Efficient DNA/RNA sequence clustering

Using *k*-mers as an approximation for sequence similarity

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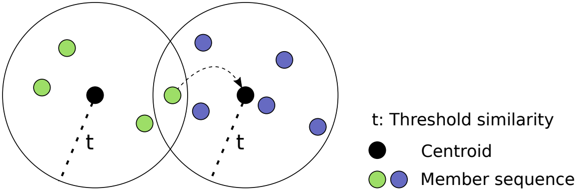
Introduction

Defining the clustering problem to be solved

Partitioning of sequences into a minimal number of clusters based on a measure of similarity between sequences.



- How to measure similarity/distance between sequences?
- How to cluster sequences based on such a measure?

Distance metrics

There are various distance metrics:

- edit distance, Levenshtein
- sequence alignment
- feature based distance, k-mer counting
    - K-DIST uses a kind of k-mer counting

The simple *d*2 algorithm

$$S_1 = ACTACAC$$
$$S_2 = ACAGAT$$

- Fill vectors with *k*-mer counts

	AC	AG	AT	CA	CT	GA	TA
S_1	3			1	1		1
S_2	1	1	1	1		1	

- Calculate the Euclidean distance

$$d2_2(S_1, S_2) = \sqrt{(3-1)^2 - 1^2 - 1^2 + (1-1)^2 + 1^2 - 1^2 + 1^2}$$
$$= \sqrt{9} = 3$$

The K-DIST algorithm

A variant of the simple *d*2 algorithm:

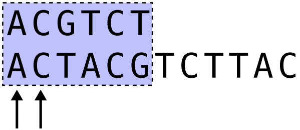
- a single *k*-mer vector
- Manhattan distance

$$\sum_{i=1}^{n} |u_i - v_i|$$

- window calculation

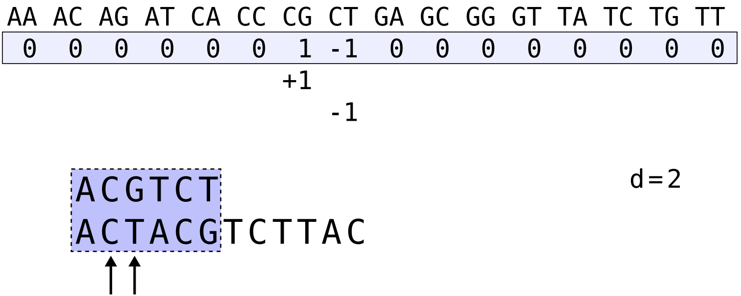
The K-Dist algorithm

AA	AC	AG	AT	CA	CC	CG	CT	GA	GC	GG	GT	TA	TC	TG	TT
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	+1														
	-1														


ACGTCT
ACTACGTCTTAC

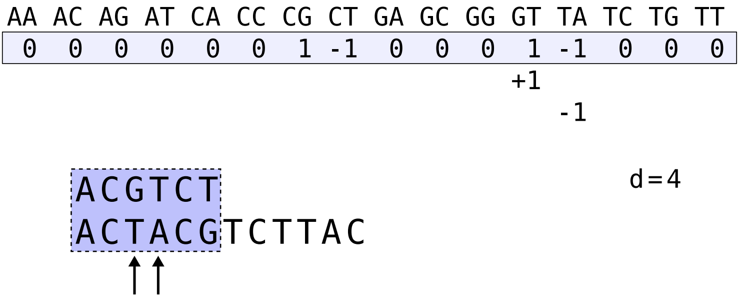
d=0

The K-DIST algorithm



AA	AC	AG	AT	CA	CC	CG	CT	GA	GC	GG	GT	TA	TC	TG	TT
0	0	0	0	0	0	1	-1	0	0	0	0	0	0	0	0

The K-DIST algorithm



AA	AC	AG	AT	CA	CC	CG	CT	GA	GC	GG	GT	TA	TC	TG	TT
0	0	0	0	0	0	1	-1	0	0	0	1	-1	0	0	0

+1

-1

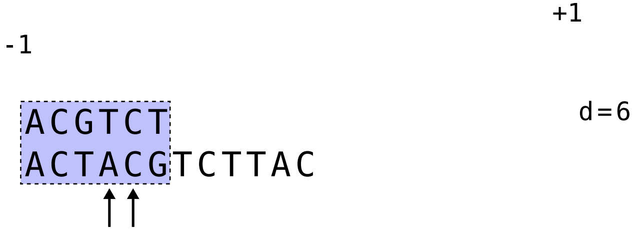
ACGTCT

ACTACGTCTTAC

d=4

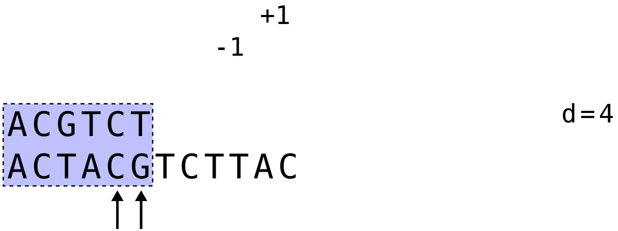
The K-DIST algorithm

AA	AC	AG	AT	CA	CC	CG	CT	GA	GC	GG	GT	TA	TC	TG	TT
0	-1	0	0	0	0	1	-1	0	0	0	1	-1	1	0	0



The K-Dist algorithm

AA	AC	AG	AT	CA	CC	CG	CT	GA	GC	GG	GT	TA	TC	TG	TT
0	-1	0	0	0	0	0	0	0	0	0	1	-1	1	0	0



The K-DIST algorithm

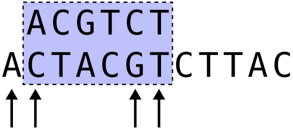
AA	AC	AG	AT	CA	CC	CG	CT	GA	GC	GG	GT	TA	TC	TG	TT
0	-1	0	0	0	0	0	0	0	0	0	1	-1	1	0	0

```
ACGTCT
ACTACGTCTTAC
```

win	0	1	2	3	4	5	6
d	4						

The K-DIST algorithm

AA	AC	AG	AT	CA	CC	CG	CT	GA	GC	GG	GT	TA	TC	TG	TT
0	0	0	0	0	0	0	0	0	0	0	0	-1	1	0	0
	+1														
											-1				



ACGTCT

ACTACGTCTTAC

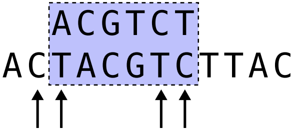
win	0	1	2	3	4	5	6
d	4	2					

The K-DIST algorithm

AA	AC	AG	AT	CA	CC	CG	CT	GA	GC	GG	GT	TA	TC	TG	TT
0	0	0	0	0	0	0	1	0	0	0	0	-1	0	0	0

+1

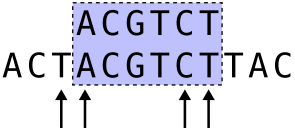
-1



win	0	1	2	3	4	5	6
d	4	2	2				

The K-DIST algorithm

AA	AC	AG	AT	CA	CC	CG	CT	GA	GC	GG	GT	TA	TC	TG	TT
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
												+1			
							-1								



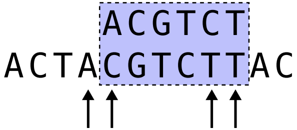
ACGTCT

ACTACGTCTTAC

win	0	1	2	3	4	5	6
d	4	2	2	0			

The K-DIST algorithm

AA	AC	AG	AT	CA	CC	CG	CT	GA	GC	GG	GT	TA	TC	TG	TT
0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	-1
	+1														
															-1



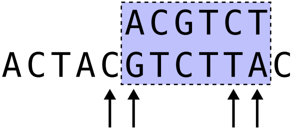
win	0	1	2	3	4	5	6
d	4	2	2	0	2		

The K-DIST algorithm

AA	AC	AG	AT	CA	CC	CG	CT	GA	GC	GG	GT	TA	TC	TG	TT
0	1	0	0	0	0	1	0	0	0	0	0	-1	0	0	-1

+1

-1



ACGTCT

ACTACGTCTTAC

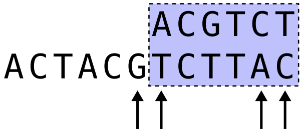
win	0	1	2	3	4	5	6
d	4	2	2	0	2	4	

The K-DIST algorithm

AA	AC	AG	AT	CA	CC	CG	CT	GA	GC	GG	GT	TA	TC	TG	TT
0	0	0	0	0	0	1	0	0	0	0	1	-1	0	0	-1

+1

-1



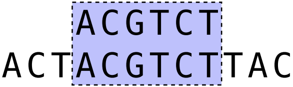
ACGTCT

ACTACGTCTTAC

win	0	1	2	3	4	5	6
d	4	2	2	0	2	4	4

The K-DIST algorithm

AA	AC	AG	AT	CA	CC	CG	CT	GA	GC	GG	GT	TA	TC	TG	TT
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0



```
   ACGTCT
ACTACGTCTTAC
```

win	0	1	2	3	4	5	6
d	4	2	2	0	2	4	4

Time complexity of K-Dist

```
for $i \leftarrow 0$ to $|s| - k$ do
    $s_i \leftarrow s.substring(i, k)$
    $t_i \leftarrow t.substring(i, k)$
    update $cur\_dist$, kmers[$s_i$] and kmers[$t_i$]
```

$\Theta\left(|s| - k\right)$

$+$

```
for $i \leftarrow 0$ to $|t| - |s|$ do
    $kmer_{out} \leftarrow t.substring(i, k)$
    $kmer_{in} \leftarrow t.substring(|s| - k + i + 1, k)$
    update $cur\_dist$, kmers[$kmer_{out}$] and kmers[$kmer_{in}$]
    $min\_dist \leftarrow min(min\_dist, cur\_dist)$
```

$\Theta\left(|t| - |s|\right)$

Total: $\Theta\left(|t| - k\right)$

Cluster analysis algorithms

Various approaches to clustering:

- hierarchical clustering
- graph-based clustering
- greedy clustering

A greedy approach is necessary due to the sizes of the data.

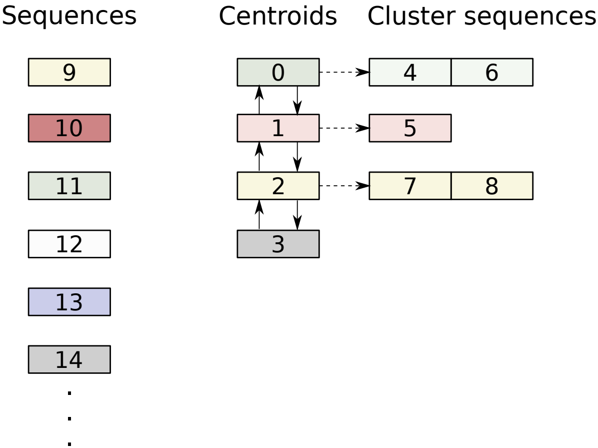
The K-Clust algorithm

The clustering algorithm used in `klust`.

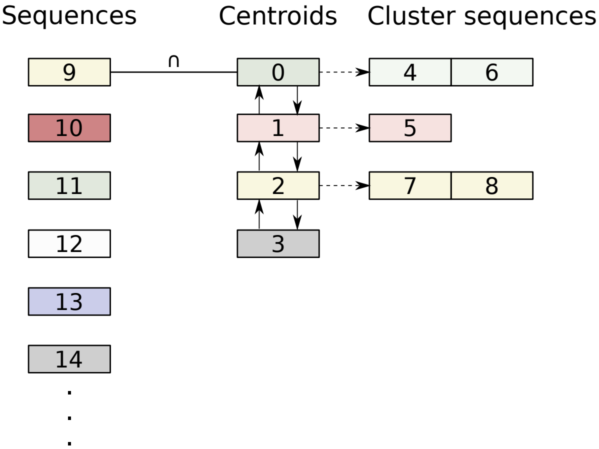
The intersection criterion

$$|K(s) \cap K(c)| \geq |K(c)| \cdot id$$

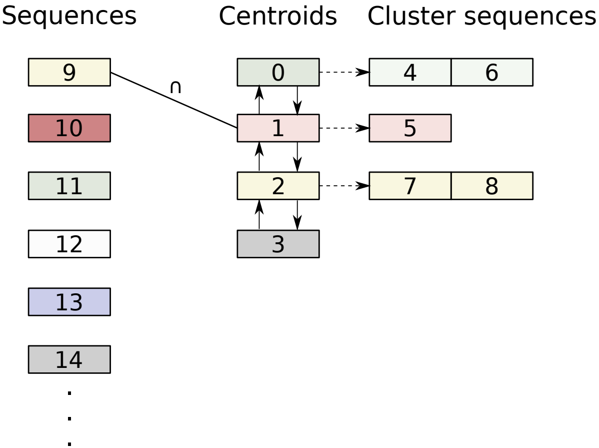
The K-CLUST algorithm



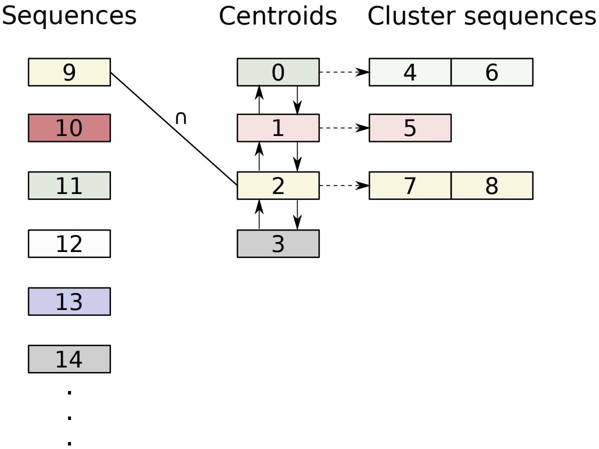
The K-Clust algorithm



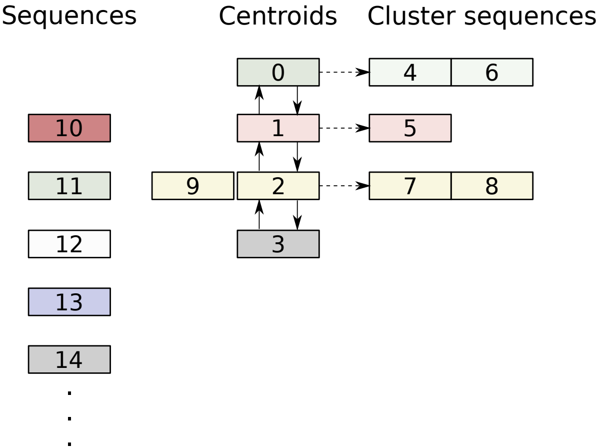
The K-Clust algorithm



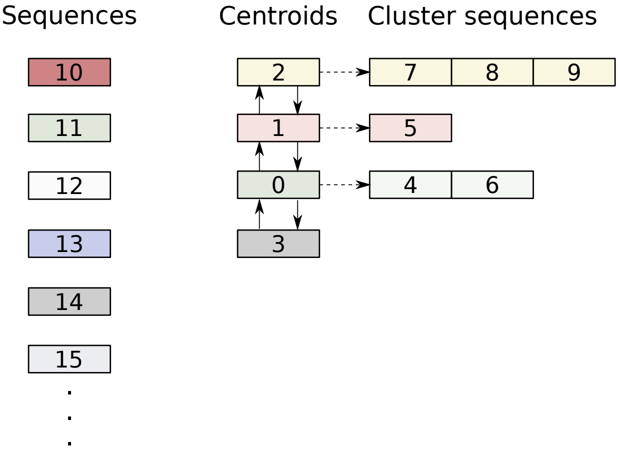
The K-Clust algorithm



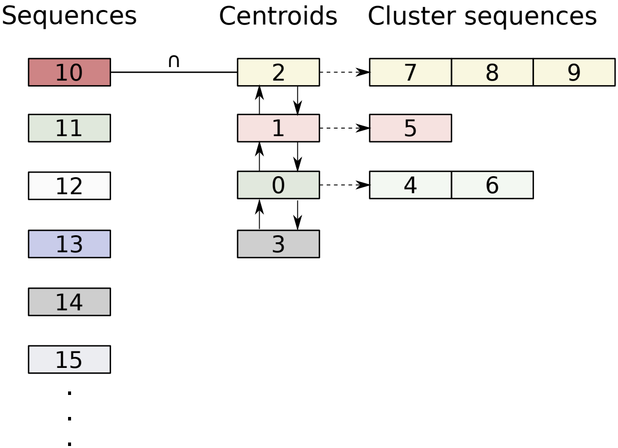
The K-Clust algorithm



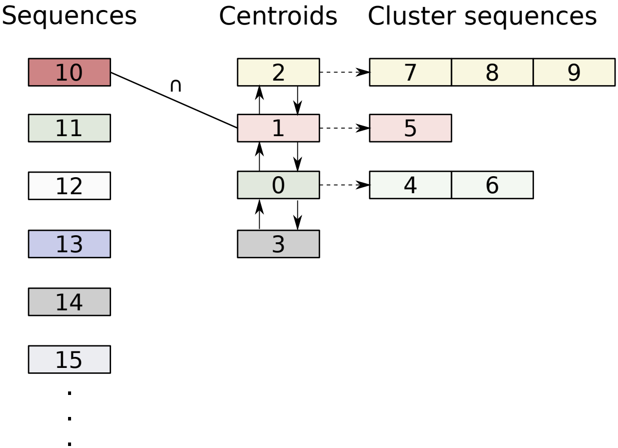
The K-CLUST algorithm



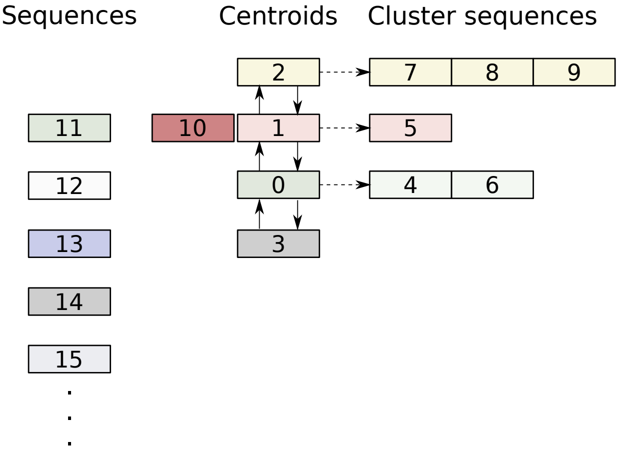
The K-Clust algorithm



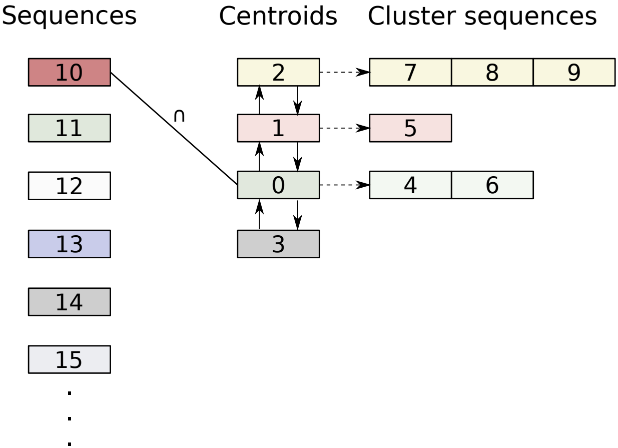
The K-Clust algorithm



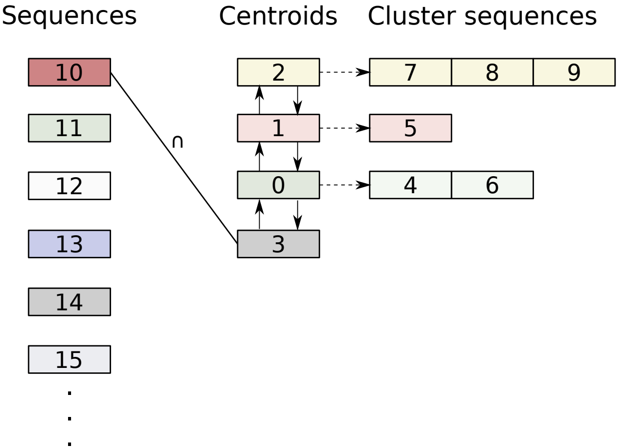
The K-Clust algorithm



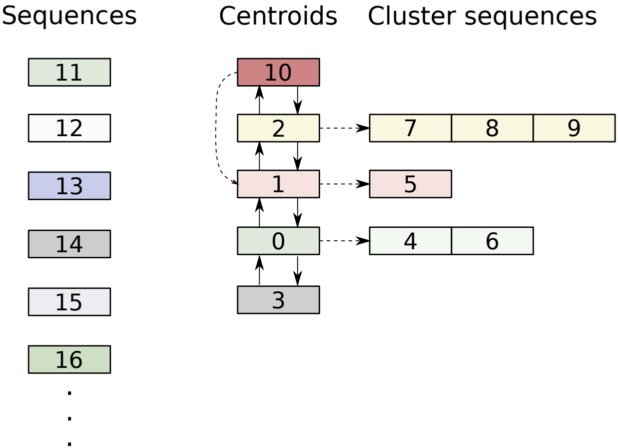
The K-Clust algorithm



The K-Clust algorithm

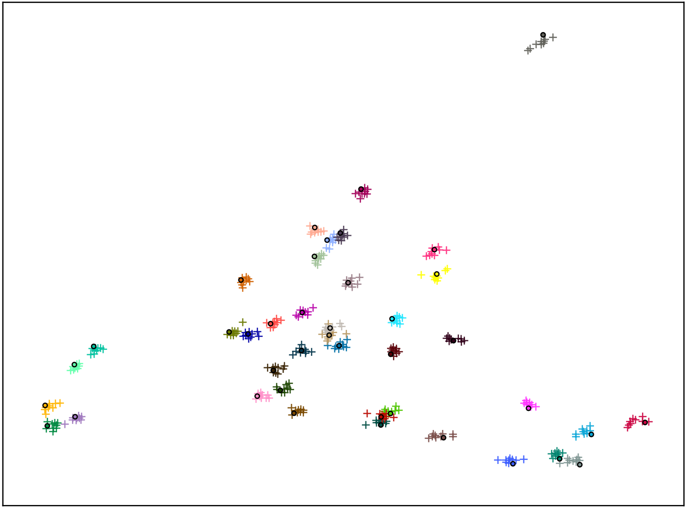


The K-CLUST algorithm



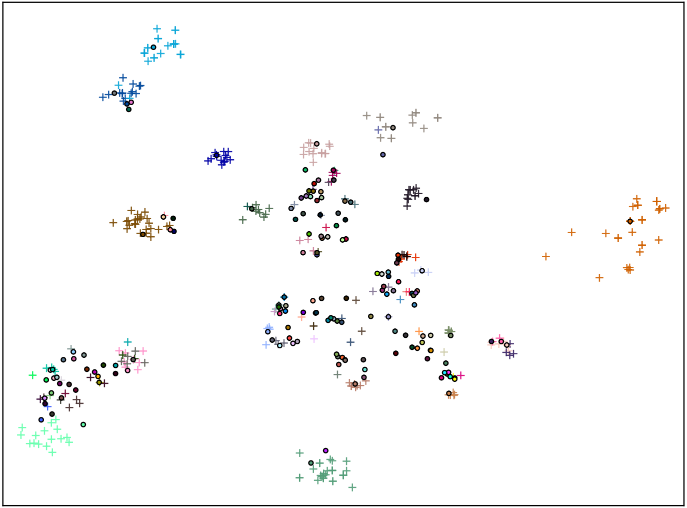
Clustering the SILVA RNA dataset

Clustering program	Time	Throughput (seqs./sec.)	Clusters	Max. memory
klust, $id = 0.90$, $k = 5$	0:42:59	614.16	159,812	$\approx$ 1021 MB
USEARCH, $id = 0.97$, -cluster_smallmem	1:04:10	411.10	221,040	$\approx$ 2048 MB



K-CLUST
$k = 5$
$id = 0.85$

clusters: 40
max. size: 10
avg. size: 10
singletons: 0



K-Clust
$k = 5$
$id = 0.85$
sort: incr.

clusters: 157
max. size: 43
avg. size: 3.18
singletons: 89