Fast lightweight accurate xenograft sorting

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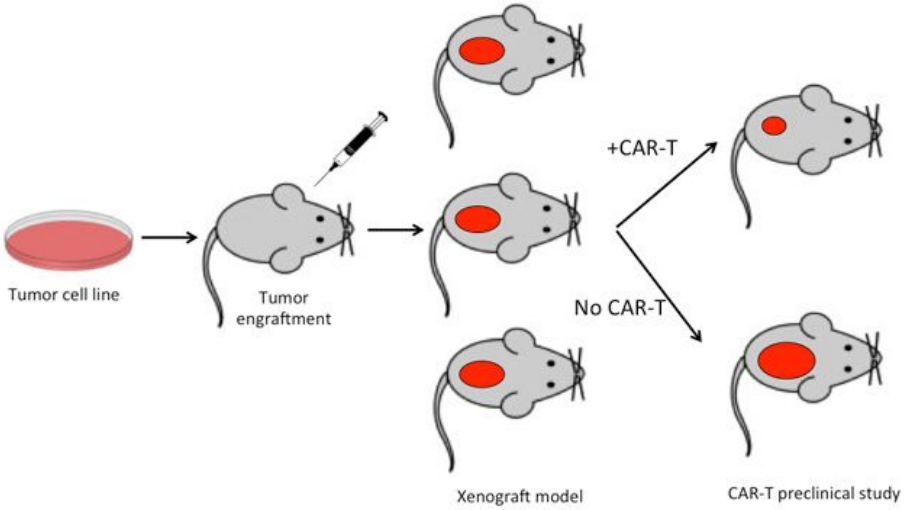
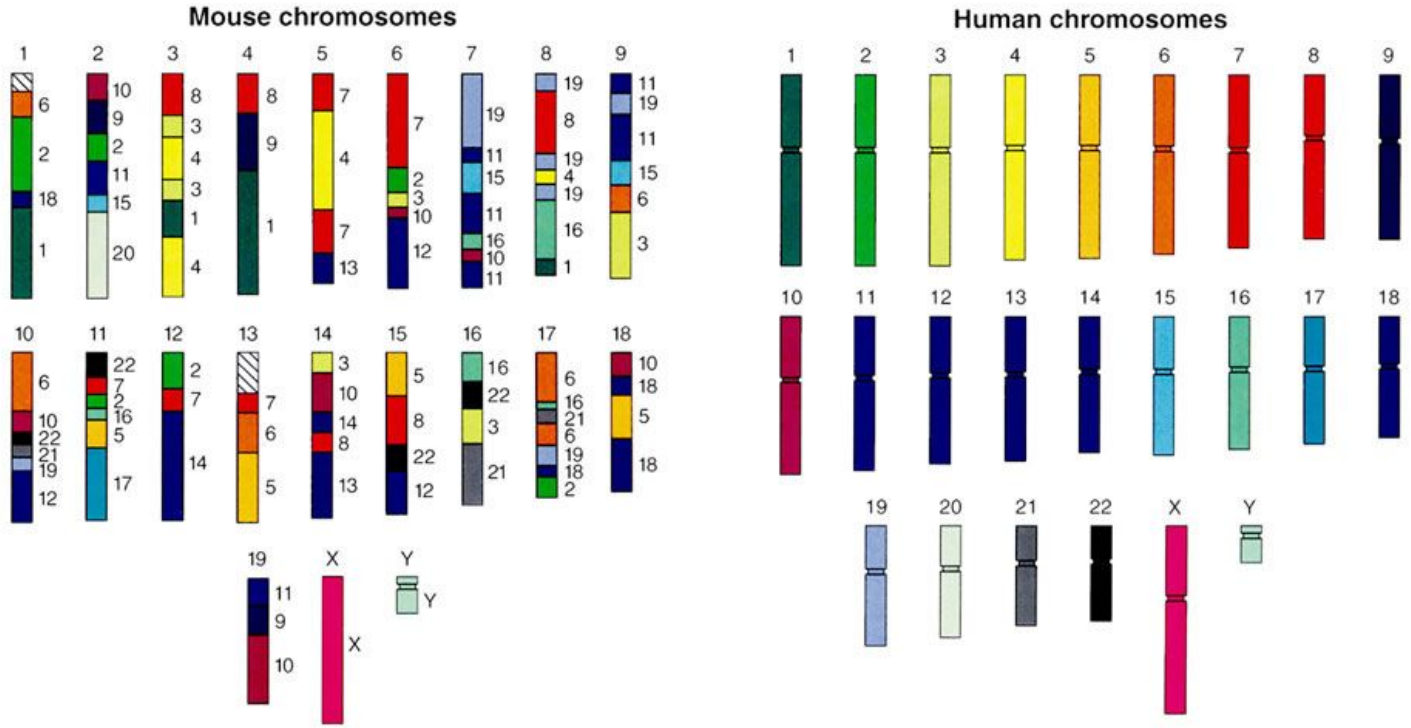
(Patient-derived) xenografts



- 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

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

Mouse and Human Genetic Similarities



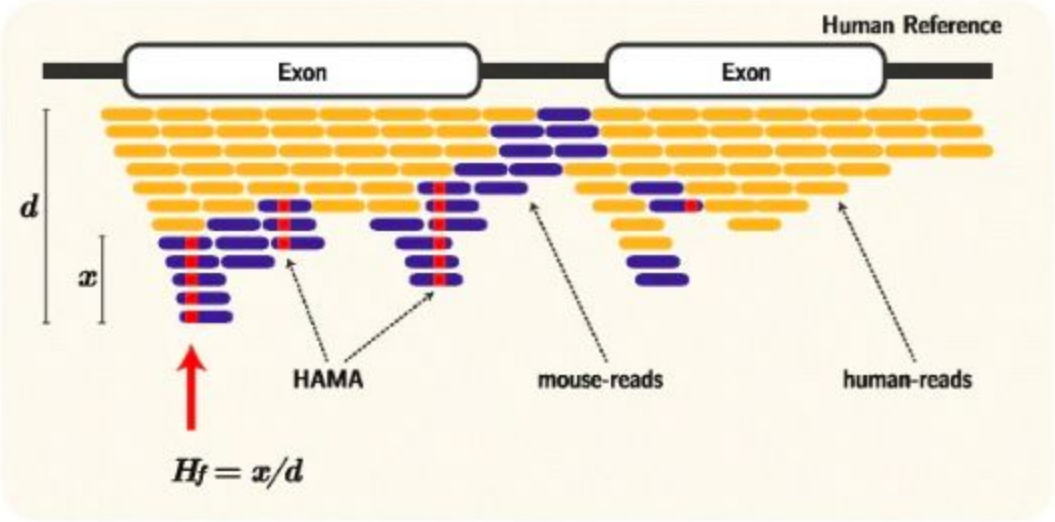
Source: https://public.ornl.gov/site/gallery/originals/Mouse_and_Human_Genetic_Similarities_-_original.jpg

Courtesy Lisa Stubbs
Oak Ridge National Laboratory

YGA 98-075R2

Problem: Human-Aligned Mouse Alleles (HAMAs)

- mouse reads may align to human genome
- may lead to false human (tumor) variant calls
- oncogenes particularly prone to this effect



S. Y. Jo, E. Kim, and S. Kim.
Impact of mouse contamination in genomic profiling of patient-derived models and best practice for robust analysis.
Genome Biology, 20(1):Article 231, Nov 2019.

The xenograft sorting problem

Given: sequenced xenograft sample (reads from two species),
paired-end or single-end,
genomic or transcriptomic reads,

sort the reads into five categories according to species of origin:
host (mouse), graft (human), both, neither, ambiguous

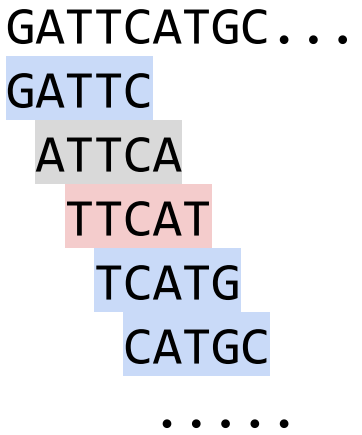
or: partially sort using fewer categories (host, graft, other),
or: count how many reads are in each category,
or: filter (select) only graft (human) reads.

Two kinds of tools: aligned BAM vs. raw FASTQ input

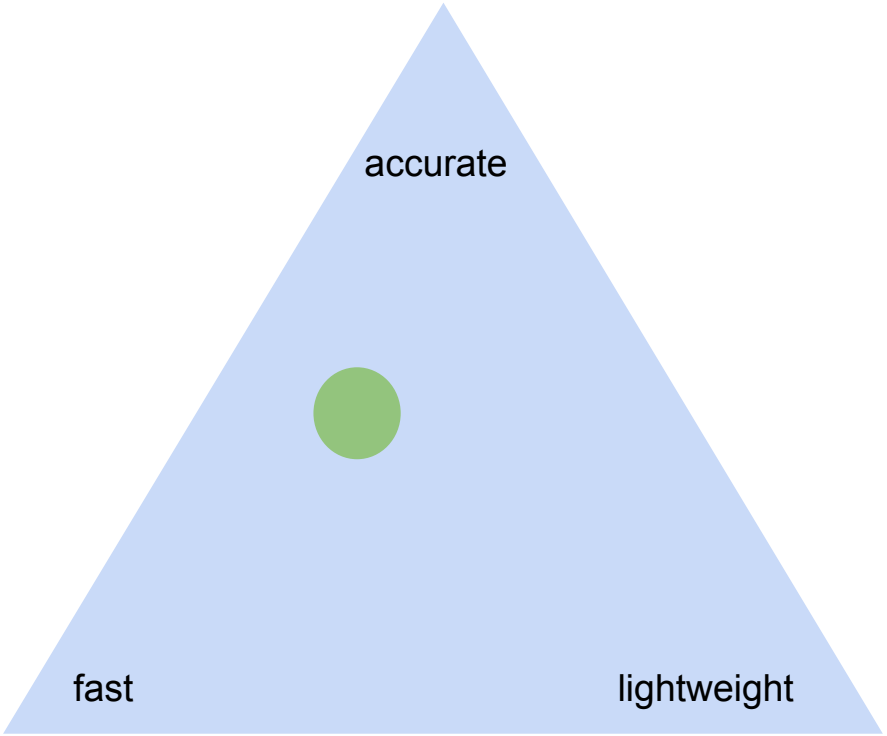
Tool	Input	Operations	Lang.
XenofilteR	aligned BAM	filter	R
Xenosplit	aligned BAM	filter, count	Python
Bamcmp	aligned BAM	partial sort	C++
Disambiguate	aligned BAM	partial sort	Python or C++
BBsplit	raw FASTQ	partial sort	Java
xenome	raw FASTQ	count, sort	C++
xengsort	raw FASTQ	count, sort	Python

k-mer methods for xenograft sorting

- Partition each read into its *k*-mers
- Look up information on each *k*-mer in a table [*k*-mer ↦ human | mouse | both]
- Absent k-mers occur in neither species.
- Aggregate *k*-mer information into a statement about the read [e.g., majority vote]



Goal: "Fast lightweight accurate xenograft sorting"



fast:

- slow random memory accesses
- 3-way bucketed Cuckoo hashing
- buckets fit within a cache line

lightweight (small memory footprint):

- 4.5 billion 25-mers + values
- high load (little wasted space)
- quotienting

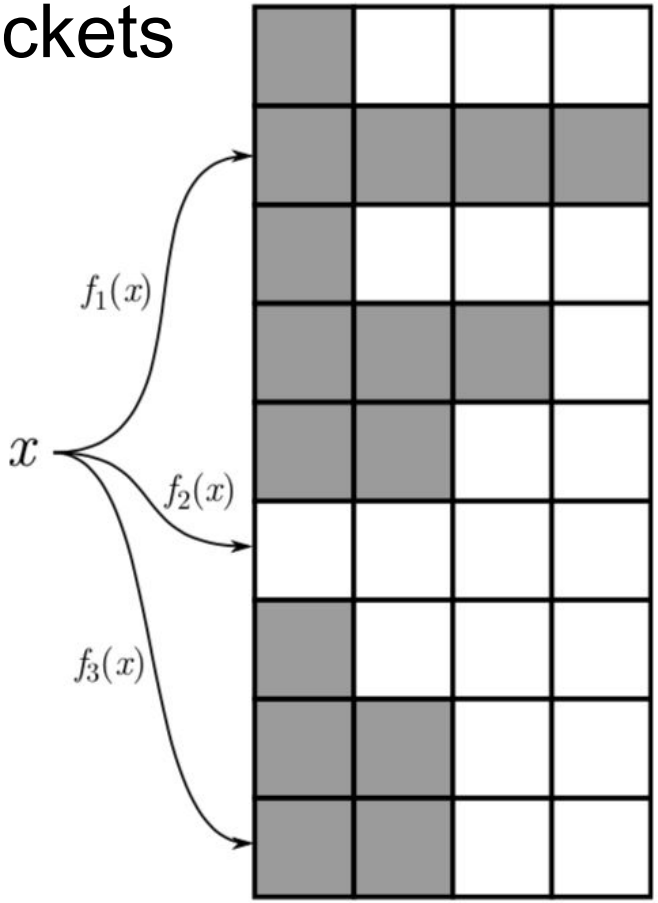
accurate:

- identical + highly similar sequences
- "weak" *k*-mers
- multi-level decision rule

3-way Cuckoo hashing with 4-buckets

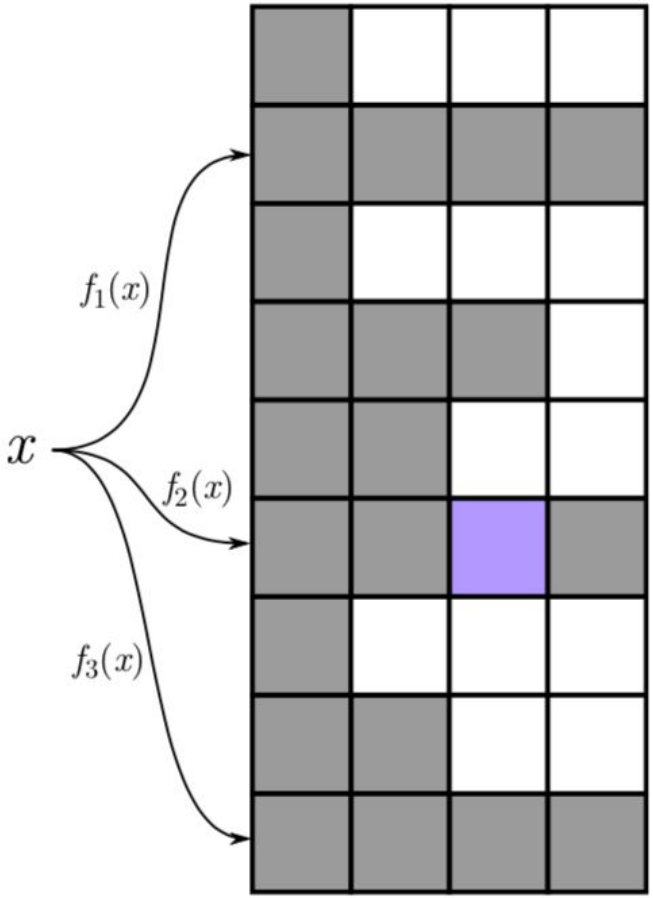
- 3 hash functions:
- each maps a *k*-mer to a bucket.
- Each bucket can store up to 4 elements.
- Idea: bucket fits within a cache line.

- 12 possible locations for each element.
- At worst 3 memory lookups (cache misses), often only 1 or 2.



Insertion by random walk

- Insert x: try buckets $f_1(x)$, $f_2(x)$, $f_3(x)$ in order; insert into first bucket with space available.
- If all full, evict a random element, place current element into now free slot.
- Re-insert evicted element into different slot.
- May cause another eviction…
  ⇒ random walk through table.
- Limit length of walk (e.g. 500 steps). Fail if limit reached.



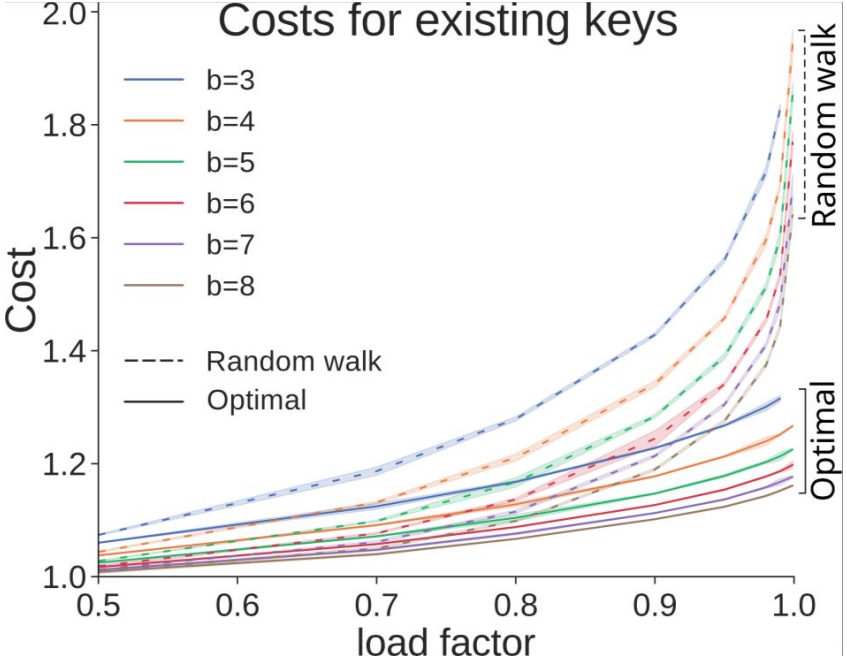
Speed vs. space: High vs. very high loads

(*h*,*b*) = (3,4) allows loads up to 99.9%.

Lower loads offer better choice distribution:
more elements at their first choice;
lower average cost (cache misses).

Placement can be optimized exactly
(Zentgraf et al., ALENEX 2020).

Random walk degrades near 100%.
At 88%, random walk performs ok.



Weak *k*-mers

Host or graft *k*-mers with a close neighbor (Hamming distance 1)
in the other species are not as reliable ("**weak**"):
A single nucleotide variation suffices to switch species.

After building the hash table, we mark weak *k*-mers.

Value set of size 5: host, weak host, graft, weak graft, both.
Each *k*-mer in the table has exactly one of these values (3 bits).

Fast method to find the Hamming-1 neighbors of each *k*-mer (see paper).

Xenome: similar concept with 4 values: host, graft, both, marginal.

Saving space with quotienting

Keys: canonical codes of 25-mers (50 bits)
Values: species (5 classes: 3 bits)

4.5 billion k-mers: reference genomes, alternative alleles, cDNA transcripts:
53 bits per entry, load 0.88: **33.88 GB** for hash table 😩

Quotienting to the rescue:

- Do not store full keys (k-mers), but only "quotients" (here 20 bits),
  plus hash function choice (2 bits) plus values (3 bits) → 25 bits per entry:

15.98 GB for hash table 😀
(could be slightly reduced by higher load, value compression, etc.)

Quotienting: Details

Keys are encoded canonical k-mers (half of set $[4^k] := \{0, .., 4^k\text{-}1\}$).

Step 1: Bijective randomizing function $[4^k] \to [4^k]$ with a odd

$$g_{a,b}(x) := [a \cdot (\mathrm{rot}_k(x) \text{ xor } b)] \bmod 4^k$$

Step 2: Map to buckets (simply mod p: number of buckets). Define

$$f(x) := g_{a,b}(x) \bmod p \quad \text{and} \quad q(x) := g_{a,b}(x) \mathbin{//} p \ .$$

Then x can be uniquely reconstructed
from $f(x)$ ("hash value, "bucket number") and $q(x)$ ("fingerprint", "quotient").
Sufficient to store $q(x)$ in bucket $f(x)$ (and which hash function was chosen).

Build time [min] & space [GB]

tool	k	build CPU	build wall	mark CPU	mark wall	total CPU	total wall	mem final	size peak
xengsort	23	50	50	591	176	641	226	12.8	17.3
xengsort	25	53	53	437	158	490	211	15.9	20.4
xengsort	27	51	51	495	214	546	265	17.3	21.8
xenome	25	992	151	2338	356	3626	552	31.2	57.1
XenofilteR	–	528	658	–	–	528	658	13.0	22.0

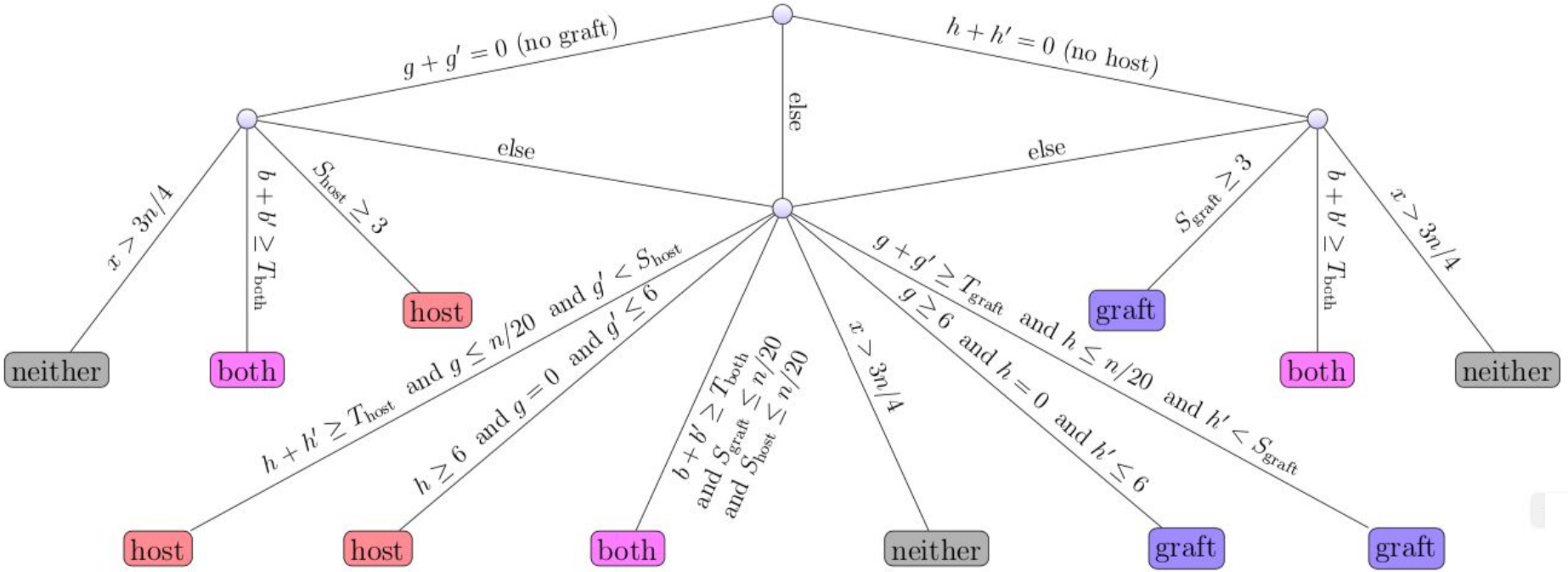
xengsort: 1 thread for build, 8 for mark
xenome: 8 (9) threads for build and mark
XenofilteR: 8 threads (bwa index)

Read classification

- Partition read into its *n* valid k-mers

- Look up class of each *k*-mer and count:
  - *h*, *h*': *k*-mers in read belonging to "host", "weak host"
  - *g*, *g*': *k*-mers in read belonging to "graft", "weak graft"
  - *b*: *k*-mers in read belonging to both species
  - *x*: *k*-mers in read belonging to neither species

Read classification using (*h*, *h*', *g*, *g*', *b*, *x*; *n*)



Quick mode heuristic

(inspired by a similar shortcut in kallisto)

- Examine 3rd and 3rd-last *k*-mer in read and look up classes.
- If classes agree, classify read accordingly.
- Otherwise, count all k-mers and use decision rule tree.

Results: Comparison of tools

Tool	Input	Operations	Lang.
XenofilteR	aligned BAM	filter	R
Xenosplit	aligned BAM	filter, count	Python
Bamcmp	aligned BAM	partial sort	C++
Disambiguate	aligned BAM	partial sort	Python or C++
BBsplit	raw FASTQ	partial sort	Java
xenome	raw FASTQ	count, sort	C++
xengsort	raw FASTQ	count, sort	Python

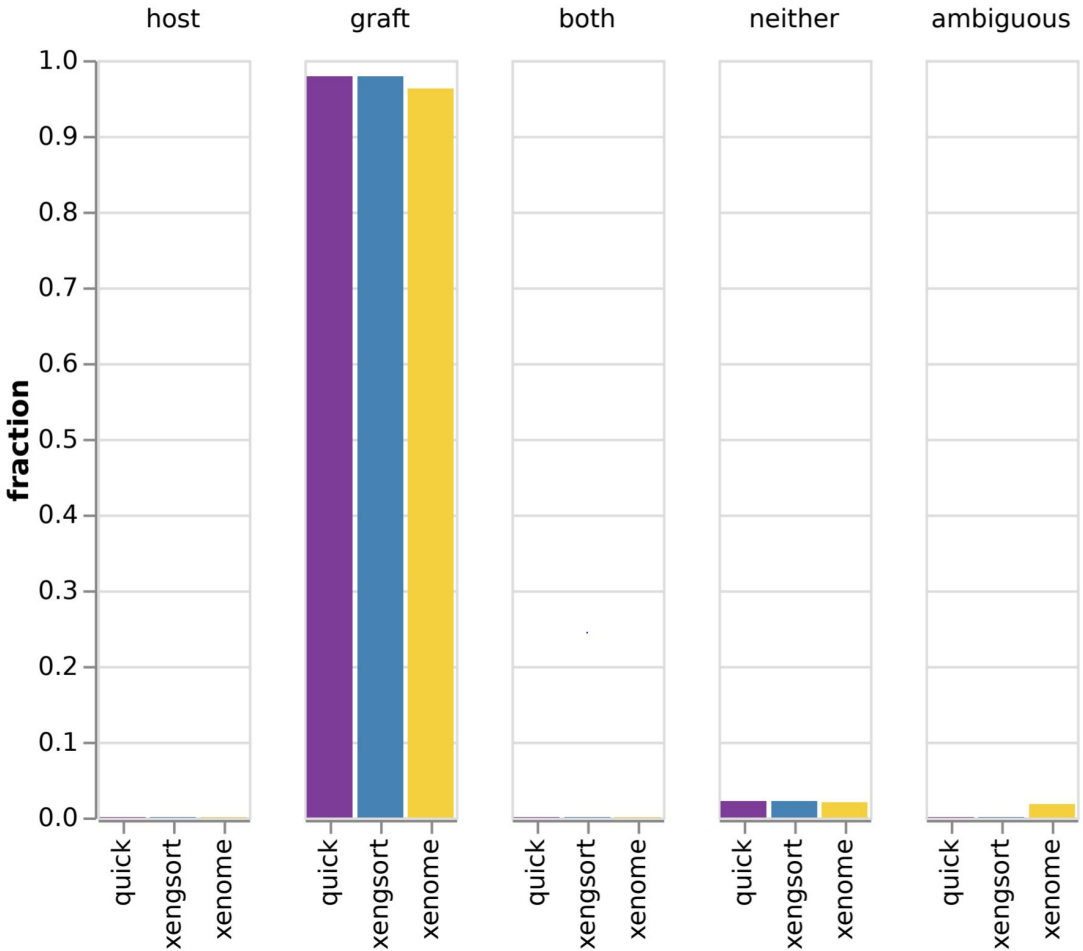
Human dataset

GIAB human matepair dataset (Ashkenazim trio; 1258 million read pairs).

Almost all graft (correct). "Neither" is mostly PhiX.

Quick mode gives almost identical results.

Xenome sometimes says "ambiguous".



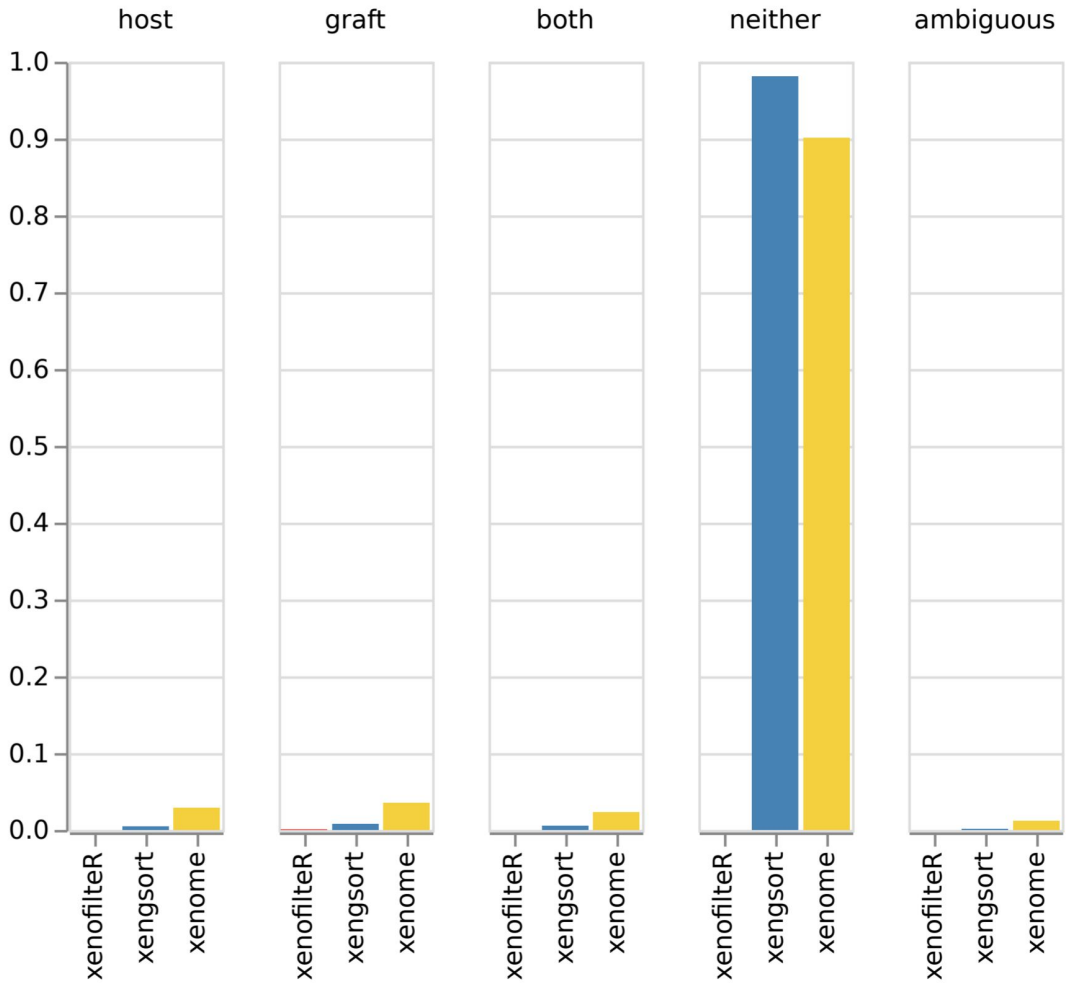
Chicken dataset

Illumina-sequenced chicken genome.

XenofilteR only extracts graft (human) reads, remainder not classified. Finds none (correct).

xengsort:
Almost all neither (correct).

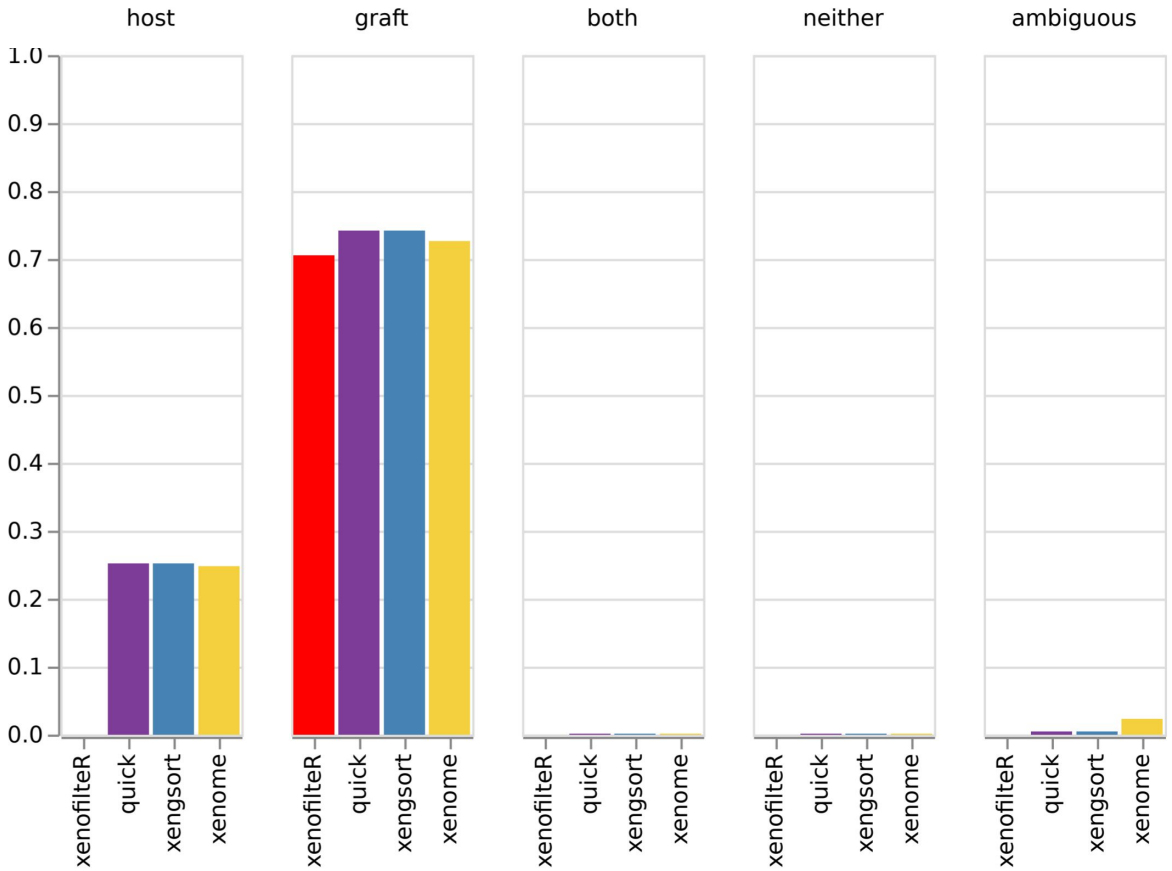
xenome: 10% host, graft, both (lower specificity).



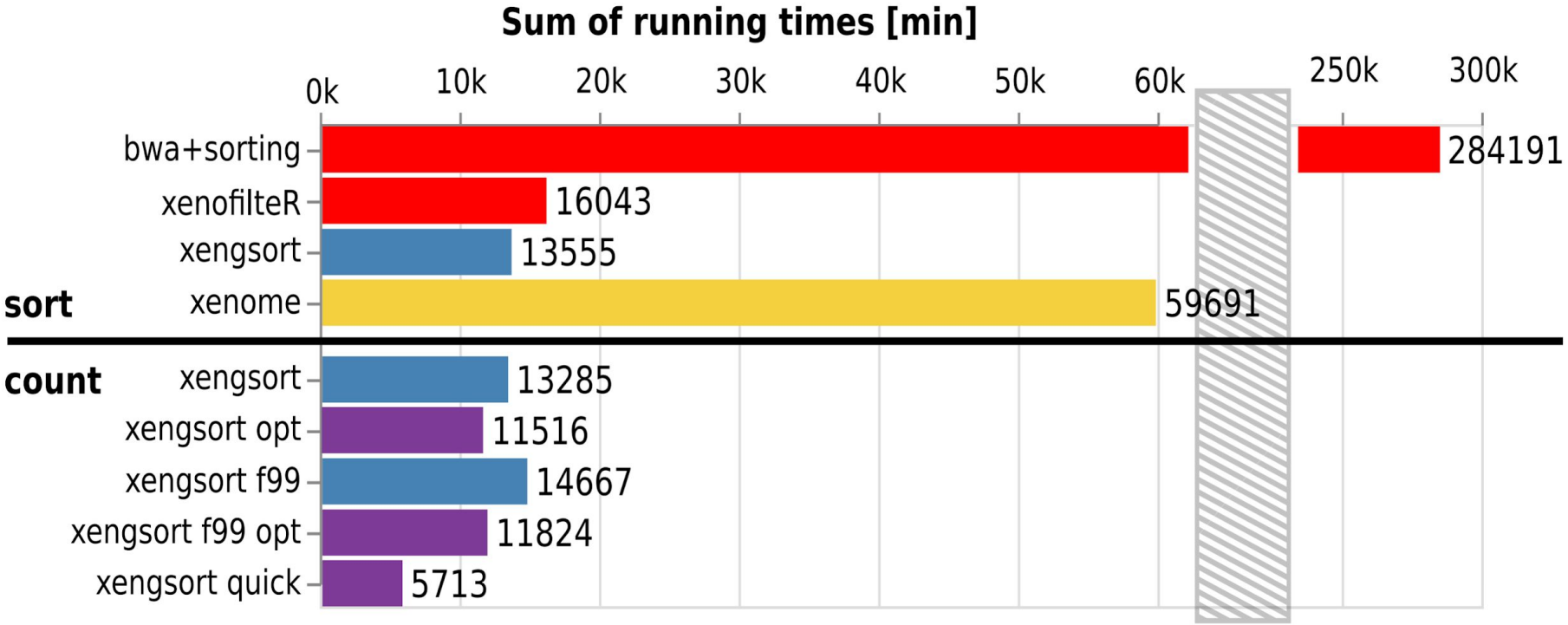
PDX dataset

174 RNA-seq PDX samples (human tumor in mouse) from Jens Siveke, University Hospital Essen.

XenofilteR only extracts graft (human) reads, remainder not classified.



174 PDX datasets: Running times [CPU minutes]



Summary: Fast lightweight xenograft sorting

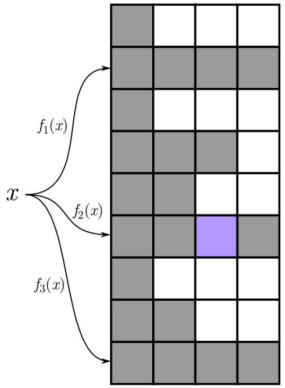
- alignment-free approach using 25-mers and decision rule
- lightweight on CPU resources, using 3-way bucketed Cuckoo hashing
- Implementation *xengsort* outperforms *xenome*;
  ⅙ of the CPU work, ⅓ of the wall clock time (both 8 threads)
- Typically it takes the same time just to scan the BAM files (*XenofilteR*)
- 25-mer table fits into 16 GB RAM, could be made smaller
  (higher load, compacted values and choice indicators).

- More times [CPU min]
  (see paper for datasets)

dataset / tool	size	XfR +	*bwa* +	sort	*xenome*	*xengsort*
mouse exomes	307 M	310 +	8291 +	179	1823	368
human matepair	1258 M	N/A +	222939 +	940	9845	2463
chicken genome	251 M	76 +	6976 +	118	1273	592
leukemia RNA	1760 M	778 +	22111 +	521	5188	1680
PDX RNA	9742 M	16043 +	278329 +	5862	59692	13555

Summary: Algorithm Engineering for xenograft sorting

- Hash table
- 3-way bucketed Cuckoo hashing
  (with bucket size 4)
- Keys reduced using quotienting
  (part of key stored in bucket number)
- Interesting trade offs:
  Small buckets = small quotients,
  but lower maximum load,
  and fewer keys at first hash choice.
- Several further engineering opportunities
- Find xengsort at gitlab: https://gitlab.com/genomeinformatics/xengsort/



$$g_{a,b}(x) := [a \cdot (\mathrm{rot}_k(x) \text{ xor } b)] \bmod 4^k$$

Appendix

Why k = 25 ?

k-mers	$k = 23$	(%)	$k = 25$	(%)	$k = 27$	(%)
total	4 396 323 491	(100)	4 496 607 845	(100)	4 576 953 994	(100)
host	1 924 087 512	(43.8)	2 050 845 757	(45.6)	2 105 520 461	(46.0)
graft	2 173 923 063	(49.4)	2 323 880 612	(51.7)	2 395 147 724	(52.3)
both	18 701 862	(0.4)	12 579 160	(0.3)	9 627 252	(0.2)
wk host	132 469 231	(3.0)	52 063 110	(1.2)	32 445 717	(0.7)
wk graft	147 141 823	(3.4)	57 239 206	(1.3)	34 212 840	(0.7)

Why (*h*,*b*) = (3,4) ?

More hash functions (*h*), larger buckets (*b*) have ⊕ and ⊖ effects:

⊕ higher load limit

[only 50% for standard (2,1)]
[over 99.9% for (3,4),
less w/ random walk]

⊖ more worst case cache misses (*h*)

⊖ more search effort per bucket (*b*)

- (3,4) is a good compromise; maybe also (2,8).

b | *h*	2	3
1	0.5	0.9179352767
2	0.8970118682	0.9882014140
3	0.9591542686	0.9972857393
4	0.9803697743	0.9992531564

S. Walzer. Load thresholds for cuckoo hashing with overlapping blocks. ICALP 2018, LIPIcs 107:102.