UNIVERSITÄT DES SAARLANDES

SAARLAND UNIVERSITY
SAARBRÜCKEN GRADUATE SCHOOL OF COMPUTER SCIENCE

CBI CENTER FOR BIOINFORMATICS

Algorithmic Bioinformatics

Xengsort2
Ultrafast accurate xenograft sorting

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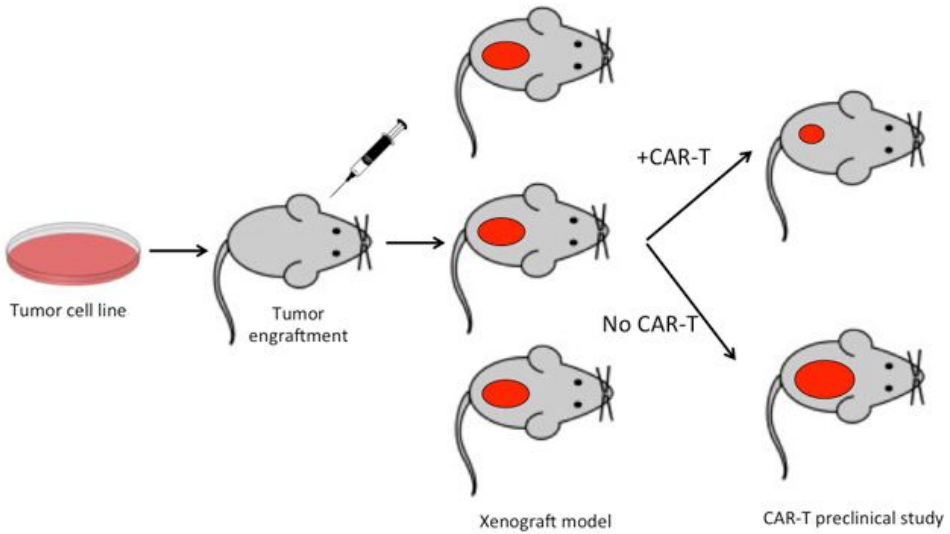
https://gitlab.com/genomeinformatics/xengsort

Xenograft data



- tumor cell lines
  or patient tumor samples
  implanted in mice
- study tumor heterogeneity, evolution
- sequencing of samples
- mixture of human+mouse DNA
- First task: separate/sort reads ("xenograft sorting"), or: extract graft (human) reads
- Problem: Human and mouse genomes are similar

Source: Creative AniModel,
https://www.creative-animodel.com/Featured-Service/Human-Tumor-Xenograft-Model.html

Xenograft sorting

- **Given**: Xenograft sample (Mixed reads from two species, host and graft)
- Sort reads into five categories: host, graft, both, neither, ambiguous

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Alignment-based approach

- xenofilteR
- align each read against host and graft reference
- classify each read based on alignment %identity

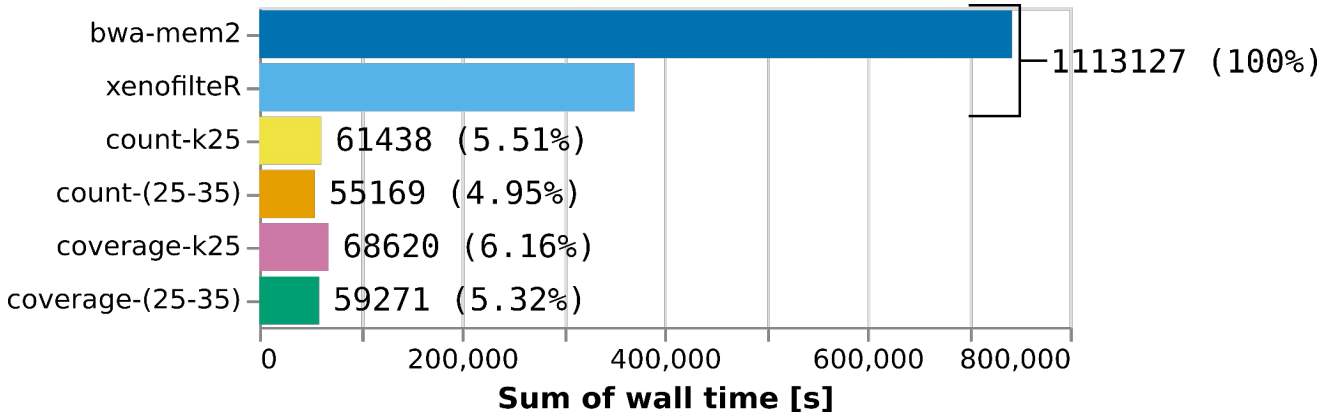
Alignment-free approach

- xengsort / xengsort2
- split each read into *k*-mers
- check how many *k*-mers belong to each species
- classify each read based on membership of *k*-mers to host and graft

Classification speed: Patient-derived xenograft RNA-seq data

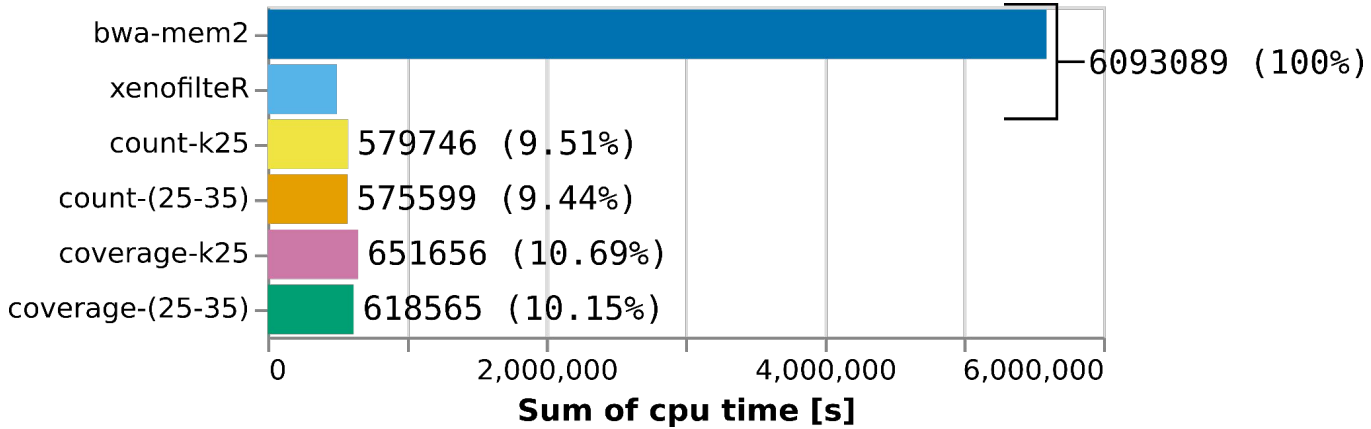
Classification time
(wall clock seconds)

bwa-mem: 8 threads
xenofilteR: 1 thread
xengsort: 8 threads



Classification time
(CPU seconds)

Evaluation on a "good gaming PC":
(AMD Ryzen 9 5950X,
16 cores / 32 threads,
128 GB DDR4 RAM,
16 TB HDD)



xengsort 2 improves many aspects of xengsort

Version 1

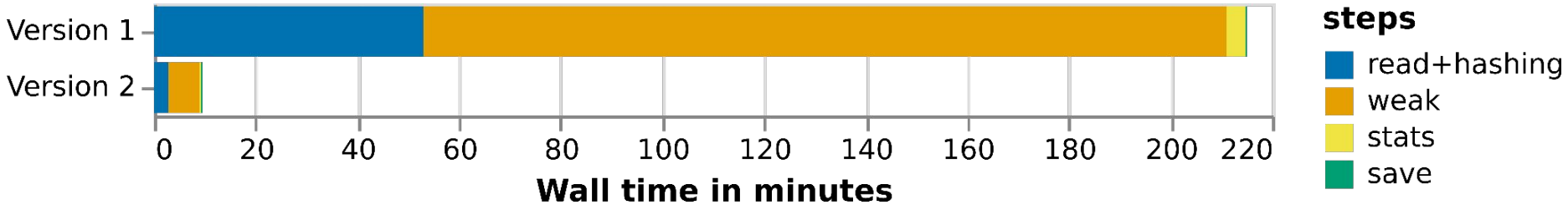
- **contiguous *k*-mers** only
- index: huge single table, **single-threaded** indexing
- identification of weak k-mers
- classification: **count-based** only
- **hard-coded** thresholds, optimized for **short reads** (100bp)
- compressed input (via shell), **uncompressed** FASTQ output
- 1000s of samples: each run loads hash table into memory **again**

Version 2

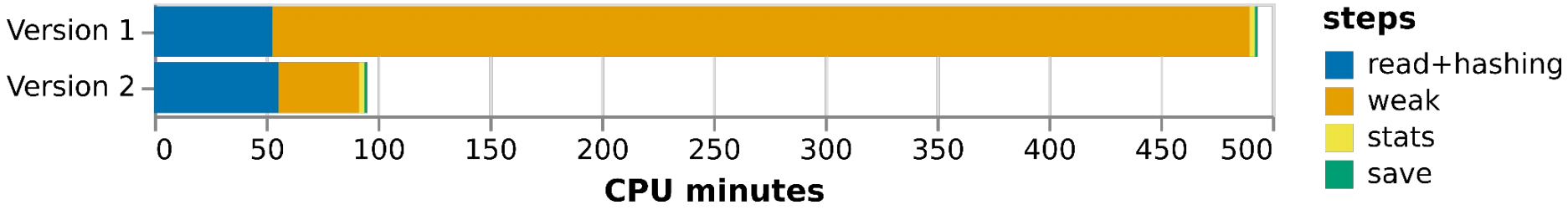
- contiguous and **gapped *k*-mers**
- index: many **subtables**, faster **parallelized** indexing
- **fast** identification of weak k-mers
- classification: count-based or **coverage-based**
- **config files** for different settings, support for **short and long reads**
- **compressed in- and output**, different formats (.gz, .bz2, .xz)
- 1000s of samples: load hash table **once** into **shared memory**

Speed: Index building (v1 vs. v2)

Indexing time (wall clock minutes)
with 15 subtables (v2)

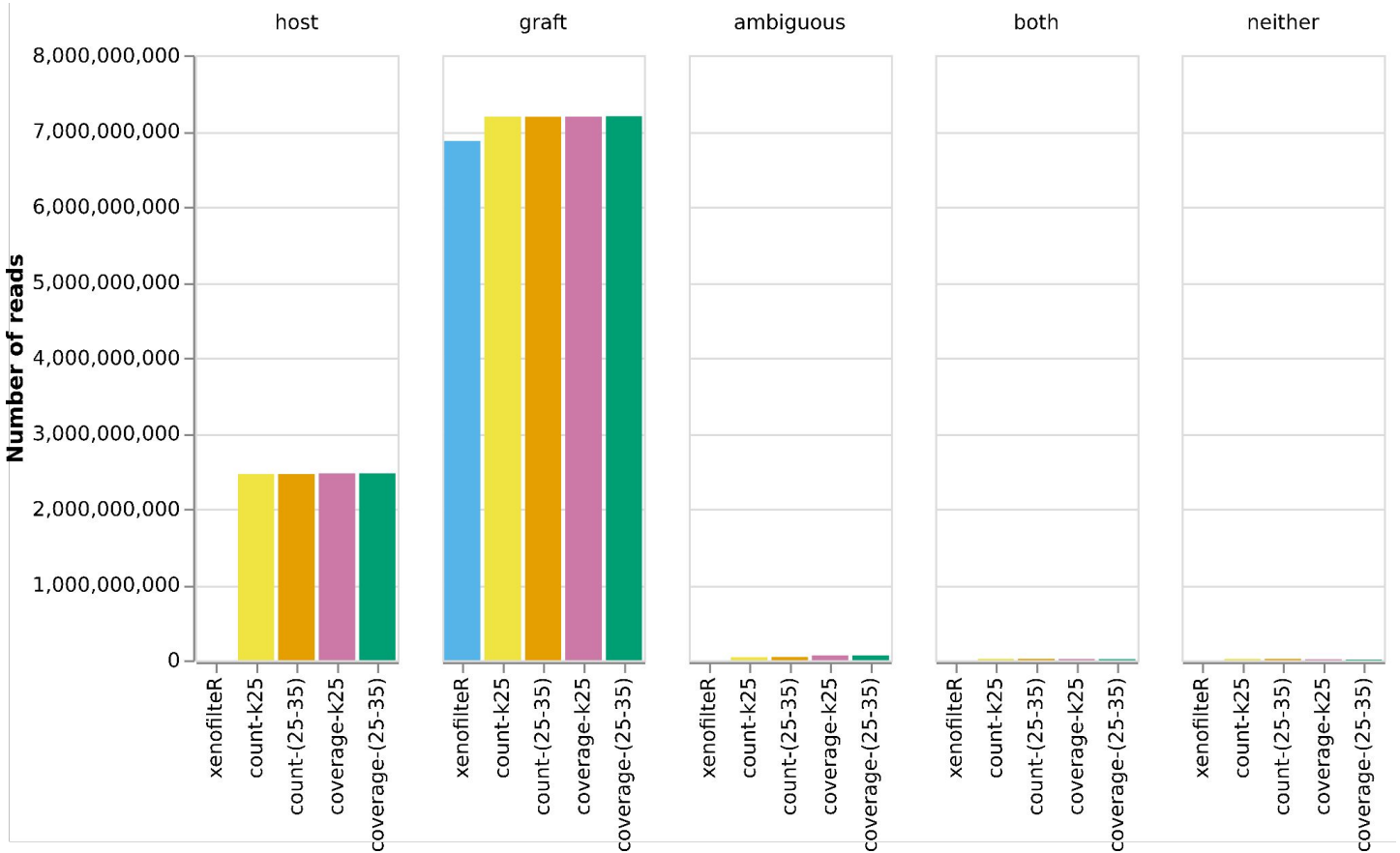


Indexing time (CPU minutes)



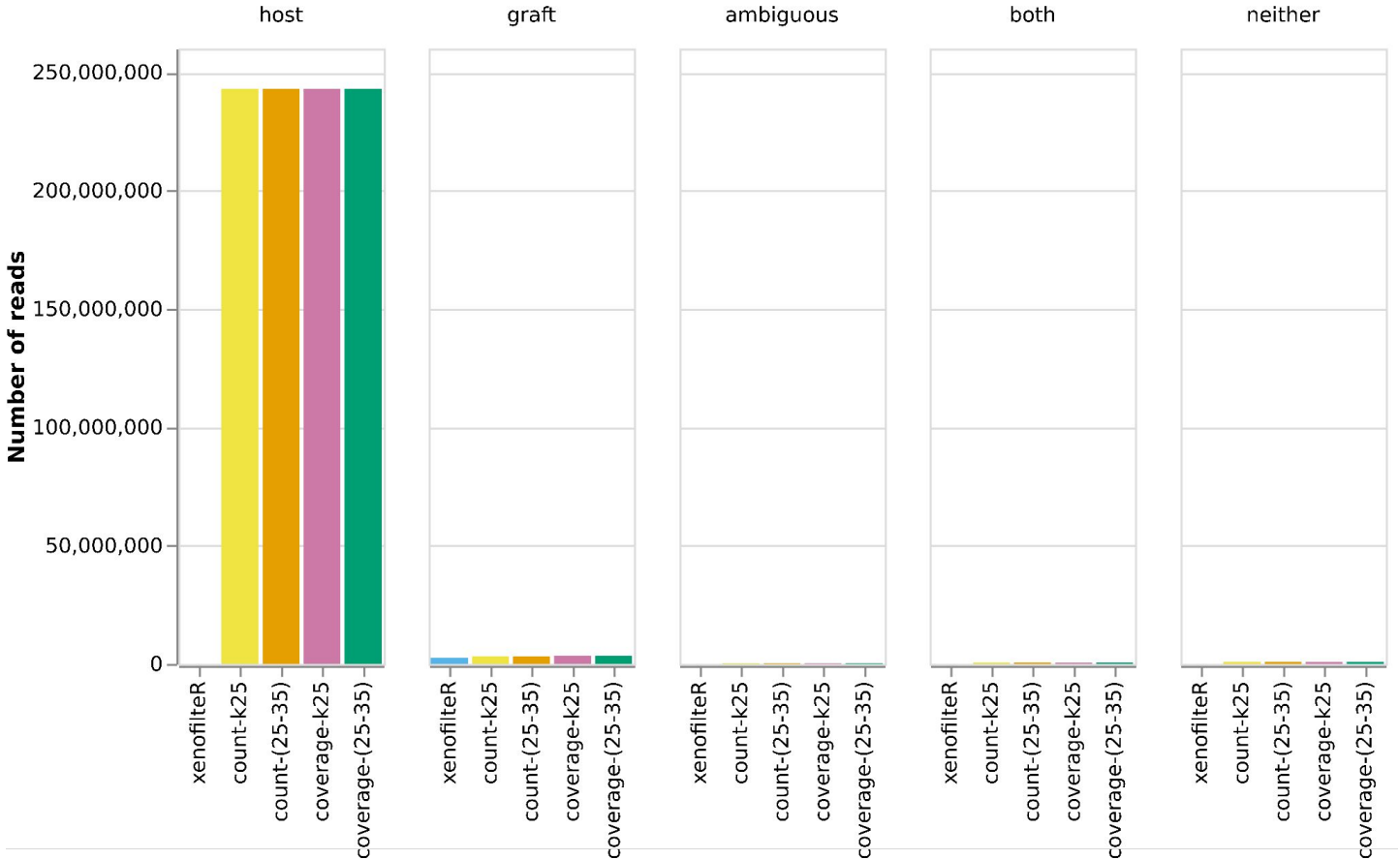
Accuracy: Patient-derived xenografts (graft, some host)

- 174 samples
- RNA-seq
- xenografts of pancreatic tumors
- total: 872 GB .fastq.gz
- almost 10 billion reads



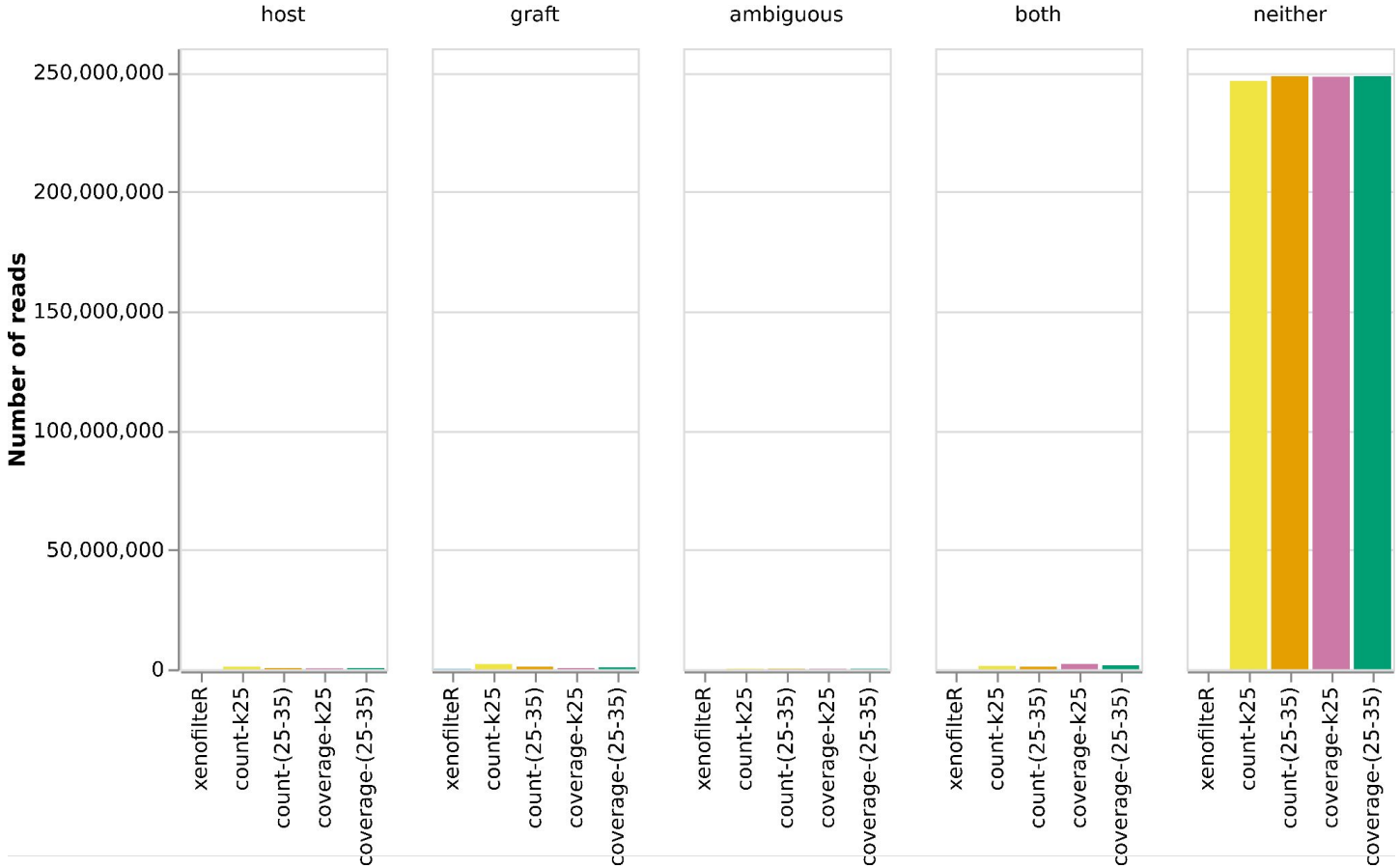
Accuracy: Human-captured mouse exomes (all host)

- Mouse exomes
- Human exome capture kit
- 4 samples
- paired-end reads
- total 35GB .fastq.gz



Accuracy: Chicken (all neither)

- 4 samples
- paired-end
- total 47 GB .fastq.gz



Selected method highlights

- gapped *k*-mers
- index: multi-way bucketed Cuckoo hash table, parallelized with subtables
- fast identification of weak k-mers
- classification: count-based or coverage-based
- config files supporting short and long reads
- compressed in- and output, different formats (.gz, .bz2, .xz)
- use shared memory for classification

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Contiguous *k*-mers

- Single parameter *k*
- Easy to handle
- One error changes
  k **consecutive** *k*-mers

```
###
TACAGATATA
TAC GAT
 ACA ATA
  CAG TAT
   AGA ATA
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###
TACAGATATA
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  CAG TAT
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```

(*k*=3, *w*=5, symmetric)

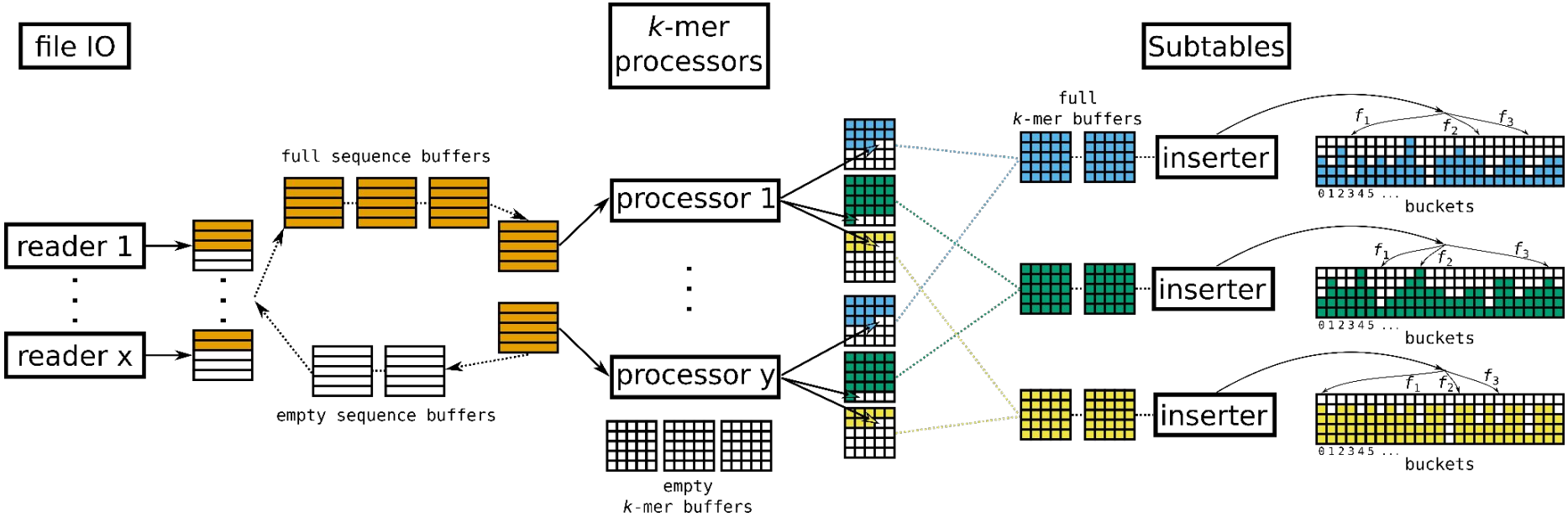
```
#_#_#
TACAGATATA
T_C_G
 A_A_A
  C_G_T
   A_A_A
    G_T_T
     A_A_A
```

Gapped *k*-mers

- Window size *w*
- Significant positions: *k* (#)
- Ignored gap positions: *w* - *k* (_)
- Mask or tuple of offsets
  - `Mask: #_#_#`
  - `Tuple: (0,2,4)`
- We use symmetric masks only.
- One error changes
  k **distributed** *k*-mers.
- This can lead to better
  error tolerance for the same *k*.

Parallelization with subtables

- Parallel file IO
- Producer: ***k*-mer Processor**
  - Extract *k*-mers of reads
  - Distribute *k*-mers to subtable
- Consumer: **inserter**
  - One inserter per subtable
  - Insert all *k*-mers in a subtable
  - Random walk



Xenograft classify count based

TTCAGAACAGGTTCTA…

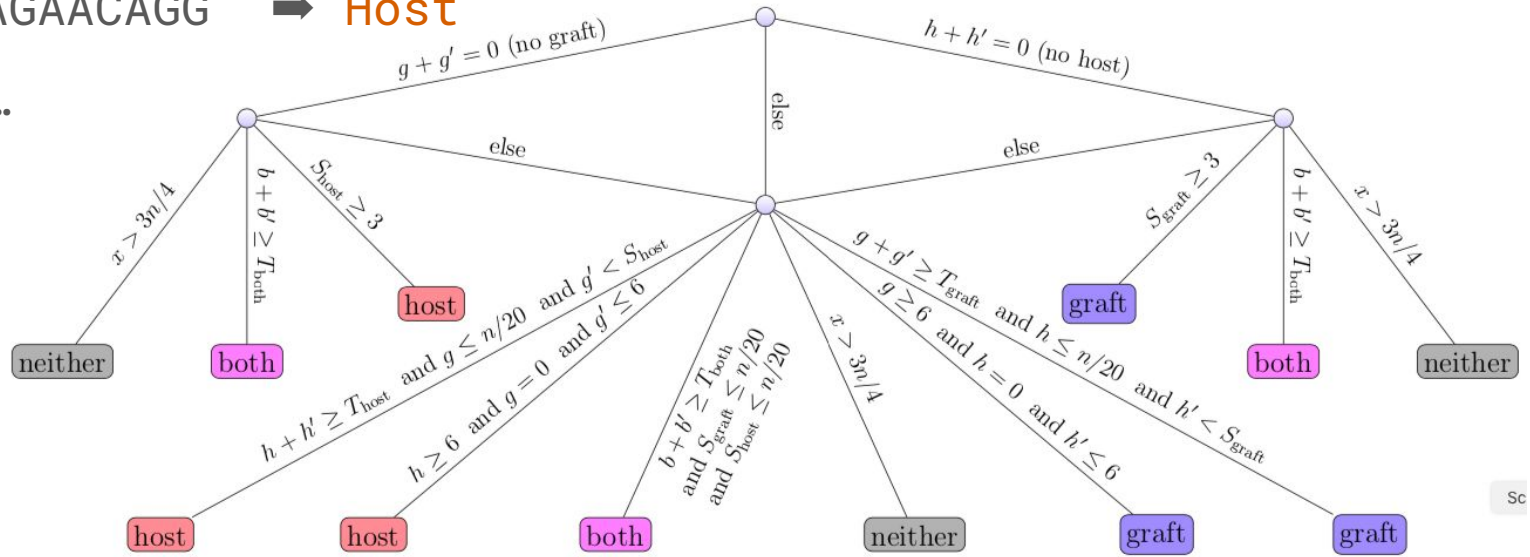
TTCAGAACA ➡ Host

TCAGAACAG ➡ Host

CAGAACAGG ➡ Host

…

host	weak host	graft	weak graft	both	neither
67	3	0	0	1	0



Disadvantage count based

TTCAGAACAGGTTCTACTACTGTCAAATGACCCCCCATACTTCCTCAAAGGCTGTGGTAAGTTTTGCACAGGTGAGGGCAGCAGAAAGGGGGTAGTTAC

TTCAGAACAGGTTCTACTACTGTCA

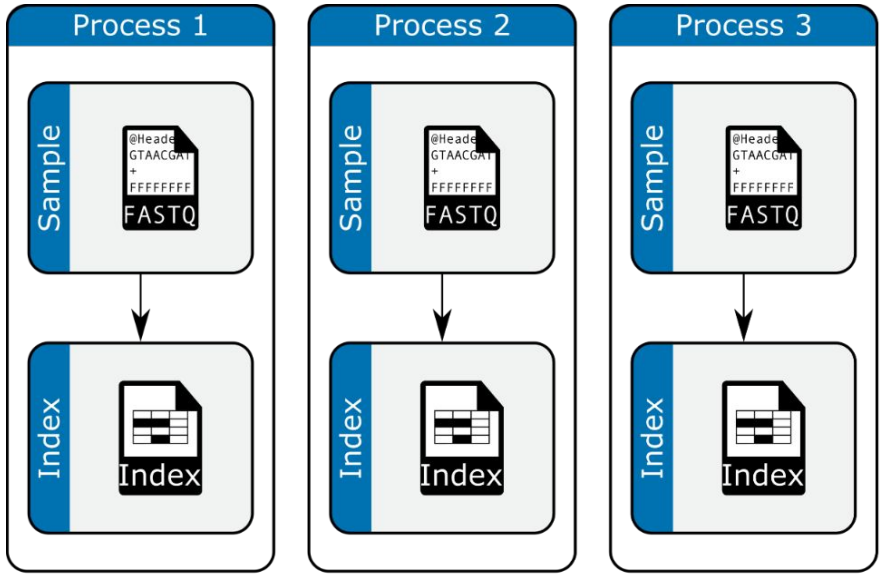
TCAGAACAGGTTCTACTACTGTCAA

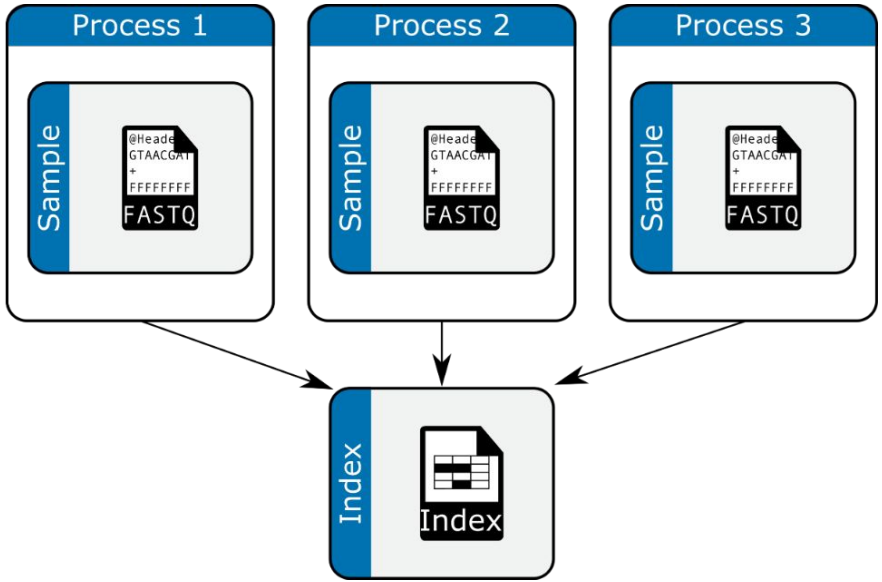
CAGAACAGGTTCTACTACTGTCAAA

TTCAGAACAGGTTCTACTACTGTCAATGACCCCCCATACTTCCTCAAAGGCTGTGGTAAGTTTTGCACAGGTGAGGGCAGCAGAAAGGGGGTAGTTAC

TTCAGAACAGGTTCTACTACTGTCA TACTTCCTCAAAGGCTGTGGTAAGT AGGGCAGCAGAAAGGGGGTAGTTAC

Shared memory





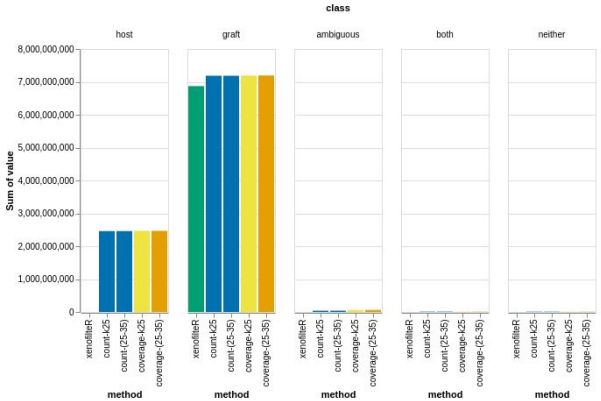
Summary

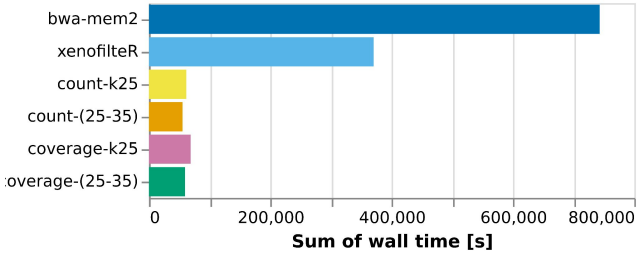
- **xengsort2**: ultrafast alignment-free xenograft sorting
- comparable classification results like alignment-based methods
- much faster (parallel index building even faster in v2)
- classification based on *k*-mer counts or covered bp
- support for parameter config files
- support for compressed input and output

Open PhD/postdoc position
Talk to me or to Sven Rahmann

python™ Package Index

BIOCONDA





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