Alignment-free analyses of next-generation sequencing data

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Acknowledgments

Algorithms
J Matthew Holt
Leonard McMillan

Applications
John Didion
Fernando Pardo-Manuel de Villena

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Jim Crowley

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Goals of next-generation sequencing (NGS)

Discovery of molecular variation

- DNAseq
- RNAseq
- metagenomics

Quantification

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- other exotic *-seq
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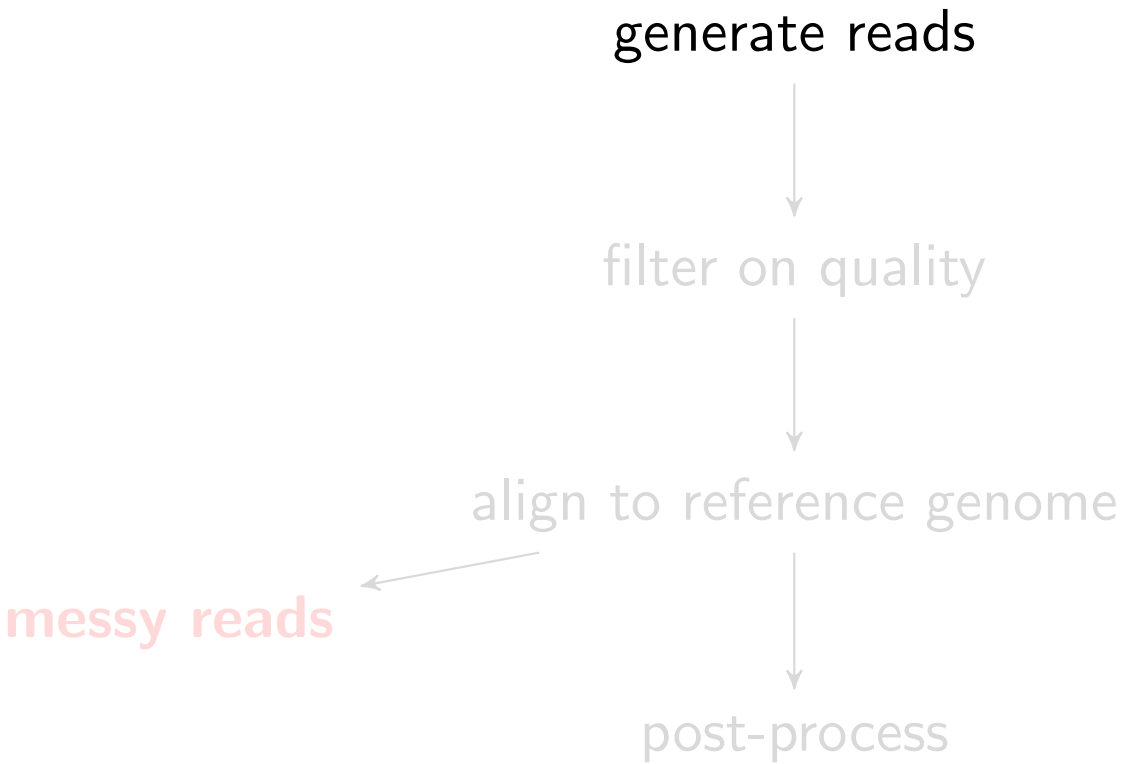

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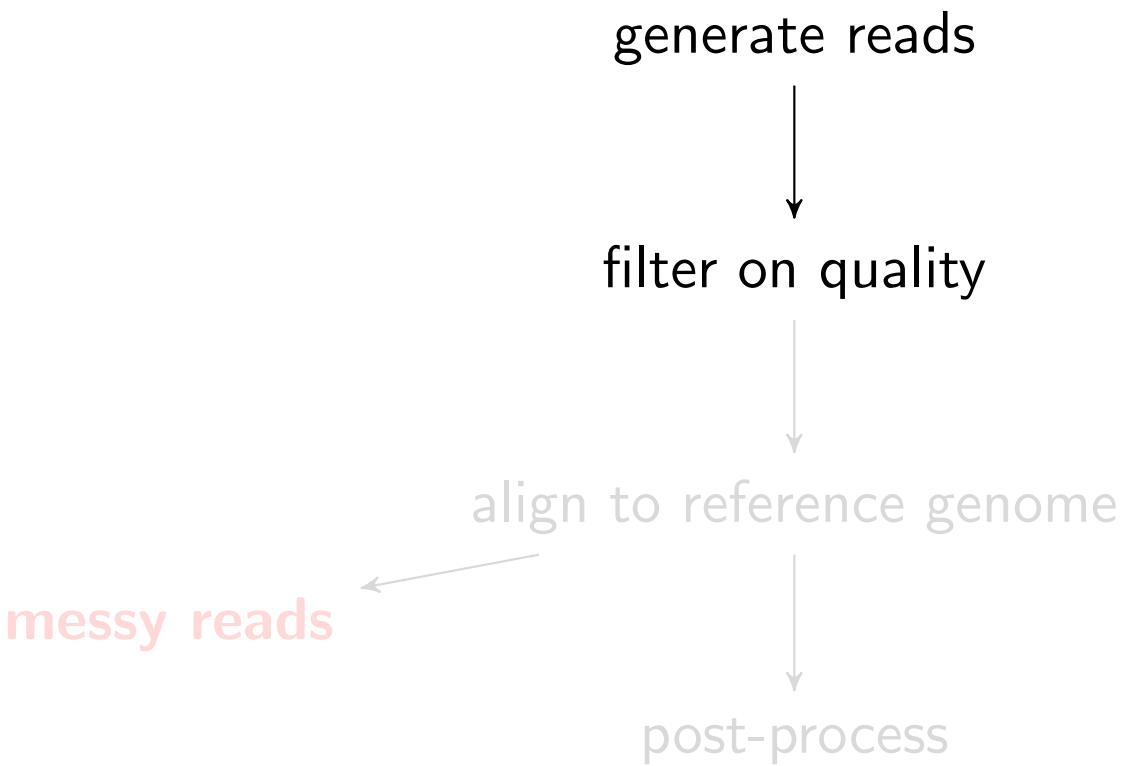

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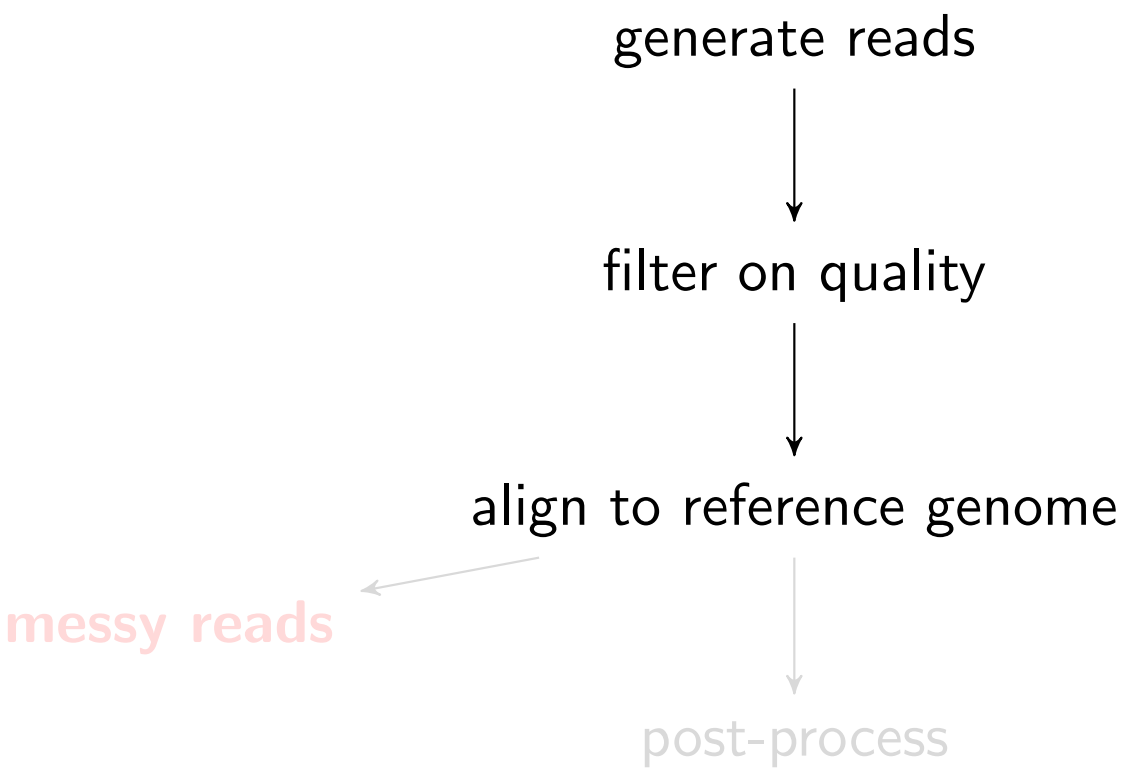

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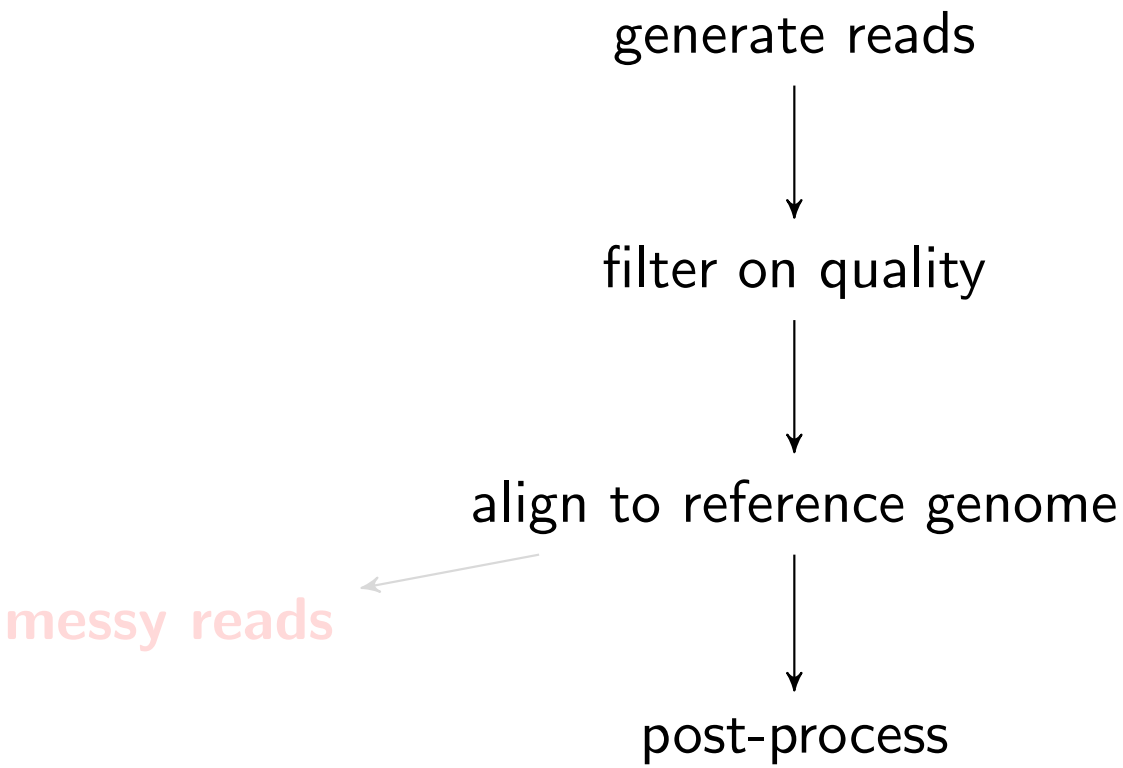

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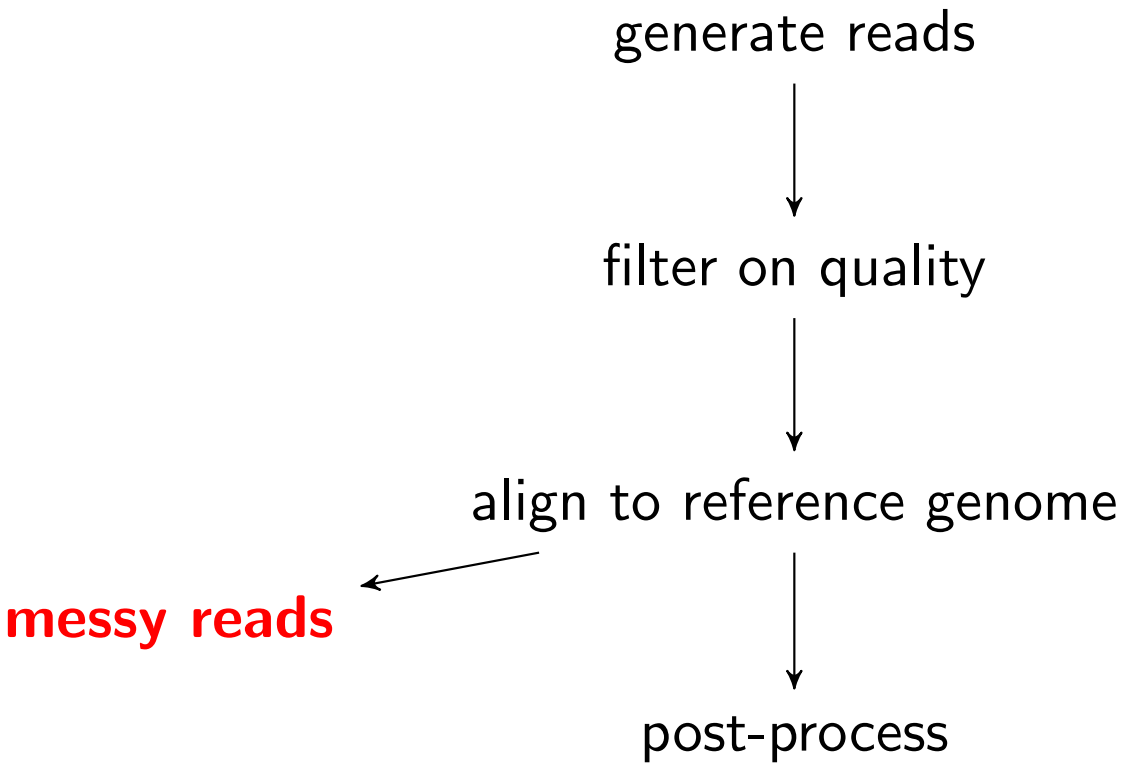

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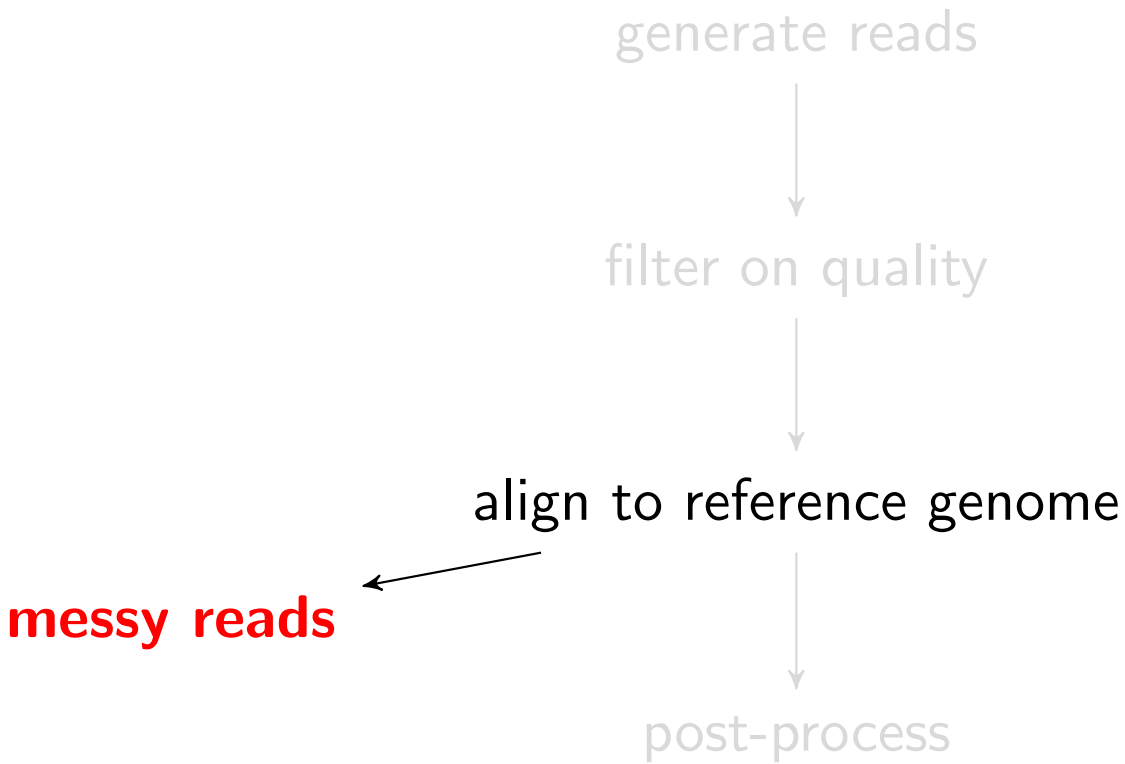
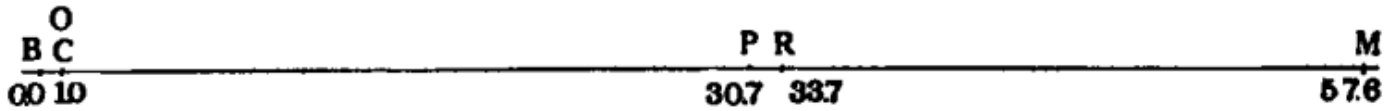


What is a reference genome?

THE LINEAR ARRANGEMENT OF SIX SEX-LINKED FACTORS IN DROSOPHILA, AS SHOWN BY THEIR MODE OF ASSOCIATION

A. H. STURTEVANT

From the Zoölogical Laboratory, Columbia University



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Insight: genomes of multiple individuals are collinear.

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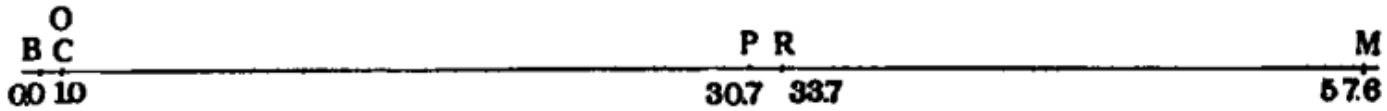

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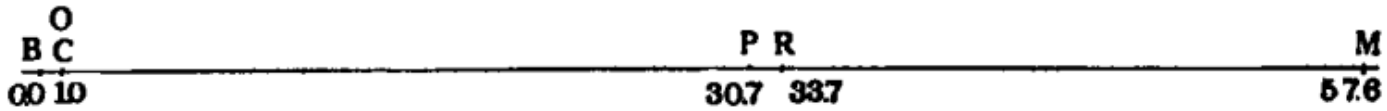

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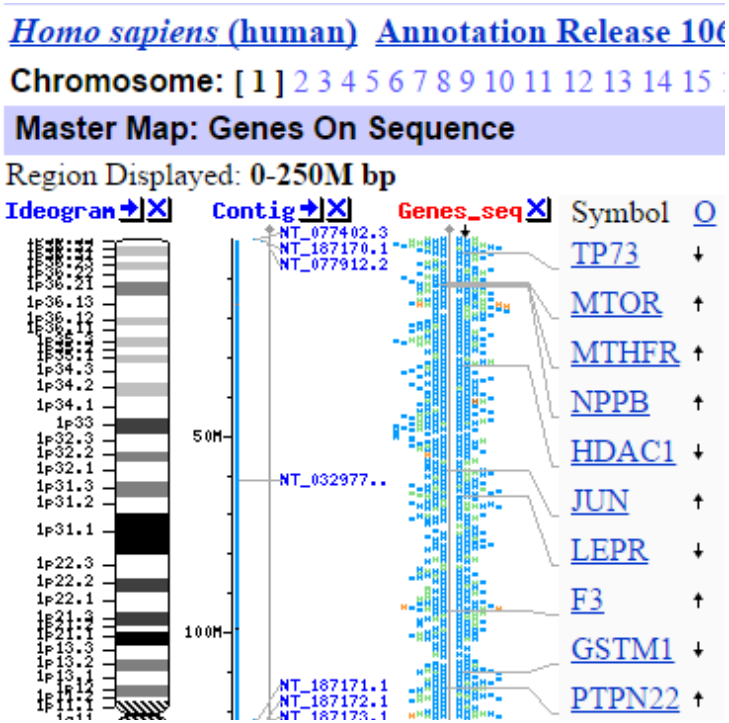

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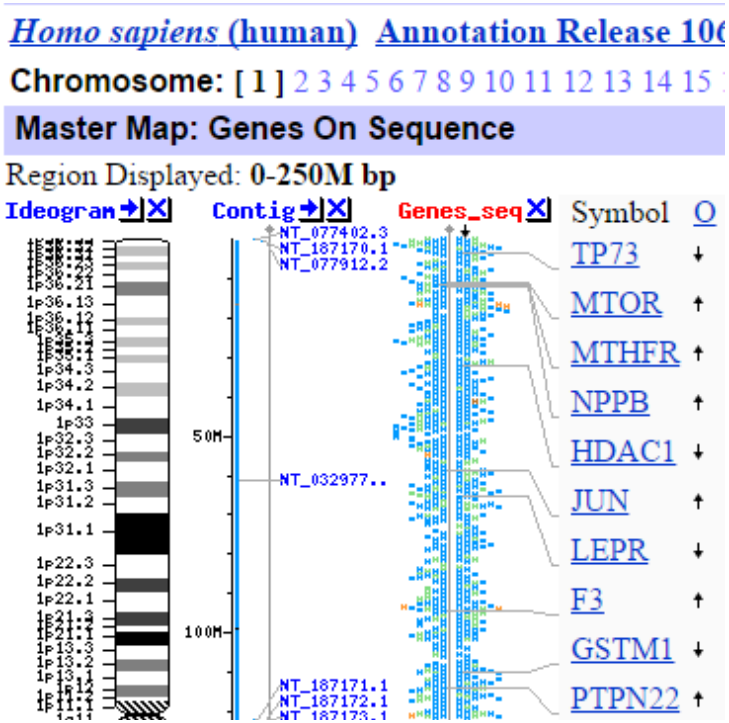

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Why use a reference?

Practical reasons

- Convenience
- Efficiency
- Compression/summary
- Generalizability

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The reads are the data.

An unruly beast

Challenges:

- *De novo* assembly is hard
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Enter the Burrows-Wheeler transform (BWT)

Constructing the BWT for the string `APPLE`.

rotation	sorted	BWT
⋆APPLE$	APPLE$⋆	**⋆**
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Extending BWT to many strings, efficiently

BIOINFORMATICS ORIGINAL PAPER

2014, pages 1–8
doi:10.1093/bioinformatics/btu584

Sequence analysis

Advance Access publication August 28, 2014

Merging of multi-string BWTs with applications

James Holt* and Leonard McMillan

Department of Computer Science, 201 S. Columbia St. UNC-CH, Chapel Hill, NC 27599, USA

Associate Editor: Michael Brudno

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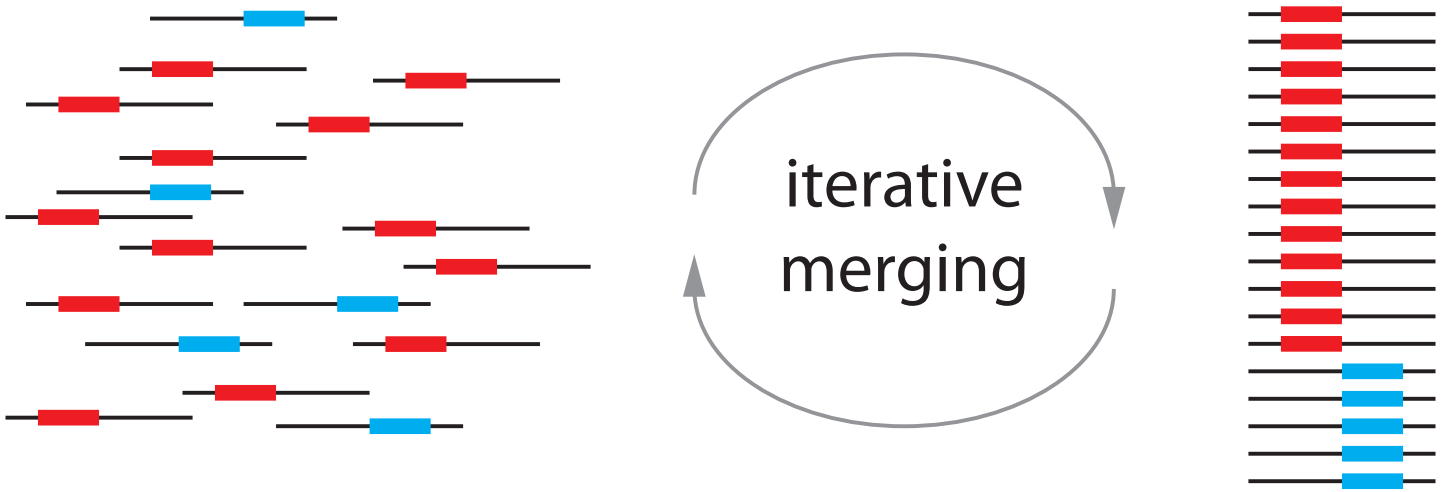

Theoretical properties of merged msBWT

Define

ℓ = length of longest common substring among strings

n = total number of strings

N = sum of length of all n strings

m = total number of msBWTs being merged

k = word size (for later)

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- Final msBWT requires $O(N \log_2 m)$ space
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Basic operations:

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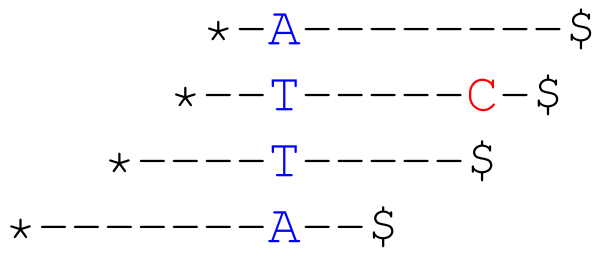

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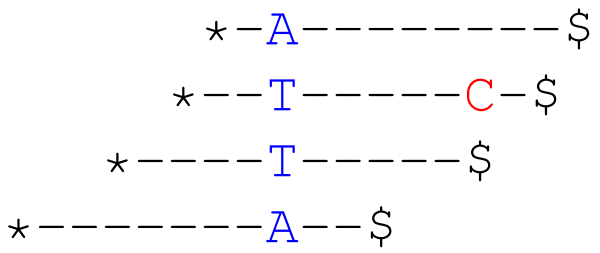

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so $\mathbb{E}[w] = 1/\varepsilon$.

Let π be the pairwise sequence divergence between the templace and the reference, and s the distance between variants.

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Note that

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. . . and can choose k sufficiently large that $\mathbb{E}[c|k] \approx X$ for "nice" queries.

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Example uses of the msBWT

1. Assembling the mouse mitochondrial genome
2. A complex structural variant in mouse
3. Profiling the gut microbiota

Example 1: assembling the 17kbp mouse mitochondrial genome

pick a seed sequence ("bait")

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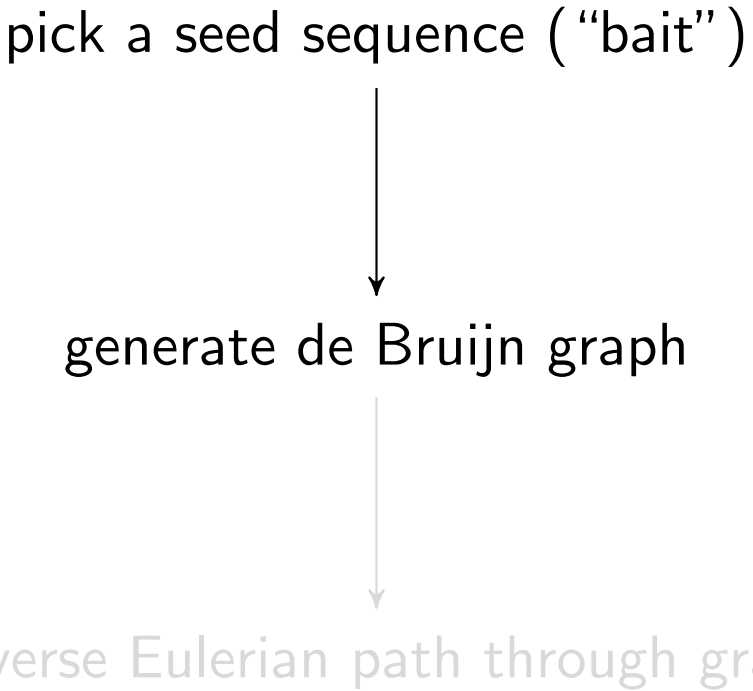

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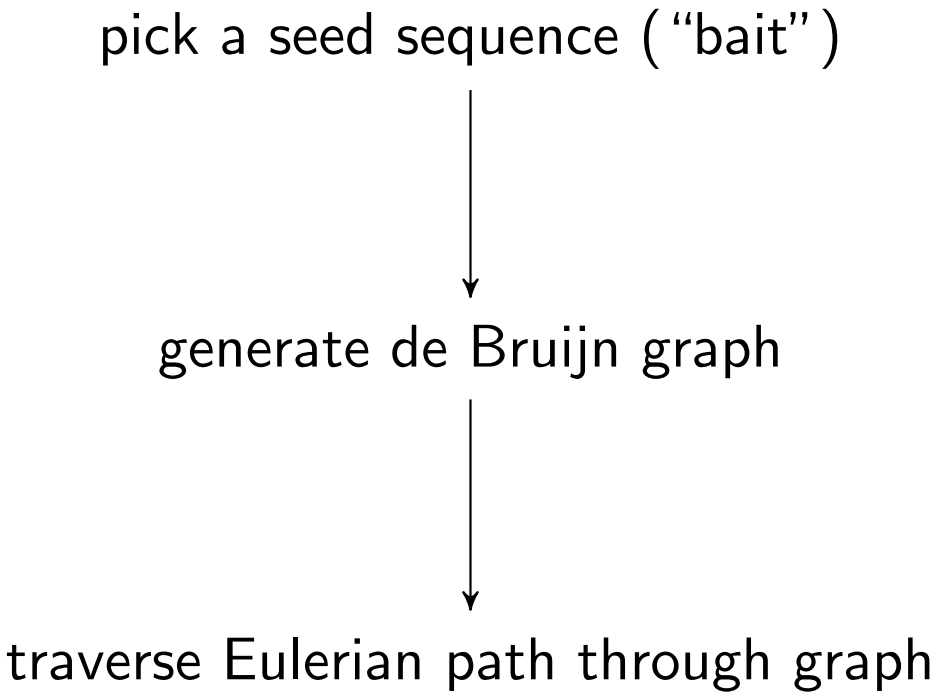

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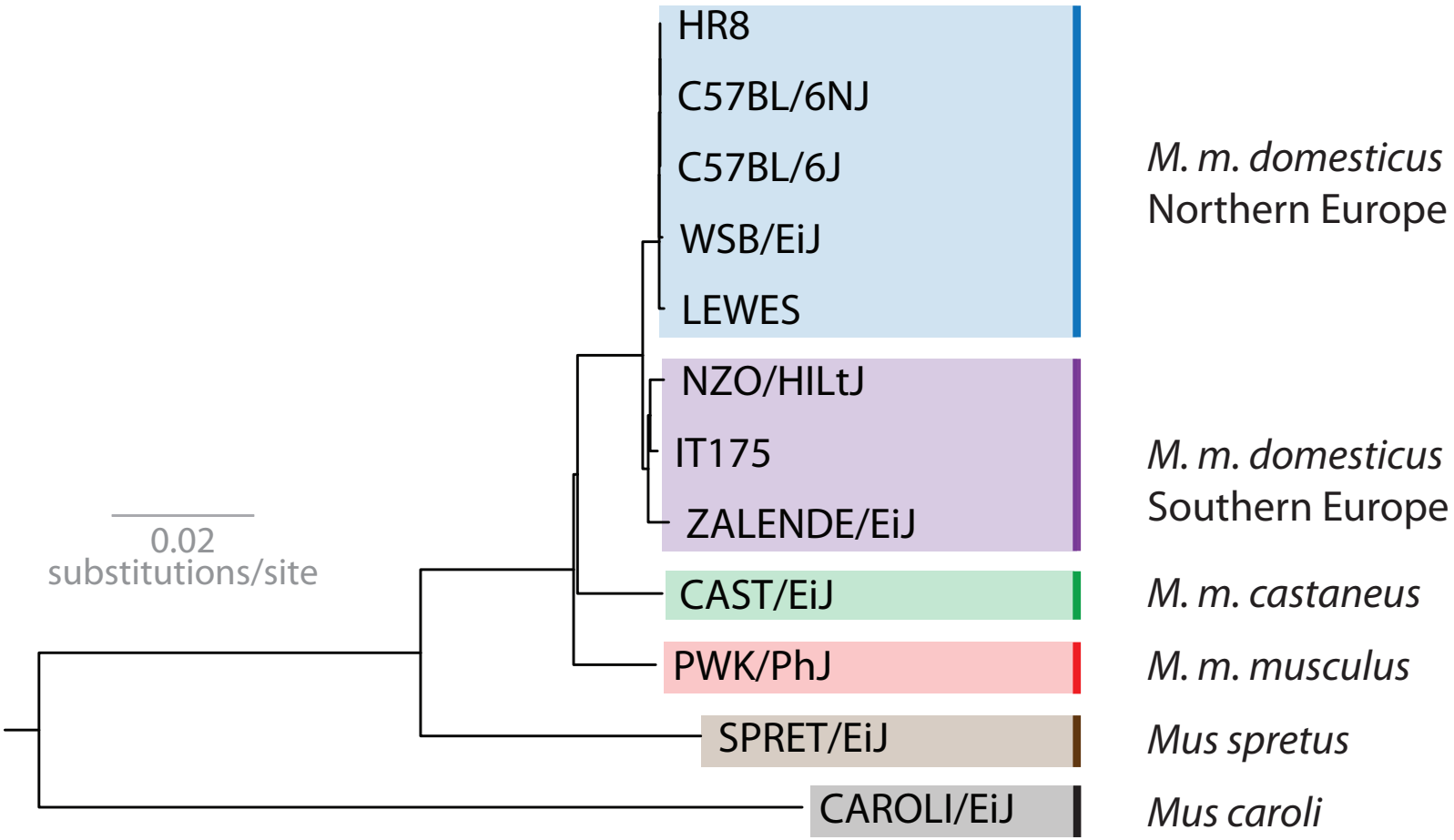

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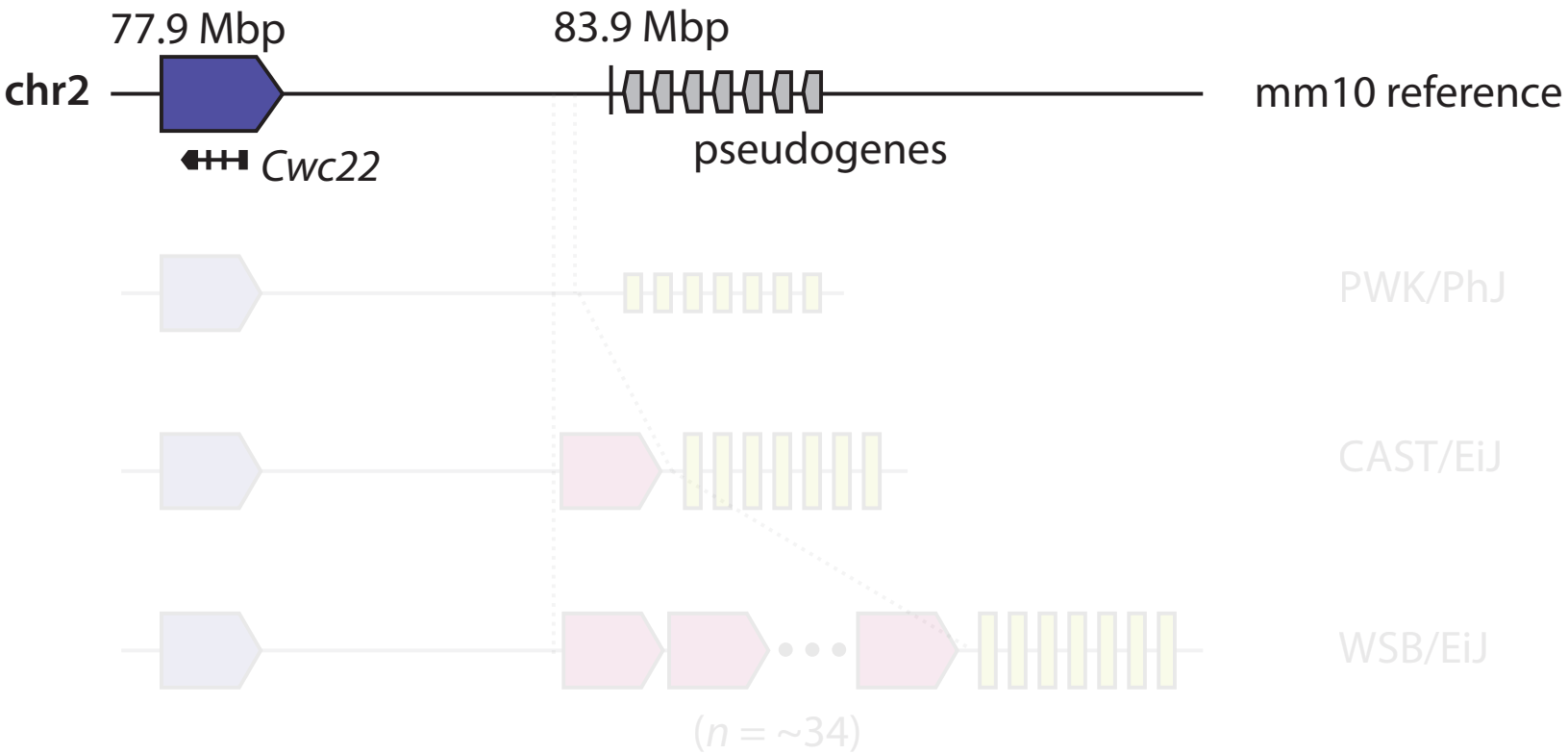

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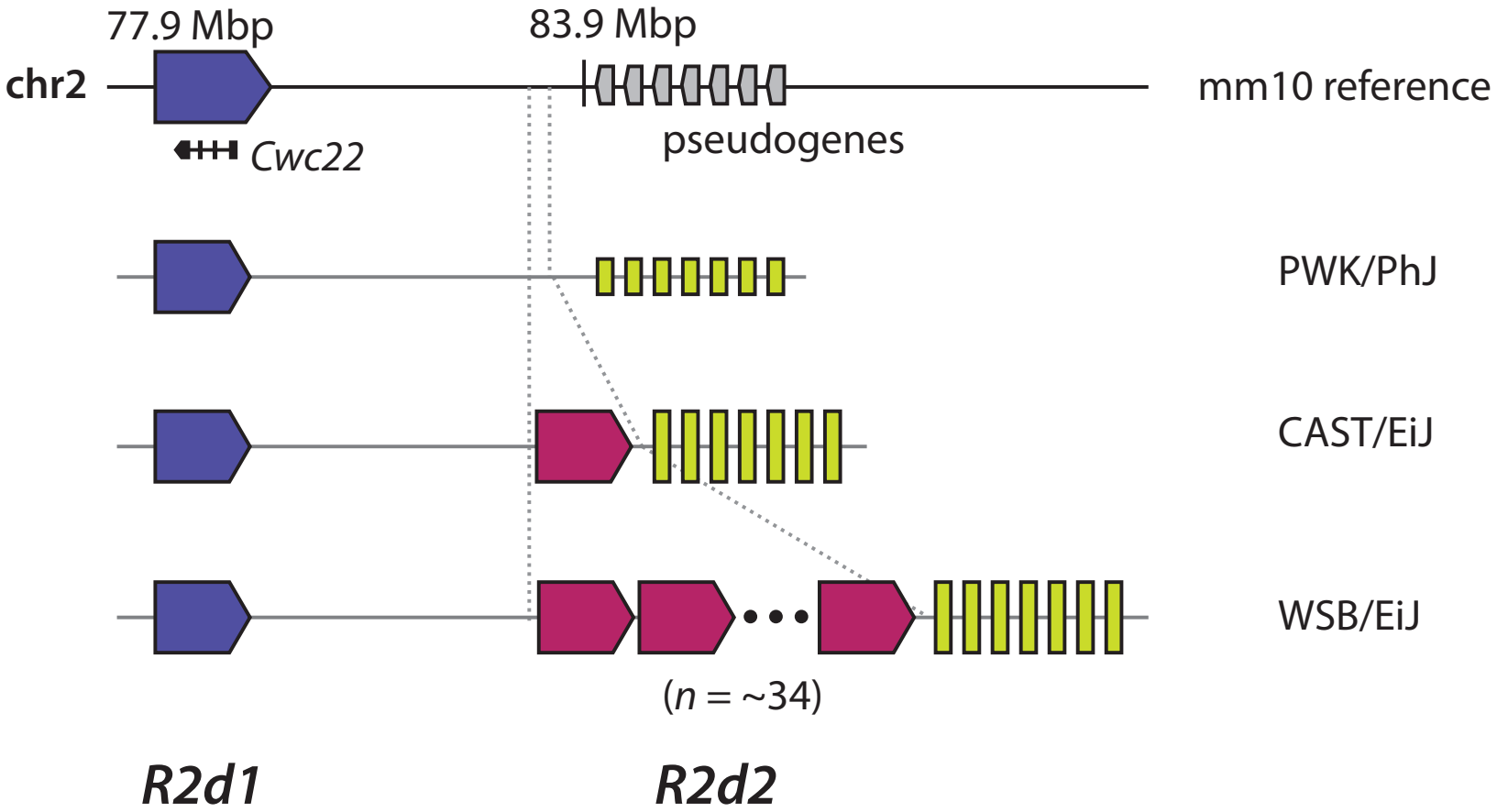

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Interactive *de novo* assembly

[online demo]

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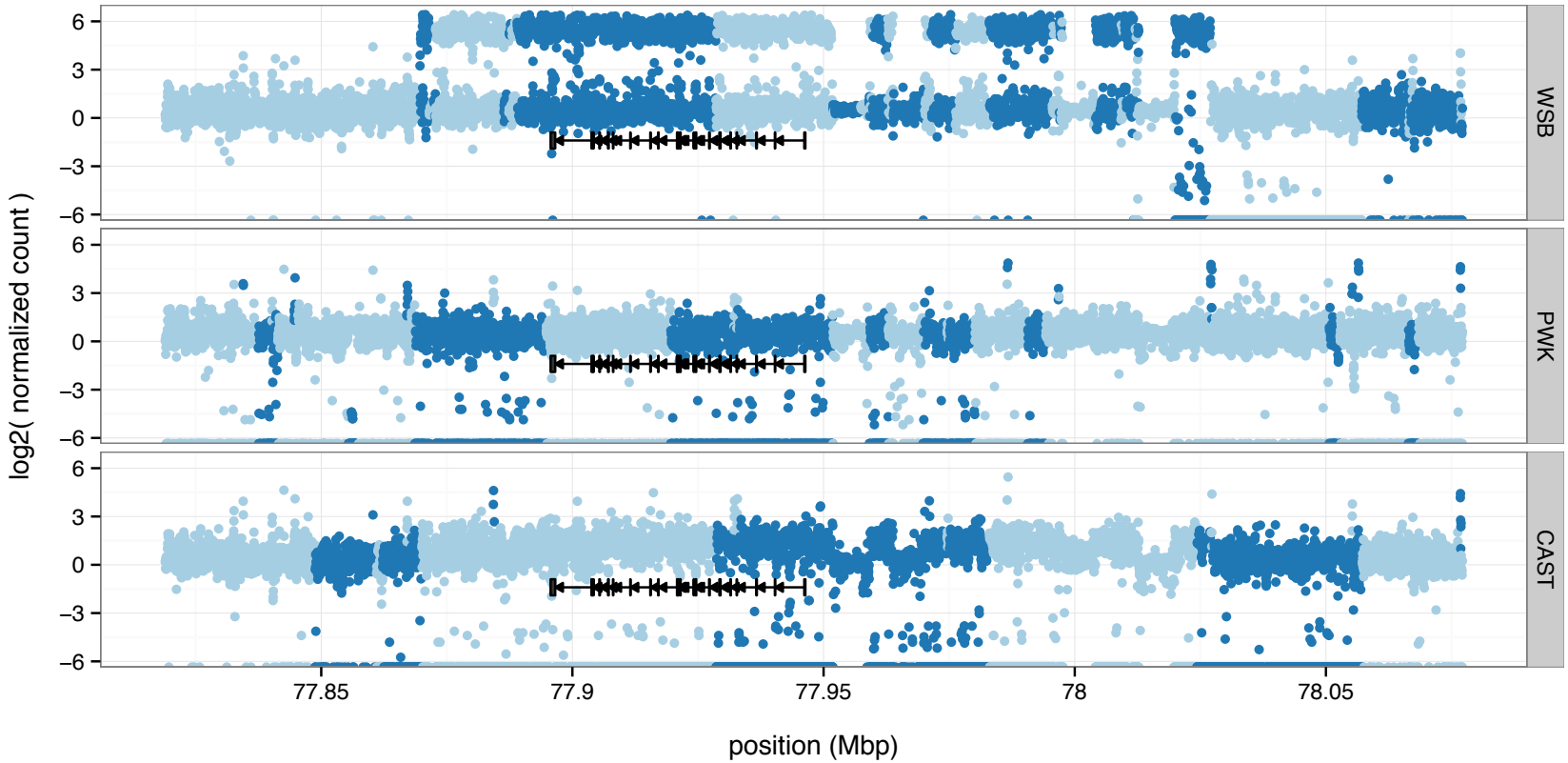
"Confetti plots"



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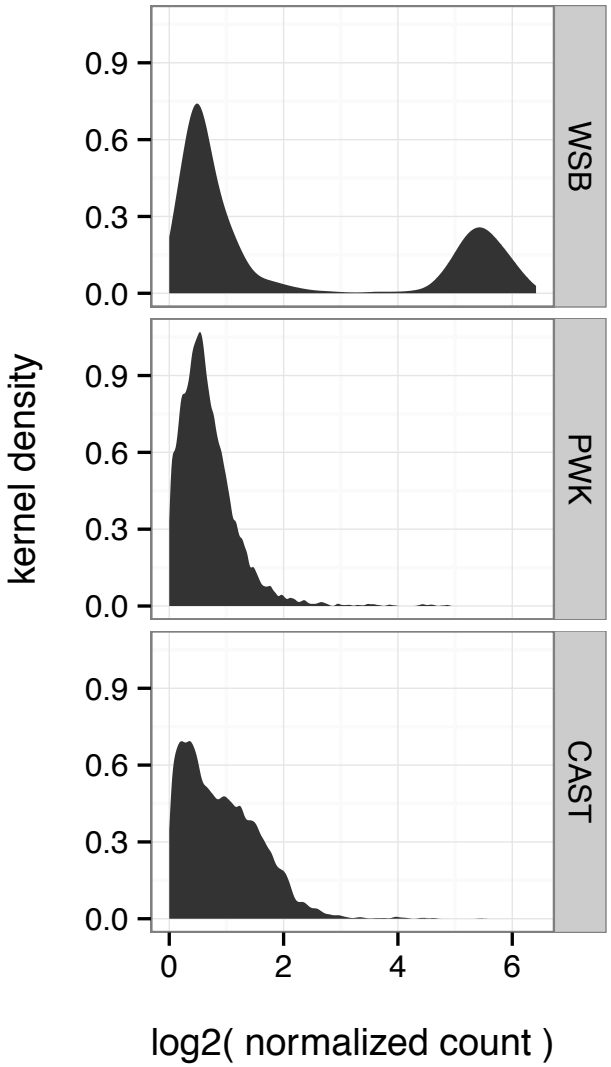
Untangling multiple copies



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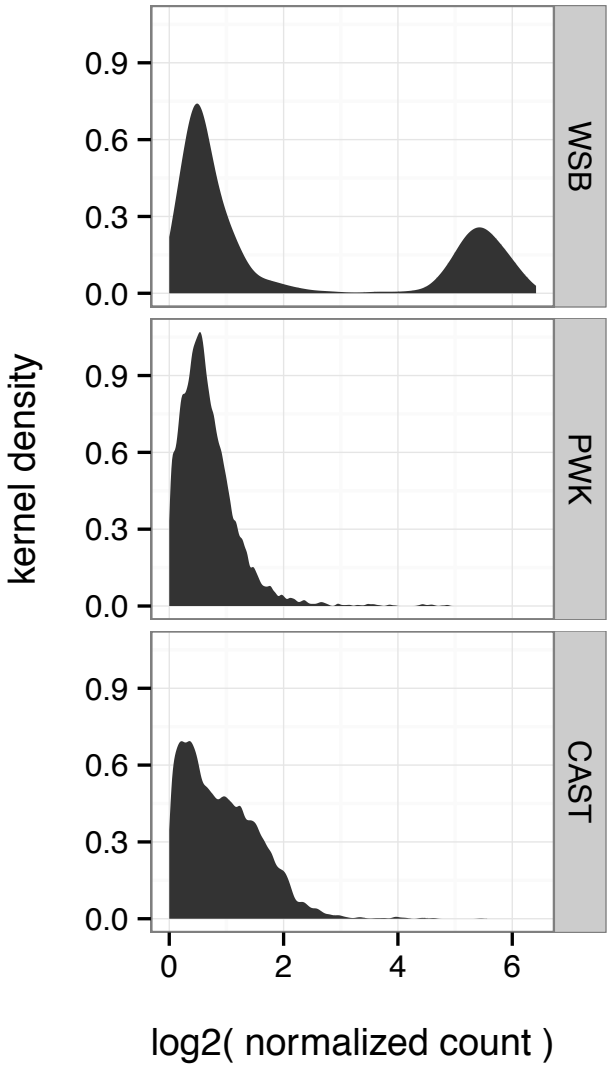

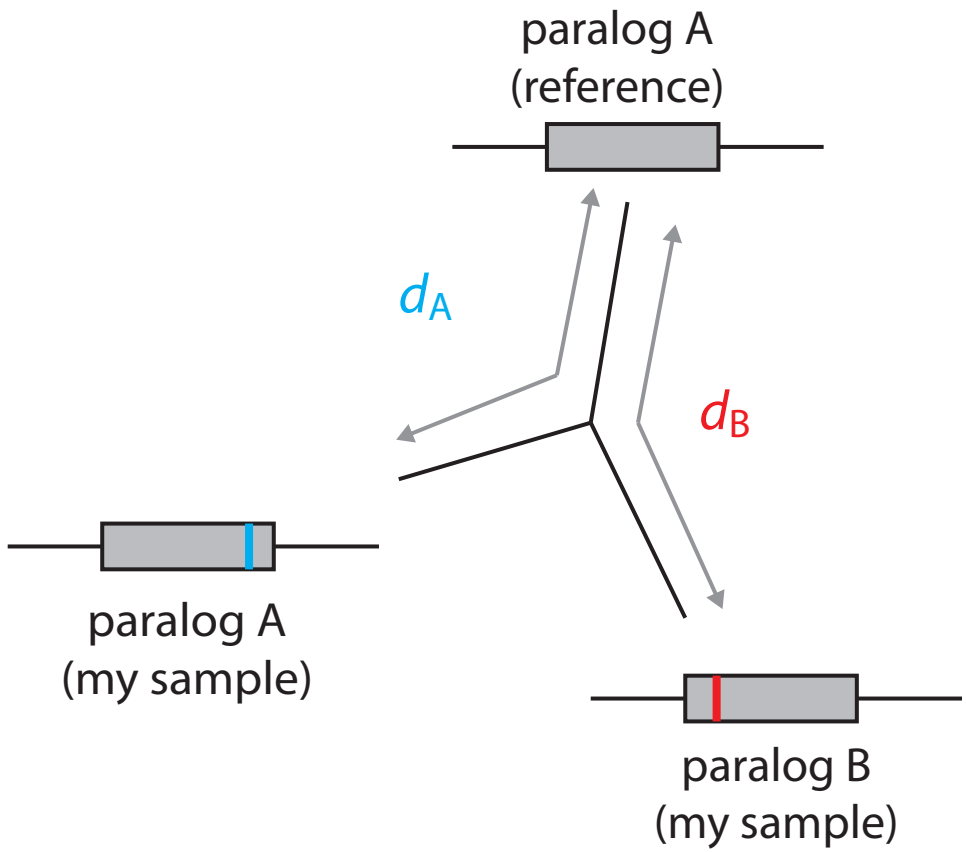

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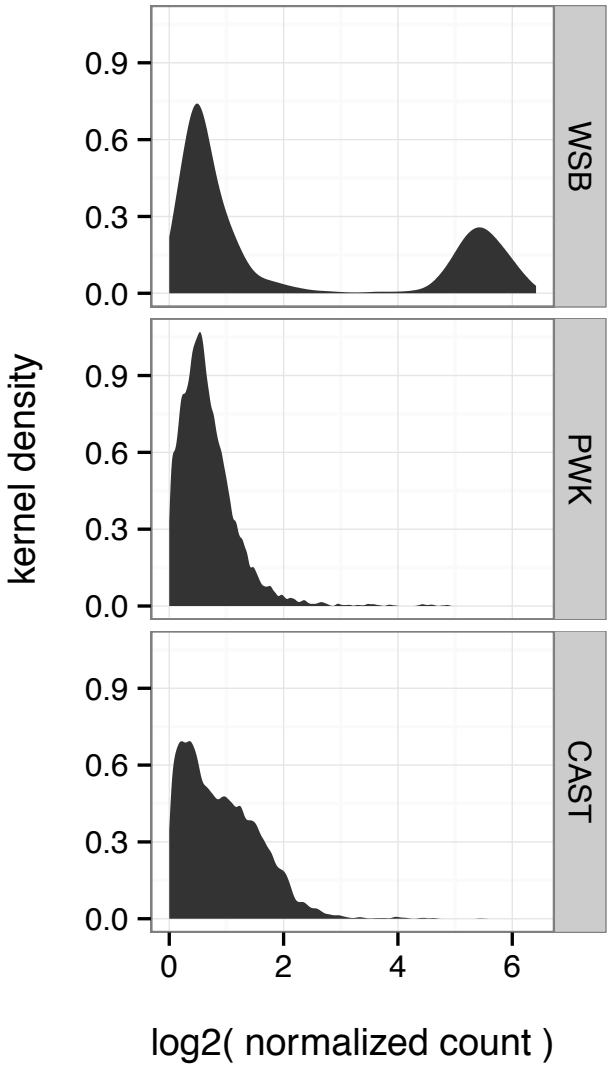

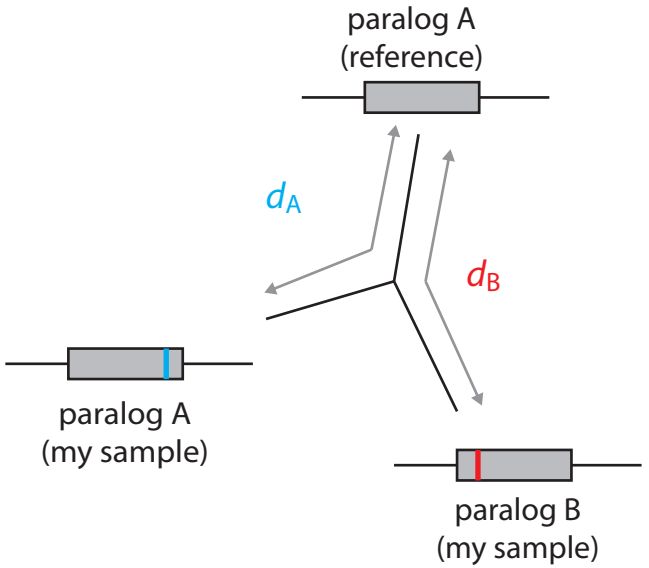

Can assign k-mers to copies using mixture model. Define mixing proportions π_i; then

$$\frac{\pi_A}{\pi_B} \approx \frac{d_A}{d_B}$$

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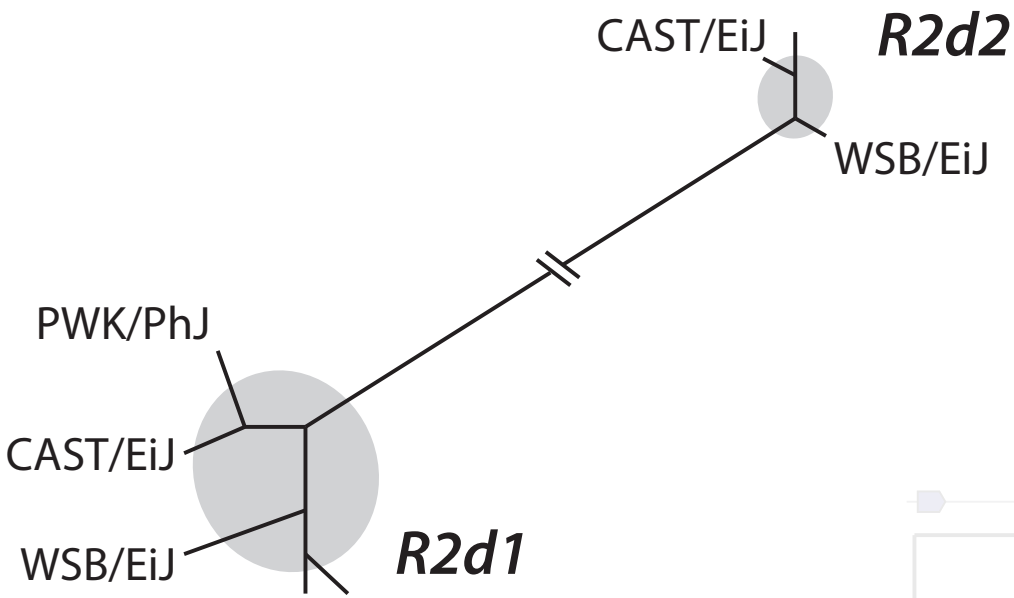
Phylogeny with novel sequence



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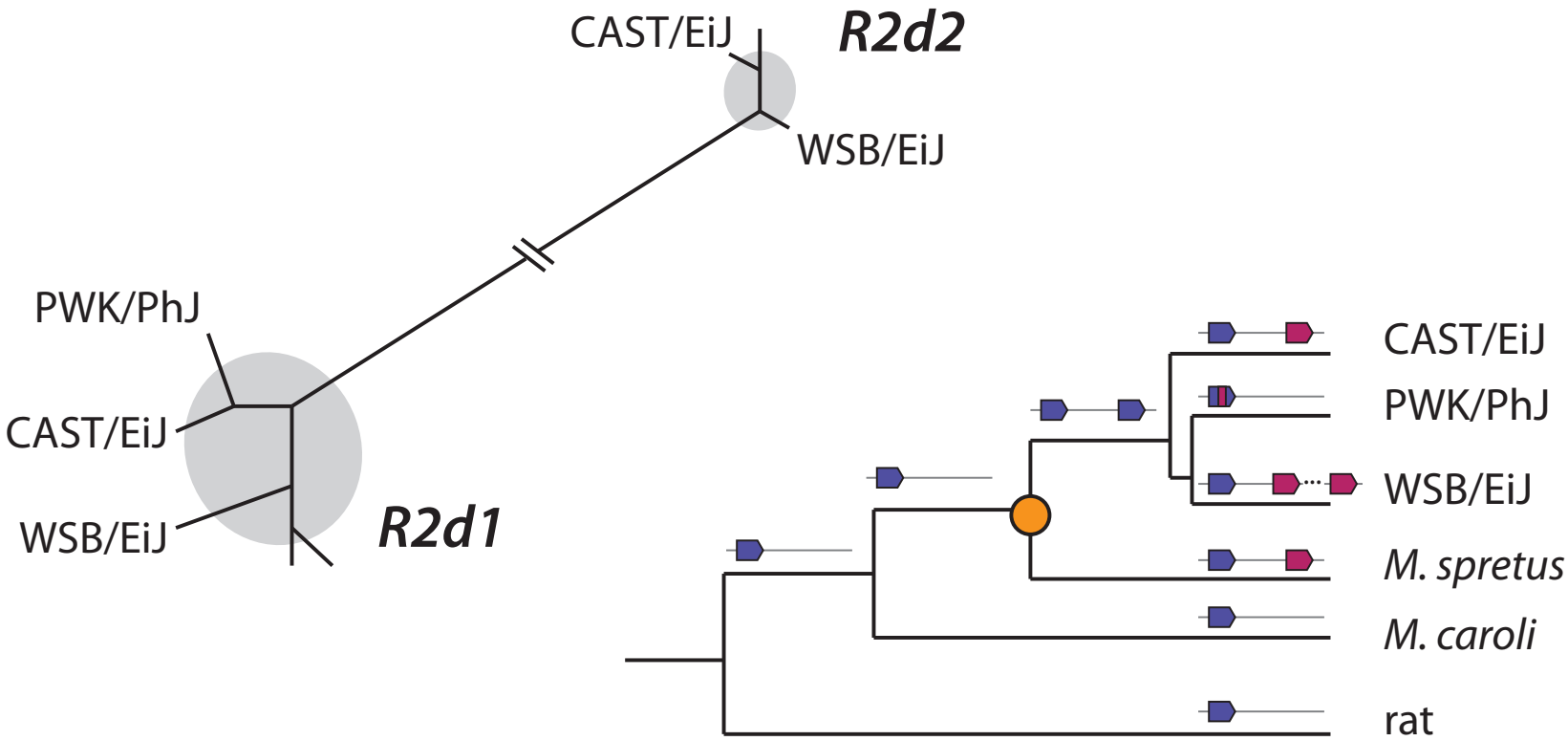
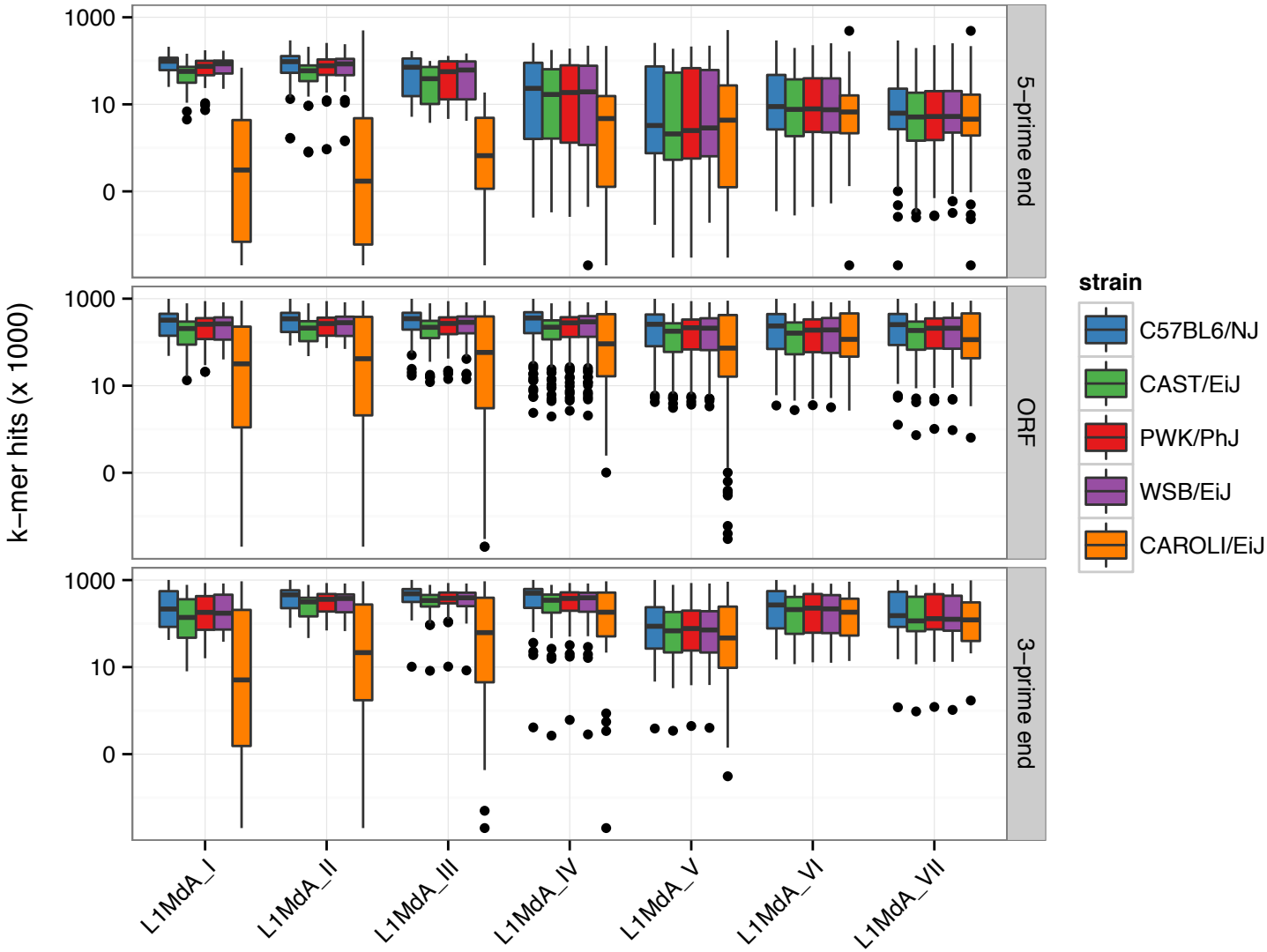


Aside: age of repeat elements



Applications of msBWT for molecular biology

- design of PCR assays
- design of oligonucleotide probes (qPCR, microarray)
- direct query of structural variants
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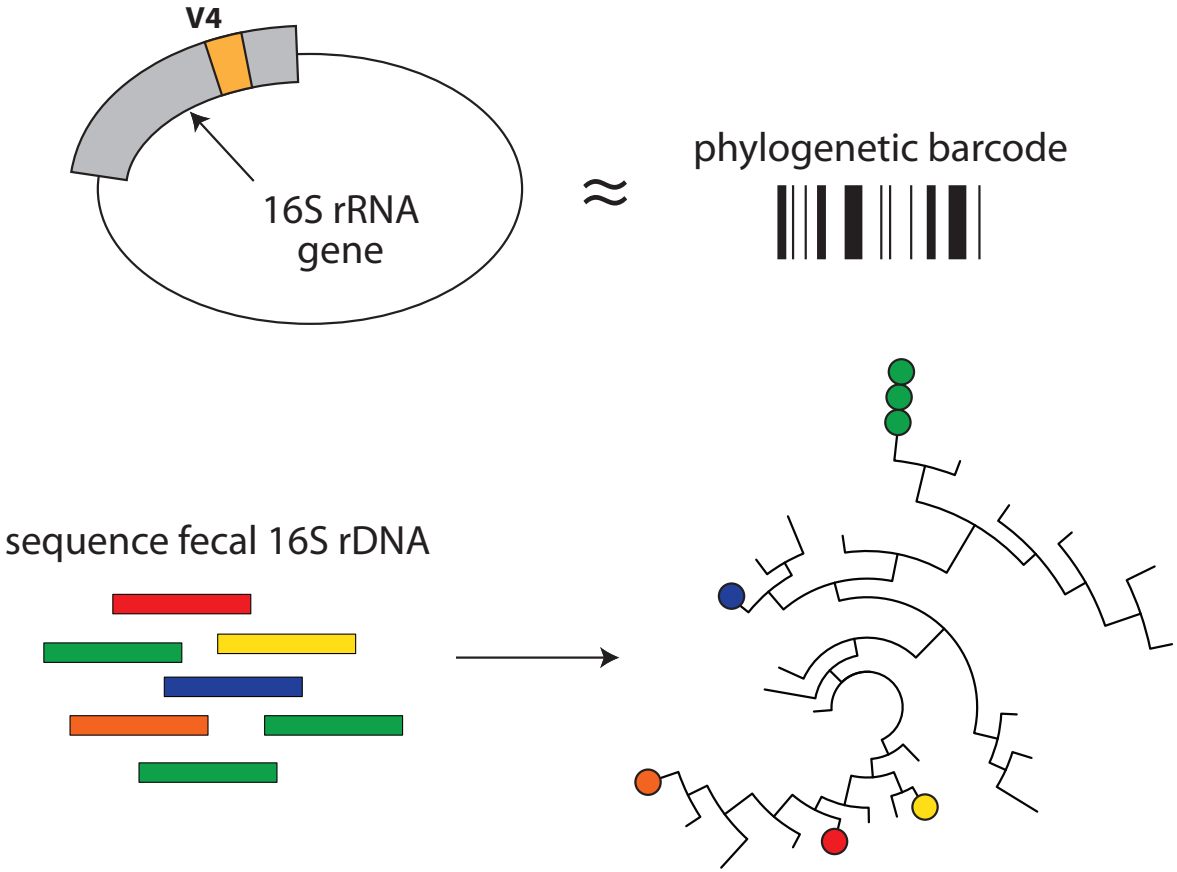
Example 3: Profiling the gut microbiota



Example 3: Profiling the gut microbiota

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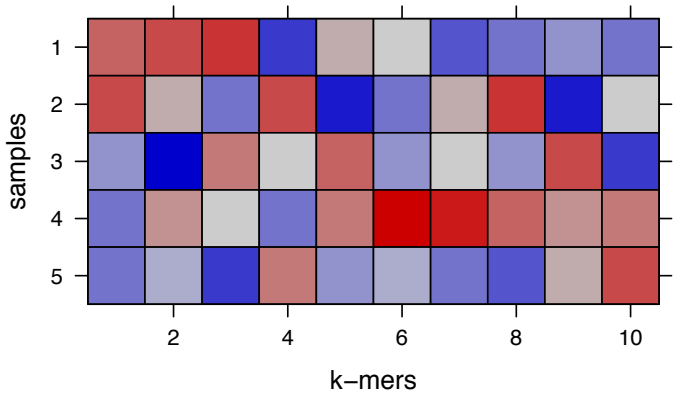
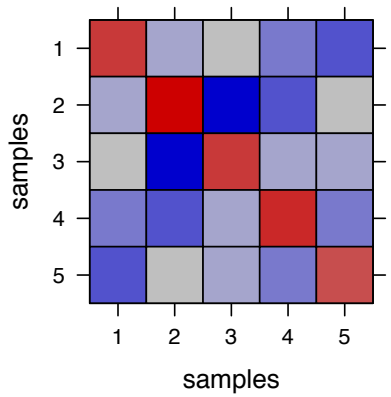


$$\frac{1}{n}\mathbf{Y}\mathbf{Y}^T = \mathbf{D} =$$



Multivariate ANOVA, given a matrix $\mathbf{X}_{n \times q} = [x_1 \ldots x_q]$ of covariates:

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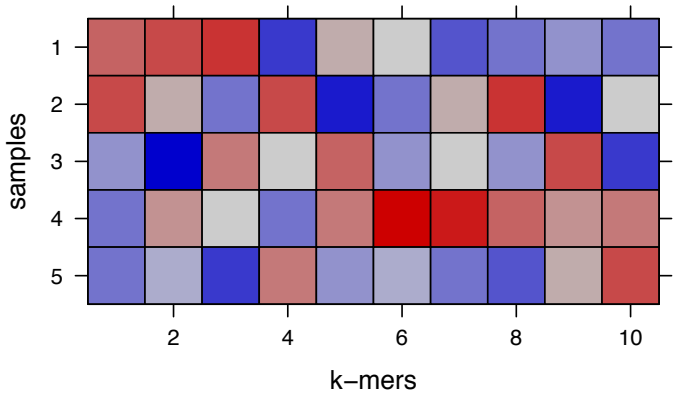

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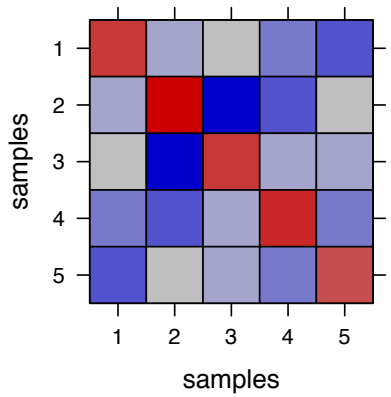

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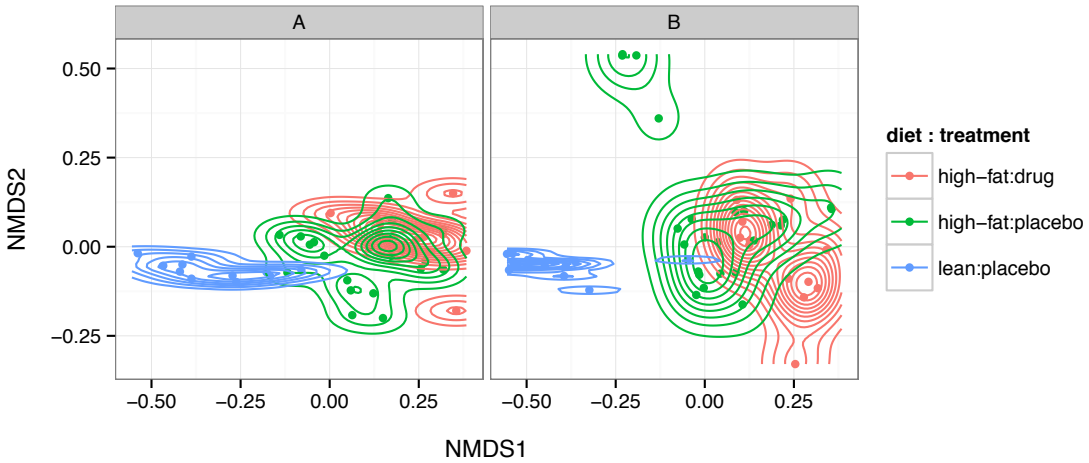

Using naïve *k*-mer frequencies from msBWT

Time: ∼ 2 hours

Morgan, Crowley et al. *PLoS One*, in process.

Using `MTToolbox` + `qiime` + `UniFrac`

Time: ∼ 8 hours

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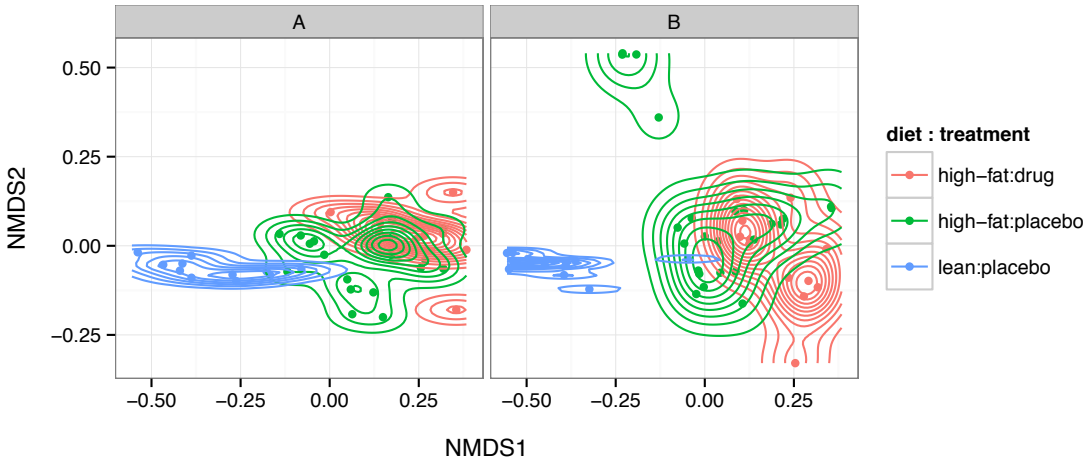

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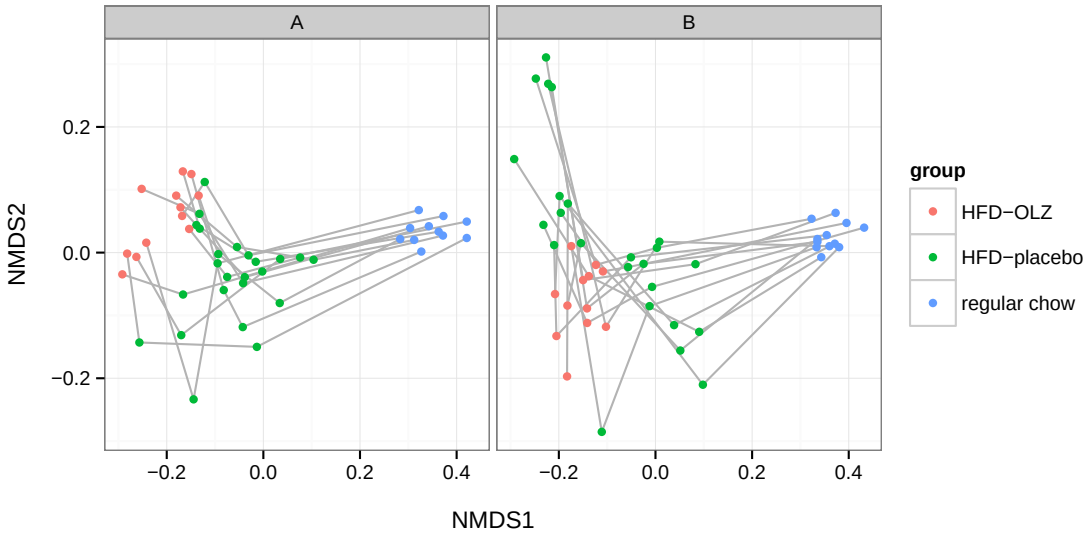

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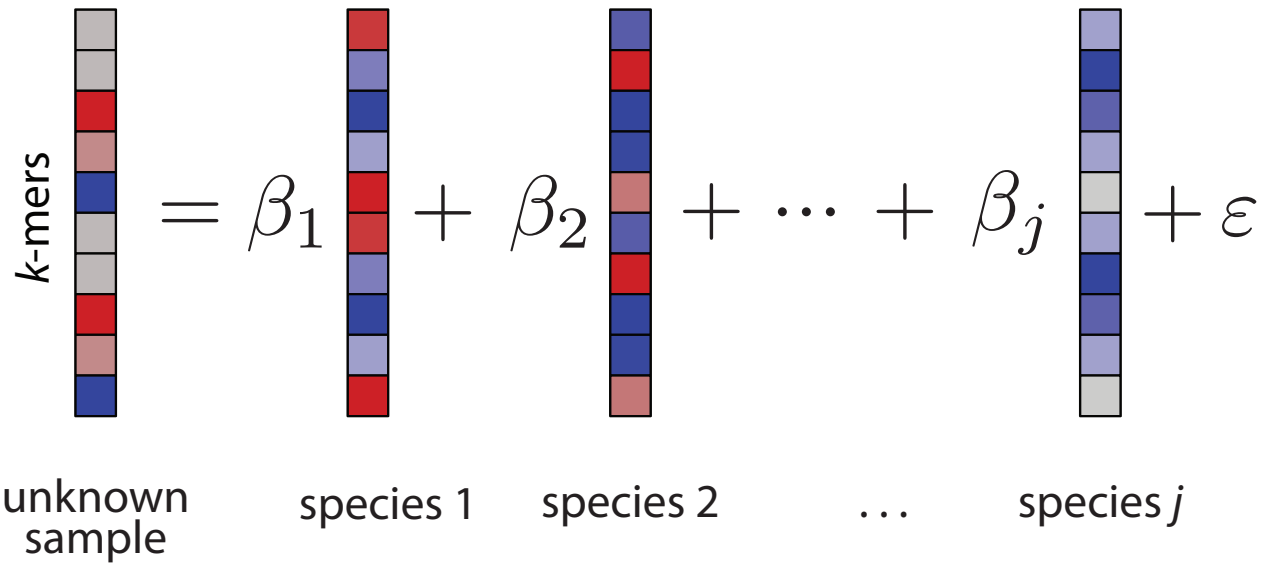
Extension: microbial abundance estimation from *k*-mers



Future directions (among many)

- Unsupervised structural variant detection
- Unsupervised *de novo* assembly, starting from arbitrary seed
- Joint analysis of RNAseq and DNAseq from same sample
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  - RNA editing
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Try it yourself

Interactive queries hosted on `csbio` cluster

`www.csbio.unc.edu/CEGSseq/?run=msBWT`

Download the `msbwt` Python package

`pypi.python.org/pypi/msbwt/0.2.4`

See my (rudimentary) scripts

`github.com/andrewparkermorgan/snoop`

Questions?