Mapping-friendly sequence reductions:

Going beyond homopolymer compression

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A little bit of context

Context - Long read mapping

- Sequencing **errors complicate mapping** *(Gusfield, 1997)*
- **Long read** sequencing errors *(Dohm et al. 2020)*:
  - Short **indels**
  - Particularly **in homopolymers**

10.1145/270563.571472
10.1093/nargab/lqaa037

Context - what is HPC ?

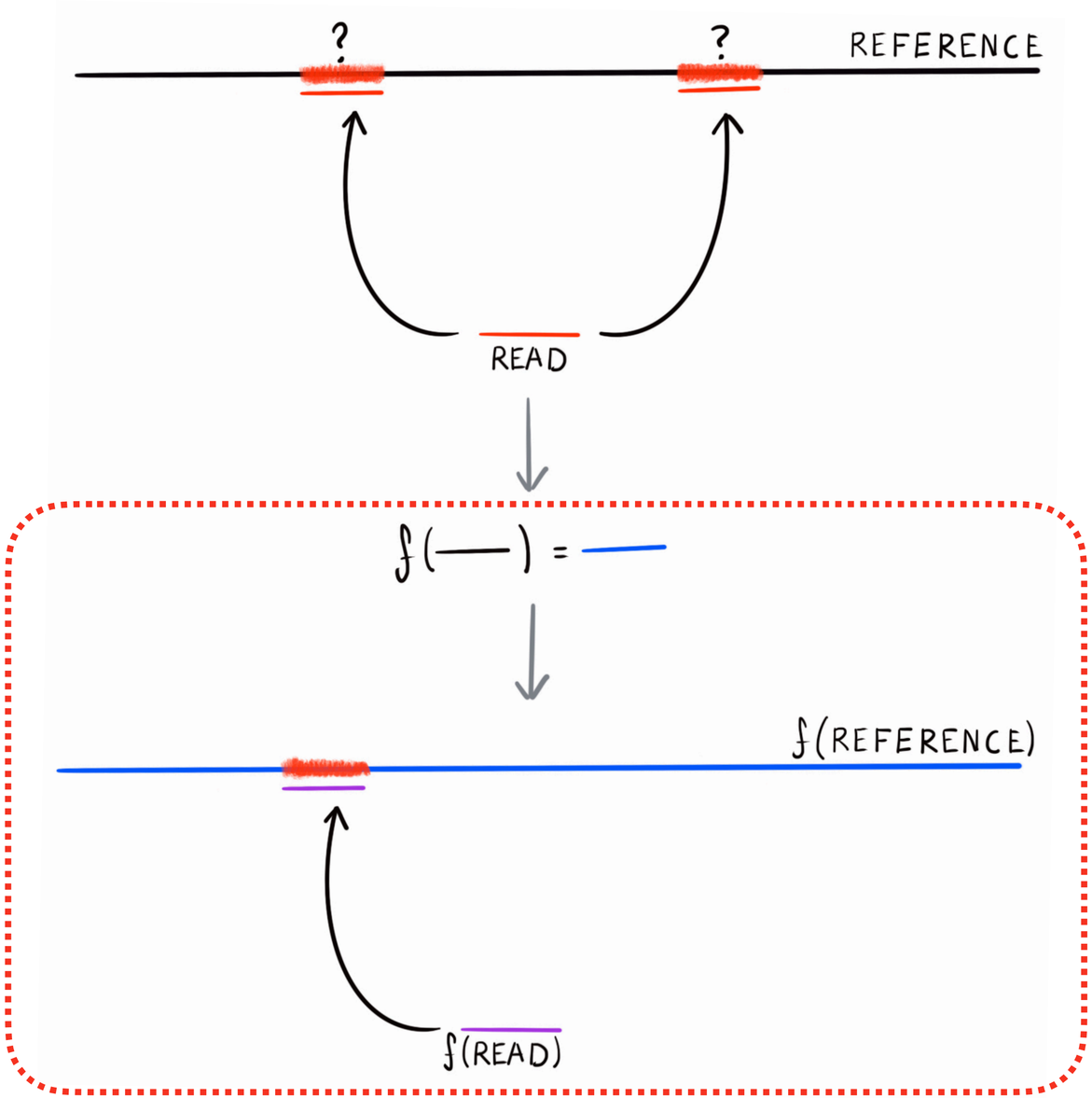
- **Homopolymer compression** (HPC) transforms sequences:

HPC(AAAATTTGGGCCCCCGGGTA) → ATGCGTA

- HPC is a **function** that **transforms strings** of characters into other strings

- Empirically it **improves mapping**, but **no guarantee it's the best**

Goals



Can we find functions f that **improve mapping more than HPC** ?

Mapping-friendly sequence reductions

A formal definition of HPC

- Let us define $\Sigma = \{A, C, G, T\}$ and ε the empty character
- Let $g^{HPC} : \Sigma^2 \rightarrow \Sigma \cup \{\varepsilon\}$ s.t. $\forall (x_1, x_2) \in \Sigma^2$

$$g^{HPC}(x_1 \cdot x_2) = \begin{cases} x_2 & if\ x_1 \neq x_2 \\ \varepsilon & if\ x_1 = x_2 \end{cases}$$

- HPC(x) = applying g^{HPC} on a sliding window of size 2 along x and concatenating outputs.
- Different g = MSR

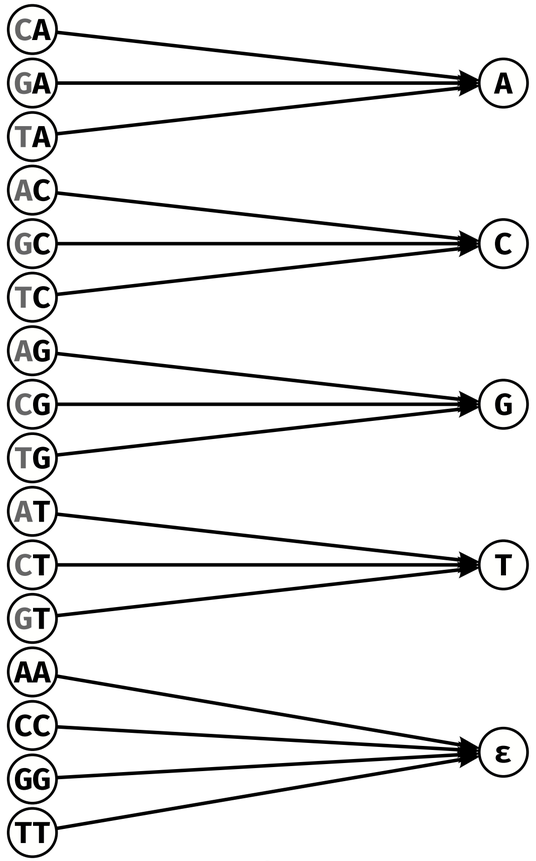
The DAG visualisation

- Each g function can be visualised as a directed graph defined by a mapping between $|\Sigma^{\ell}|$ inputs and $|\Sigma| + 1$ outputs

- HPC as a directed graph

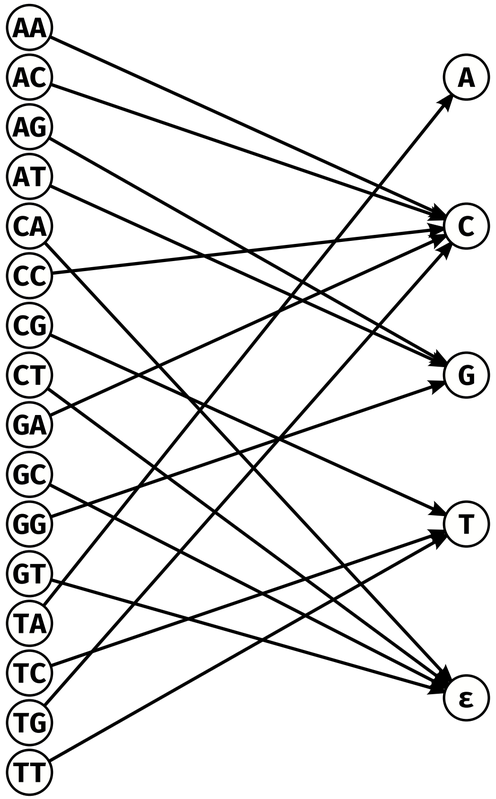
  (n=16 inputs k=5 outputs)

- There are 5^{16} functions $g : \Sigma^2 \rightarrow \Sigma \cup \{\varepsilon\}$



Reducing the search space - RC

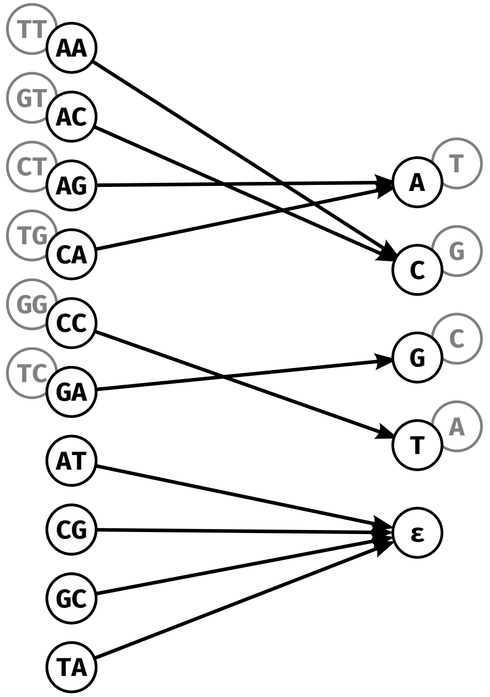
Random MSR f_r ❌



$$x = TAAGTTGA$$
$$f_r(x) = TACGTCC$$
$$RC(x) = TCAACTTA$$

$$f_r(RC(x)) = TTCCTA$$
$$RC(f_r(x)) = GGACGTA$$

RC-core-insensitive MSR f ✅



$$x = TAAGTTGA$$
$$f(x) = TCAGGTG$$
$$RC(x) = TCAACTTA$$

$$f(RC(x)) = TCACCTG$$
$$RC(f(x)) = CACCTGA$$

Reducing the search space - RC

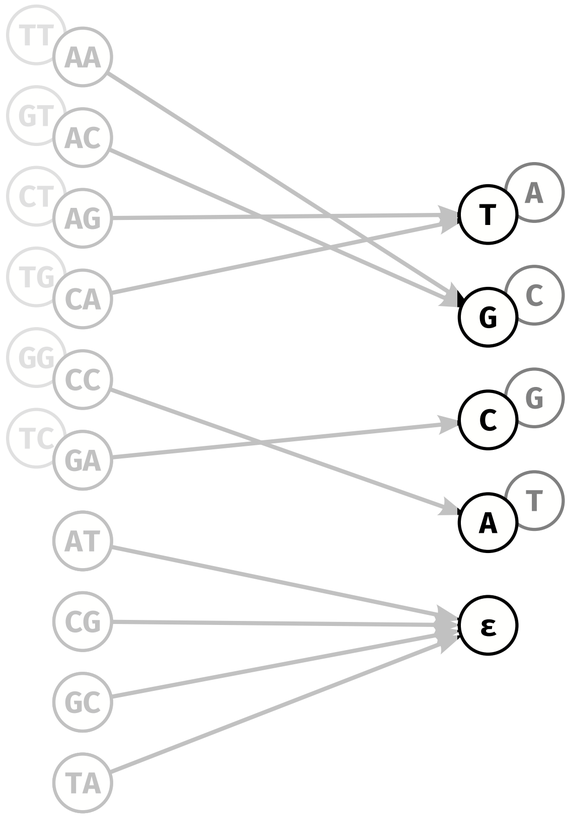
- There are $5^6 \approx 1.5 \cdot 10^4$ RC-core-insensitive MSRs
- Mapping is **computationally expensive**

We need to **reduce** the search space **even more**

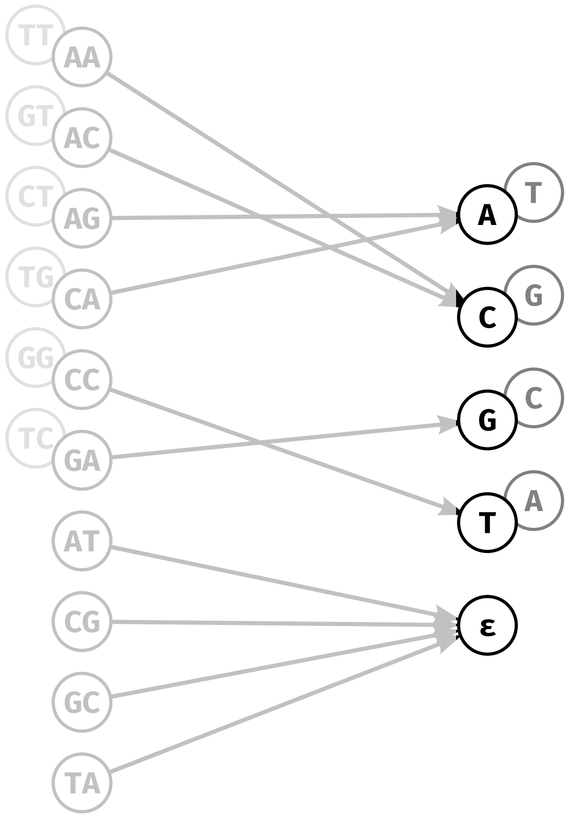
Reducing the search space - equivalence

- Symmetries:
  - $A \Leftrightarrow T$ and $G \Leftrightarrow C$
  - $(G/C)_{pair} \Leftrightarrow (A/T)_{pair}$
- We define **equivalence classes** from them

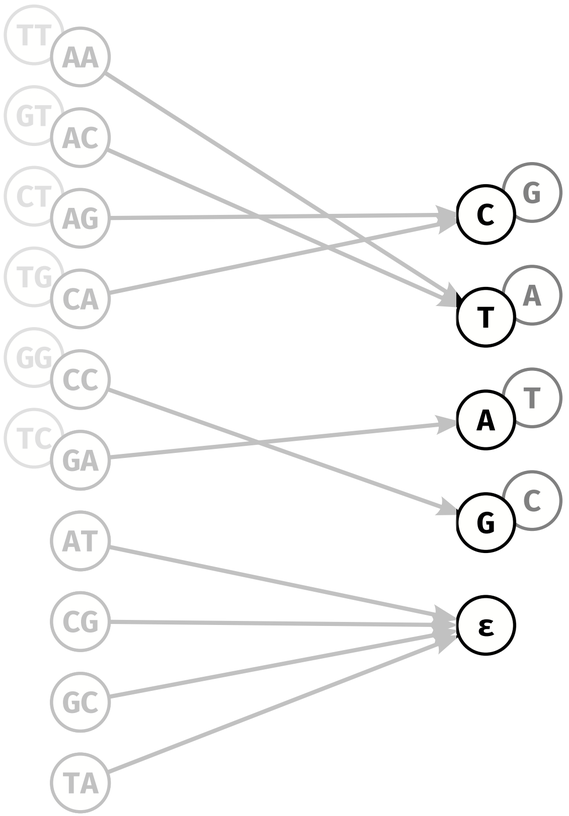
Reducing the search space - equivalence



$$A \leftrightarrow T$$
$$C \leftrightarrow G$$



$$A/T \leftrightarrow C/G$$



Reducing the search space - final words

5^{16}

↓

5^{6}

↓

2135 MSRs to look at

How do we evaluate MSRs ?

Evaluating MSRs - datasets

- Simulate ONT reads, with **`nanosim`**, on 4 references:
  - **Whole human genome**, CHM13hTERT human cell line by the T2T
  - **Whole *Drosophila* genome**, *Adams et al. (2022)*
  - **Whole *Escherichia coli* genome**, *Blattner et al. (1977)*
  - **Synthetic human centromeric sequence**, *Mikheenko et al. (2020)* `tandemtools` mapper test data

Can MSRs **improve mapping** of simulated reads?

Evaluating MSRs - evaluation pipeline

- For **each (MSR, reference) pair** (and no MSR i.e. *raw*):
  1. **Transform** the reference and reads with the MSR
  2. **Map** transformed reads to transformed reference with `minimap2`
  3. **Evaluate** mapping with `paftools mapeval`

Evaluating MSRs - evaluation pipeline

- Mapping quality *(**mapq**)* is a measure of how confident the aligner is in its read placement. 0 *(worse)* ≤ mapq ≤ 60 *(best)*

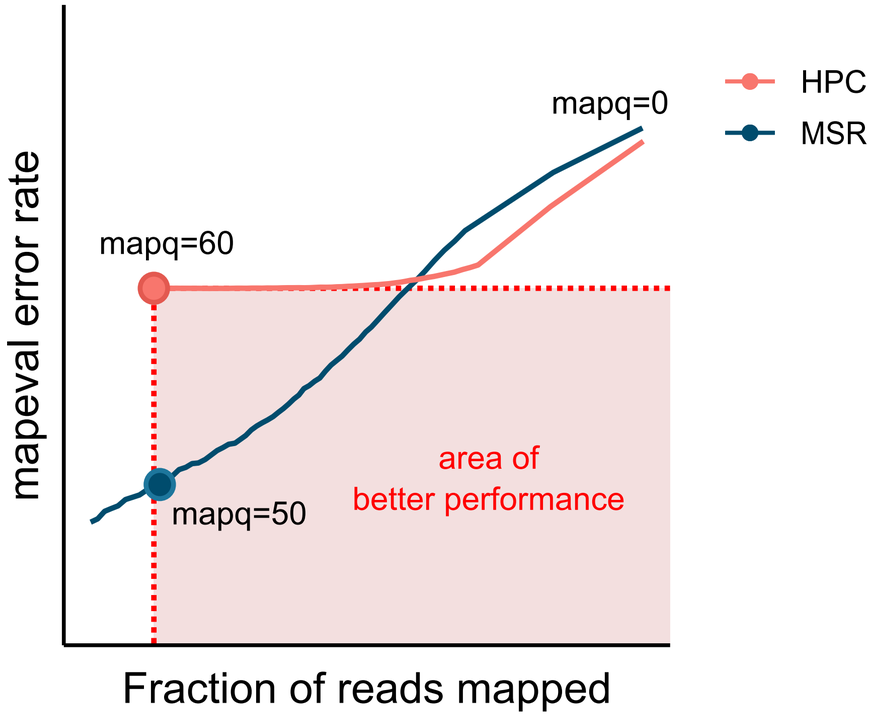
- **`mapeval`** gives results for **mapq thresholds**
  i.e. sets of mapped reads with mapq ≥ than a given value

- **`mapeval`** reports for each threshold:

  - **Number of reads** mapped

  - Mapping **error rate**

Evaluating MSRs - MSR selection

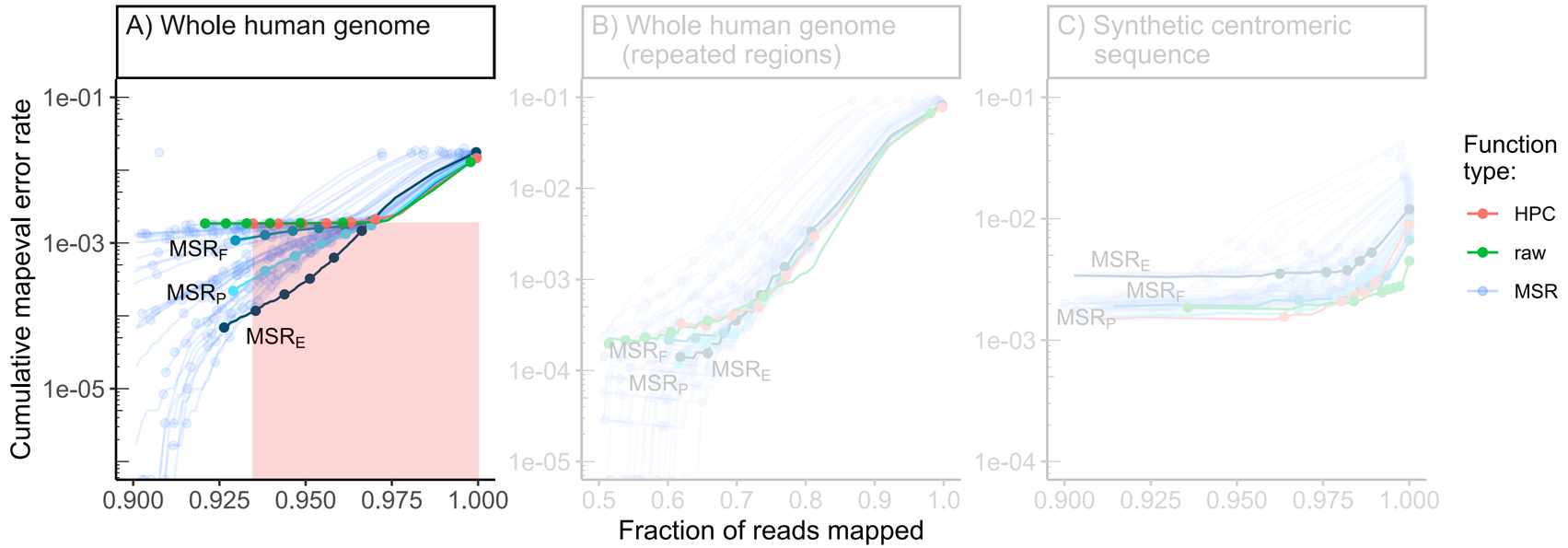


We compare **MSRs** to **HPC at mapq 60**

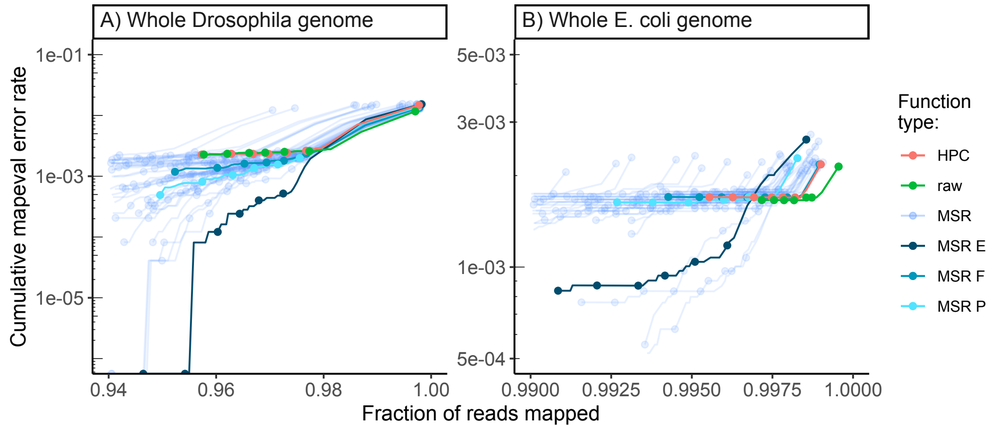
We **select top MSRs** *(error, %mapped, %in shaded area)*

Some results

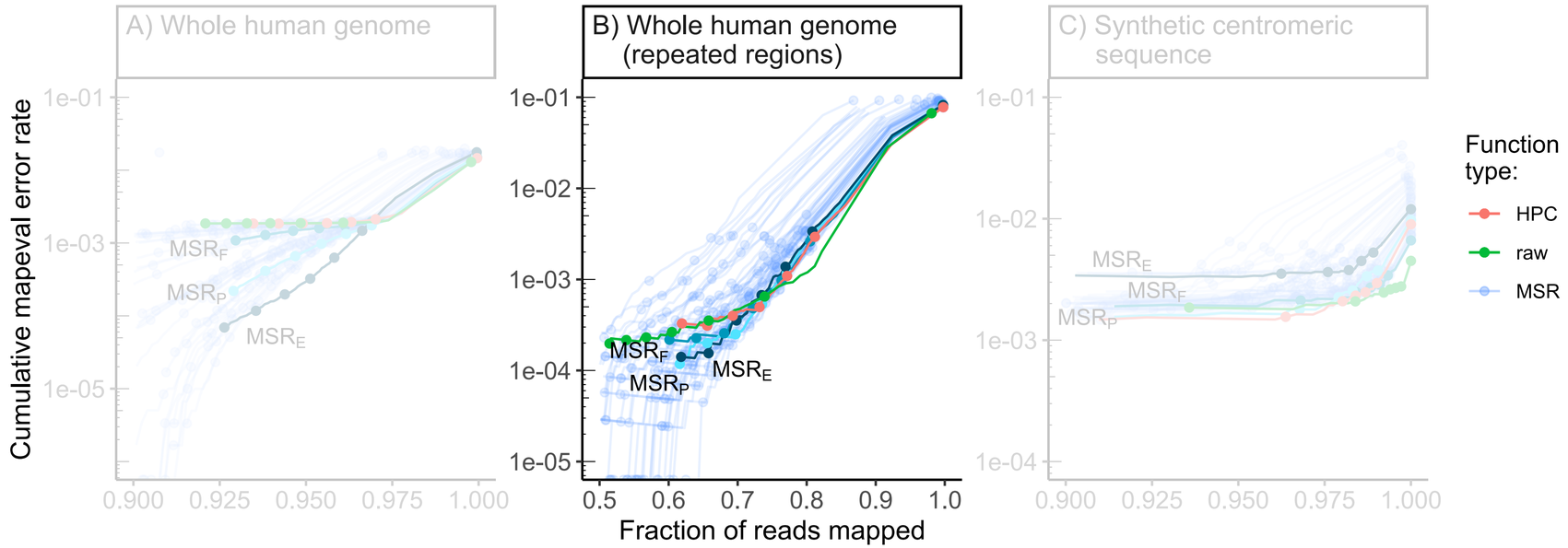
Results - across whole genomes



Many MSRs are **better** than HPC60

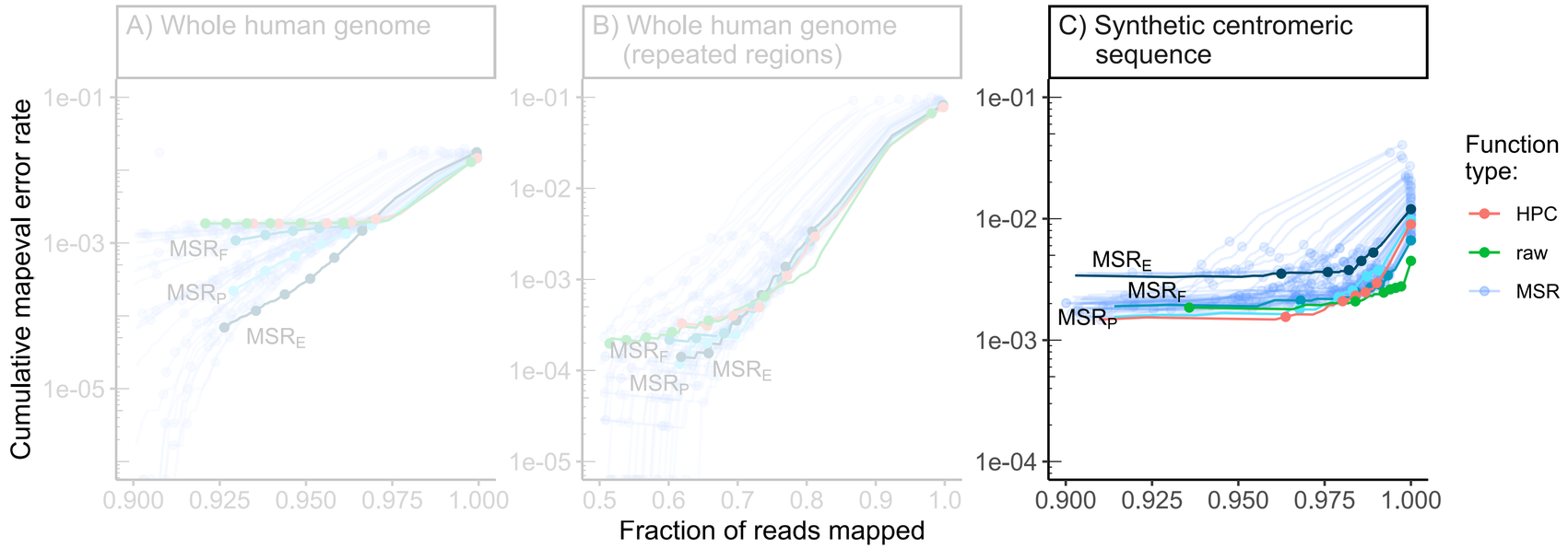


Results - repeated regions



MSRs are still **better** than HPC60
but performance **gap is smaller**

Results - centromeric sequence



Mapping to **centromeres** is **hard**,
best **not to apply any function**

Take home message

- Some **MSRs** are **better than HPC**
- In some cases, the **mapping error rate** goes from 10^{-3} to $\mathbf{10^{-6}}$
- **MSRs** are **easy to implement** in existing aligners,
  i.e. **cheap performance gains**

Where do we go now ?

Perspectives

- MSRs work on simulated data → How do we evaluate on **real datasets** ? *(fraction of mapped reads, mismatch rate, …)*
- Explore **higher-order MSRs** ($N(3) \approx 3 \cdot 10^{21}$ and $N(4) \approx 10^{85}$):
  - **Reduce** the search space
  - **Explore** search space **better**:
    - Define objective function and **optimise**
    - **“Learn”** MSRs (connections, sequence to sequence models, …)

Thank you !

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What is an MSR ?

- An order-ℓ MSR is defined by a function $g : \Sigma^{\ell} \rightarrow \Sigma \cup \{\varepsilon\}$ applied to a sliding window of length ℓ along the sequence to transform
- The output sequence of the MSR is the concatenation of the first $\ell - 1$ characters of the original sequence plus all the outputs of g
- Let x be a string and f an MSR defined by g:
  $f(x) = x[1,\ell - 1] \cdot g(x[1,\ell]) \cdot g(x[2,\ell + 1]) \cdots g(x[|x| - \ell + 1, |x|])$
- By exploring the space of g functions we can explore different sequence transformation functions and see how the impact mapping
- We explore order-2 MSRs in this project

Reducing the search space - RC

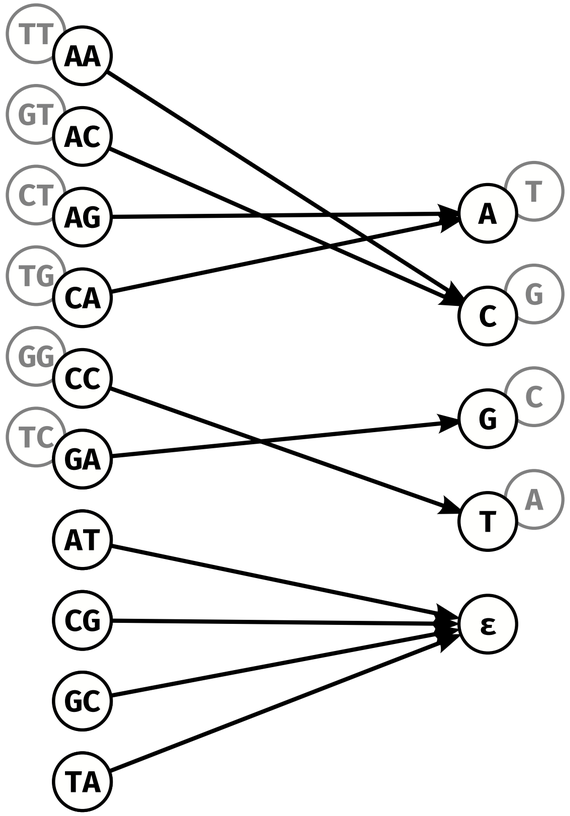
- Since we want to apply MSRs to biological sequences we want it to be **commutative** with reverse complementation *(RC)*.
  i.e. for an MSR f we want $RC(f(x)) = f(RC(x))$

- We define *RC-core-insensitive* MSRs by restricting g. Let x be an ℓ-mer and y its RC; then either $g(x)$ must be the RC of $g(y)$ or $g(x) = g(y) = \varepsilon$

- For order-2 MSRs, there are 16 ℓ-mers, 4 are their own RC (AT,TA,GC,CG) so they must be mapped to ε.
  The remaining 12 ℓ-mers can be grouped into 6 pairs of RCs.

- defining g amounts to choosing an output for each pair. So we have 5^6 RC-core-insensitive MSRs.

Reducing the search space - equivalence



$\rightarrow$

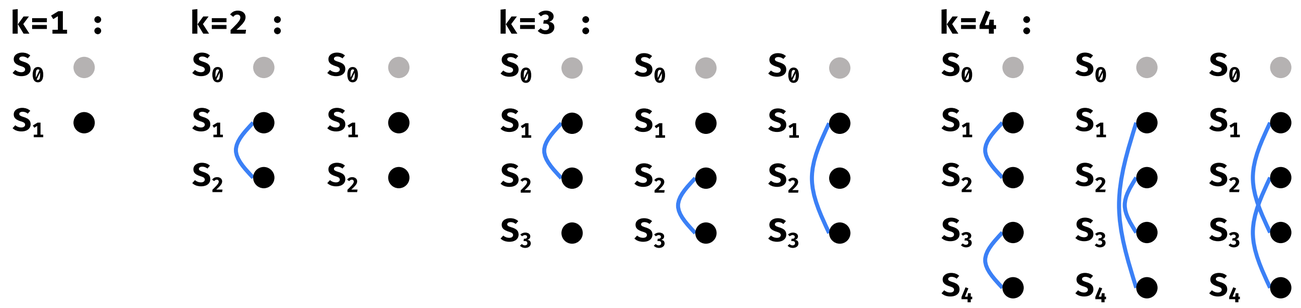
$$S_0 = \{AT, TA, CG, GC\}$$
$$S_1 = \{AG, CA\}$$
$$S_2 = \{CC\}$$
$$S_3 = \{AA, AC\}$$
$$S_4 = \{GA\}$$

and

$$t(1) = A, \;\; t(2) = T, \;\; t(3) = C, \;\; t(4) = G$$

Reducing the search space - equivalence

- For 2 MSRs defined by $S_0, \ldots, S_k, t$ and $S'_0, \ldots, S'_k, t'$ respectively.
- They are equivalent iff:
  - $S_0 = S'_0$
  - there is a permutation π of $[[1; k]]$ such that $\forall i \in [[1; k]]\ S_i = S'_{\pi(i)}$
  - $\forall (i, j) \in [[1; k]]^2$ we have $IsComp(t(i), t(j)) = IsComp(t'(\pi(i)), t'(\pi(j)))$



Number of equivalence classes for fixed partitions with output cardinality k

Reducing the search space - equivalence

- In preliminary tests, when swapping $A \leftrightarrow T$, $G \leftrightarrow C$ or whole G/C pairs with A/T pairs in the MSR outputs, it did not affect performance of the MSRs.
- We can reduce the search space even more by defining MSR equivalence classes.
- Let's consider an MSR defined by g, it's output cardinality k is the number of distinct nucleotides *(i.e. $\neq \varepsilon$)* that can be output by g
- Since g maps all ℓ-mers to $k+1$ values, we can view it as a partition of the set of all ℓ-mers into $k+1$ sets $S_0, \ldots, S_k$ with an injection $t : \{1,\ldots,k\} \to \Sigma$ That assigns an output letter to each set (S_0 is assigned ε)

Reducing the search space - final words

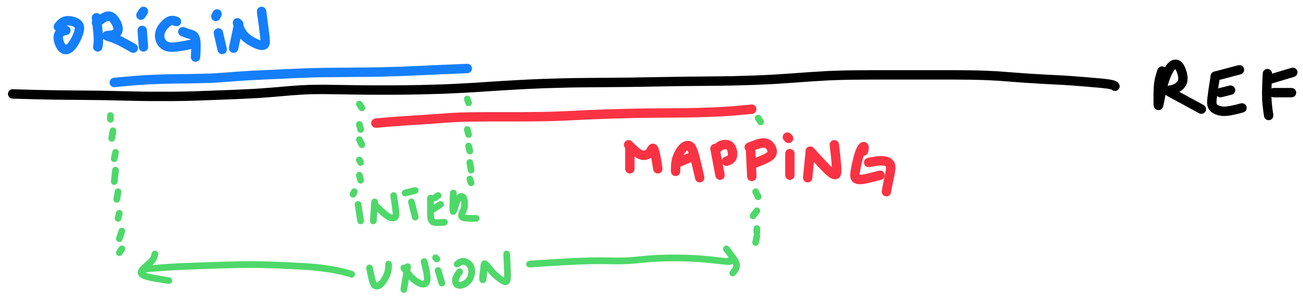
- Let $i(\ell)$ be the number of inputs necessary to define an order-ℓ RC-core-insensitive MSR, and $o(k)$ the number of equivalence classes for a partition with output cardinality k.
- Let $C(\ell, k)$ be the number of ways we can partition $i(\ell)$ ℓ-mers into $k+1$ sets ($S_1, \ldots, S_k \neq \emptyset$).
- Then the total number of MSRs of "interest" is:

$$N(\ell) = \sum_{k=1}^{4} C(\ell, k) \cdot o(k)$$

- For order-2 MSRs we have $N(2) = 2135 \ll 5^6 \ll 5^{16}$

Evaluating MSRs - evaluation pipeline

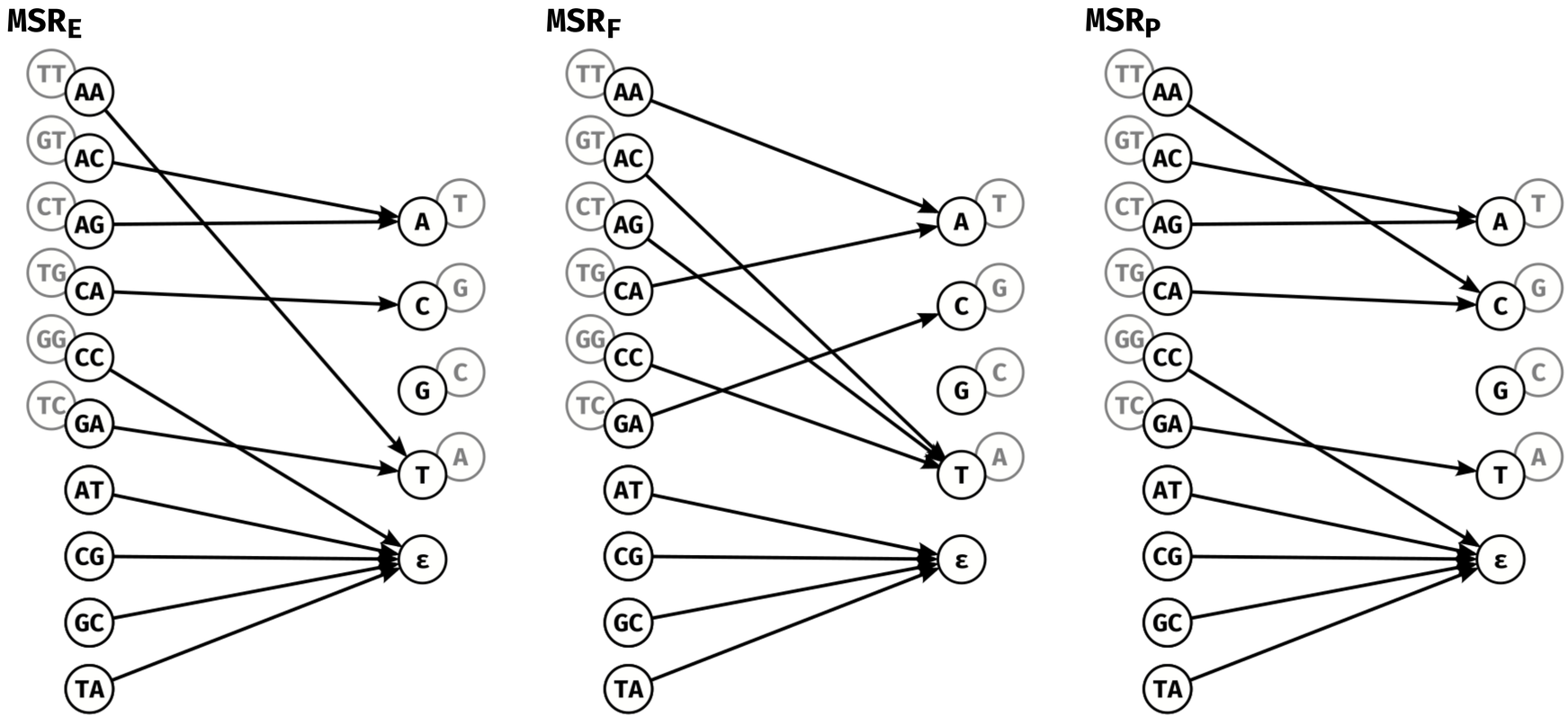
- For each evaluated MSR (and no MSR i.e. *raw*) and each reference we:
  1. Transform the reference and reads with the MSR
  2. Map transformed reads to transformed reference with `minimap2`
  3. Evaluate mapping with `paftools mapeval`
- `mapeval` considers a read correctly mapped if the intersection of the mapped interval and the origin interval on the reference is $\geq 10\,\%$ of the union



Evaluating MSRs - simulation pipeline

- On each reference sequence we simulate ONT long reads using **`nanosim`**
- We aimed for a theoretical coverage of 1.5x (more for centromeric sequence):
    - Whole human genome: $5.5 \cdot 10^5$ reads
    - Whole *Drosophila* genome: $2.6 \cdot 10^4$ reads
    - Synthetic centromere: $1.3 \cdot 10^4$ reads *(i.e. 100x theoretical coverage)*

Results - The top MSRs

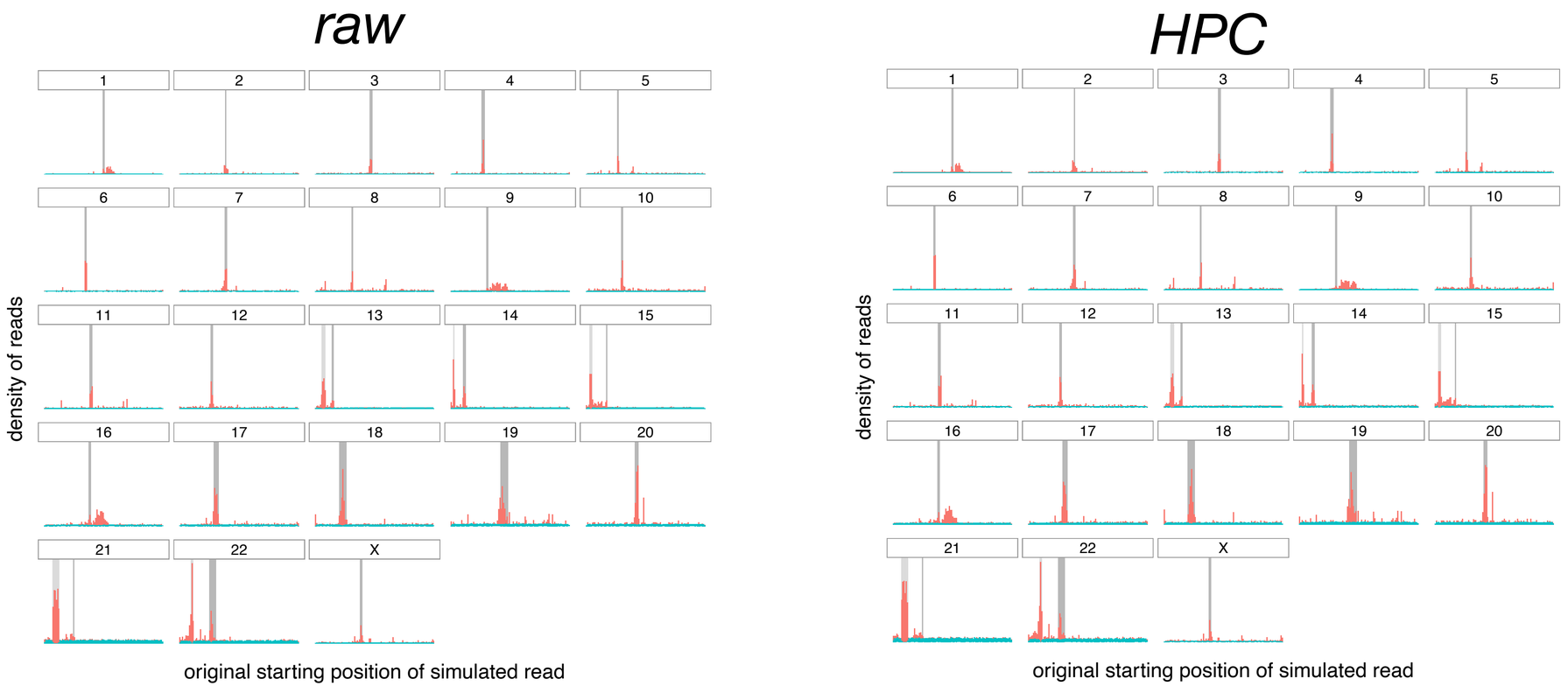


Results - where do MSRs go wrong ?

Origin of incorrectly mapped reads on chromosome

- Incorrectly mapped reads with mapq ≥ 50 for MSRs and 60 for HPC & *raw*
- These reads originate from across the whole genome
- Overall, there is a lower number of these mappings for MSRs (E:549, F:970, P:261) than HPC (1130) or *raw* (1118)

Results - where do MSRs go wrong ?



- We looked at all reads, correctly (blue) and incorrectly (red) mapped
- Most of the mistakes come from centromeres and highly repetitive regions

Why not use PacBio reads ?

- We tried simulating PacBio reads with PBSim2:
- We were getting identical read error profiles with: R94 (ONT) and PC6 (PB) pre-trained models
- We decided not to investigate further
- It might be worth it to test other simulators