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Designing small universal k-mer hitting sets for improved analysis of high throughput sequencing

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Outline

- Background
- Methods and results
- Conclusion

Background

· Sequencing datasets are larger and larger.
· New computational ideas are essential to manage and analyze data.

Minimizer

· Michael Roberts, Wayne Hayes, Brian R. Hunt, Stephen M. Mount, James A. Yorke;
Reducing storage requirements for biological sequence comparison,
Bioinformatics, Volume 20, Issue 18, 12 December 2004, Pages 3363–3369

· Given a sequence of length L, the minimizer is the lexicographically smallest k-mer in it.

· Given a sequence S of any length, the minimizer set is the set of minimizers of every L-long subsequence in S.

$\implies$ Every L-long subsequence in S is represented in the set.

Application of Minimizers

- Hashing for read overlapping
- Sparse suffix arrays
- Bloom filters to speed up sequence search

Hashing for read overlapping

$L = 6$, $k = 3$

R1：CATCGACA

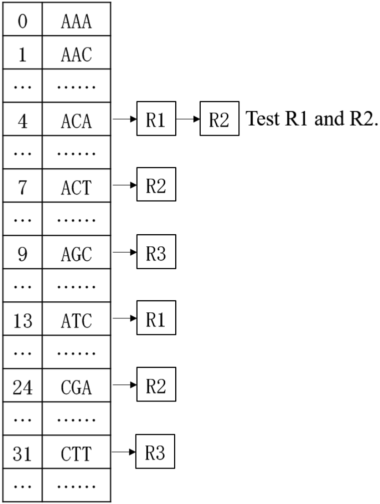
minimizers：ATC, ACA

R2：ACTCGACA

minimizers：ACT, CGA, ACA

R3：GAGCTTGC

minimizers：AGC, CTT



Sparse suffix arrays

1	2	3	4	5	6	7	8
A	G	T	C	G	A	C	T

AGTCGACT	1
GTCGACT	2
TCGACT	3
CGACT	4
GACT	5
ACT	6
CT	7
T	8

Suffix Array

ACT	6
AGTCGACT	1
CGACT	4
CT	7
GACT	5
GTCGACT	2
T	8
TCGACT	3

Sparse Suffix Array

ACT	6
CGACT	4
GTCGACT	2
T	8

$s = 2$

To query a string q, perform at most s queries starting from indices 0, ..., s - 1 in q.

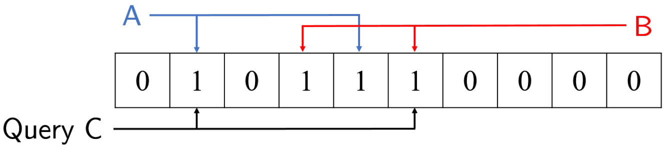
ACT	6
AGTCGACT	1
CGACT	4

When $L = 6$, $k = 3$,
minimizers: AGT, CGA, ACT

To query a string q, find q's minimizers and search strings starting with these minimizers.

Bloom filter

- A bit array.
- A constant number of different hash functions are defined to map elements to the array.
- Supports two operations: “storing an element in the set” and “checking if an element is in the set.”
- Can generate false positives during querying.



Universal hitting set(UHS)

· For integers k, L, a set $U_{k,L}$ is called a UHS of k-mers if every possible sequence of length L must contain at least one k-mer in $U_{k,L}$.

· For example, the set of all k-mers is a trivial UHS.

· ***Problem 1***. Given k and L, find a smallest UHS of k-mers.

Hits

- A k-mer w hits string S, denoted $w \subseteq S$, if w is a substring in S.
- k-mer set X hits string S if there exists $w \in X$ such that $w \subseteq S$.
- The UHS in Problem 1 is a set of k-mers $U_{k,L}$ which hits every possible sequence of length L.

Advantages of UHS over minimizers

· The set of minimizers may be as large as the complete set of k-mers. The method in this paper can often generate UHSs smaller by a factor of nearly k.

· UHS is universal.

$\Longrightarrow$ For any k and L, a UHS needs to be computed only once for every dataset.

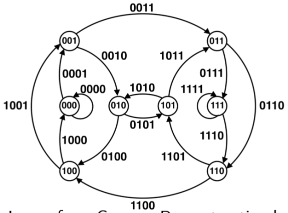
$\Longrightarrow$ The data structures created for different datasets will contain a comparable set of k-mers.

Using de Bruijn graphs to find UHSs

· ***Problem 2.*** Given a complete de Bruijn graph D_k of order k and an integer L, find a smallest set of vertices $U_{k,L}$ such that any path in D_k of length $l = L - k$ passes through at least one vertex of $U_{k,L}$.

Complete de Bruijn graph

- A complete de Bruijn graph of order k over alphabet Σ:
  V: $|\Sigma|^k$ vertices, each labelled with a unique k-mer.
  E: If there is an edge (u, v) with a $(k+1)$-mer label l, then the label of vertex u is the k-suffix of l and the label of vertex v is the k-prefix of l. A complete de Bruijn graph contains all possible $|\Sigma|^{k+1}$edges of this type.



A complete de Bruijn graph of order 3 over alphabet $\{0, 1\}$ $= B(2, 4)$
A complete de Bruijn graph of order k over alphabet Σ $= B(|\Sigma|, k+1)$

Image from Genome Reconstruction by Phillip E. C. Compeau and Pavel A. Pevzner

How to find the UHS?

- NP-hard in general(supporting information in the paper).
- Heuristic approaches.(DOCKS, DOCKSany, DOCKSanyX)

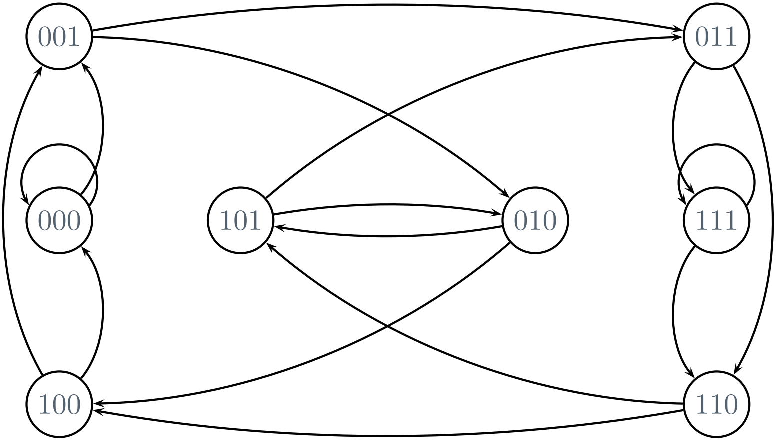
How to find UHS?

1. Generate a complete de Bruijn graph of order k, set $l = L - k$.
2. Find the decycling vertex set(V set), X.
3. Remove X from the graph, result in G'.
4. Remove vertices from G' and add them to S to hit the remained L length sequences.
(i) DOCKS
(ii) DOCKSany
(iii) DOCKSanyX
5. X is the universal hitting set we're searching for.

Decycling de Bruijn graph

- Vertices labeling
- Factor
- Pure cycling register(PCR_k)
- V-set

Decycling de Bruijn graph



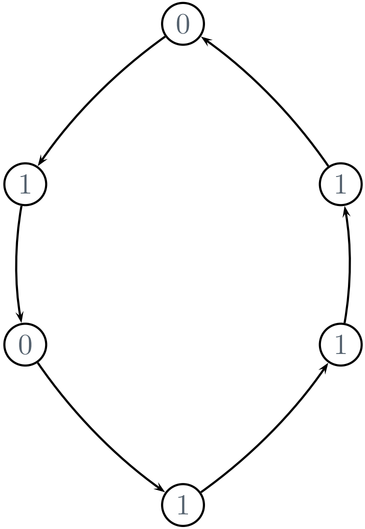
Vertices labeling

For a vertex $v(s_0, s_1, \ldots, s_{k-1})$, calculate the center of mass.

According to the center of mass position in the coordinate system, label the vertex I if $x = 0$, L if $x < 0$, R if $x > 0$,

Vertex labeling example

$v = 010111$, the center of mass' x value > 0. $\implies$ *R*.



Factor

· A factor is a set of cycles such that all vertices in the graph are in exactly one of the cycles.
· Each cycle has a unique feedback function $f(s_0, s_1, \ldots, s_{k-1}) = s_k$.

Pure cycling register(PCR_k)

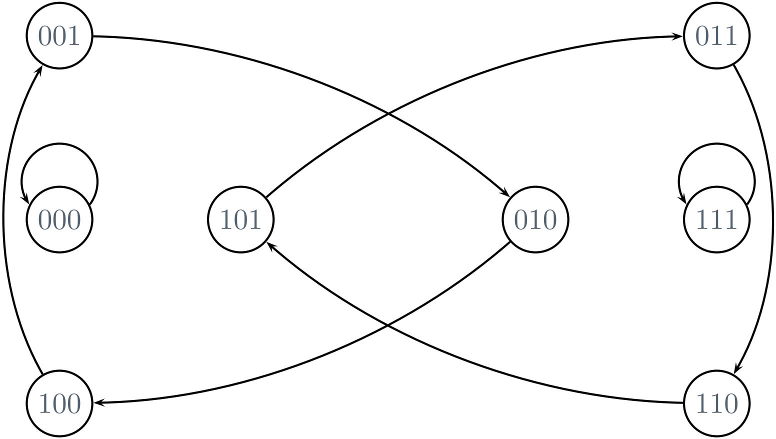
· PCR_k is a factor.

· Each cycle has a unique function $f(s_0, s_1, \ldots, s_{k-1}) = s_k = s_0$, that is, for every arc $< u, v >$, $u = (s_0, s_1, \ldots, s_{k-1}) \implies v = (s_1, s_2, \ldots, s_k) = (s_1, s_2, \ldots, s_0)$.

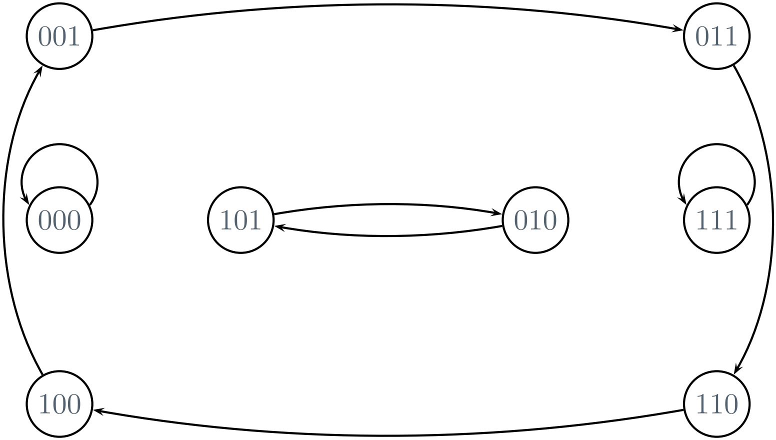
· The number of cycles in PCR_k is $Z(k)$, which converges to $\frac{|\Sigma|^k}{k}$.

· It is proved that any circle in the PCR_k must be either all I's or a block of L's and a block of R's separated by at most two I's.

PCR_k example



Factor but not PCR_k example



Why PCR_k?

Lemmas tell us:

· All cycles are in the form of all I's or at least a L and a R.

· Cycles with all I's are in PCR_k.

· For each cycle with at least a L and a R, there exist exactly one cycle in PCR_k such that the first vertex of L block of the two cycles are the same one.

$\Longrightarrow$ We only need to deal with cycles in PCR_k.

V-set

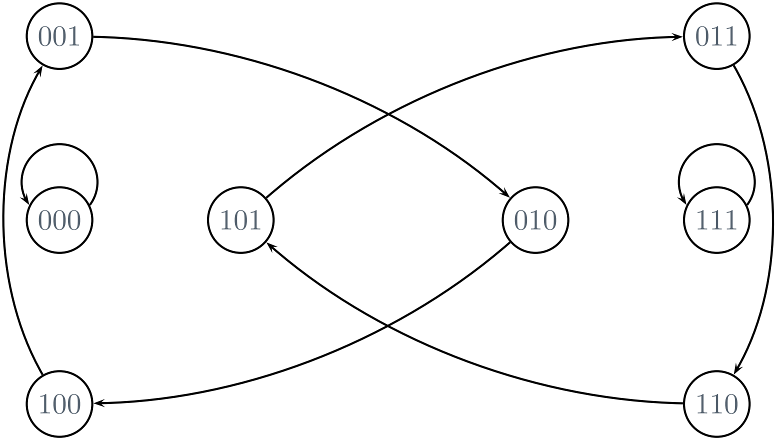
A minimum set of vertices which when removed leaves a graph with no cycles.

V-set

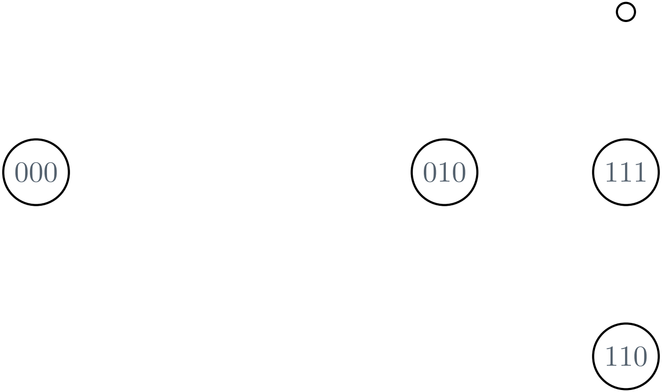
Naïve algorithm:

1. Choose a vertex v, find the cycle belongs to PCR_k that contains v.
2. Choose a certain vertex u and add it to the V-set:
   Arbitrary one, if the cycle is all I's.
   The first vertex in the L block, otherwise.
3. Remove the cycle from the graph.
4. Repeat until all cycles belong to PCR_k are tested.

V-set example



V-set example



Time complexity analysis

There are $Z(k)$ iterations. Find the vertex to be added with $O(k)$ time cost in every iteration.
$\implies O(kZ(k)) = O(|\Sigma|^k)$ in total.

How to find Minimum UHS?

1. Generate a complete de Bruijn graph of order k, set $l = L - k$.
2. Find the decycling vertex set(V set), X.
3. Remove X from the graph, result in G'.
4. Remove vertices from G' and add them to S to hit the remained L length sequences.
(i) DOCKS
(ii) DOCKSany
(iii) DOCKSanyX
5. X is the universal hitting set we're searching for.

DOCKS

Define:

$$D(v,i) = \text{the number of } i\text{-long paths starting at } v$$
$$F(v,i) = \text{the number of } i\text{-long paths ending at } v$$

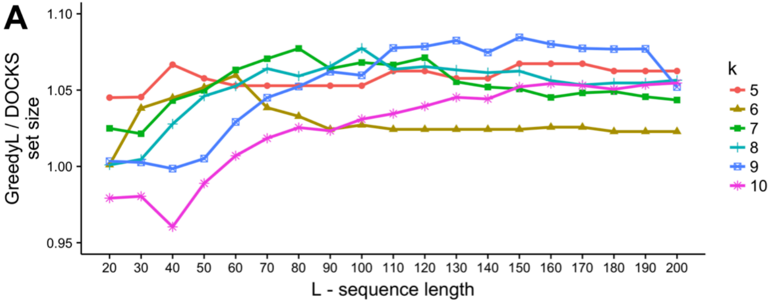
$\Longrightarrow$

$$T(v,l) = \text{the number of } l\text{-long paths through } v$$
$$= \Sigma_{i=0}^{l} F(v,i) \cdot D(v,l-i)$$

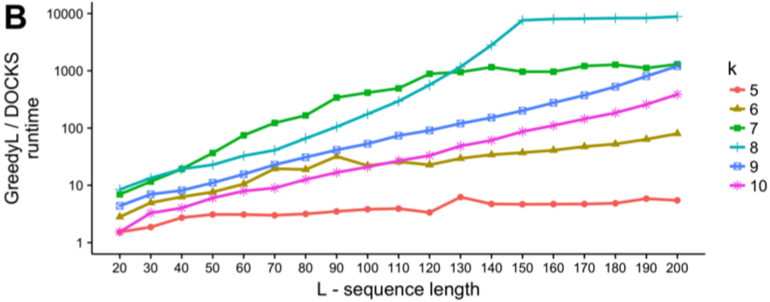
- Calculate $D(-,-)$, $F(-,-)$ to find $T(-,l)$.
- Choose the one has the largest $T(-,l)$ and extract it.
- Repeat until no such vertex(p iterations).
- $O((1+p)|\Sigma|^{k+1} \cdot l)$

DOCKS performance(set size)

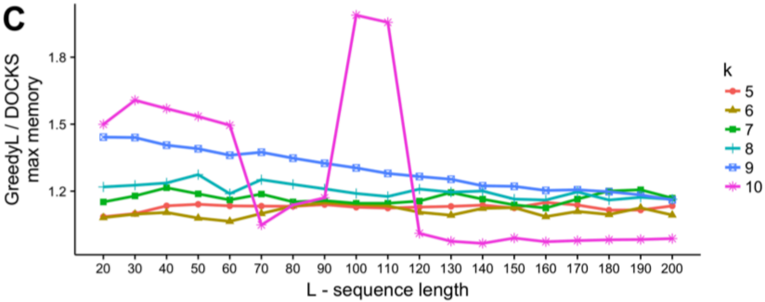
Fig A



DOCKS performance(runtime)



DOCKS performance(memory)



DOCKSany

Define:

$$D(v) = \text{the number of paths start at } v$$
$$F(v) = \text{the number of paths end at } v$$

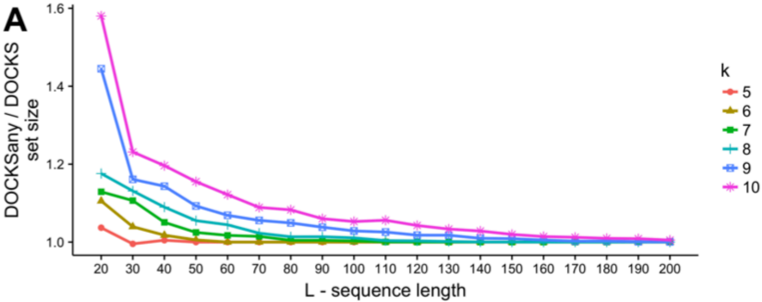
$\Longrightarrow$

$$T(v) = \text{the number of paths through } v$$
$$= F(v) \cdot D(v)$$

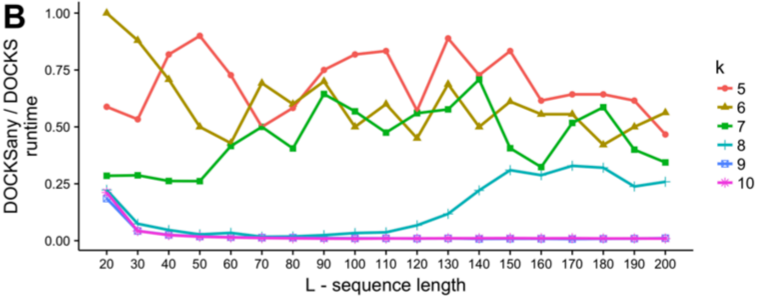
- Calculate $D(-), F(-)$ to find $T(-)$.
- Choose the one has the largest $T(-)$ and extract it.
- Repeat until no paths of length l (p iterations).
- $O((1+p)|\Sigma|^{k+1})$

DOCKSany performance(set size)

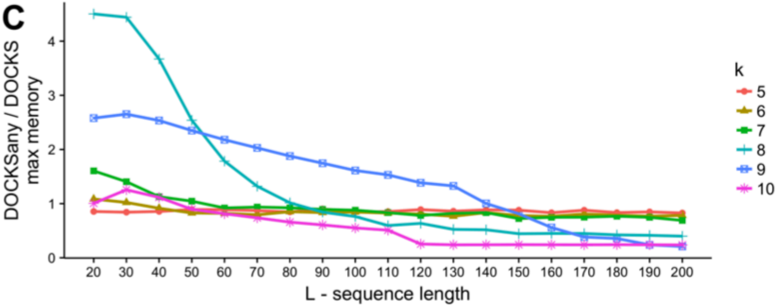
Fig C



DOCKSany performance(runtime)



DOCKSany performance(memory)

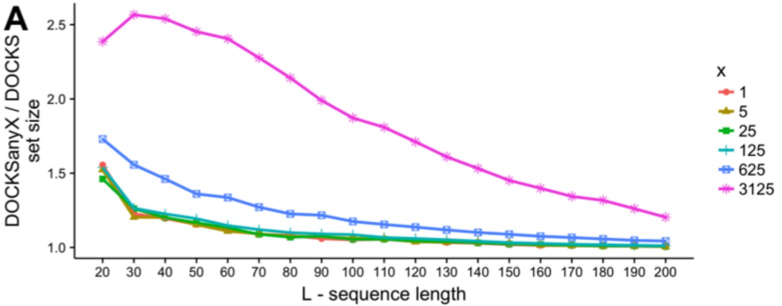


DOCKSanyX

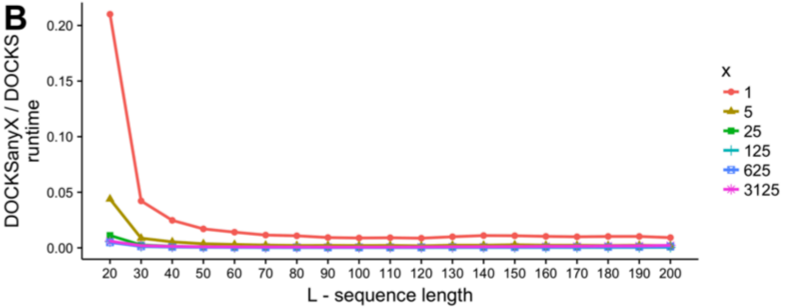
Same calculation as DOCKSany.
Extract at most x such vertices instead of just one.

DOCKSanyX performance(set size)

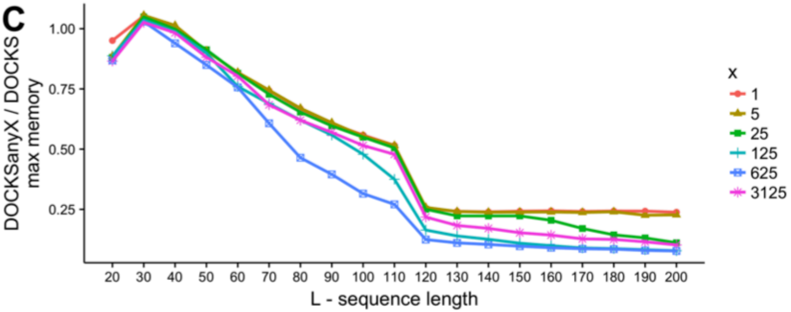
Fig D



DOCKSanyX performance(runtime)



DOCKSanyX performance(memory)



Conclusion

· DOCKS can generate compact sets of k-mers that hit all L-long sequences for any $k \leq 13$ and L.

· These compact sets can improve many of the applications that currently use minimizers.