TwoPaCo

An efficient algorithm to build the compacted de Bruijn graph from many complete genomes

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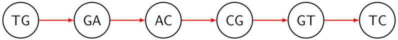
Parte I

Introduction

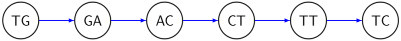
A pan-genomic algorithm

de Bruijn graph

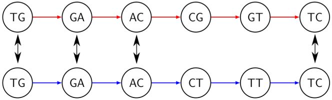
Sequence #1: TGACGTC (2-mers: TG GA AC CG GT TC)



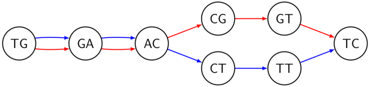
Sequence #2: TGACGTC (2-mers: TG GA AC CT TT TC)



Find common vertices



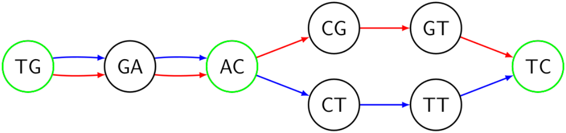
Merge them



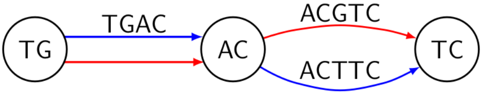
Compacted de Bruijn graph

To compact the de Bruijn graph we need to find the junctions

A vertex is a junction if :
Is the initial or final part of some sequence
Has more than 1 in-edge or more than 1 out-edge



Remove simple paths by connect junctions



The problem

How to construct the compacted de Bruijn graph,
from many complete genomes,
avoiding the ordinary graph construction?

↓

Which k-mers are junctions?

+

How to construct the compacted de Bruijn graph given the junctions?

Parte II

The algorithm

Naive algorithm

- Store all $(k+1)$-mers in a hash table
- For each k-mers query the possible edges
- If only 1 in and 1 out edge, unmark as a junction

Algorithm 1: FILTER-JUNCTIONS

```
Input  : $S = \{s_1, ..., s_m\}$ genoma sequences
         $k$ integer, size of $k$-mers
         $E$ empty set data structure
         $C$ Candidate set of junctions (naively all positions are marked)
Output: A reduce candidate set of junctions $C$

foreach $s \in S$ do
    for $1 \leq i < |s| - k$ do
        if $C[s,i] = marked$ then
            $E \leftarrow E \cup s[i..i+k] \cup s[i-1..i+k-1]$    ▷ Store all $(k+1)$-mers

foreach $s \in S$ do
    for $1 \leq i < |s| - k$ do
        if $C[s,i] = marked$ then
            $(in, out) \leftarrow (0,0)$    ▷ Count in/out edges
            foreach $c \in \{A, C, G, T\}$ do
                if $v \cdot c \in E$ then
                    $in \leftarrow in + 1$
                if $c \cdot v \in E$ then
                    $out \leftarrow out + 1$
            if $(in, out) = (1,1)$ then
                $C[s,i] = unmarked$    ▷ surely not a junction
return $C$
```

The memory issue

First part of the naive algorithm:

```
foreach $s \in S$ do
    for $1 \leq i < |s| - k$ do
        if $C[s, i] = marked$ then
            $E \leftarrow E \cup s[i..i + k] \cup s[i - 1..i + k - 1]$
```

We don't really need and, in almost all pratical cases, we can't store all the possible $(k + 1)$-mers.

Mainly because **only a little percentual** of them are junction in the de Bruijn graph.

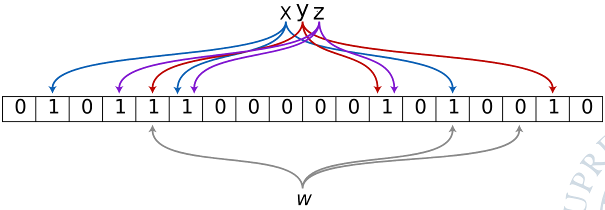
Bloom filter

A space-efficient probabilistic hash table

Bitmap V of size b, h hash functions $f_0, f_1, ..., f_{h-1} : U \to [0, b-1]$

insertion(x) $\to V[f_i(x)] = 1$, $\forall\ 0 \leq i < h$

contains(x) $\to$ **probabily yes** if $V[f_i(x)] == 1$, $\forall\ 0 \leq i < h$



0	1	0	1	1	1	0	0	0	0	0	1	0	1	0	0	1	0

Probability of false positive, after n insertion: $p_{FP} \simeq (1 - e^{-hn/m})^h$

Two Pass version

- First pass: Select a set of junction candidates by insert all the $(k+1)$-mers in a bloom filter of choosing size
- Second pass: Filter out the false positive by storing the reduce sets of $(k+1)$-mers in an hash table

Algorithm 2: FILTER-JUNCTIONS-TWO-PASS

```
Input  : strings $S = \{s_1, ..., s_m\}$ genoma sequences
         integer $k$, size of $k$-mers
         integer $b$, size of bloom filter
         Candidate set of junctions $C_{in}$ (naively all positions are marked)
Output: A reduce candidate set of junctions $C_{out}$
$F \leftarrow$ empty bloom filter of size $b$                    ▷ First pass
$C_{temp} \leftarrow$ FILTER-JUNCTIONS$(S, k, F, C_{in})$
$H \leftarrow$ empty hash table                                  ▷ Second pass
$C_{out} \leftarrow$ FILTER-JUNCTIONS$(S, k, H, C_{in})$
return $C_{out}$
```

The memory issue[2]

How much memory do we use now?

- First pass: Bloom filter of size b (of our decision)
- Second pass: Hash table containing $(k+1)$-mers of junction candidates

We don't know the possible size of the hash table in the second pass.
What if the hash table is not small enough?

Solution: Split the input k-mers in chunks and analyze them in multiple rounds.

k-mers splitting

Algorithm 3: Round-Splitting

```
Input  : strings $S = \{s_1, ..., s_m\}$ genoma sequences
         integer $k$, size of $k$-mers
         integer $b$, size of bloom filter
         integer $l$, number of rounds
         function $f(x)$, from $k$-mers to integers
Output: $(V_0, V_1, \ldots, V_{l-1})$ chunks of $k$-mers

$V_0 \leftarrow \emptyset, V_1 \leftarrow \emptyset, \ldots, V_{l-1} \leftarrow \emptyset$        ▷ Chunks, initially empty
$c_0 \leftarrow 0, c_1 \leftarrow 0, \ldots, c_{q-1} \leftarrow 0$        ▷ Counters, for balancing $k$-mers
$F \leftarrow$ empty Bloom filter of size $b$
foreach $s \in S$ do
    for $1 \leq i < |s| - k$ do
        if $s[i..i+k-1]$ not in $F$ then
            Insert $s[i..i+k-1]$ in $F$
            $c_{f(s[i..i+k-1])} \leftarrow c_{f(s[i..i+k-1])} + 1$
$T \leftarrow \sum_{0 \leq t < q} c_t / l$        ▷ Average size of a chunk
$acc \leftarrow 0, idx \leftarrow 0$        ▷ Create nearly balanced chunks
for $0 \leq i < q$ do
    $V_{idx} = V_{idx} \cup \{i\}$
    $acc \leftarrow acc + c_i$
    if $acc > T$ then
        $acc \leftarrow 0$
        $idx \leftarrow \min(idx + 1, l - 1)$        ▷ Next chunk, until reach $l - 1$
return $(V_0, V_1, \ldots, V_{l-1})$
```

Multiple rounds: dealing with memory restrictions

- Partitionate the input with ROUND-SPLITTING
- Analyse each partition with FILTER-JUNCTIONS-TWO-PASS
- Merge the results of each rounds and output the real junctions

Algorithm 4: TWOPACO

```
Input  : strings $S = \{s_1, ..., s_m\}$ genoma sequences
         integer $k$, size of $k$-mers
         integer $b$, size of bloom filter
         integer $l$, number of rounds
         function $f(x)$, from $k$-mers to integers
Output: $C_{final}$ all the junctions in the compacted de Bruijn graph
$(V_0, V_1, \dots, V_{l-1}) \leftarrow$ ROUND-SPLITTING($S$, $k$, $b$, $l$, $f$)
$C_{init} \leftarrow$ Boolean array with every position unmarked
for $0 \leq i < l$ do
    $C_i \leftarrow$ mark every position of $C_{init}$ that starts a $k$-mer with hash in $V_i$
    $C_i \leftarrow$ FILTER-JUNCTIONS-TWO-PASS($S$, $k$, $b$, $C_i$)
$C_{final} = \bigcup C_i$
return $C_{final}$
```

Parallelization scheme

As far we focused in reducing memory. What about the time?

TwoPaCo can be easily parallelizable:

- Parallel for
- Concurrent bloom filter (parallel insert and search)
- Concurrent hash table (parallel insert and search)

We made a clever usage of the concurrent data structs:

Parte III

Results

Source code & Dataset

Original source code by medvedev group available on:

https://github.com/medvedevgroup/TwoPaCo

Personal implementation available on:

https://github.com/GaspareG/TwoPaCo

Dataset for experiments:

62 Escherichia coli (∼300Mb)
5 humans (∼21Gb)
8 primates (∼23Gb)
100 simulated human (∼400Gb)

Memory complexity

The memory complexity is the maximum
among the first and the second pass of TwoPaCo

- First pass: insert all k-mers in a bloom filter of size b
- Second pass: store all **junction candidates** in a hash table

How many junction candidates?

- Real junction, J
- False positive induced from the bloom filter, FP

Result: $\mathcal{O}(\max\{b, (J + FP)k\})$

Time complexity

The memory complexity is the sum
between the first and the second pass of TwoPaCo

- First pass: insert all k-mers in a bloom filter of size b using h hash functions
- Second pass: iterate over all **candidate positions** and query the hash table

How many k-mers?
$\mathcal{O}(m)$, where $m = \Sigma_{s \in S}|s|$ is the total input size

How many candidate positions?

- Real positions, $|G_c|$
- False positive induced from the bloom filter, FP

Result: $\mathcal{O}(mh + (|G_c| + FP)k)$

Complexity comparison

State of the art for compressed de Bruijn graph construction:

- Sibelia (Minkin, Patel, Kolmogorov, Vyahhi, Pham, 2013)
- SplitMEM (Marcus, Lee, Schatz, 2014)
- bwt-based (Baier, Beller, Ohlebusch, 2015)

Algorithm	Time complexity	Memory complexity
Sibelia	$\mathcal{O}(m)$	$\mathcal{O}(m)$
SplitMEM	$\mathcal{O}(m \log g)$	$\mathcal{O}(m + \lvert G_c \rvert)$
bwt-based	$\mathcal{O}(m)$	$\mathcal{O}(m)$
TwoPaCo	$\mathcal{O}(mh + (\lvert G_c \rvert + FP)k)$	$\mathcal{O}(\max\{b, (J + FP)k\})$

- $m = \Sigma_{s \in S} |s|$, total input size
- $g = \max_{s \in S} |s|$, size of the biggest genoma
- J and $|G_c|$, number of vertex and edge in the de Bruijn Graph
- b and h, size of the bloom filter table and number of hash functions
- FP, number of false positives in first pass

Running time comparison

What are the practical performances of TwoPaCo against the state of the art?

Dataset	Sibelia	SplitMEM	bwt-based	TwoPaCo	
				1 thread	15 thread
62 E.coli (k=25)	0 (12.2)	70 (178.0)	8 (0.85)	4 (0.16)	2 (0.39)
62 E.coli (k=100)	8 (7.6)	67 (178.0)	8 (0.50)	4 (0.19)	2 (0.39)
7 humans (k=25)	-	-	867 (100.30)	436 (4.40)	63 (4.84)
7 humans (k=100)	-	-	807 (46.02)	317 (8.42)	57 (8.75)
8 primates (k=25)	-	-	-	914 (34.36)	111 (34.36)
8 primates (k=100)	-	-	-	756 (56.06)	101 (61.68)
50 humans (k=25)	-	-	-	-	705 (69.77)
50 humans (k=100)	-	-	-	-	927 (70.21)

Running times in minutes and memory usage in gigabytes in parenthesis

- - on Sibelia = out of time
- - on SplitMEM = out of memory
- - on bwt-based = out of memory
- - on TwoPaCo = experiment not done

Fixed memory

In how many rounds do we need to split the input
to not exceeding a given memory threshold?

Threshold	Used memory	Bloom filter size	Running time	Rounds
10GB	8.62GB	8.59GB (2^{33})	4h	1
8GB	6.73GB	4.29GB (2^{32})	7h	3
6GB	5.98GB	4.29GB (2^{32})	9h	4
4GB	3.51GB	2.14GB (2^{31})	11h	6

Experiments on 5 simulated human genomes, $k = 25$, 8 threads.

Result: we can trade-off memory for time.

Bloom Filter false positive

Is the Bloom Filter really efficient in reducing junction candidates?

		Junction candidates		
Dataset	k	Initial	First pass	Second pass
62 E.coli	25	310 157 564 (100%)	24 649 489 (7.94%)	24 572 562 (7.92%)
62 E.coli	100	310 157 489 (100%)	22 848 018 (7.36%)	9 492 091 (3.06%)
7 humans	25	21 201 290 922 (100%)	3 489 946 013 (16.46%)	2 974 098 154 (14.02%)
7 humans	100	21 201 290 847 (100%)	1 374 287 870 (6.48%)	188 224 214 (0.88%)
8 primates	25	24 540 556 921 (100%)	5 423 003 377 (22.09%)	5 401 587 503 (22.01%)
8 primates	100	24 540 556 846 (100%)	1 174 160 336 (4.78%)	502 441 107 (2.04%)

- Initial: total number of k-mers in dataset
- First pass: number of junction candidates (using a bloom filter)
- Second pass: real number of junction (using an hash table)

References

[1] Minkin, I., Pham, S., Medvedev, P. (2016).
TwoPaCo: An efficient algorithm to build the compacted de Bruijn graph from many complete genomes

[2] Minkin, I., Patel, A., Kolmogorov, M., Vyahhi, N., Pham, S. (2013).
Sibelia: a scalable and comprehensive synteny block generation tool for closely related microbial genomes.

[3] Marcus, S., Lee, H., Schatz, M. C. (2014).
SplitMEM: a graphical algorithm for pan-genome analysis with suffix skips.

[4] Baier, U., Beller, T., Ohlebusch, E. (2015).
Graphical pan-genome analysis with compressed suffix trees and the **Burrows-Wheeler transform**.

Conclusion

Thanks for your attention!

Questions?