Genome Skimming Workshop:

Using Low Coverage WGS to Estimate Distances and Sequencing Parameters.

by Eduardo Charvel and Siavash Mirarab

UCSD

ALFRED P. SLOAN FOUNDATION

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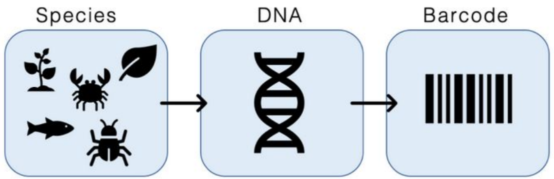
Cloning GitHub and Installing Tools:

```
git clone https://github.com/echarvel3/skimming_scripts-echarvel.git
```

```
cd skimming_scripts-echarvel
bash ./install.sh
```

Intro: Evolutionary Ecology Approaches

Genetic Markers



marker genes, mtDNA, microsatellites

PROS:

- cost-effective (single genes)
- no recombination (mtDNA)

CONS:

- expensive (larger bait sets)
- limited resolution (single genes)
- reference dependency
- homoplasy

SNP based

RAD-seq, ANGSD, GATK

PROS:

- genome-wide resolution (WGS)

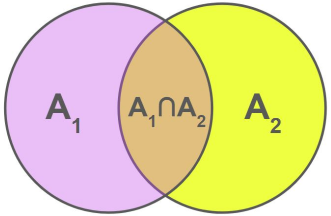
CONS:

- reference-based (WGS)
- higher coverage (>10x) (WGS)
- restriction-enzymes limit resolution (RAD-seq)

SNP

Genotype	1	2	3	4	5	6
1	0	1	1	1	0	1
2	0	1	0	0	0	0
3	1	0	0	1	1	1

k-mer based



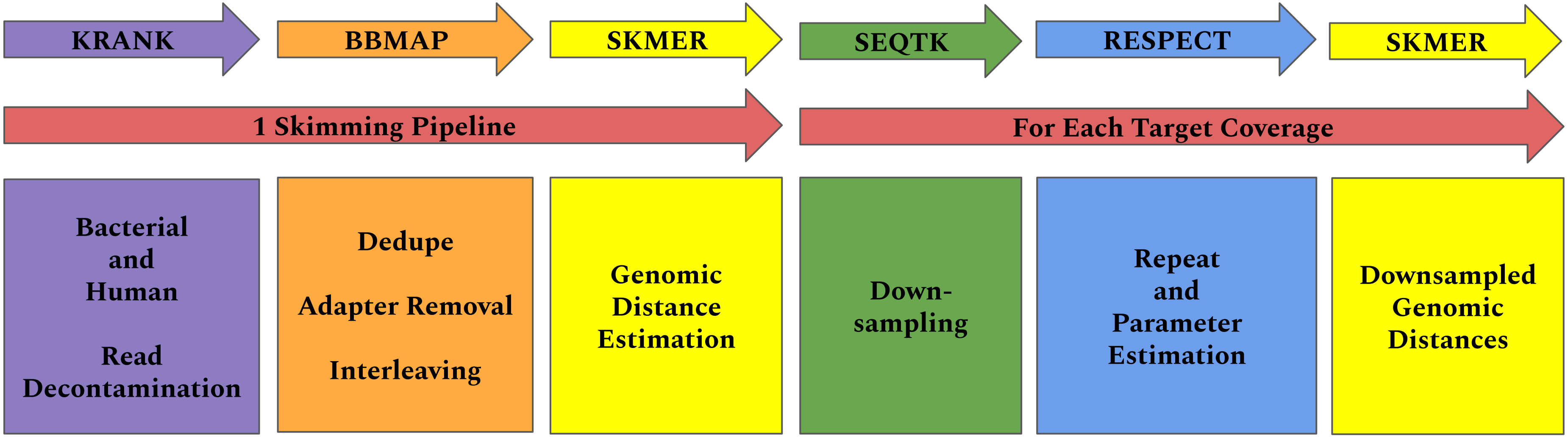
PROS:

- genome-wide resolution
- low coverage (< 1x .. 8x)
- alignment-free

CONS:

- sensitive to sequencing quality:
  - contamination *(addressed in this workshop)*
  - repetitiveness *(active area of research)*
  - hybridization

Skimming Pipeline Overview



KRANK

BBMAP

SKMER

SEQTK

RESPECT

SKMER

1 Skimming Pipeline

For Each Target Coverage

Bacterial and Human Read Decontamination

Dedupe

Adapter Removal

Interleaving

Genomic Distance Estimation

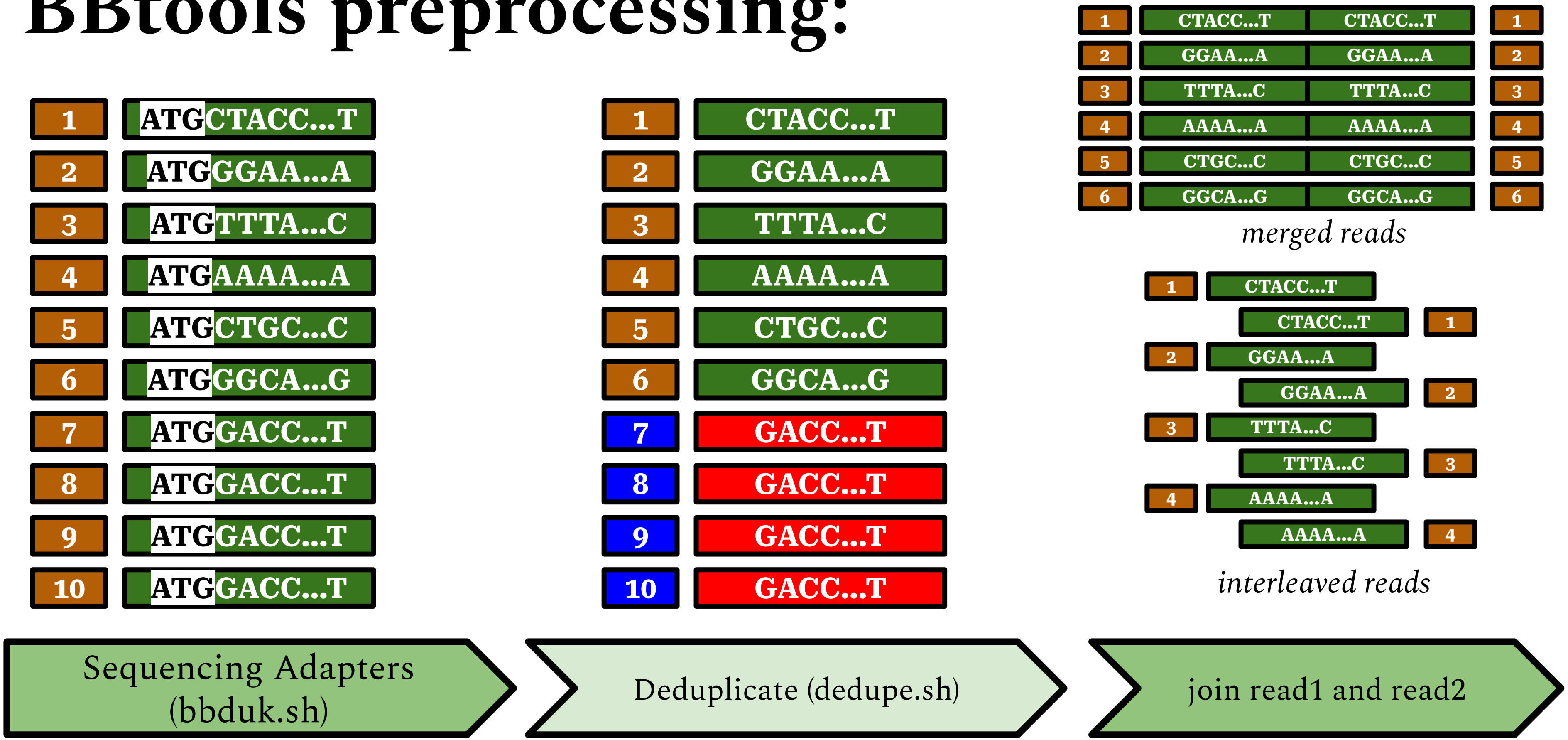
Down-sampling

Repeat and Parameter Estimation

Downsampled Genomic Distances

- Meant to easily perform all operations necessary for accurate genomic distance and sequencing parameter estimation.

BBtools preprocessing:



1	ATGCTACC...T
2	ATGGGAA...A
3	ATGTTTA...C
4	ATGAAAA...A
5	ATGCTGC...C
6	ATGGGCA...G
7	ATGGACC...T
8	ATGGACC...T
9	ATGGACC...T
10	ATGGACC...T

1	CTACC...T
2	GGAA...A
3	TTTA...C
4	AAAA...A
5	CTGC...C
6	GGCA...G
7	GACC...T
8	GACC...T
9	GACC...T
10	GACC...T

1	CTACC...T	CTACC...T	1
2	GGAA...A	GGAA...A	2
3	TTTA...C	TTTA...C	3
4	AAAA...A	AAAA...A	4
5	CTGC...C	CTGC...C	5
6	GGCA...G	GGCA...G	6

merged reads

1	CTACC...T	
	CTACC...T	1
2	GGAA...A	
	GGAA...A	2
3	TTTA...C	
	TTTA...C	3
4	AAAA...A	
	AAAA...A	4

interleaved reads

Sequencing Adapters (bbduk.sh)

Deduplicate (dedupe.sh)

join read1 and read2

Running BBMAP

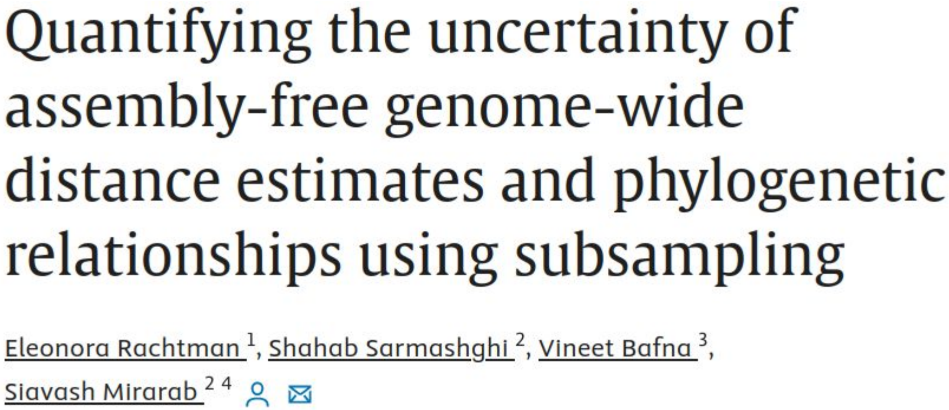
```
cd skimming_scripts-echarvel
conda activate ${CONDA_ENV} #replace with env name
cd ./test/
gunzip ./skims/*gz
mkdir bbmap_reads
bash ../bbmap_pipeline.sh \
       ./skims/read1.fq \
       ./skims/read2.fq \
       ./bbmap_reads/read_out.fq
```

Main Skmer Paper

Skmer: assembly-free and alignment-free sample identification using genome skims

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

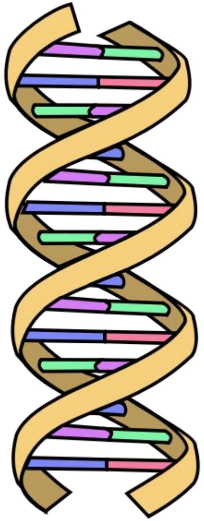
Skmer Bootstrapping Approach



Quantifying the uncertainty of assembly-free genome-wide distance estimates and phylogenetic relationships using subsampling

Eleonora Rachtman[1], Shahab Sarmashghi[2], Vineet Bafna[3], Siavash Mirarab[2 4]

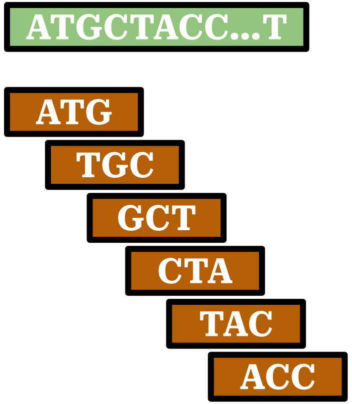
SKMER Theory:

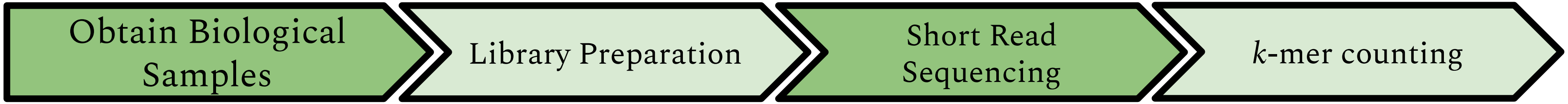


collection of
reads ~ 150 bp

1	ATGCTACC...T
2	CTAGGAA...A
3	CCCTTTA...C
4	GATAAAA...A
5	TATACTGC...C
6	GTCGGCA...G
7	GGACTGC...C
8	CTTATCC...G
9	TAAGTGG...C
10	CAGGACC...T

each can be decomposed
into a set of *k*-mers





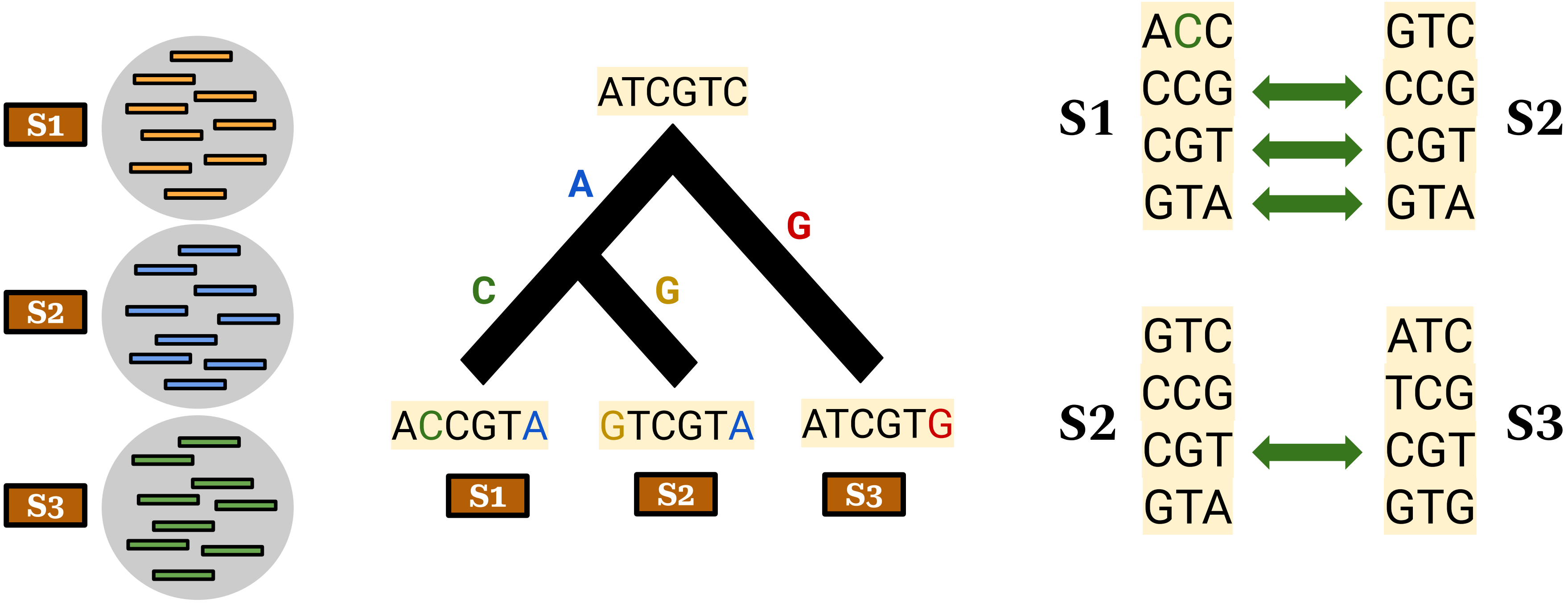
Obtain Biological Samples

Library Preparation

Short Read Sequencing

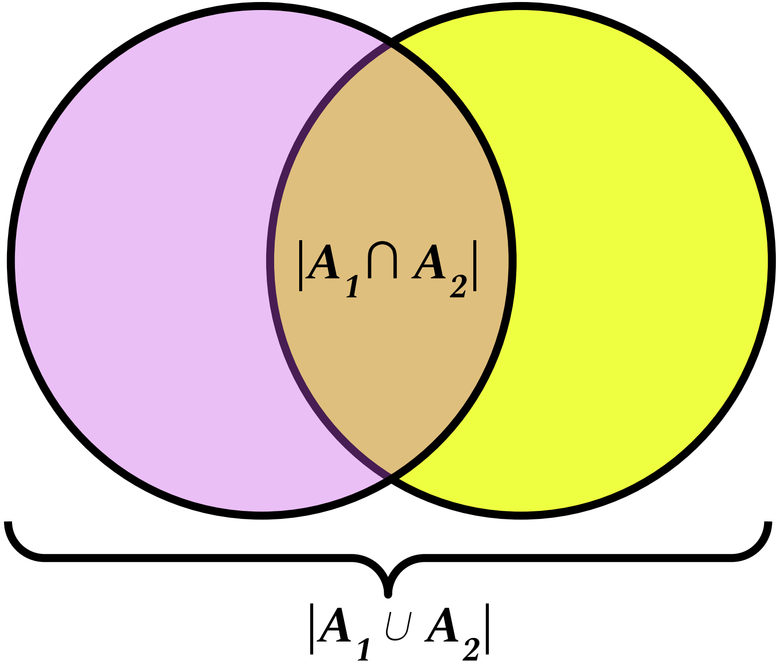
k-mer counting

SKMER Theory:



k-mer set for each sample → Mutations accumulate → Compare *k*-mer similarities

Mash (2016): *k*-mer Based Distances



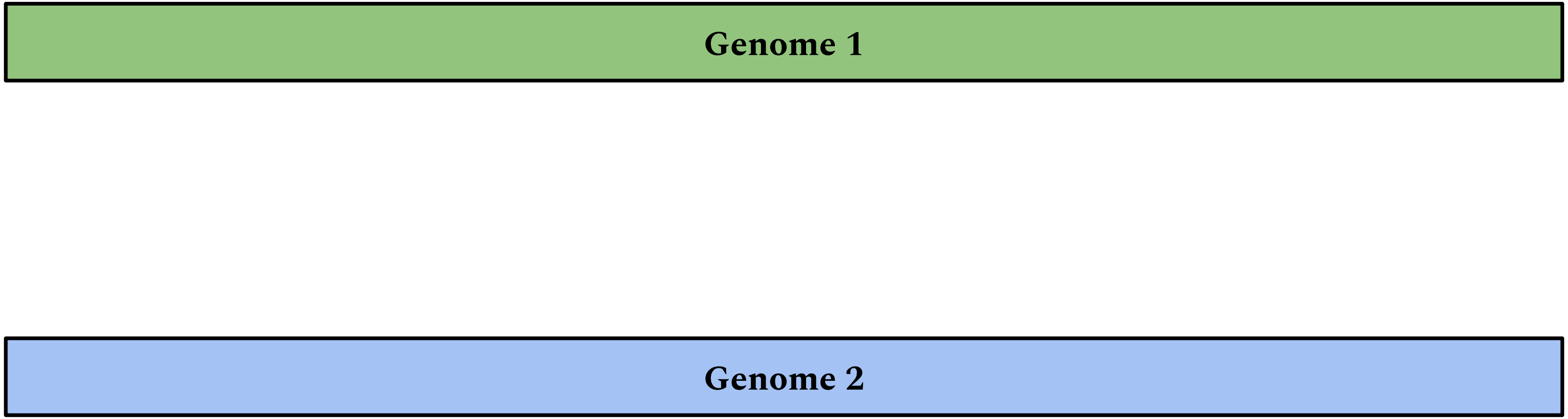
shared *k*-mers = intersection = $|A_1 \cap A_2|$

set of all *k*-mers = union = $|A_1 \cup A_2|$

Jaccard Similarity Index = $J = \dfrac{|A_1 \cap A_2|}{|A_1 \cup A_2|}$

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

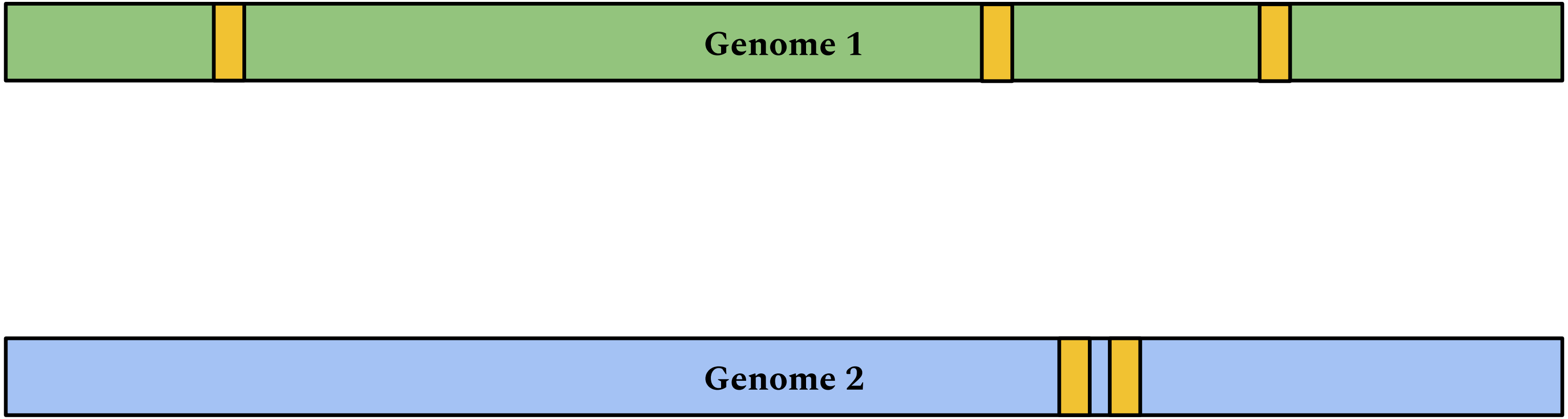
SKMER: Using Low Coverage Skims



Genome 1

Genome 2

SKMER: Using Low Coverage Skims



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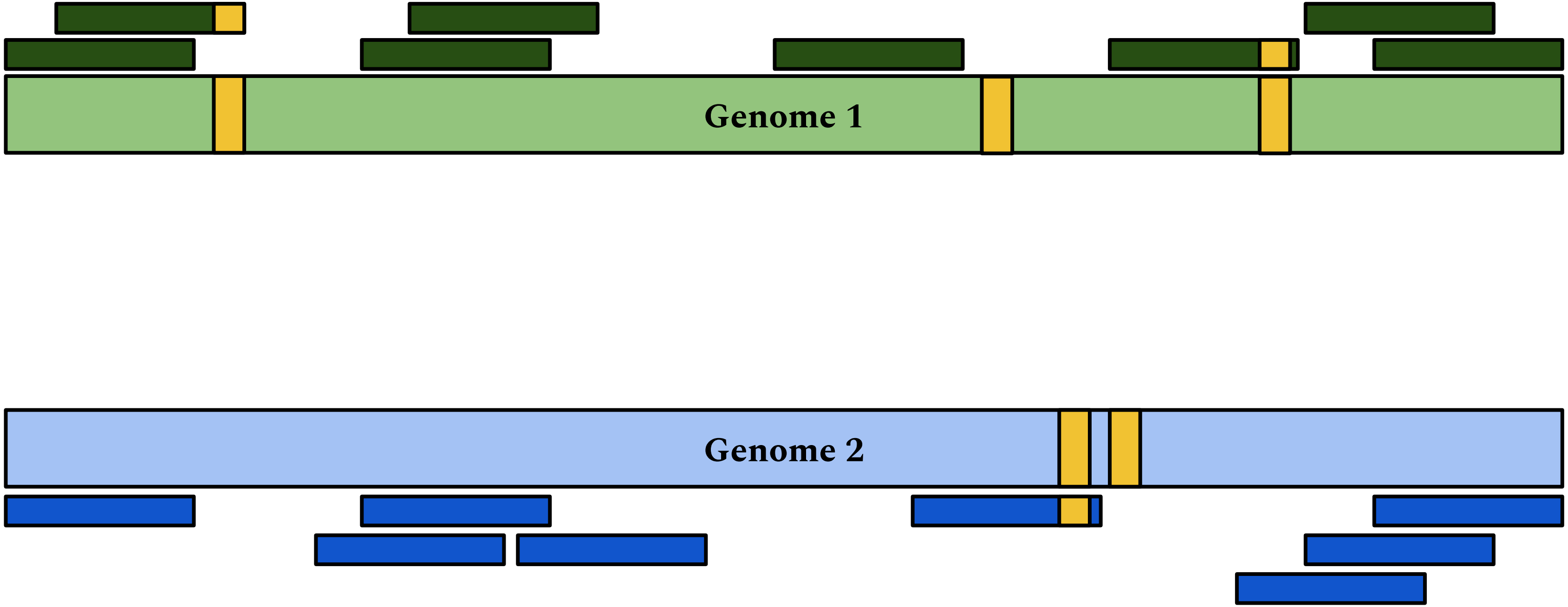

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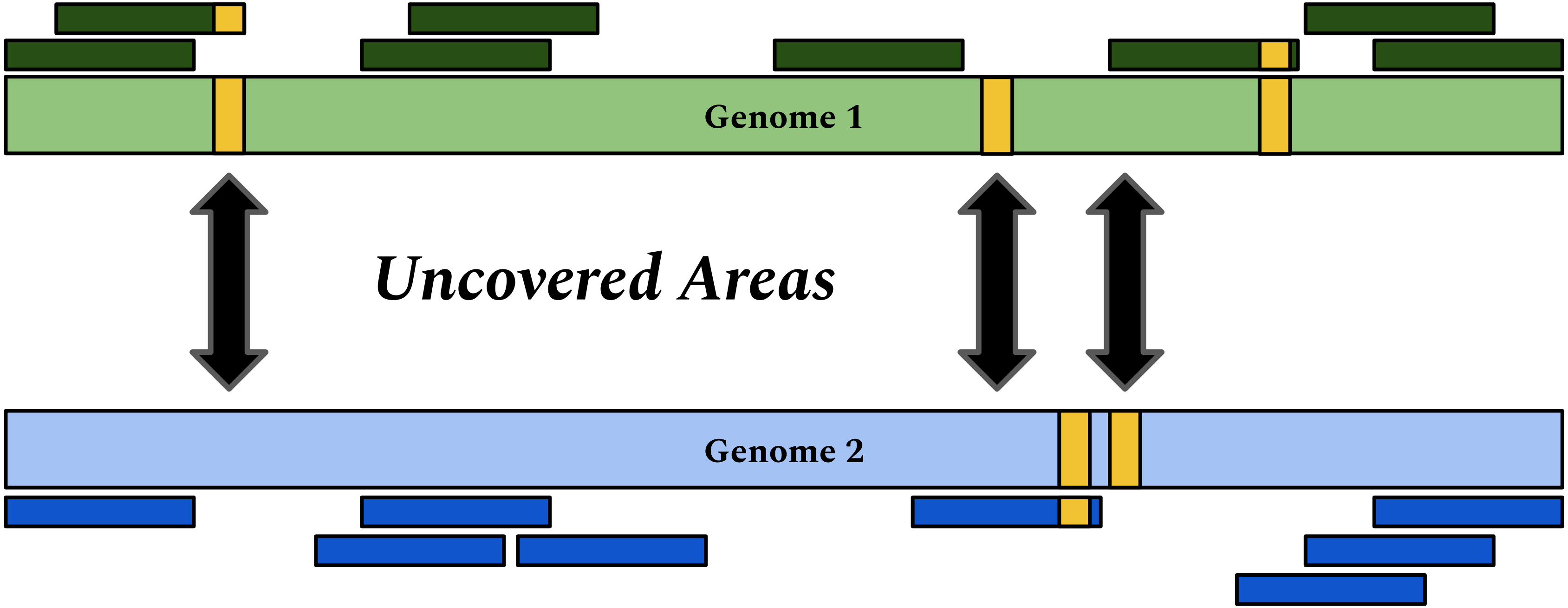
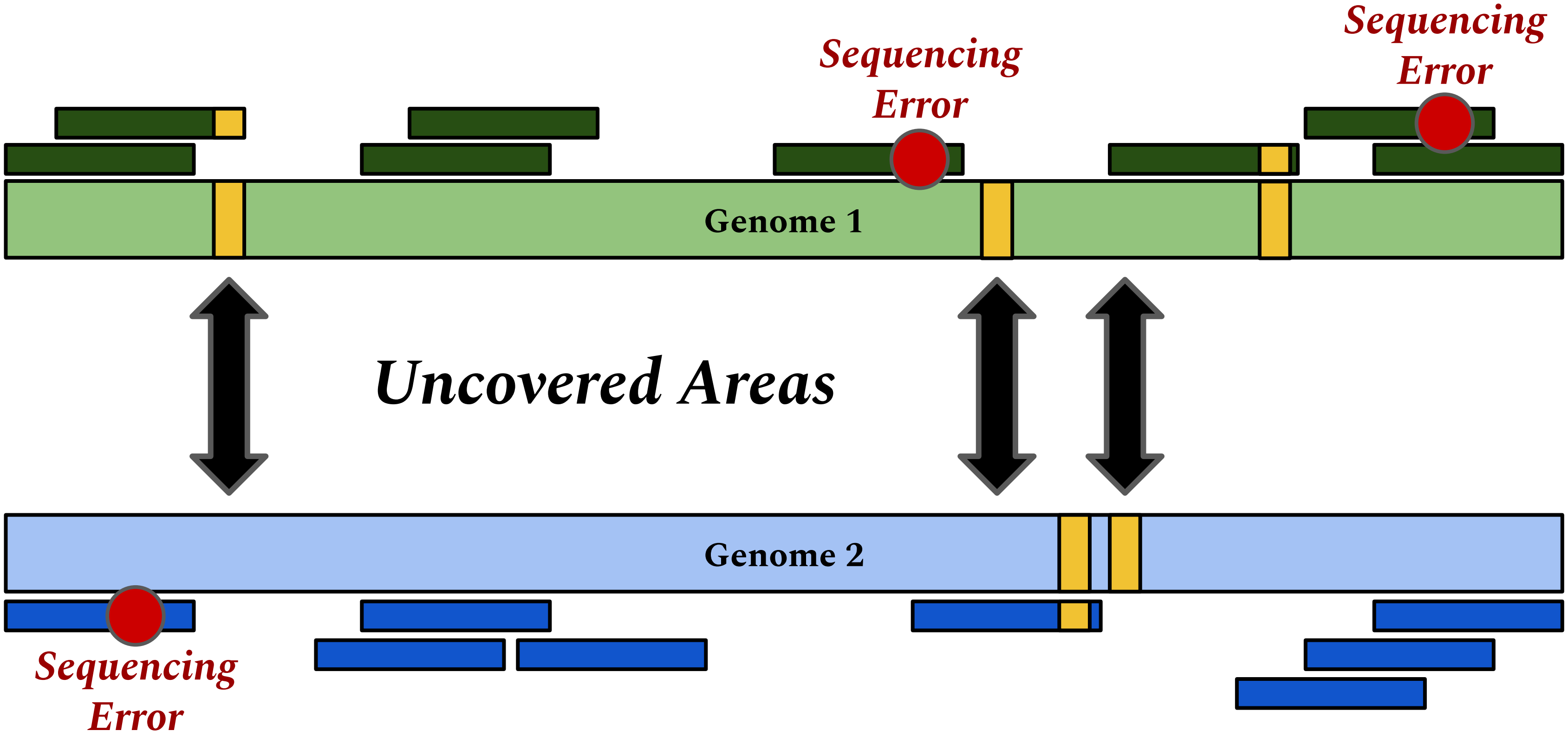


SKMER: Using Low Coverage Skims



SKMER: Using Low Coverage Skims

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

→

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

η_i (eta) = probability a k-mer is covered at least once without error.

ζ_i (zeta) = the total number of k-mers observed from both genomes.

L_i = genome size.

→ Both **eta** and **zeta** are functions of **coverage** and **error**.

SKMER: Estimating Parameters

$\boldsymbol{\lambda}$ (lambda) = how many times a *k*-mer is covered

$\boldsymbol{\xi}$ (xi) = number of error free reads covering a *k*-mer

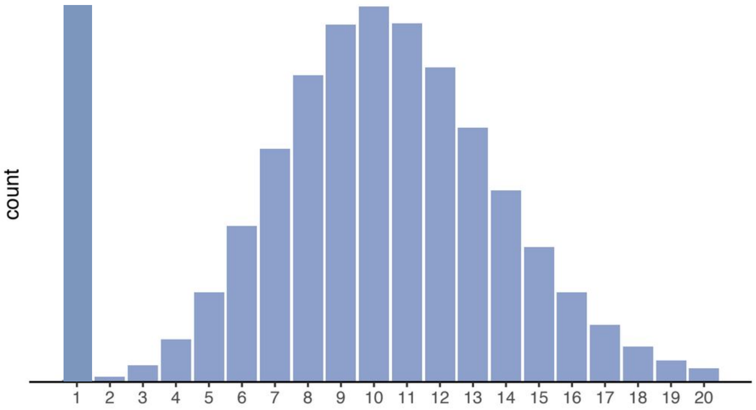
$\boldsymbol{\varepsilon}$ (epsilon) = sequencing error rate

$\mathbf{|A \cap B|}$ (obs. intersection) = shared *k*-mers between samples.

- Use *k*-mer frequency spectrum to estimate of parameters.

- Use *MASH* to compute observed intersection.

Estimating Error and Coverage:



$$P(X) = \frac{\lambda^x e^{-\lambda}}{X!}$$

WGS follows a Poisson Distribution

$$h = \operatorname{argmax}_{i \geq 2} M_i$$ = ***mode***

$$\xi = \frac{M_{h+1}}{M_h}(h+1)$$ = ***number of error-free reads covering a k-mer***

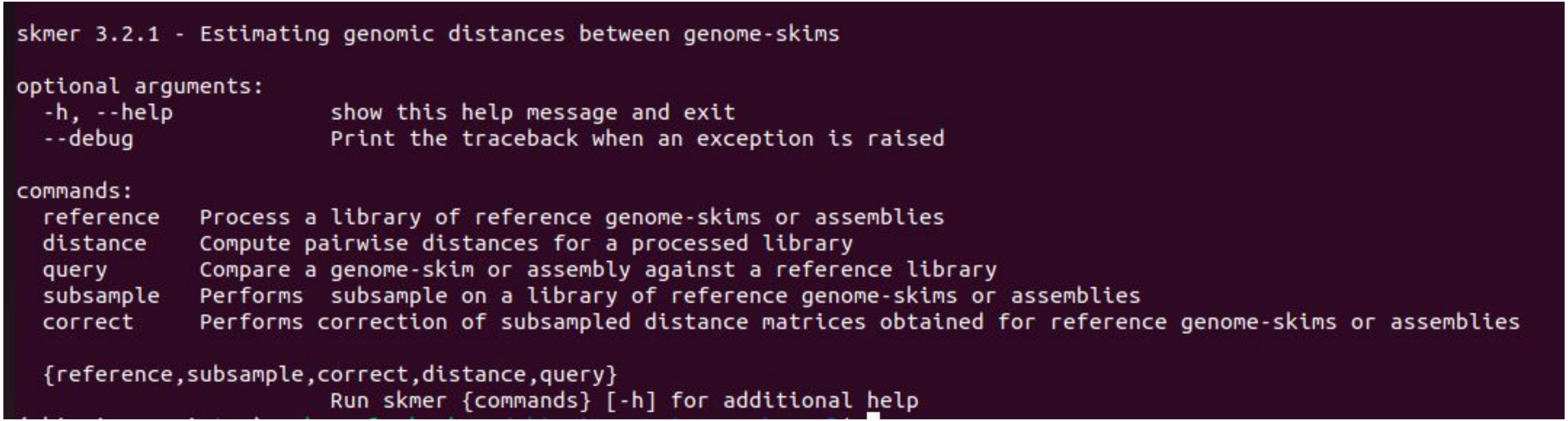
$$\lambda \quad = \quad \frac{M_1}{M_h}\frac{\xi^h}{h!}e^{-\xi} + \xi(1 - e^{-\xi})$$ = ***k-mer coverage***

$$\epsilon \quad = \quad 1 - (\xi/\lambda)^{1/k}$$ = ***sequencing error rate***

Running Skmer Reference

```
cd skimming_scripts-echarvel
conda activate ${CONDA_ENV} #replace with env name
gunzip ./test/skims/*gz
skmer reference ./skims/bbmap_reads -p1
```

Other Skmer Utilities:



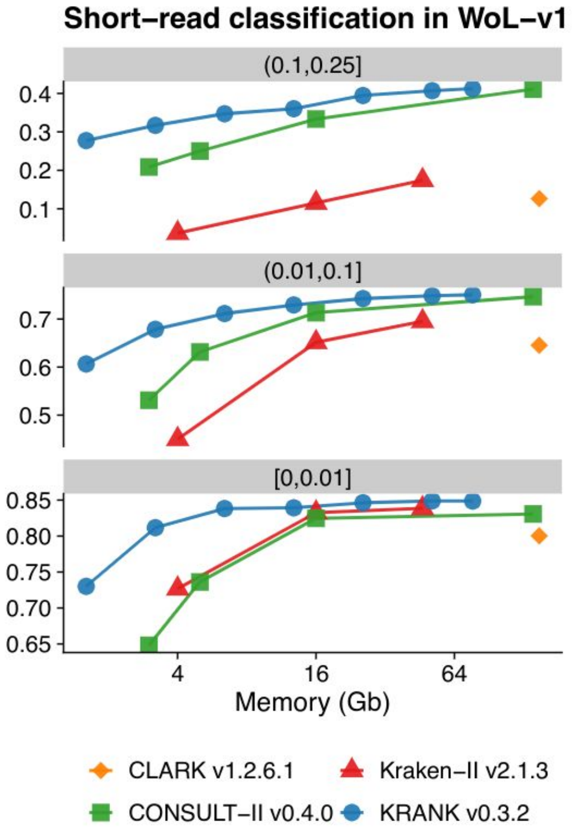
```
skmer 3.2.1 - Estimating genomic distances between genome-skims

optional arguments:
  -h, --help            show this help message and exit
  --debug               Print the traceback when an exception is raised

commands:
  reference   Process a library of reference genome-skims or assemblies
  distance    Compute pairwise distances for a processed library
  query       Compare a genome-skim or assembly against a reference library
  subsample   Performs  subsample on a library of reference genome-skims or assemblies
  correct     Performs correction of subsampled distance matrices obtained for reference genome-skims or assemblies

  {reference,subsample,correct,distance,query}
                        Run skmer {commands} [-h] for additional help
```

distance *== generate distance matrix (automatic for reference)*
query *== add new skim/assembly to library*
subsample *== perform bootstrap approach*

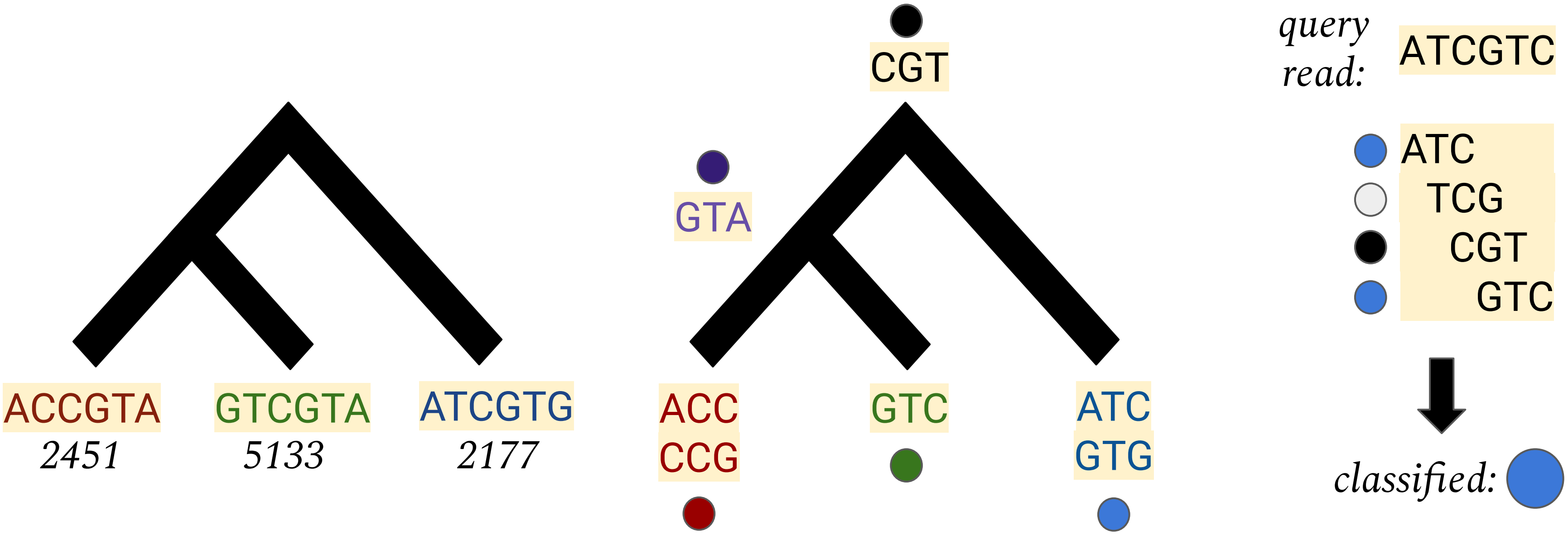
Memory-bound *k*-mer selection for large and evolutionary diverse reference libraries

Ali Osman Berk Şapcı, Siavash Mirarab

doi: https://doi.org/10.1101/2024.02.12.580015



KRANK: Decontamination



genomes and taxonomy IDs

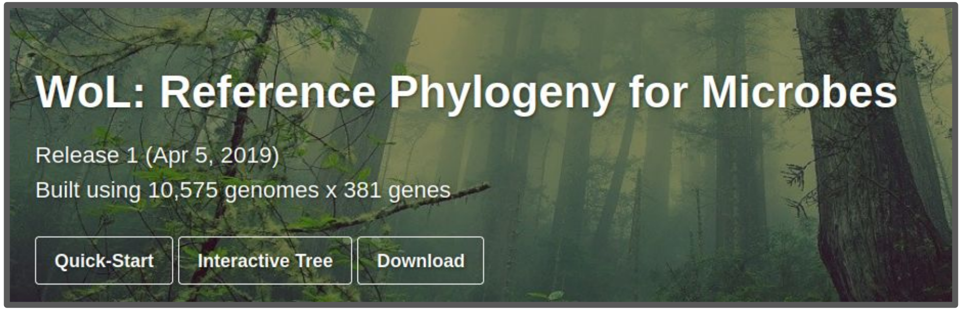
chooses a subset of representative *k*-mers

classifying reads

KRANK: Decontamination

Most Common Sources of Contamination:

- **Bacterial** → WoL-v1 dataset - archaeal and bacterial genomes



WoL: Reference Phylogeny for Microbes

Release 1 (Apr 5, 2019)

Built using 10,575 genomes x 381 genes

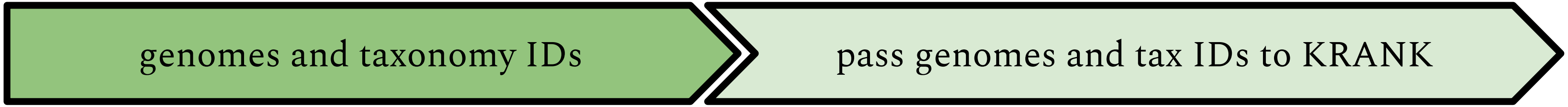
- **Human** → T2T Pangenome [05-2024]

KRANK: Decontamination

	genomes		tax IDs
1	ATGCTACC...T		52631
2	CTAGGAA...A		23100
3	CCCTTTA...C		23100
4	GATAAAA...A	×	48019
5	TATACTGC...C		5001
6	GTCGGCA...G		662
7	GGACTGC...C		80919
8	CTTATCC...G		80919

```
krank build
        -l $LIBRARY_DIRECTORY
        -t $TAXONOMY_DIRECTORY
        -i $MAPPING_FILE
        --from-library
        --batch-size 8
        --target-batch 0
        --num-threads $NUM_THREADS
```

https://github.com/bo1929/KRANK



Running KRANK build

```
cd ./skimming_scripts-echarvel/test/KRANK_test
```

```
krank build \
  -l ${LIBDIR} -t ./taxonomy/ \
  -i ./input_map.tsv  \
  -k 27 -w 35 -h 12 -b 8 -s 2\
  --from-scratch --input-sequences \
  --kmer-ranking representative --adaptive-size --lca soft \
  --num-threads ${NTHREADS} \
```

```
krank build \
  -l ${LIBDIR} -t ./taxonomy/ \
  -i ./input_map.tsv  \
  -k 27 -w 35 -h 12 -b 8 -s 2\
  --target-batch 0 --fast-mode --from-library --input-sequences --keep-intermediate \
  --kmer-ranking representative --adaptive-size --lca soft \
  --num-threads ${NTHREADS}
```

Running KRANK query

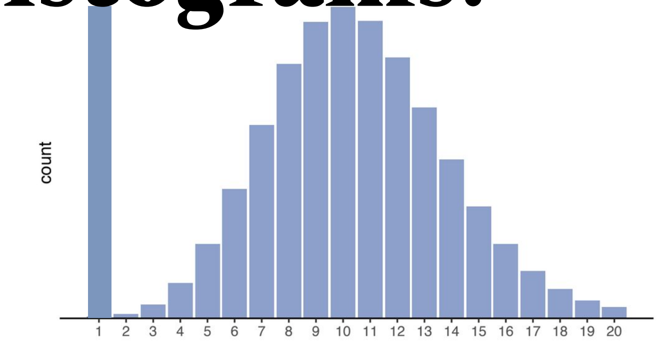
```
cd ./skimming_scripts-echarvel/test/
```

```
${SCRIPT_DIR}/KRANK/krank query \
        --library-dir ${LIBRARIES} \
        --query-file ./bbmap_read.fq  \
        --max-match-distance 5 \
        --total-vote-threshold 0.03 \
        --num-threads ${NUM_THREADS} \
        --output-dir "${OUTPUT_DIRECTORY}/krank_output/krank_reports/"
```

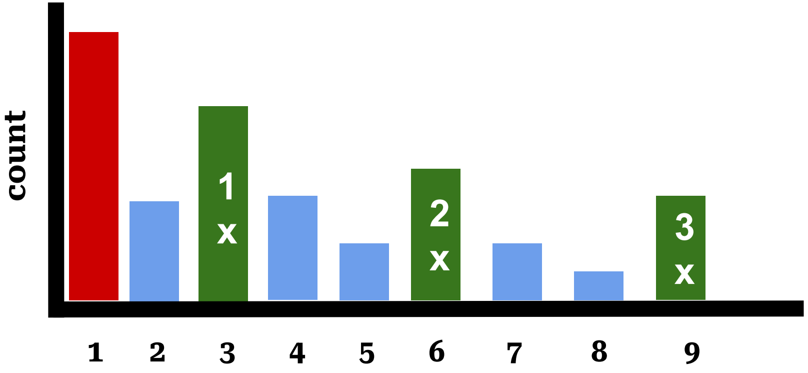
Estimating repeat spectra and genome length from low-coverage genome skims with RESPECT

Shahab Sarmashghi, Metin Balaban, Eleonora Rachtman, Behrouz Touri, Siavash Mirarab, Vineet Bafna ✉

How Repeats Affect *k*-mer Histograms:



simple genome skim

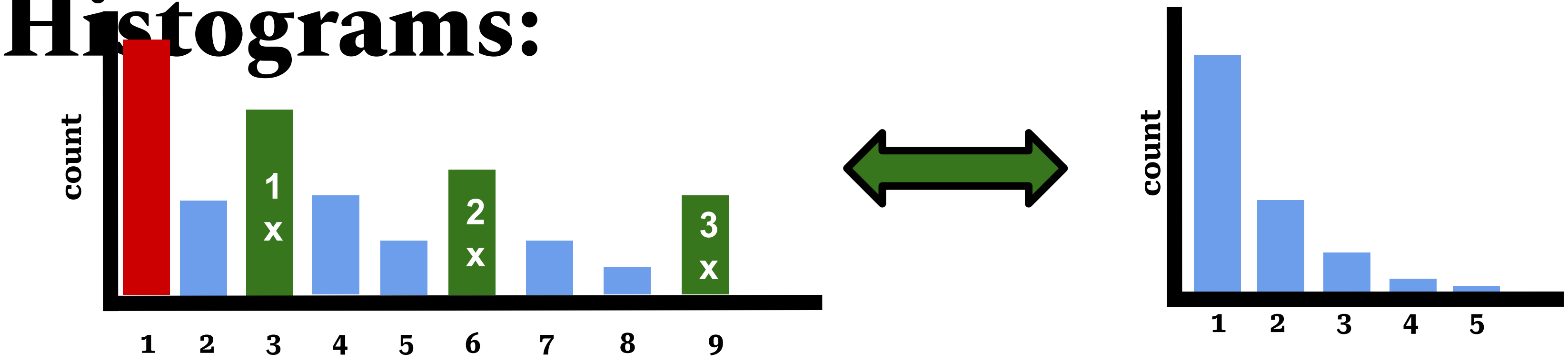


repetitive genome skim

Multiple peaks and coverages.

Can we get measures of repetitiveness using this?

How Repeats Affect *k*-mer Histograms:



repetitive genome

Repeat spectra of an assembly

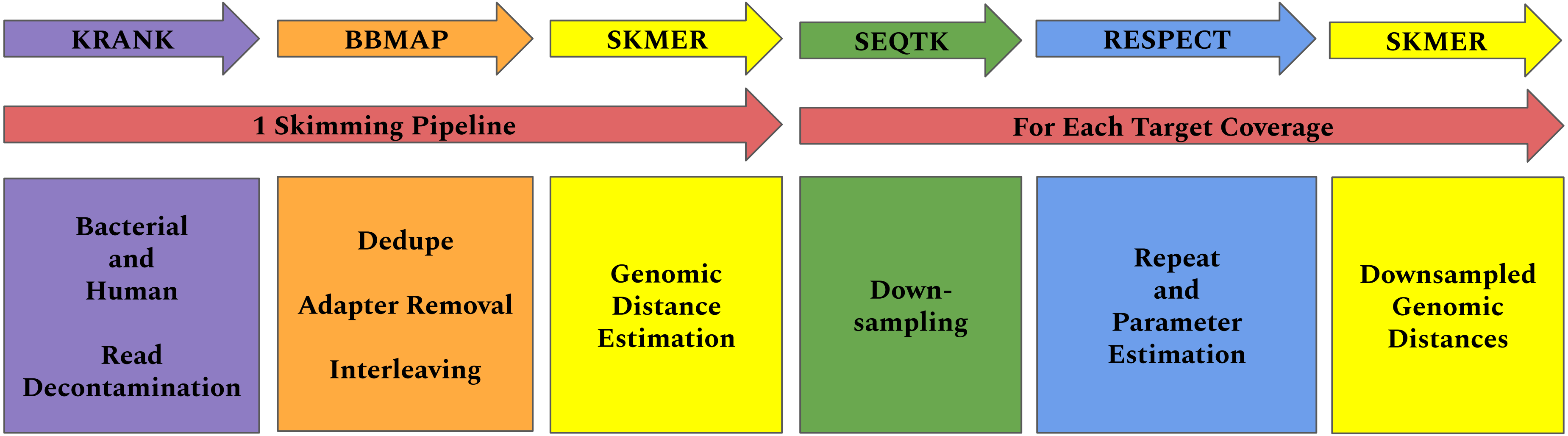
RESPECT output:

- repeat-informed coverage, error, and genome length estimates
- repeat spectra of a genome
- repetitiveness metrics:
  - Uniqueness Ratio, High Copy Repeats per Million

Running RESPECT

```
cd ./skimming_scripts-echarvel/test/
respect -d ./bbmap_reads/ -N 2 --threads 1
```

Using the Skimming Pipeline



KRANK

BBMAP

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SEQTK

RESPECT

SKMER

1 Skimming Pipeline

For Each Target Coverage

Bacterial and Human

Read Decontamination

Dedupe

Adapter Removal

Interleaving

Genomic Distance Estimation

Down-sampling

Repeat and Parameter Estimation

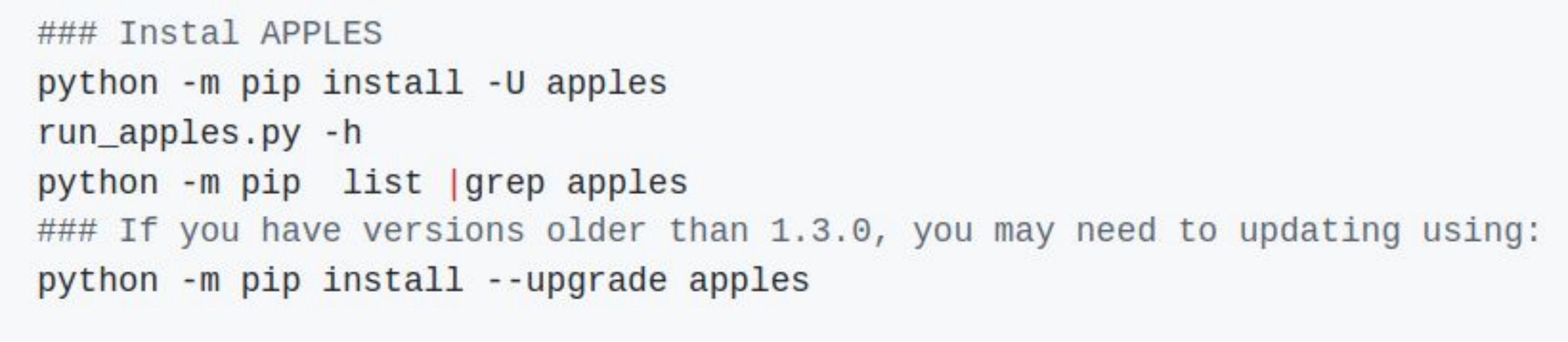
Downsampled Genomic Distances

```
bash ./skimming_pipeline.sh -h
```

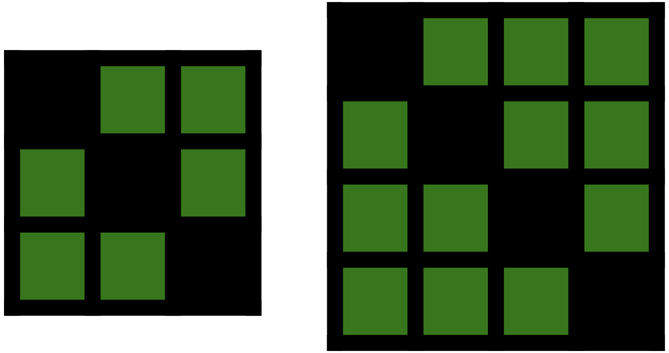
APPLES: Scalable Distance-Based Phylogenetic Placement with or without Alignments FREE

Metin Balaban, Shahab Sarmashghi, Siavash Mirarab ✉

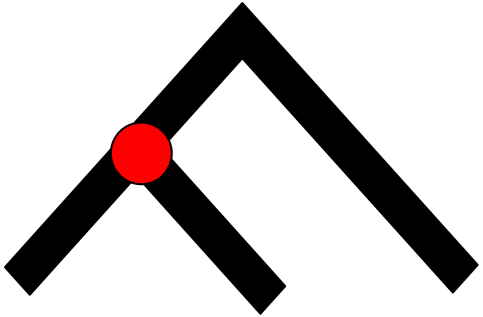
Installing Apples:



```
### Instal APPLES
python -m pip install -U apples
run_apples.py -h
python -m pip  list |grep apples
### If you have versions older than 1.3.0, you may need to updating using:
python -m pip install --upgrade apples
```



skmer query



Apples placement

Thank you!

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Center

MINDEROO

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Mirarab Lab:
Isaac, Daira, Homere, Ali, Shayasteh, Nora, and Yueyu

Field Museum: Grainger Bioinformatics Center
Minderoo Foundation