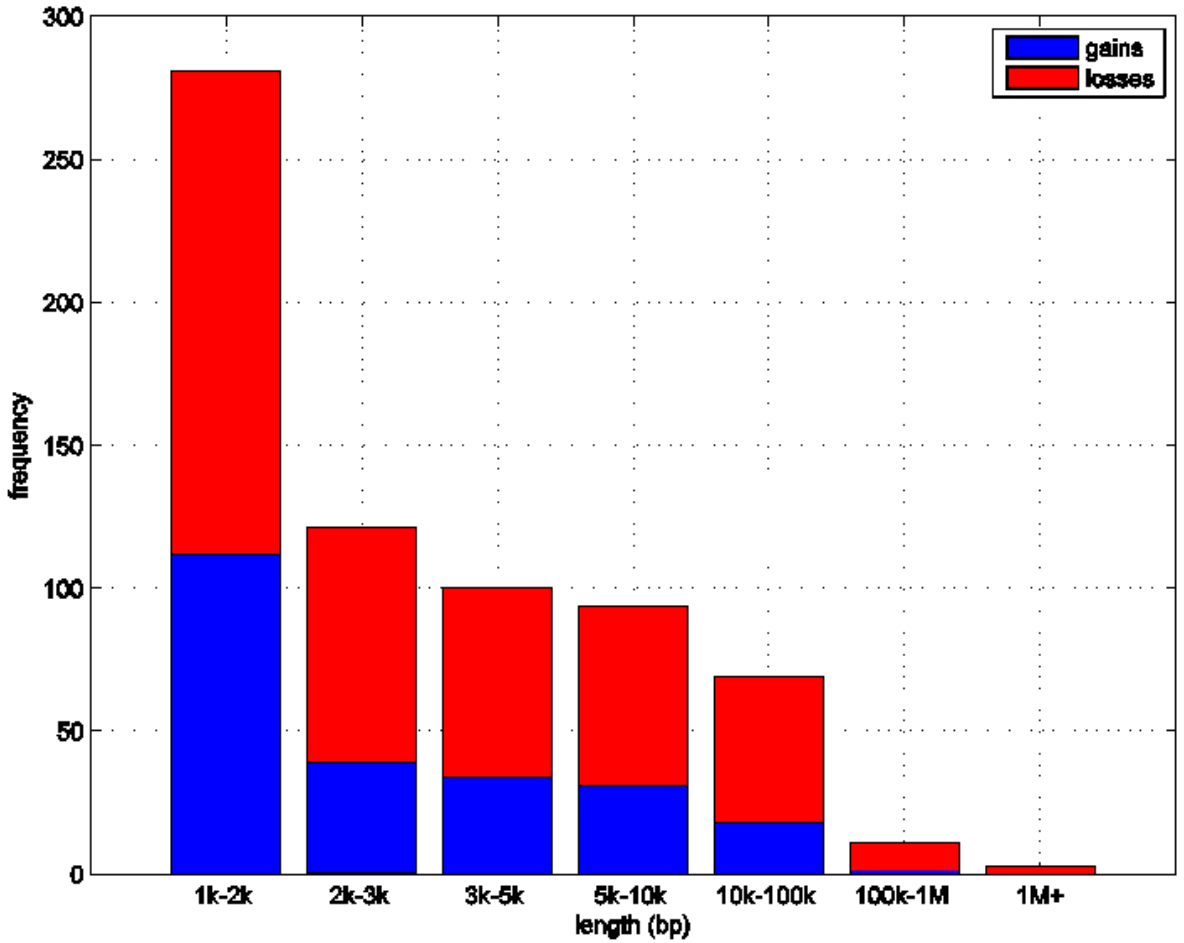
- Large # of indels (~32%) overlapped with Mills et al. results (>=20bp)
- Compared sizes of Mills et al. and MoDIL
  - Pearson's correlation coefficient, r^2=0.96
  - (Mills et al. minus MoDIL) overlaps with Gaussian with STD=4 (expected STD for a cluster with 20 matepairs)



Preliminary Results

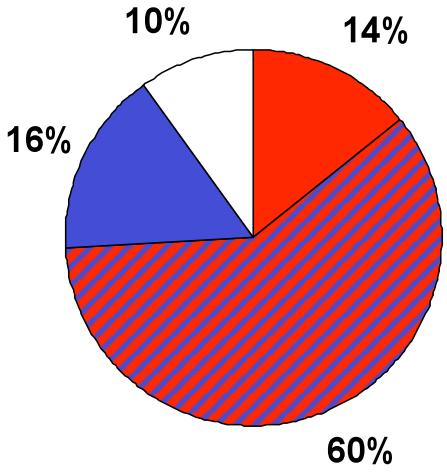
- NA18507 individual sampled with Illumina

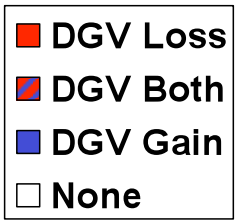
- Total of 9909 CNV calls
- 5795 losses, 4114 gains



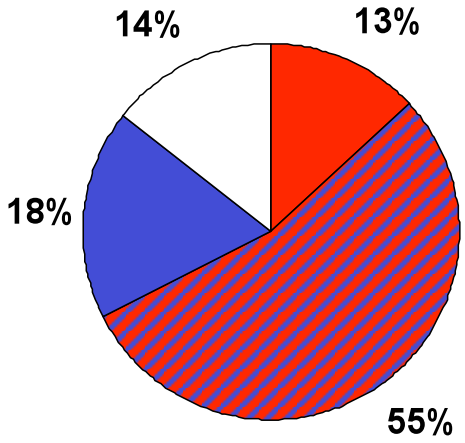
Preliminary Results (Specificity)

Percent of our GAIN calls that overlap with DGV:

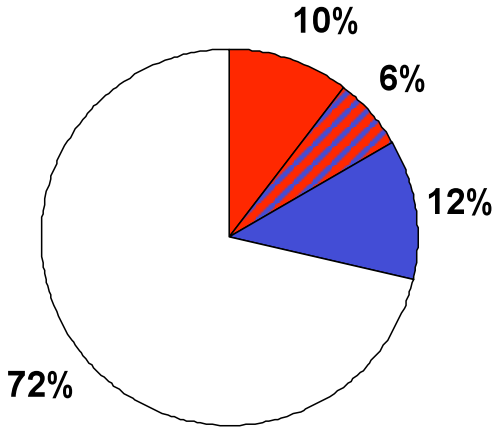


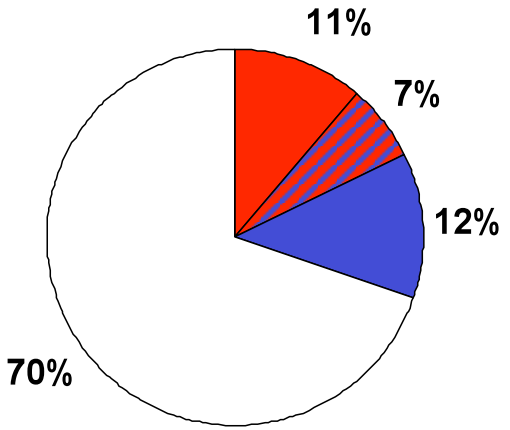


Percent of our LOSS calls that overlap with DGV:



After shuffle:





Diversity of Humans

- Humans are diverse
  - Genomic Variation

- Single Nucleotide Polymorphisms
  - SNPs occur ~1/1000 positions
  - Find by comparing reads from one individual to the reference human genome

```
G: 798   GAACCCCTTACAACTGAACCCCTTAC
         |||||||||| |||||||||||||||
R:       GAACCCCTTATAACTGAACCCCTTAC
```

- Structural variations are large scale genomic alterations
  - Insertions, deletions, inversions, translocations and changes in copy numbers