sourmash

a tutorial on the FracMinHash sketch approach for metagenomic data

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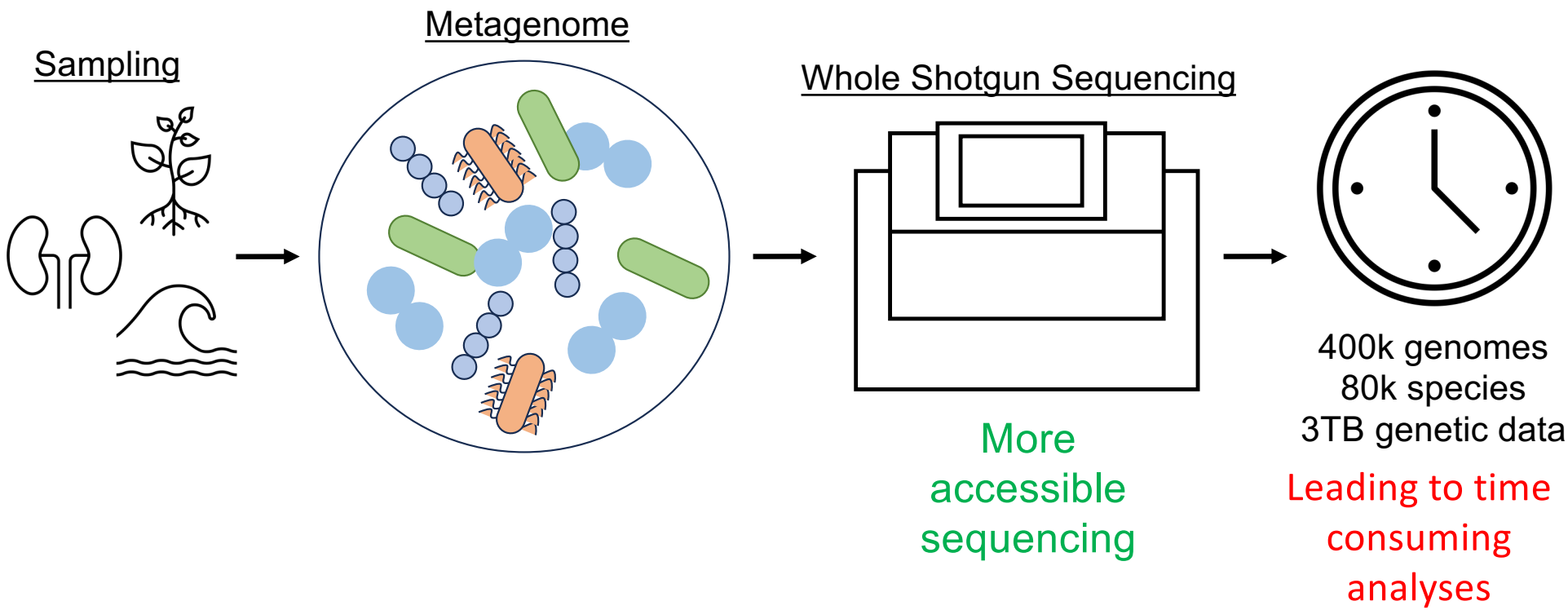
Genomics and metagenomics research can be **computationally overwhelming**



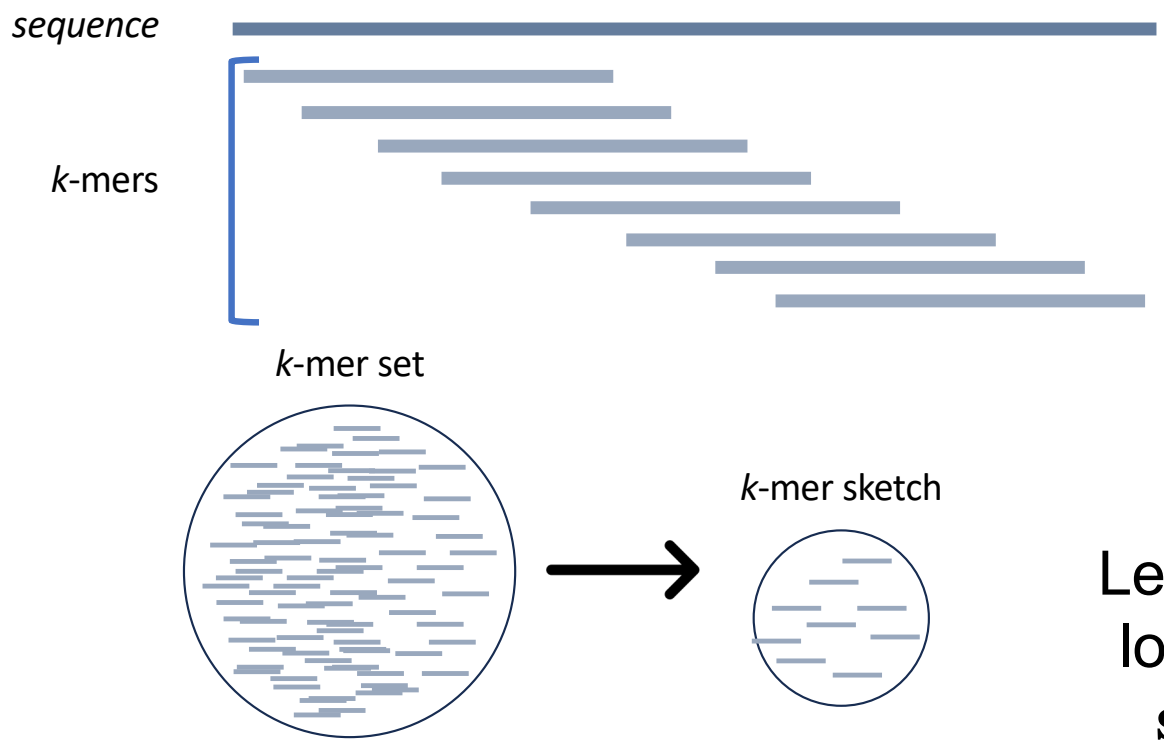
sketching approaches can help with big data

Do we recognize who this is?

→

Condense data to a simpler representation

sourmash facilitates sketching and analyses of large sequencing data



Let's take a closer look into utilizing **sourmash**…

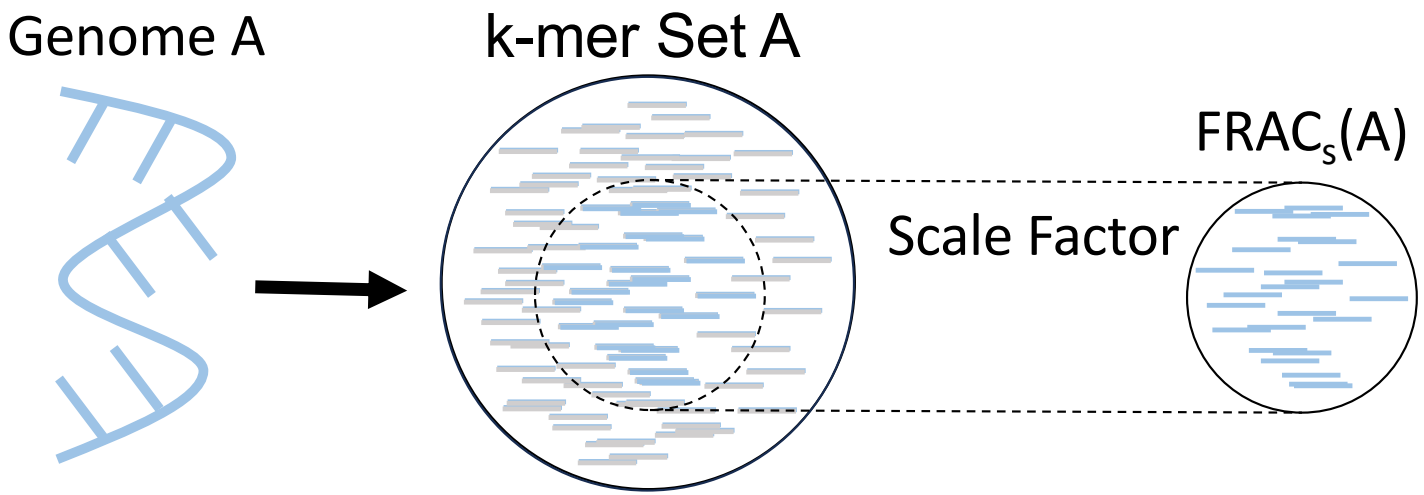
https://sourmash.readthedocs.io/

Brown, C. T., & Irber, L. (2016). sourmash: a library for MinHash sketching of DNA. Journal of open source software, 1(5), 27.

sourmash sketch

first step in
sourmash analyses

The **FracMinHash** sketch

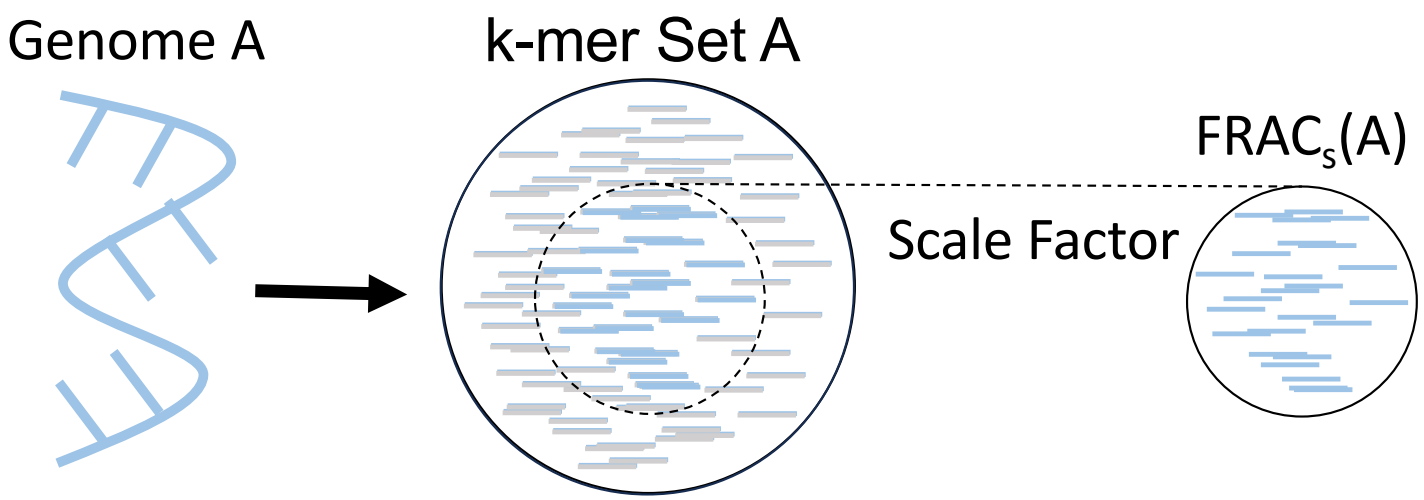


$$FRAC_s(A) = \{h(a) | a \in A \text{ and } h(a) \leq Hs\}$$

Hera, M. R., Pierce-Ward, N. T., & Koslicki, D. (2023). Deriving confidence intervals for mutation rates across a wide range of evolutionary distances using FracMinHash. Genome research, 33(7), 1061-1068.

sourmash sketch

producing a FracMinHash sketch

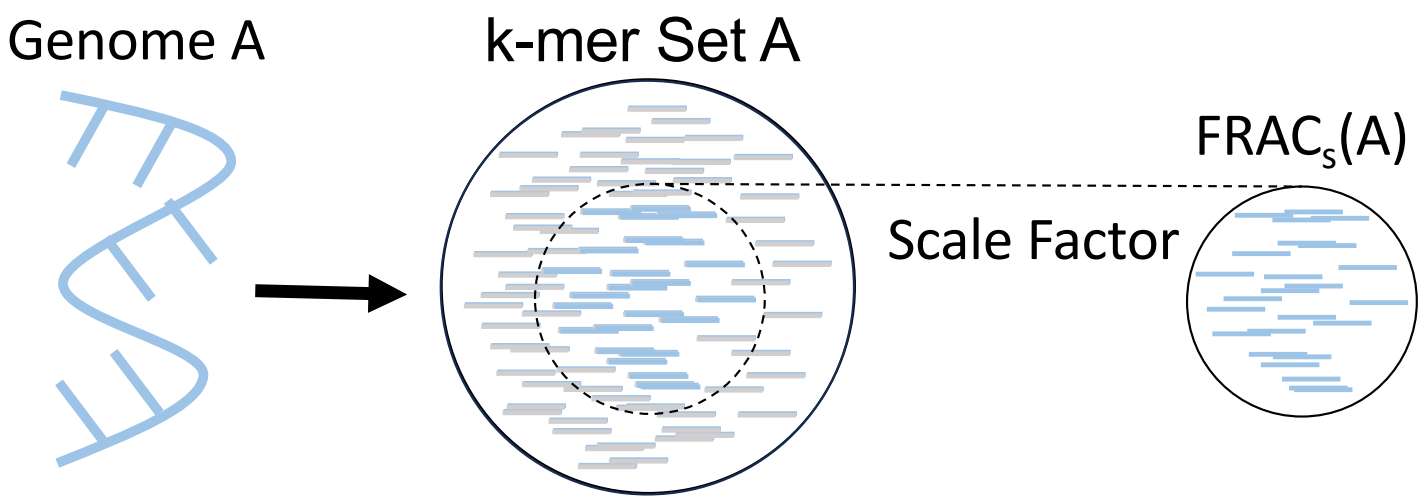



```
sourmash sketch <dna, protein, translate> <FASTA/Q> -p k=<ksize>,scaled=<int>
```

sourmash command

sourmash sketch

producing a FracMinHash sketch

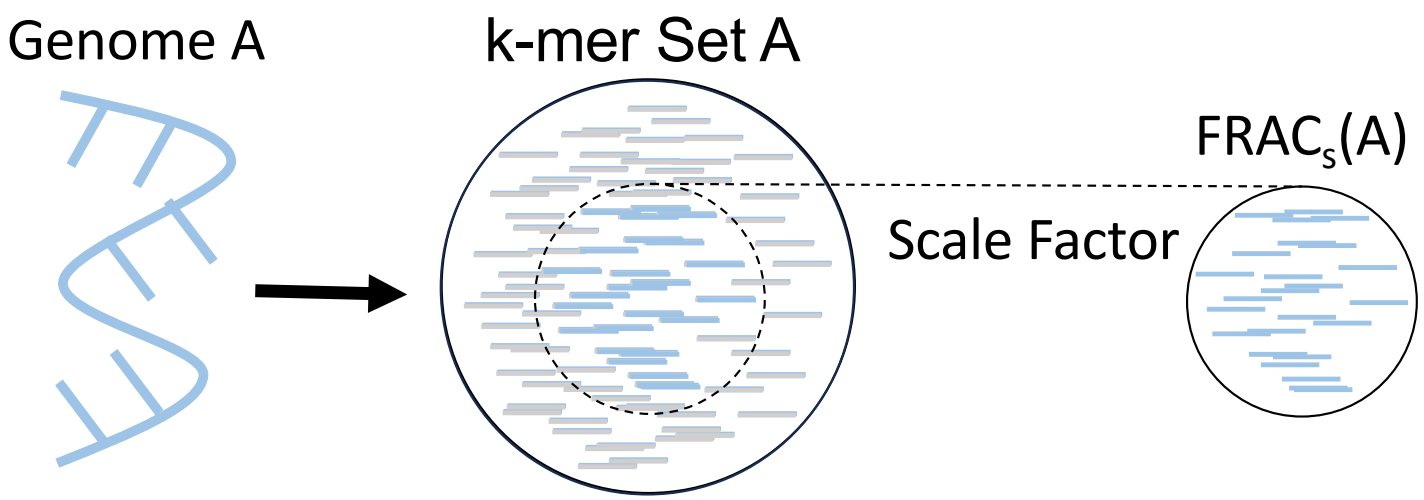



```
sourmash sketch <dna, protein, translate> <FASTA/Q> -p k=<ksize>,scaled=<int>
```

identify the type of input sequences

sourmash sketch

producing a FracMinHash sketch

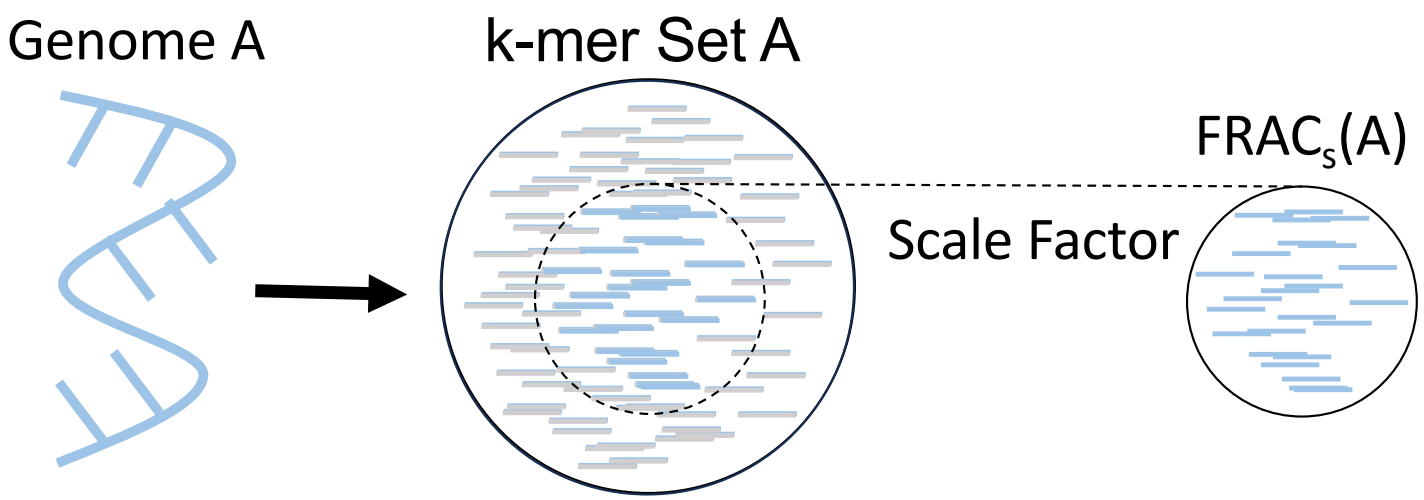



```
sourmash sketch <dna, protein, translate> <FASTA/Q> -p k=<ksize>,scaled=<int>
```

Identify the filename with sequences

sourmash sketch

producing a FracMinHash sketch

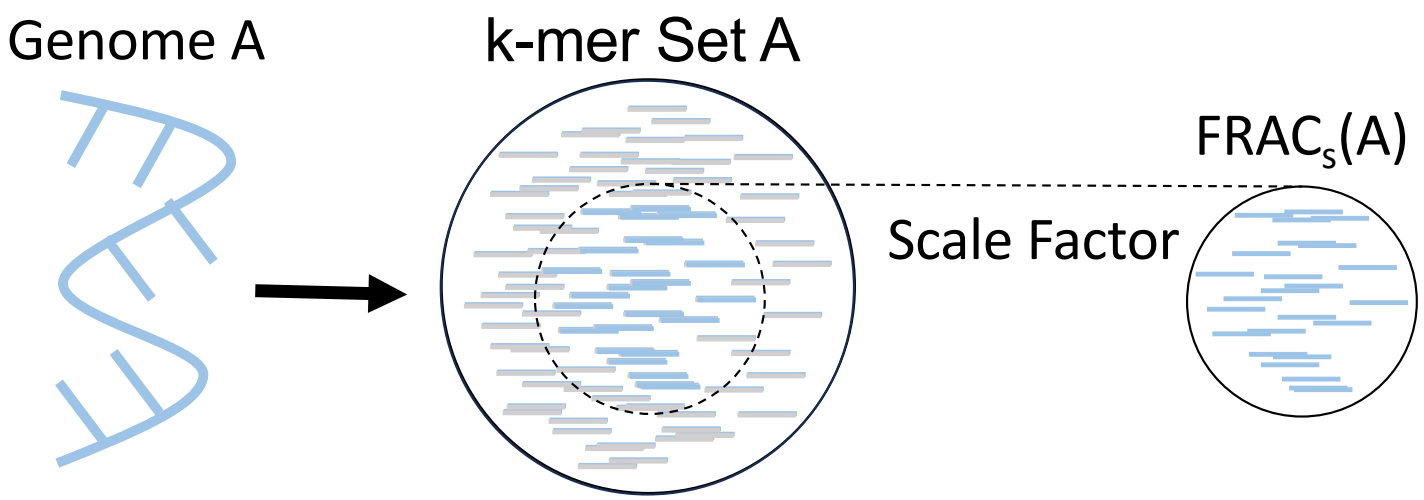



```
sourmash sketch <dna, protein, translate> <FASTA/Q> -p k=<ksize>,scaled=<int>
```

Flag required to tune parameters

sourmash sketch

producing a FracMinHash sketch

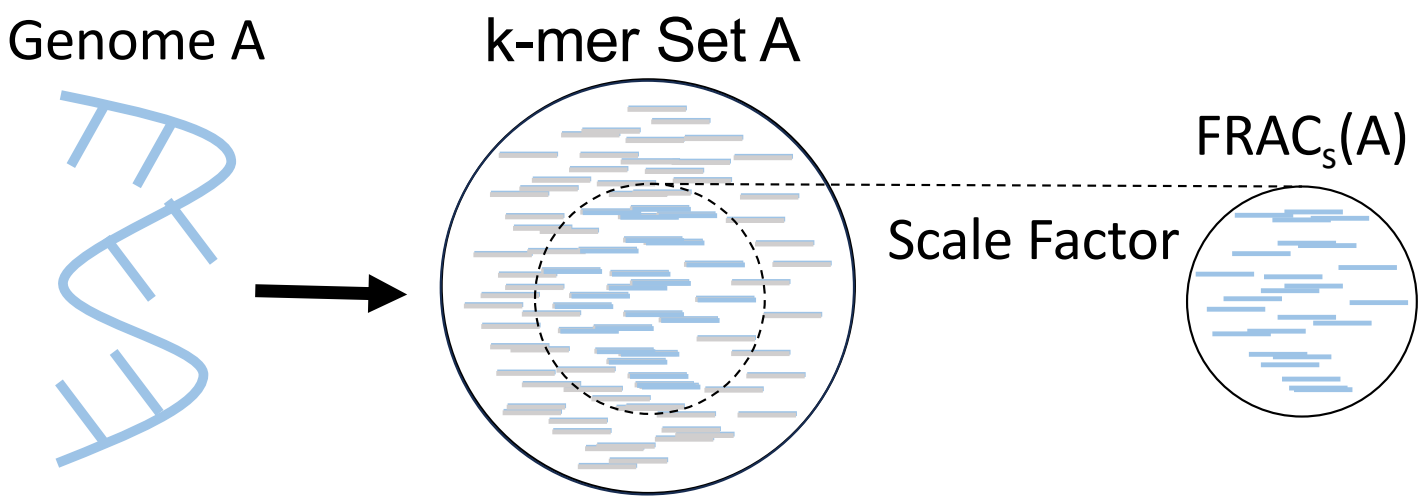



```
sourmash sketch <dna, protein, translate> <FASTA/Q> -p k=<ksize>,scaled=<int>
```

Identify the filename with sequences

sourmash sketch

producing a FracMinHash sketch

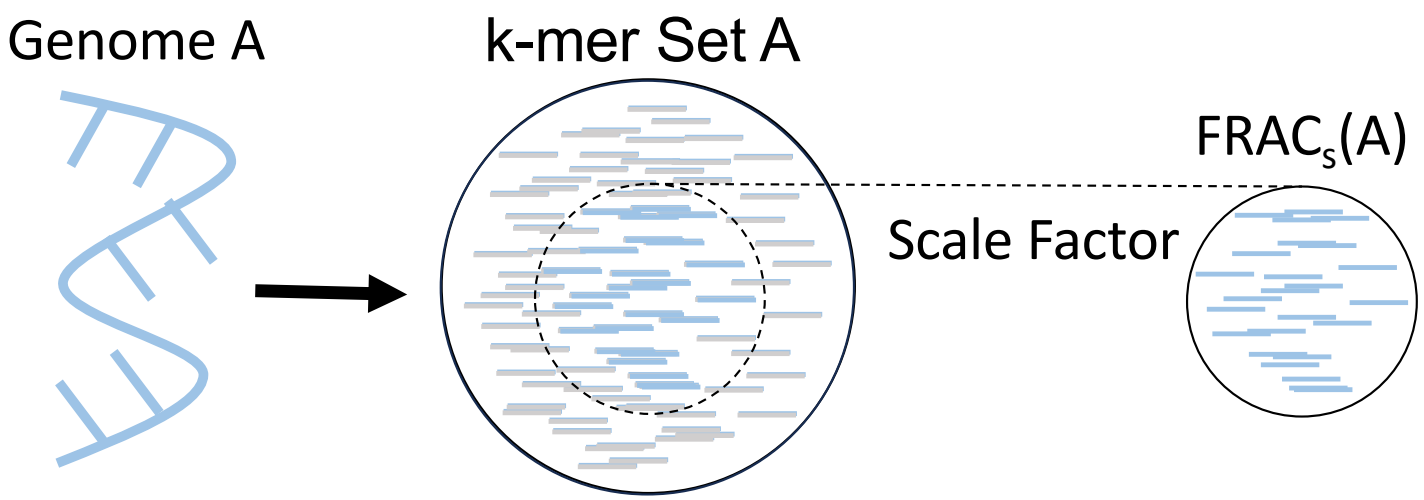



```
sourmash sketch <dna, protein, translate> <FASTA/Q> -p k=<ksize>,scaled=<int>
```

scales down sketch

sourmash sketch

producing a FracMinHash sketch




```
sourmash sketch <dna, protein, translate> <FASTA/Q> -p k=<ksize>,scaled=<int>
```

Example: scaled=10
will keep 1 of 10 *k*-mers

sourmash compare

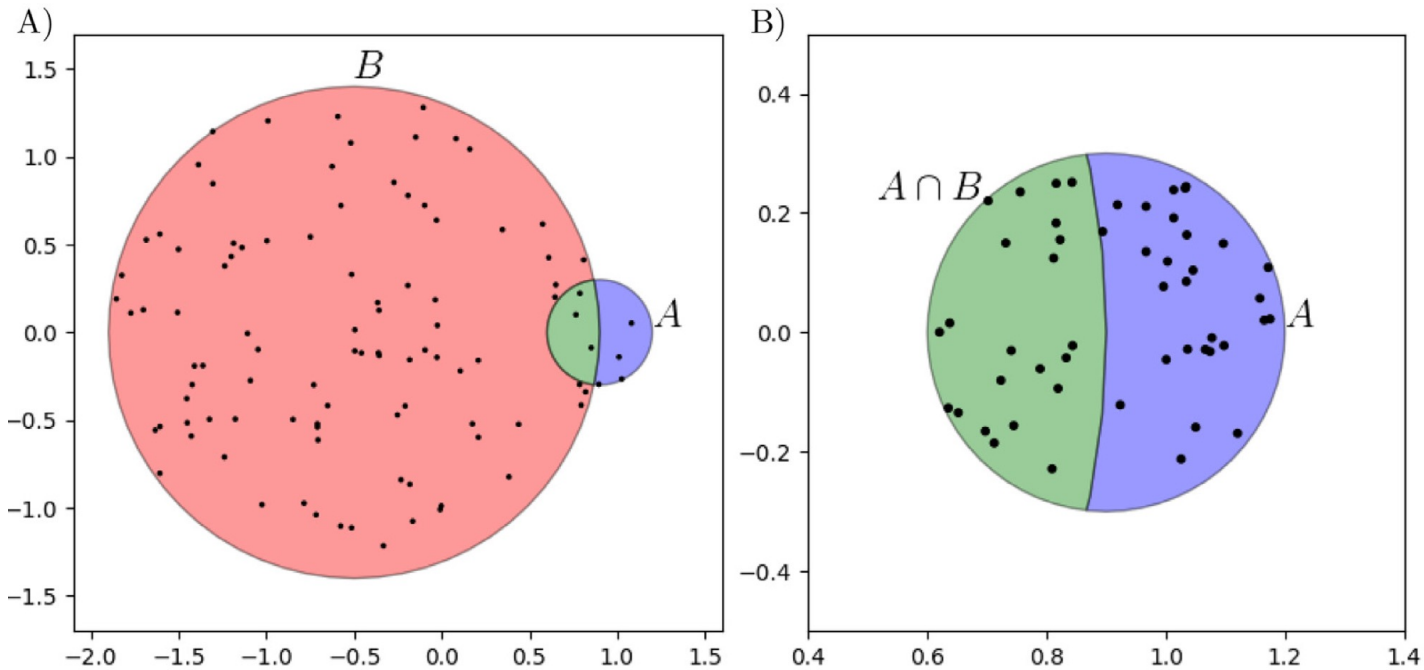
perform pairwise similarity estimations between metagenomic samples

Estimating similarity indexes: **jaccard**

$$J(A,B) = \frac{|A \cap B|}{|A \cup B|} \approx \frac{|S(A \cup B) \cap S(A) \cap S(B)|}{|S(A \cup B)|}$$

Indexes close to 1 are interpreted as more similar

However, the **jaccard** estimate becomes worse with differing set sizes

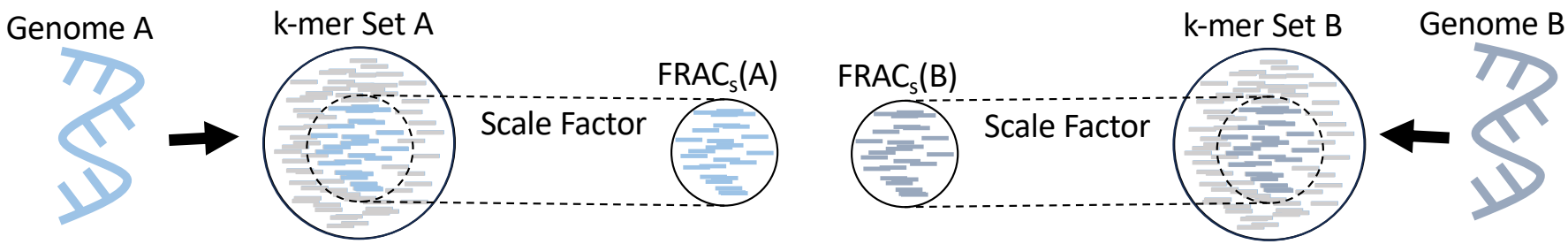


…further, bloom filters are limited to smaller datasets

Koslicki and Zabeti 2019

Estimating similarity indexes: **containment**

…addresses issues using in jaccard

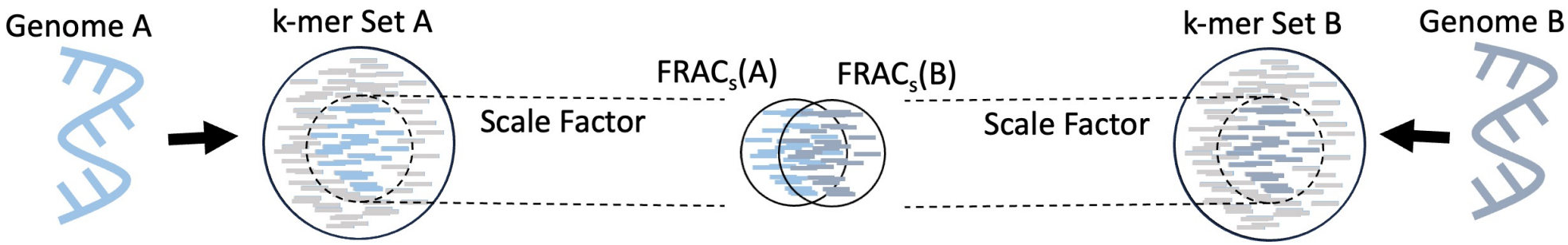


$$C_{frac}(A,B) := \frac{|\boldsymbol{FRAC_s}(A) \cap \boldsymbol{FRAC_s}(B)|}{|\boldsymbol{FRAC_s}(A)|(1-(1-s)^{|A|})} \mathbb{1}_{|\boldsymbol{FRAC_s}(A)|>0}$$

Hera and colleagues 2023

sourmash compare

Estimate the containment between two genomes

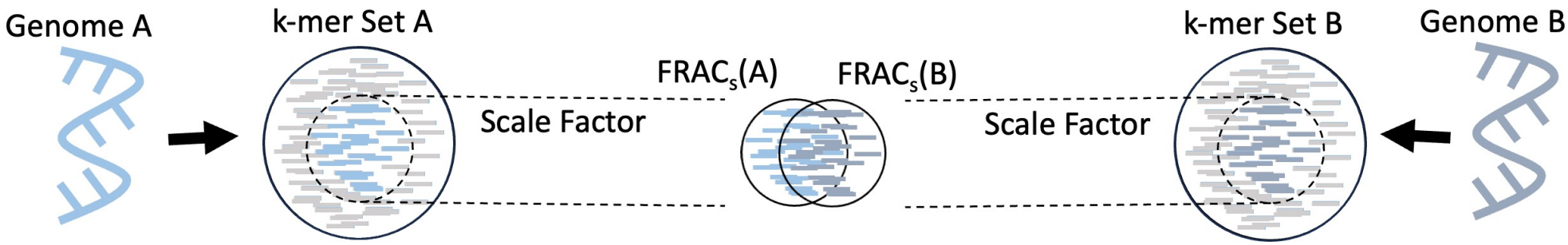



```
sourmash compare <ref signature> <query signature> --containment --ksize <ksize>
```

sourmash command

sourmash compare

Estimate the containment between two genomes

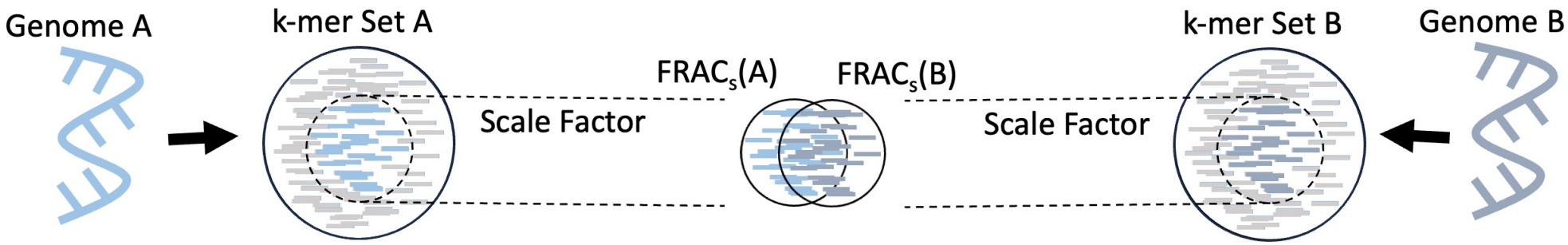



```
sourmash compare <query signature> <ref signature> --containment --ksize <ksize>
```

sample of interest

sourmash compare

Estimate the containment between two genomes

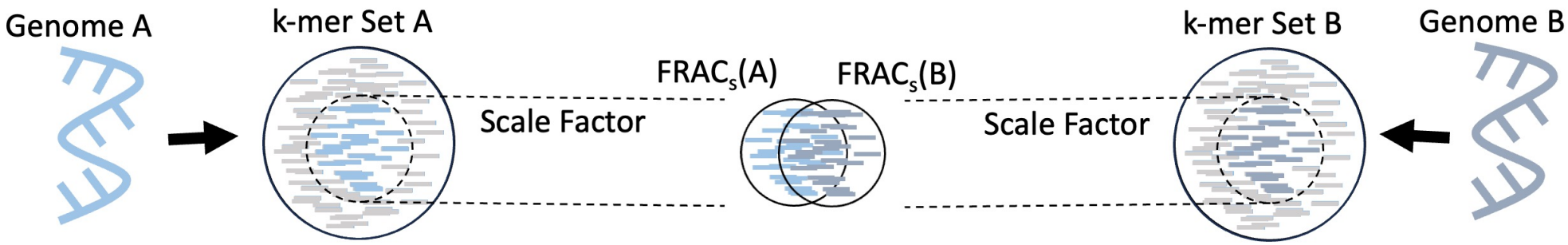



```
sourmash compare <query signature> <ref signature> --containment --ksize <ksize>
```

compared to another sample

sourmash compare

Estimate the containment between two genomes

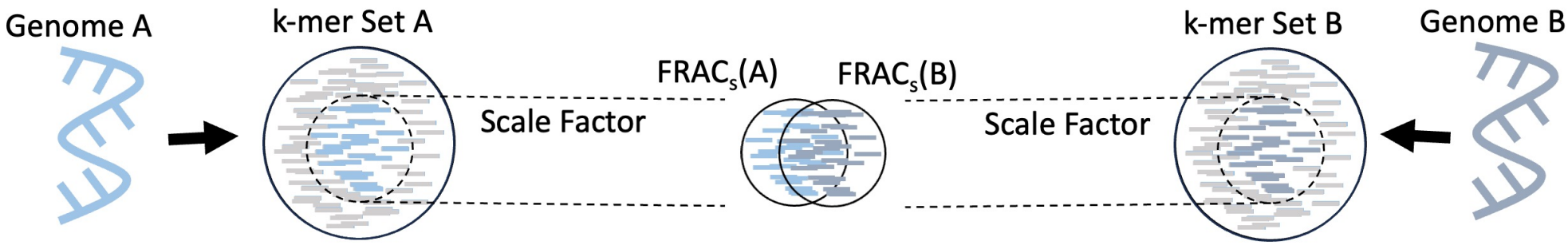



```
sourmash compare <query signature> <ref signature> --containment --ksize <ksize>
```

indicate the similarity index

sourmash compare

Estimate the containment between two genomes




```
sourmash compare <query signature> <ref signature> --containment --ksize <ksize>
```

sourmash search

report highly similar sequences data between samples

sourmash search

First, produce a MANIFEST.csv to search using an md5 identifier as the query

```
sourmash sig manifest <signature file> -o MANIFEST.csv
```

command for further options to information from signatures

sourmash search

First, produce a MANIFEST.csv to search using an md5 identifier as the query

```
sourmash sig manifest <signature file> -o MANIFEST.csv
```

option to create MANIFEST

sourmash search

First, produce a MANIFEST.csv to search using an md5 identifier as the query

```
sourmash sig manifest <signature file> -o MANIFEST.csv
```

input signature query of interest

sourmash search

First, produce a MANIFEST.csv to search using an md5 identifier as the query

```
sourmash sig manifest <signature file> -o MANIFEST.csv
```

output name flag

sourmash search

First, produce a MANIFEST.csv to search using an md5 identifier as the query

```
sourmash sig manifest <signature file> -o sample_001.manifest.csv
```

```
# SOURMASH-MANIFEST-VERSION: 1.0
internal_location,md5,md5short,ksize,moltype,num,scaled,n_hashes,with_abundance,name,filename
sample_001.fna.singleton.sig,d3513280b35b2a918a7181875c0683c8,d3513280,31,DNA,0,500,22,0,genome_A,sample_001.fna
sample_001.fna.singleton.sig,2d96ee330b6b295a06b70fdbfb75af34,2d96ee33,31,DNA,0,500,21,0,genome_B,sample_001.fna
sample_001.fna.singleton.sig,364c20a1ca43d3ae3e9ae7d9e9a6c837,364c20a1,31,DNA,0,500,19,0,genome_C,sample_001.fna
```

Column Name	Description
internal_location	Name of signature file
md5	Name for sketch
ksize	Size used to produce *k*-mers
moltype	Type of sequence: DNA, Protein
scaled	Parameter used for sourmash sketch to reduce *k*-mer set
n_hashes	Total k-mers in final sketch
name	Sequence name in FASTA/Q
filename	Input FASTA/Q name

sourmash search

First, produce a MANIFEST.csv to search using an md5 identifier as the query

```
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```

```
# SOURMASH-MANIFEST-VERSION: 1.0
internal_location,md5,md5short,ksize,moltype,num,scaled,n_hashes,with_abundance,name,filename
sample_001.fna.singleton.sig,d3513280b35b2a918a7181875c0683c8,d3513280,31,DNA,0,500,22,0,genome_A,sample_001.fna
sample_001.fna.singleton.sig,2d96ee330b6b295a06b70fdbfb75af34,2d96ee33,31,DNA,0,500,21,0,genome_B,sample_001.fna
sample_001.fna.singleton.sig,364c20a1ca43d3ae3e9ae7d9e9a6c837,364c20a1,31,DNA,0,500,19,0,genome_C,sample_001.fna
```

```
sourmash search <signature query> <signature ref> --md5 d3513280b35b2a918a7181875c0683c8 --containment
```

command to searching signatures

sourmash search

First, produce a MANIFEST.csv to search using an md5 identifier as the query

```
sourmash sig manifest <signature file> -o sample_001.manifest.csv
```

```
# SOURMASH-MANIFEST-VERSION: 1.0
internal_location,md5,md5short,ksize,moltype,num,scaled,n_hashes,with_abundance,name,filename
sample_001.fna.singleton.sig,d3513280b35b2a918a7181875c0683c8,d3513280,31,DNA,0,500,22,0,genome_A,sample_001.fna
sample_001.fna.singleton.sig,2d96ee330b6b295a06b70fdbfb75af34,2d96ee33,31,DNA,0,500,21,0,genome_B,sample_001.fna
sample_001.fna.singleton.sig,364c20a1ca43d3ae3e9ae7d9e9a6c837,364c20a1,31,DNA,0,500,19,0,genome_C,sample_001.fna
```

```
sourmash search <signature query> <signature ref> --md5 d3513280b35b2a918a7181875c0683c8 --containment
```

signature file where md5 of interest is found

sourmash search

First, produce a MANIFEST.csv to search using an md5 identifier as the query

```
sourmash sig manifest <signature file> -o sample_001.manifest.csv
```

```
# SOURMASH-MANIFEST-VERSION: 1.0
internal_location,md5,md5short,ksize,moltype,num,scaled,n_hashes,with_abundance,name,filename
sample_001.fna.singleton.sig,d3513280b35b2a918a7181875c0683c8,d3513280,31,DNA,0,500,22,0,genome_A,sample_001.fna
sample_001.fna.singleton.sig,2d96ee330b6b295a06b70fdbfb75af34,2d96ee33,31,DNA,0,500,21,0,genome_B,sample_001.fna
sample_001.fna.singleton.sig,364c20a1ca43d3ae3e9ae7d9e9a6c837,364c20a1,31,DNA,0,500,19,0,genome_C,sample_001.fna
```

```
sourmash search <signature query> <signature ref> --md5 d3513280b35b2a918a7181875c0683c8 --containment
```

signature file that will be searched against

sourmash search

First, produce a MANIFEST.csv to search using an md5 identifier as the query

```
sourmash sig manifest <signature file> -o sample_001.manifest.csv
```

```
# SOURMASH-MANIFEST-VERSION: 1.0
internal_location,md5,md5short,ksize,moltype,num,scaled,n_hashes,with_abundance,name,filename
sample_001.fna.singleton.sig,d3513280b35b2a918a7181875c0683c8,d3513280,31,DNA,0,500,22,0,genome_A,sample_001.fna
sample_001.fna.singleton.sig,2d96ee330b6b295a06b70fdbfb75af34,2d96ee33,31,DNA,0,500,21,0,genome_B,sample_001.fna
sample_001.fna.singleton.sig,364c20a1ca43d3ae3e9ae7d9e9a6c837,364c20a1,31,DNA,0,500,19,0,genome_C,sample_001.fna
```

```
sourmash search <signature query> <signature ref> --md5 d3513280b35b2a918a7181875c0683c8 --containment
```

md5 identifier of interest indicated

sourmash search

First, produce a MANIFEST.csv to search using an md5 identifier as the query

```
sourmash sig manifest <signature file> -o sample_001.manifest.csv
```

```
# SOURMASH-MANIFEST-VERSION: 1.0
internal_location,md5,md5short,ksize,moltype,num,scaled,n_hashes,with_abundance,name,filename
sample_001.fna.singleton.sig,d3513280b35b2a918a7181875c0683c8,d3513280,31,DNA,0,500,22,0,genome_A,sample_001.fna
sample_001.fna.singleton.sig,2d96ee330b6b295a06b70fdbfb75af34,2d96ee33,31,DNA,0,500,21,0,genome_B,sample_001.fna
sample_001.fna.singleton.sig,364c20a1ca43d3ae3e9ae7d9e9a6c837,364c20a1,31,DNA,0,500,19,0,genome_C,sample_001.fna
```

```
sourmash search <signature query> <signature ref> --md5 d3513280b35b2a918a7181875c0683c8 --containment
```

indicate the similarity index

sourmash search

First, produce a MANIFEST.csv to search using an md5 identifier as the query

```
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```

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internal_location,md5,md5short,ksize,moltype,num,scaled,n_hashes,with_abundance,name,filename
sample_001.fna.singleton.sig,d3513280b35b2a918a7181875c0683c8,d3513280,31,DNA,0,500,22,0,genome_A,sample_001.fna
sample_001.fna.singleton.sig,2d96ee330b6b295a06b70fdbfb75af34,2d96ee33,31,DNA,0,500,21,0,genome_B,sample_001.fna
sample_001.fna.singleton.sig,364c20a1ca43d3ae3e9ae7d9e9a6c837,364c20a1,31,DNA,0,500,19,0,genome_C,sample_001.fna
```

```
sourmash search <signature query> <signature ref> --md5 d3513280b35b2a918a7181875c0683c8 --containment
```

```
select query k=31 automatically.
loaded query: genome_A (k=31, DNA)
-
loaded 10 total signatures from 1 locations. after selecting signatures compatible with
search, 10 remain.

9 matches above threshold 0.080; showing first 3:
similarity match
---------- -----
100.0% ref_gene
90.9% genome_A_2
86.4% genome_A_1
```

sourmash overview

sourmash overview

Task
sketch a signature file

Commands
```
sourmash sketch
```

Output
```
sketch.sig
```

Produce a scaled down representation of your sequencing data

Task
similarity index matrix

Commands
```
sourmash compare
```

Output
```
similarity_matrix.csv
```

Report similarity between sequencing data

Task
report similarity highly similar sketches

Commands
```
sourmash search
```

Output
```
similarity match
---------- -----
100.0% ref_gene
90.9% genome_A_2
86.4% genome_A_1
```

Match highly similar sequences data

sourmash tutorial

https://github.com/KoslickiLab/sourmash_tutorial