

Efficient representation of de Bruijn Graphs

Assembly data is big

For very large datasets, even after filtering, a hash table over all k-mers may be too big.

Why is a hash table big?

How can we do better?

What if we just want to know “if” a k-mer is present?



What if we just wanted “approximate” occurrence?

Bloom Filters

Originally designed to answer *probabilistic* membership queries:

Is element e in my set S ?

If yes, **always** say yes

If no, say no **with large probability**

False positives can happen; false negatives cannot.

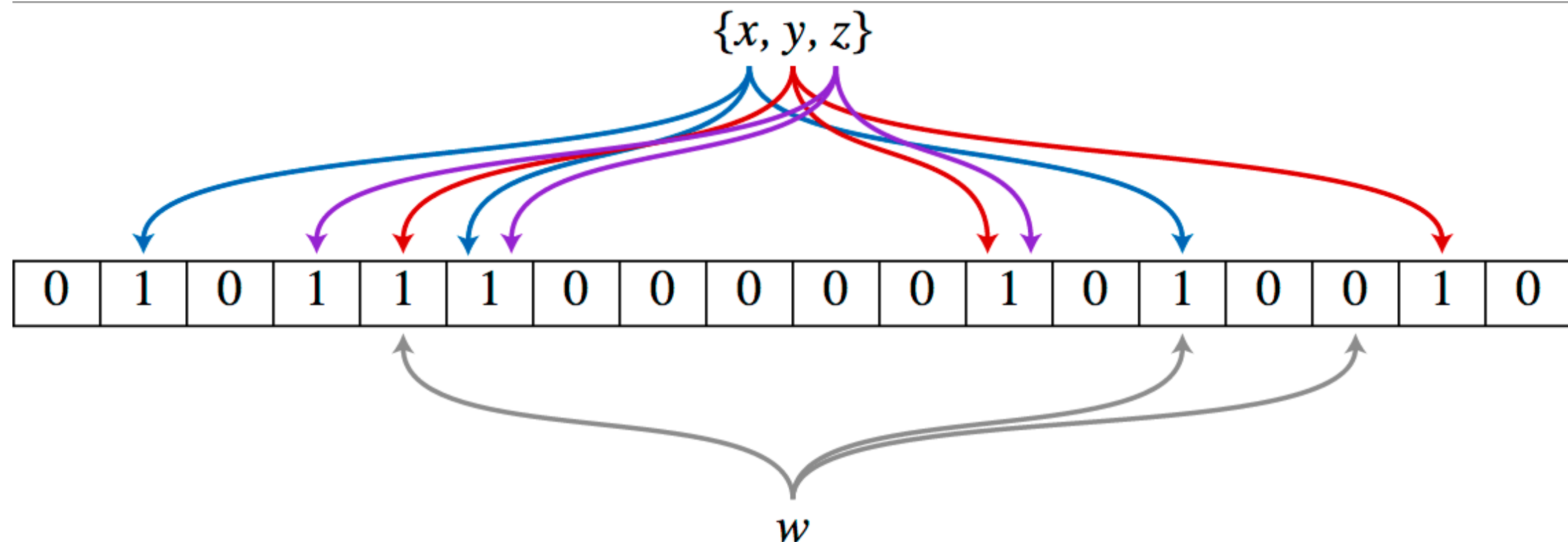
Bloom Filters

For a set of size N , store an array of M bits

Use k different hash functions, $\{h_0, \dots, h_{k-1}\}$

To insert e , set $A[h_i(e)] = 1$ for $0 < i < k$

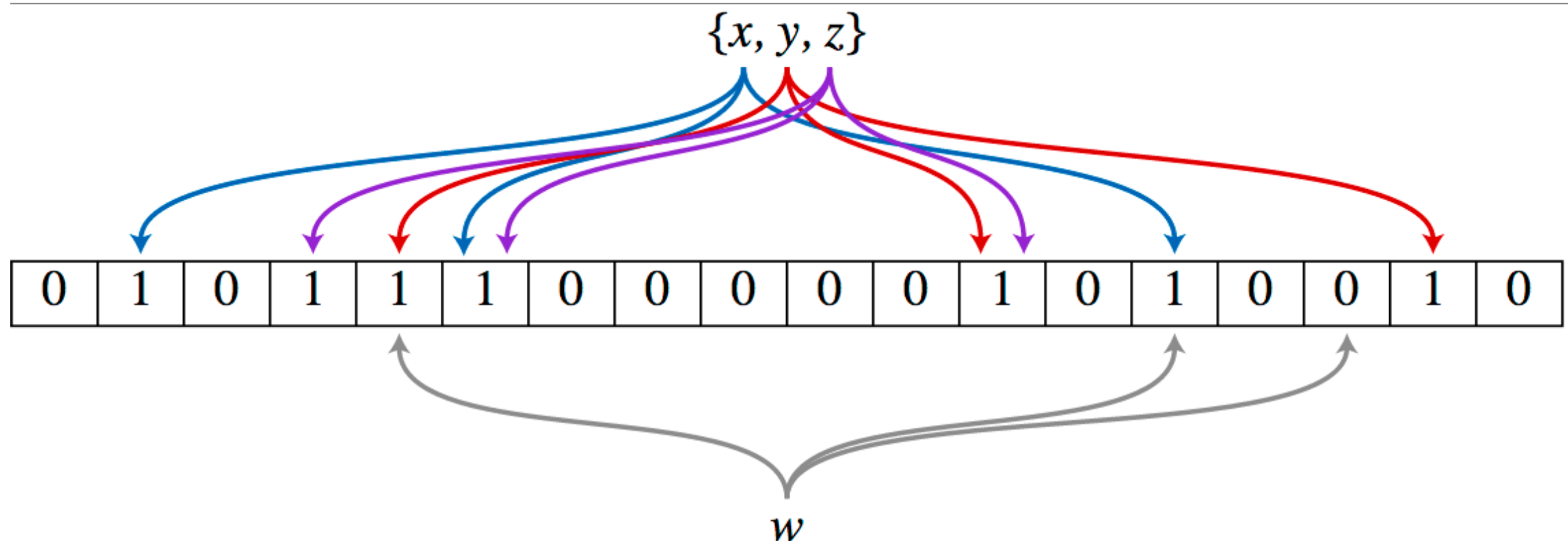
To query for e , check if $A[h_i(e)] = 1$ for $0 < i < k$



Bloom Filters

If hash functions are good and sufficiently independent, then the probability of false positives is low and controllable.

How low?



False Positives

Let q be the fraction of the m -bits which remain as 0 after n insertions.

The probability that a randomly chosen bit is 1 is $1-q$.

But we need a 1 in the position returned by k different hash functions; the probability of this is $(1-q)^k$

We can derive a formula for the expected value of q , for a filter of m bits, after n insertions with k different hash functions:

$$E[q] = (1 - 1/m)^{kn}$$

False Positives

Mitzenmacher & Upfal used the Azuma-Hoeffding inequality to prove (without assuming the probability of setting each bit is independent) that

$$\Pr(|q - E[q]| \geq \frac{\lambda}{m}) \leq 2\exp(-2\frac{\lambda^2}{m})$$

That is, the random realizations of q are highly concentrated around $E[q]$, which yields a false positive prob of:

$$\sum_t \Pr(q = t)(1 - t)^k \approx (1 - E[q])^k = \left(1 - \left[1 - \frac{1}{m}\right]^{kn}\right)^k \approx (1 - e^{-\frac{kn}{m}})^k$$

False Positives

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This lets us choose optimal values to achieve a target false positive rate. For example, assume m & n are given. Then we can derive the optimal k

$$k = (m/n) \ln 2 \Rightarrow 2^{-k} \approx 0.6185^{m/n}$$

We can then compute the false positive prob

$$p = \left(1 - e^{-\left(\frac{m}{n} \ln 2\right) \frac{n}{m}}\right)^{\left(\frac{m}{n} \ln 2\right)} \Rightarrow$$

$$\ln p = -\frac{m}{n} (\ln 2)^2 \Rightarrow$$

$$m = -\frac{n \ln p}{(\ln 2)^2}$$

False Positives

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given an **expected**
elems

and a **desired**
false positive rate

we can compute
the **optimal size** and
of has functions

Detour: Bloom Filters & De Bruijn Graphs

How could this data structure be useful for representing a De Bruijn graph?



A given $(k-1)$ -mer can only have $2 * |\Sigma|$ neighbors;
 $|\Sigma|$ incoming and $|\Sigma|$ outgoing neighbors — for
genomes $|\Sigma| = 4$

To navigate in the De Bruijn graph, we can simply
query all possible successors, and see which are
actually present.

Bloom Filters & De Bruijn Graphs

How could this data structure be useful for representing a De Bruijn graph?

Say we have a bloom filter B , for all of the k -mers in our data set, and say I give you one k -mer that is truly present.

We now have a “navigational” representation of the De Bruijn graph (can return the set of neighbors of a node, but not select/iterate over nodes); why?

Bloom Filters & De Bruijn Graphs

But, a Bloom filter still has false-positives, right?

May return some neighbors that are not actually present.

Pell et al., PNAS 2012, use a lossy Bloom filter directly

Chikhi & Rizk, WABI 2012, present a *loss/less* datastructure
based on Bloom filters

Salikhov et al., WABI 2013 extend this work and introduce
the concept of “cascading” Bloom filters

First, some bounds

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Research Articles

On the Representation of De Bruijn Graphs

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JARED T. SIMPSON,⁵ and PAUL MEDVEDEV^{1,2,6}

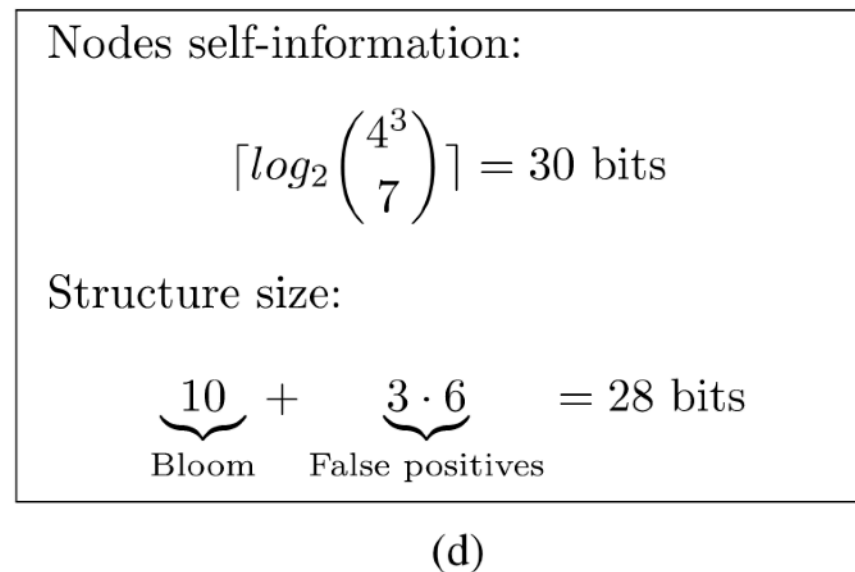
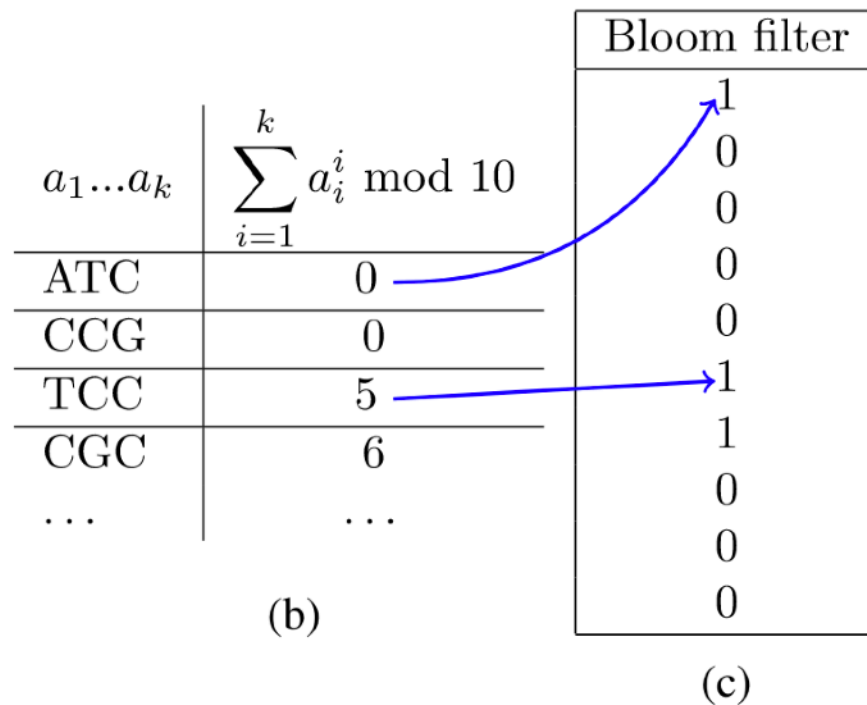
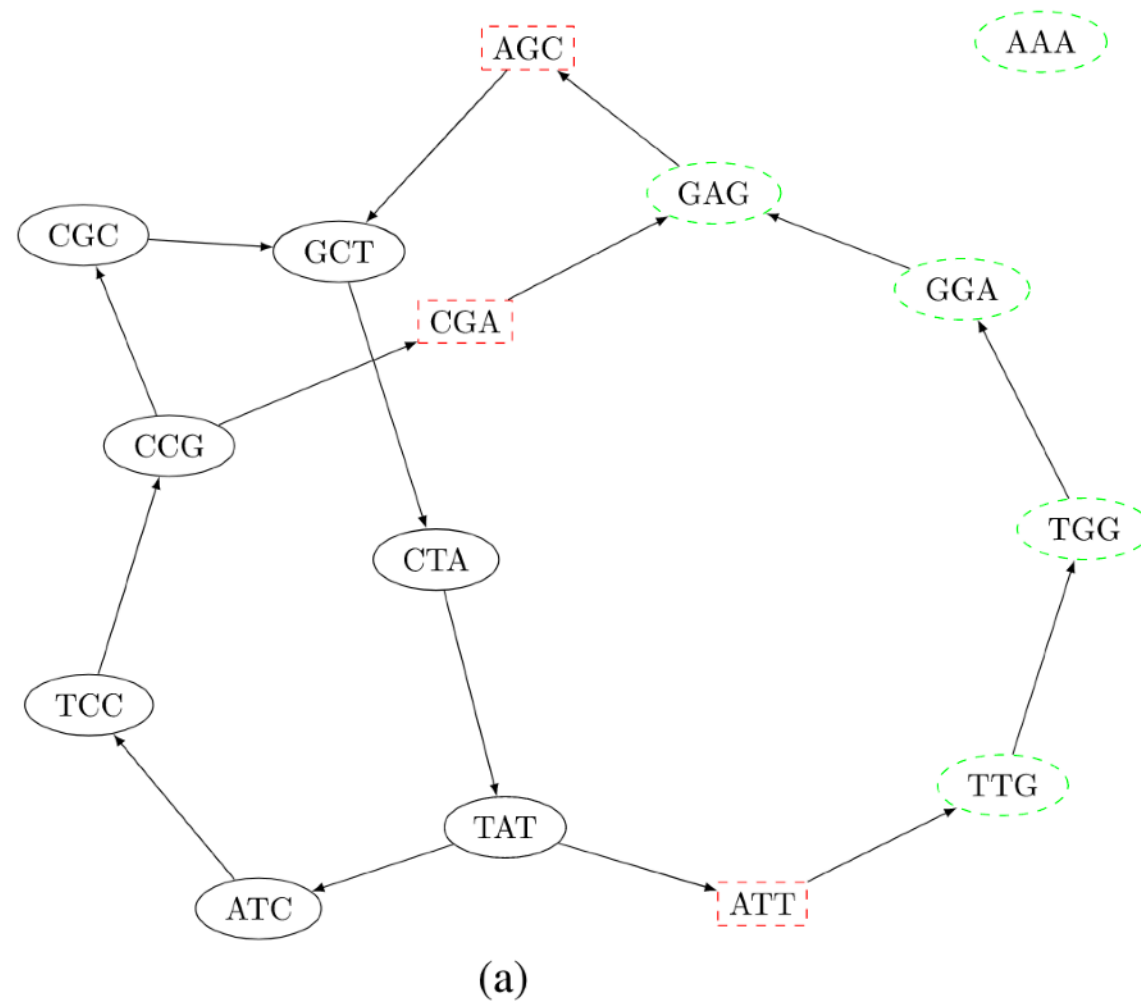
We use the term membership data structure to refer to a way of representing a dBG and answering k -mer membership queries. We can view this as a pair of algorithms: (CONST, MEMB). The CONST algorithm takes a set of k -mers S (i.e., a dBG) and outputs a bit string. We call CONST a constructor, since it constructs a representation of a dBG. The MEMB algorithm takes as input a bit string and a k -mer x and outputs true or false. Intuitively, MEMB takes a representation of a dBG created by CONST and outputs whether a given k -mer is present. Formally, we require that for all $x \in \Sigma^k$, $\text{MEMB}(\text{CONST}(S), x)$ is true if and only if $x \in S$.

An NDS is a pair of algorithms, CONST and NBR. As before, CONST takes a set of k -mers and outputs a bit string. NBR takes a bit string and a k -mer and outputs a set of k -mers. The algorithms must satisfy that for every dBG S and a k -mer $x \in S$, $\text{NBR}(\text{CONST}(S), x) = \text{ext}(x) \cap S$. Note that if $x \notin S$, then the behavior of $\text{NBR}(\text{CONST}(S), x)$ is undefined. We observe that a membership data structure immediately implies an NDS because an NBR query can be reduced to eight MEMB queries.

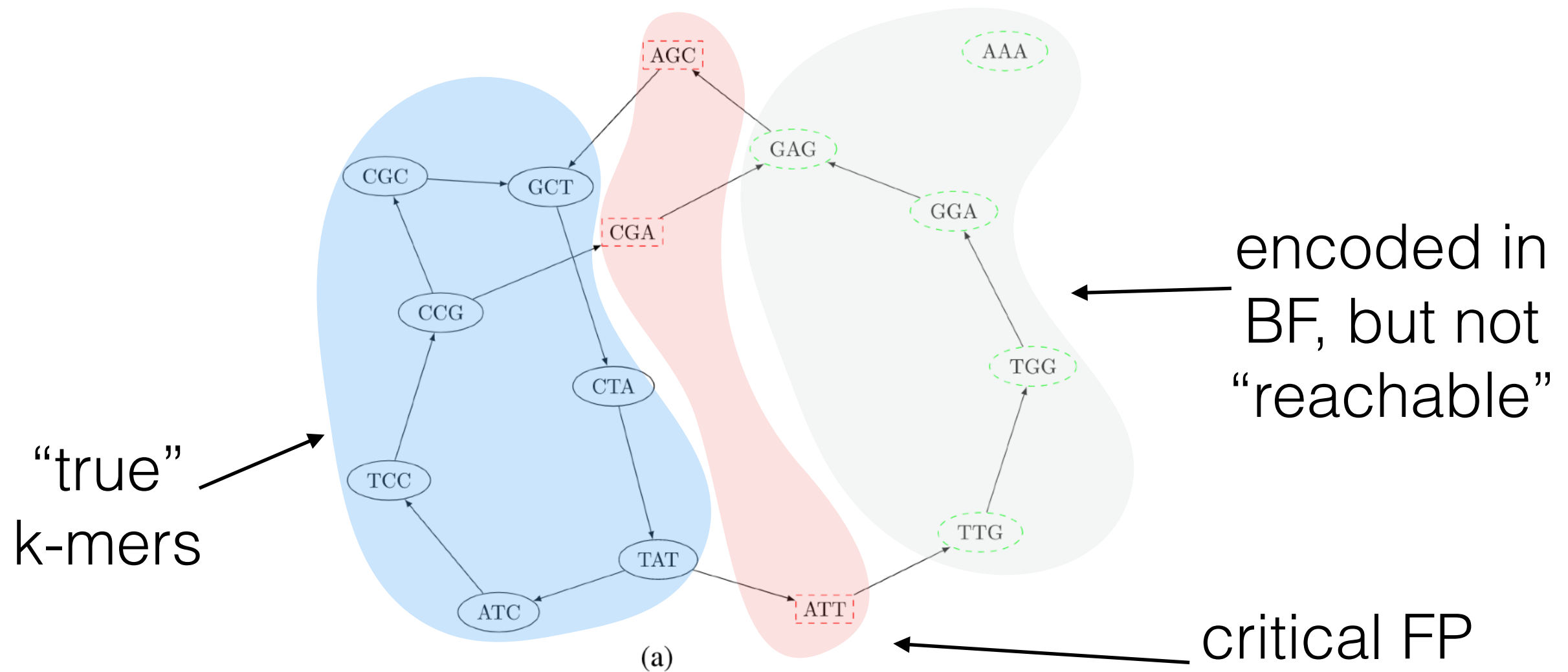
In this section, we prove that a navigational data structure on de Bruijn graphs needs at least 3.24 bits per k -mer to represent the graph:

Theorem 1. *Consider an arbitrary NDS and let CONST be its constructor. For any $0 < \epsilon < 1$, there exists a k and $x \in \Sigma^k$ such that $|\text{CONST}(x)| \geq |x| \cdot (c - \epsilon)$, where $c = 8 - 3 \lg 3 \approx 3.25$.*

Critical False Positives



Critical False Positives



$a_1 \dots a_k$	$\sum_{i=1}^k a_i^i \bmod 10$	Bloom filter
ATC	0	1
CCG	0	0
TCC	5	0
CGC	6	0
...	...	0

(b)

(c)

Nodes self-information:

$$\lceil \log_2 \binom{4^3}{7} \rceil = 30 \text{ bits}$$

Structure size:

$$\underbrace{10}_{\text{Bloom}} + \underbrace{3 \cdot 6}_{\text{False positives}} = 28 \text{ bits}$$

(d)

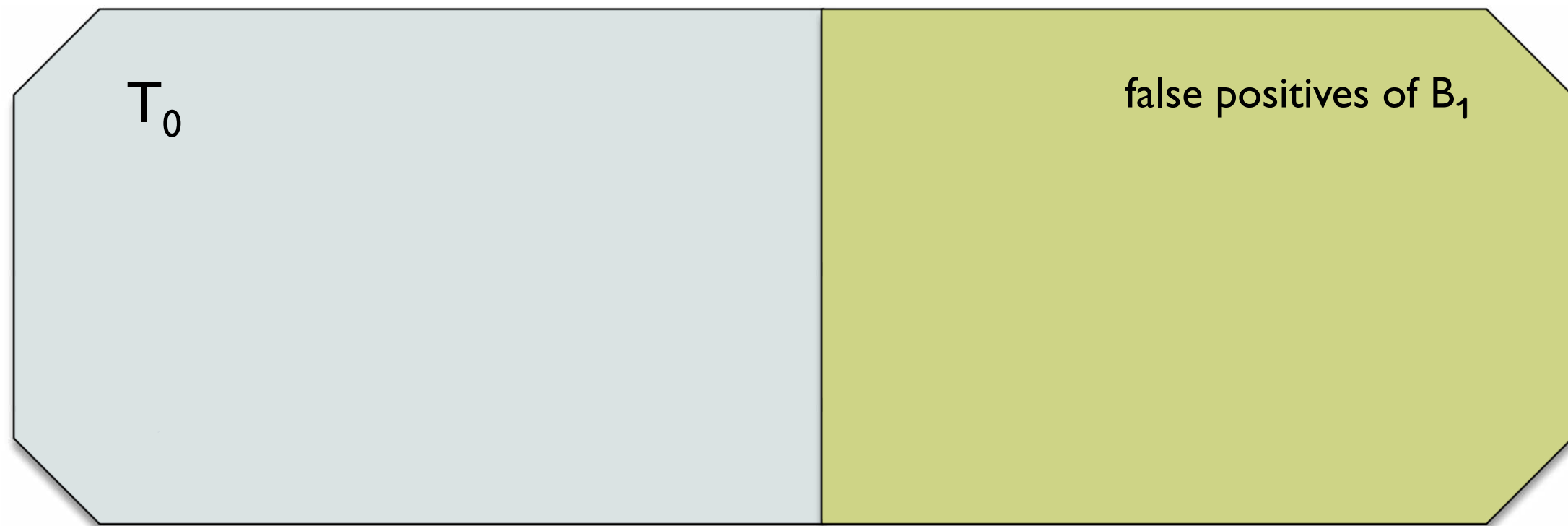
Idea of Chkhi and Rizk

Assume we want to represent specific set T_0 of k -mers with a Bloom filter B_1

Key observation: in assembly, not all k -mers can be queried, only those having $k-1$ overlap with k -mers known to be in the graph.

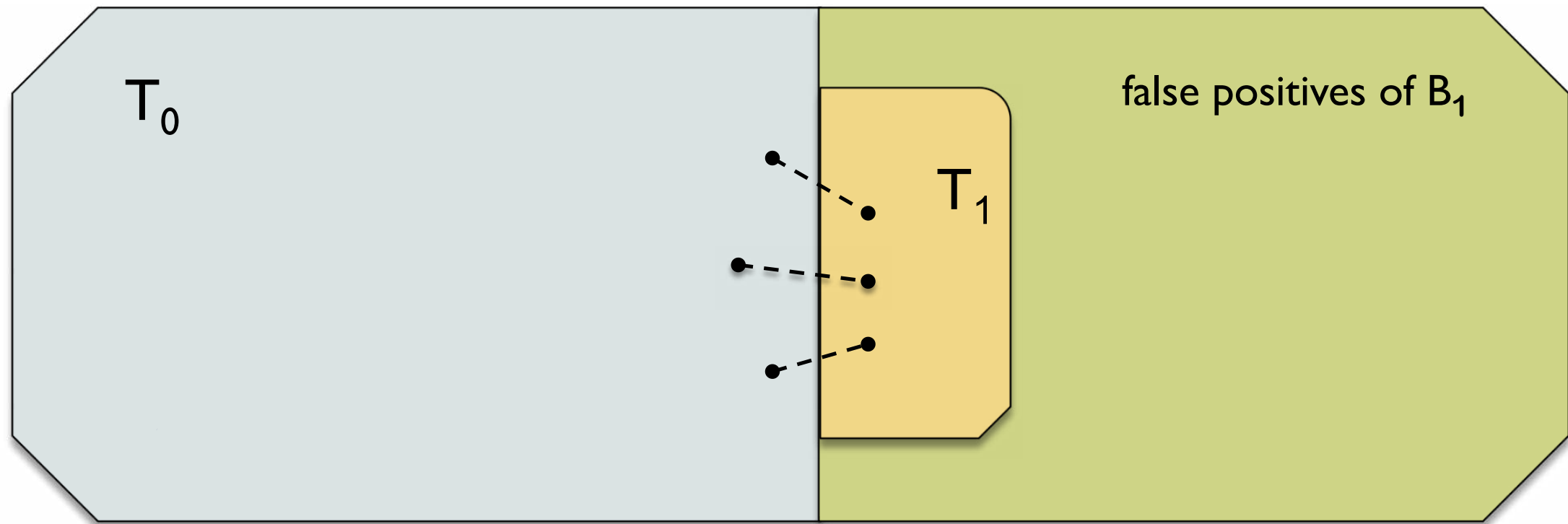
The set T_1 of “critical false positives” (false neighbors of true k -mers) is *much* smaller than the set of all false positives and can be stored explicitly

Storing B_1 and T_1 is much more space efficient than other exact methods for storing T_0 . Membership of w in T_0 is tested by first querying B_1 , and if $w \in B_1$, check that it is *not* in T_1 .



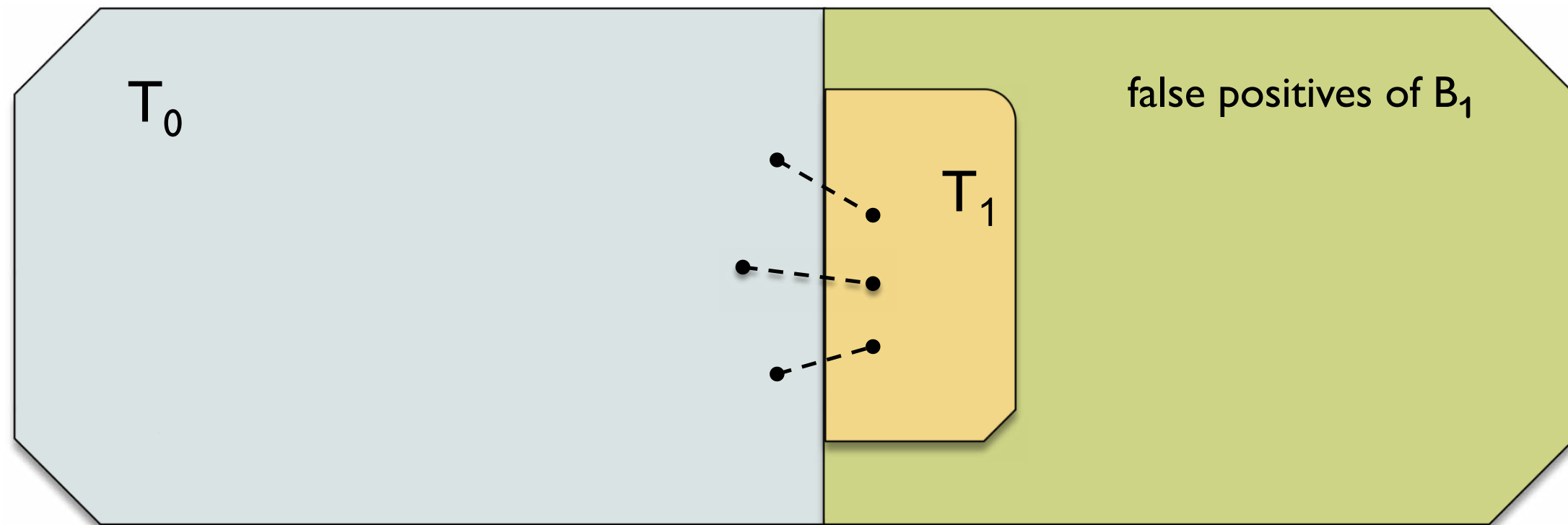
- Represent T_0 by Bloom filter B_1





- ▶ Represent T_0 by Bloom filter B_1
- ▶ Compute T_1 ('critical false positives') and represent it e.g. by a hash table




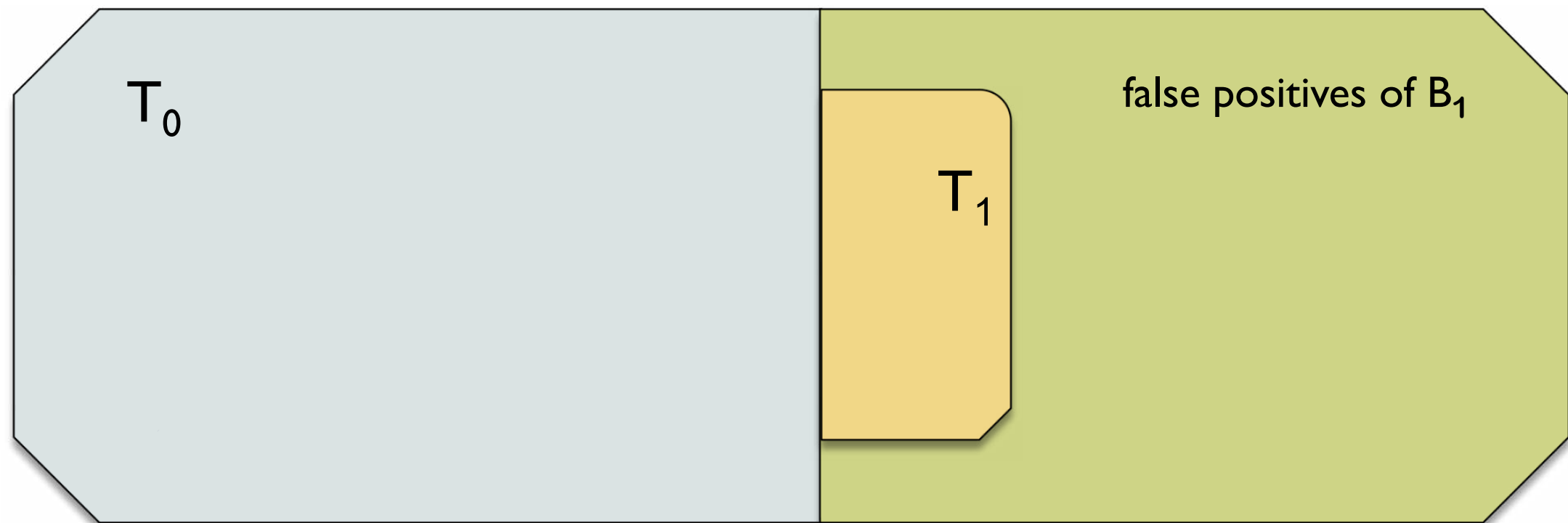


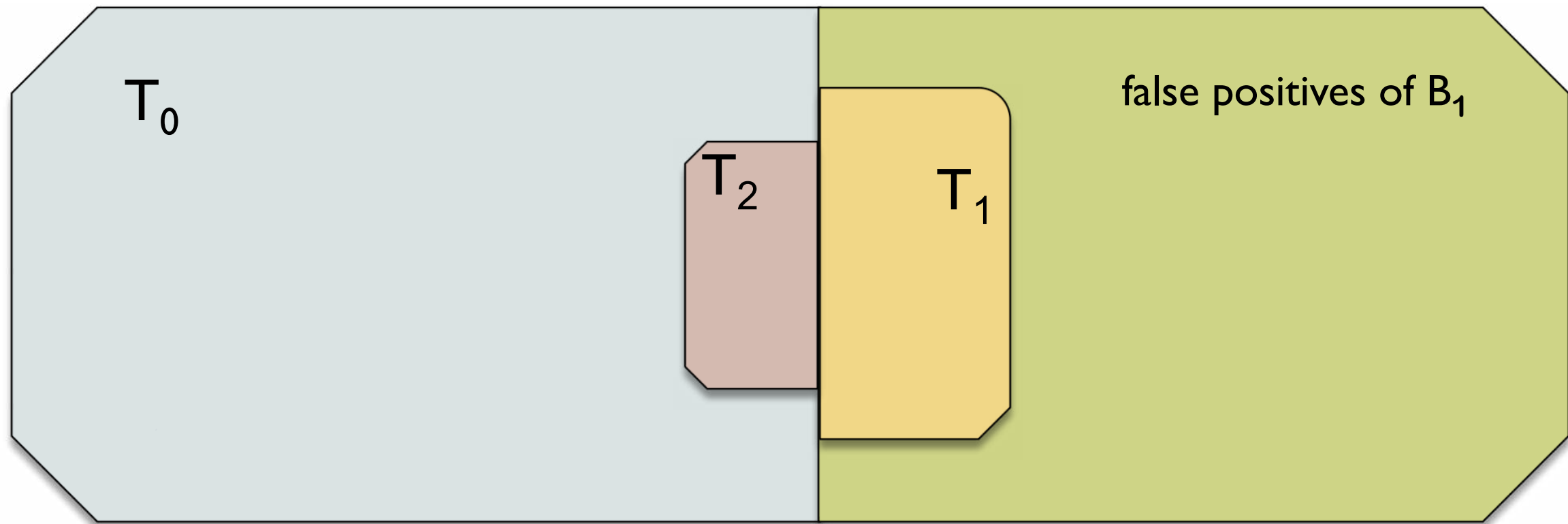
- ▶ Represent T_0 by Bloom filter B_1
- ▶ Compute T_1 ('critical false positives') and represent it e.g. by a hash table
- ▶ Result (example): 13.2 bits/node for $k=27$ (of which 11.1 bits for B_1 and 2.1 bits for T_1)



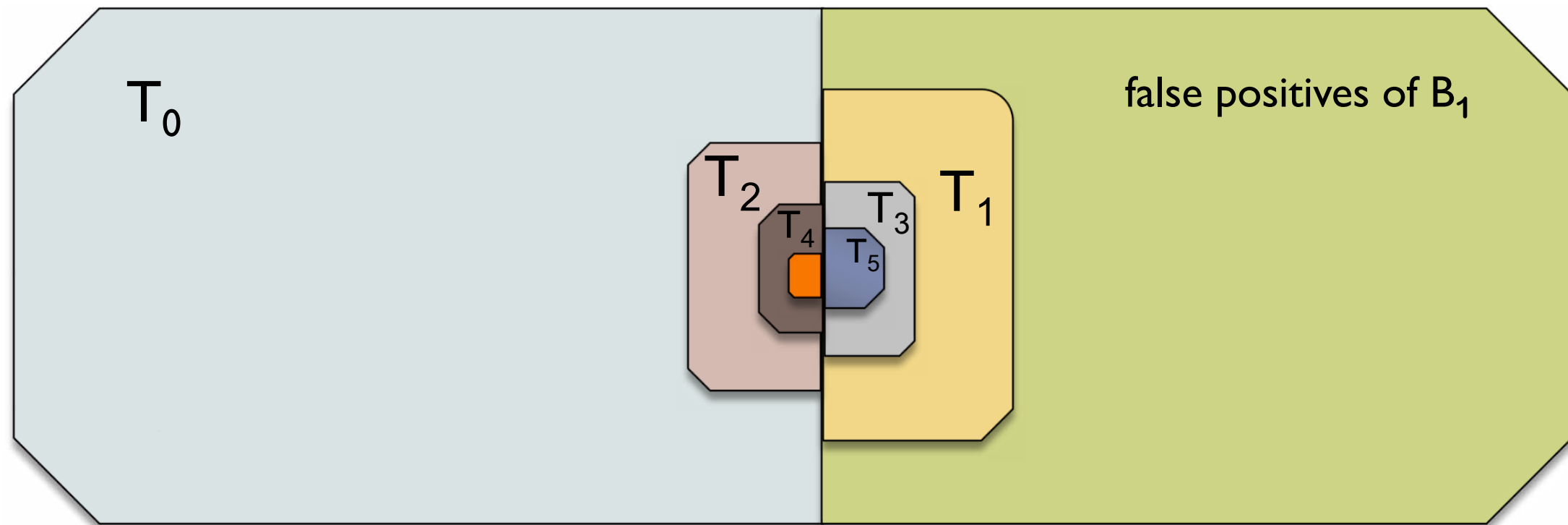
Improving on Chikhi and Rizk's method

- ▶ *Main idea*: iteratively apply the same construction to T_1 i.e. encode T_1 by a Bloom filter B_2 and set of 'false-false positives' T_2 , then apply this to T_2 etc.
- ▶  *cascading Bloom filters*

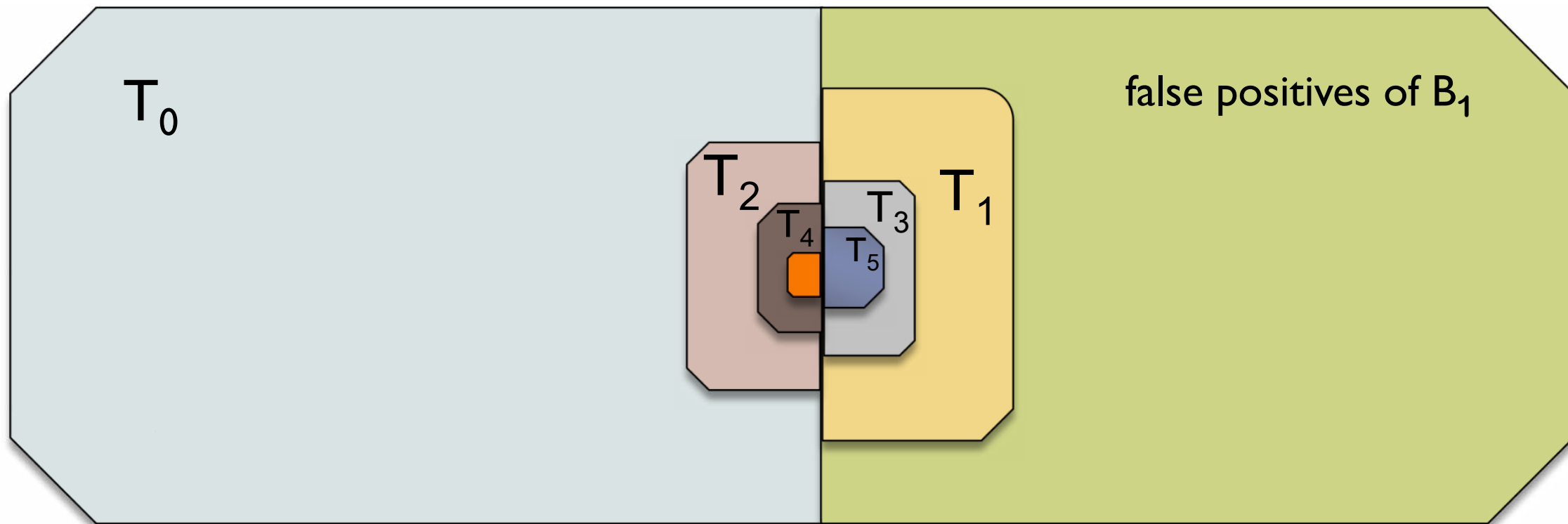




- ▶ further encode T_1 via a Bloom filter B_2 and set T_2 , where $T_2 \subseteq T_0$ is the set of k -mers stored in B_2 by mistake ('false² positives')



- ▶ further encode T_1 via a Bloom filter B_2 and set T_2 , where $T_2 \subseteq T_0$ is the set of k -mers stored in B_2 by mistake ('false² positives')
- ▶ iterate the construction on T_2
- ▶ we obtain a sequence of sets $T_0, T_1, T_2, T_3, \dots$ encode by Bloom filters $B_1, B_2, B_3, B_4, \dots$ respectively
- ▶ $T_0 \supseteq T_2 \supseteq T_4 \supseteq \dots, T_1 \supseteq T_3 \supseteq T_5 \supseteq$



Lemma [correctness]: For a k -mer w , consider the smallest i such that $w \notin B_{i+1}$. Then $w \in T_0$ if i is odd and $w \notin T_0$ if i is even.

- ▶ if $w \notin B_1$ then $w \notin T_0$
- ▶ if $w \in B_1$, but $w \notin B_2$ then $w \in T_0$
- ▶ if $w \in B_1$, $w \in B_2$, but $w \notin B_3$ then $w \notin T_0$
- ▶ etc.

Assuming infinite number of filters

Let $N=|T_0|$ and $r=m_i/n_i$ is the same for every B_i . Then the total size is

$$\underbrace{rN}_{|B_1|} + \underbrace{6rNc^r}_{|B_2|} + \underbrace{rNc^r}_{|B_3|} + \underbrace{6rNc^{2r}}_{|B_4|} + \underbrace{rNc^{2r}}_{|B_5|} + \dots = N(1+6c^r) \frac{r}{1-c^r}$$

The minimum is achieved for $r=5.464$, which yields the memory consumption of **8.45** bits/node

Infinity difficult to deal with ;)

- In practice we will store only a small finite number of filters B_1, B_2, \dots, B_t together with the set T_t stored explicitly
- $t=1 \Rightarrow$ Chkhi&Rizk's method
- The estimation should be adjusted, optimal value of r has to be updated, example for $t=4$

k	optimal r	bits per k -mer
16	5.776737	8.555654
32	6.048557	8.664086
64	6.398529	8.824496
128	6.819496	9.045435

Table: Estimations for $t=4$. Optimal r and corresponding memory consumption

Compared to Chikhi&Rizk's method

k	“Optimal” (infinite) Cascading Bloom Filter	Cascading Bloom Filter with $t = 4$	Data structure of Chikhi & Rizk
16	8.45	8.555654	12.0785
32	8.45	8.664086	13.5185
64	8.45	8.824496	14.9585
128	8.45	9.045435	16.3985

Table: Space (bits/node) compared to Chikhi&Rizk
for $t=4$ and different values of k .

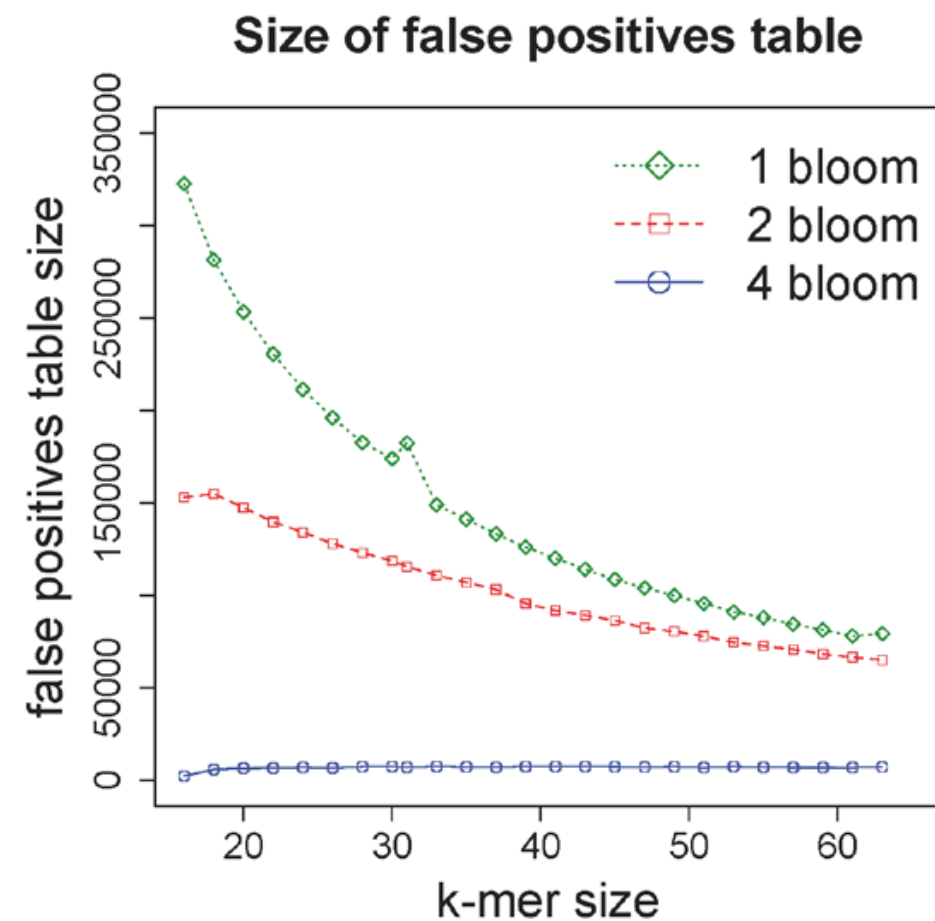
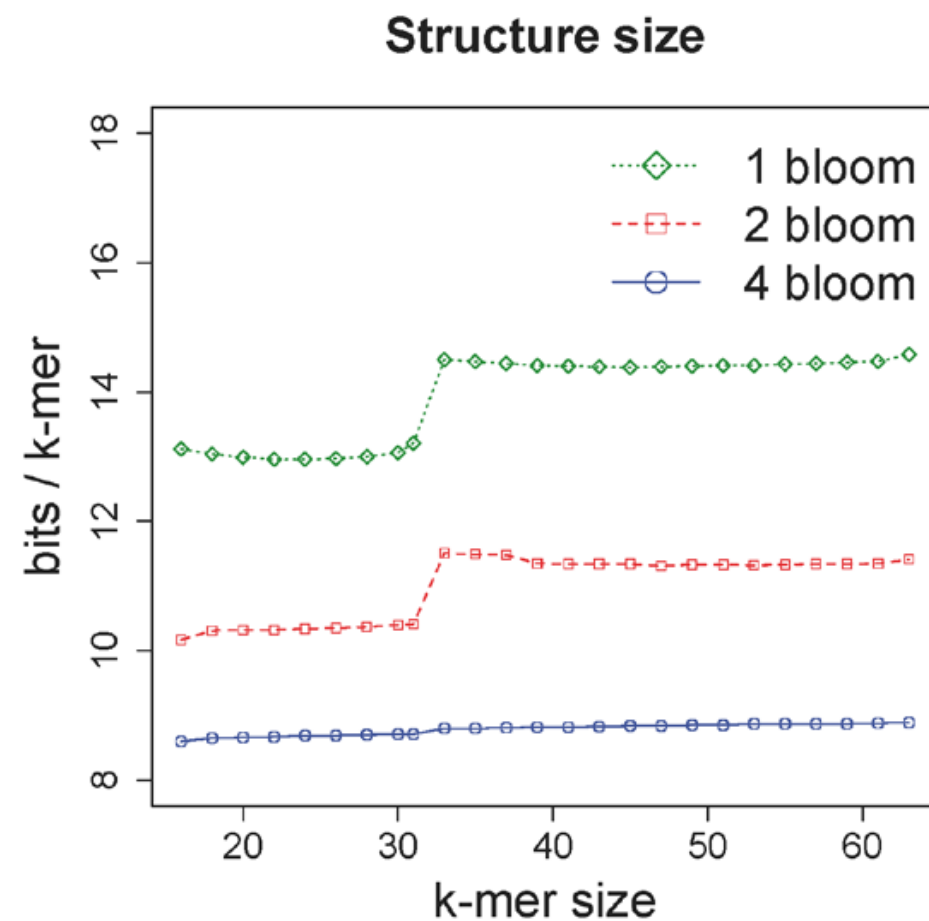
We can cut down a bit more ...

- Rather than using the same r for all filters B_1, B_2, \dots , we can use different properly chosen coefficients r_1, r_2, \dots
- This allows saving another 0.2 – 0.4 bits/k-mer



Experiments I: E.Coli, varying k

- 10M E.Coli reads of 100bp
- 3 versions compared: 1 Bloom (=Chikhi&Rizk), 2 Bloom ($t=2$) and 4 Bloom ($t=4$)

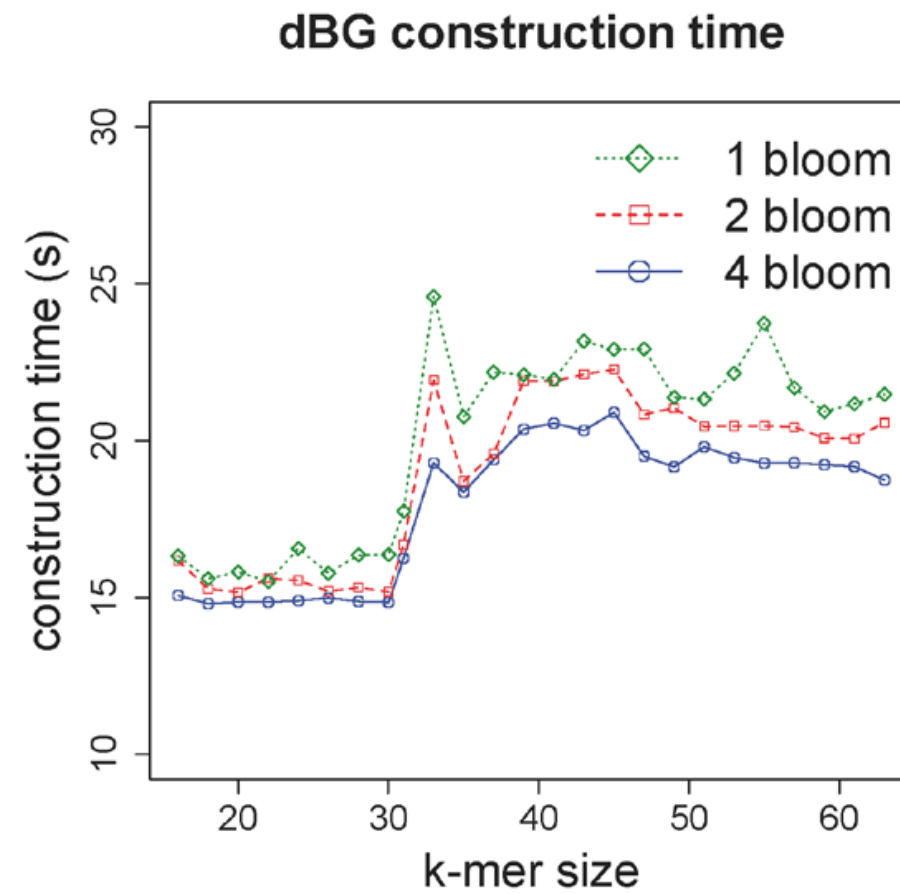
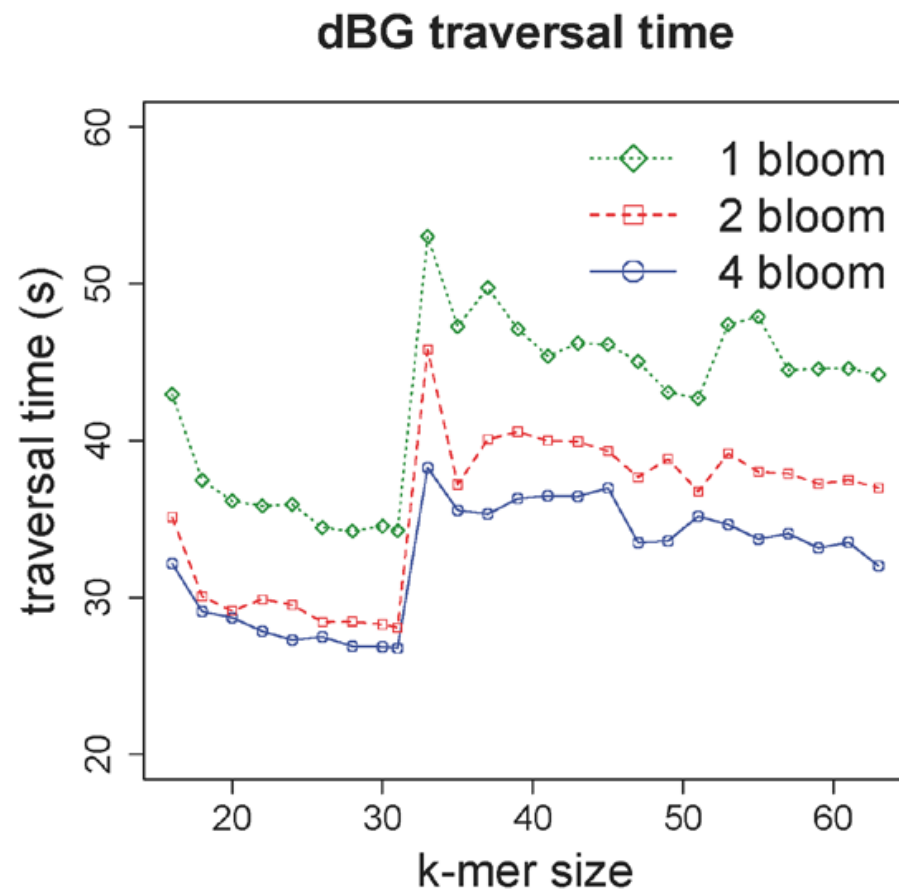


Experiments II: Human dataset

- 564M Human reads of 100bp (~17X coverage)

Method	1 Bloom	2 Bloom	4 Bloom
Construction time (s)	40160.7	43362.8	44300.7
Traversal time (s)	46596.5	35909.3	34177.2
r (bits)	11.10	8.10	6.56
Bloom filters size (MB)	$B_1 = 3250.95$	$B_1 = 2372.51$ $B_2 = 292.65$	$B_1 = 1921.20$ $B_2 = 496.92$ $B_3 = 83.39$ $B_4 = 21.57$
False positive table size (MB)	$T_1 = 545.94$	$T_2 = 370.96$	$T_4 = 24.07$
Total size (MB)	3796.89	2524.12	2547.15
Size (bits/ k -mer)	12.96	10.37	8.70

Experiments I (cont)



Efficiently enumerating cFP

Algorithm 1 Constant-memory enumeration of critical false positives

- 1: **Input:** The set \mathcal{S} of all nodes in the graph, the Bloom filter constructed from \mathcal{S} , the maximum number M of elements in each partition (determines memory usage)
 - 2: **Output:** The set cFP
 - 3: Store on disk the set \mathcal{P} of extensions of \mathcal{S} for which the Bloom filter answers *yes*
 - 4: Free the Bloom filter from memory
 - 5: $D_0 \leftarrow \mathcal{P}$
 - 6: $i \leftarrow 0$
 - 7: **while** end of \mathcal{S} is not reached **do**
 - 8: $P_i \leftarrow \emptyset$
 - 9: **while** $|P_i| < M$ **do**
 - 10: $P_i \leftarrow P_i \cup \{\text{next } k\text{-mer in } \mathcal{S}\}$
 - 11: **for each** k -mer m in D_i **do**
 - 12: **if** $m \notin P_i$ **then**
 - 13: $D_{i+1} \leftarrow D_{i+1} \cup \{m\}$
 - 14: Delete D_i, P_i
 - 15: $i \leftarrow i + 1$
 - 16: cFP $\leftarrow D_i$
-

Bloom filters & De Bruijn Graphs

So, we can make very small representation of the dBG.
But it's navigational! We can also make them:

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Original Paper



Sequence analysis

Practical dynamic de Bruijn graphs

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Dynamic &
membership



and even
weighted



deBGR: an efficient and near-exact representation of the weighted de Bruijn graph

Prashant Pandey¹, Michael A. Bender¹, Rob Johnson^{1,2} and Rob Patro^{1,*}

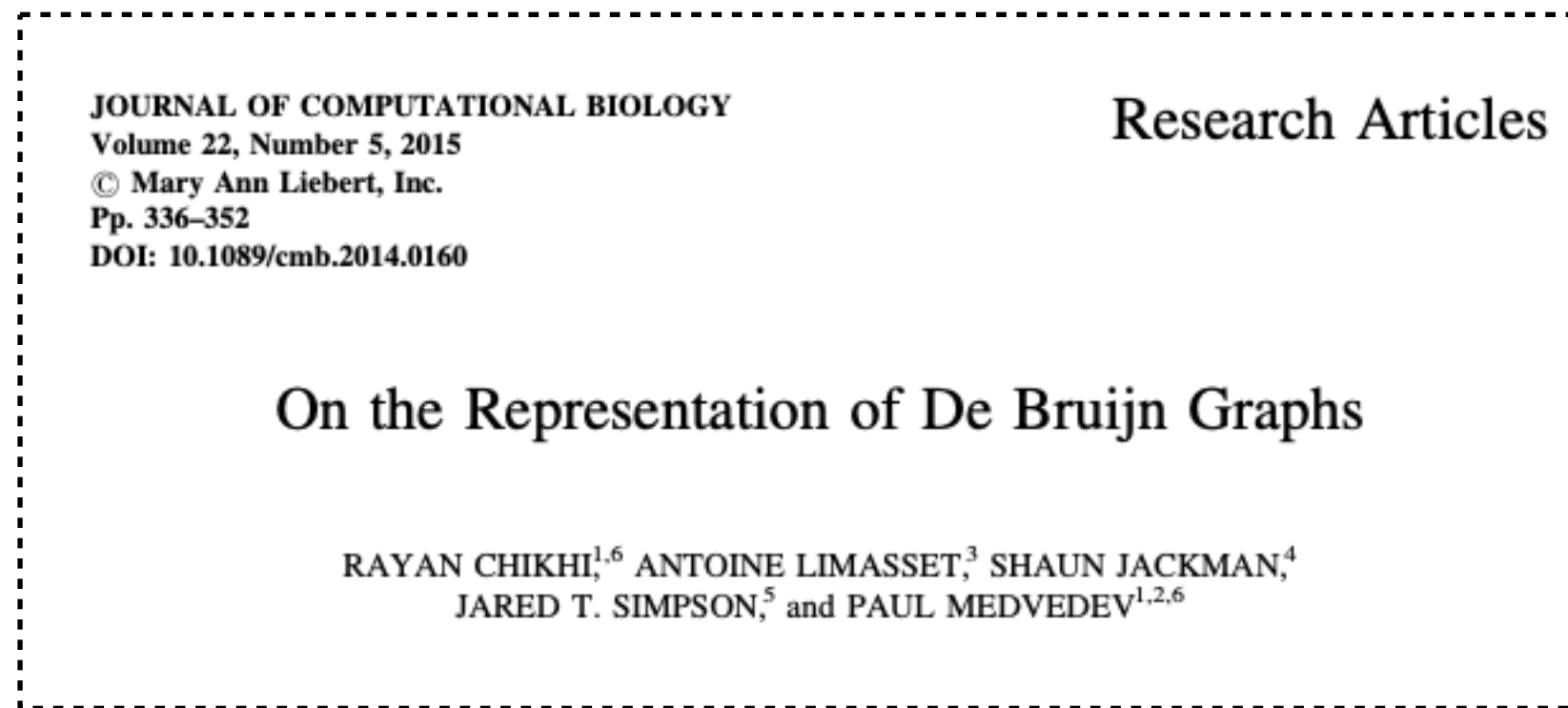
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Bioinformatics, 33, 2017, i133–i141
doi: 10.1093/bioinformatics/btx261
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Other efficient representations as well



In addition to the theoretical bounds, this paper introduced an algorithm for constructing the contigs of the compacted dBG efficiently (bcalm), and an efficient representation based on building the FM-index over these contigs (dbgFM).