GSNAP: Fast and SNP-tolerant detection of complex variants and splicing in short reads

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Computational Methods for High-Throughput Omics Data, WS 2011

Outline

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- "Why should we be happy with millions of reads, when we can have...

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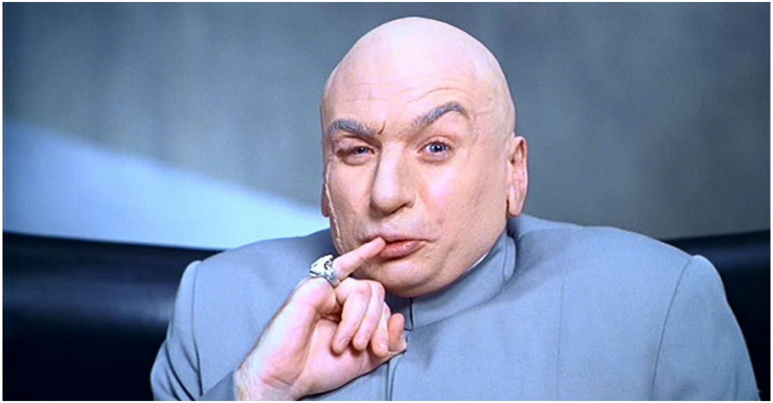

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- Current (Feb 2010) read mappers tend to either be very fast (BWA, Bowtie, SOAP2) or sensitive to variants (SOAP)
- GSNAP is intended to be fast and able to handle complex variants

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- Still pretty fast

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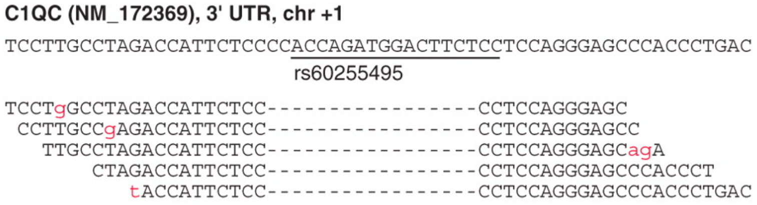
Long deletion of 17nt plus mismatches

- 17nt deletion matching an entry in dbSNP, including mismatches:



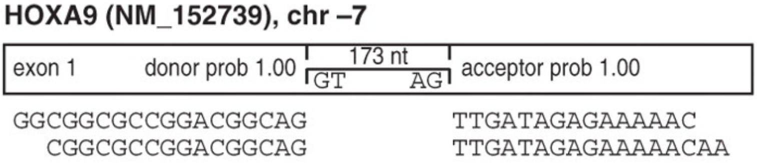
C1QC (NM_172369), 3' UTR, chr +1

```
TCCTTGCCTAGACCATTCTCCCCACCAGATGGACTTCTCCTCCAGGGAGCCCACCCTGAC
                       rs60255495

TCCTgGCCTAGACCATTCTCC-----------------CCTCCAGGGAGC
 CCTTGCCgAGACCATTCTCC-----------------CCTCCAGGGAGCC
   TTGCCTAGACCATTCTCC-----------------CCTCCAGGGAGCagA
       CTAGACCATTCTCC-----------------CCTCCAGGGAGCCCACCCT
          tACCATTCTCC-----------------CCTCCAGGGAGCCCACCCTGAC
```

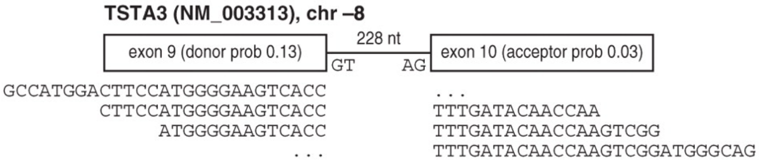
Splicing identified using the probabilistic model

- An intron within exon 1 of HOXA9. Is also experimentally supported.



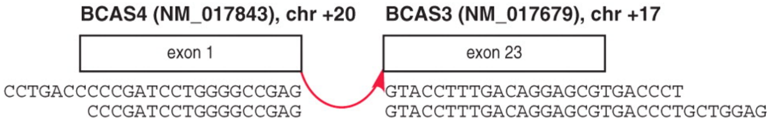
Splicing identified using a database of known splice sites

- Splicing sites identified despite having low probabilistic scores.


TSTA3 (NM_003313), chr –8
exon 9 (donor prob 0.13)
228 nt
GT
AG
exon 10 (acceptor prob 0.03)
GCCATGGACTTCCATGGGAAGTCACC
CTTCCATGGGAAGTCACC
ATGGGAAGTCACC
...
...
TTTGATACAACCAA
TTTGATACAACCAAGTCGG
TTTGATACAACCAAGTCGGATGGGCAG

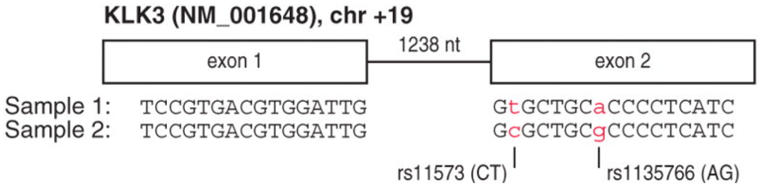
Interchromosomal splicing (gene fusion)

- Splicing between BCAS4 (chr 20) and BCAS3 (chr 17).


BCAS4 (NM_017843), chr +20
BCAS3 (NM_017679), chr +17
exon 1
exon 23
CCTGACCCCCGATCCTGGGCCGAG
CCCGATCCTGGGCCGAG
GTACCTTTGACAGGAGCGTGACCCT
GTACCTTTGACAGGAGCGTGACCCTGCTGGAG

SNP-tolerant alignment

- SNP-tolerance allows both genotypes to align well


KLK3 (NM_001648), chr +19
exon 1
1238 nt
exon 2
Sample 1: TCCGTGACGTGGATTG GtGCTGCaCCCCTCATC
Sample 2: TCCGTGACGTGGATTG GcGCTGCgCCCCTCATC
rs11573 (CT)
rs1135766 (AG)

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- Look at resulting position lists for each element and see if they support a common target location and have a reasonable number of mismatches
- Verify the number of mismatches by checking the whole read against the reference

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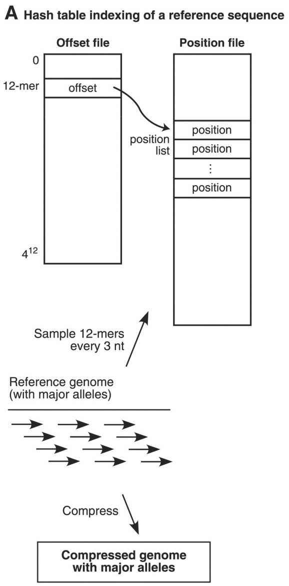
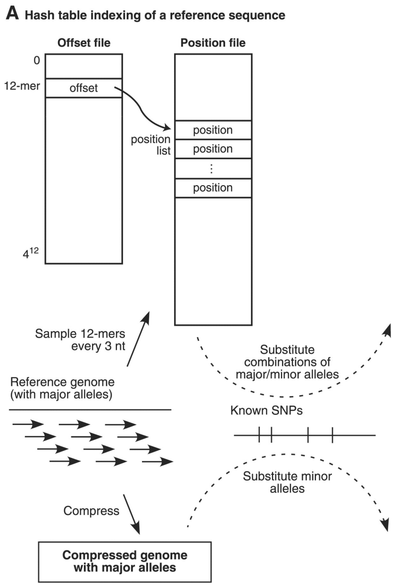
- We only hash 12mers every 3nt (approx. 4GB)
- Optional SNP-tolerance only adds a small amount to total memory requirements (3.8 GB $\rightarrow$ 4.0 GB)
- Entire table only needs to be in memory during construction. Afterwards it is mmap'd and only part is loaded into memory.

Hashing the Reference Genome


A Hash table indexing of a reference sequence
Offset file
Position file
0
12-mer
offset
4^{12}
position list
position
position
⋮
position
Sample 12-mers every 3 nt
Reference genome (with major alleles)
Compress
Compressed genome with major alleles

Including SNPs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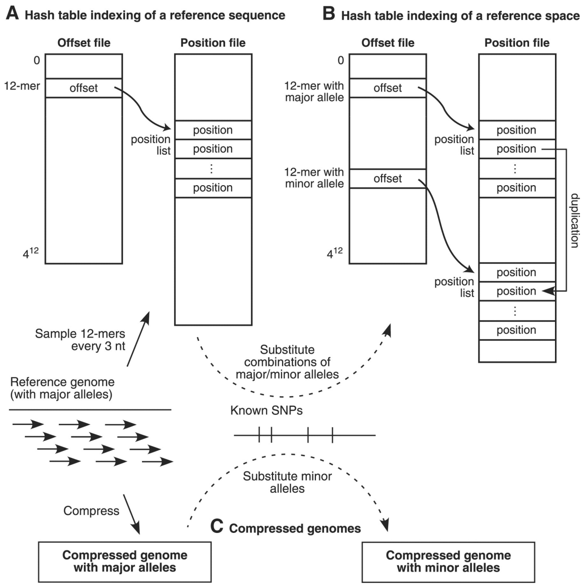
Substitute combinations of major/minor alleles
Known SNPs
Substitute minor alleles
Compress
Compressed genome with major alleles

Resulting Reference "Space"



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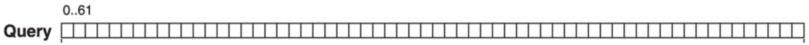
Spanning Set Generation and Filtering



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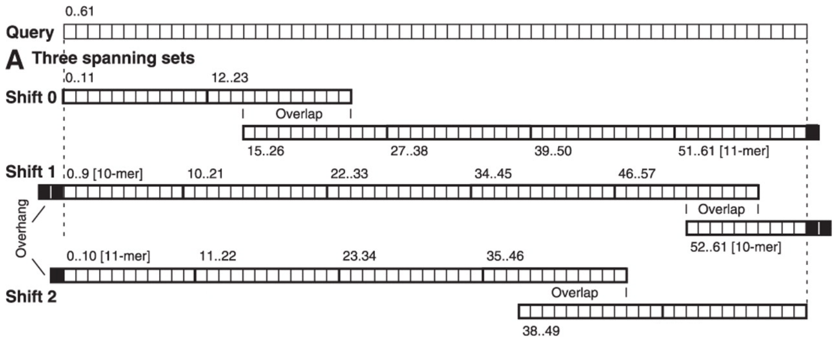

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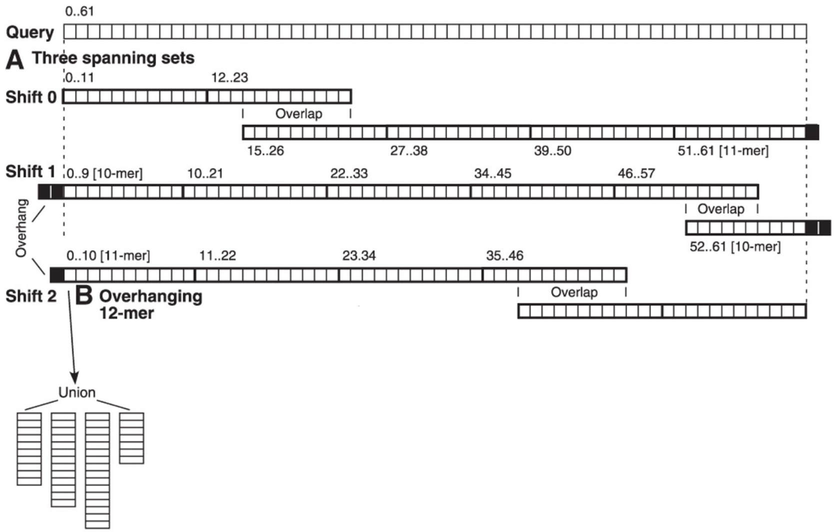

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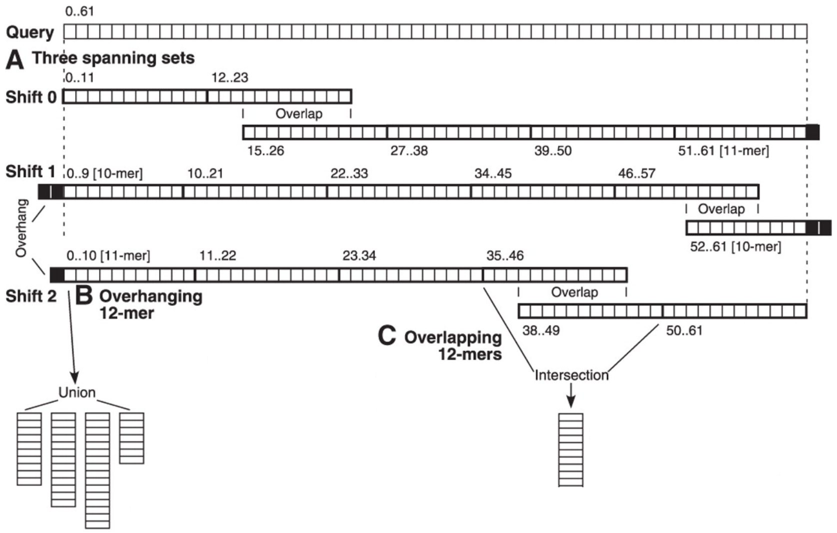

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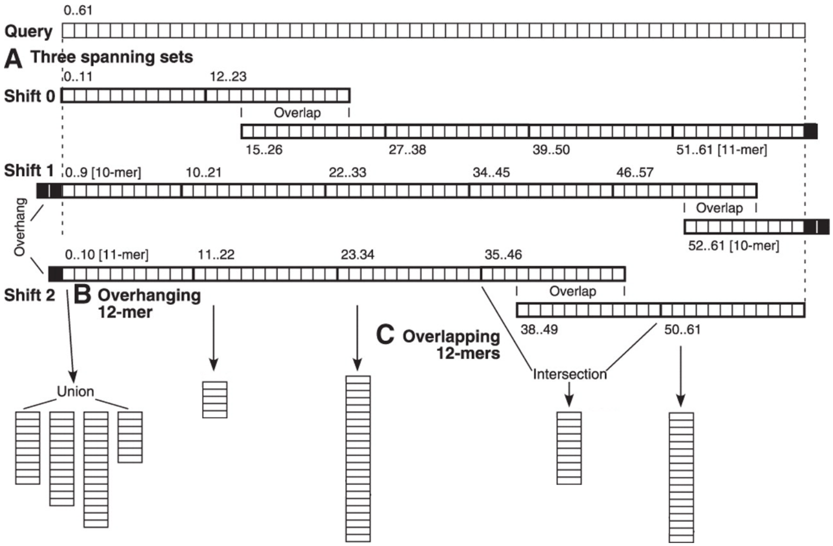

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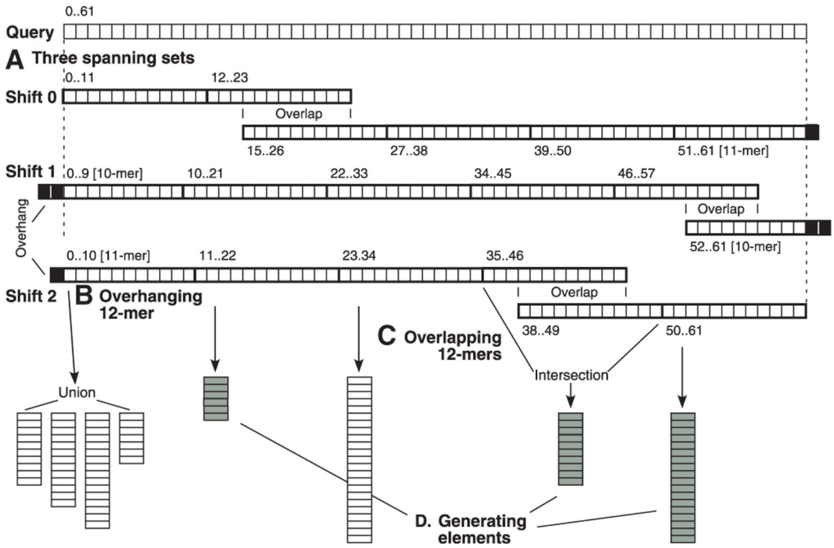

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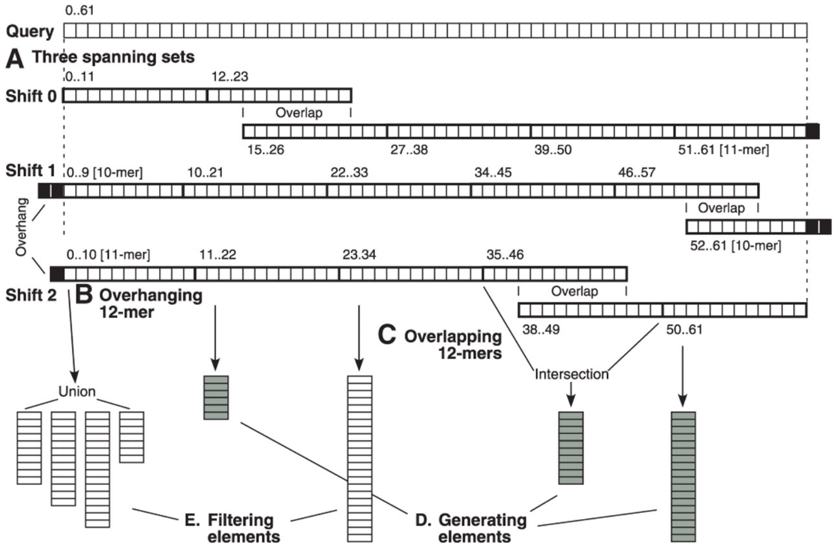

Spanning Set Generation and Filtering

- Generating elements: used to find supporting candidate locations
  - Uses a *multiway merging* procedure (Knuth TAOCP Vol 3)
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- They choose $K + 2$ generating sets, where K is the constraint score (= max number of mismatches)

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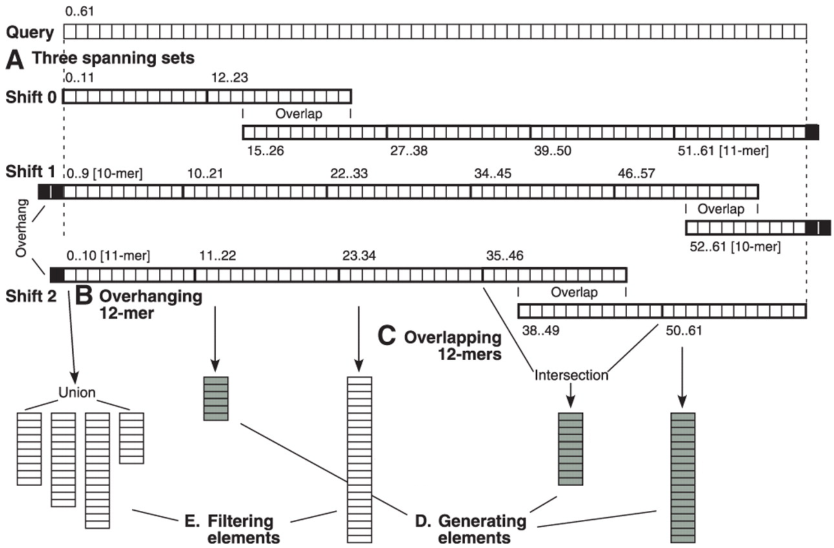

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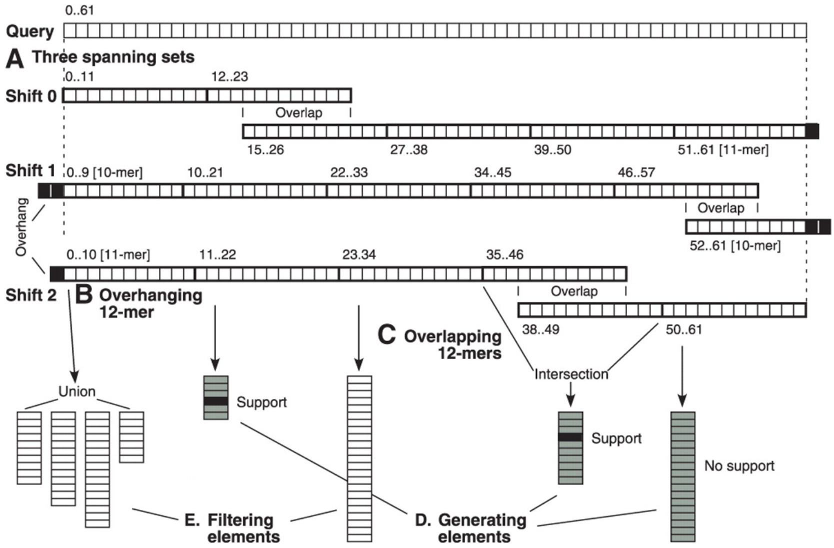

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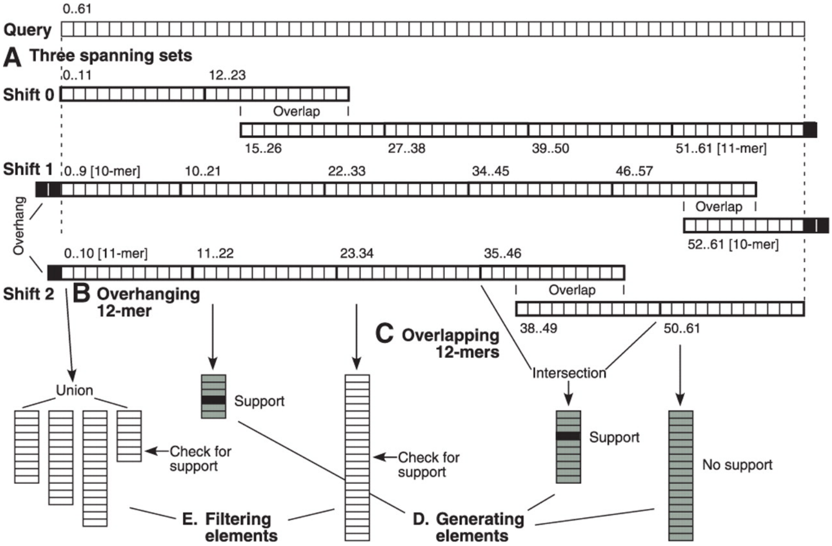

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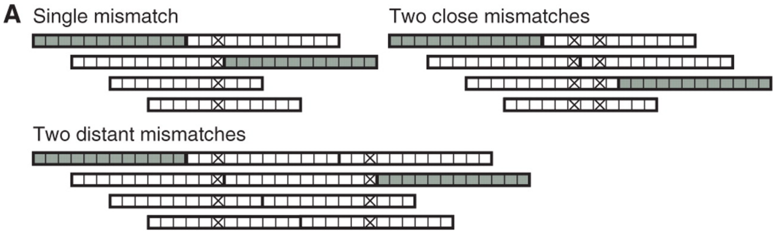
- For reads of length 400 (454), the we can allow a maximum of 31 mismatches.
- If we want to allow larger numbers of mismatches or the same number of mismatches in shorter reads, we need to use another method...

Outline

Complete set generation and filtering

- Uses the complete set of overlapping 12mers.
- Works for any number of mismatches as long as read and target have ≥ 14 consecutive matches (12mer out of phase by up to two bases)
- Exhaustive for $K \leq \lfloor L/14 \rfloor - 1$

Complete set generation and filtering



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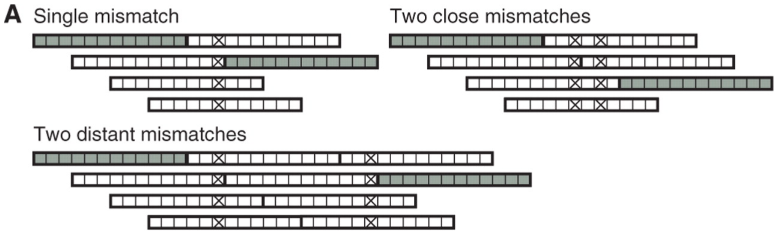
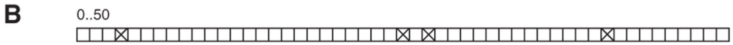
Complete set generation and filtering

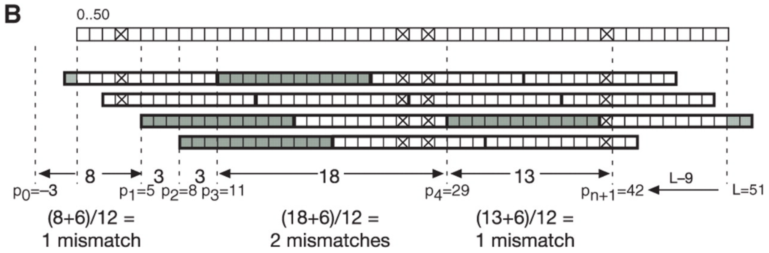


- Lower bound on mismatches: $\lfloor (\Delta p + 6)/12 \rfloor$
  where Δp is the distance between start locations of consecutive supporting 12mers.

Complete set example



Complete set generation and filtering

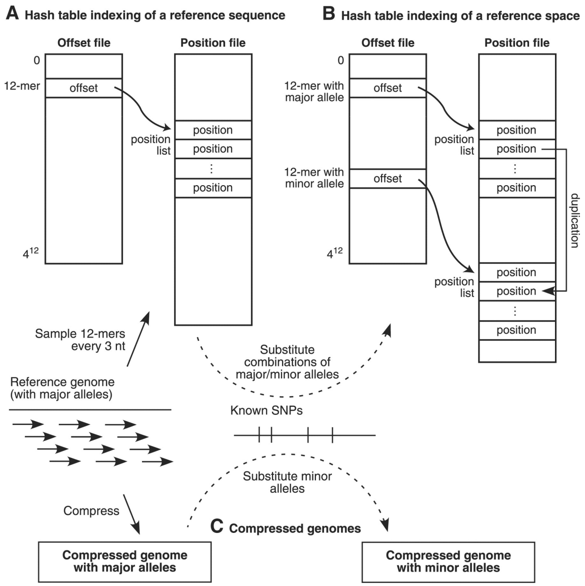


Outline

Verification of Candidate Regions

- The spanning set and complete set methods generate candidate regions for which we know a **lower bound** on the number of mismatches.
- These regions need to be verified to check the **exact number** of mismatches.

Remember: Resulting Reference "Space"



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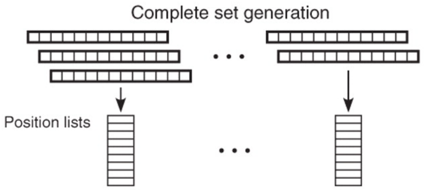
- Because we have a reduced alphabet the reference is stored as 3 bits per character: 2 bits for the nt + a flag
  - Flag in major-allele genome: indicates unknown or ambiguous nt
  - Flag in minor-allele genome: indicates a SNP

Verification of Candidate Regions

- Query sequence converted to the same compressed representation as the reference
- Shifted into position and bitwise XOR combined with the major- and minor-allele genomes separately
- Resulting arrays are bitwise AND'd, so mismatches at a SNP only occur if both alleles do not match

Outline

Detecting Insertions and Deletions



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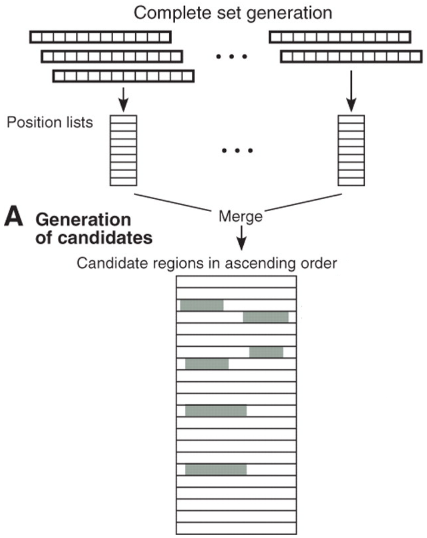

Complete set generation
Position lists
A Generation of candidates
Merge
Candidate regions in ascending order

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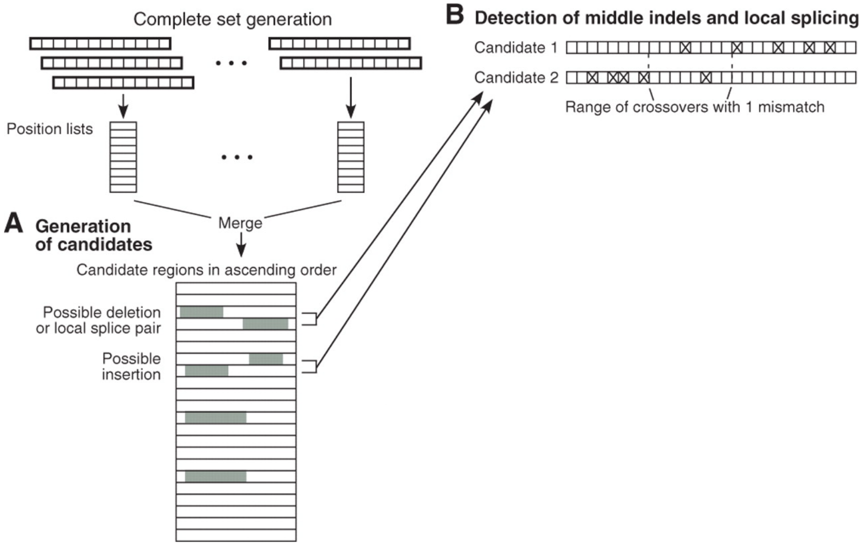
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Candidate 1
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Range of crossovers with 1 mismatch

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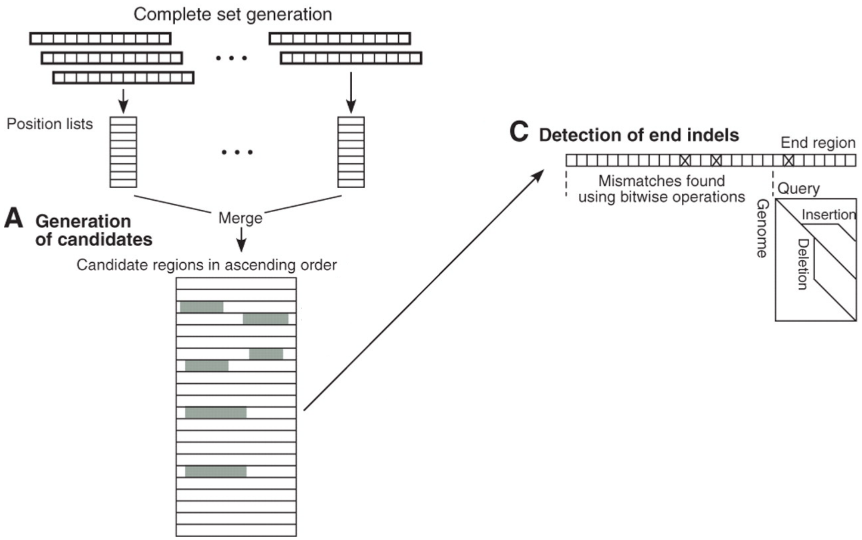

Complete set generation
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C Detection of end indels
End region
Mismatches found using bitwise operations
Query
Genome
Insertion
Deletion

Outline

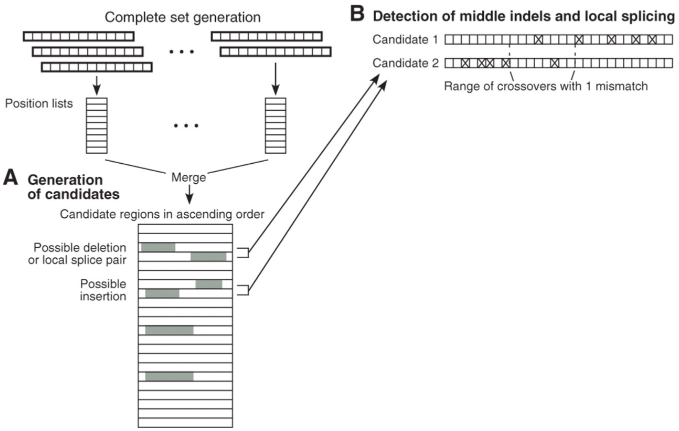
Detecting Splice Junctions

- Known splice sites: user-provided database
- Novel splice sites: maximum entropy probabilistic model from Yeo and Burge, 2004

Detecting Splice Junctions

- Short-distance splice sites are on the same chromosome and < some distance apart (default: 200,000 nt)
- Method similar to the one we used to find middle deletions earlier. . .

Detecting Splice Junctions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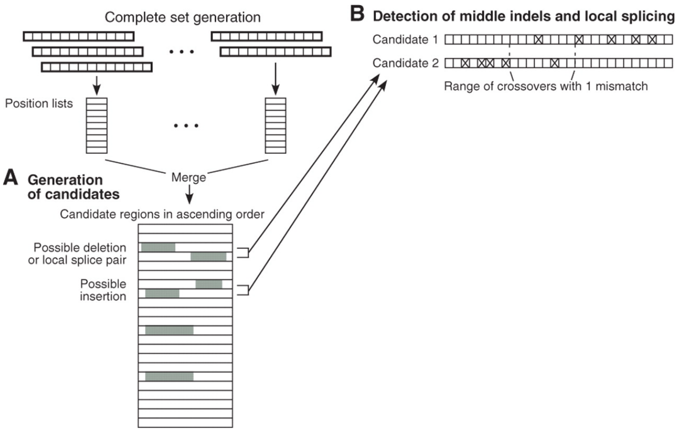
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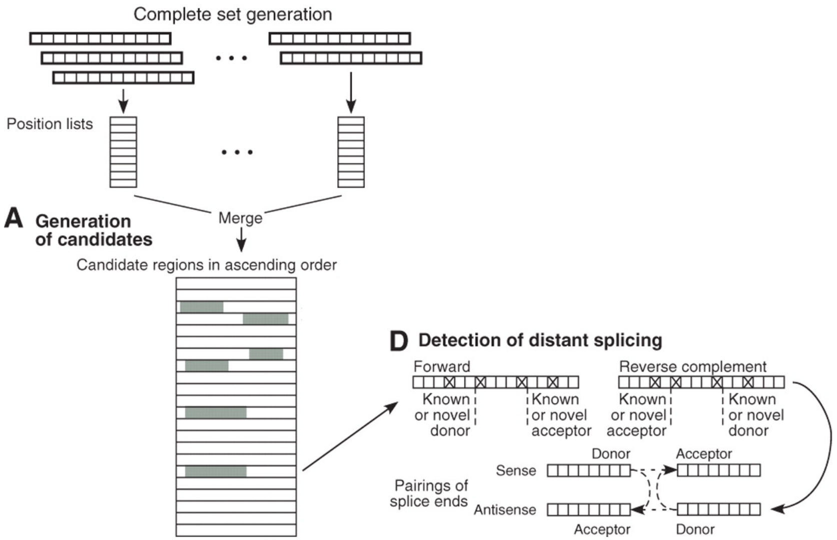
- Crossover area is then searched for donor or acceptor sites (either known or novel with high probability).

Detecting Splice Junctions

- Long-distance splice sites can be on different chromosomes
- Require higher probability scores for novel splice sites than short-distance splice sites
- Candidates with matching breakpoints on the read are matched

Detecting Splice Junctions



Detecting Splice Junctions

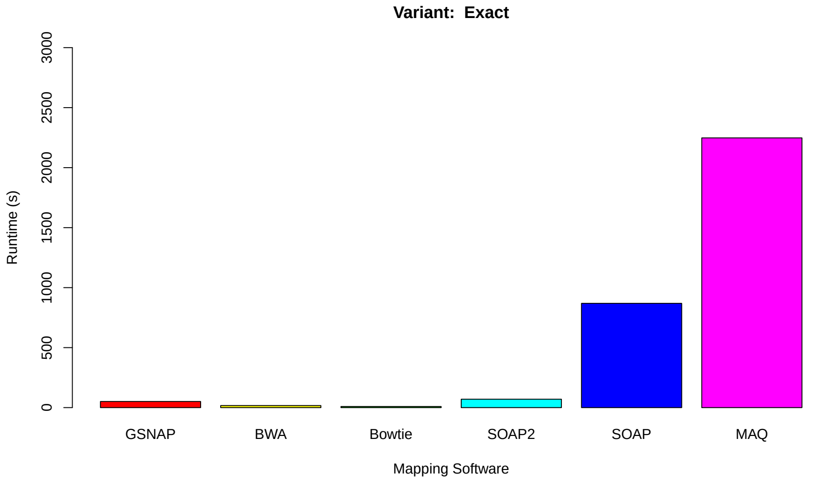
- If both splice sites can not be found then GSNAP will return one site (a "half-intron")

Outline

Simulated Reads

- Runtime comparison between GSNAP and other alignment tools (for 100,000 reads)
- Simulated increasingly complicated variants
  - exact matches only
  - 1 - 3 mismatches
  - short insertions and deletions
  - longer insertions and deletions

Simulated Reads: 36nt



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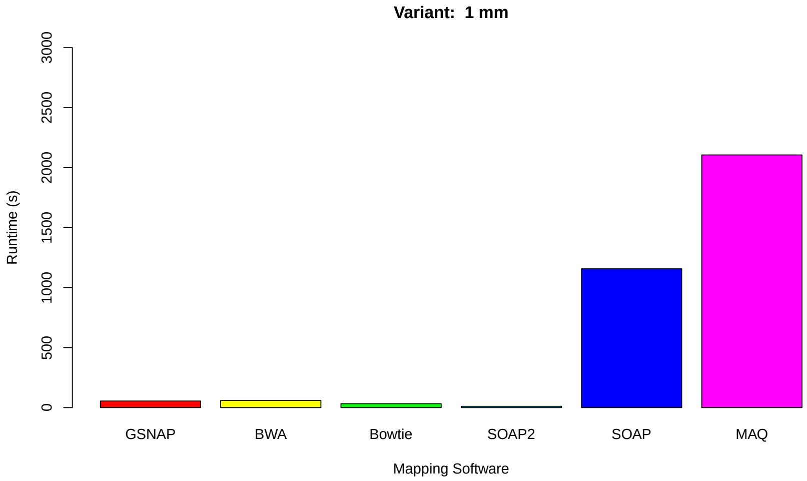

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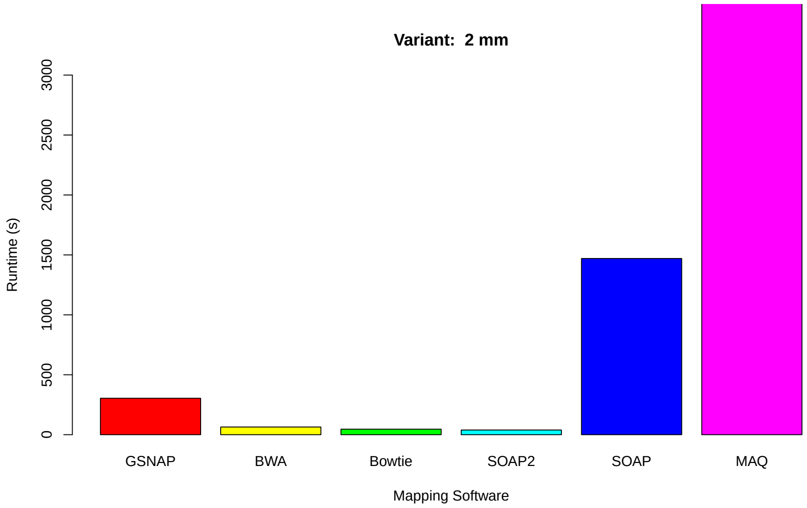
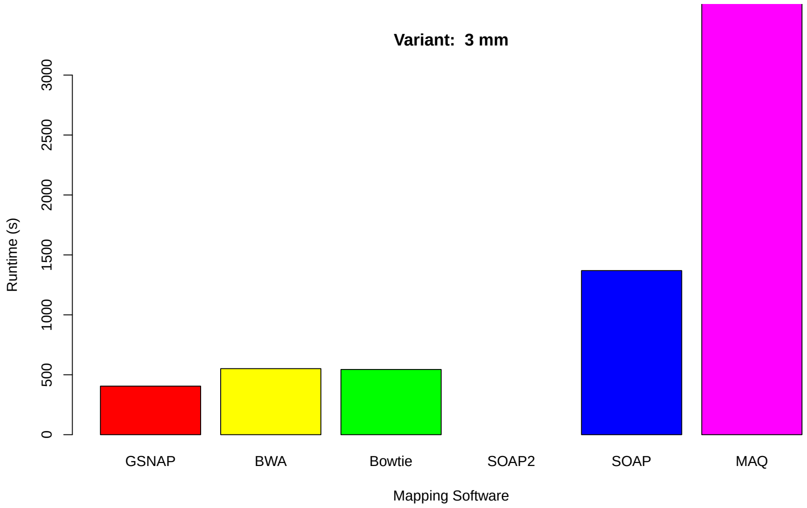


Simulated Reads: 36nt



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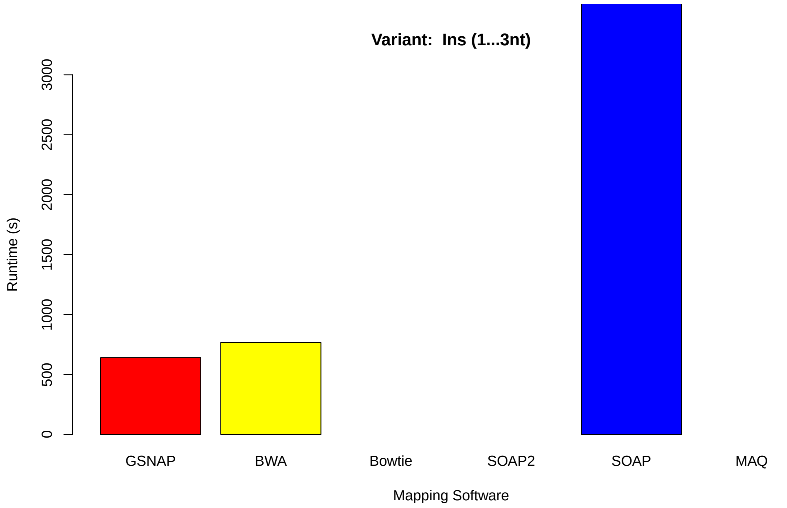
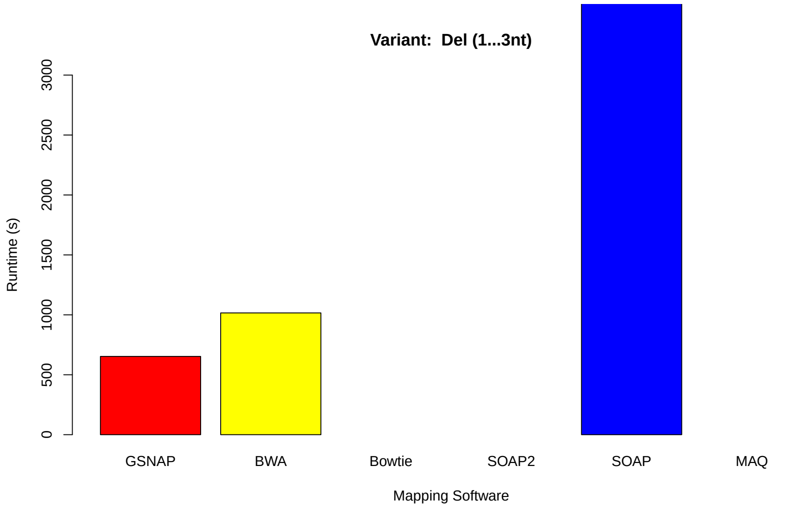
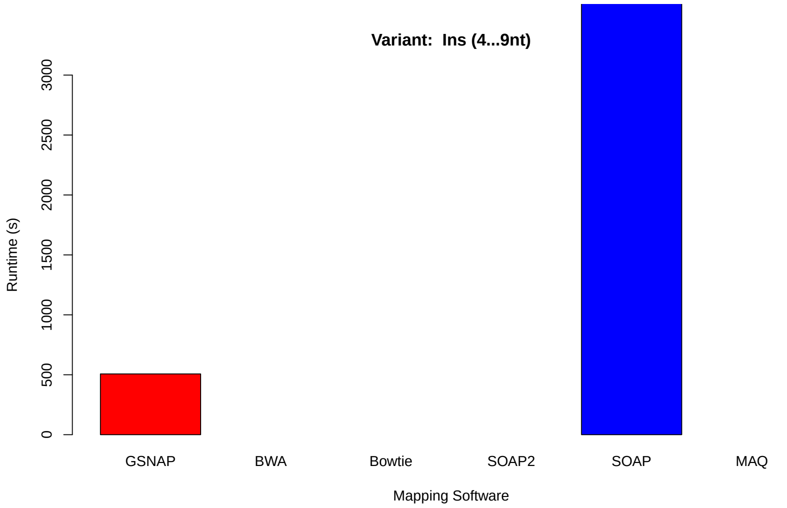


Simulated Reads: 36nt



Simulated Reads: 36nt



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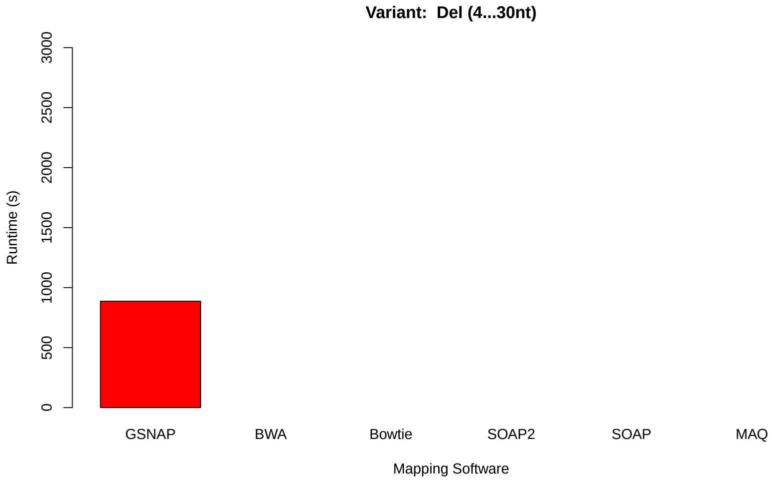
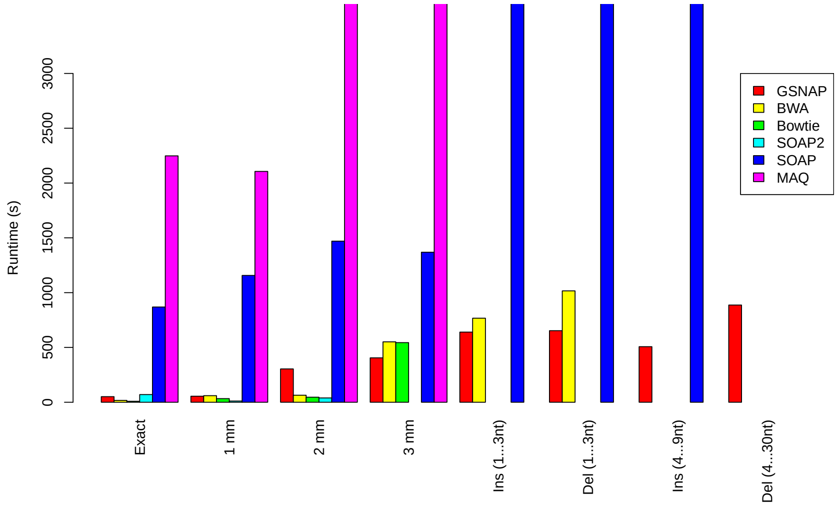
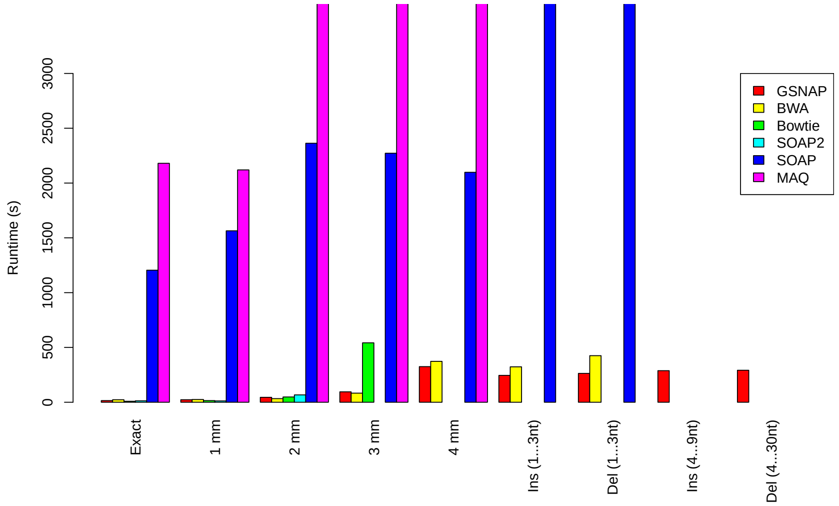


Simulated Reads: 36nt



Simulated Reads: 70nt



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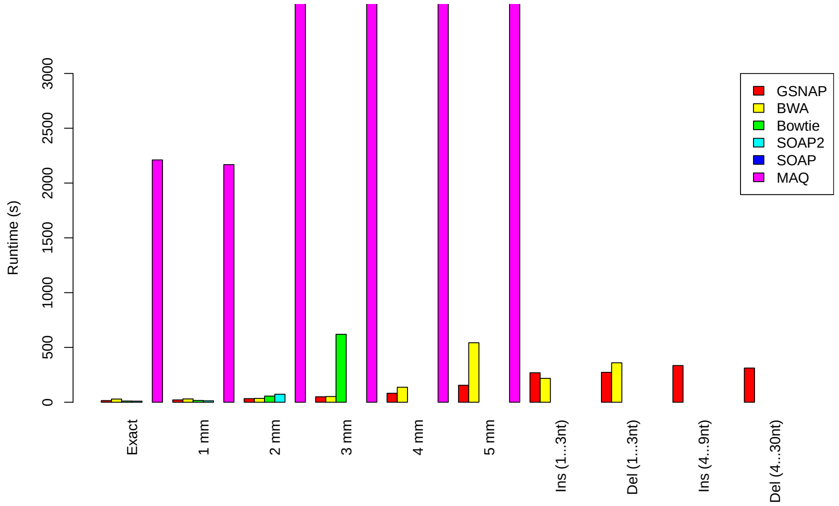
Simulated Reads: 100nt



Simulated Reads: Percent misses (on unique reads)

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- SOAP: 15% misses for 36nt reads with 3 mismatches, around 5% for 1-3nt indels
- BWA: 1-5% misses in 1-3nt indels

Memory Requirements

- GSNAP: should have access to 5 GB of memory, otherwise it will run slowly
- BWA: 2.2 GB
- Bowtie: 1.1 GB (exact matches) or 2.2 GB (allowing mismatches)
- MAQ: 302 MB
- SOAP: 14 GB
- SOAP2: unknown ("only provided as a binary and did not have the required compile time flag")

Outline

Transcriptional Reads

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- Limited to one indel or splice site per read
- Does not use read quality scores
- Does not work with ABI SOLiD data

TL;DR

- Comparable to other fast read alignment algorithms in terms of speed, but can handle more complex variants and splicing

For Further Reading I

Knuth D.E.
The Art of Computer Programming: Sorting and Searching. Vol 3.
Addison-Wesley, 1973

Thomas D. Wu and Serban Nacu
Fast and SNP-tolerant detection of complex variants and splicing in short reads
Bioinformatics, 2010 Apr 1;26(7):873-81.

Yeo G and Burge CB.
Maximum entropy modeling of short sequence motifs with applications to RNA splicing signals
J Comput Biol. 2004;11(2-3):377-94.

Aligning Paired-End Reads

- Optional

Bisulfite-converted DNA

- Optional