

Efficient DNA/RNA sequence clustering

Using k -mers as an approximation for sequence similarity

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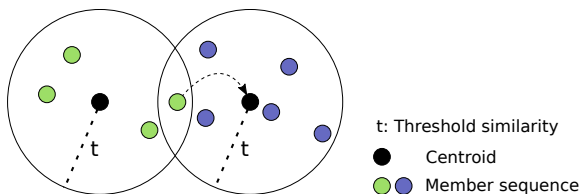
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Introduction

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

$$\begin{aligned}
 d_{22}(S_1, S_2) &= \sqrt{(3-1)^2 - 1^2 - 1^2 + (1-1)^2 + 1^2 - 1^2 + 1^2} \\
 &= \sqrt{9} = 3
 \end{aligned}$$

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	1	-1	0	0	0	0	0	0	0	0

+1

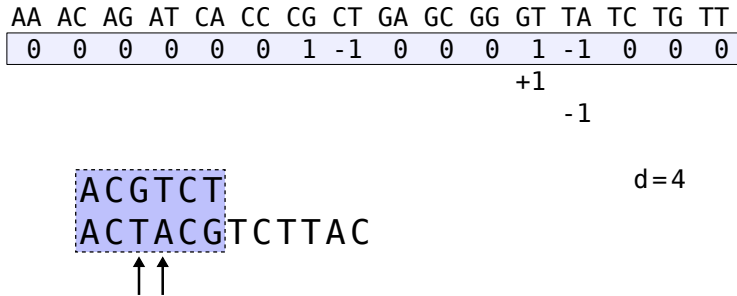
-1

ACGTCT
 ACTACGTCTTAC

↑↑

$d=2$

The K-DIST algorithm



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

-1

+1

ACGTCT
 ACTACGTCTTAC

↑↑

$d=6$

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

+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	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
 AACTACGTCTTAC
 ↑↑ ↑↑

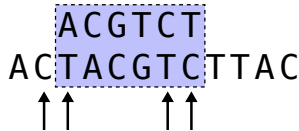
win	0	1	2	3	4	5	6
d	4	2					

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

ACT ACGTCT TAC
 ↑ ↑ ↑ ↑

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

ACTA **ACGTCT**TTAC

↑↑ ↑↑

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

ACTAC **ACGTCT**
 GTCTTAC

↑↑ ↑↑

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

ACTACG ACGTCT

 ↑↑ ↑↑

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

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.

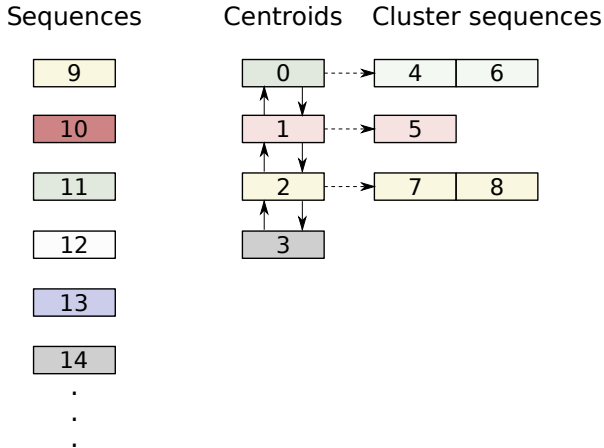
The K-CLUST algorithm

- centroid based
- the clustering algorithm used in `klust`

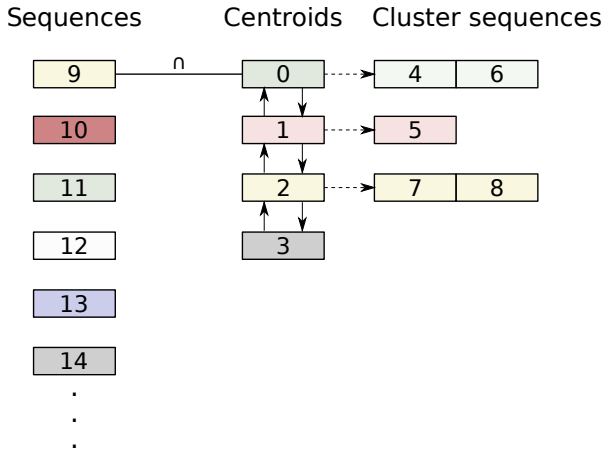
Intersection criterion

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

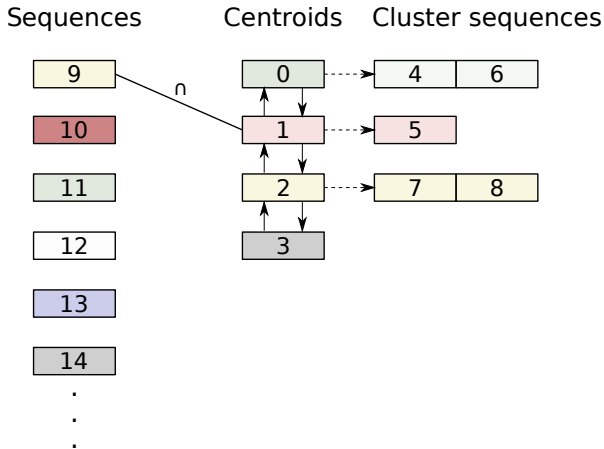
The K-CLUST algorithm



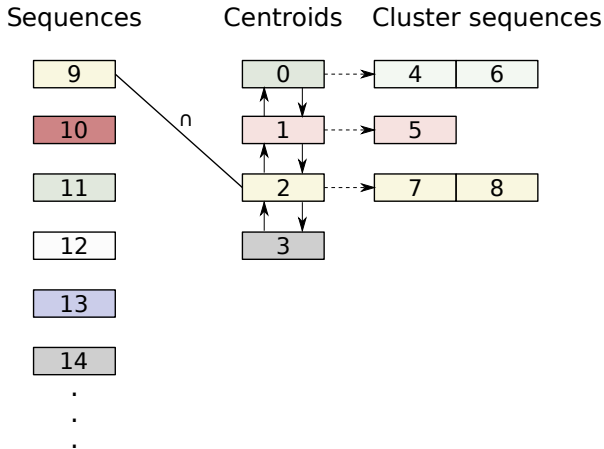
The K-CLUST algorithm



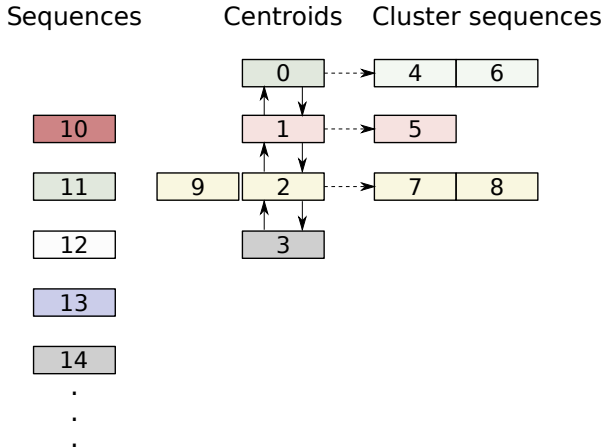
The K-CLUST algorithm



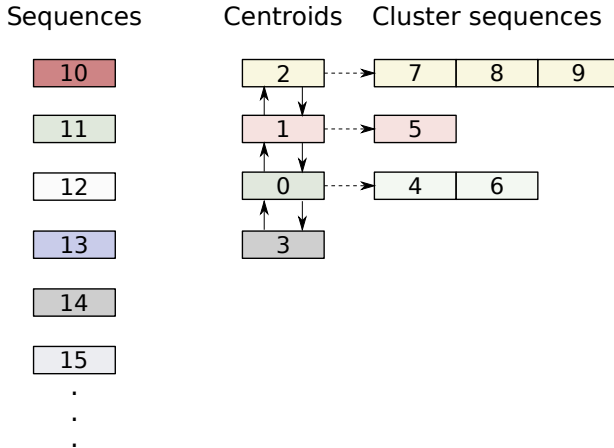
The K-CLUST algorithm



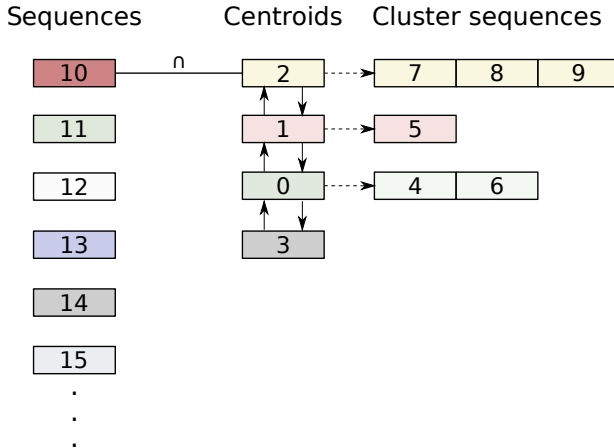
The K-CLUST algorithm



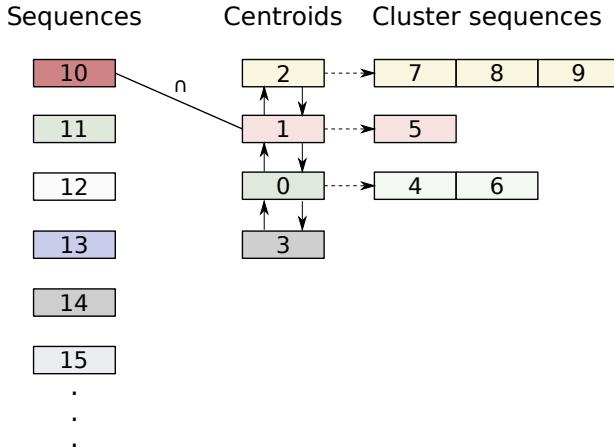
The K-CLUST algorithm



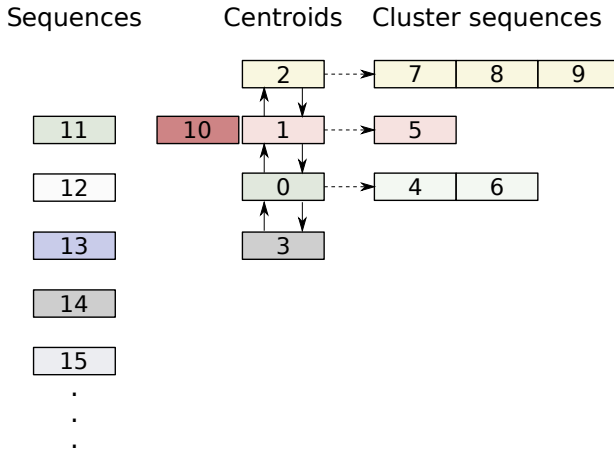
The K-CLUST algorithm



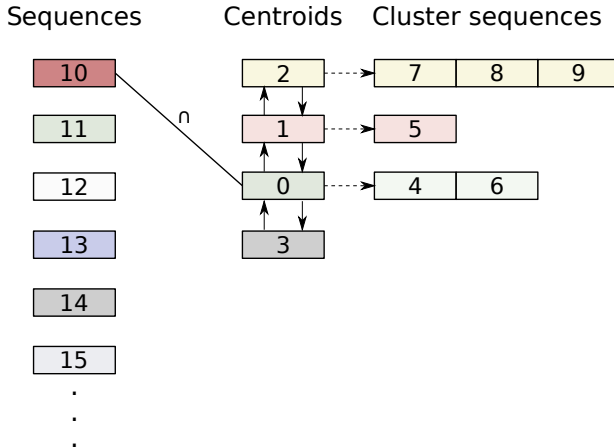
The K-CLUST algorithm



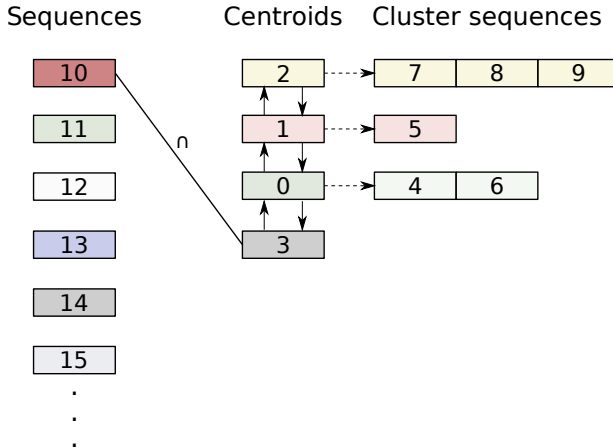
The K-CLUST algorithm



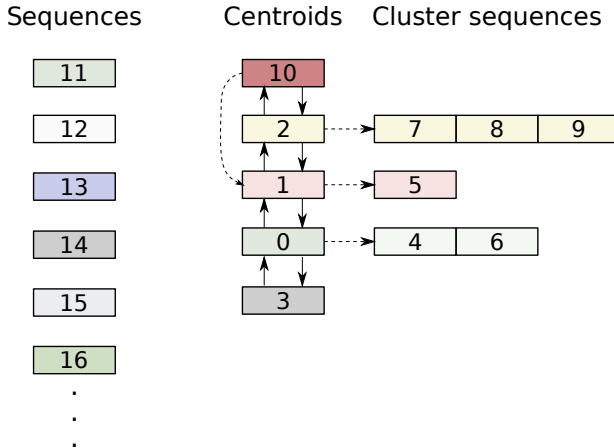
The K-CLUST algorithm



The K-CLUST algorithm

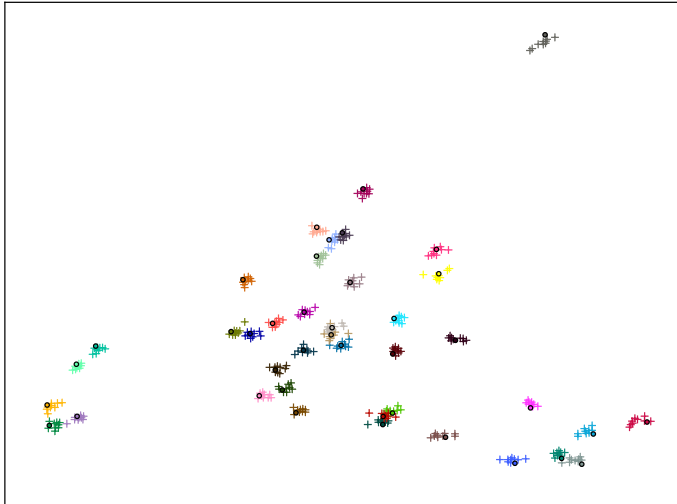


The K-CLUST algorithm



Clustering the SILVA RNA dataset

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



K-CLUST

$k = 5$

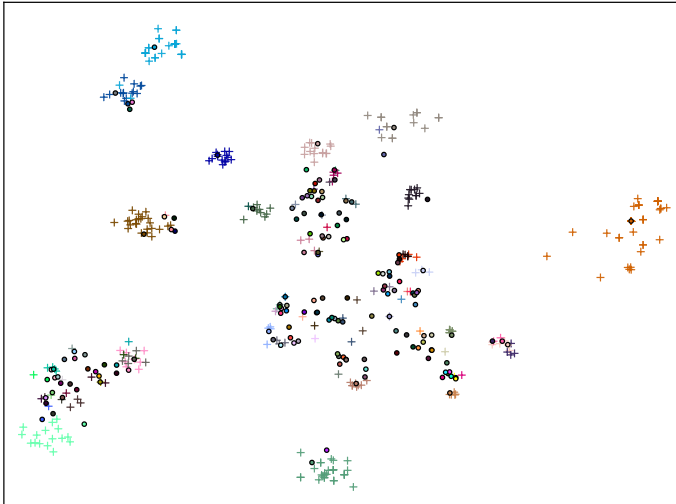
$id = 0.85$

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