RNA-seq read mapping

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Enabler for Life Sciences

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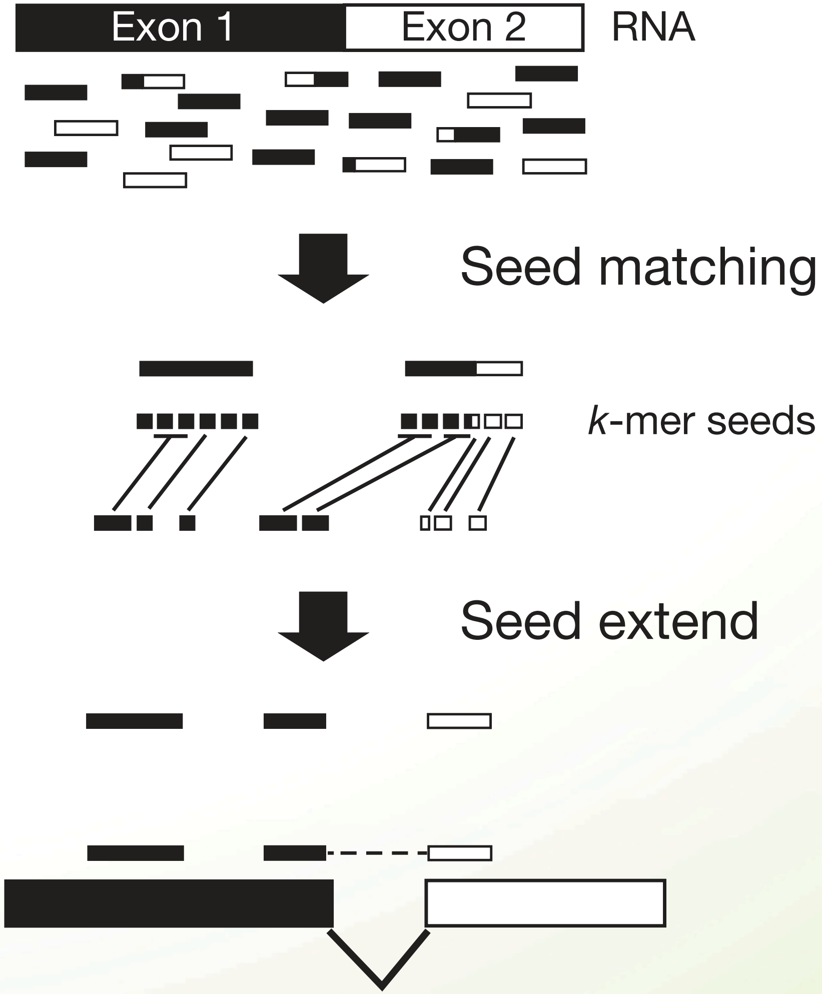
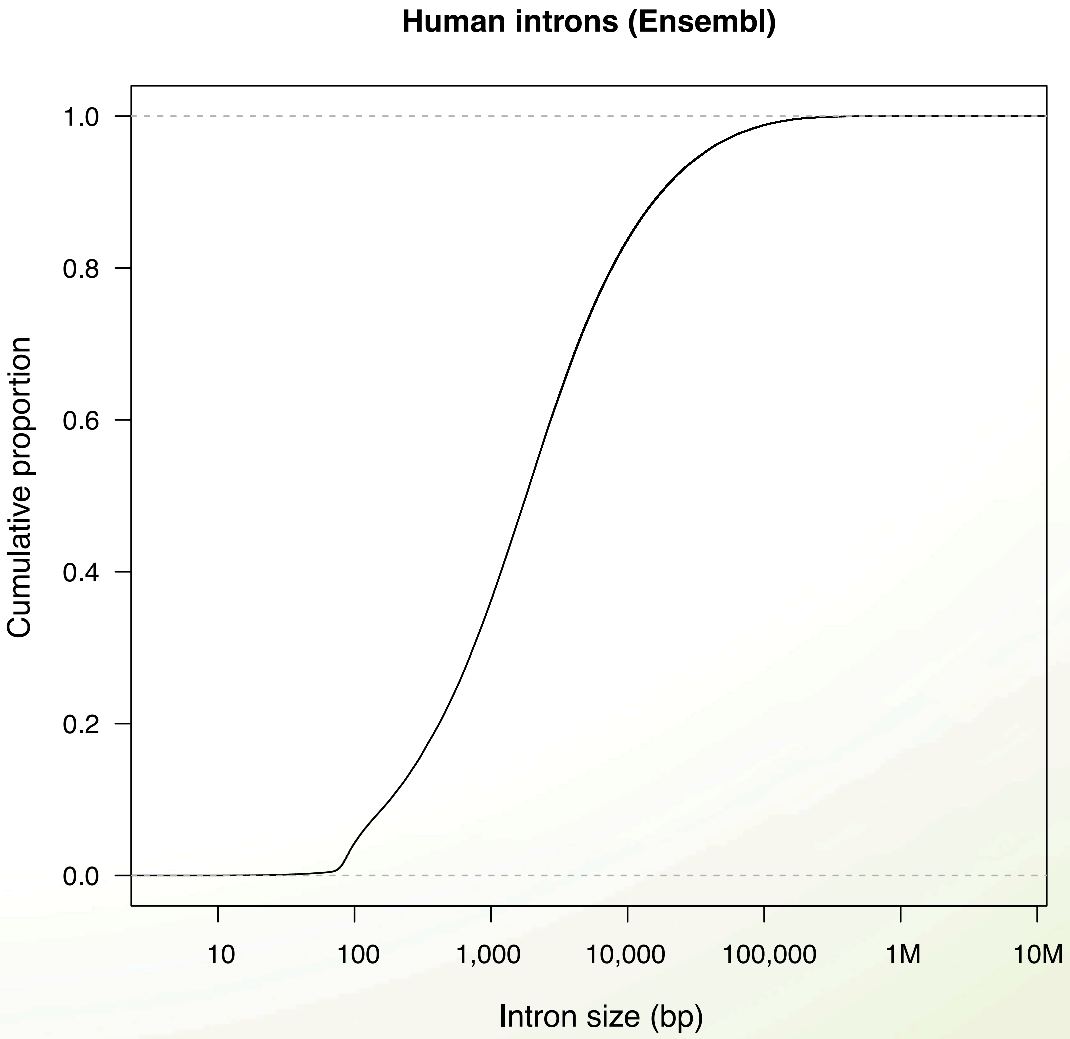
Spliced alignment

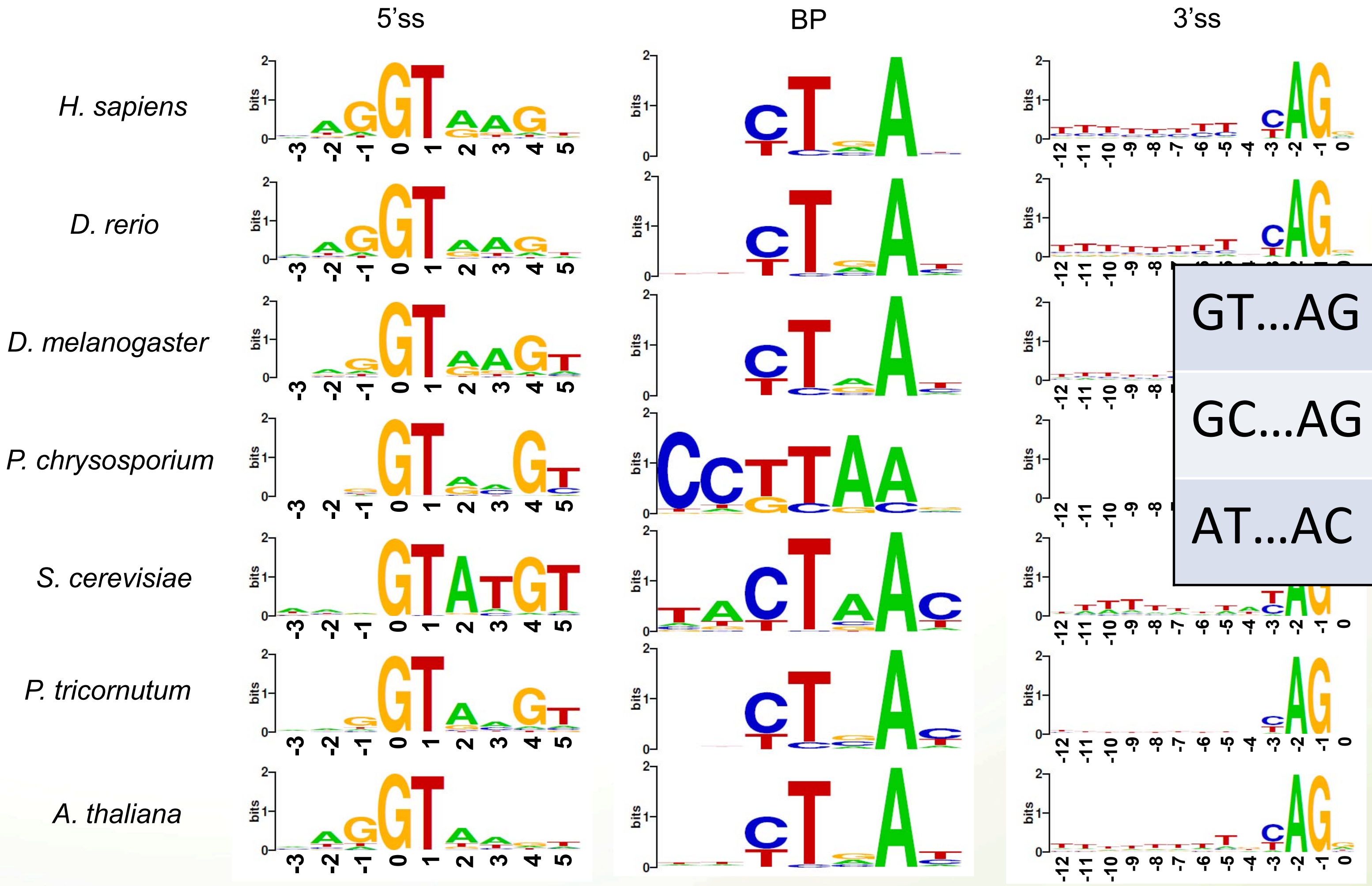


Garber et al. *Nature Methods* 2011

Introns can be very large!



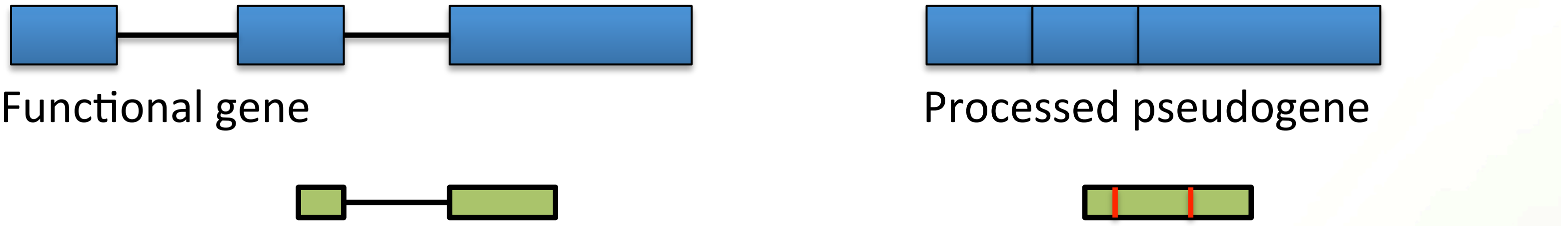
Limited sequence signals at splice sites



GT…AG	98.6%
GC…AG	1.3%
AT…AC	0.1%

Iwata and Gotoh *BMC Genomics* 2011

Multi-mapping reads and pseudogenes



Functional gene

Processed pseudogene

Correct read alignment
Identical, spliced

Incorrect read alignment
Mismatches, not spliced

Note:

- An aligner may report both alignments or either
- Some search strategies and scoring schemes give preference to unspliced alignments

Current RNA-seq aligners

TopHat2	Kim et al. *Genome Biology* 2013	
HISAT	Kim et al. *Nature Methods* 2015	
STAR	Dobin et al. *Bioinformatics* 2013	
GSNAP	Wu and Nacu *Bioinformatics* 2010	
OLego	Wu et al. *Nucleic Acids Research* 2013	
MapSplice2	http://www.netlab.uky.edu/p/bioinfo/MapSplice2	
HPG aligner	https://github.com/opencb/hpg-aligner	

The predecessor: BLAT

“In the process of assembling and annotating the human genome, I was faced with two very large-scale alignment problems: aligning three million ESTs and aligning 13 million mouse whole-genome random reads against the human genome. These alignments needed to be done in less than two weeks’ time on a moderate-sized (90 CPU) Linux cluster in order to have time to process an updated genome every month or two. To achieve this I developed a very-high-speed mRNA/DNA and translated protein alignment algorithm. “

(Kent *Genome Research* 2002)

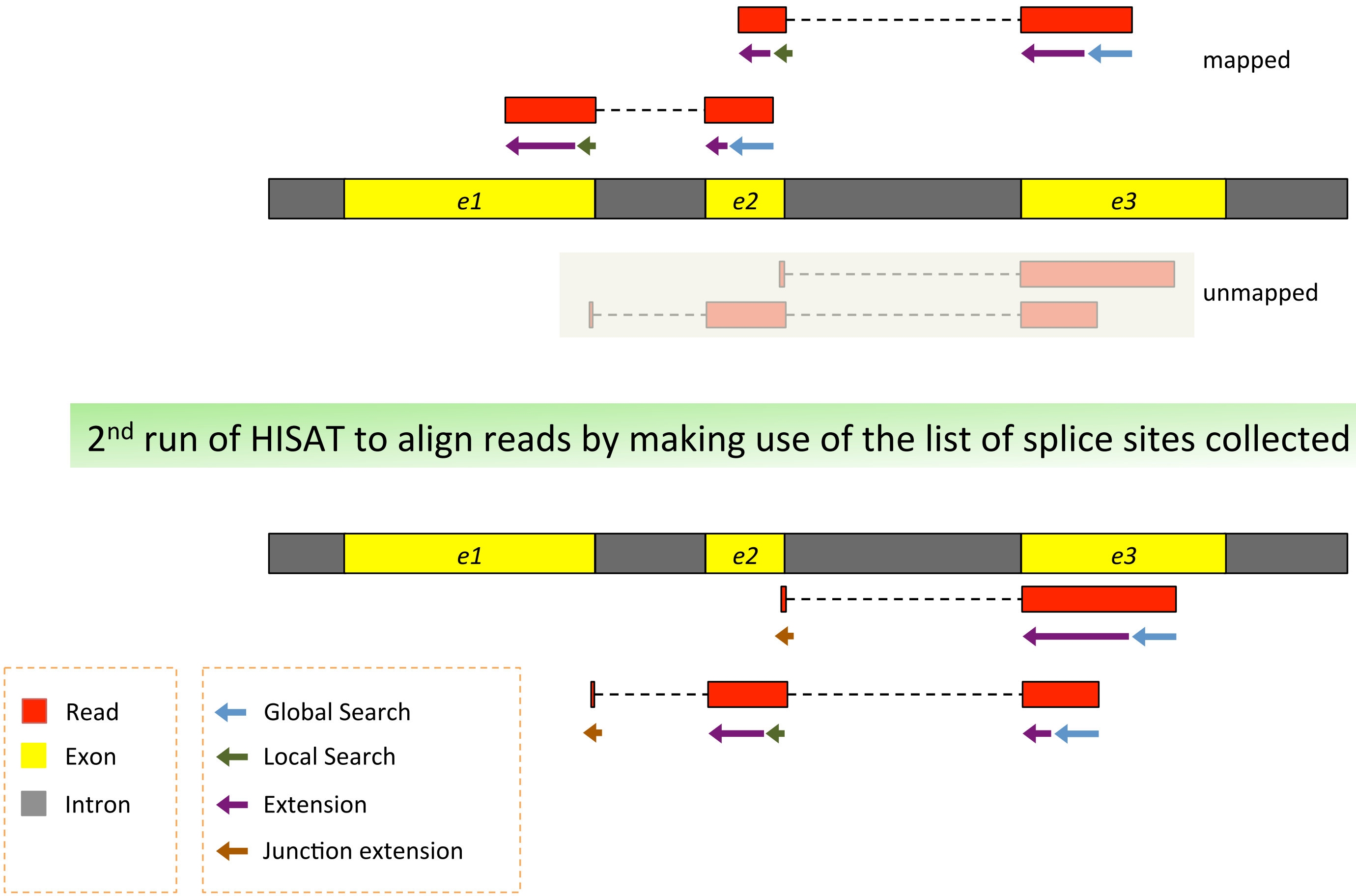
Innovations in RNA-seq alignment software

- Read pair alignment
- Consider base call quality scores
- Sophisticated indexing to decrease CPU and memory usage
- Resolve multi-mappers using regional read coverage
- Consider junction annotation
- Two-step approach (junction discovery & final alignment)

Two-step RNA-seq read mapping

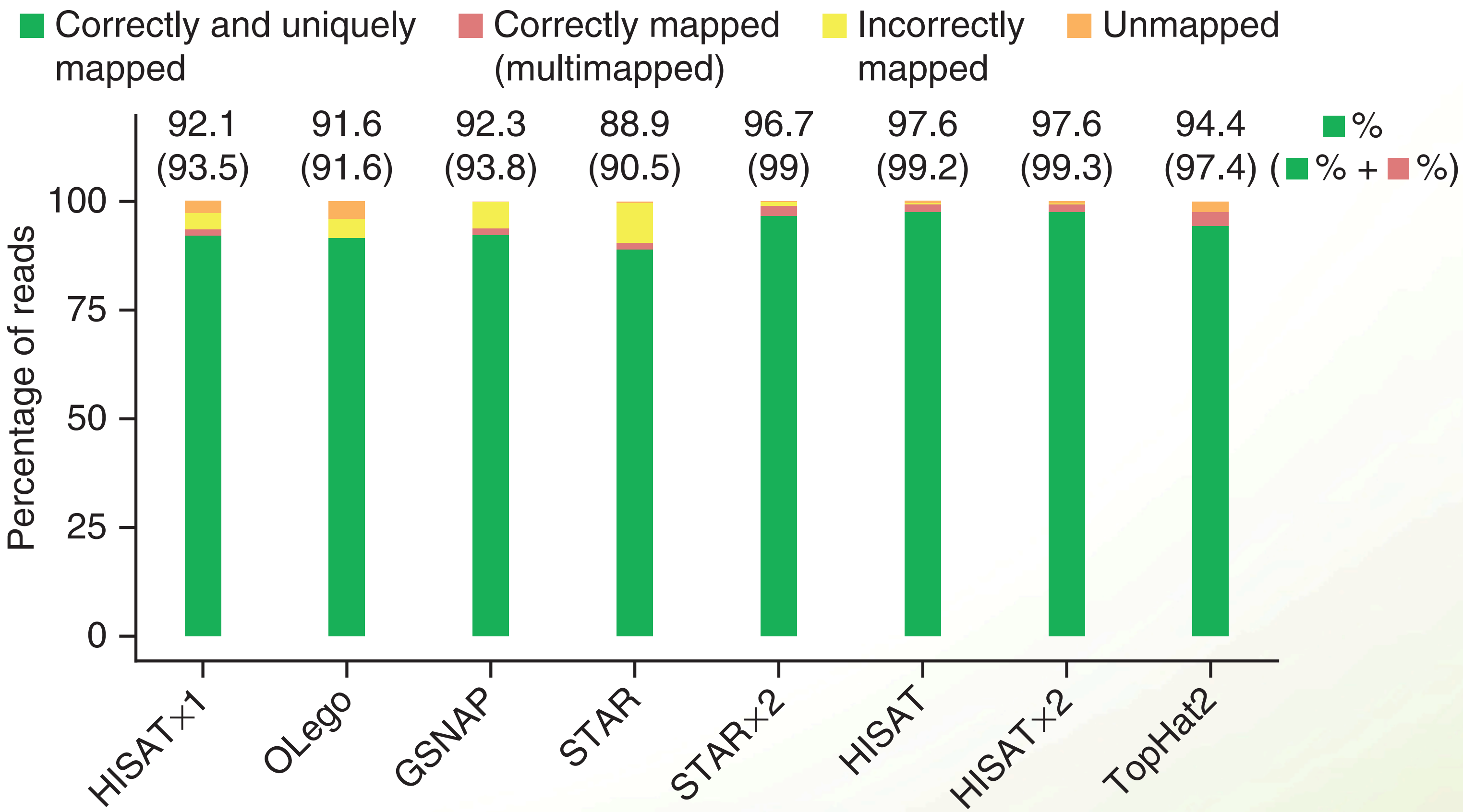
1st run of HISAT to discover splice sites

2nd run of HISAT to align reads by making use of the list of splice sites collected above



Kim et al. *Nature Methods* 2015

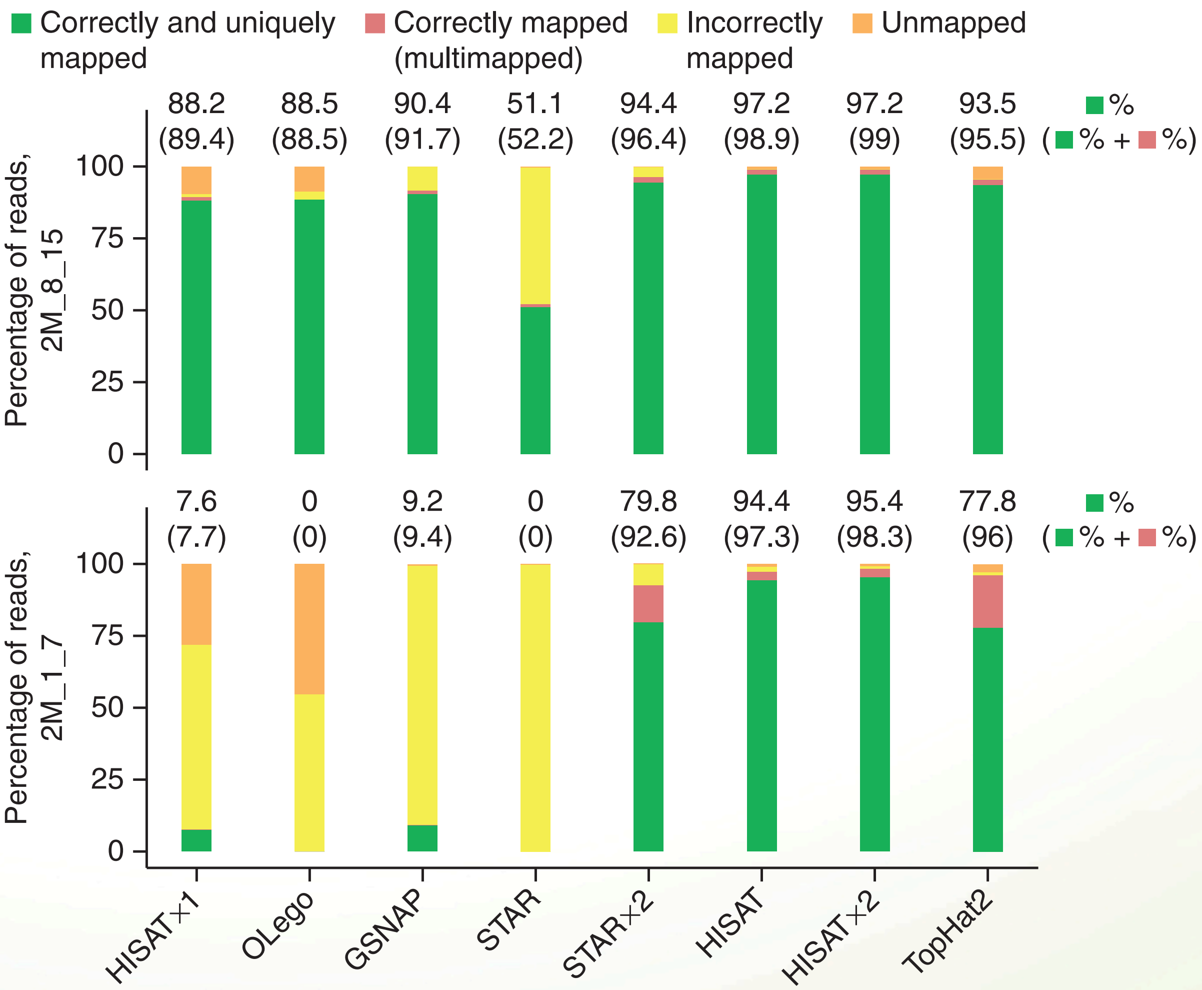
Mapping accuracy

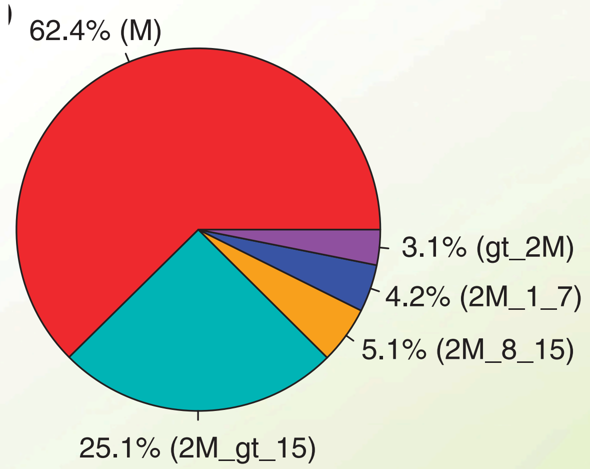


Accuracy for 20 million simulated human 100 bp reads with 0.5% mismatch rate

Kim et al. *Nature Methods* 2015

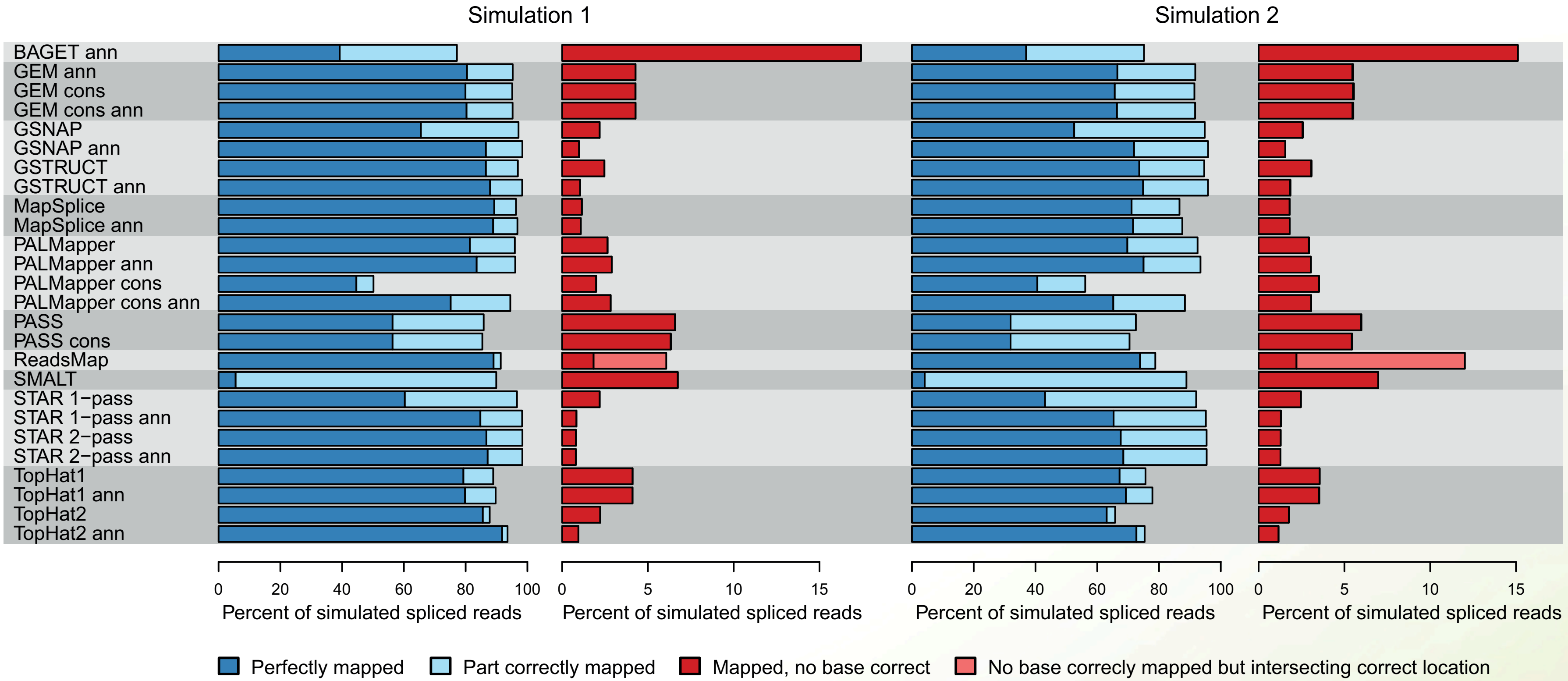
Mapping accuracy for reads with small anchors





Kim et al. *Nature Methods* 2015

Mapping accuracy for spliced RNA-seq reads

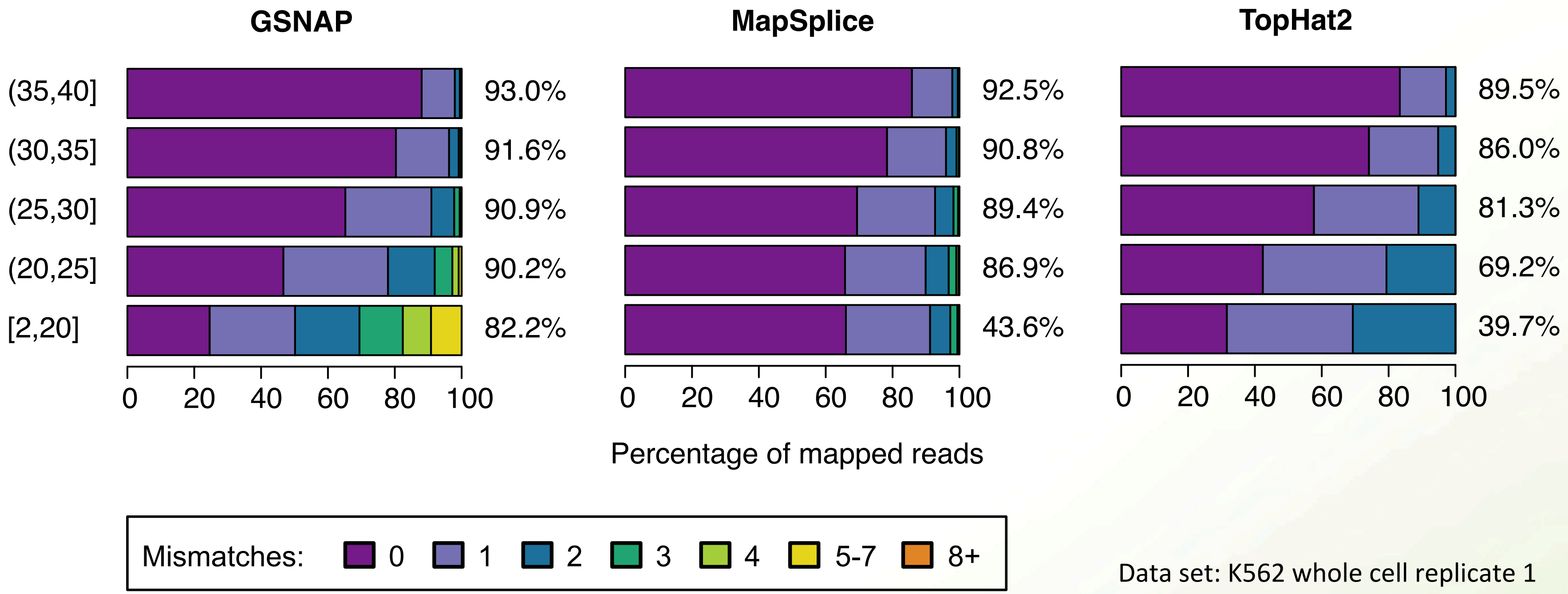


High accuracy at mapping to correct locus: GSNAP, GSTRUCT, MapSplice, STAR

High rate of perfect spliced alignments: ReadsMap, TopHat2 ann

Engström et al. *Nature Methods* 2013

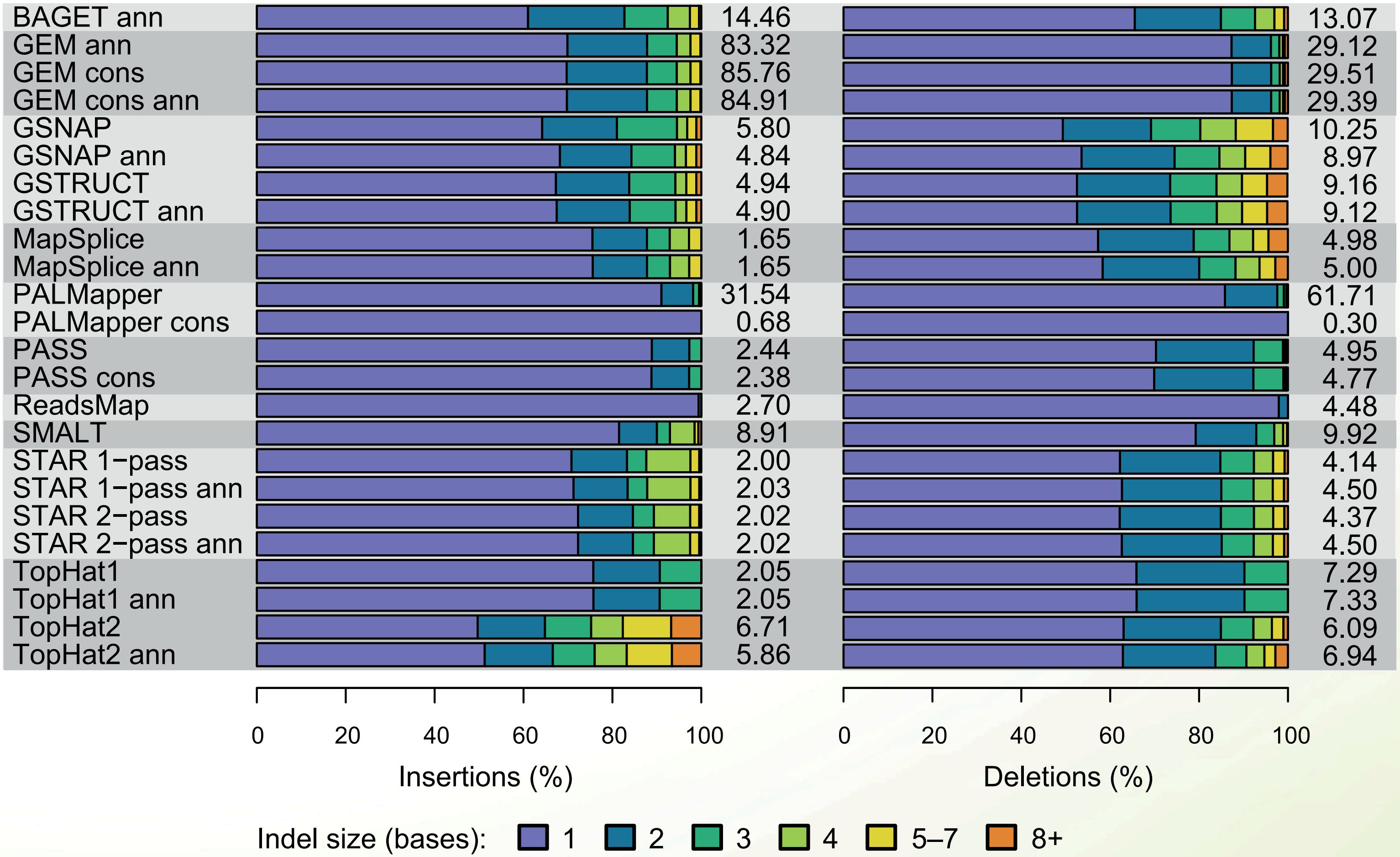
Large differences in mapping rate for low-quality reads



Data set: K562 whole cell replicate 1

Engström et al. *Nature Methods* 2013

Major differences in indel frequencies



Indel frequencies are tabulated (number of indels per thousand sequenced reads). Data set: K562 (mean).

Engström et al. *Nature Methods* 2013

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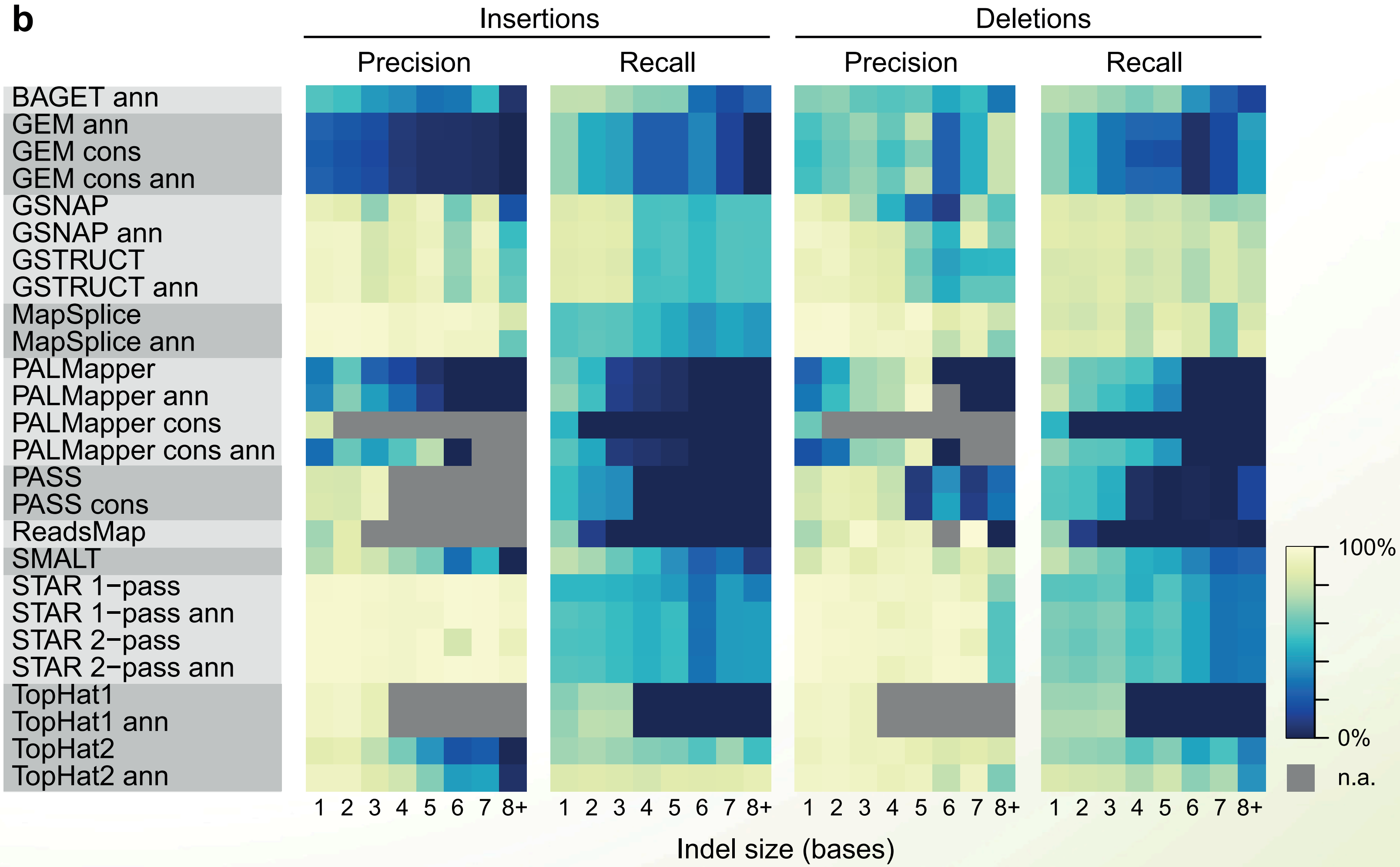
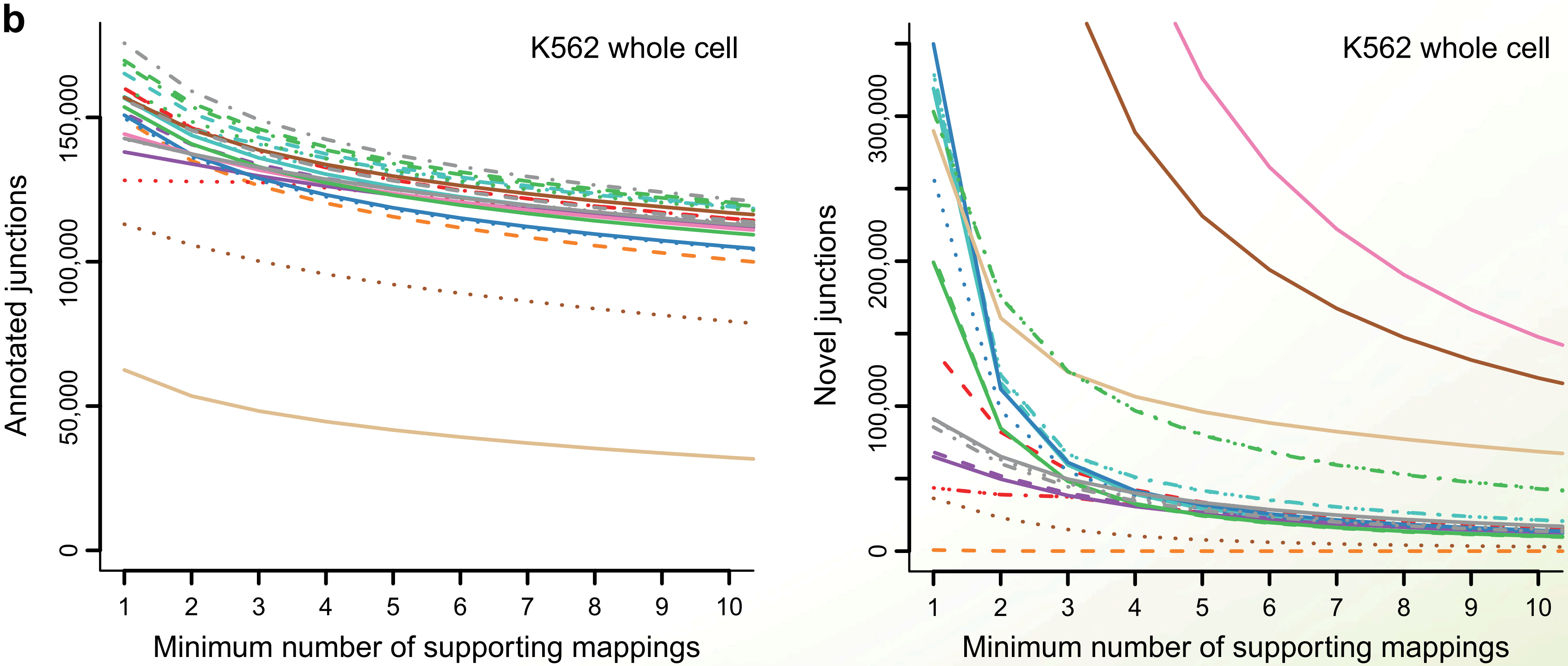
Indel accuracy on simulated data



- GSNAP and GSTRUCT exhibit high sensitivity for both long and short deletions
- TopHat2 ann is most sensitive for long insertions

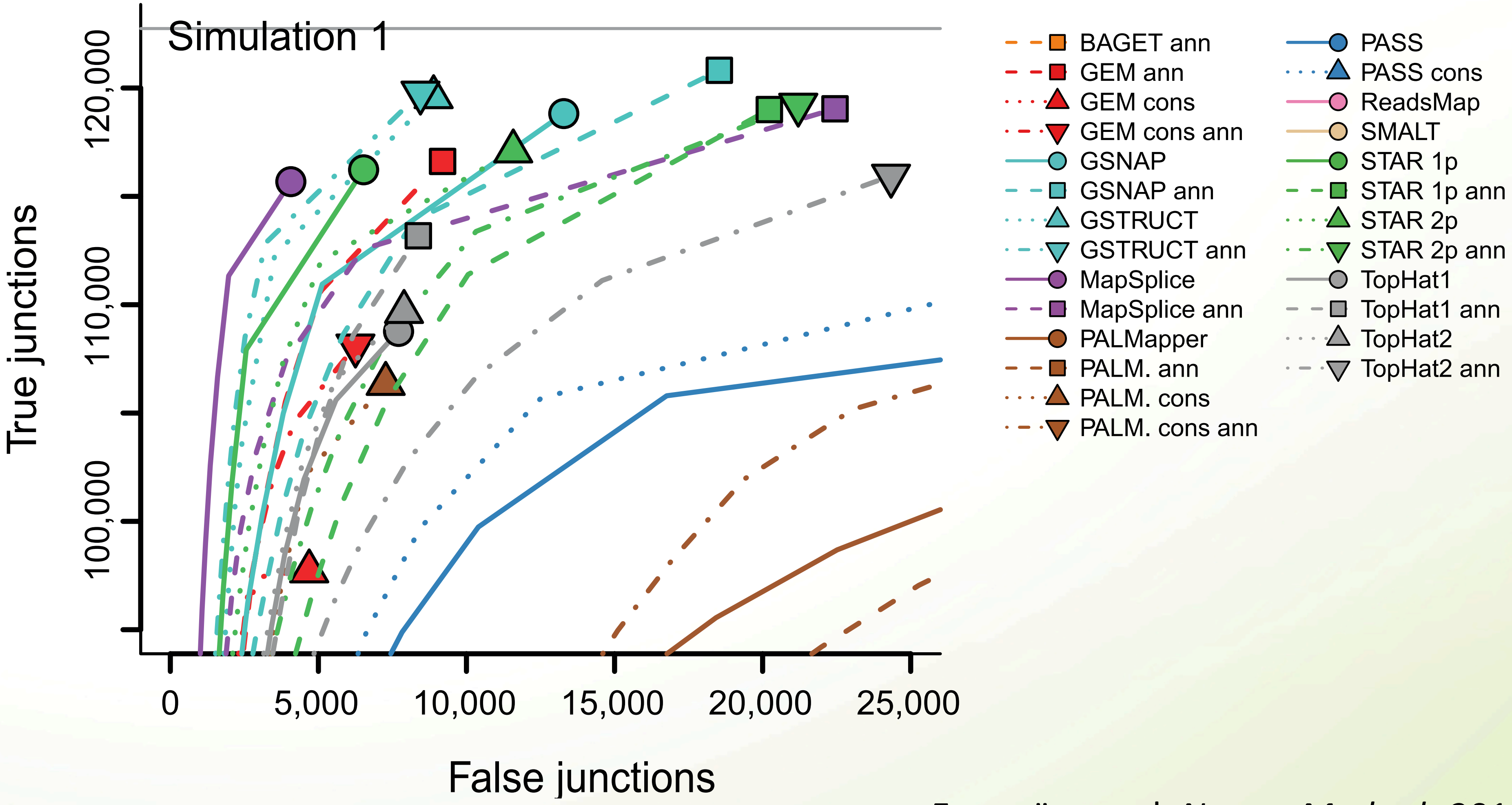
Engström et al. *Nature Methods* 2013

Novel junctions are typically supported by few alignments



Engström et al. *Nature Methods* 2013

Improved junction accuracy by filtering on coverage



Engström et al. *Nature Methods* 2013

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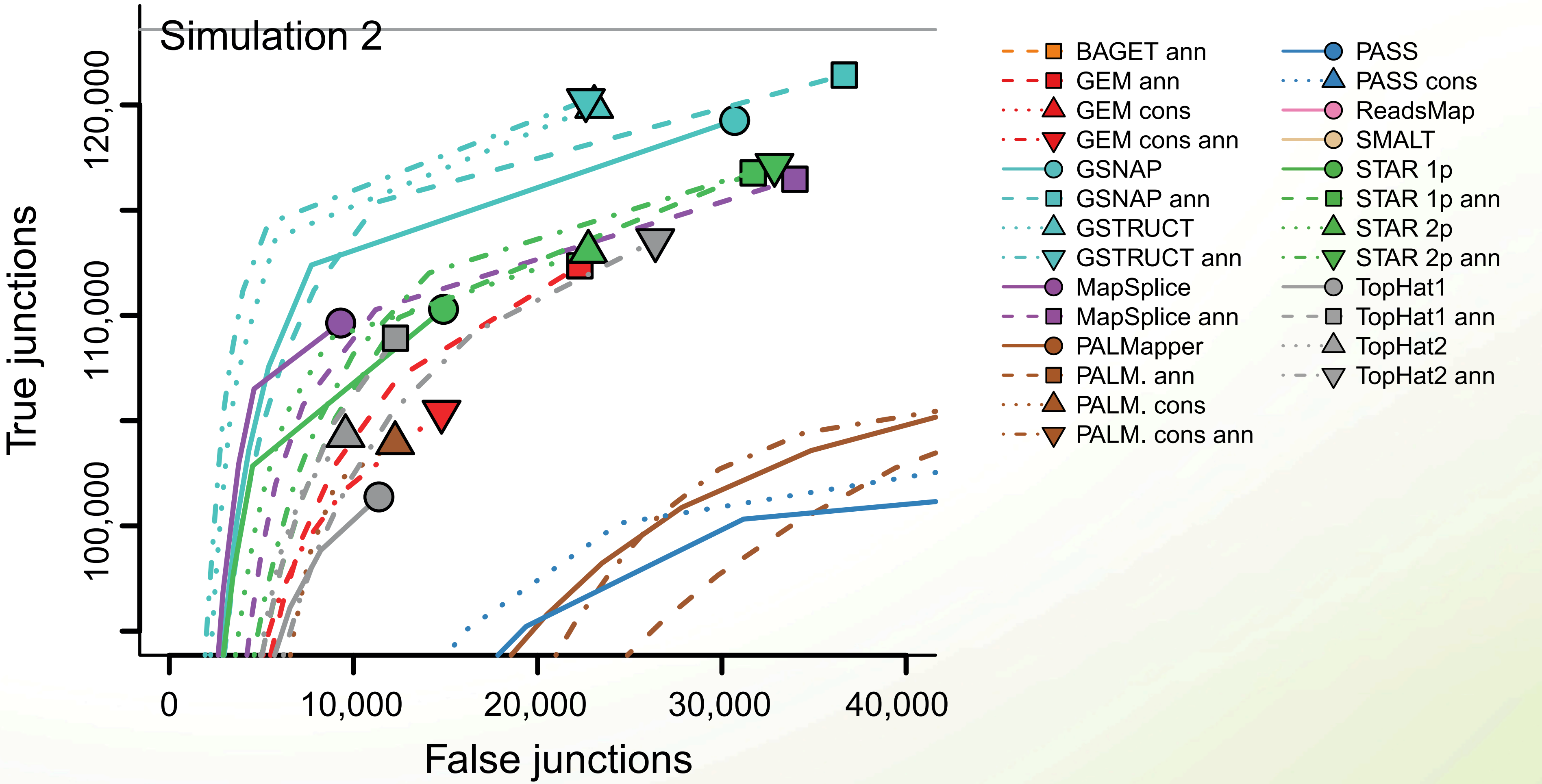
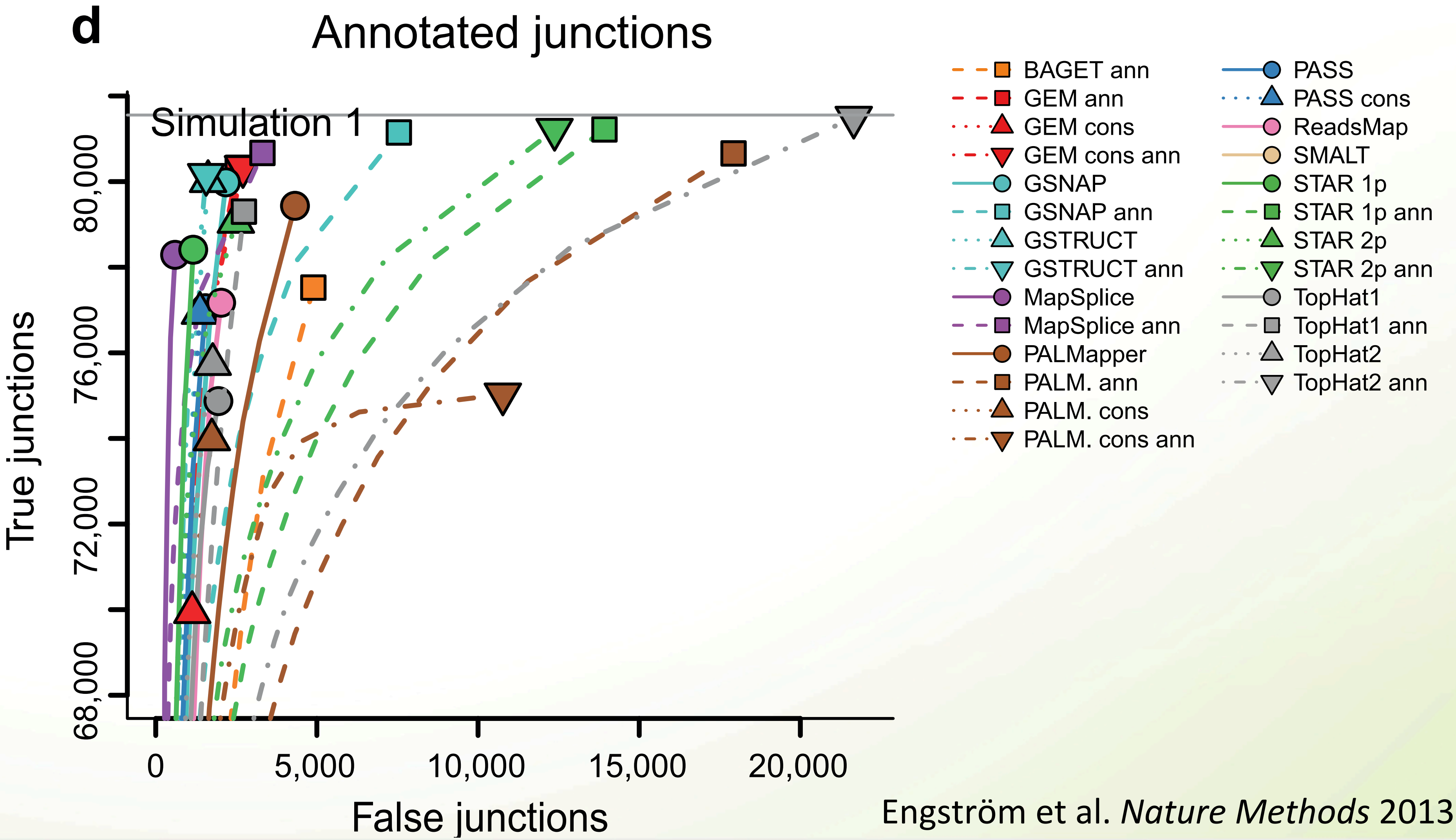


Engström et al. *Nature Methods* 2013

Several methods show over-confidence in annotation



Engström et al. *Nature Methods* 2013

Top performers (RGASP)

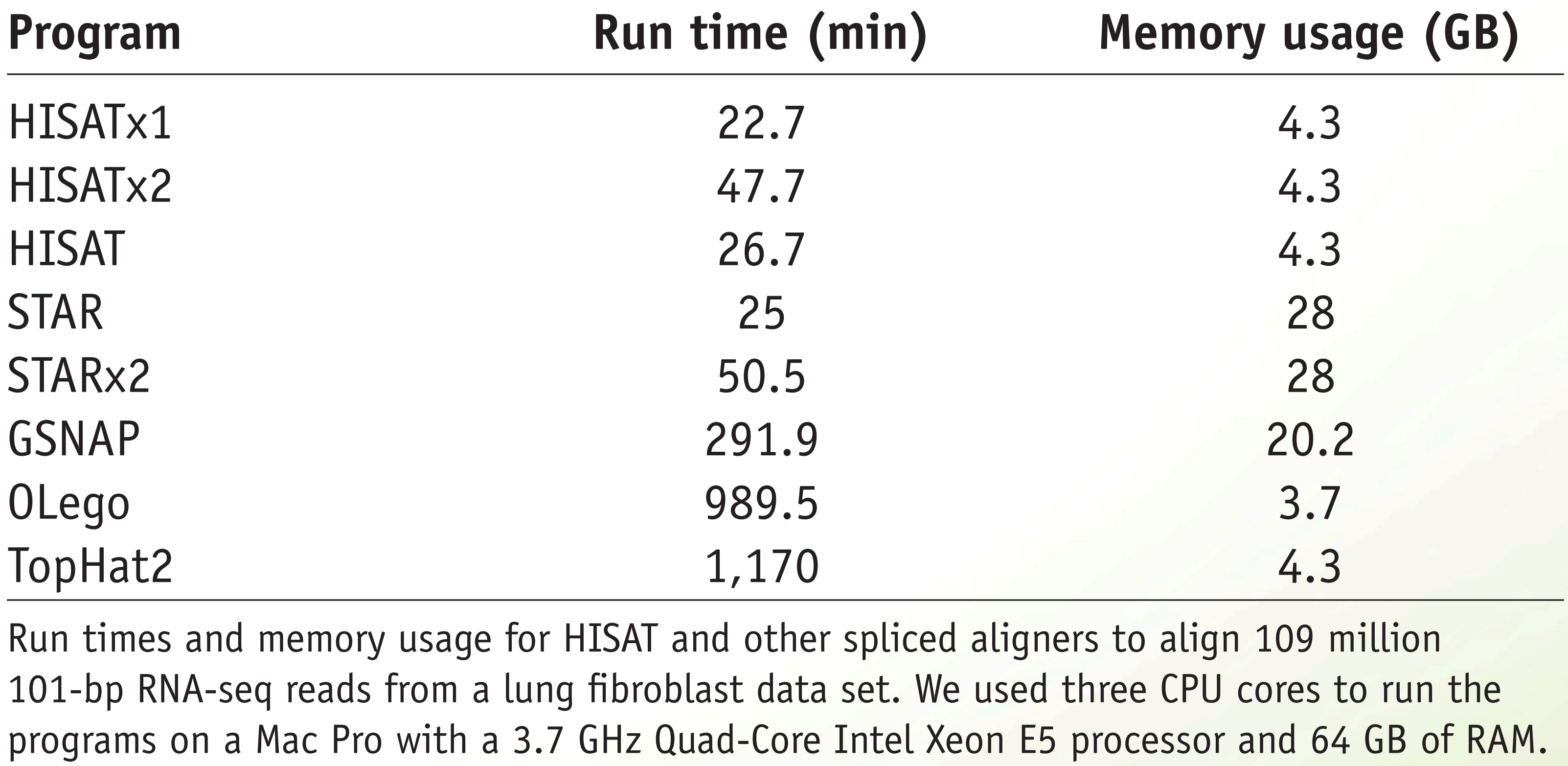
In general, GSNAP, GSTRUCT, MapSplice and STAR compared favorably to the other methods, but also displayed certain weaknesses:

- MapSplice is a conservative aligner, both with respect to mismatch frequency, indel calls and exon junction calls.

- The largest issue with GSNAP, GSTRUCT and STAR is the presence of many false exon junctions in the output.

Engström et al. *Nature Methods* 2013

Compute requirements



Program	Run time (min)	Memory usage (GB)
HISATx1	22.7	4.3
HISATx2	47.7	4.3
HISAT	26.7	4.3
STAR	25	28
STARx2	50.5	28
GSNAP	291.9	20.2
OLego	989.5	3.7
TopHat2	1,170	4.3

Run times and memory usage for HISAT and other spliced aligners to align 109 million 101-bp RNA-seq reads from a lung fibroblast data set. We used three CPU cores to run the programs on a Mac Pro with a 3.7 GHz Quad-Core Intel Xeon E5 processor and 64 GB of RAM.

Kim et al. *Nature Methods* 2015

Recommendations

- Use a two-pass workflow
- STAR and GSNAP generally perform well
- HISAT also seems to do well (or better)
- HISAT and STAR are the fastest
- GSNAP has a SNP-tolerant mode and may give higher sensitivity
- HiSAT2 also has SNP-tolerant mode
- If you want to run Cufflinks, use TopHat or HISAT
- For long (PacBio) reads, STAR, BLAT or GMAP can be used

Important SAM fields

Command:

```
samtools view -X file.bam
```

Perfectly and uniquely aligned read pair:

```
HWI-ST1018:3:1305:21090:45397#0  pPR1  chr1  4426  255  101M       =  4435   110  GT…  C@…
NH:i:1  HI:i:1  AS:i:200  nM:i:0

HWI-ST1018:3:1305:21090:45397#0  pPr2  chr1  4435  255  101M       =  4426  -110  CG…  5<…
NH:i:1  HI:i:1  AS:i:200  nM:i:0
```

Problematic read pair:

```
HWI-ST1018:3:2109:6170:66353#0  pPR2s  chr1  5058    3  65M36S     =  5058    95  CA…  B@…
NH:i:2  HI:i:2  AS:i:135  nM:i:9

HWI-ST1018:3:2109:6170:66353#0  pPr1s  chr1  5058    3  7S73M1D21M =  5058   -95  CC…  ##…
NH:i:2  HI:i:2  AS:i:135  nM:i:9
```

Beyond mapping reads to reference

- Kallisto is a near optimal RNA-seq quantification program
  - Insted of aligning reads to reference Kallisto identifyies which transcripts a read is compatible with
  - Makes it much faster and need much less cpu demanding and much less memory is needed
  - The quantification of 78.6 million reads takes 14 minutes on a standard desktop using a single CPU core
  - Ho

Thanks for listening!