# 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

# Compacted de Bruijn graph

# Junctions

# The problem

# Parte II

# The algorithm

# Naive algorithm

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

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**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.

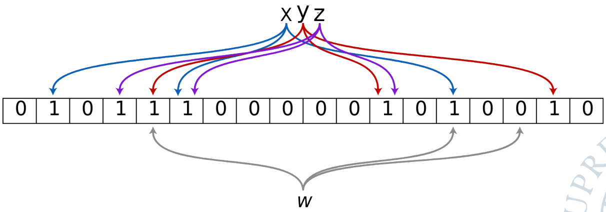
# Bloom filter

A space-efficient probabilistic hash table

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

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

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




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 ← empty bloom filter of size b                        ▷ First pass
C_temp ← FILTER-JUNCTIONS(S, k, F, C_in)
H ← empty hash table                                    ▷ Second pass
C_out ← 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, ..., V_{l-1}) ← ROUND-SPLITTING(S, k, b, l, f)
C_init ← Boolean array with every position unmarked
for 0 ≤ i < l do
    C_i ← mark every position of C_init that starts a k-mer with hash in V_i
    C_i ← FILTER-JUNCTIONS-TWO-PASS(S, k, b, C_i)
C_final = ⋃ 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)

# Parte III

# Results

# Source code & Dataset

Original source code by medvedev group available on:

https://github.com/medvedevgroup/TwoPaCo

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Personal implementation available on:

https://github.com/GaspareG/TwoPaCo

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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 | $\mathcal{O}(m \log g)$ | $\mathcal{O}(m + \|G_c\|)$ |
| bwt-based | $\mathcal{O}(m)$ | $\mathcal{O}(m)$ |
| TwoPaCo | $\mathcal{O}(mh + (\|G_c\| + FP)k)$ | $\mathcal{O}(\max\{b, (J + FP)k\})$ |

- $m = \Sigma_{s \in S} |s|$, 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?