# Meta-colored compacted de Bruijn graphs

**Giulio Ermanno Pibiri**

Ca’ Foscari University of Venice

@giulio_pibiri

@jermp

**28-th RECOMB**
Boston, USA, 30 April 2024

Joint work with
**Jason Fan** and **Rob Patro**
University of Maryland

# The colored k-mer indexing problem

- A **k-mer** is a sub-string of length k of some string $R$.
- We are given a collection $\mathscr{R} = \{R_1, \ldots, R_N\}$ of reference sequences.
  Each $R_i$ is a (long) sequence over the DNA alphabet {A,C,G,T}.
- **Problem.** We want to build an *index* for $\mathscr{R}$ so that we can retrieve the set $\text{Color}(x) = \{i \,|\, x \in R_i\}$ efficiently for any k-mer $x$. Note that $\text{Color}(x) = \emptyset$ if $x \notin \mathscr{R}$.

# The colored k-mer indexing problem

- A **k-mer** is a sub-string of length k of some string $R$.
- We are given a collection $\mathscr{R} = \{R_1, \ldots, R_N\}$ of reference sequences.
  Each $R_i$ is a (long) sequence over the DNA alphabet {A,C,G,T}.
- **Problem.** We want to build an *index* for $\mathscr{R}$ so that we can retrieve the set $\text{Color}(x) = \{i \,|\, x \in R_i\}$ efficiently for any k-mer $x$. Note that $\text{Color}(x) = \emptyset$ if $x \notin \mathscr{R}$.

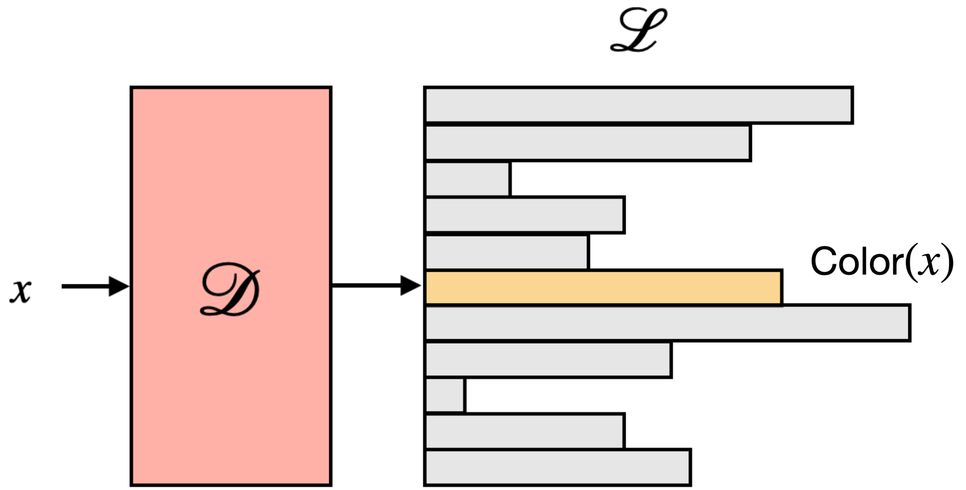
- A lot of hype in the indexing community for the case where $\mathscr{R}$ is a **pangenome**, i.e., a collection of related genomes.
- **Applications.** This problem is relevant for applications where sequences are first matched against known references (i.e., mapping/alignment algorithms): single-cell RNA-seq, metagenomics, etc.

# Modular indexing layout

- All the distinct k-mers in $\mathscr{R} = \{R_1, \ldots, R_N\}$ are stored in the **dictionary** $\mathscr{D}$.
- What we want for a k-mer $x$ is the map:

$$x \to \text{Color}(x) = \{i \,|\, x \in R_i\}.$$

The collection of all Color$(x)$ is the **inverted index** $\mathscr{L}$.

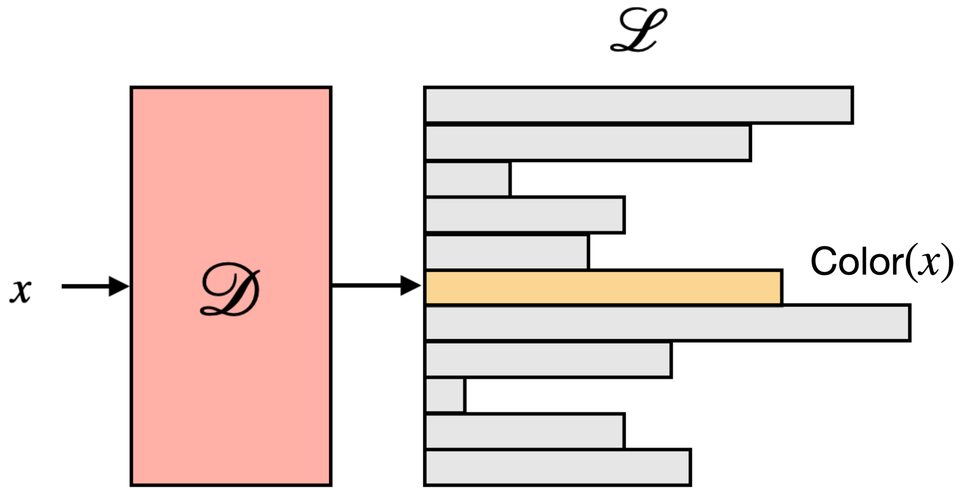


# Modular indexing layout

- All the distinct k-mers in $\mathscr{R} = \{R_1, \ldots, R_N\}$ are stored in the **dictionary** $\mathscr{D}$.
- What we want for a k-mer $x$ is the map:

$$x \rightarrow \text{Color}(x) = \{i \,|\, x \in R_i\}.$$

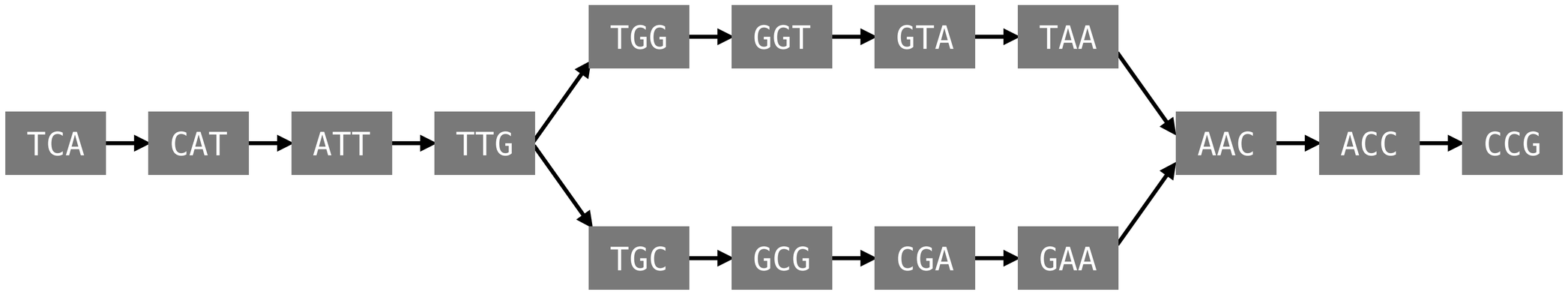
The collection of all Color$(x)$ is the **inverted index** $\mathscr{L}$.




- Our problem reduces to that of **representing two data structures**, $\mathscr{D}$ and $\mathscr{L}$.

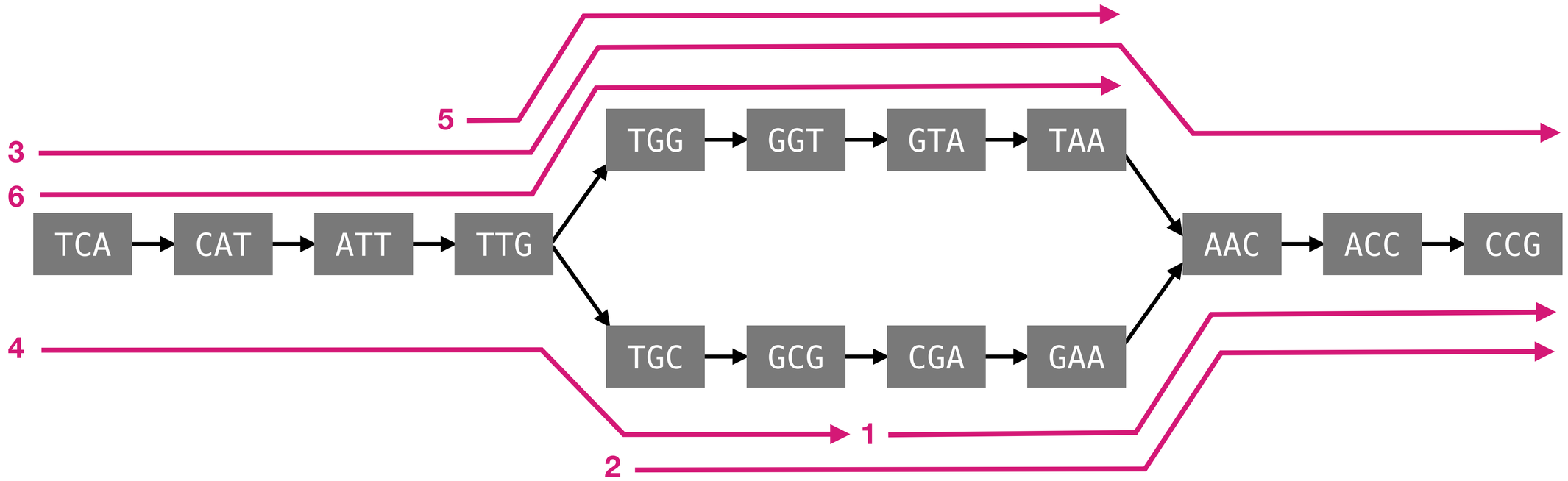
# de Bruijn graphs

- The dictionary $\mathscr{D}$ is a set of k-mers with (k-1)-symbol overlaps.
- One-to-one correspondence between $\mathscr{D}$ and a *de Bruijn* graph (dBG).
- Example for **k = 3**.



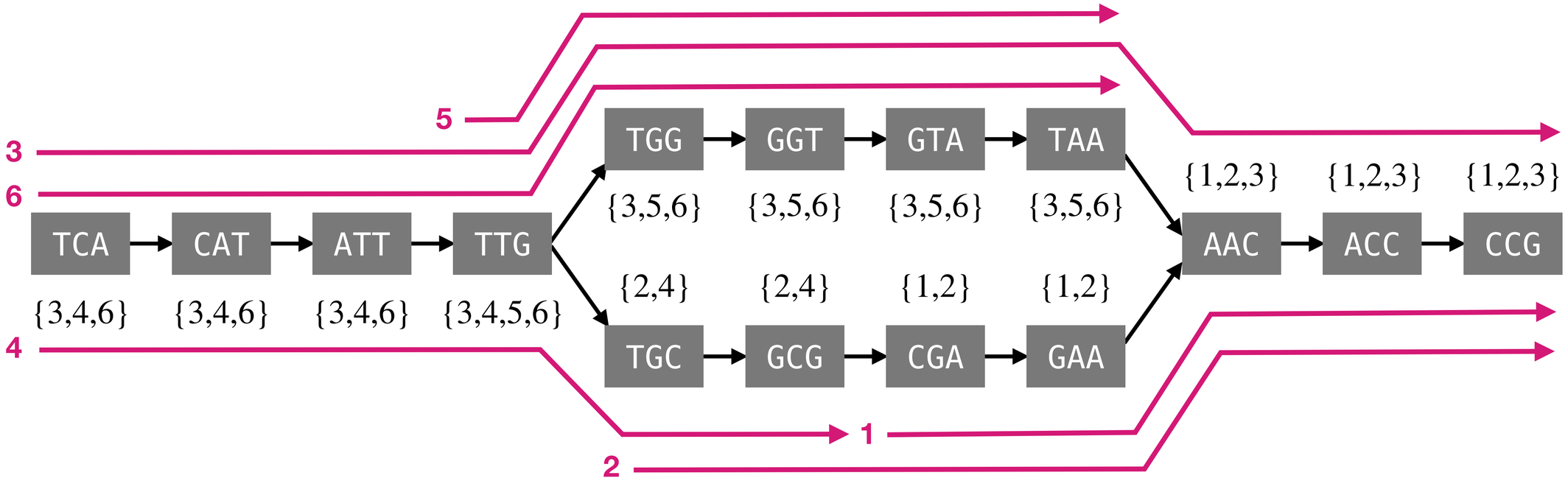
# Colored de Bruijn graphs

- Example for k = 3 and **N = 6 references.** References in $\mathscr{R}$ are spelled by **paths** in the graph.



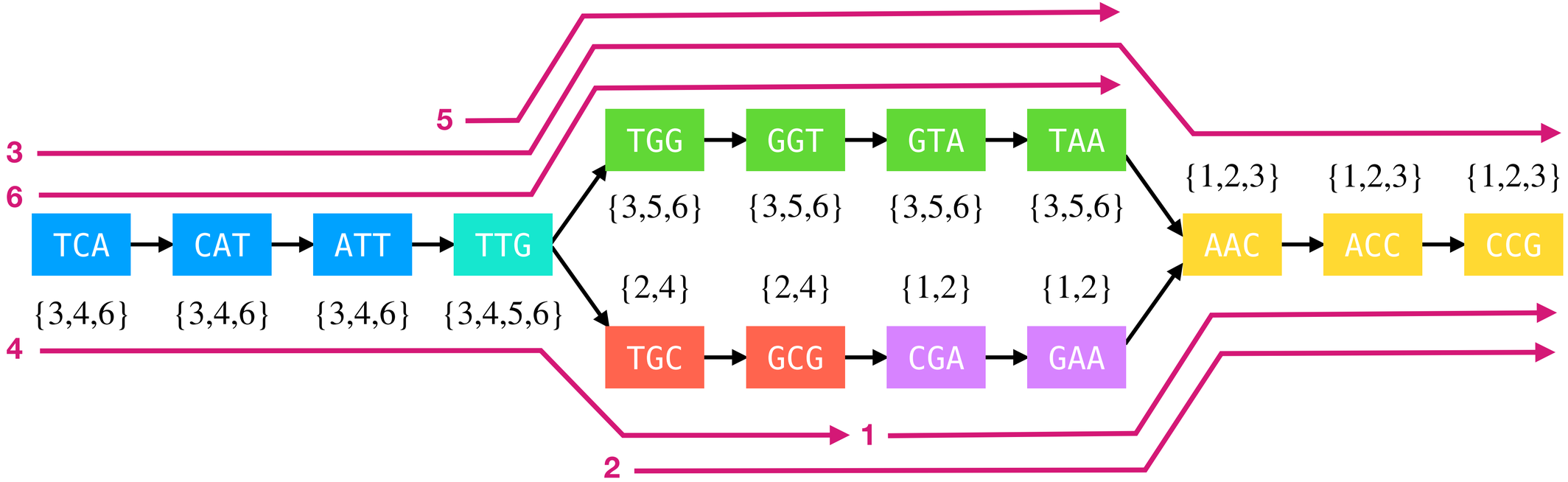
# Colored de Bruijn graphs

- Example for k = 3 and **N = 6 references.** References in $\mathscr{R}$ are spelled by **paths** in the graph.



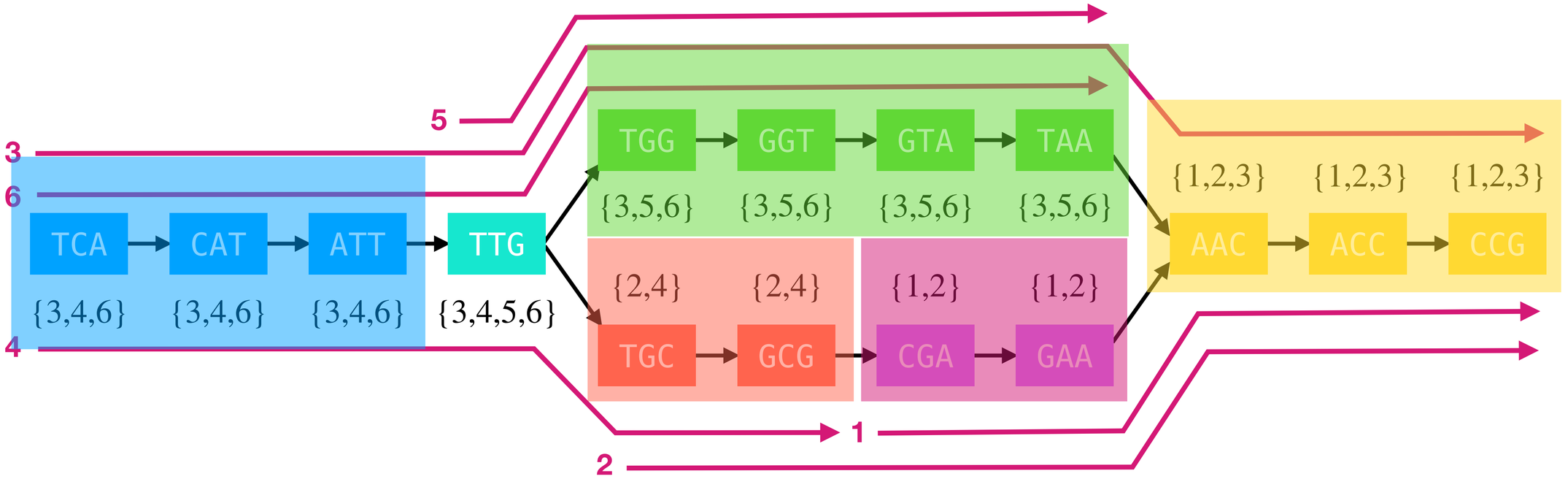
# Colored de Bruijn graphs

- Example for k = 3 and **N = 6 references.** References in $\mathscr{R}$ are spelled by **paths** in the graph.



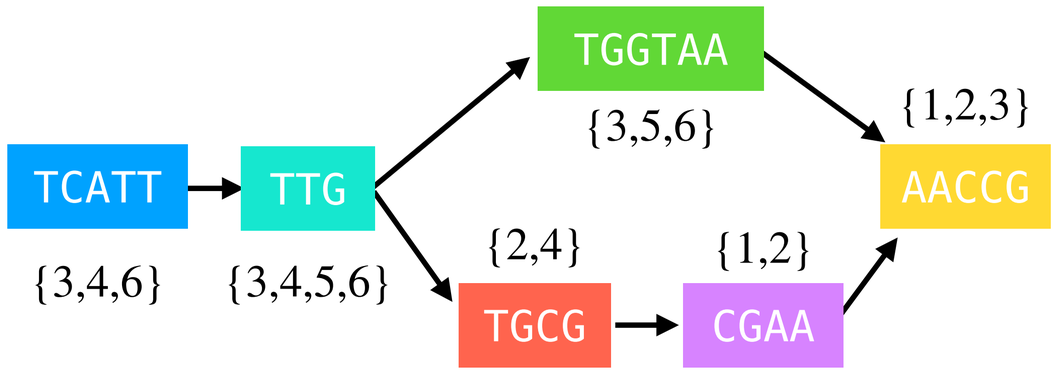
# Colored de Bruijn graphs

- Example for k = 3 and **N = 6 references.** References in $\mathscr{R}$ are spelled by **paths** in the graph.



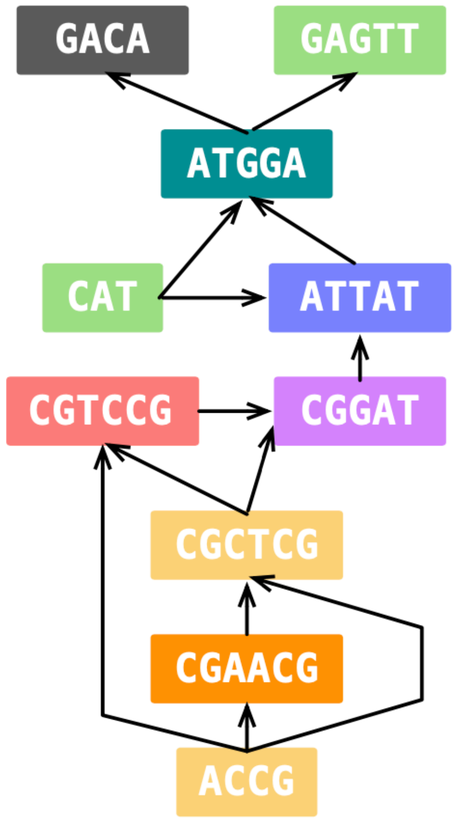
# Colored compacted de Bruijn graphs

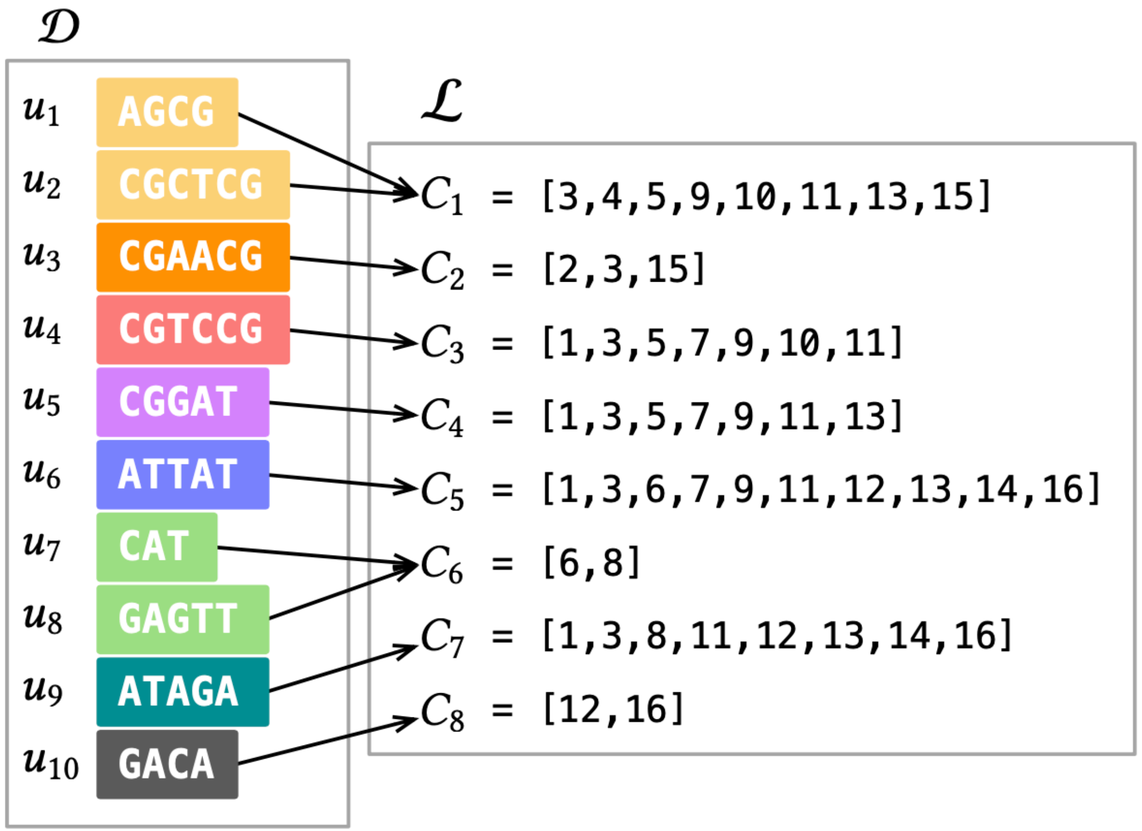
- Example for k = 3 and **N = 6 references.**
- Nodes having the **same color along non-branching paths** are collapsed into (monochromatic) **unitigs**.



# Colored compacted de Bruijn graphs

- Another, larger, example for **N = 16** references.



# Properties of colored compacted dBGs

1. **Unitigs spell references in $\mathscr{R}$.** → We can represent the set of unitigs instead of the set of k-mers. Better space and cache locality.

2. **Unitigs are monochromatic**. → We store a color set for each unitig, rather than for each k-mer. We need an efficient map from k-mers to unitigs.

3. **Unitigs co-occur.** → Distinct unitigs often have the same color, i.e., they co-occur in the same subset of references. We have way less distinct colors than unitigs.
   We need an efficient map from unitigs to colors.

# Properties of colored compacted dBGs

**SSHash** [P., 2022] → **Fulgor** [Fan et al., ALMOB 2024]

1. **Unitigs spell references in $\mathscr{R}$.** → We can represent the set of unitigs instead of the set of k-mers. Better space and cache locality.

2. **Unitigs are monochromatic**. → We store a color set for each unitig, rather than for each k-mer. We need an efficient map from k-mers to unitigs.

3. **Unitigs co-occur.** → Distinct unitigs often have the same color, i.e., they co-occur in the same subset of references. We have way less distinct colors than unitigs.
   We need an efficient map from unitigs to colors.

# Properties of colored compacted dBGs

**SSHash** [P., 2022] → **Fulgor** [Fan et al., ALMOB 2024]

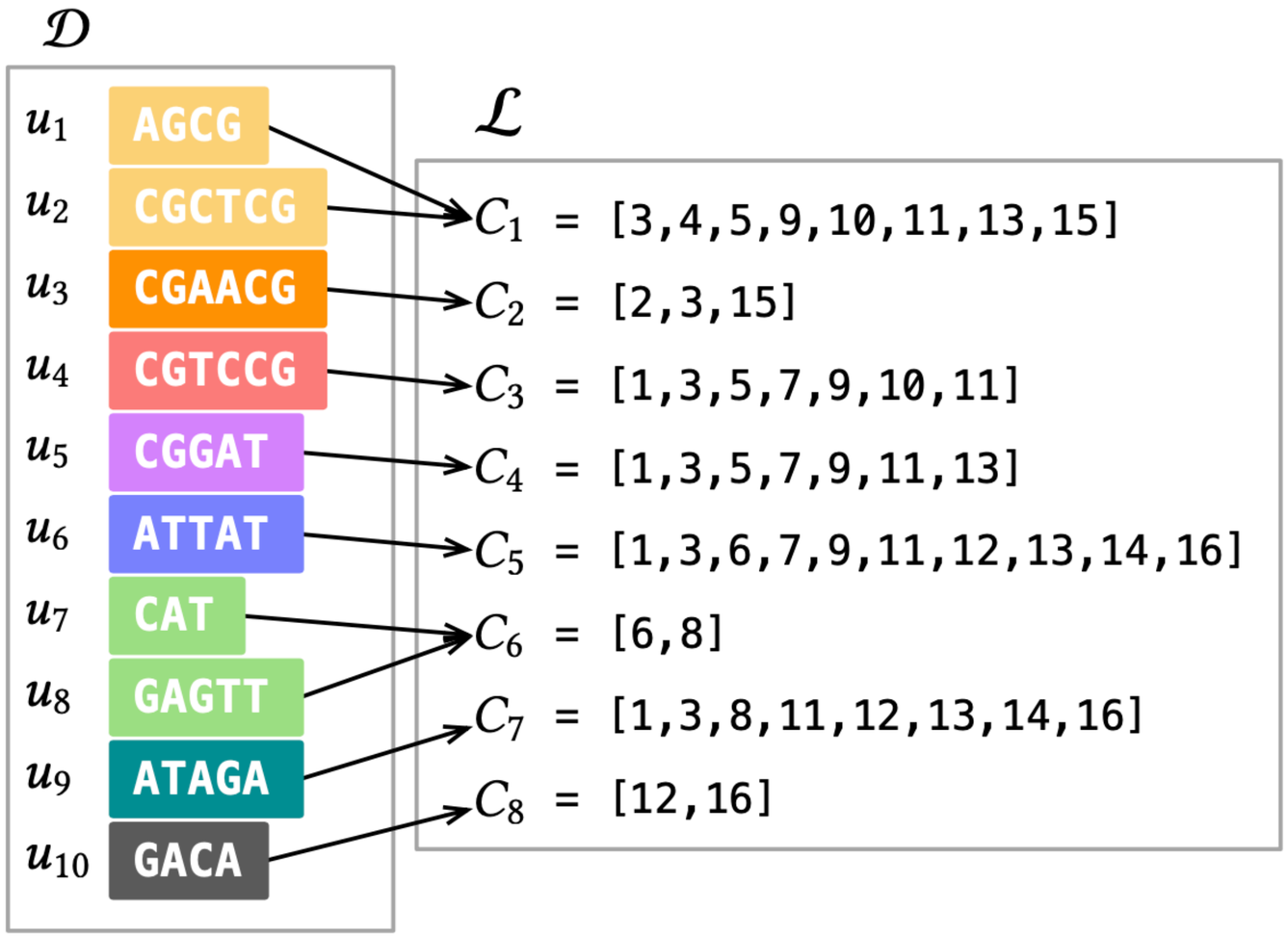
1. **Unitigs spell references in $\mathscr{R}$.** → We can represent the set of unitigs instead of the set of k-mers. Better space and cache locality.

2. **Unitigs are monochromatic**. → We store a color set for each unitig, rather than for each k-mer. We need an efficient map from k-mers to unitigs.

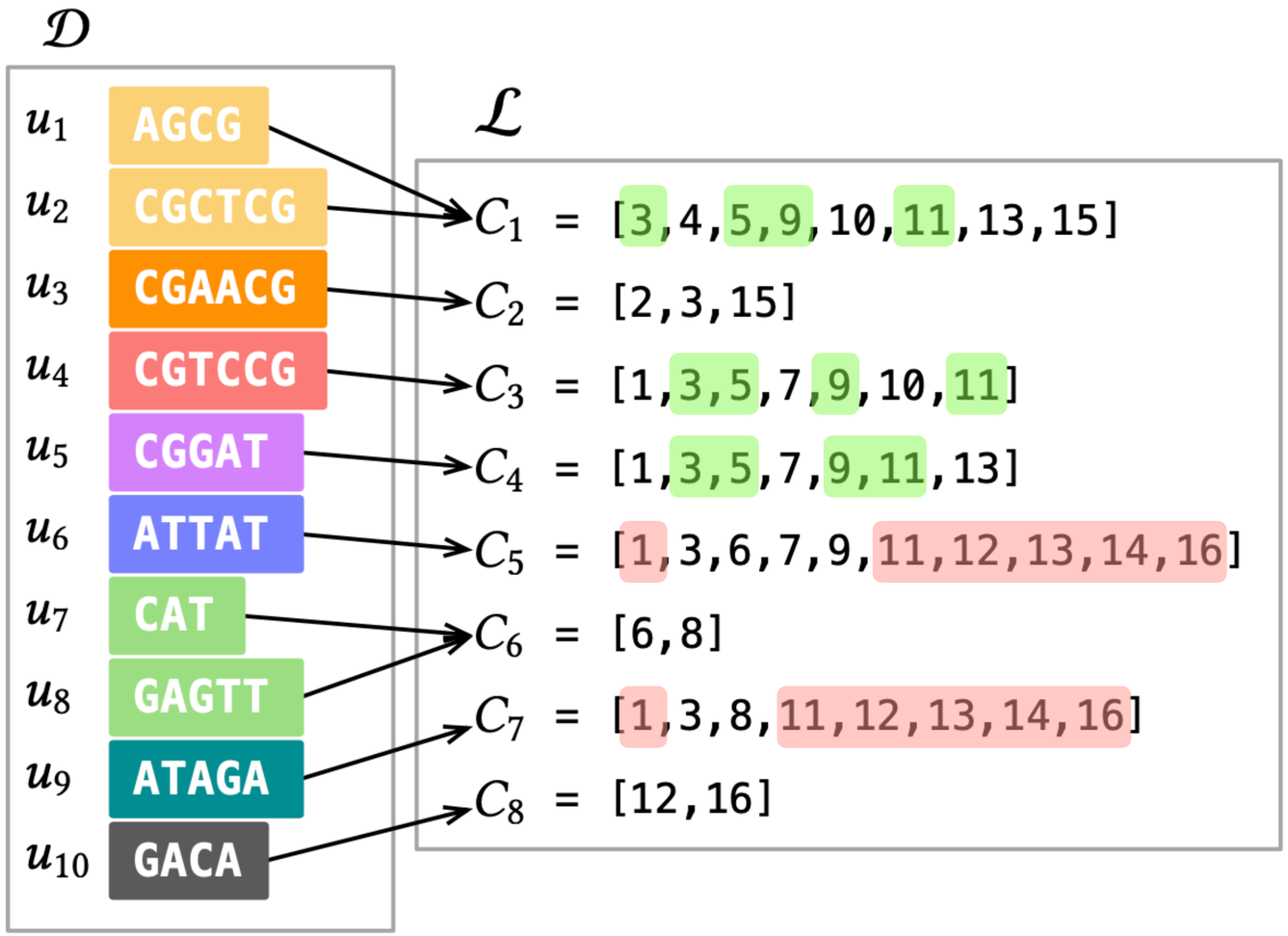
3. **Unitigs co-occur.** → Distinct unitigs often have the same color, i.e., they co-occur in the same subset of references. We have way less distinct colors than unitigs.
   We need an efficient map from unitigs to colors.

4. **Colors are similar when indexing pangenomes.** → Opportunity to achieve much better compression if colors are not compressed *individually* (each set independently of the others) but *common patterns are factored out and compressed once*.

# Colors are similar when indexing pangenomes

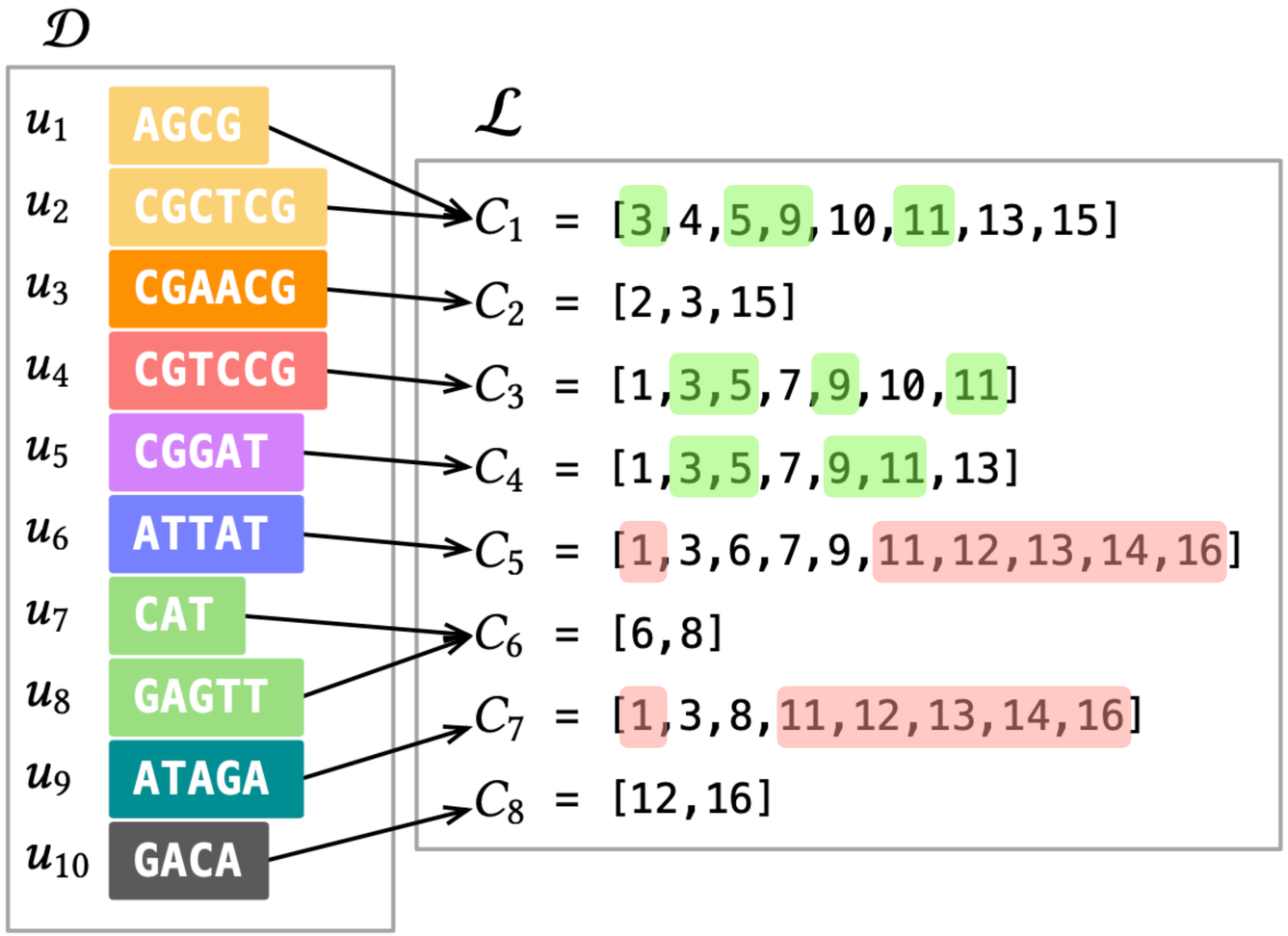


# Colors are similar when indexing pangenomes




- The pattern $\{3,5,9,11\}$ is currently represented three times.
- The pattern $\{1,11,12,13,14,16\}$ is represented twice.

# Colors are similar when indexing pangenomes




- The pattern $\{3,5,9,11\}$ is currently represented three times.
- The pattern $\{1,11,12,13,14,16\}$ is represented twice.
- **Q.** How to factor out this redundancy?

# Introducing meta and partial colors

- Recall that $N$ is the number of references in the collection $\mathscr{R}$.
- Two steps:

  1. We determine a **partition** of $[N] = \{1, \ldots, N\}$ so that references in the same partition are *similar*.

     - **Intuition:** Similar references induce similar colors and thus *share patterns in the colors* → the number of **distinct partial colors** in a partition is small → factor out the redundancy.

  2. We render each original color as a sequence of references — or **meta colors** — to those **partial** colors.

# Meta and partial colors — Example

- Example for **N = 16** references and **4** partitions.

$$\begin{array}{rl} & \{\ 1\ 12\ 13\ 14\ 16\ \}\{\ 3\ 5\ 9\ \}\{\ 7\ 11\ \}\{\ 2\ \ 4\ \ 6\ \ 8\ \ 10\ 15\ \} \\ new\ identifiers \rightarrow & 1\ \ 2\ \ 3\ \ 4\ \ 5\ \ \ \ 6\ 7\ 8\ \ \ \ 9\ 10\ \ \ \ 11\ 12\ 13\ 14\ 15\ 16 \end{array}$$

this defines a **permutation $\pi$**

# Meta and partial colors — Example

- Example for **N = 16** references and **4** partitions.

$$\begin{array}{rl} & \{\ 1\ 12\ 13\ 14\ 16\ \}\{\ 3\ 5\ 9\ \}\{\ 7\ 11\ \}\{\ 2\ \ 4\ \ 6\ \ 8\ \ 10\ 15\ \} \\ new\ identifiers \rightarrow & 1\ \ 2\ \ 3\ \ 4\ \ 5\ \ \ \ 6\ 7\ 8\ \ \ \ 9\ 10\ \ \ \ 11\ 12\ 13\ 14\ 15\ 16 \end{array}$$

this defines a **permutation $\pi$**

$\mathcal{L}$

$C_1$ = [3,4,5,9,10,11,13,15]

$C_2$ = [2,3,15]

$C_3$ = [1,3,5,7,9,10,11]

$C_4$ = [1,3,5,7,9,11,13]

$C_5$ = [1,3,6,7,9,11,12,13,14,16]

$C_6$ = [6,8]

$C_7$ = [1,3,8,11,12,13,14,16]

$C_8$ = [12,16]

$\pi$

$C_1 = [3|6,7,8|10|12,15,16]$

$C_2 = [6|11,16]$

$C_3 = [1|6,7,8|9,10|15]$

$C_4 = [1,3|6,7,8|9,10]$

$C_5 = [1,2,3,4,5|6,8|9,10|13]$

$C_6 = [13,14]$

$C_7 = [1,2,3,4,5|6|10|14]$

$C_8 = [2,5]$

# Meta and partial colors — Example

- Example for **N = 16** references and **4** partitions.

$$\begin{array}{rl} & \{\ 1\ 12\ 13\ 14\ 16\ \}\{\ 3\ 5\ 9\ \}\{\ 7\ 11\ \}\{\ 2\ \ 4\ \ 6\ \ 8\ \ 10\ 15\ \} \\ new\ identifiers \rightarrow & 1\ \ 2\ \ 3\ \ 4\ \ 5\ \ \ 6\ 7\ 8\ \ \ 9\ 10\ \ \ 11\ 12\ 13\ 14\ 15\ 16 \end{array}$$

partition 2

this defines a **permutation** $\pi$

$\mathcal{L}$

$C_1$ = [3,4,5,9,10,11,13,15]
$C_2$ = [2,3,15]
$C_3$ = [1,3,5,7,9,10,11]
$C_4$ = [1,3,5,7,9,11,13]
$C_5$ = [1,3,6,7,9,11,12,13,14,16]
$C_6$ = [6,8]
$C_7$ = [1,3,8,11,12,13,14,16]
$C_8$ = [12,16]

$\pi$

$C_1 = [3|6,7,8|10|12,15,16]$
$C_2 = [6|11,16]$
$C_3 = [1|6,7,8|9,10|15]$
$C_4 = [1,3|6,7,8|9,10]$
$C_5 = [1,2,3,4,5|6,8|9,10|13]$
$C_6 = [13,14]$
$C_7 = [1,2,3,4,5|6|10|14]$
$C_8 = [2,5]$

**distinct** partial colors from partition 2

# Meta and partial colors — Example

- Example for **N = 16** references and **4** partitions.

$$\begin{array}{rl} & \{\ 1\ 12\ 13\ 14\ 16\ \}\{\ 3\ 5\ 9\ \}\{\ 7\ 11\ \}\{\ 2\ \ 4\ \ 6\ \ 8\ \ 10\ 15\ \} \\ new\ identifiers \rightarrow & 1\ \ 2\ \ 3\ \ 4\ \ 5\ \ \ 6\ 7\ 8\ \ \ 9\ 10\ \ \ 11\ 12\ 13\ 14\ 15\ 16 \end{array}$$

partition 2

this defines a **permutation** $\pi$

$\mathcal{L}$

$C_1$ = [3,4,5,9,10,11,13,15]
$C_2$ = [2,3,15]
$C_3$ = [1,3,5,7,9,10,11]
$C_4$ = [1,3,5,7,9,11,13]
$C_5$ = [1,3,6,7,9,11,12,13,14,16]
$C_6$ = [6,8]
$C_7$ = [1,3,8,11,12,13,14,16]
$C_8$ = [12,16]

$\pi$

$C_1 = [3|6,7,8|10|12,15,16]$
$C_2 = [6|11,16]$
$C_3 = [1|6,7,8|9,10|15]$
$C_4 = [1,3|6,7,8|9,10]$
$C_5 = [1,2,3,4,5|6,8|9,10|13]$
$C_6 = [13,14]$
$C_7 = [1,2,3,4,5|6|10|14]$
$C_8 = [2,5]$

**distinct** partial colors from partition 2

# Meta and partial colors — Example

- Example for **N = 16** references and **4** partitions.

$$\begin{array}{rl} & \{\ 1\ 12\ 13\ 14\ 16\ \}\{\ 3\ 5\ 9\ \}\{\ 7\ 11\ \}\{\ 2\ \ 4\ \ 6\ \ 8\ \ 10\ 15\ \} \\ new\ identifiers \rightarrow & 1\ \ 2\ \ 3\ \ 4\ \ 5\ \ \ 6\ 7\ 8\ \ \ 9\ 10\ \ \ 11\ 12\ 13\ 14\ 15\ 16 \end{array}$$

partition 2

this defines a **permutation** $\pi$

$\mathcal{L}$

$C_1$ = [3,4,5,9,10,11,13,15]
$C_2$ = [2,3,15]
$C_3$ = [1,3,5,7,9,10,11]
$C_4$ = [1,3,5,7,9,11,13]
$C_5$ = [1,3,6,7,9,11,12,13,14,16]
$C_6$ = [6,8]
$C_7$ = [1,3,8,11,12,13,14,16]
$C_8$ = [12,16]

$\pi$

$C_1 = [3|6,7,8|10|12,15,16]$
$C_2 = [6|11,16]$
$C_3 = [1|6,7,8|9,10|15]$
$C_4 = [1,3|6,7,8|9,10]$
$C_5 = [1,2,3,4,5|6,8|9,10|13]$
$C_6 = [13,14]$
$C_7 = [1,2,3,4,5|6|10|14]$
$C_8 = [2,5]$

**distinct** partial colors from partition 2

[6,7,8]
[6]
[6,8]

# Meta and partial colors — Example

- Example for **N = 16** references and **4** partitions.

$$\begin{array}{l} \{\ 1\ 12\ 13\ 14\ 16\ \}\{\ 3\ 5\ 9\ \}\{\ 7\ 11\ \}\{\ 2\ \ 4\ \ 6\ \ 8\ \ 10\ 15\ \} \\ new\ identifiers \rightarrow 1\ \ 2\ \ 3\ \ 4\ \ 5\ \ \ 6\ 7\ 8\ \ \ 9\ 10\ \ \ 11\ 12\ 13\ 14\ 15\ 16 \end{array}$$

partition 2

this defines a **permutation** $\pi$

$\mathcal{L}$

$C_1$ = [3,4,5,9,10,11,13,15]
$C_2$ = [2,3,15]
$C_3$ = [1,3,5,7,9,10,11]
$C_4$ = [1,3,5,7,9,11,13]
$C_5$ = [1,3,6,7,9,11,12,13,14,16]
$C_6$ = [6,8]
$C_7$ = [1,3,8,11,12,13,14,16]
$C_8$ = [12,16]

$\pi$

$C_1 = [3|6,7,8|10|12,15,16]$
$C_2 = [6|11,16]$
$C_3 = [1|6,7,8|9,10|15]$
$C_4 = [1,3|6,7,8|9,10]$
$C_5 = [1,2,3,4,5|6,8|9,10|13]$
$C_6 = [13,14]$
$C_7 = [1,2,3,4,5|6|10|14]$
$C_8 = [2,5]$

**distinct** partial colors from partition 2

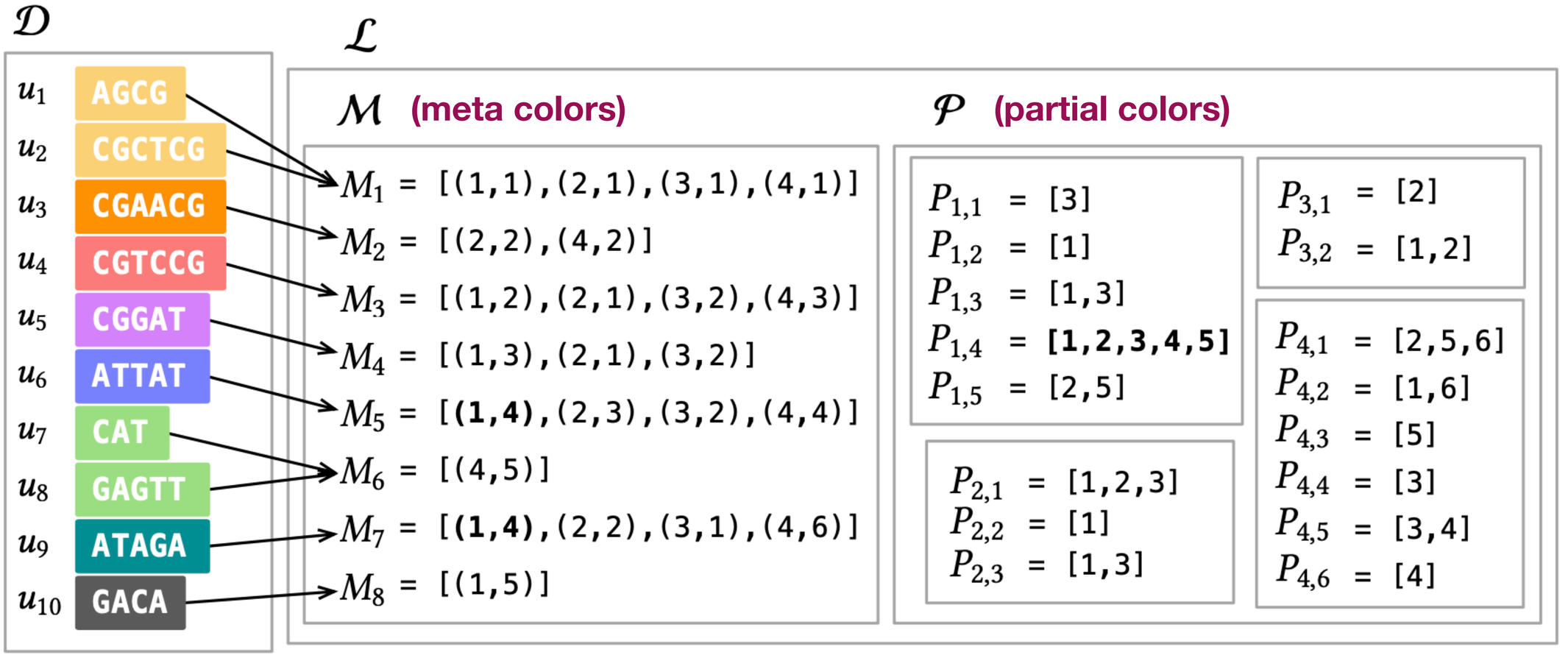
[6,7,8]
[6]
[6,8]

-5

[1,2,3]
[1]
[1,3]

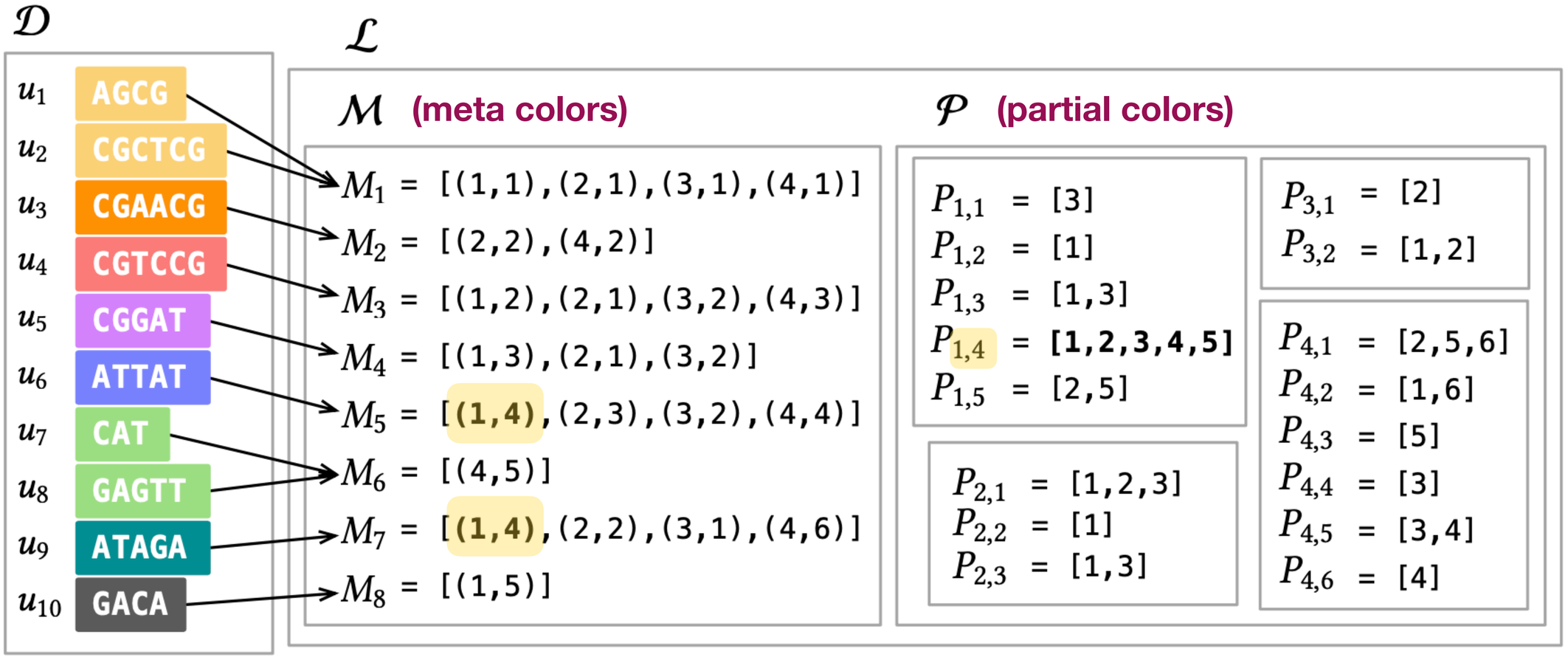
# Meta and partial colors — Example

- Example for **N = 16** references and **4** partitions.



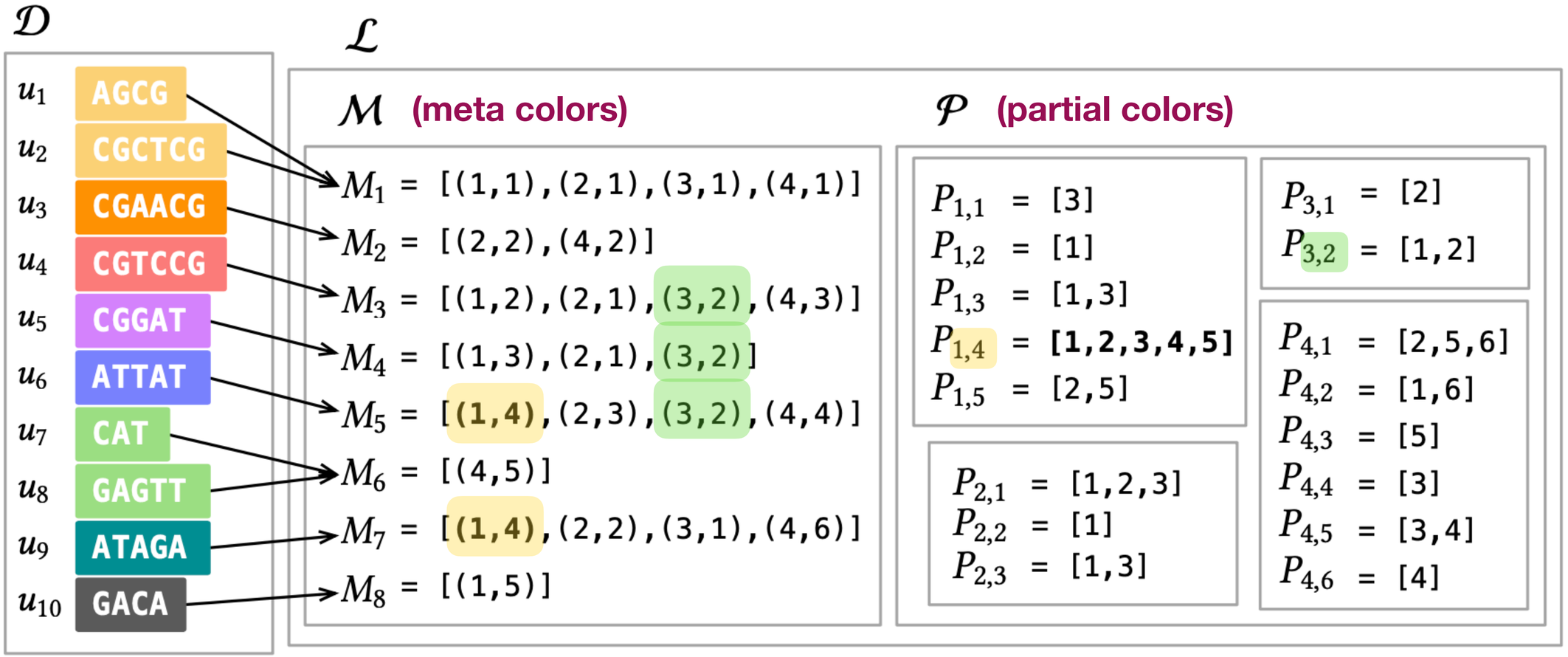
# Meta and partial colors — Example

- Example for **N = 16** references and **4** partitions.



# Meta and partial colors — Example

- Example for **N = 16** references and **4** partitions.



# Results

- We applied the meta/partial color optimisation to **Fulgor** [Fan et al., ALMOB 2024].
- We call it the *meta-colored* compacted dBG (**Mac-dBG**, or **Fulgor-v2**).
- Code: https://github.com/jermp/fulgor.
- Results on some large pangenomes of different complexities.

| | *E. Coli* (EC) | *S. Enterica* (SE) | | | | Gut bacteria (GB) |
|---|---|---|---|---|---|---|
| Genomes | 3,682 | 5,000 | 10,000 | 50,000 | 100,000 | 150,000 | 30,691 |
| Distinct colors ($\times 10^6$) | 5.59 | 2.69 | 4.24 | 13.92 | 19.36 | 23.61 | 227.80 |
| Integers in colors ($\times 10^9$) | 5.74 | 5.77 | 15.68 | 133.49 | 303.53 | 490.04 | 10.04 |
| $k$-mers in dBG ($\times 10^6$) | 170.65 | 104.69 | 239.88 | 806.23 | 1,018.69 | 1,194.44 | 13,936.86 |
| Unitigs in dBG ($\times 10^6$) | 9.31 | 4.95 | 8.24 | 30.64 | 41.16 | 49.60 | 566.39 |

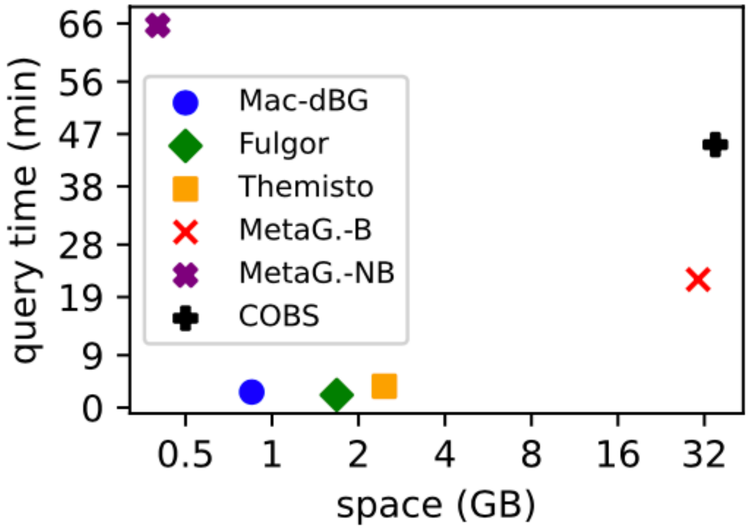
# Index space in GB

| | Genomes | Mac-dBG | | | Fulgor | | | Themisto | | | MetaGraph | | | COBS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | dBG | Colors | Total | dBG | Colors | Total | dBG | Colors | Total | dBG | Colors | Total | Total |
| EC | 3,682 | 0.29 | 0.52 | 0.81 | 0.29 | 1.36 | 1.65 | 0.22 | 1.85 | 2.08 | 0.10 | 0.23 | 0.33 | 7.53 |
| SE | 5,000 | 0.16 | 0.16 | 0.32 | 0.16 | 0.59 | 0.75 | 0.14 | 1.29 | 1.43 | 0.07 | 0.19 | 0.26 | 9.11 |
| | 10,000 | 0.35 | 0.33 | 0.68 | 0.35 | 1.66 | 2.01 | 0.32 | 3.50 | 3.81 | 0.13 | 0.38 | 0.51 | 18.68 |
| | 50,000 | 1.26 | 2.14 | 3.40 | 1.26 | 17.03 | 18.30 | 1.07 | 32.42 | 33.48 | 0.36 | 1.95 | 2.31 | 88.61 |
| | 100,000 | 1.72 | 3.83 | 5.55 | 1.72 | 40.70 | 42.44 | 1.35 | 75.94 | 77.28 | 0.45 | 3.50 | 3.95 | 173.58 |
| | 150,000 | 2.03 | 5.37 | 7.40 | 2.03 | 68.60 | 70.66 | 1.58 | 125.16 | 126.74 | — | — | — | 265.49 |
| GB | 30,691 | 21.31 | 7.85 | 29.16 | 21.31 | 15.45 | 36.85 | 18.33 | 30.88 | 49.21 | 5.23 | 4.77 | 10.00 | 21.23 |

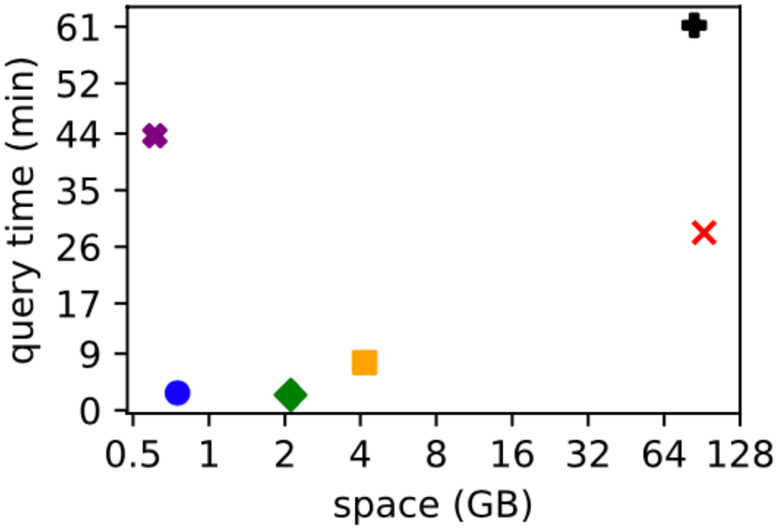
# Pseudoalignment efficiency

| | Genomes | Rate | Mac-dBG | | Fulgor | | Themisto | | MetaG.-B | | MetaG.-NB | | COBS | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | mm:ss | GB | mm:ss | GB | h:mm:ss | GB | mm:ss | GB | h:mm:ss | GB | h:mm:ss | GB |
| EC | 3,682 | 98.99 | 2:40 | 0.85 | 2:10 | 1.68 | 0:03:40 | 2.46 | 22:00 | 30.44 | 1:05:41 | 0.40 | 0:45:11 | 34.93 |
| SE | 5,000 | 89.49 | 1:16 | 0.37 | 1:16 | 0.82 | 0:03:50 | 1.82 | 14:14 | 36.54 | 0:20:32 | 0.33 | 0:38:34 | 41.93 |
| | 10,000 | 89.71 | 2:45 | 0.75 | 2:26 | 2.11 | 0:07:35 | 4.16 | 28:15 | 92.18 | 0:43:40 | 0.61 | 1:01:14 | 84.20 |
| | 50,000 | 91.25 | 14:00 | 3.65 | 19:15 | 18.53 | 0:42:02 | 33.14 | — | — | 4:30:03 | 2.72 | 3:54:18 | 408.82 |
| | 100,000 | 91.41 | 26:48 | 6.29 | 27:30 | 42.78 | 1:22:00 | 75.93 | — | — | 9:40:06 | 4.82 | 8:07:29 | 522.56 |
| | 150,000 | 91.52 | 41:30 | 8.51 | 42:30 | 70.55 | 2:00:13 | 124.27 | — | — | — | — | 7:47:14 | 522.63 |
| GB | 30,691 | 92.91 | 01:03 | 28.51 | 01:10 | 30.02 | 0:01:20 | 48.47 | 28:55 | 15.86 | 0:22:05 | 9.91 | 0:34:45 | 225.57 |

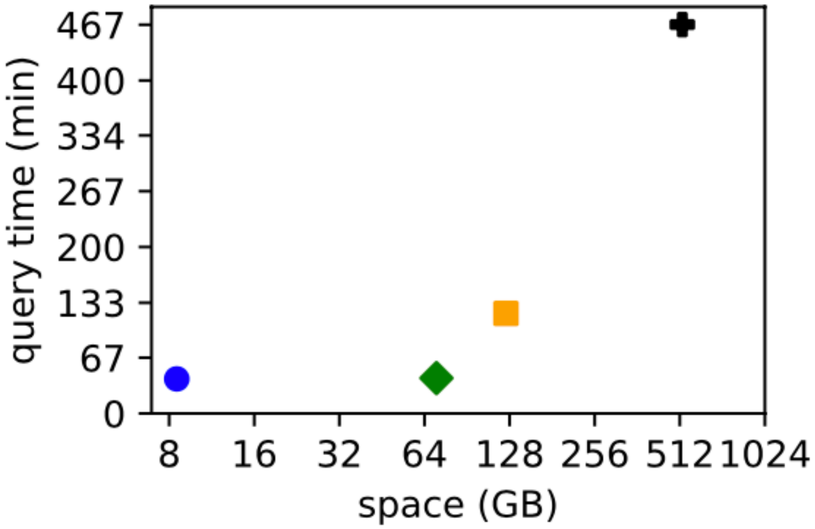
# Overall space vs. time trade-off




(a) EC




(b) SE 10,000




(c) SE 150,000

# Conclusions

- SSHash to obtain an **efficient map from k-mers to unitigs**.
- Permute unitigs in color order to enable a **space-efficient mapping from unitigs to colors**.
- Factorize the redundancy in large color matrixes via **meta/partial colors**.
- **Result:** the meta-colored dBG embodies a superior space/time trade-off compared to the state of the art. Space improvement can be dramatic but query efficiency not harmed.
- **Take-away:** No reason not to use meta-colored dBGs to compress and index pangenomes!
- **Code:** https://github.com/jermp/fulgor.

# Conclusions

- SSHash to obtain an **efficient map from k-mers to unitigs**.
- Permute unitigs in color order to enable a **space-efficient mapping from unitigs to colors**.
- Factorize the redundancy in large color matrixes via **meta/partial colors**.
- **Result:** the meta-colored dBG embodies a superior space/time trade-off compared to the state of the art. Space improvement can be dramatic but query efficiency not harmed.
- **Take-away:** No reason not to use meta-colored dBGs to compress and index pangenomes!
- **Code:** https://github.com/jermp/fulgor.

## Thank you for the attention!