The unrealized potential of genome skimming for sample identification

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Joint work with Shahab Sarmashghi, Metin Balaban, Nora Rachtman, Kristine Bohmann, Vineet Bafna, Tom Gilbert

Identifying a biological sample is essential to many applications: monitoring biodiversity and conservation to tracing food provenance

Sample identification is not easy!

The New York Times

Survey Finds That Fish Are Often Not What Label Says

SEATTLE — Many Europeans are fretting these days over horse meat, and whether it might have adulterated their shepherd's pie. Over here, it's all about the red snapper.

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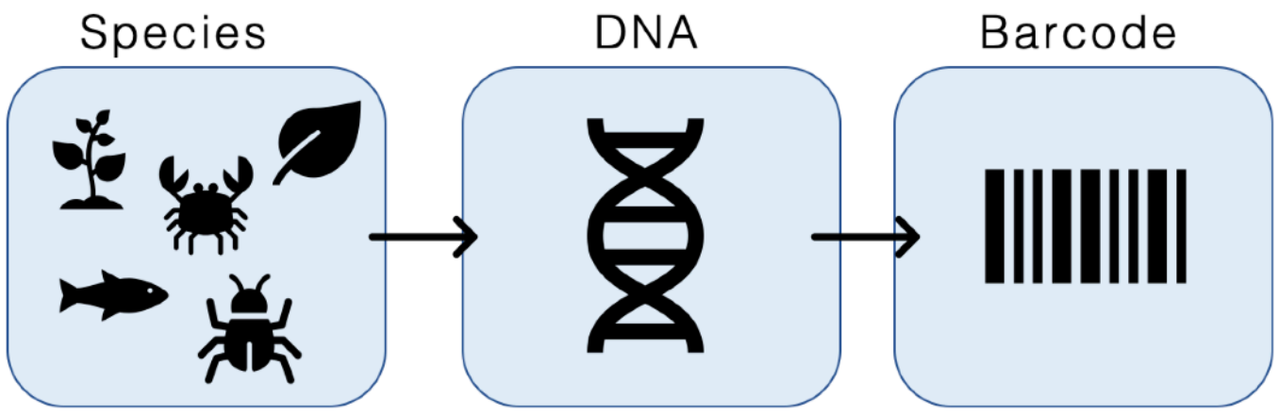
NEWS · 06 MAY 2019 · UPDATE 06 MAY 2019

Humans are driving one million species to extinction

Landmark United Nations-backed report finds that agriculture is one of the biggest threats to Earth's ecosystems.

Existing DNA barcoding

- Represent each species by its DNA sequence, like a barcode. Compare a query to a library of known barcodes (e.g., BOLD) to find matches
  - Uses the cheap PCR amplification of marker genes (e.g., COI)
  - Supposed to be varied enough to distinguish species



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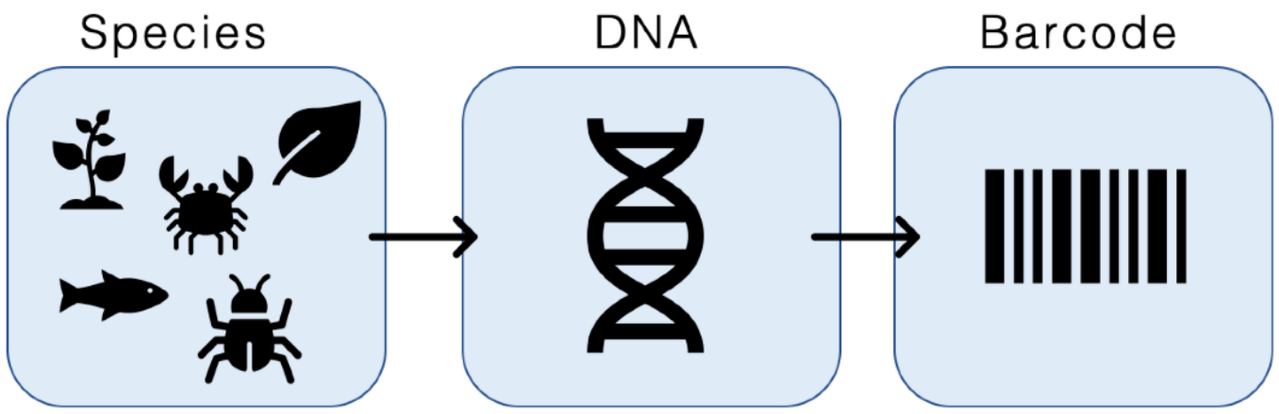
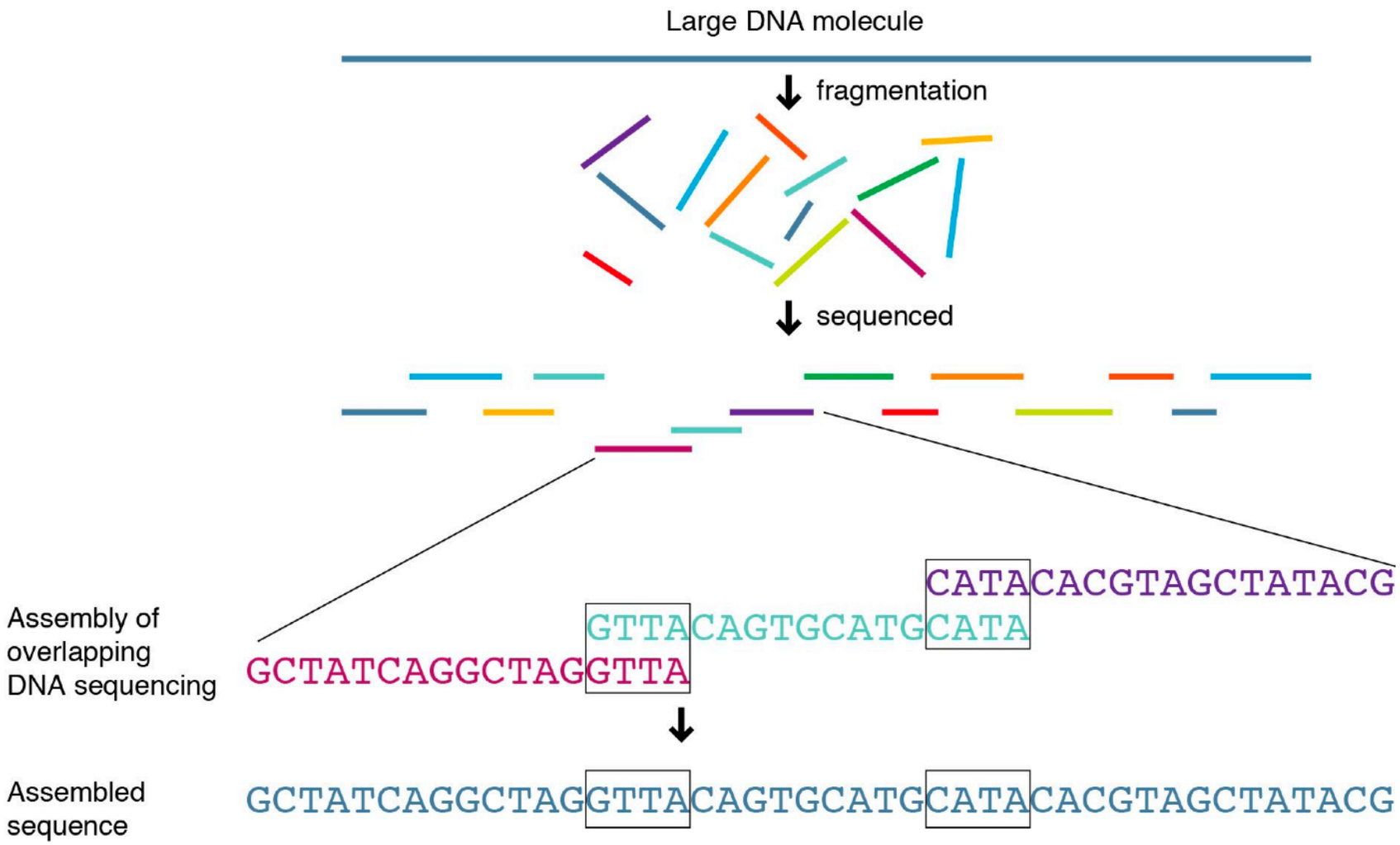
  - Supposed to be varied enough to distinguish species
- Barcodes are short, so have limited resolution
  - Often fail to distinguish species of the same genus

Hebert *et al.*, 2003, Proc. Royal Soc. B

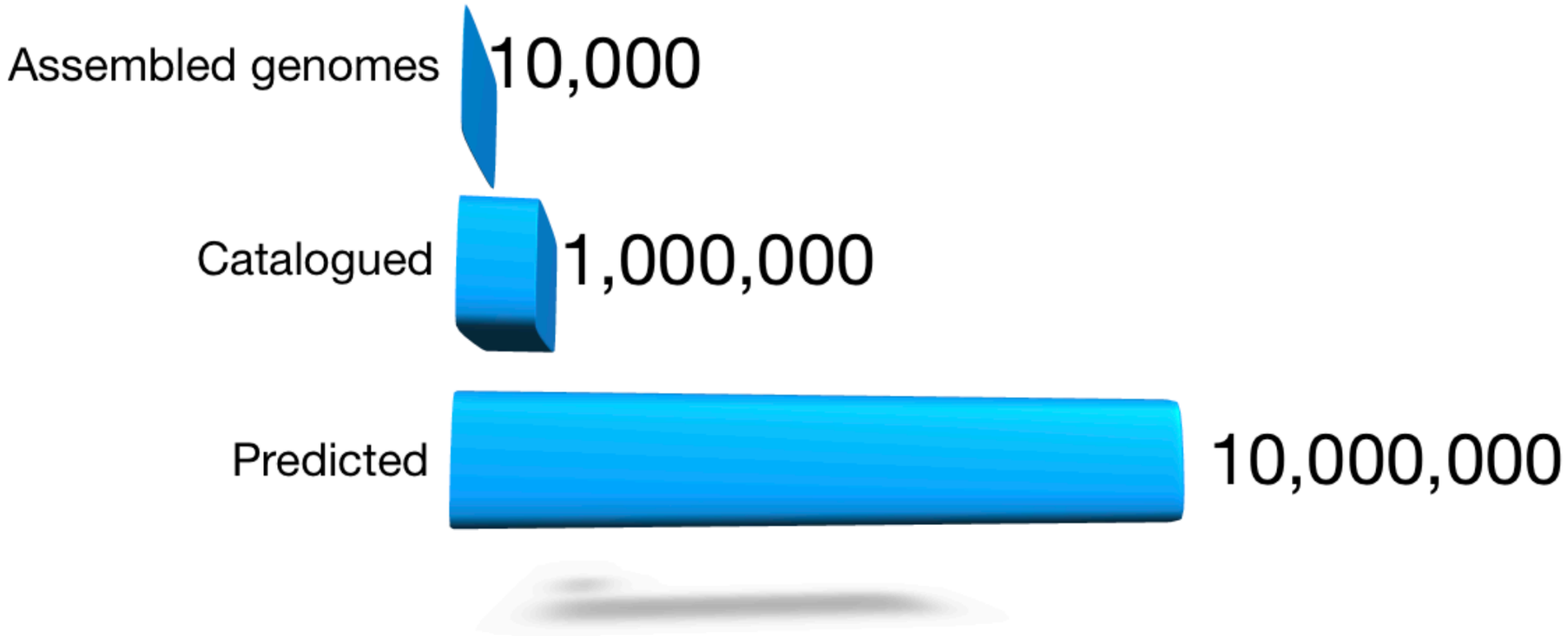


How about sequencing the whole genomes? Isn't that cheap these days?



Assembly is challenging and costly: it requires a high coverage of genome (>30X)

- Eukaryotic organisms



Mora *et al.*, 2011, Plos Biology

Genome skimming: low-coverage NGS

Quicke *et al.*, 2012, Mol. Eco. Resources

Coissac *et al.*, 2016, Molecular Ecology

Genome skimming: low-coverage NGS

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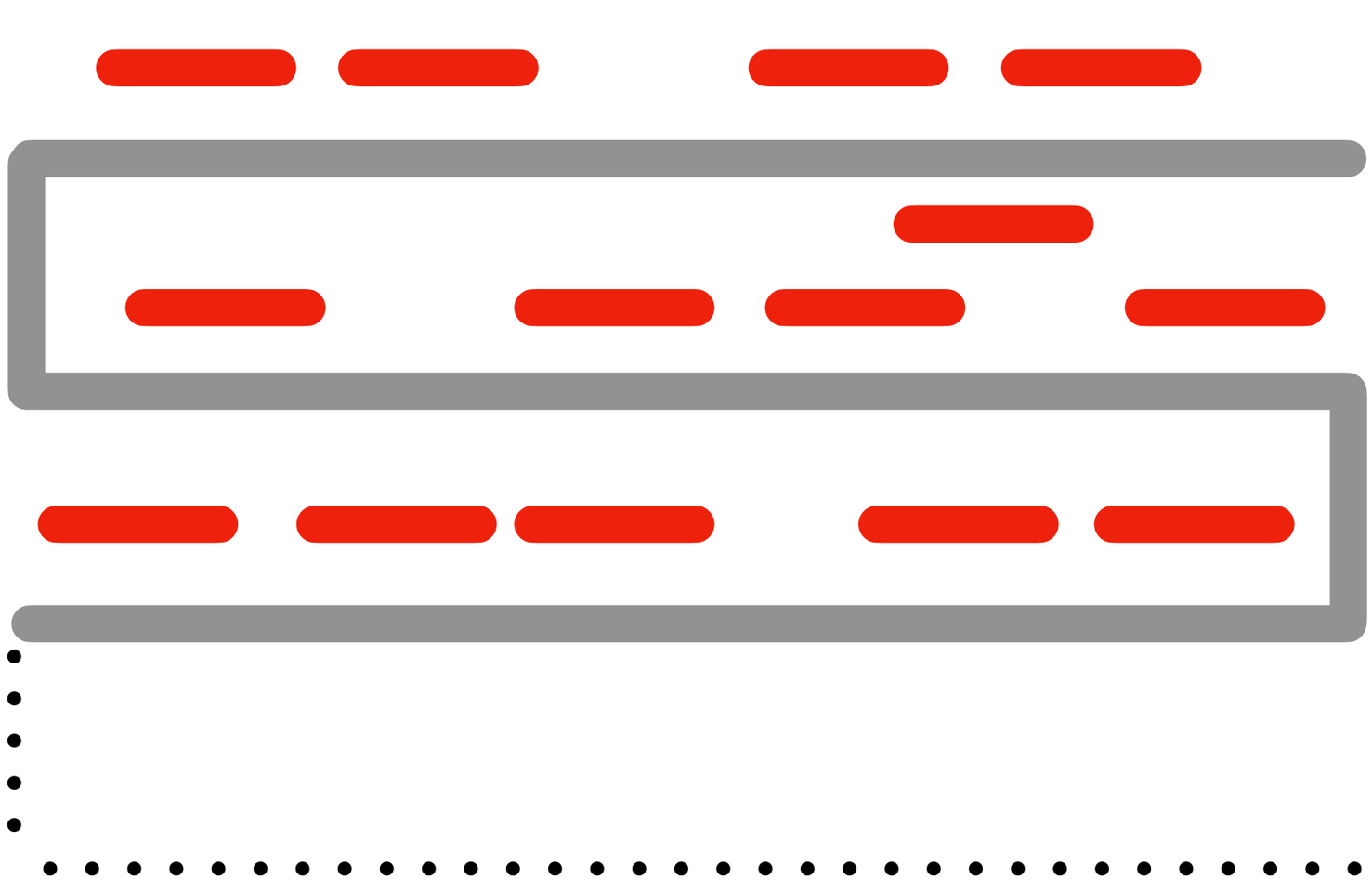
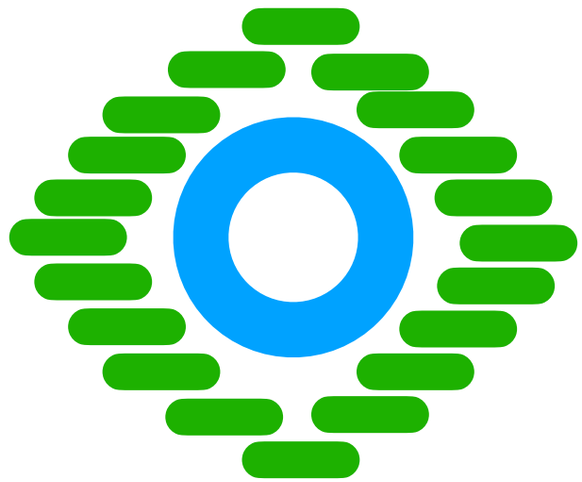
Coissac *et al.*, 2016, Molecular Ecology

Can we use all reads in a genome skim?

Nuclear genome

Organelle genome

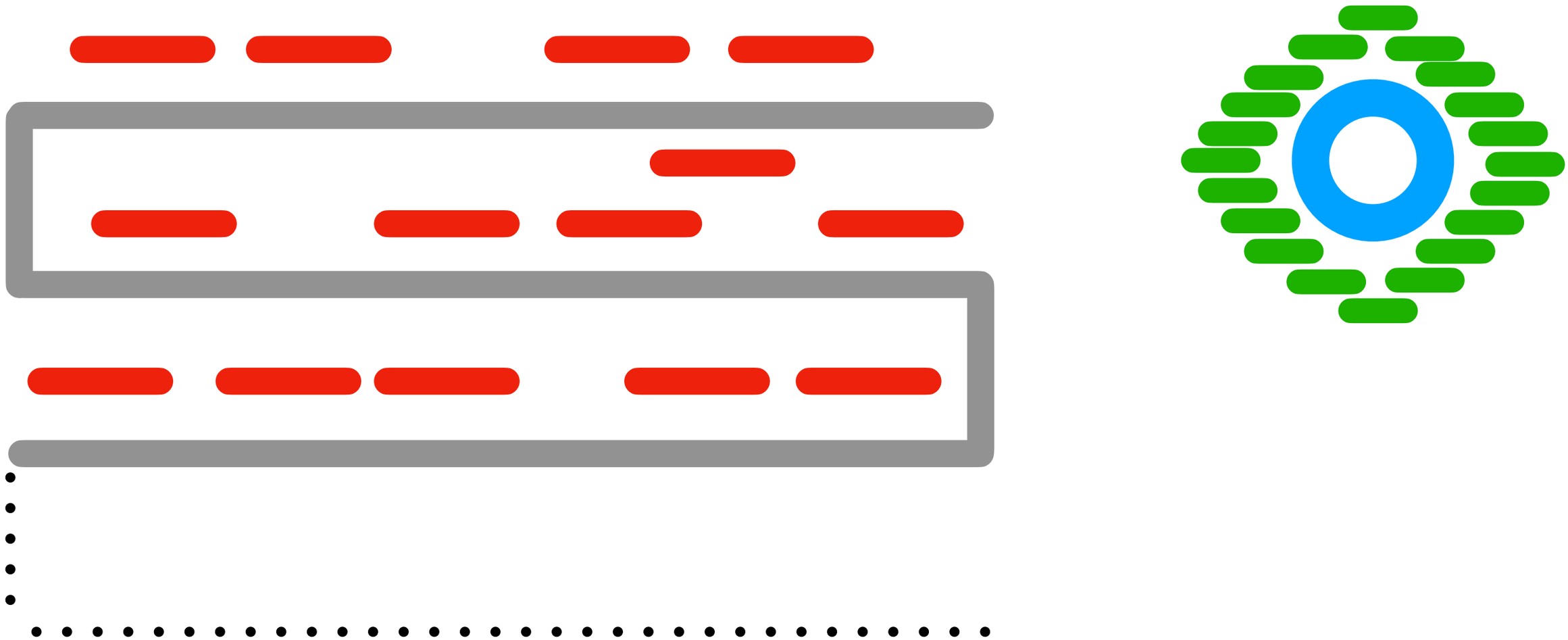




Can we use all reads in a genome skim?

Low read depth → Assembly is not possible

e genome

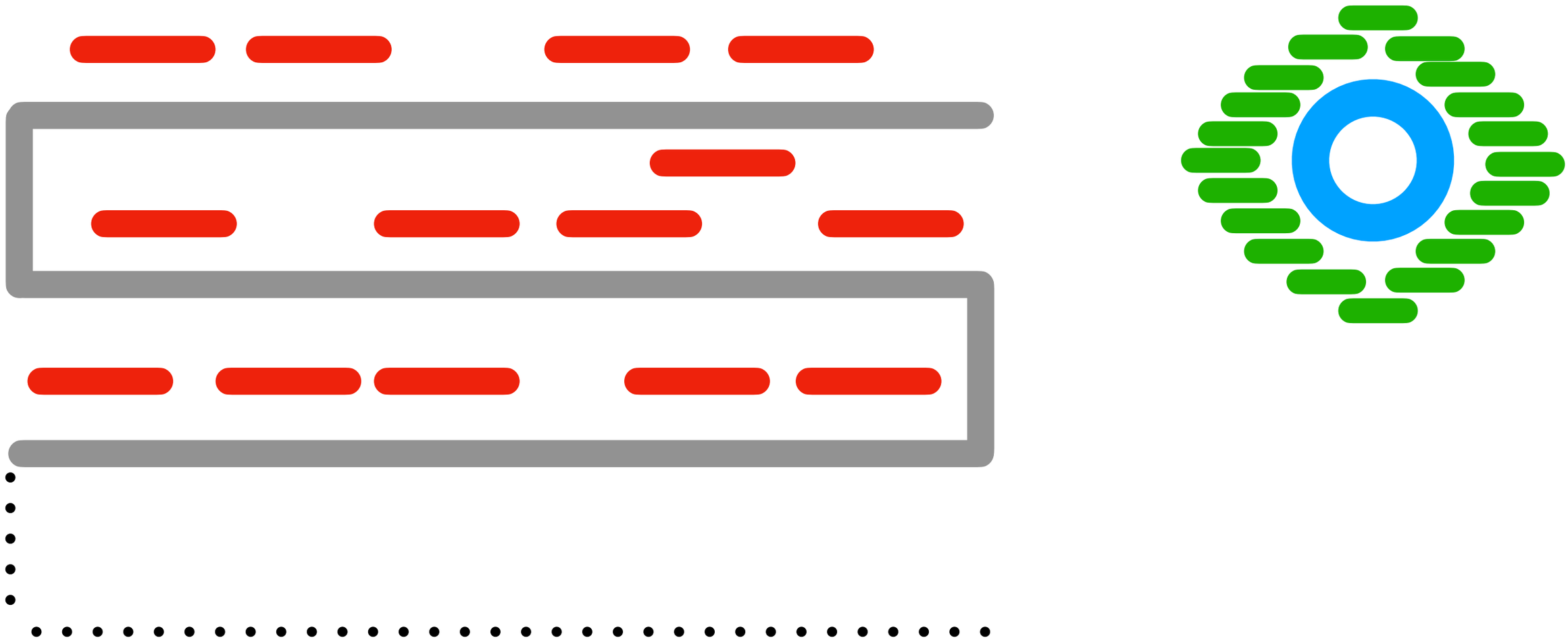


Can we use all reads in a

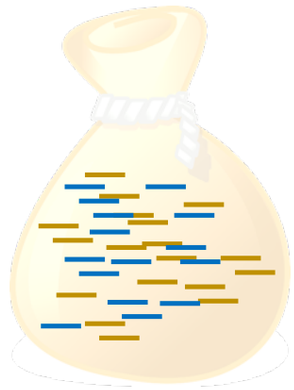
No reference → Alignment is not easy

Low read depth → Assembly is not possible

e genome



What if we barcode species as bags of reads?



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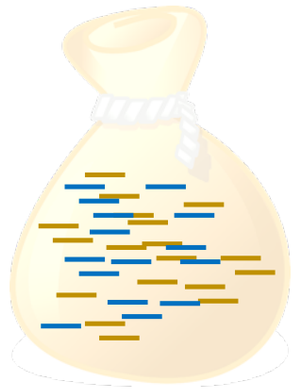
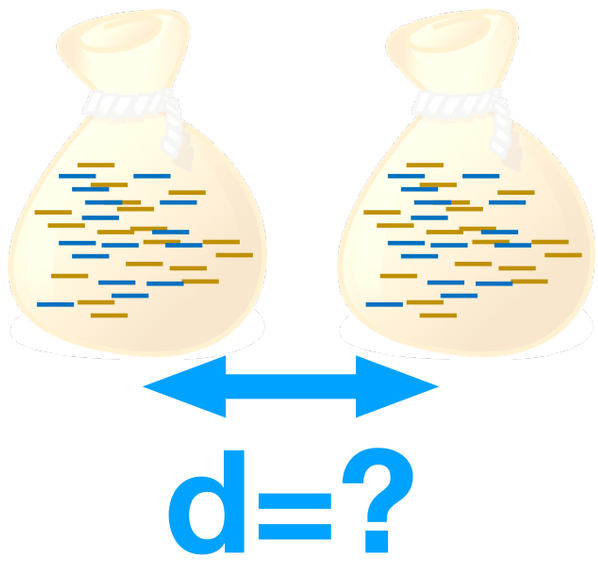


We need to quantify the distance between two genomes skims from these bags of reads



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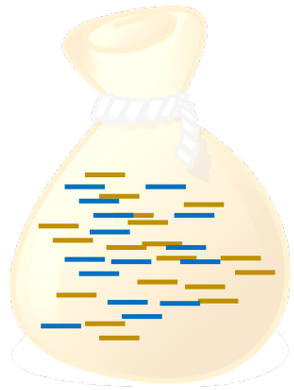

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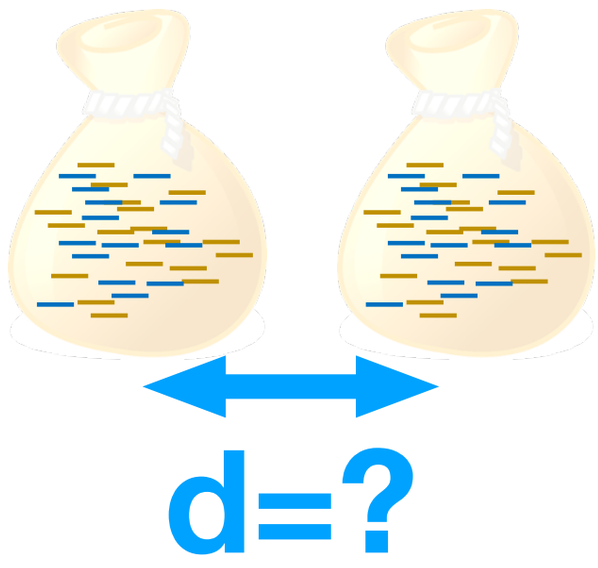
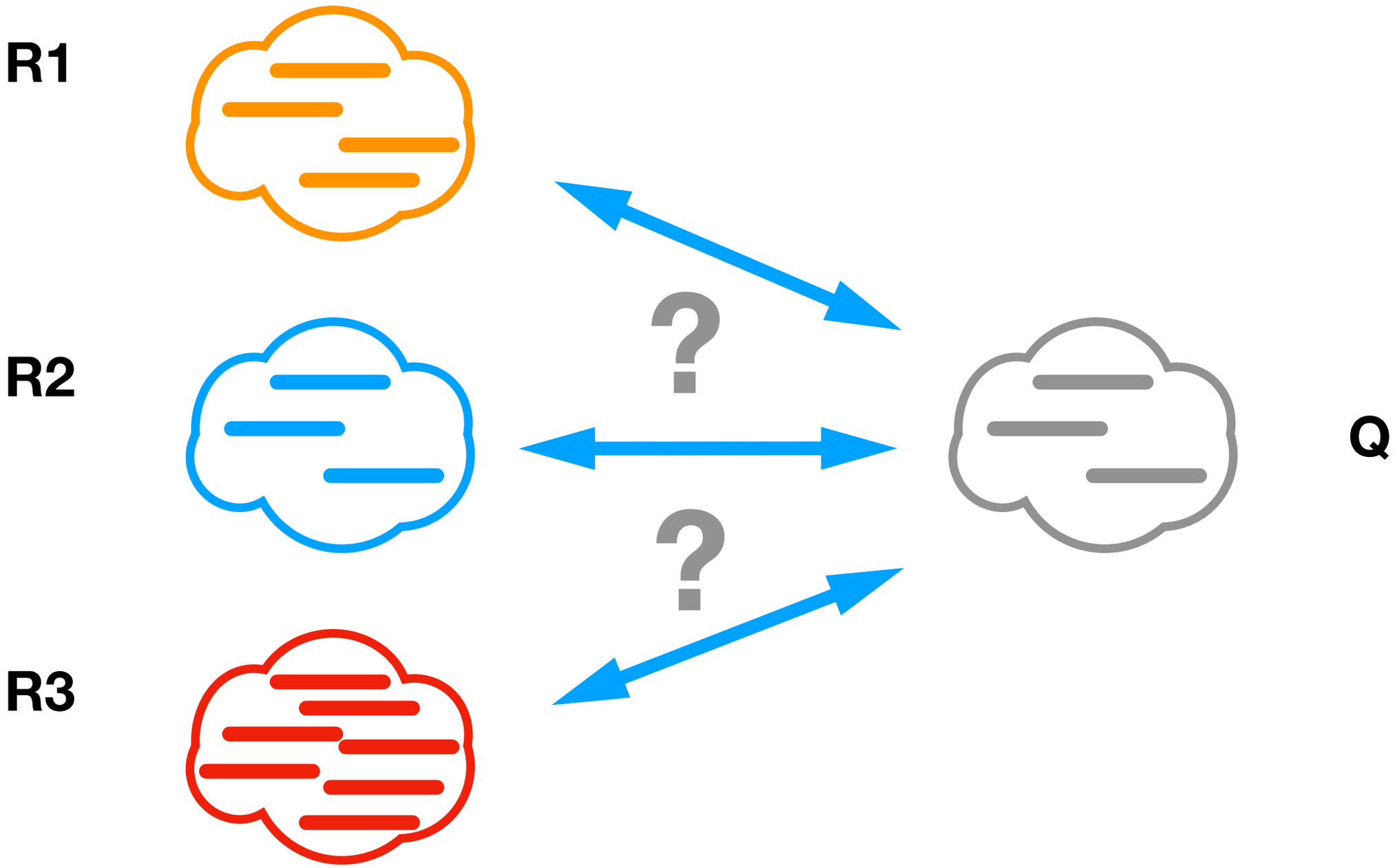


Can we do this when the bags of reads provides a low coverage of the genome (e.g., 1X)?

Compare to references



Genomic nucleotide distance

- Distance is defined as the fraction of sites that would not match if we had perfectly correct alignments of the fully assembled genomes (i.e., hamming distance)

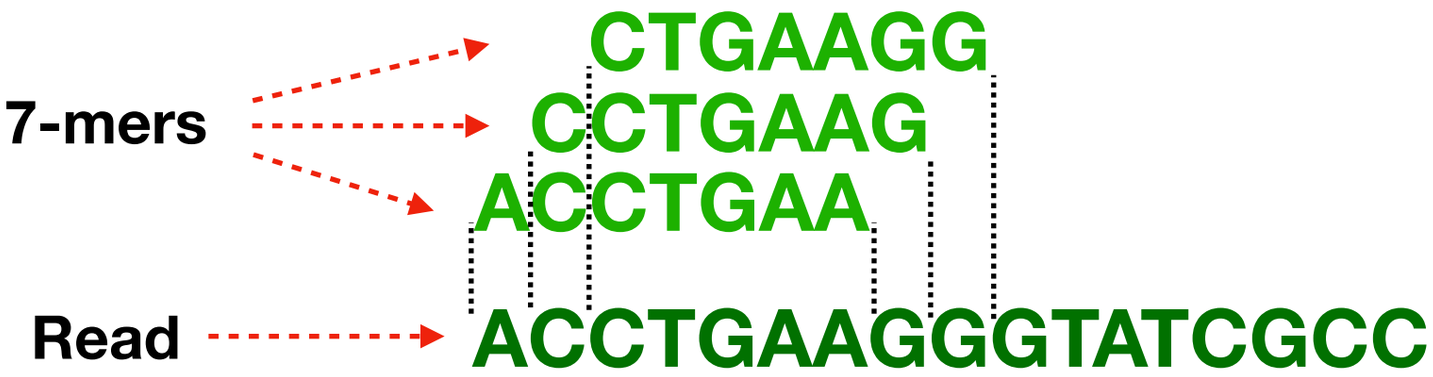
Genome1 …ACCTGAAGGGTATCGCC…

Genome2 …ACCTGAGGGGTATAGCC…

d=2/16

k-mer representation

- Reads are decomposed into consecutive k-mers



Alignment-free comparison of reads

- Sets of k-mers for two genome-skims are compared

Genome skim 1

CTGAA
CCTGA
ACCTG

ACCTGAAGGGTATC

TCGCC
ATCGC
TATCG

TATCGCCAAAAGCG

Genome skim 2

CTGAA
CCTGA
ACCTG

ACCTGAAGGGTATC

TCGCC
ATCGC
TATCG

TATCGCCAAAAGCG

Alignment-free comparison of reads

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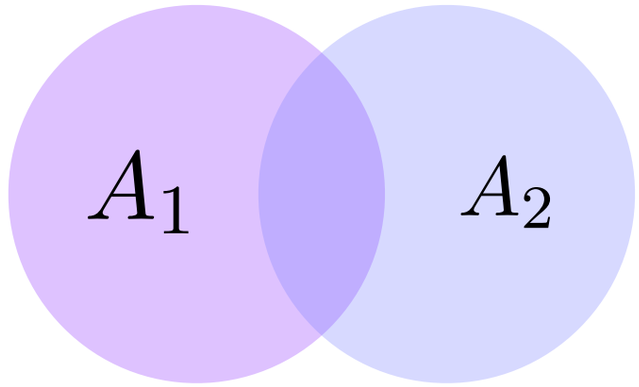
Jaccard index

- Using Jaccard index to compute distances
- Measure of similarity between k-mer sets

$$J = \frac{|A_1 \cap A_2|}{|A_1 \cup A_2|}$$



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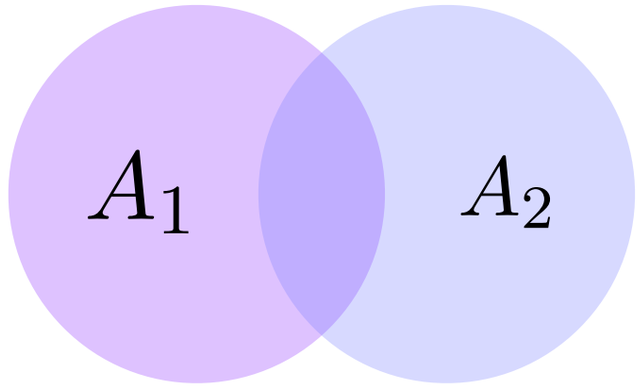


- Can be efficiently computed with a MinHash technique (Mash)

Ondov *et al.*, 2016, Genome Biology

Efficient Jaccard estimation

- Mash computes the Jaccard index efficiently via MinHash

Input size	Running time
100Mb	1min
500Mb	2min
1Gb	3min

Ondov *et al.*, 2016, Genome Biology

Jaccard to distance

- Distance (D) can be estimated from the Jaccard (J) using a simple Poisson model

- W = The number of shared k-mers

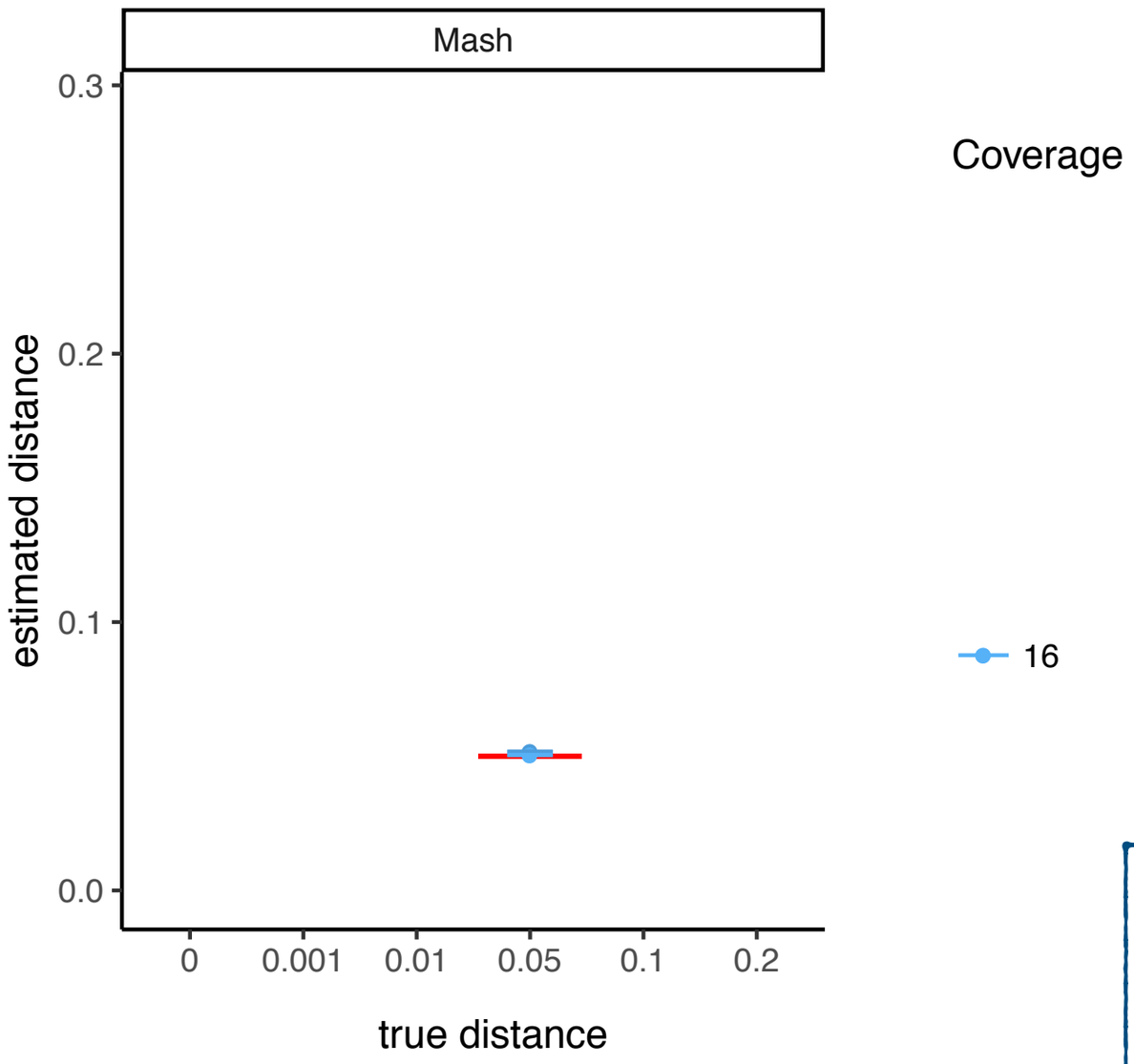
$$J = \frac{W}{L + L - W}$$

$$\rightarrow J = \frac{L(1-D)^k}{2L - L(1-D)^k}$$

$$\rightarrow \boxed{D = 1 - (\frac{2J}{1+J})^{1/k}}$$

Mash accuracy

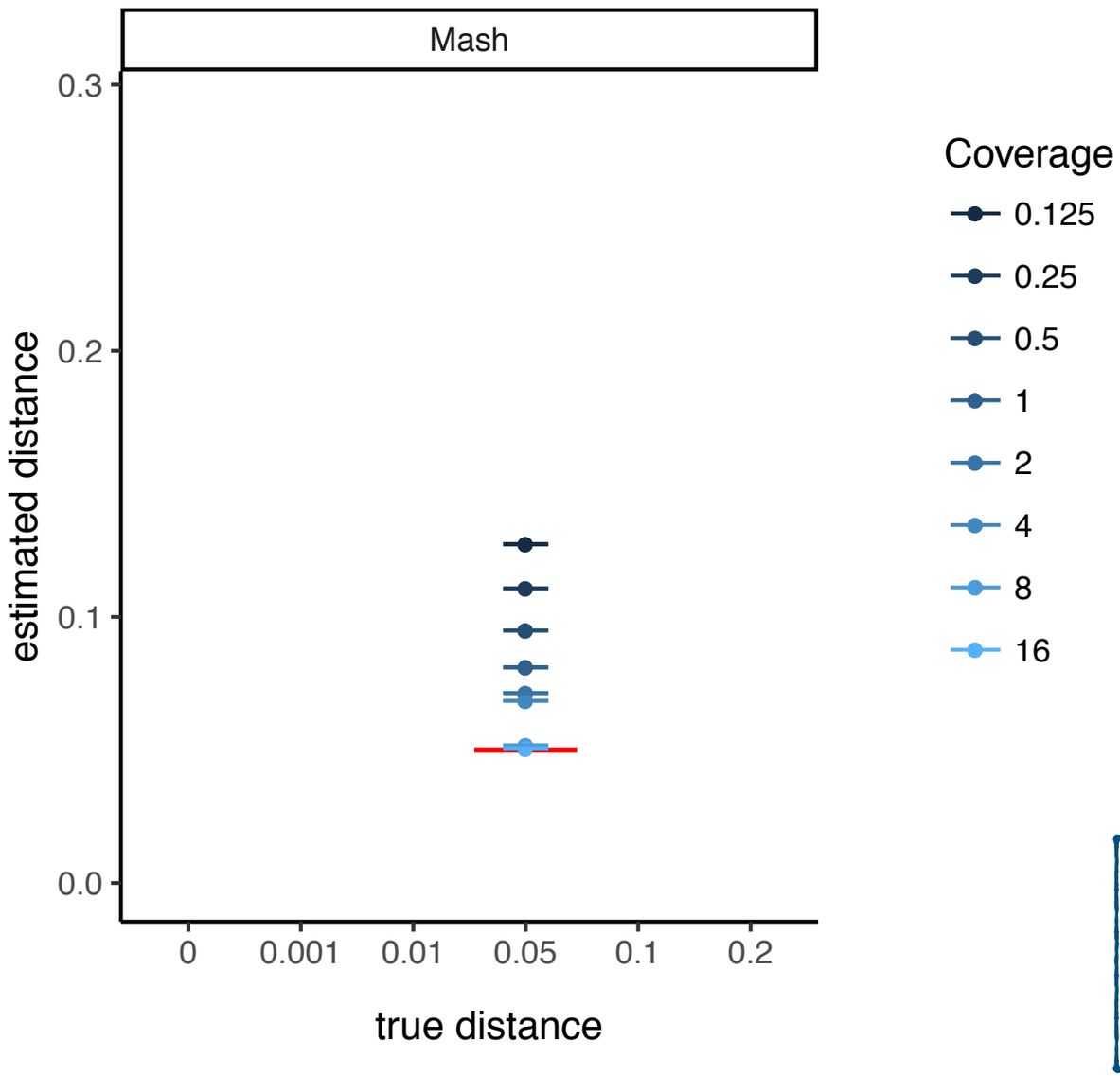
- Works well at high coverage



5% random mutations added to an insect genome

Mash accuracy

- Inaccurate when coverage is < 4X



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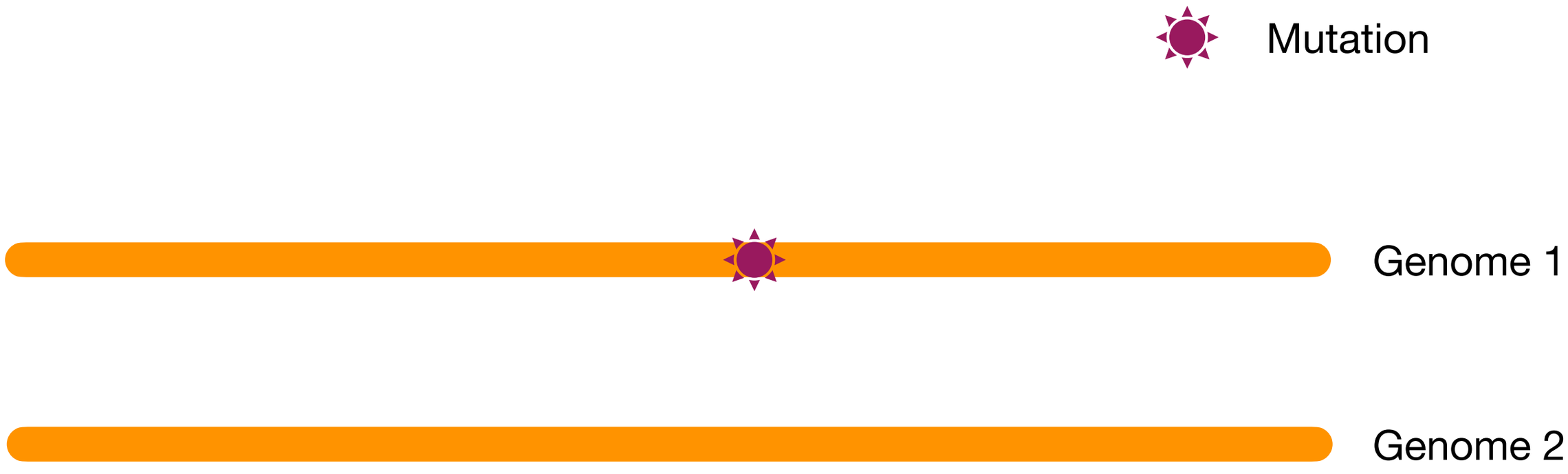
What happens when coverage is low?

- The Jaccard is reduced as some parts are not covered by any error-free read



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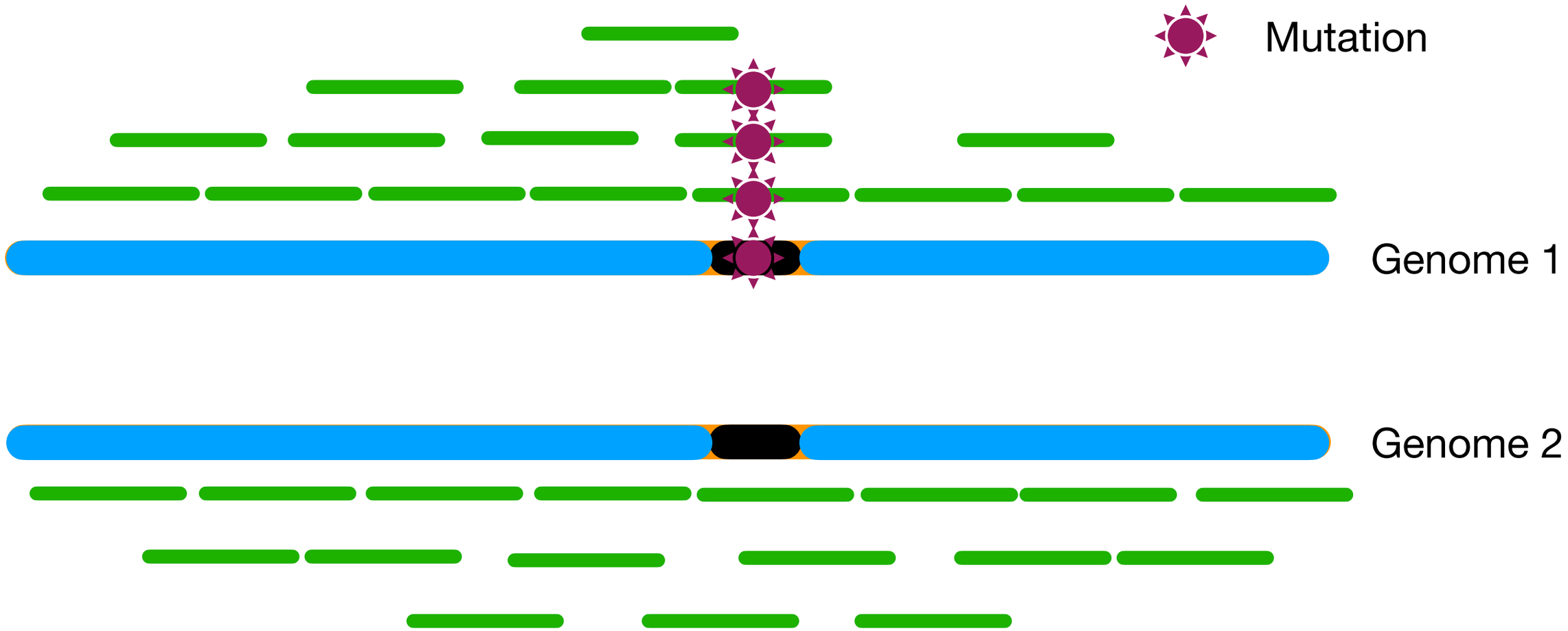

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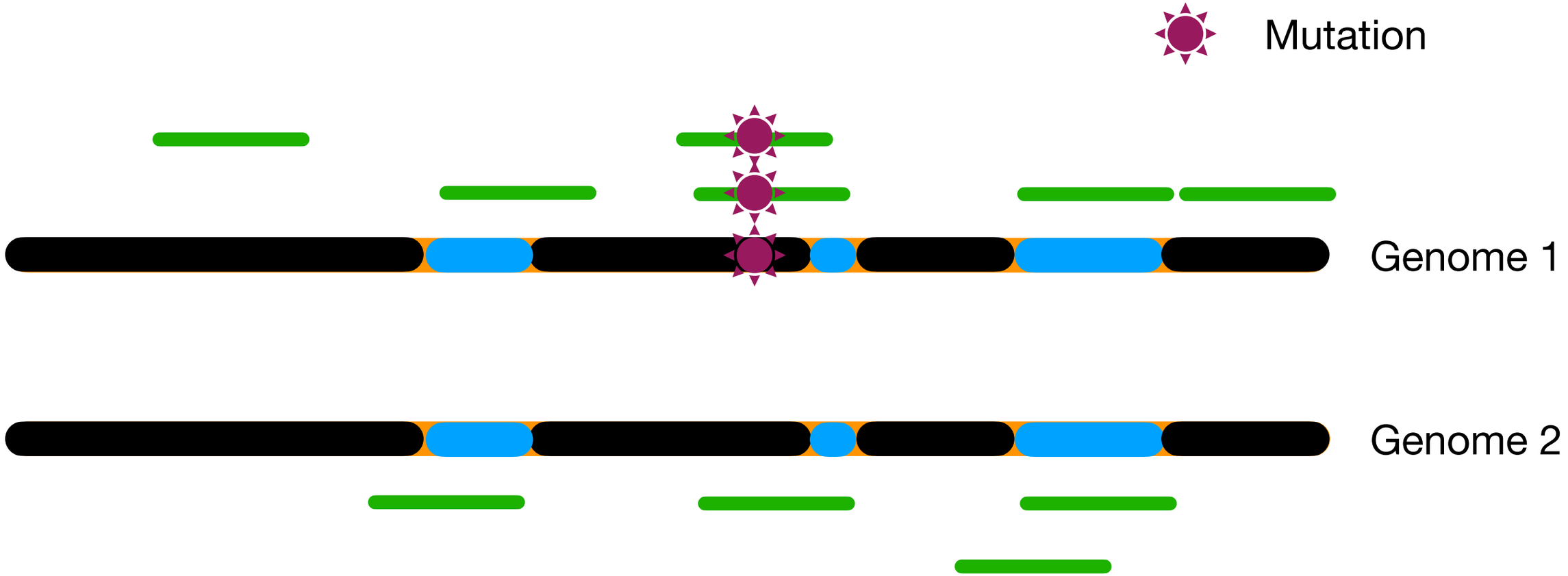

What happens with sequencing error?

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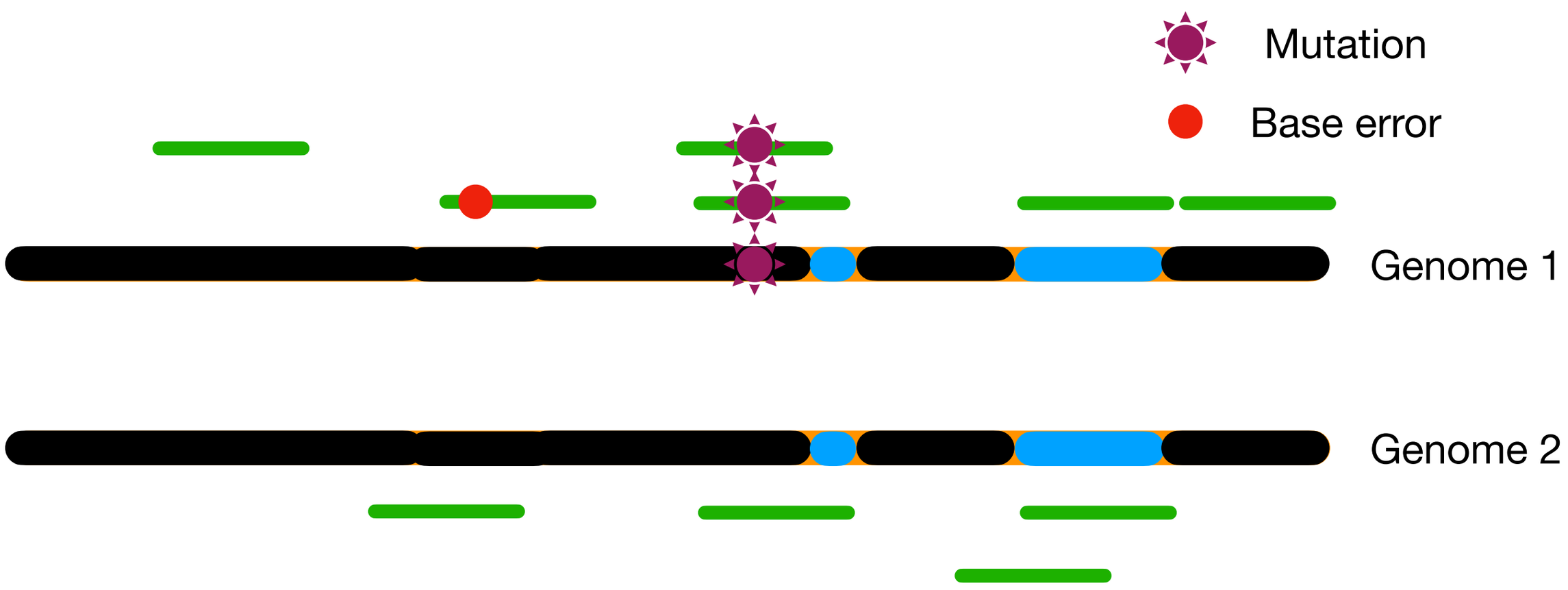

Jaccard to distance with low coverage and error

- Write Jaccard as a function of genomic distance and sequencing parameters

- Number of reads covering a k-mer follows a Poisson distribution

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$$D = 1 - \left(\frac{2(\zeta_1 L_1 + \zeta_2 L_2)J}{\eta_1 \eta_2 (L_1 + L_2)(1 + J)} \right)^{1/k}$$

Average number of k-mers per locus

Probability that a k-mer is covered

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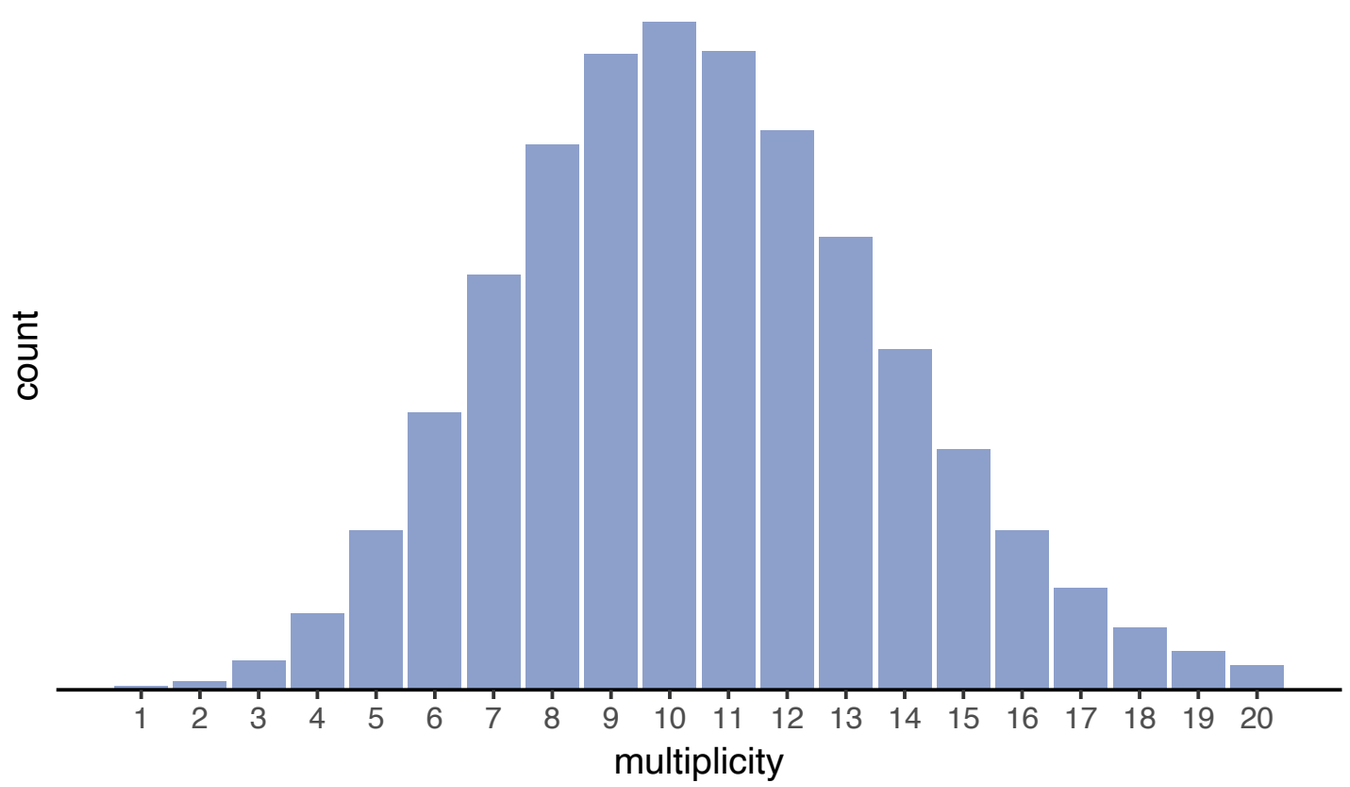
Estimating the sequencing parameters (λ_i and ϵ_i)

- Using k-mer profile (i.e., spectrum)
- For high coverage and zero error



Interpreting k-mer counts at low coverage

- Just using the mode won't work
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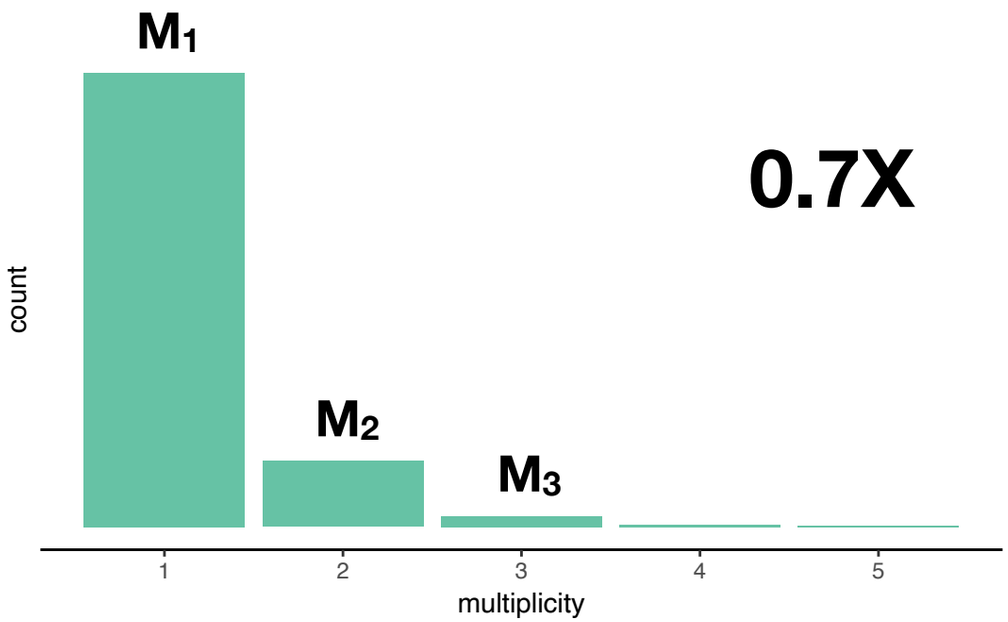

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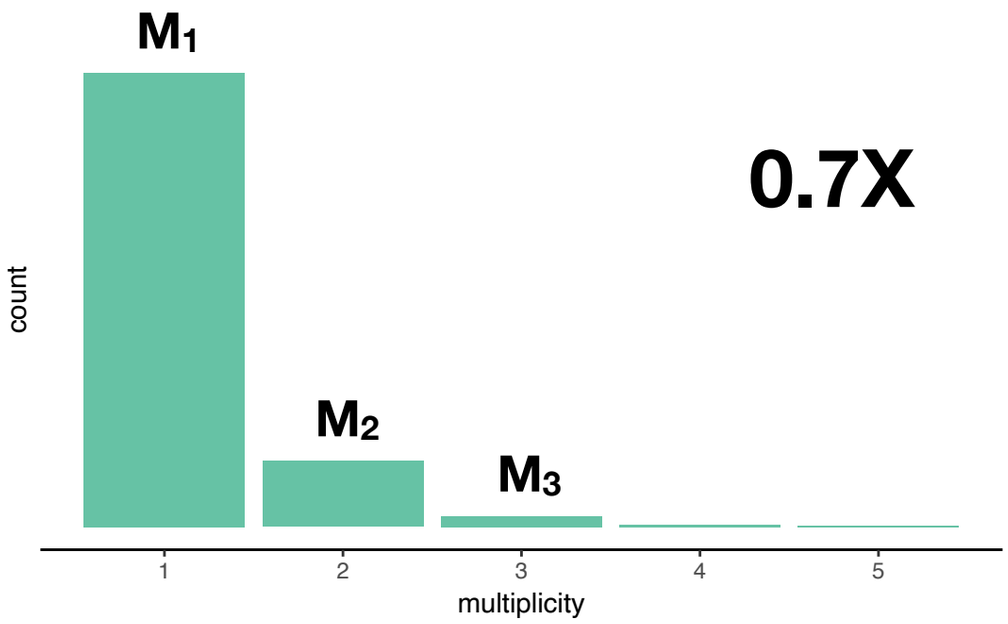


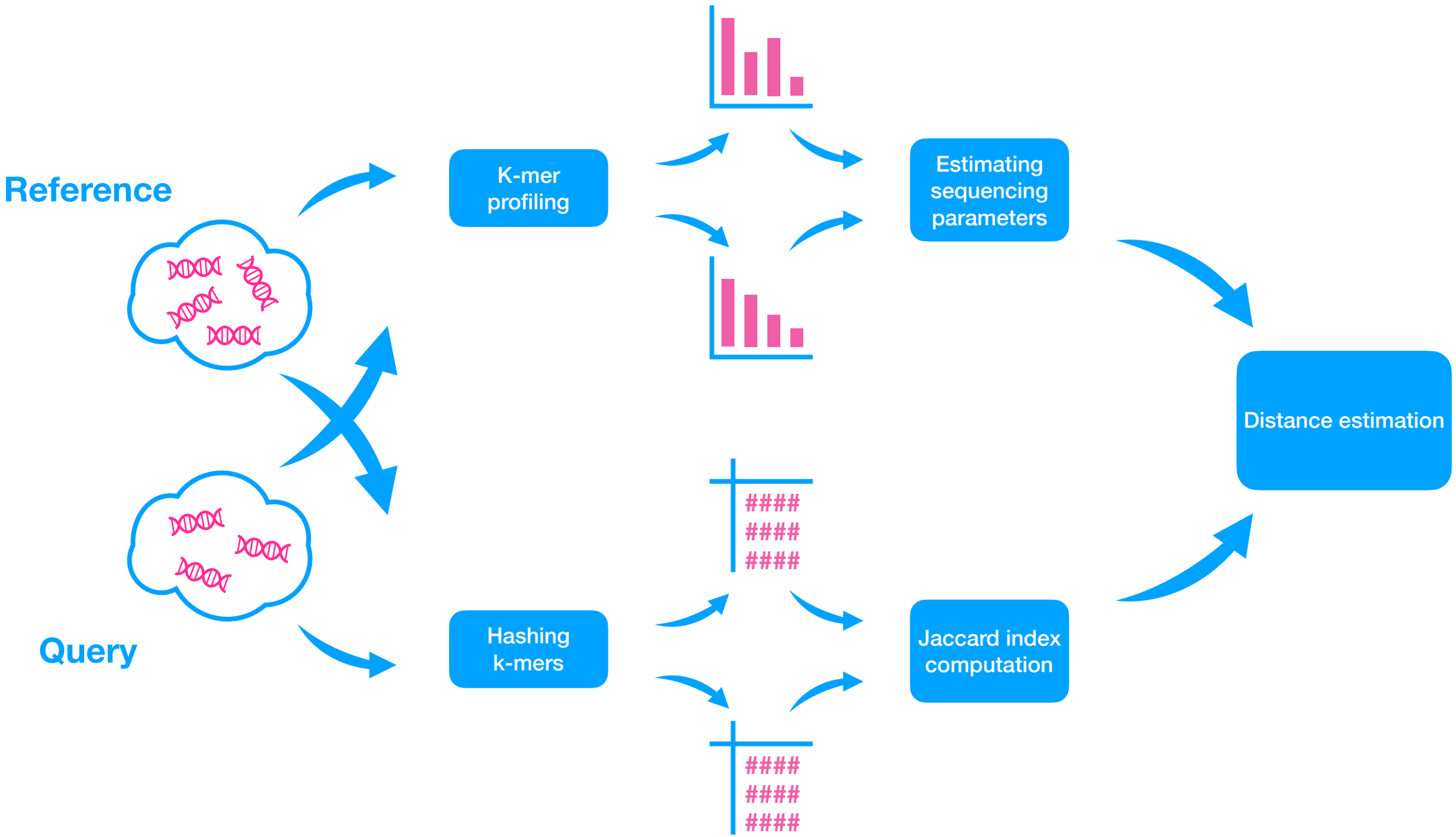
$$\mathbb{E}(M_i) = f(\lambda, \epsilon, L)$$

Coverage

Error rate

Genome length

Skmer


Reference
Query
K-mer profiling
Hashing k-mers
Estimating sequencing parameters
Jaccard index computation
Distance estimation
####
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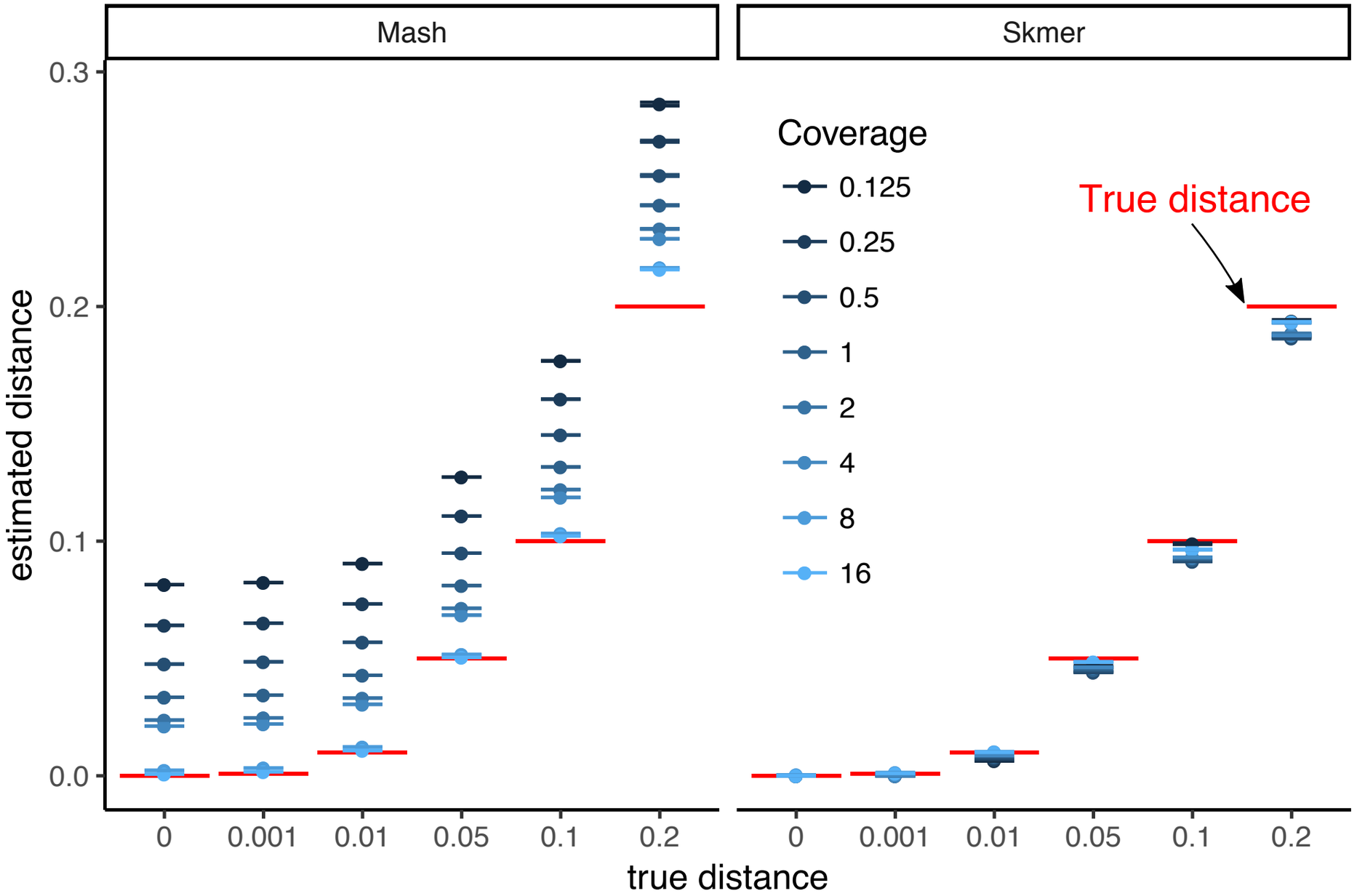
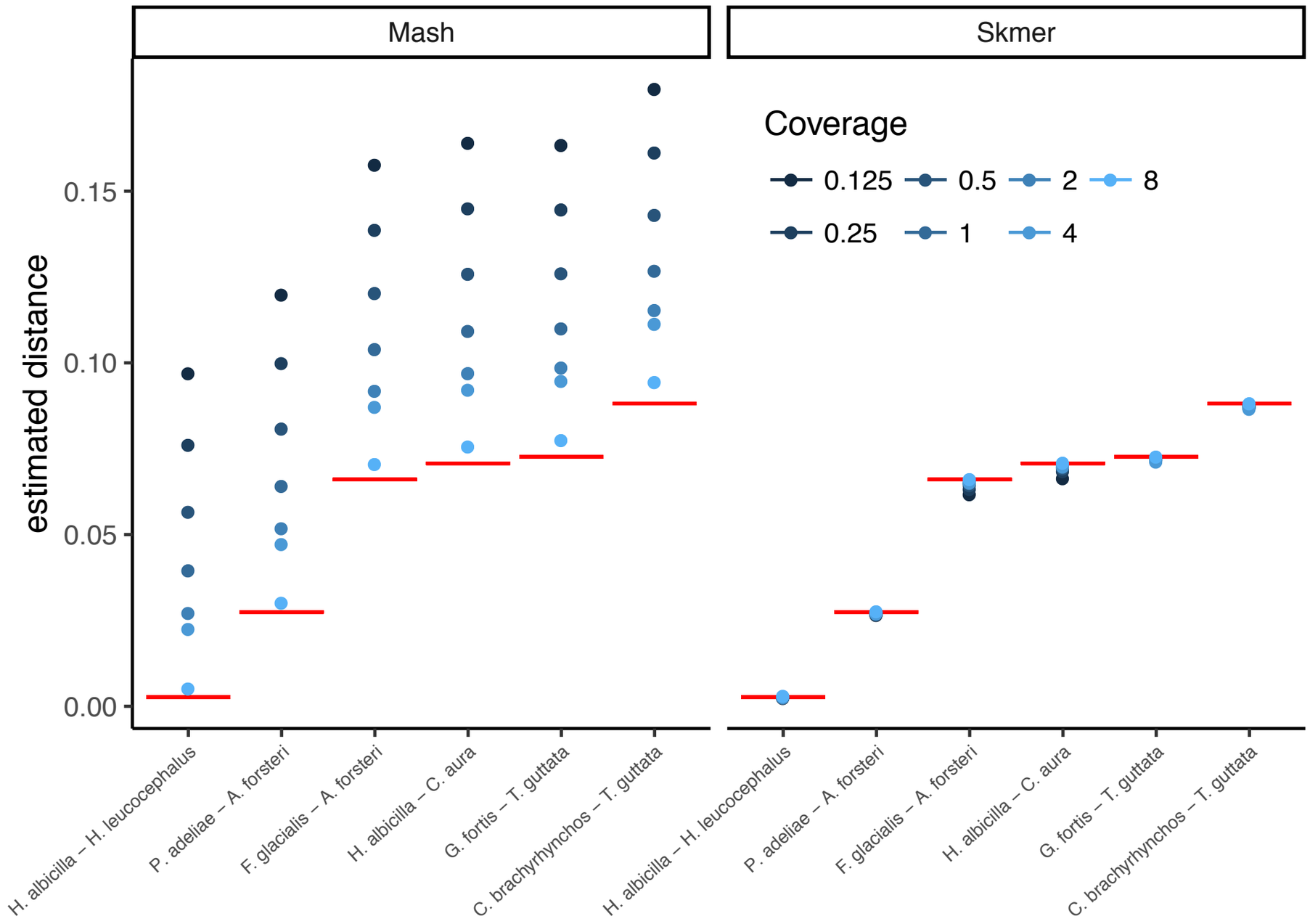
Skmer accuracy: simulations



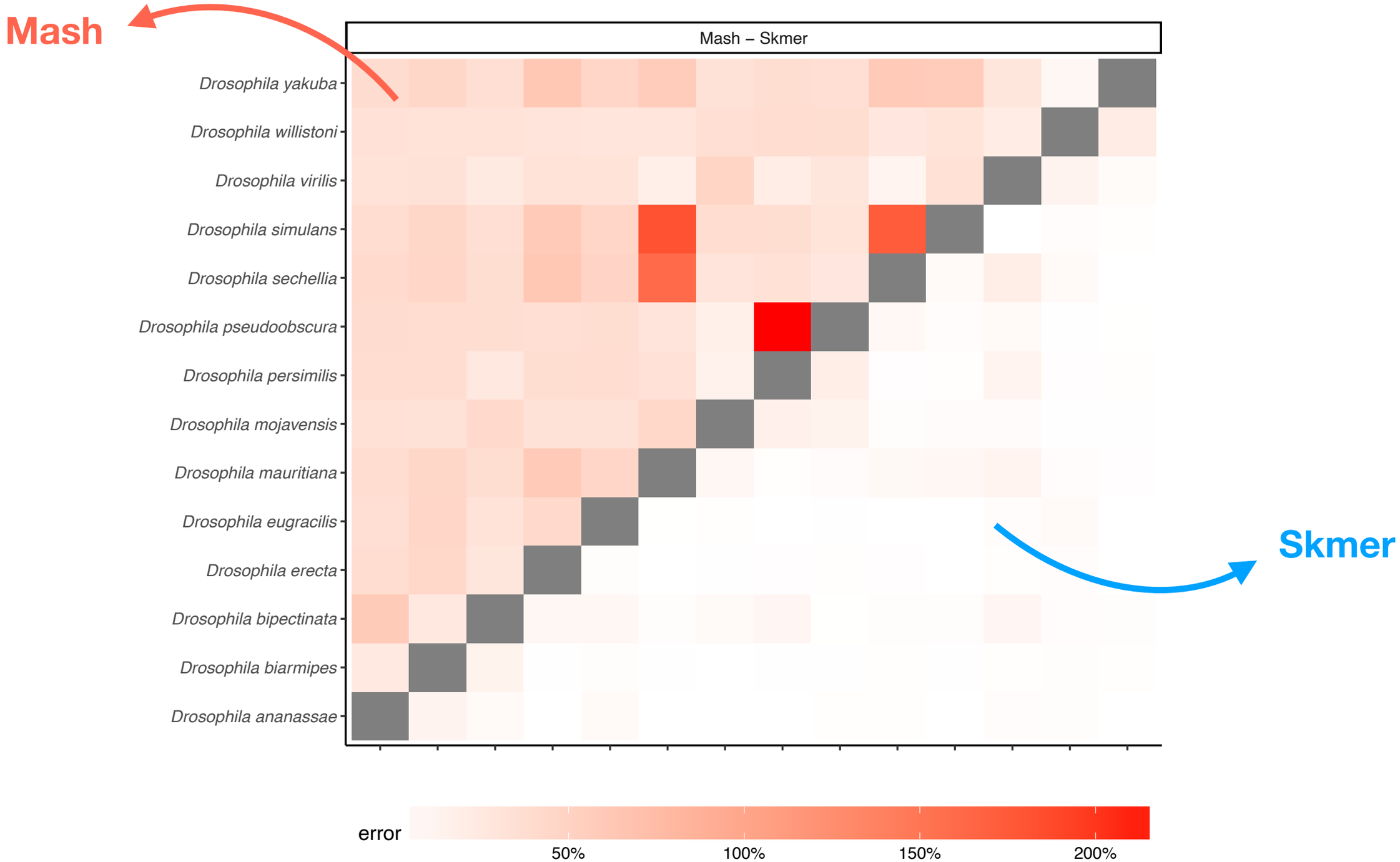
Random mutations added to an insect genome

Skmer accuracy: real data

Real pairs of bird genomes

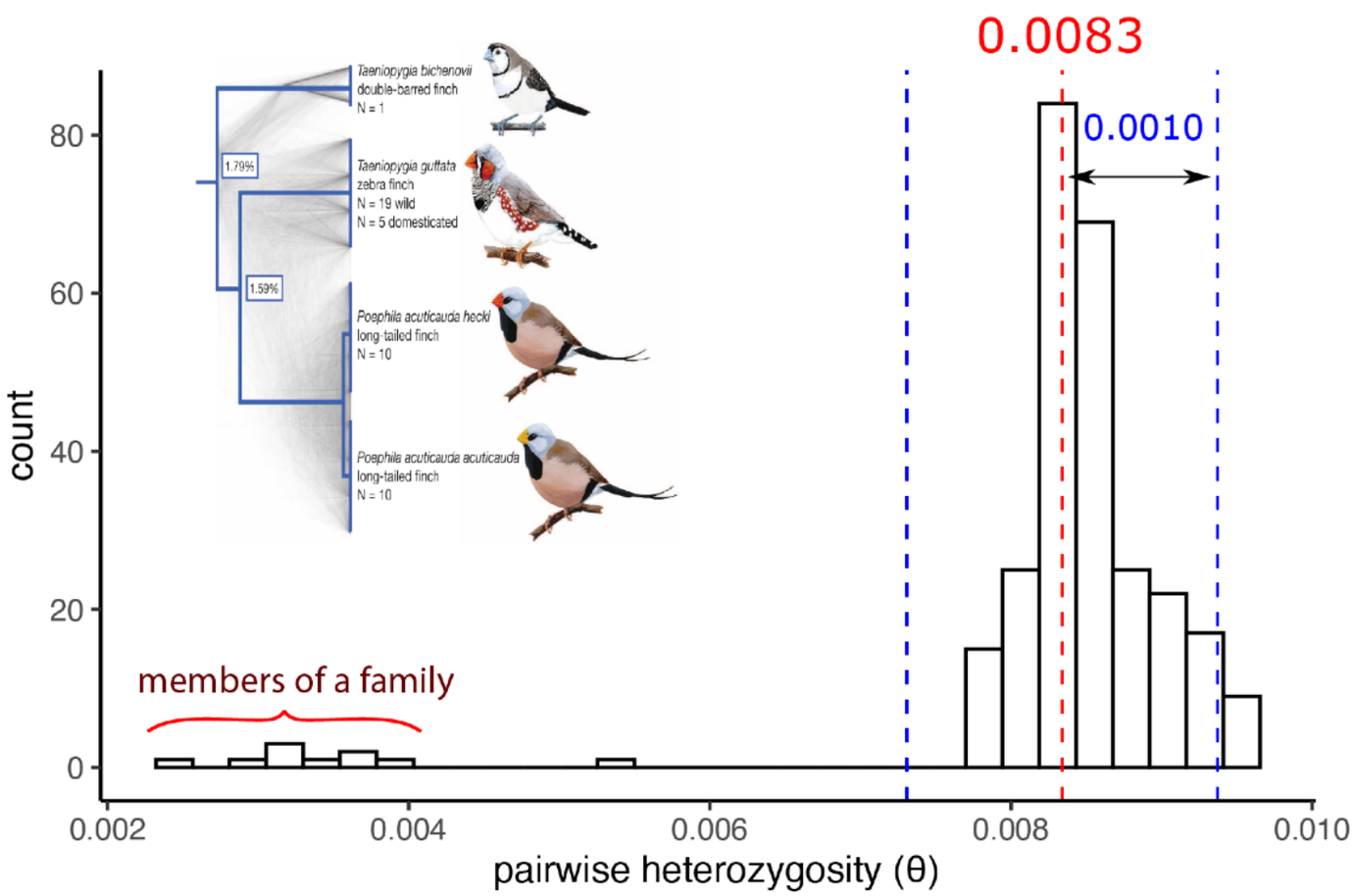


Results on real skims (~1/2X)



Stable recombination hotspots in birds

Sonal Singhal,[1,2]*† Ellen M. Leffler,[3,4]* Keerthi Sannareddy,[3] Isaac Turner,[4] Oliver Venn,[4] Daniel M. Hooper,[5] Alva I. Strand,[1] Qiye Li,[6] Brian Raney,[7] Christopher N. Balakrishnan,[8] Simon C. Griffith,[9] Gil McVean,[4] Molly Przeworski[1,2]†



Autosomal nucleotide diversity	Assembly	Skmer (2X)	Skmer+ RESPECT (2X)
Zebra finch	0.82%	0.69%	0.83%
Long-tailed finch	0.55%	0.43%	0.55%

[Unpublished work]

Research articles

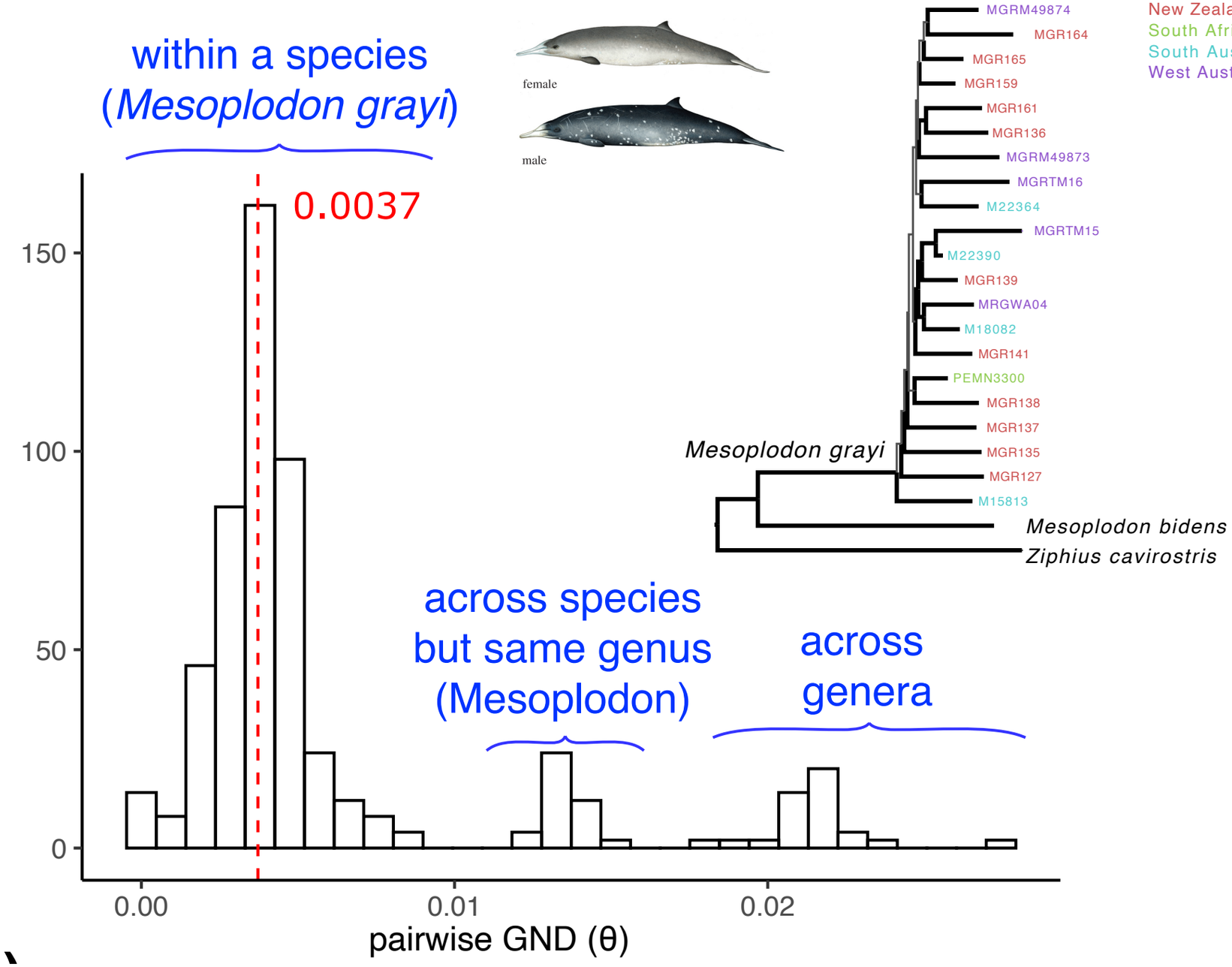
Ocean-wide genomic variation in Gray's beaked whales, *Mesoplodon grayi*

M. V. Westbury†, K. F. Thompson† ☐, M. Louis, A. A. Cabrera, M. Skovrind, J. A. S. Castruita, R. Constantine, J. R. Stevens and E. D. Lorenzen

Published: 24 March 2021 | https://doi.org/10.1098/rsos.201788

- Skims from 19 individuals of *Mesoplodon grayi* with coverage: 0.3X - 0.6X

- Two assemblies of closely-related beaked whale species (NCBI)

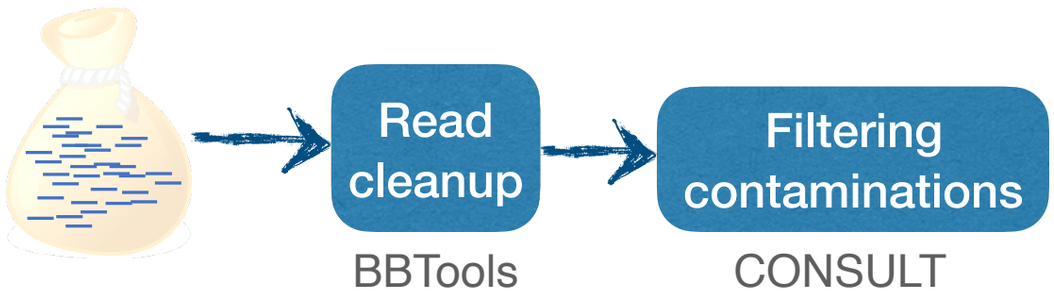


[Unpublished work]

Preprocessing of query and references

Identification of query

Preprocessing of query and references



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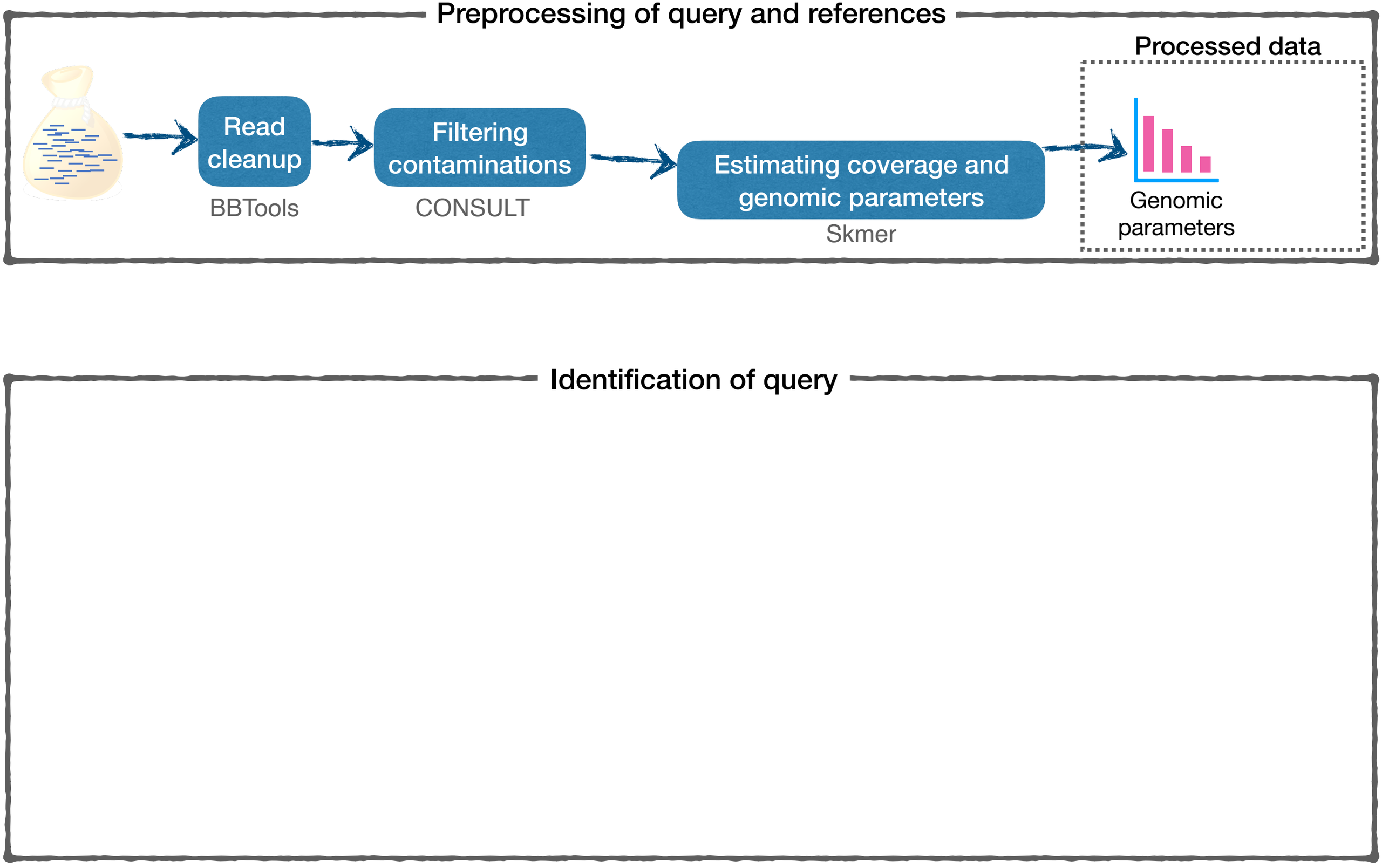
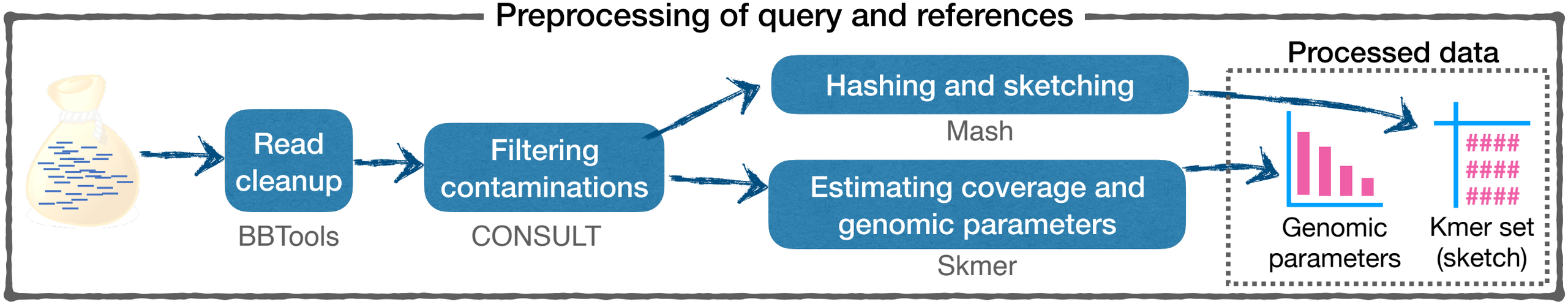

Preprocessing of query and references
Processed data
Read cleanup
BBTools
Filtering contaminations
CONSULT
Estimating coverage and genomic parameters
Skmer
Genomic parameters
Identification of query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Mash
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Skmer
Genomic parameters
Kmer set (sketch)
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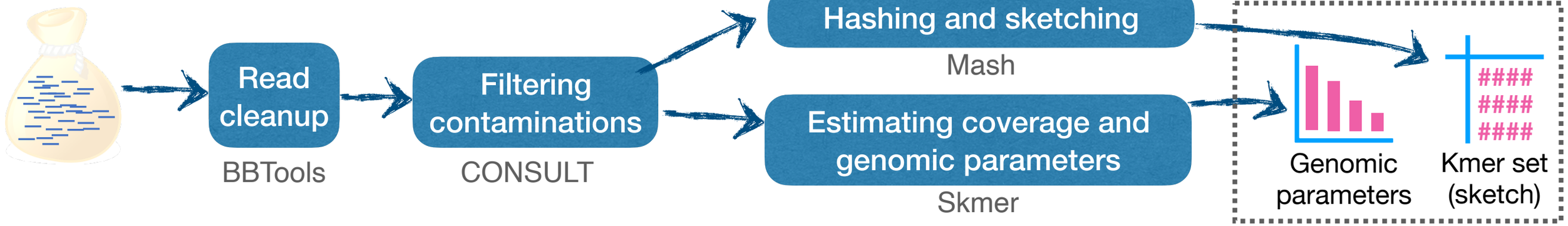
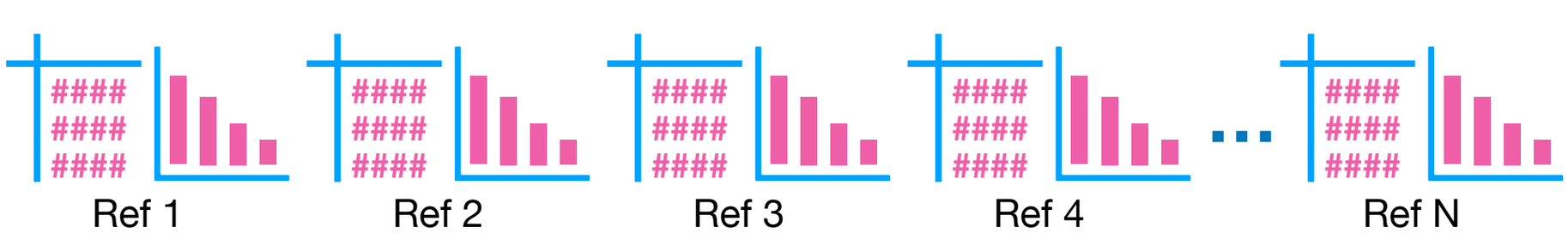
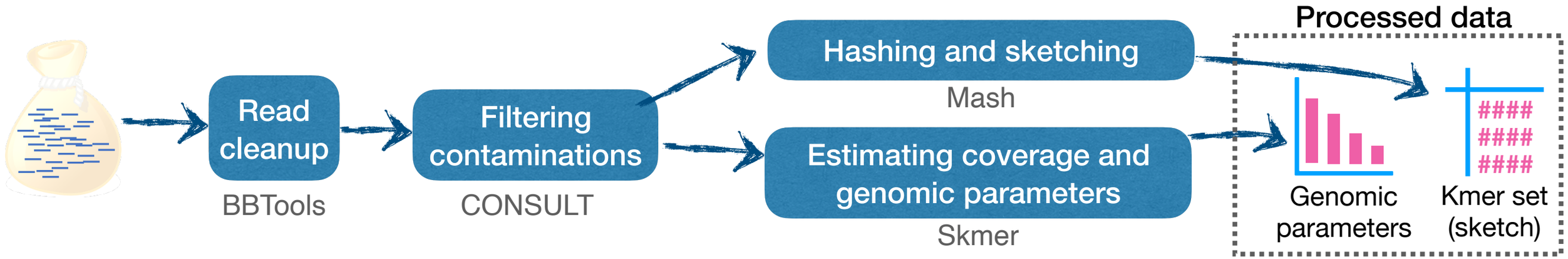


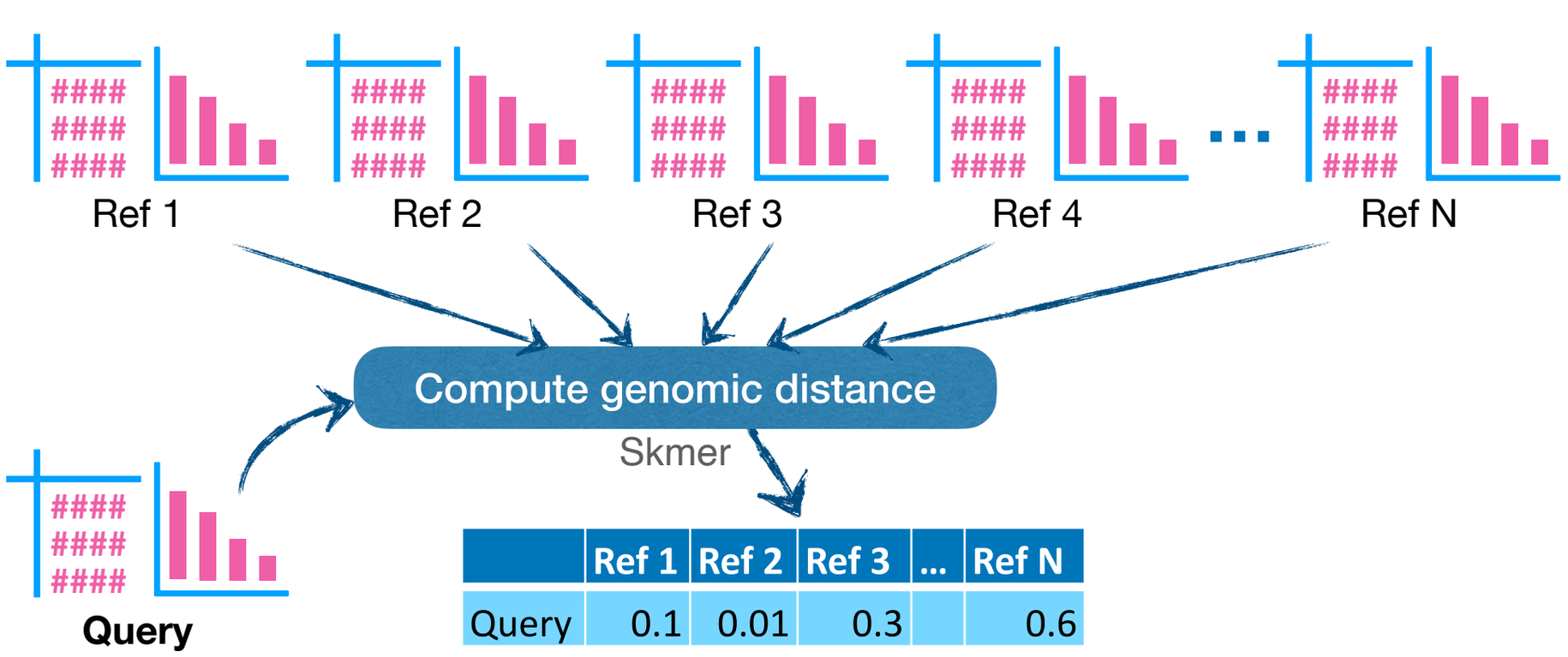
Identification of query



Preprocessing of query and references



Identification of query



	Ref 1	Ref 2	Ref 3	…	Ref N
Query	0.1	0.01	0.3		0.6

Skmer

https://github.com/shahab-sarmashghi/Skmer

```
$ conda install skmer
```

https://genomebiology.biomedcentral.com/articles/10.1186/s13059-019-1632-4

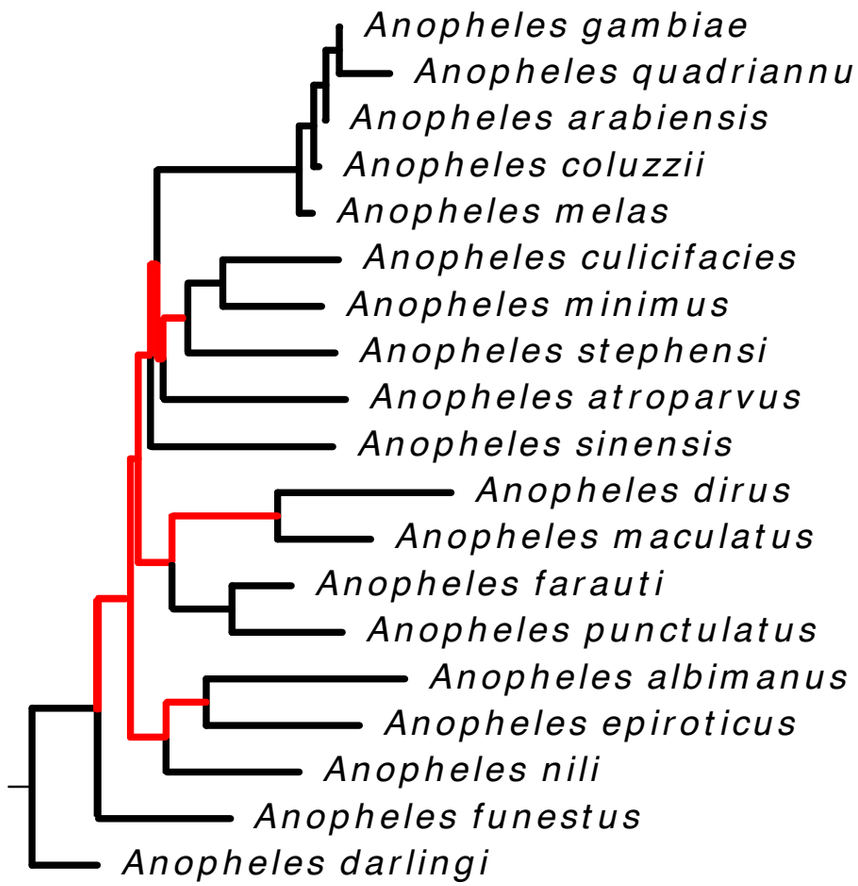
Distance-based phylogenetics

1. Compute all pairwise distance (e.g., GND)
   - $D_{i,j}$: distance of species i and j
2. Correct according to models of sequence evolution
   - Example: JC69 model:

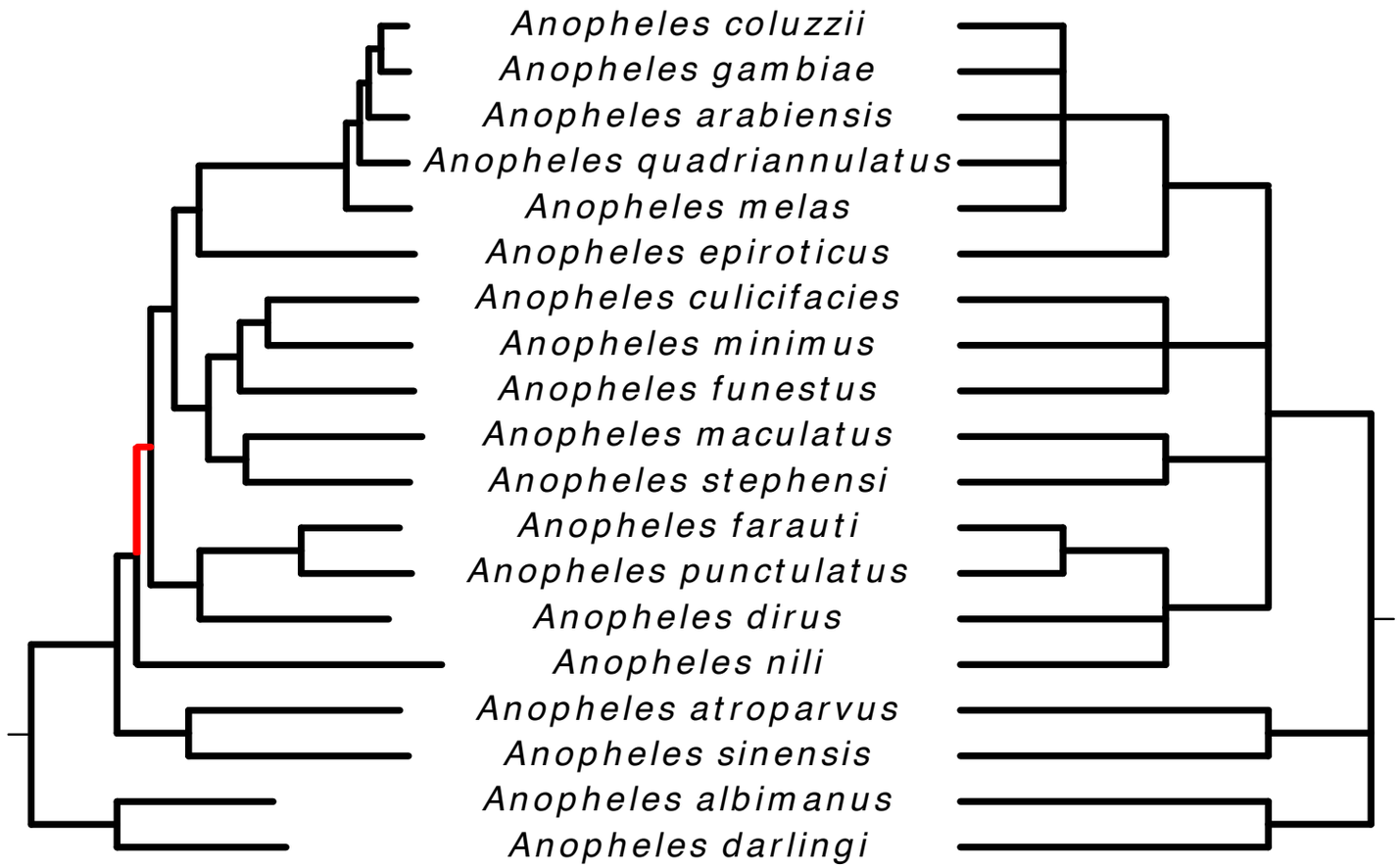
$$t_{i,j} = -\frac{3}{4}\ln(1 - \frac{4}{3}D_{i,j})$$

3. Build the phylogeny using an algorithm of choice
   - Neighbour joining, Minimum Evolution, etc.

Building Phylogeny



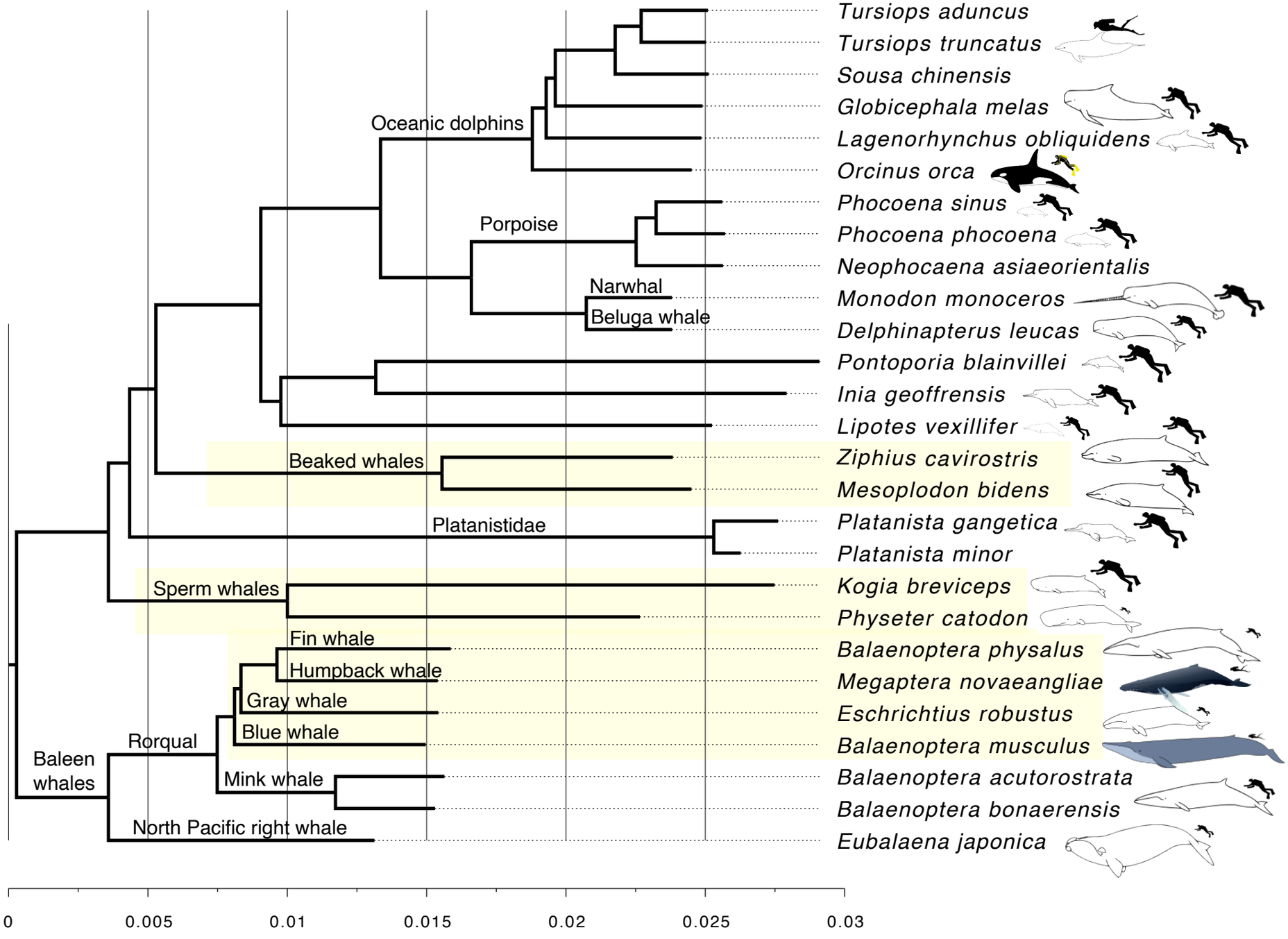
Marker Phylogeny



Skmer distances+ JC69 correction

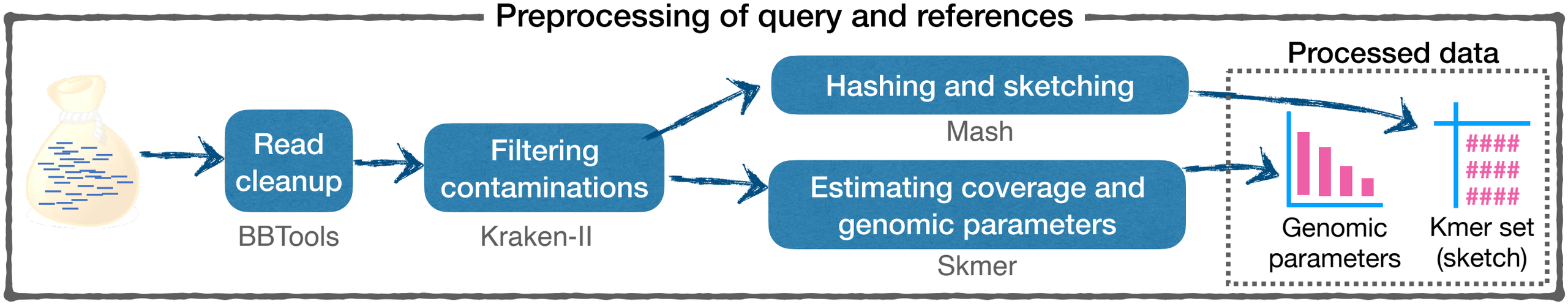
oTL Reference

Cetacean phylogeny

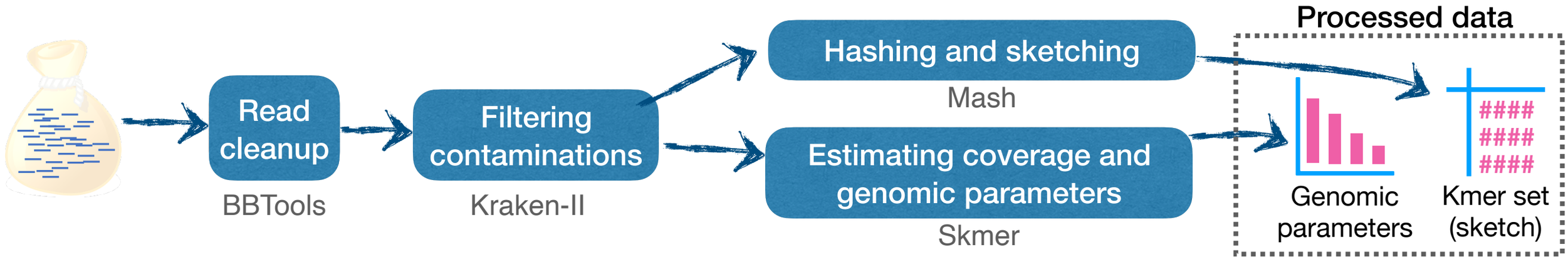


Good tools to use

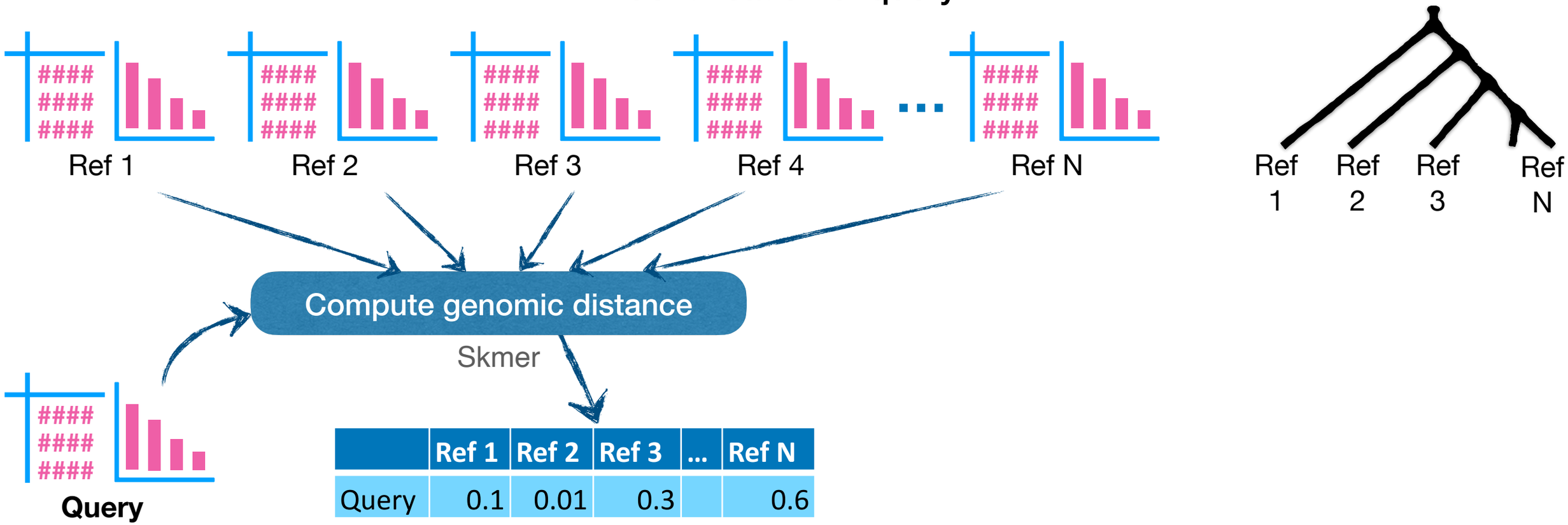
- FastME is my favorite


Preprocessing of query and references
Read cleanup
BBTools
Filtering contaminations
Kraken-II
Hashing and sketching
Mash
Estimating coverage and genomic parameters
Skmer
Processed data
Genomic parameters
Kmer set (sketch)
Identification of query
Ref 1
Ref 2
Ref 3
Ref 4
Ref N
Compute genomic distance
Skmer
Query
Ref 1 Ref 2 Ref 3 ... Ref N
Query 0.1 0.01 0.3 0.6

Preprocessing of query and references

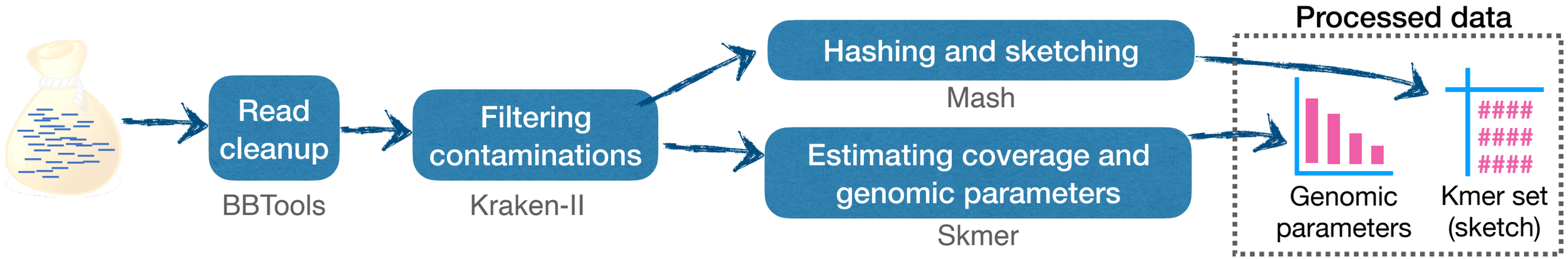


Identification of query

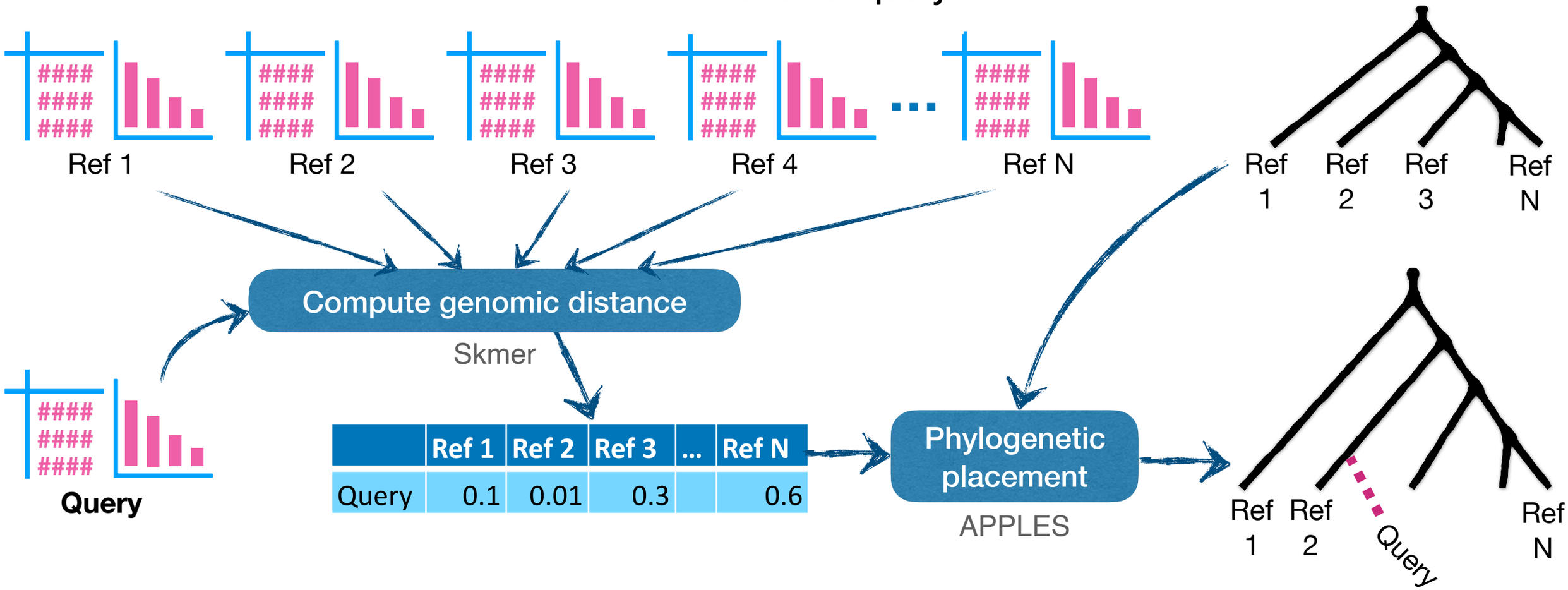


	Ref 1	Ref 2	Ref 3	...	Ref N
Query	0.1	0.01	0.3		0.6

Preprocessing of query and references

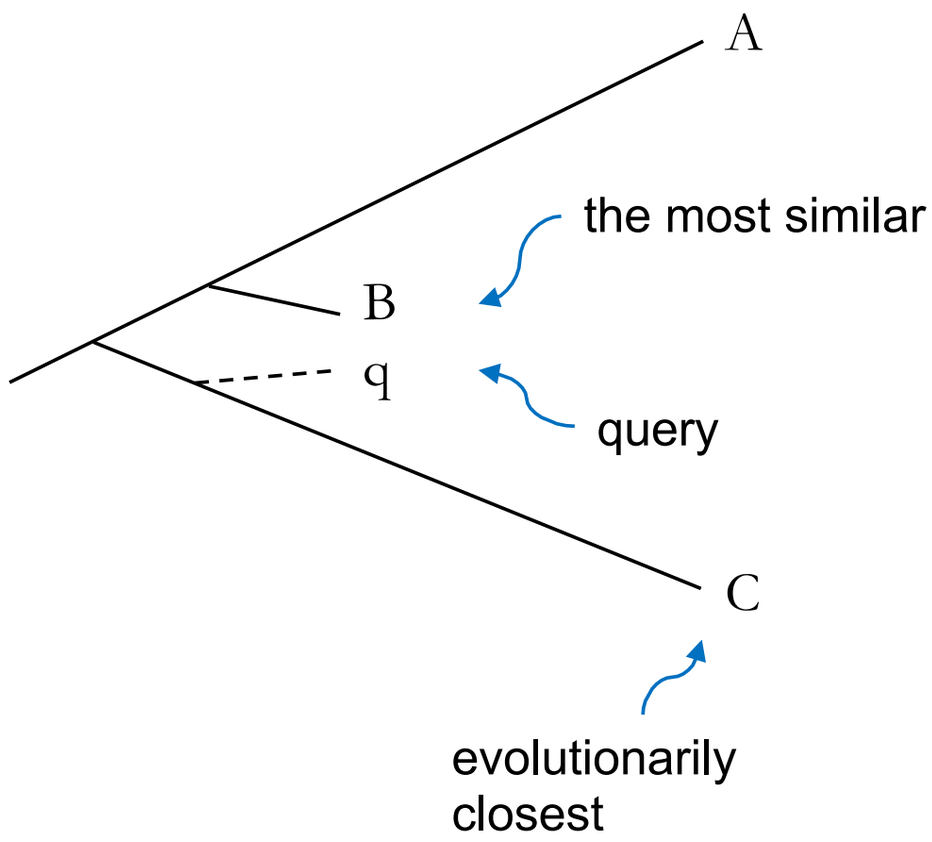


Identification of query

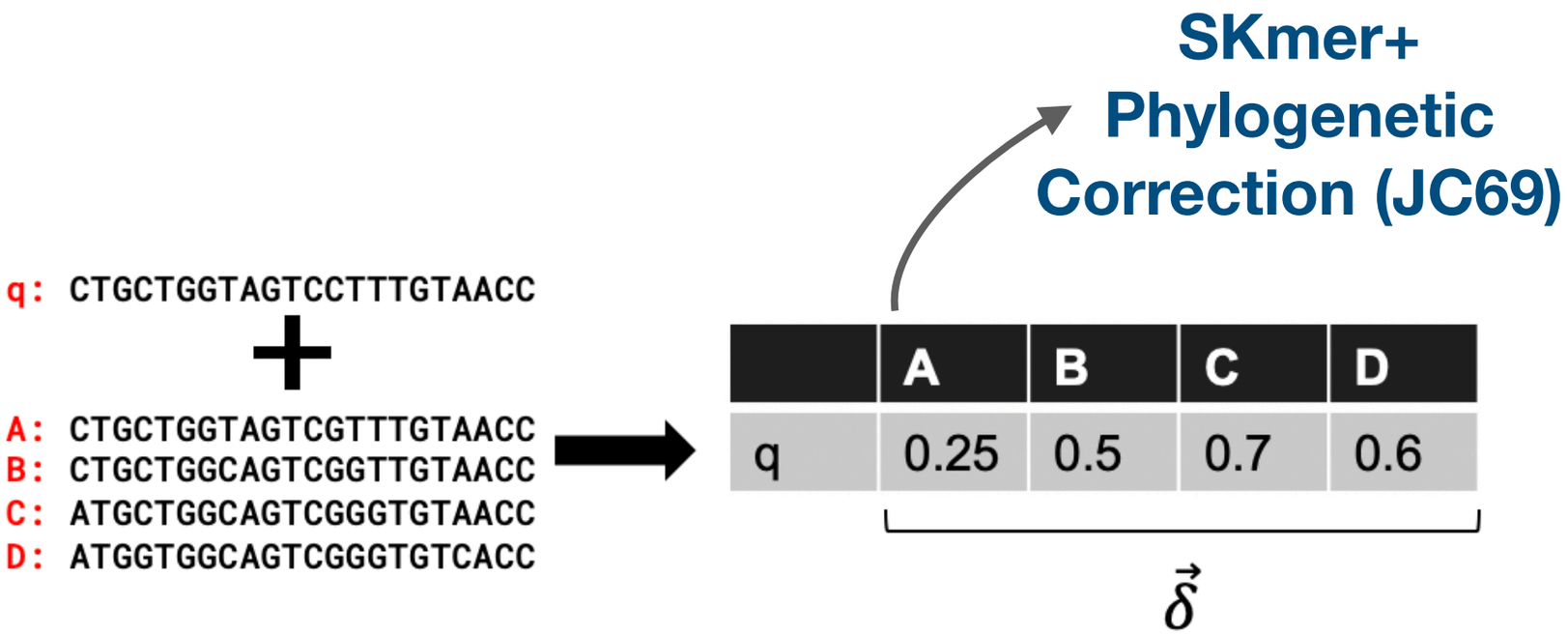


Closest match not always enough

- Alternative to placement: closest match
- For incomplete reference sets, assignment to closest is misleading
- When rate of evolution changes best match can mislead.



Distance-based Phylogenetic Placement Framework



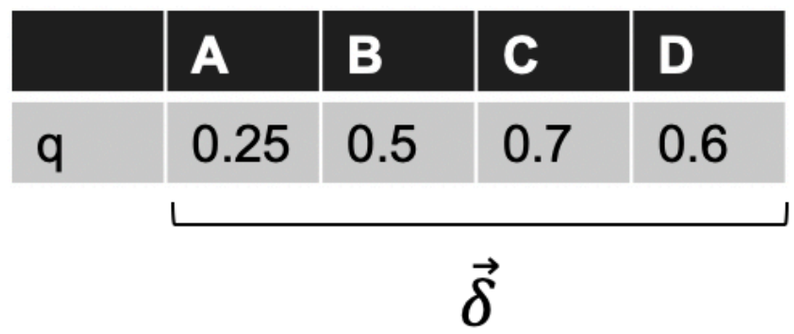
Distance-based Phylogenetic Placement Framework

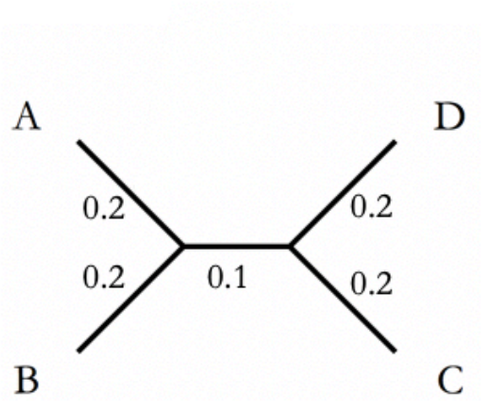
SKmer+ Phylogenetic Correction (JC69)

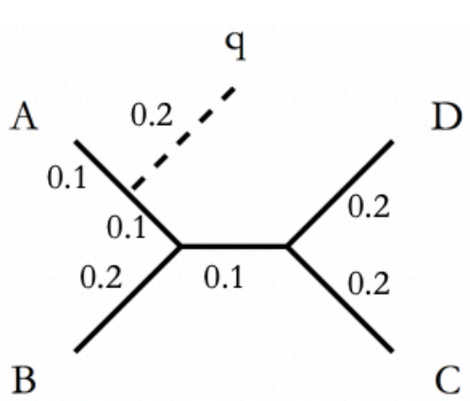
q: CTGCTGGTAGTCCTTTGTAACC

\+

A: CTGCTGGTAGTCGTTTGTAACC
B: CTGCTGGCAGTCGGTTGTAACC
C: ATGCTGGCAGTCGGGTGTAACC
D: ATGGTGGCAGTCGGGTGTCACC



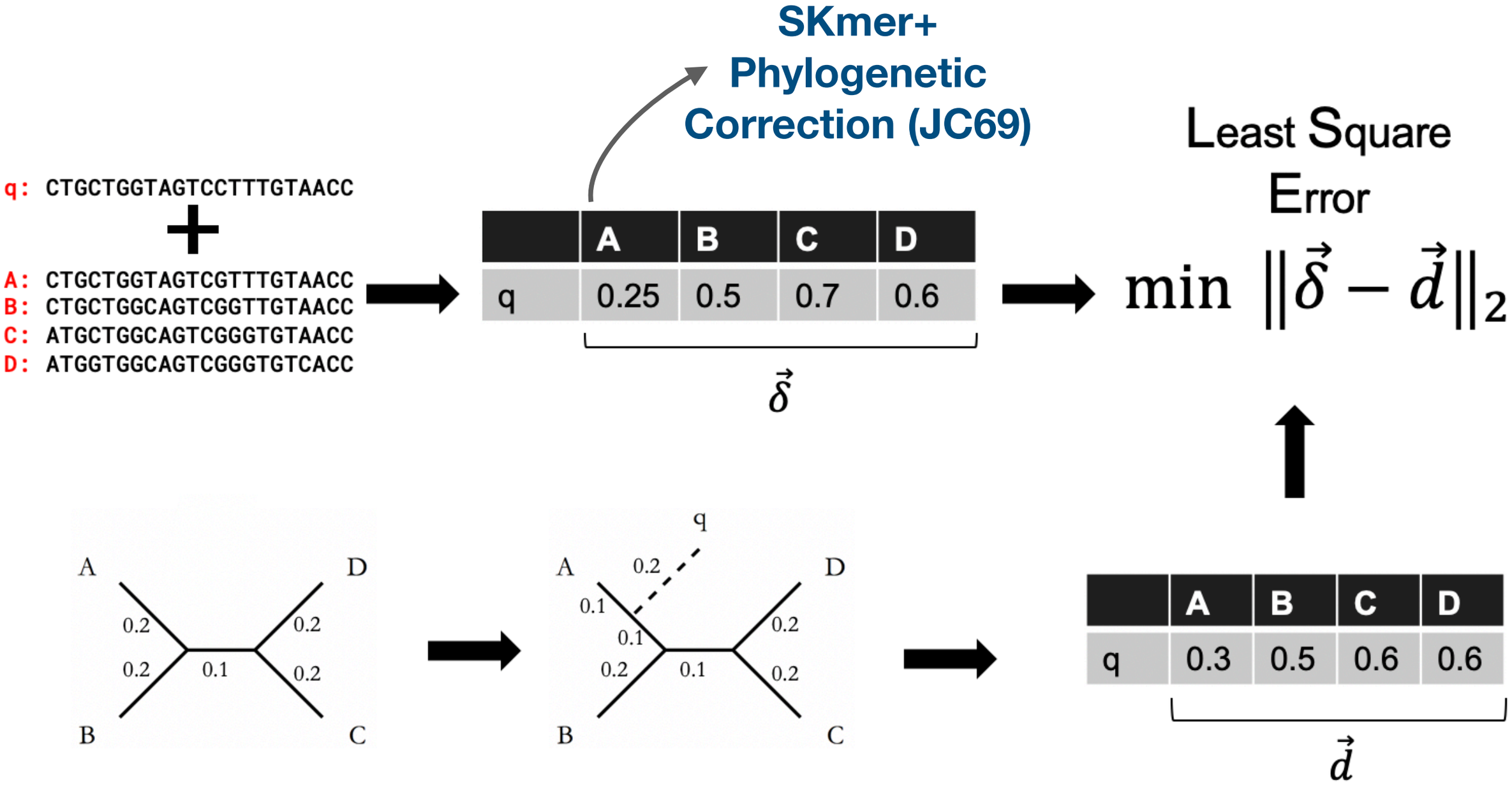




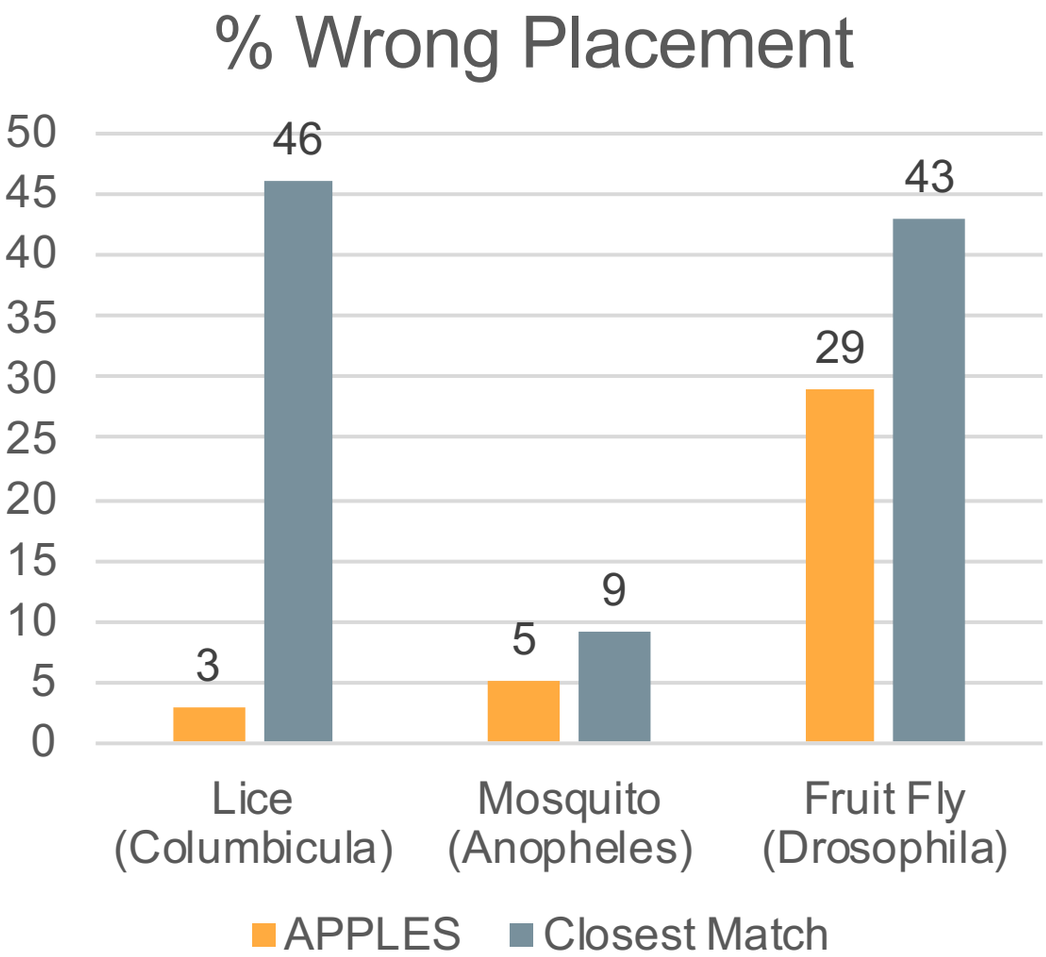
	A	B	C	D
q	0.3	0.5	0.6	0.6

$\vec{d}$

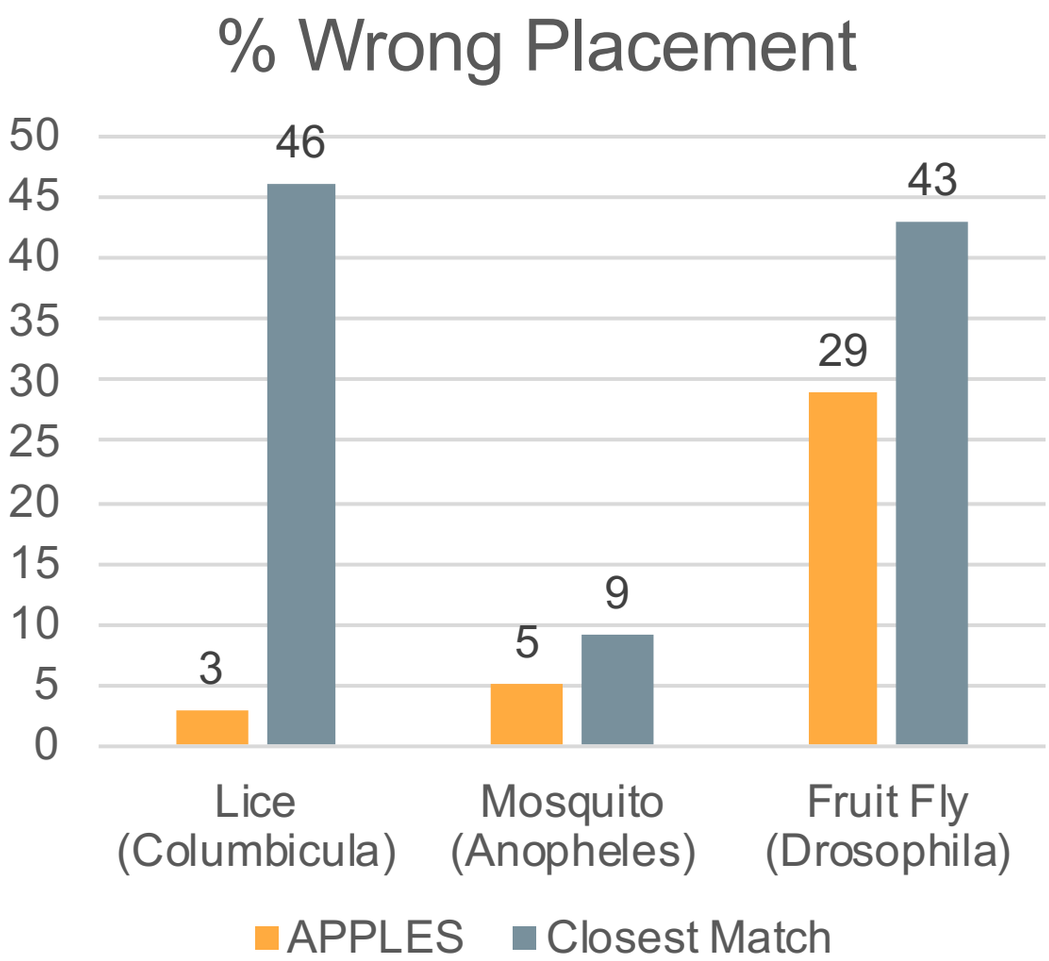
Distance-based Phylogenetic Placement Framework

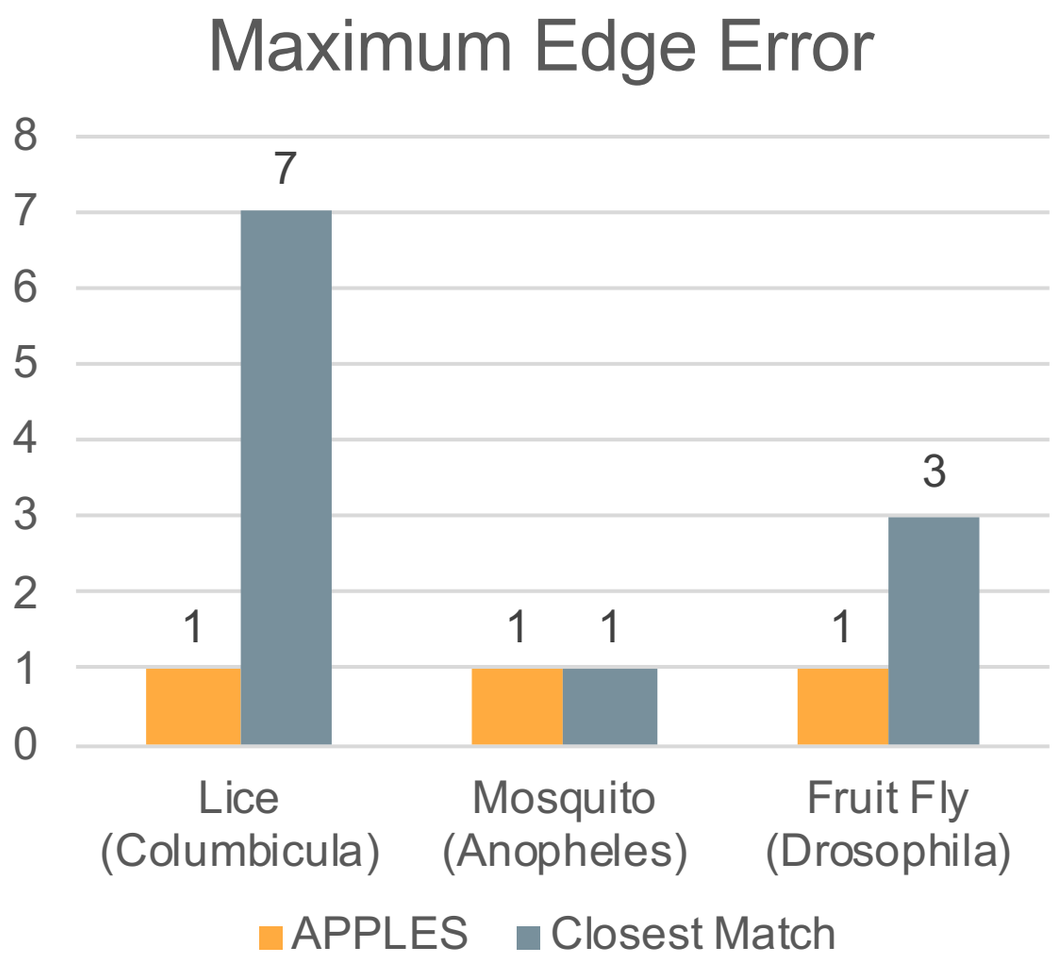


Placement accuracy results



Placement accuracy results





APPLES

https://github.com/balabanmetin/apples

```
$ pip install apples
```

Systematic Biology

https://doi.org/10.1093/sysbio/syz063

MOLECULAR ECOLOGY

NEWS AND VIEWS | Open Access

Beyond DNA barcoding: The unrealized potential of genome skim data in sample identification

Kristine Bohmann, Siavash Mirarab, Vineet Bafna, M. Thomas P. Gilbert

First published: 16 June 2020 | https://doi.org/10.1111/mec.15507

Publications

- Bohmann, Kristine, Siavash Mirarab, Vineet Bafna, and M. Thomas P. Gilbert. “Beyond DNA Barcoding: The Unrealized Potential of Genome Skim Data in Sample Identification.” Molecular Ecology 29, no. 14 (2020): 2521–34. https://doi.org/10.1111/mec.15507.

- **Skmer:** Sarmashghi, Shahab, Kristine Bohmann, M Thomas P Gilbert, Vineet Bafna, and Siavash Mirarab. “Skmer: Assembly-Free and Alignment-Free Sample Identification Using Genome Skims.” Genome Biology 20, no. 1 (2019): 34. https://doi.org/10.1186/s13059-019-1632-4.

- **Contamination:** Rachtman, Eleonora, Metin Balaban, Vineet Bafna, and Siavash Mirarab. “The Impact of Contaminants on the Accuracy of Genome Skimming and the Effectiveness of Exclusion Read Filters.” Molecular Ecology Resources 20, no. 3 (2020): 1755-0998.13135. https://doi.org/10.1111/1755-0998.13135.

- **APPLES:** Balaban, Metin, Shahab Sarmashghi, and Siavash Mirarab. “APPLES: Scalable Distance-Based Phylogenetic Placement with or without Alignments.” Edited by David Posada. Systematic Biology 69, no. 3 (2020): 566–78. https://doi.org/10.1093/sysbio/syz063

- **Mixed samples:** Balaban, Metin, and Siavash Mirarab. “Phylogenetic Double Placement of Mixed Samples.” Bioinformatics 36, no. Supplement_1 (2020): i335–43. https://doi.org/10.1093/bioinformatics/btaa489.

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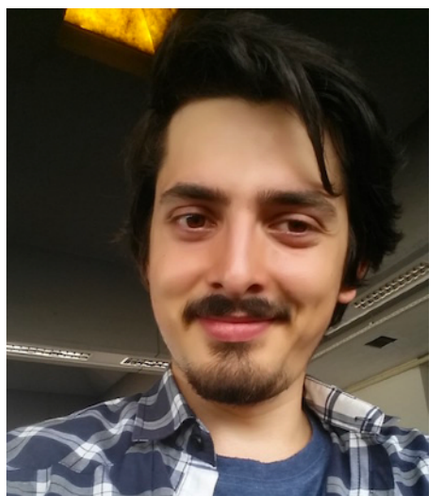
Vineet Bafna

Kristine Bohmann

Tom Gilbert



Metin Balaban

Eleonora (Nora) Rachtman

1815485 and 1565862