UNIVERSITÄT DES SAARLANDES

CLEANIFIER: Removing human DNA contamination with a pangenomic gapped *k*-mer index

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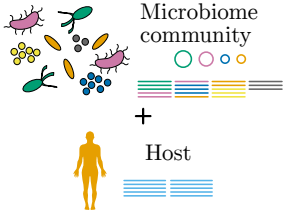
Saarland University

GCB 2025

python Package Index

BIOCONDA
Reproducible research.
Install the software from bioconda:
> conda install -c bioconda cleanifier
See bioconda.github.io

Motivation



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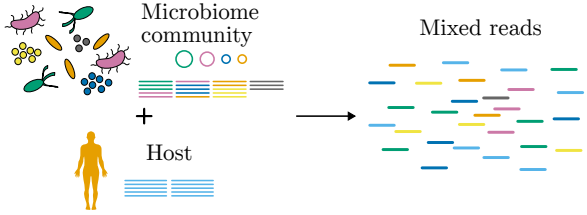
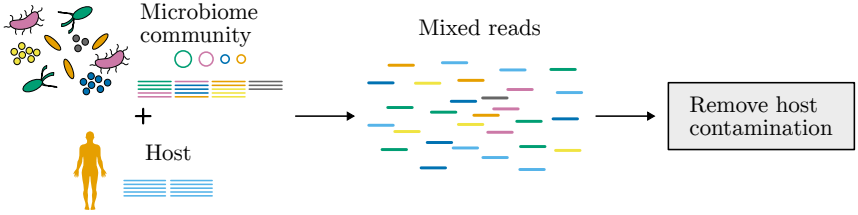
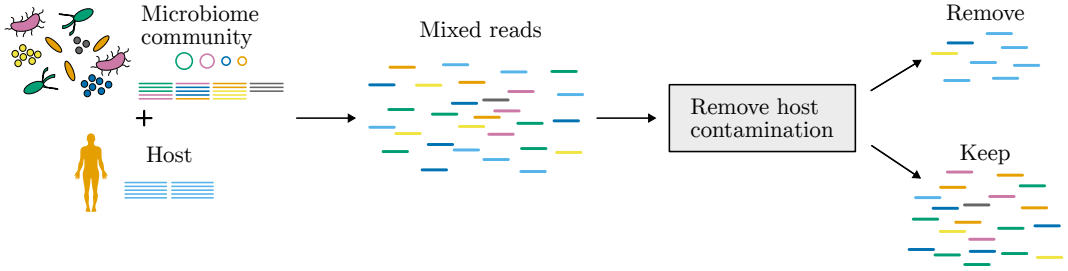



Microbiome community
+
Host
Mixed reads
Remove host contamination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Remove host contamination
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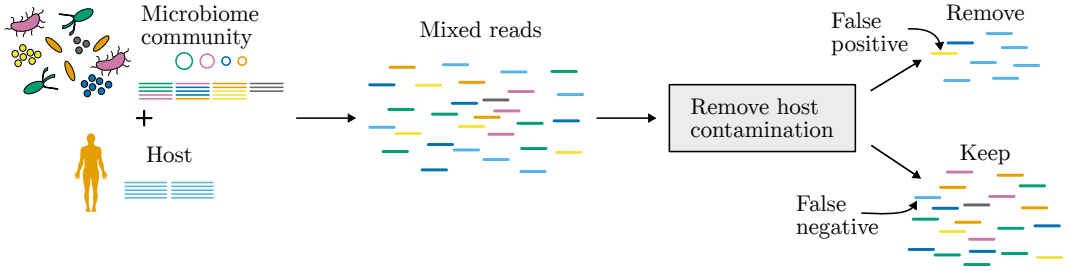
Microbiome community
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False positive
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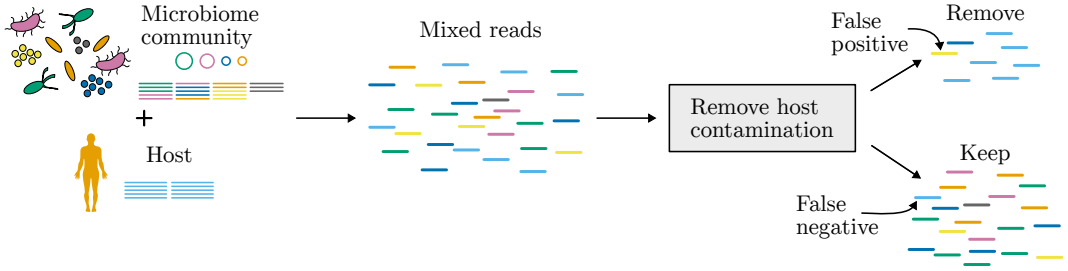
False negative



Privacy

- Human data cannot be made public

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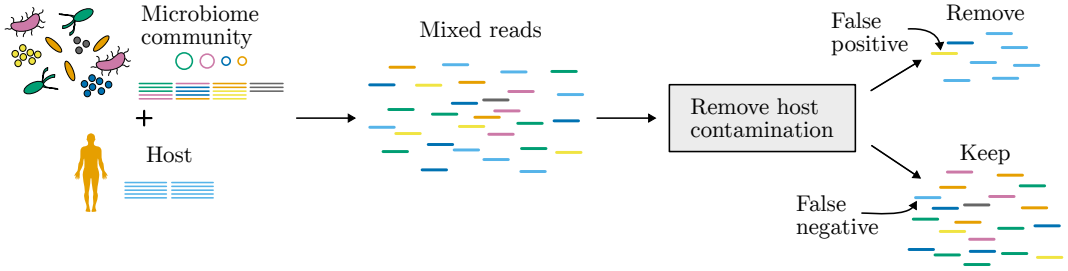

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Downstream analysis

- Reduce problems in binning and assembly

Definitions

- k-mers
  - Substrings of length k

```
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  - `A` $\leftrightarrow$ `T`, `C` $\leftrightarrow$ `G`

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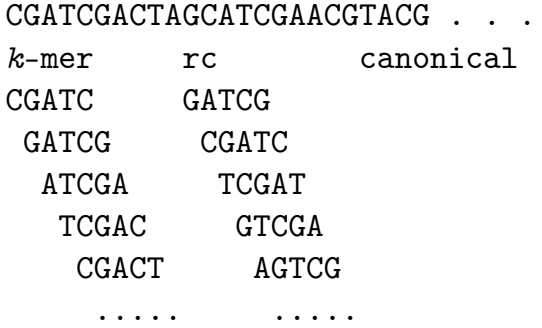

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  - `##_#_##`

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- More robust against substitutions
- *Design of Worst-Case-Optimal Spaced Seeds* at WABI 2025

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  - Alignment based
  - Human reference
  - BOWTIE2 or MINIMAP2

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  - Include all *k*-mers from human derived eukaryotic species
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1. Bucketed Cuckoo hash table
   - Exact data structure
   - Stores the k-mers
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Data structures

1. Bucketed Cuckoo hash table
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   - Size 13.85 GB
2. Windowed Cuckoo filter
   - Probabilistic set membership data structure
   - Store a fingerprint (of p bits) instead of the k-mer
   - False positive rate of $2^{-p} = 2^{-14}$
   - Size 6.9 GB

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- Store a fingerprint (of p bits) instead of the k-mer
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  - $d = 2$, $\ell = 4$ fill rate of ≈ 0.9989

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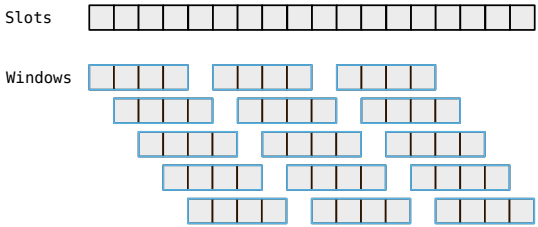

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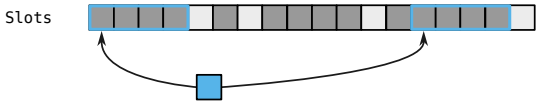

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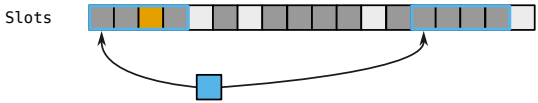

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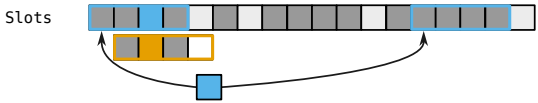

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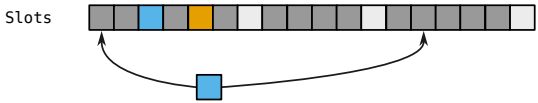

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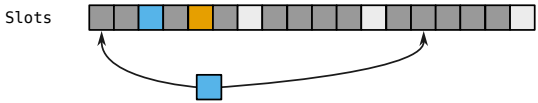
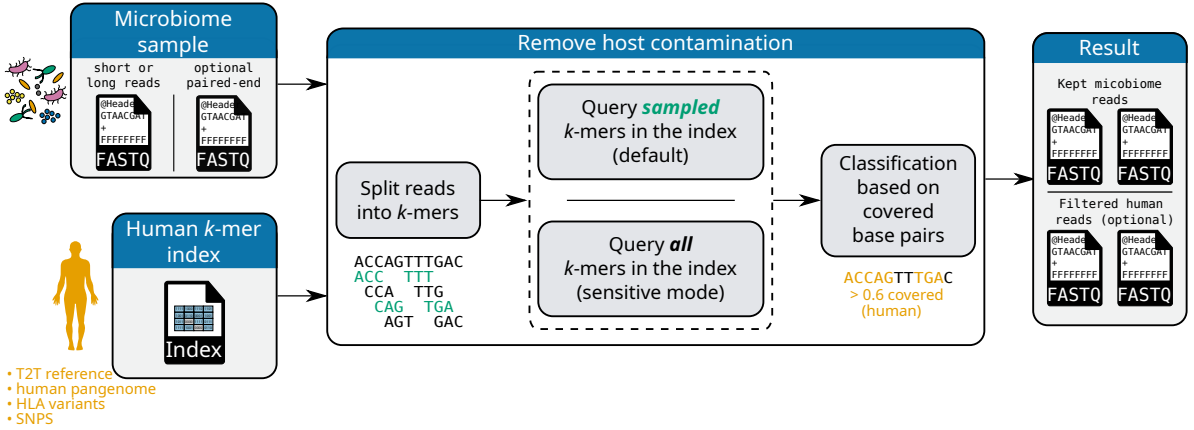


Smaller and More Flexible Cuckoo Filters
at ALENEX 2026


Microbiome sample
short or long reads
optional paired-end
@Heade
GTAACGAT
+
FFFFFFFF
FASTQ
Human k-mer index
Index
• T2T reference
• human pangenome
• HLA variants
• SNPS
Remove host contamination
Split reads into k-mers
ACCAGTTTGAC
ACC TTT
CCA TTG
CAG TGA
AGT GAC
Query sampled k-mers in the index (default)
Query all k-mers in the index (sensitive mode)
Classification based on covered base pairs
ACCAGTTTGAC
> 0.6 covered (human)
Result
Kept micobiome reads
Filtered human reads (optional)

Sensitive mode

- Query all gapped k-mers
- All k bases count as covered
- Check how many bases are covered by a human gapped k-mer
- Threshold to decide if human or not

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 GA C AC  GC T GA
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   TC A TA  AT G AC
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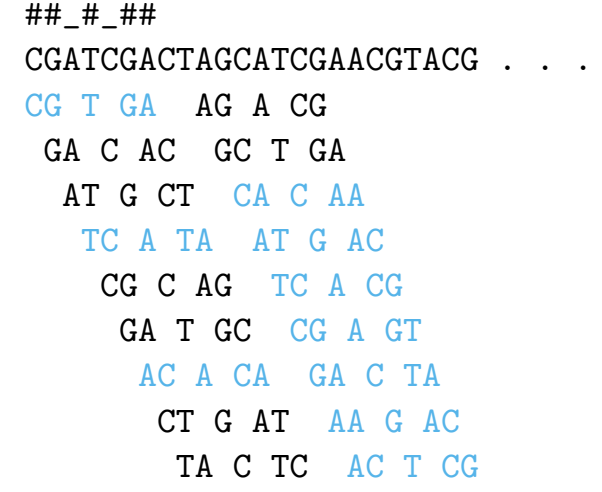

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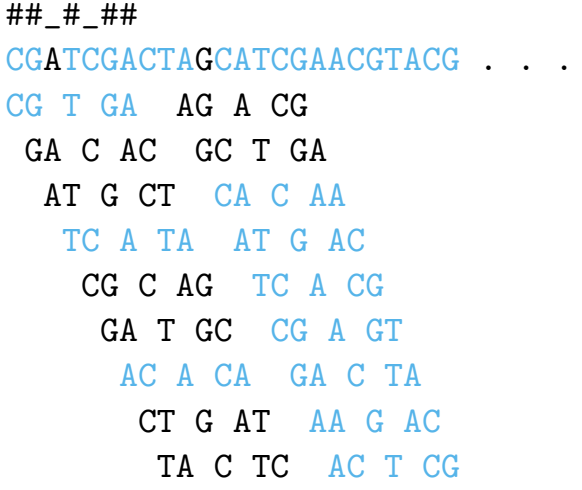

Sampling mode

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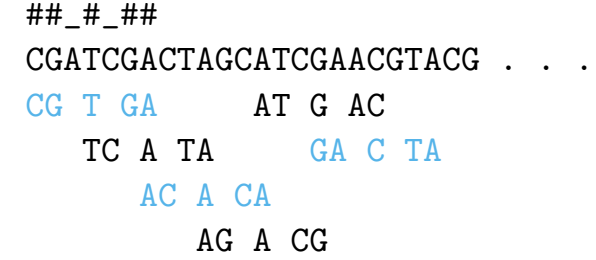

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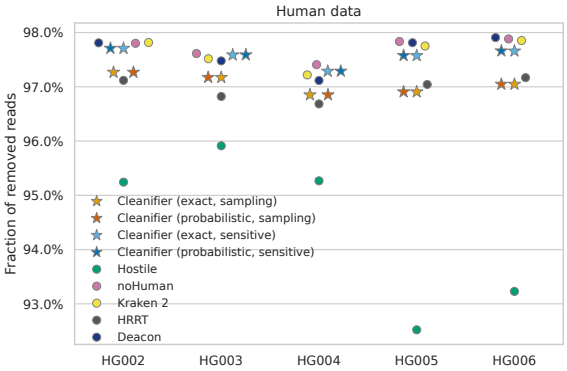
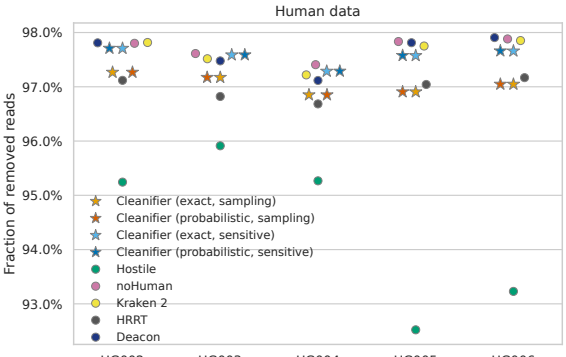

Human data
Fraction of removed reads
98.0%
97.0%
96.0%
95.0%
94.0%
93.0%
Cleanifier (exact, sampling)
Cleanifier (probabilistic, sampling)
Cleanifier (exact, sensitive)
Cleanifier (probabilistic, sensitive)
Hostile
noHuman
Kraken 2
HRRT
Deacon
HG002
HG003
HG004
HG005
HG006

Evaluation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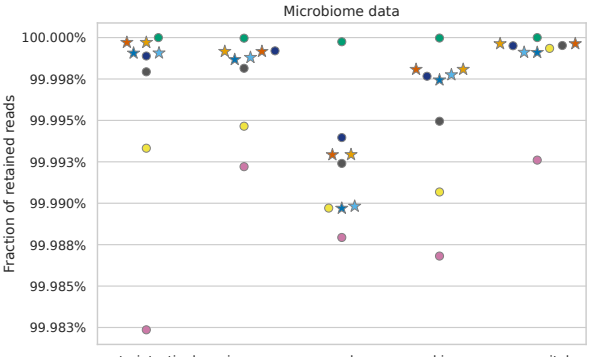
Microbiome data
Fraction of retained reads
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99.983%
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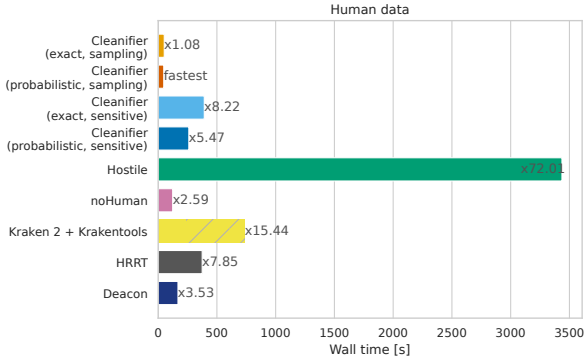
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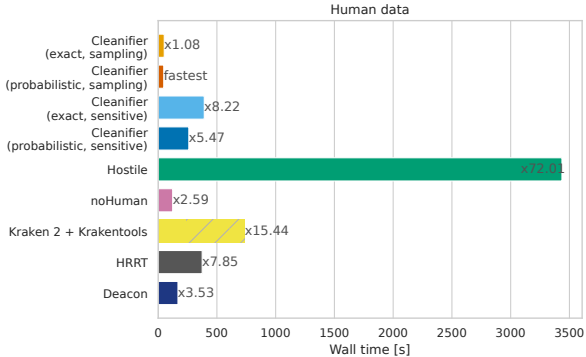
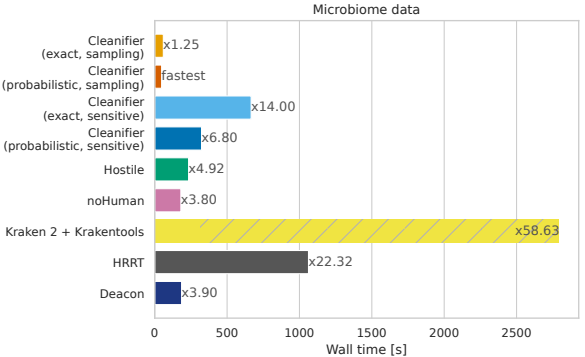
x8.22
x5.47
x72.01
x2.59
x15.44
x7.85
x3.53
0
500
1000
1500
2000
2500
3000
3500
Wall time [s]

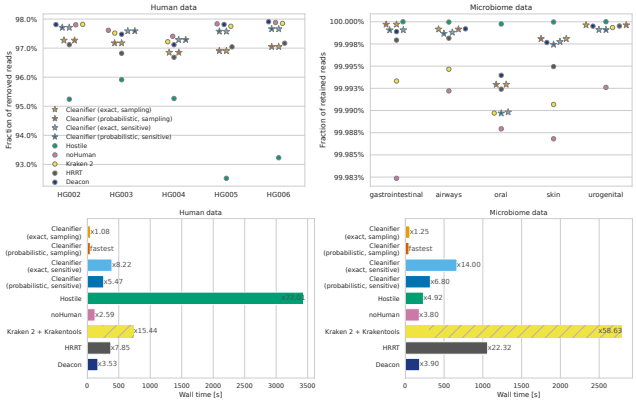
Evaluation


Human data
Cleanifier (exact, sampling)
Cleanifier (probabilistic, sampling)
Cleanifier (exact, sensitive)
Cleanifier (probabilistic, sensitive)
Hostile
noHuman
Kraken 2 + Krakentools
HRRT
Deacon
x1.08
fastest
x8.22
x5.47
x72.01
x2.59
x15.44
x7.85
x3.53
0
500
1000
1500
2000
2500
3000
3500
Wall time [s]


Microbiome data
Cleanifier (exact, sampling)
Cleanifier (probabilistic, sampling)
Cleanifier (exact, sensitive)
Cleanifier (probabilistic, sensitive)
Hostile
noHuman
Kraken 2 + Krakentools
HRRT
Deacon
x1.25
fastest
x14.00
x6.80
x4.92
x3.80
x58.63
x22.32
x3.90
0
500
1000
1500
2000
Wall time [s]

Cleanifier

- High accuracy
- Low memory footprint (supports shared memory)
- Fast filtering
- Supports short and long reads



python Package Index

BIOCONDA
Reproducible research.
Install the software from bioconda:
> conda install -c bioconda cleanifier
See bioconda.github.io

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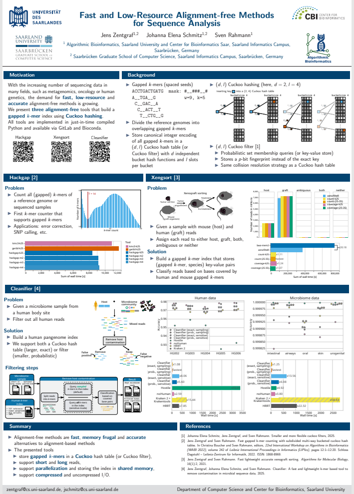

Johanna Elena Schmitz

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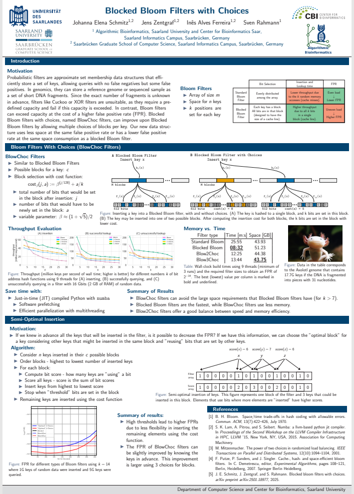

Inês Alves Ferreira

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Moein Karami