Sequence Alignment - NGS

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Outline of the Talk:

- ***Global and Local Alignment***
- ***Alignment methods***
- ***Alignment tools: BWA and Smalt***
- ***Comparison of the results***
- ***Data visualisation***

Biological Motivation

Why We Need Sequence Alignment

Inference of Homology

- Two genes are homologous if they share a common evolutionary history.
- Evolutionary history can tell us a lot about properties of a given gene
- Homology can be inferred from similarity between the genes

- Variation Detection – SNP, indel, CNV

Sequence Alignment

Global Alignment:

Goal: How similar are two sequences S_1 and S_2

Input: two sequences S_1, S_2 over the same alphabet

Output: two sequences S'_1, S'_2 of equal length

(S'_1, S'_2 are S_1, S_2 with possibly additional gaps)

Example:

- S_1= GCGCATGGATTGAGCGA
- S_2= TGCGCCATTGATGACC
- A possible alignment:

```
S'1= -GCGC-ATGGATTGAGCGA
S'2=  TGCGCCATTGAT-GACC--
```

Sequence Alignment (cont)

Local Alignment:

Goal: Find the pair of substrings in two input sequences which have the highest similarity

Input: two sequences S_1, S_2 over the same alphabet

Output: two sequences S'_1, S'_2 of equal length

(S'_1, S'_2 are substrings of S_1, S_2 with possibly additional gaps)

Example:

- $S_1 =$ GCGCATGGATTGAGCGA
- $S_2 =$ TGCGCCATTGATGACC
- A possible alignment:

$S'_1 =$ ATTGA-G

$S'_2 =$ ATTGATG

Global vs. Local Alignment

- The Global Alignment Problem tries to find the longest path between vertices *(0,0)* and (*n*,*m*) in the edit graph.

- The Local Alignment Problem tries to find the longest path among paths between **arbitrary vertices** (*i*,*j*) and (*i'*, *j'*) in the edit graph.

Global vs. Local Alignment (cont’d)

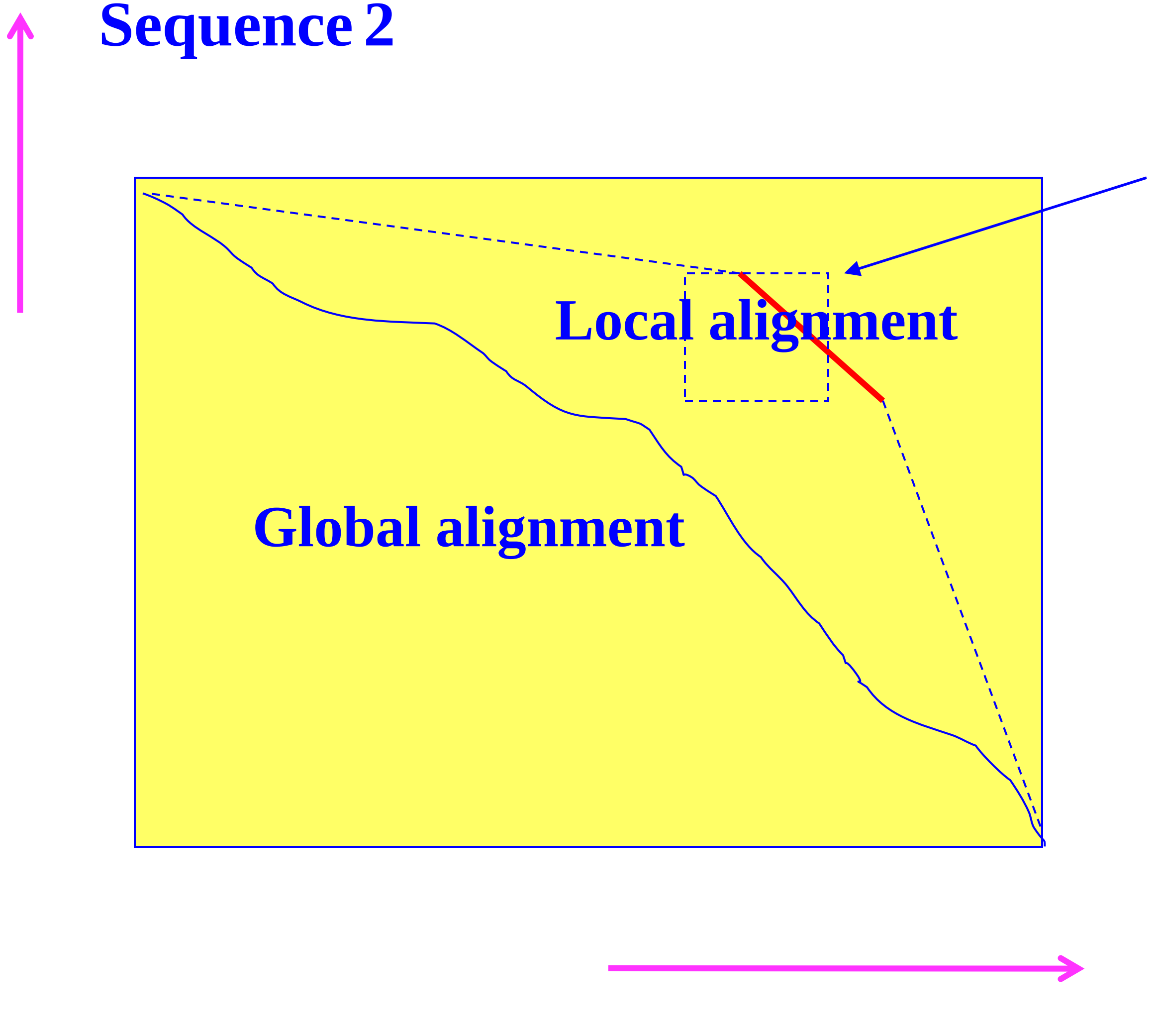
- **Global Alignment**

```
--T—-CC-C-AGT—-TATGT-CAGGGGACACG—A-GCATGCAGA-GAC
  |  || |  ||  | | | |||    || | | |  | ||||   |
AATTGCCGCC-GTCGT-T-TTCAG----CA-GTTATG—T-CAGAT--C
```

- **Local Alignment—betten alignment to find conserved segment**

```
                  tccCAGTTATGTCAGgggacacgagcatgcagagac
                     ||||||||||||
aattgccgccgtcgttttcagCAGTTATGTCAGatc
```

Local Alignment: Example



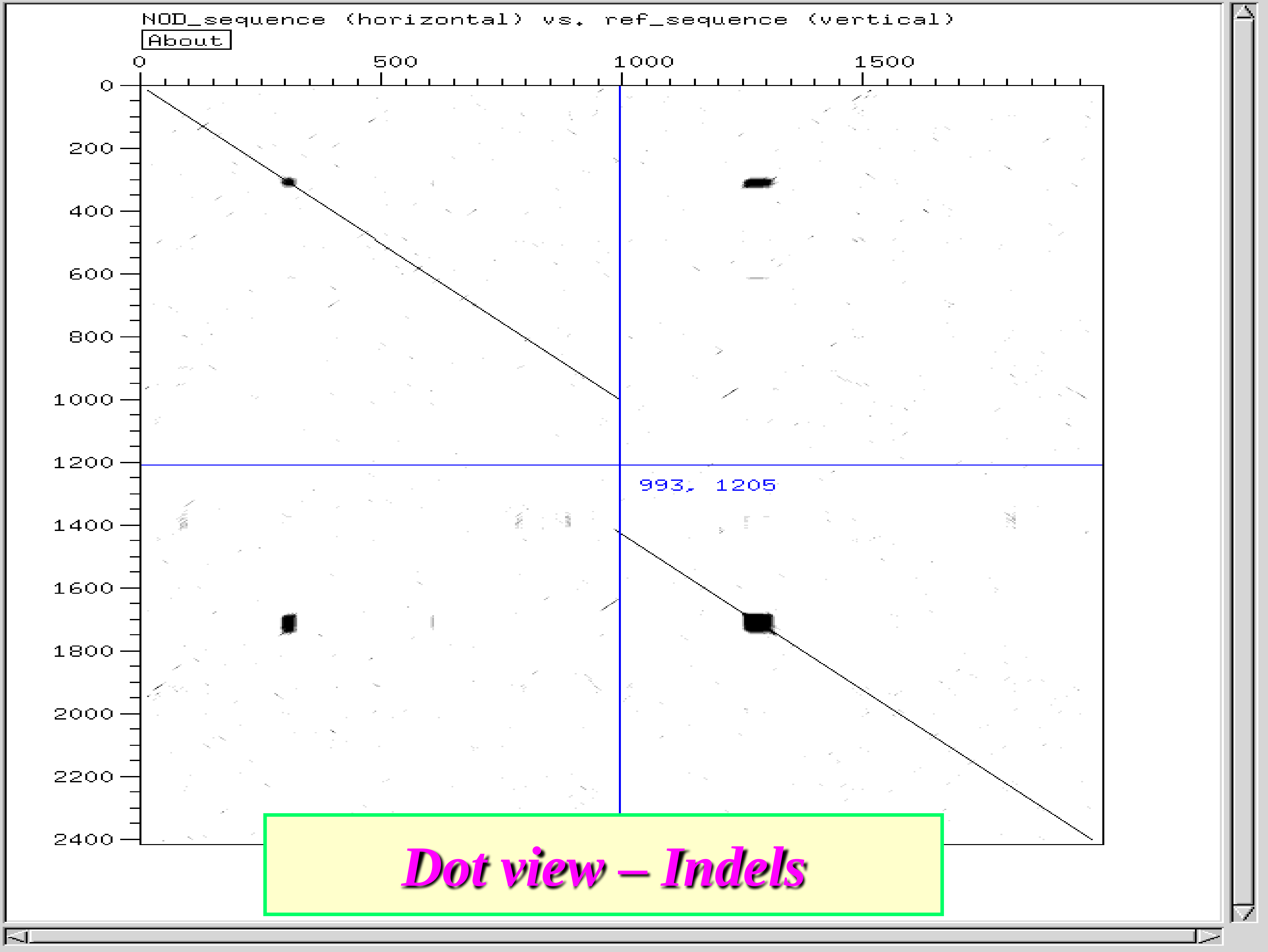
Compute a “mini” Global Alignment to get Local

Methods of DNA Sequence Alignment

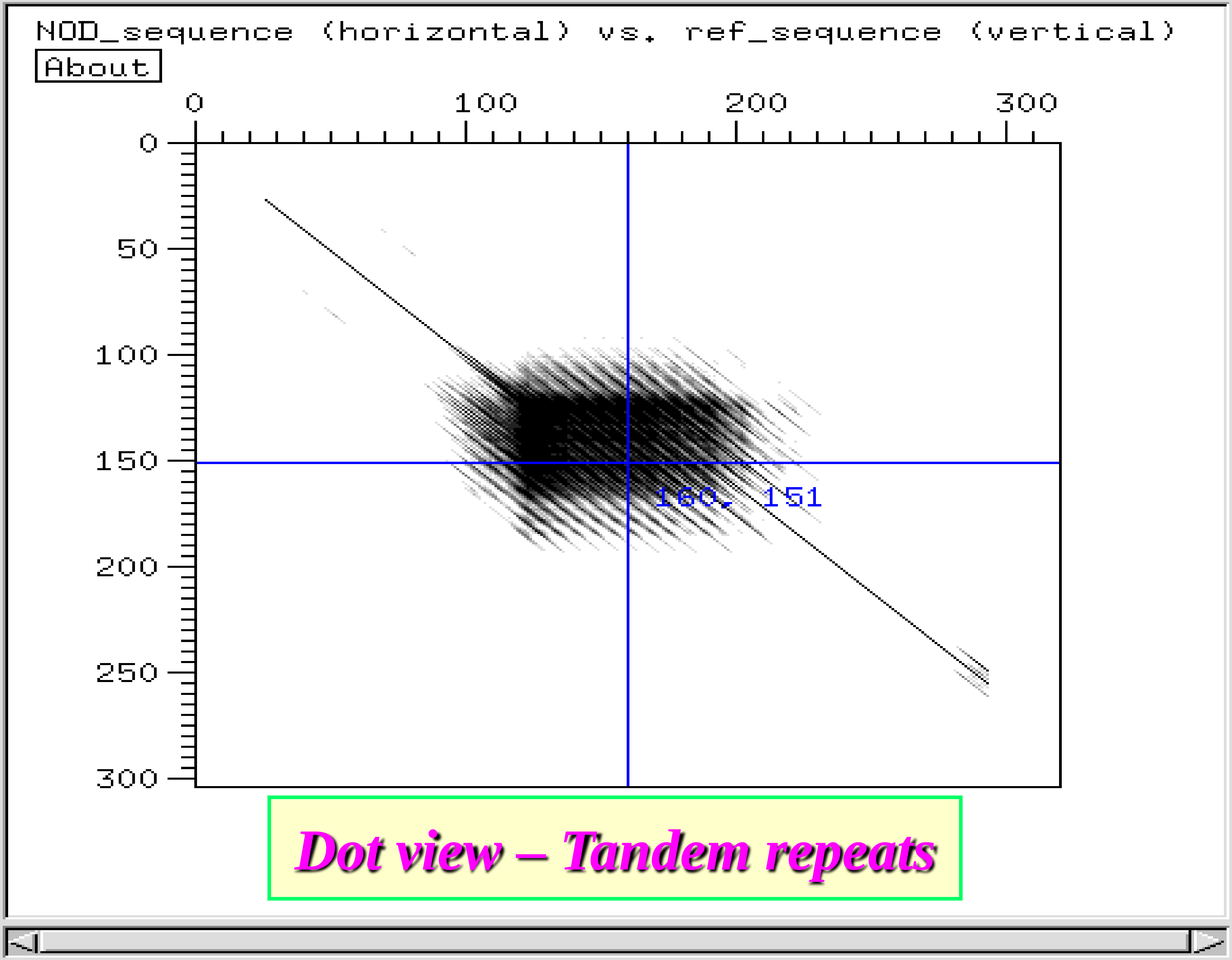
- **Dot matrix analysis**
- **The dynamic programming (DP) algorithm**
    - **Needleman-Wunsch Algorithm**
    - **Smith-Waterman Algorithm**
- **Burrows-Wheeler Index (BWA, Bowtie, SOAP2, etc)**
- **Hash table based algorithm (ssaha2, smalt, novoAlign, etc)**

Dot Matrix Analysis

- A dot matrix analysis is a method for comparing two sequences to look for possible alignment (Gibbs and McIntyre 1970)
- One sequence (A) is listed across the top of the matrix and the other (B) is listed down the left side
- Starting from the first character in B, one moves across the page keeping in the first row and placing a dot in many column where the character in A is the same
- The process is continued until all possible comparisons between A and B are made
- Any region of similarity is revealed by a diagonal row of dots
- Isolated dots not on diagonal represent random matches



Dot view – Indels


NOD_sequence (horizontal) vs. ref_sequence (vertical)

Dot view – Tandem repeats

Smith-Waterman Algorithm

- **Only works effectively when gap penalties are used**
- **In example shown**
  - **match = +1**
  - **mismatch = -1/3**
  - **gap = -1+1/3k (k=extent of gap)**
- **Start with all cell values = 0**
- **Looks in subcolumn and subrow shown and in direct diagonal for a score that is the highest when you take alignment score or gap penalty into account**

	C	A	G	C	C	T	C	G	C	T	T	A	G
A	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.0
A	0.0	1.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.7
T	0.0	0.0	0.8	0.3	0.0	0.0	0.0	0.0	0.0	1.0	1.0	0.0	0.7
G	0.0	0.0	1.0	0.3	0.0	0.0	0.7	1.0	0.0	0.0	0.7	0.7	1.0
C	1.0	0.0	0.0	2.0	1.3	0.3	1.0	0.3	2.0	0.7	0.3	0.3	0.3
C	1.0	0.7	0.0	1.0	3.0	1.7	?						
A													
T													
T													
G													
A													
C													
G													
G													

Local alignment score H = 3.0

A--GCC
ATGCC

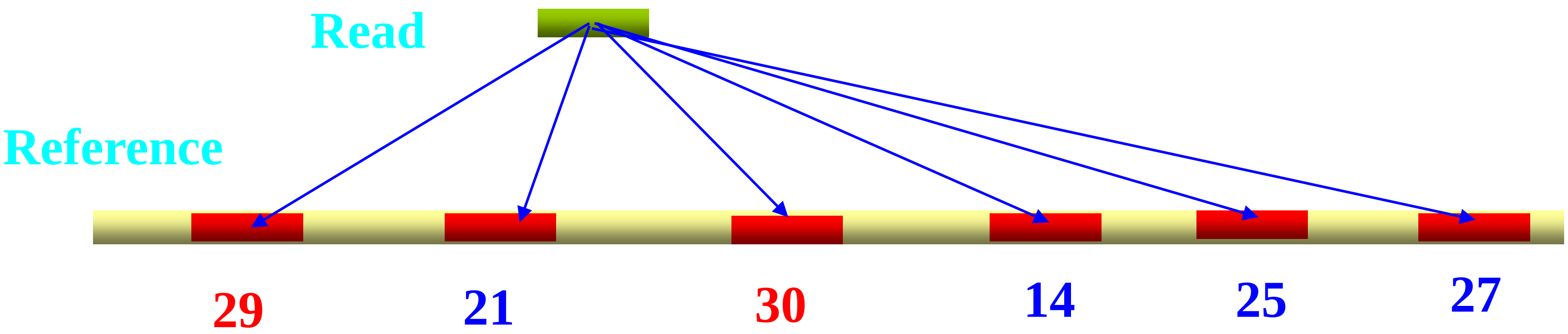
$$H_{ij}=\max\{H_{i-1,\,j-1}+s(a_i,b_j),\ \max\{H_{i-k,j}-W_k\},\ \max\{H_{i,\,j-l}-W_l\},\ 0\}$$

Mapping Score in Short Read Alignment

Read mapping score is used to assess the repetitive feature of the read in the genome. With a maximum mapping score 50, we have:

$$S_{map} = \begin{matrix} 10*(S_{\max} - S_{\max 2}) & if\,(S_{map} <= 50) \\ 50 & if\,(S_{map} > \ 50) \end{matrix}$$

S_{max} - maximum alignment score (smith-waterman) of the hits on genome; S_{max2} - second best alignment score of the hits on genome; Say you have one read of 30 bases which has a few hits on the genome: Best hit: exact match with S_{max} 30; Second best hit: one base mismatch with S_{max2} 29. The mapping score for this read is S_{map} = 10;



Short Read Alignment Tools

Bfast

BioScope

Bowtie

BWA

CLC bio

CloudBurst

Eland/Eland2

GenomeMapper

GnuMap

Karma

MAQ

MOM

Mosaik

MrFAST/MrsFAST

NovoAlign

PASS

PerM

RazerS

RMAP

Smalt

SSAHA2

Segemehl

SeqMap

SHRiMP

Slider/SliderII

SOAP/SOAP2

Srprism

Stampy

vmatch

ZOOM

……

Overview of the BWA algorithm

- **Based on FM-index (Burrows-Wheeler Transform plus auxilliary data structures) which enables fast exact matching.**
- **Short-read algorithm: alter the read sequence such that it matches the reference exactly.**
- **Long-read algorithm (BWA-SW): sample reference subsequences and perform Smith-Waterman alignment between the subsequences and the read.**
- **Work for Illumina and SOLiD single-end (SE) and paired-end (PE) reads; new component BWA-SW for 454/Sanger SE reads.**

Key Features

- □ Fast and moderate memory (<4GB)
- □ SAM output by default
- □ Gapped alignment for both SE and PE reads
- □ Effective pairing to achieve high alignment accuracy; suboptimal hits considered in pairing.
- □ Non-unique read is placed randomly with a mapping quality 0; all hits can be outputted in a concise format.
- □ The default configuration works for most typical input

  -Automatically adjust parameters based on read lengths and error rates.

  -Estimate the insert size distribution

Running BWA

- Input: ref.fa, read1.fq, read2.fq and long-read.fq
- Step 1: Index the genome (3 CPU hours for the human genome):

```
bwa index -a is ref.fa
```

- Step 2a: Generate alignments in the suffix array coordinate:

```
bwa aln ref.fa read1.fq > read1.sai
bwa aln ref.fa read2.fq > read2.sai
```

- Step 3a: Generate alignments in the SAM format:

```
bwa sampe ref.fa read1.sai read2.sai read1.fq read2.fq > aln.sam
```

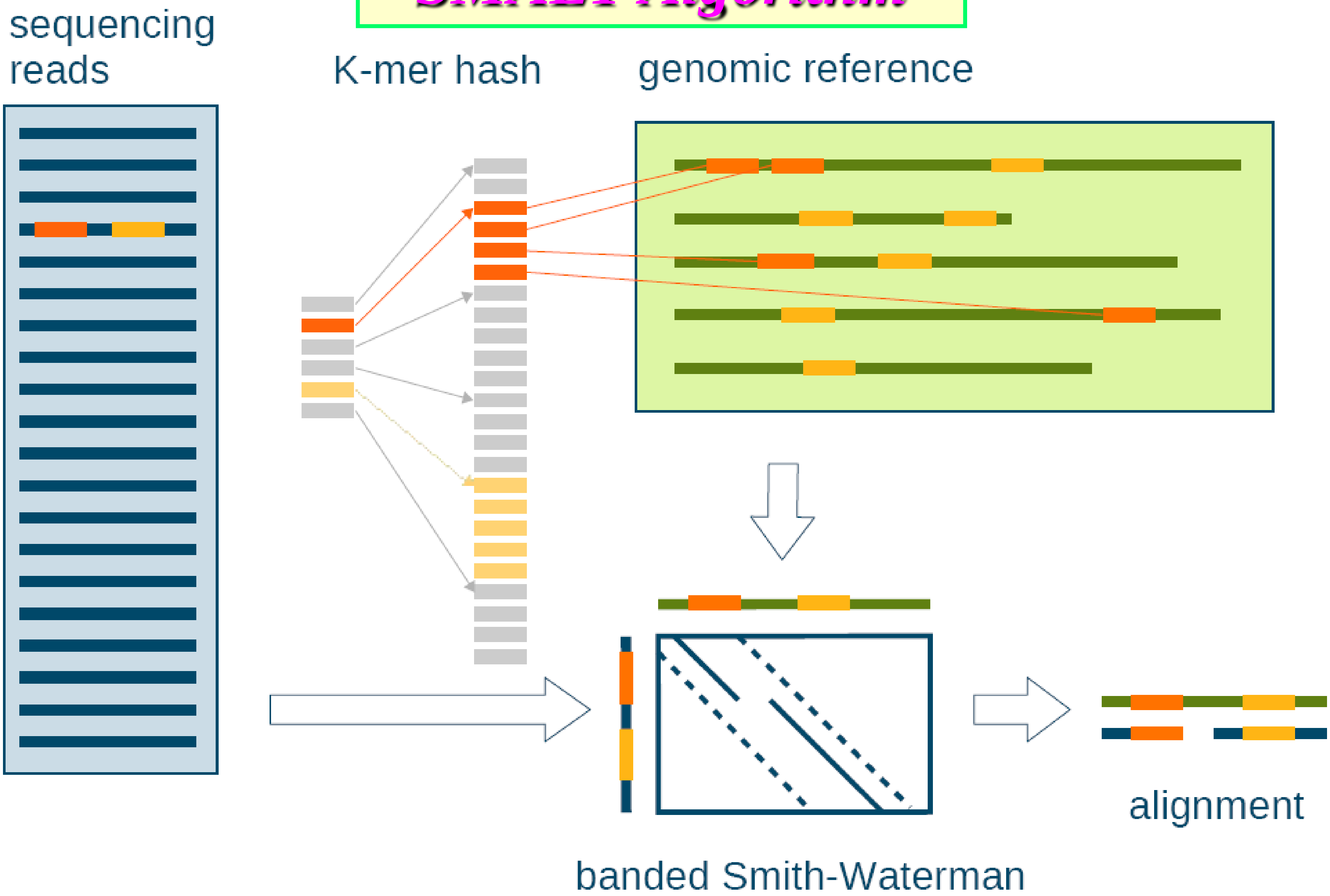
- Step 4a: Get multiple hits:

```
bwa samse -n 100 ref.fa read1.sai read1.fq
```

- Step 2b: Use BWA-SW for long reads:

```
bwa bwasw ref.fa long-read.fq > aln-long.sam
```

SMALT Algorithm



Non-overlap Hashing v Overlap Hashing

ATGGCGTGCAGTCCATGTTCGGATCA

ATGGCGTGCAGT

TGGCGTGCAGTC

GGCGTGCAGTCC

GCGTGCAGTCCA

CGTGCAGTCCAT

Overlap hashing
$W = N-k+1$
($k = 12$)

ATGGCGTGCAGTCCATGTTCGGATCATTACGTAAGC

ATGGGCAGATGT

CCATGTTCGGAT

CATTACGTAAGC

Non-overlap Hashing
$W = N/k$

Sequence Representation

Sequence S: $(s_1 s_2, \ldots, s_i, \ldots, s_m)$ $\quad i = 1, 2, \ldots, m$

K-tuple: $(s_i s_{i+1} \ldots s_{i+k-1})$

Using two binary digits for each base, we may have the following representations:

"A" =00; "C" = 01; "G" = 10; "T" = 11

For any of the m/k no-overlapping k-tuples in the sequence, an integer may be used to represent the k-tuple in a unique way

$$E = \sum_{i=1}^{2k} \beta_i 2^{i-1} \quad \text{with } E_{\max} = 2^{2\mathrm{k}} \text{ -1}$$

where β_i = 0 or 1, depending on the value of the sequence base and E_{max} is the maximum value of the possible E values.

***Hash Table**: A 2-tuple hashing table of S1, S2 and S3*

E	*k*-tuple	N_i	Indices and Offsets						
0	AA	1	2, 19						
1	AC	3	1, 9	2, 5	2, 11				
2	AG	2	1, 15	2, 35					
3	AT	2	2, 13	3, 3					
4	CA	7	2, 3	2, 9	2, 21	2, 27	2, 33	3, 21	3, 23
5	CC	4	1, 21	2, 31	3, 5	3, 7			
6	CG	1	1, 5						
7	CT	6	1, 23	2, 39	2, 43	3, 13	3, 15	3, 17	
8	GA	4	1, 3	1, 17	2, 15	2, 25			
9	GC	0							
10	GG	5	1, 25	1, 31	2, 17	2, 29	3, 1		
11	GT	6	1, 1	1, 27	1, 29	2, 1	2, 37	3, 19	
12	TA	1	3, 25						
13	TC	6	1, 7	1, 11	1, 19	2, 23	2, 41	3, 11	
14	TG	3	1, 13	2, 7	3, 9				
15	TT								

S1=(GTGACGTCACTCTGAGGATCCCCTGGGTGTGG)
S2=(GTCAACTGCAACATGAGGAACATCGACAGGCCCAAGGTCTTCCT)
S3=(GGATCCCCTGTCCTCTCTGTCACATA)

Query sequence: S_q = *(TGCAACAT)*

E	*k*-tuple	N_i	Indices and Offsets						
0	AA	1	2, 19						
1	AC	3	1, 9	2, 5	2, 11				
2	AG	2	1, 15	2, 35					
3	AT	2	2, 13	3, 3					
4	CA	7	2, 3	2, 9	2, 21	2, 27	2, 33	3, 21	3, 23
5	CC	4	1, 21	2, 31	3, 5	3, 7			
6	CG	1	1, 5						
7	CT	6	1, 23	2, 39	2, 43	3, 13	3, 15	3, 17	
8	GA	4	1, 3	1, 17	2, 15	2, 25			
9	GC	0							
10	GG	5	1, 25	1, 31	2, 17	2, 29	3, 1		
11	GT	6	1, 1	1, 27	1, 29	2, 1	2, 37	3, 19	
12	TA	1	3, 25						
13	TC	6	1, 7	1, 11	1, 19	2, 23	2, 41	3, 11	
14	TG	3	1, 13	2, 7	3, 9				
15	TT								

Query sequence:

$S_q = (TGCAACAT)$

Array of index and offset data

k-tuples	*f(t)*	*F(t)*	*-(t-1)*	$F_s(t)$
TG	1, 13	1, 13	0	1, 5
	2, 7	2, 7	0	1, 13
	3, 9	3, 9	0	2, -2
GC			-1	
CA	2, 3	2, 1	-2	2, 1
	2, 9	2, 7	-2	2, 1
	2, 21	2, 19	-2	2, 4
	2, 27	2, 25	-2	2, 7
	2, 33	2, 31	-2	2, 7
	3, 21	3, 19	-2	2, 7
	3, 23	3, 21	-2	2, 7
AA	2, 19	2, 16	-3	2, 16
AC	1, 9	1, 5	-4	2, 16
	2, 5	2, 1	-4	2, 19
	2, 11	2, 7	-4	2, 21
CA	2, 3	2, -2	-5	2, 25
	2, 9	2, 4	-5	2, 28
	2, 21	2, 16	-5	2, 31
	2, 27	2, 22	-5	3, -3
	2, 33	2, 28	-5	3, 9
	3, 21	3, 16	-5	3, 16
	3, 23	3, 18	-5	3, 18
AT	2, 13	2, 7	-6	3, 19
	3, 3	3, -3	-6	3, 21

Running SMALT

- Data files: genome_ref.fa, read1.fastq, read2.fastq
- Hash the reference genome:

  smalt index –k 13 –s 6 hash_ref genome_ref.fa

- Generate alignments in the SAM format:

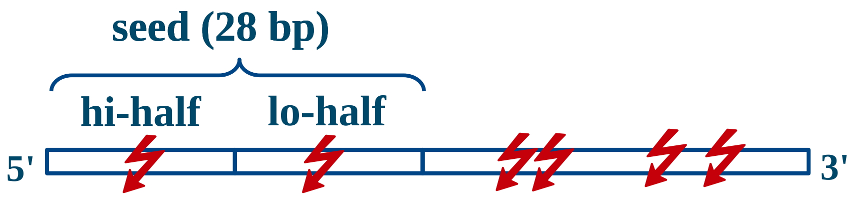
  smalt map -i 800 –j 20 –o aln.sam -f samsoft hash_ref read1.fastq read2.fq

- Where to download:

  http://www.sanger.ac.uk/resources/software/smalt/

Burrows-Wheeler *vs* Hashing

BOWTIE/TOPHAT



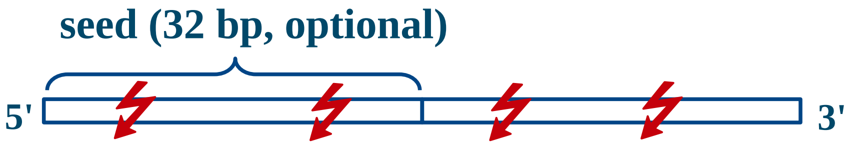
seed (28 bp)

hi-half lo-half

5' 3'

depth-1st by default, breadth-1st slower
no indels

BWA

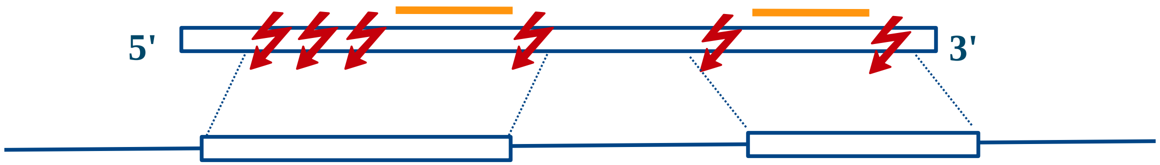


seed (32 bp, optional)

5' 3'

breadth first,
upper bound on edit distance, e.g. max 5 mismatches in 100bp read. Can deal with indels.

SMALT/SSAHA2



5' 3'

Exact matching k-segment (1 kmer) required.
Partial alignments (indels, splice junctions)

Burrows-Wheeler *vs* Hashing

- Strengths and weaknesses (trends)
  - Burrows-Wheeler, e.g. bwa, bowtie
    - Fast, esp. (multiple) exact matches
    - High sensitivity at repetitive regions
    - less robust at high genomic variation
  - Hashing (overlapping k-mer words, e.g SMALT/SSAHA2, Stampy)
    - Slower (more memory hungry)
    - Less sensitivity at repetitive regions
    - tolerate high genomic variation
    - partial alignments (junction reads) easier
    - Flexible (multiple sequencing platforms)

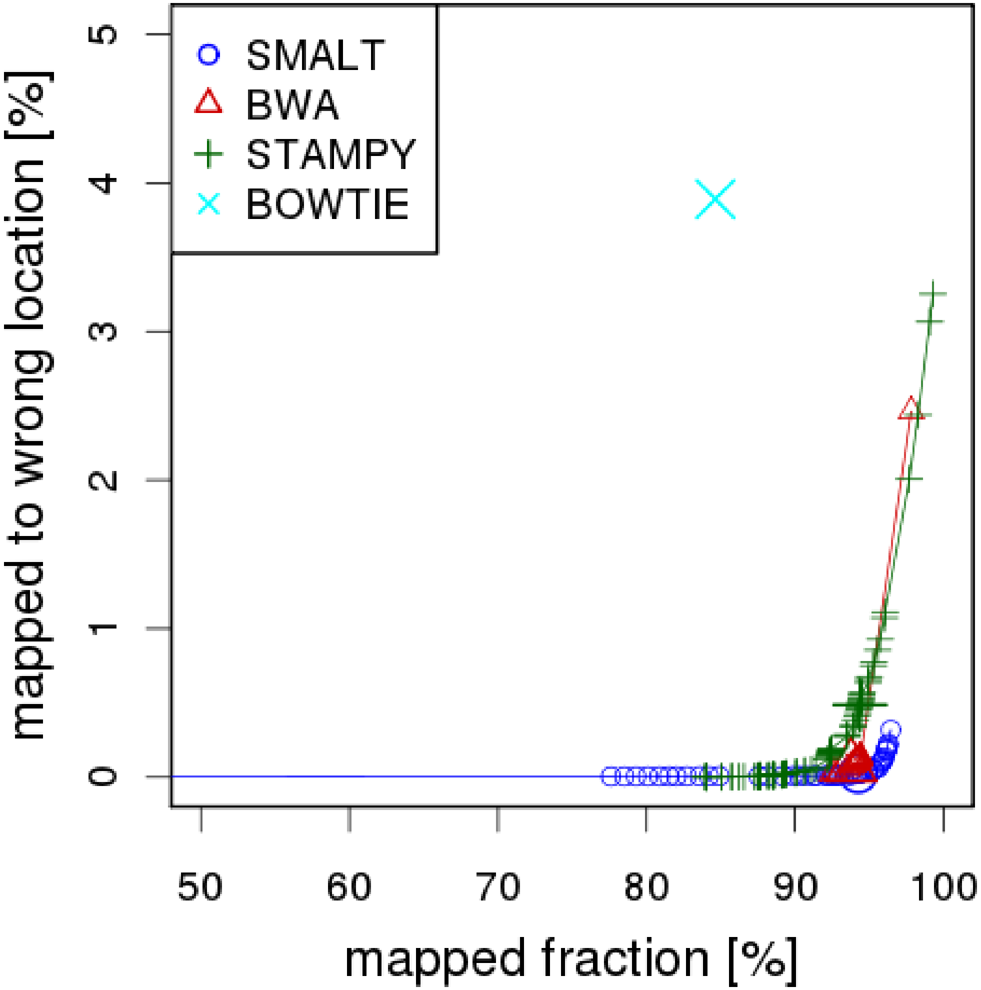
Performance Assessment

on simulated reads

	SSAHA2			SMALT			BWA		
variation	1%	2%	5%	1%	2%	5%	1%	2%	5%
rate [10^6 pairs/h]	0.34	0.22	0.18	0.71	0.60	0.47	1.35	0.74	0.63
memory [GB]	3.8	3.8	3.8	3.3	3.3	3.3	2.3	2.3	2.3
mapped [%]	97.3	97.2	96.1	97.1	97.0	96.5	95.6	89.1	48.1
error [%]	0.09	0.16	0.49	0.08	0.14	0.44	0.09	0.17	0.41

human genome
10^5 read pairs 2 x 100 bp (insert 500)
20% of variations indels (max. 10)

Performance of mappers
(genome re-sequencing)

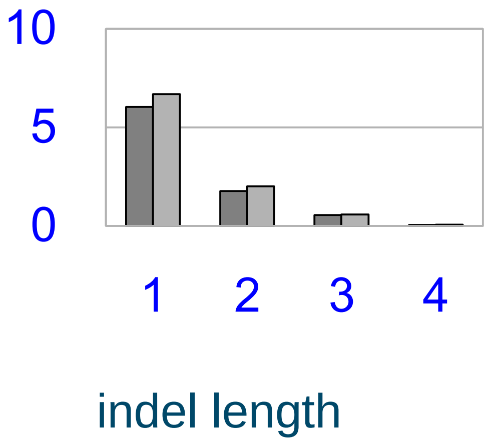


Simulated for human genome:

4×10^6 x 100 bp single reads

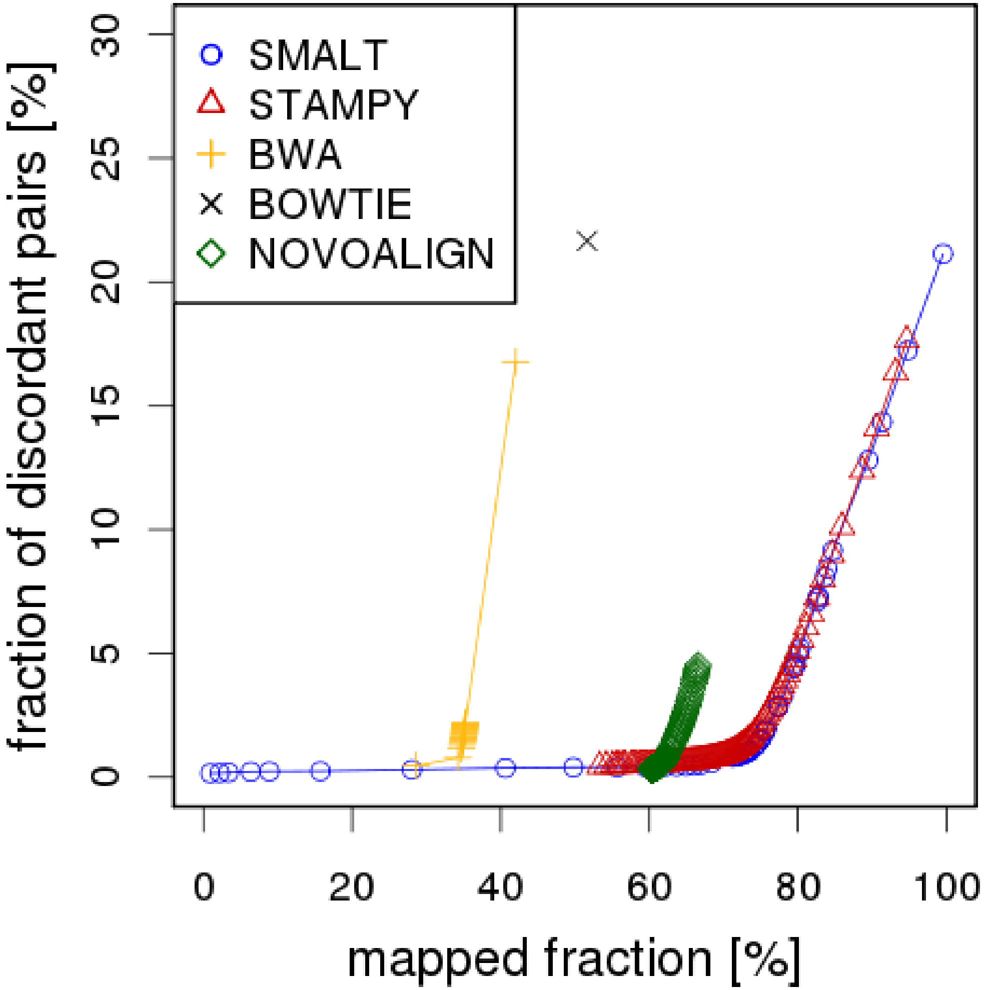
1% variation of which 20% indels

14 bp maximum indel length



Sensitivity Assessment

~ 2% genomic variation

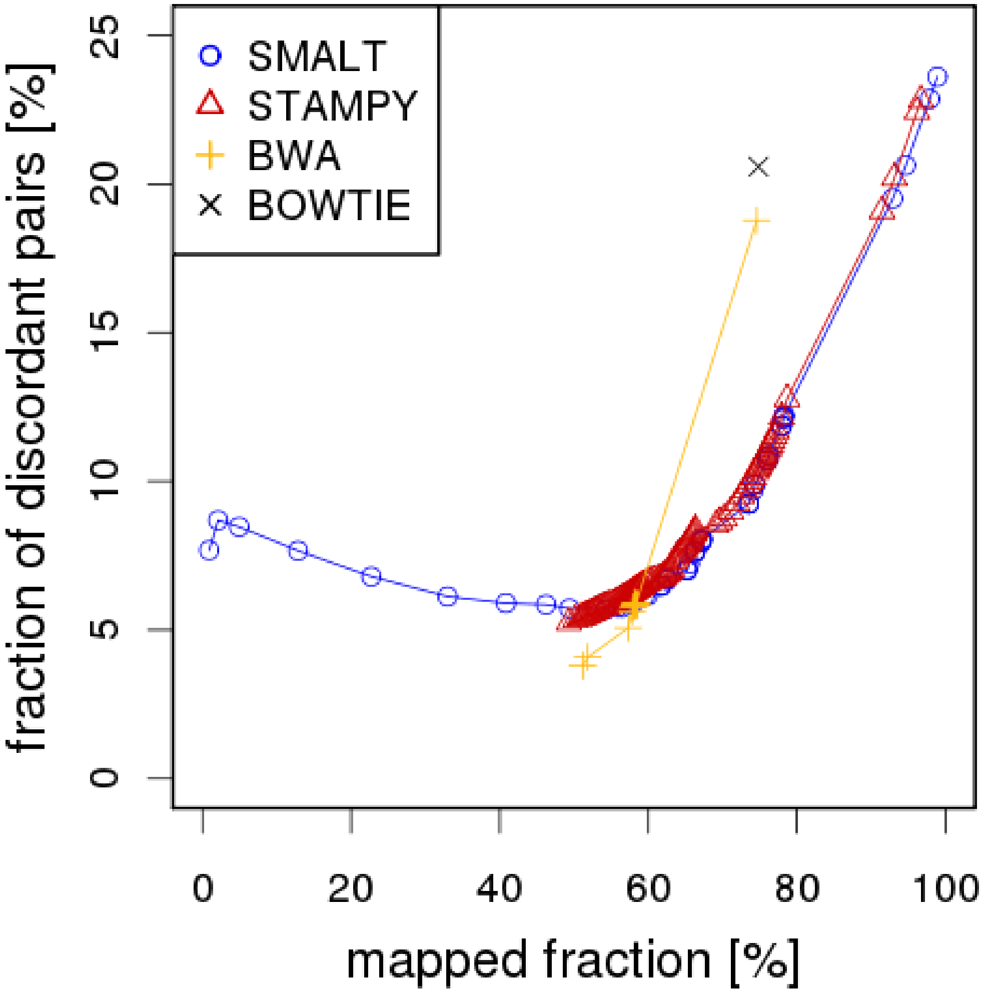


Reads:
M. spretus
whole genome shotgun
2 x 108 bp, insert 250 bp
Reference:
M. musculus
NCBI build 37

- **independently mapped reads**
- **Count discordant pairs as erroneous mappings**

Sensitivity Assessment

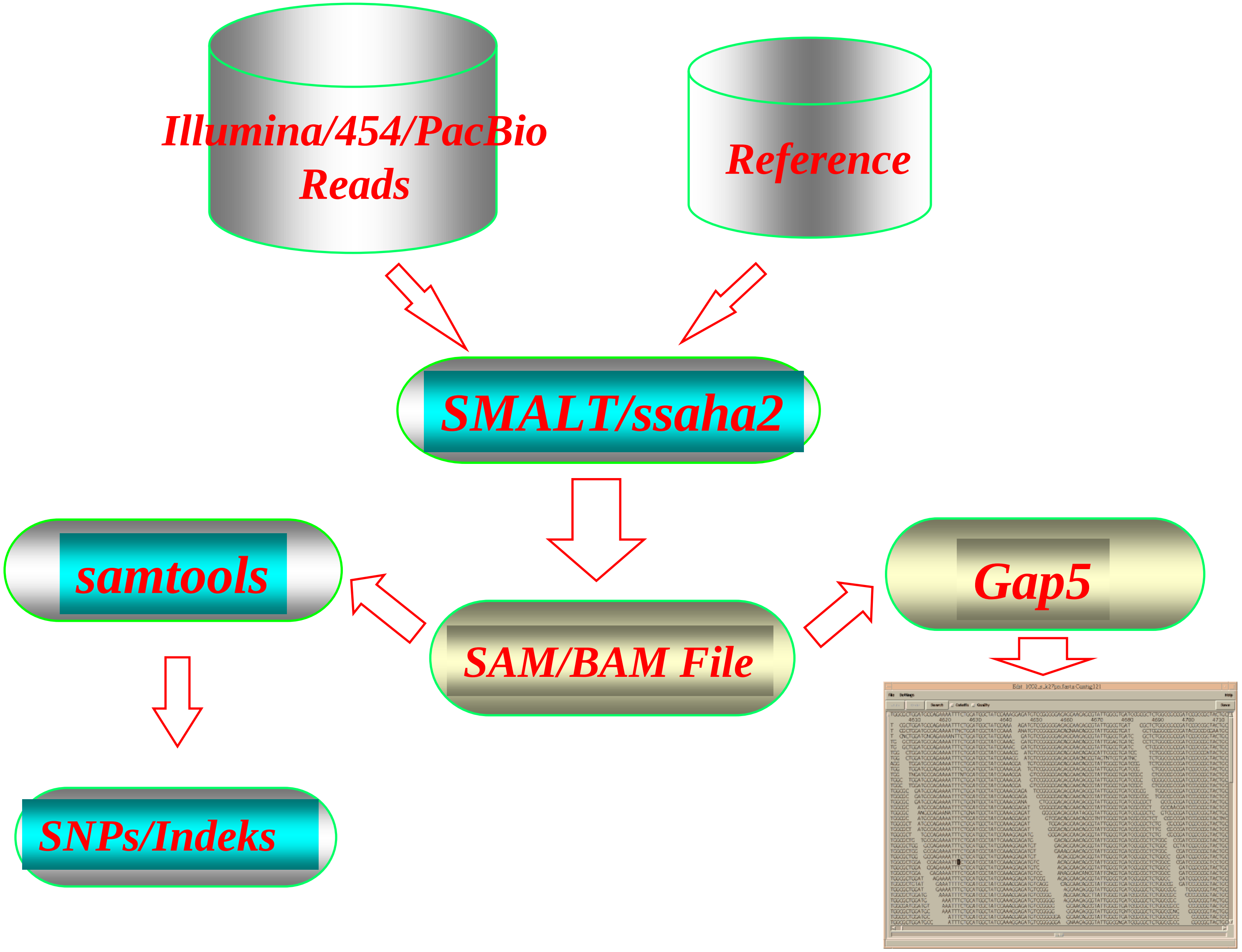
RNA-Seq data



Reads:
M. musculus C57B6
RNA-Seq data
2 x 76 bp, insert 320 bp
Reference:
M. musculus
NCBI build 37

- **independently mapped reads**
- **count discordant pairs (> 10 kbp apart) as erroneous mappings**

SMALT Workflow



Illumina/454/PacBio Reads

Reference

SMALT/ssaha2

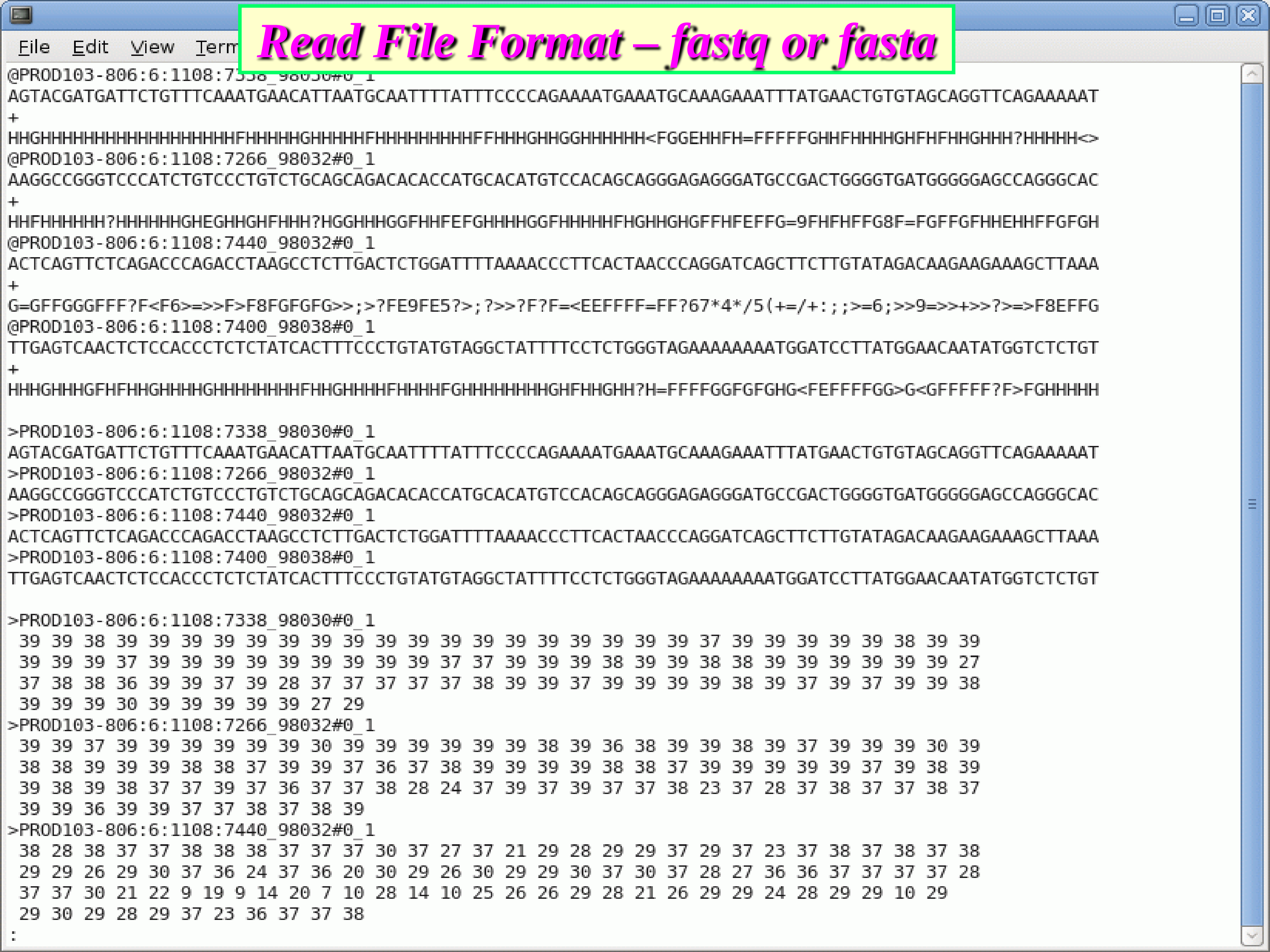
SAM/BAM File

samtools

SNPs/Indeks

Gap5

Read File Format – fastq or fasta



```
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AGTACGATGATTCTGTTTCAAATGAACATTAATGCAATTTTATTTCCCCAGAAAATGAAATGCAAAGAAATTTATGAACTGTGTAGCAGGTTCAGAAAAAT
+
HHGHHHHHHHHHHHHHHHHHHFHHHHHGHHHHHFHHHHHHHHHHFFHHHGHHGGHHHHHH<FGGEHHFH=FFFFFGHHFHHHHGHFHFHHGHHH?HHHHH<>
@PROD103-806:6:1108:7266_98032#0_1
AAGGCCGGGTCCCATCTGTCCCTGTCTGCAGCAGACACACCATGCACATGTCCACAGCAGGGAGAGGGATGCCGACTGGGGTGATGGGGGAGCCAGGGCAC
+
HHFHHHHHH?HHHHHHGHEGHHGHFHHH?HGGHHHGGFHHFEFGHHHHGGFHHHHHFHGHHGHGFFHFEFFG=9FHFHFFG8F=FGFFGFHHEHHFFGFGH
@PROD103-806:6:1108:7440_98032#0_1
ACTCAGTTCTCAGACCCAGACCTAAGCCTCTTGACTCTGGATTTTAAAACCCTTCACTAACCCAGGATCAGCTTCTTGTATAGACAAGAAGAAAGCTTAAA
+
G=GFFGGGFFF?F<F6>=>>F>F8FGFGFG>>;>?FE9FE5?>;?>>?F?F=<EEFFFF=FF?67*4*/5(+=/+:;;>=6;>>9=>>+>>?>=>F8EFFG
@PROD103-806:6:1108:7400_98038#0_1
TTGAGTCAACTCTCCACCCTCTCTATCACTTTCCCTGTATGTAGGCTATTTTCCTCTGGGTAGAAAAAAAAATGGATCCTTATGGAACAATATGGTCTCTGT
+
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>PROD103-806:6:1108:7338_98030#0_1
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>PROD103-806:6:1108:7440_98032#0_1
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 39 39 38 39 39 39 39 39 39 39 39 39 39 39 39 39 39 39 39 39 39 37 39 39 39 39 39 38 39 39
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 38 28 38 37 37 38 38 38 37 37 37 30 37 27 37 21 29 28 29 29 37 29 37 23 37 38 37 38 37 38
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 29 30 29 28 29 37 23 36 37 37 38
:
```

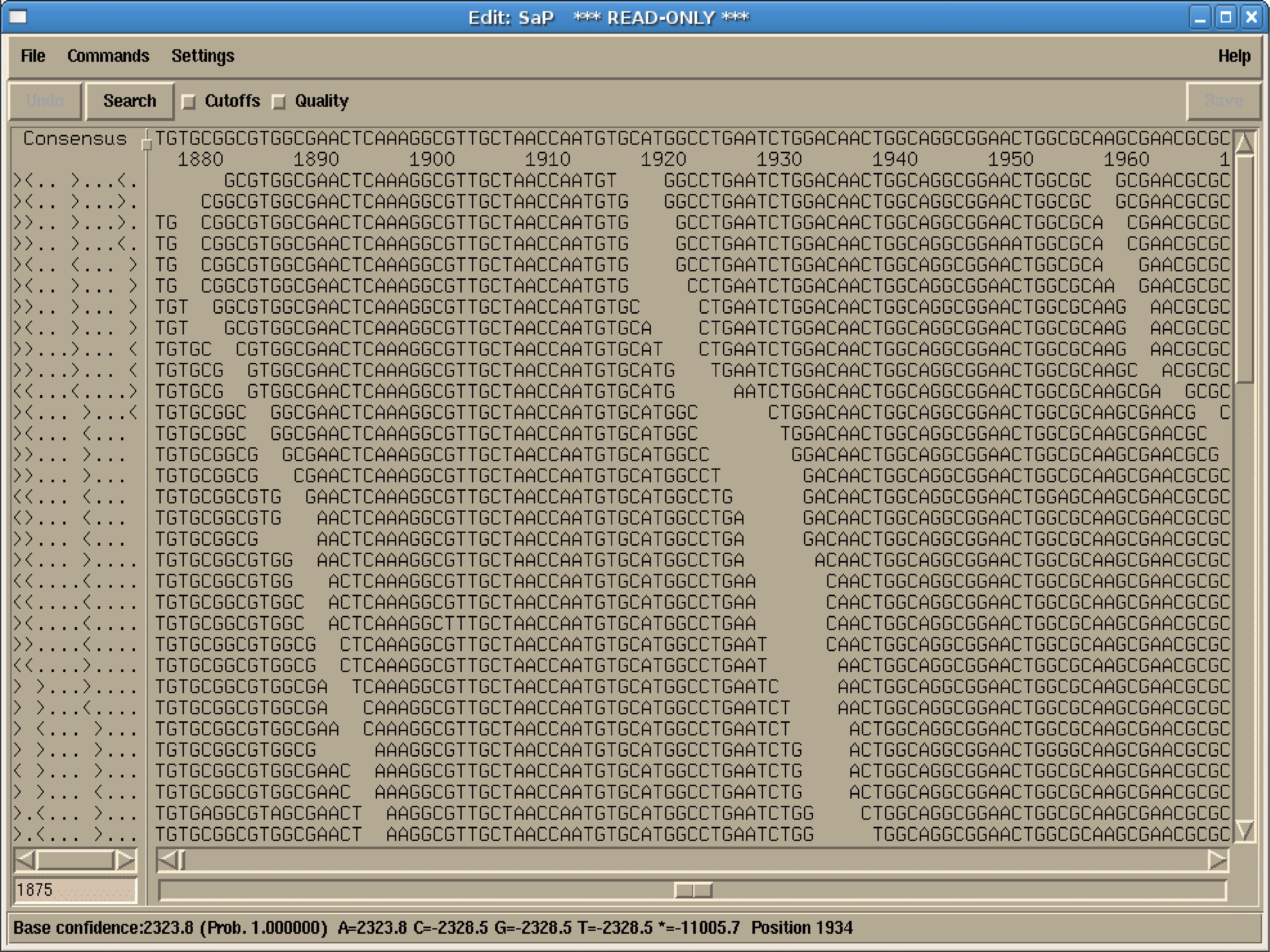

```
 Consensus  TGTGCGGCGTGGCGAACTCAAAGGCGTTGCTAACCAATGTGCATGGCCTGAATCTGGACAACTGGCAGGCGGAACTGGCGCAAGCGAACGCGC
              1880      1890      1900      1910      1920      1930      1940      1950      1960      1
><.. >...<.       GCGTGGCGAACTCAAAGGCGTTGCTAACCAATGT    GGCCTGAATCTGGACAACTGGCAGGCGGAACTGGCGC  GCGAACGCGC
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1875

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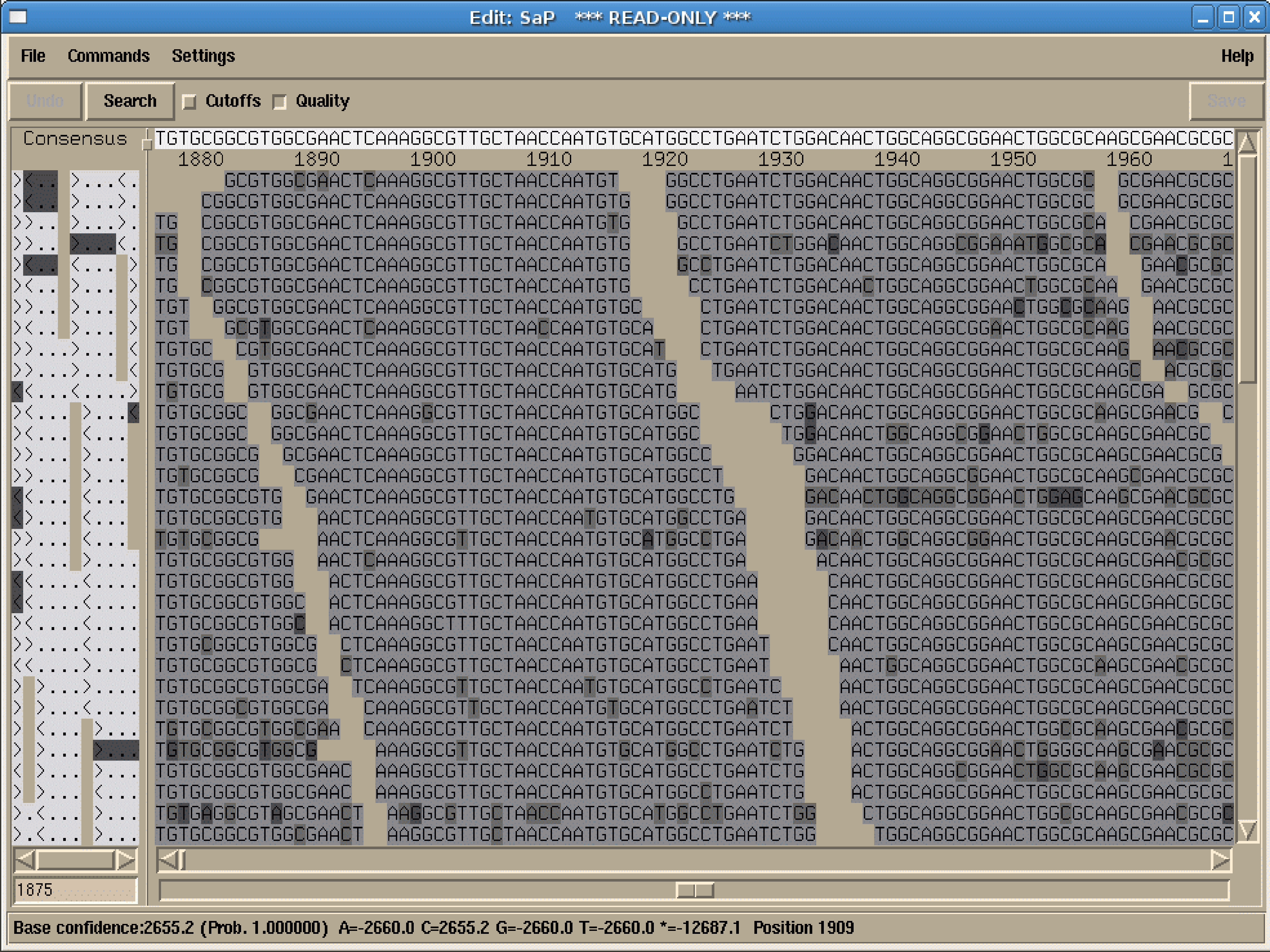

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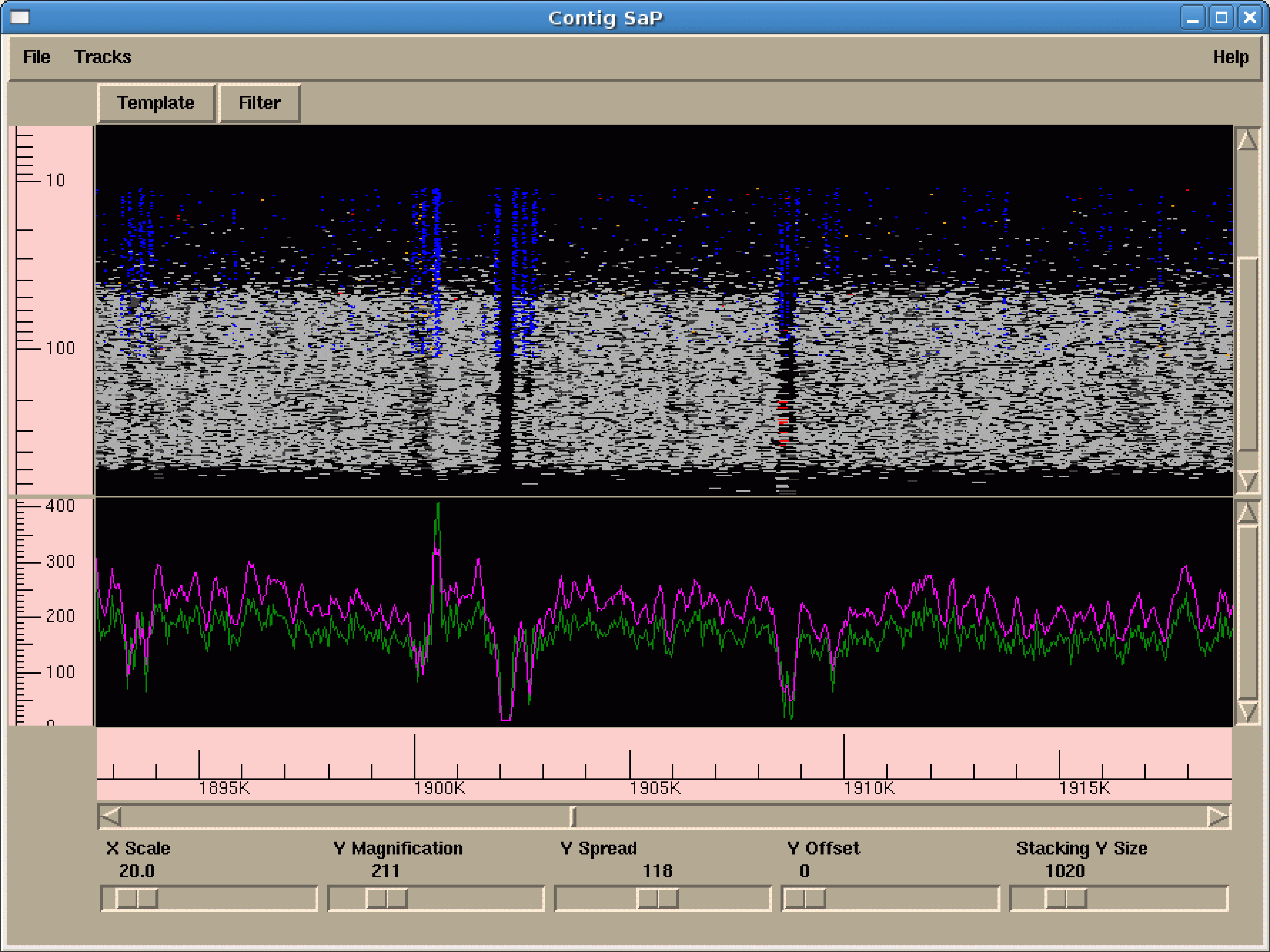

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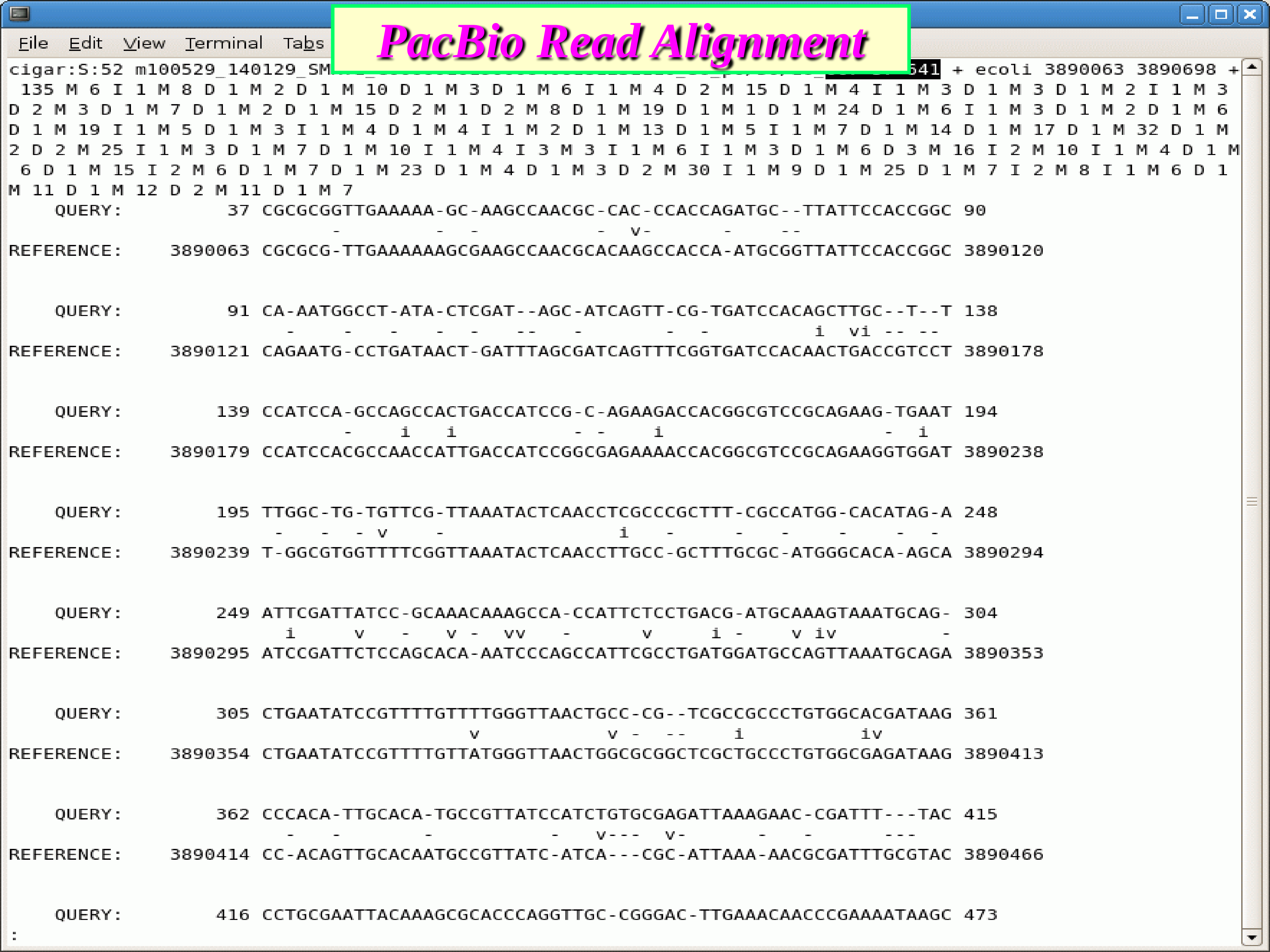

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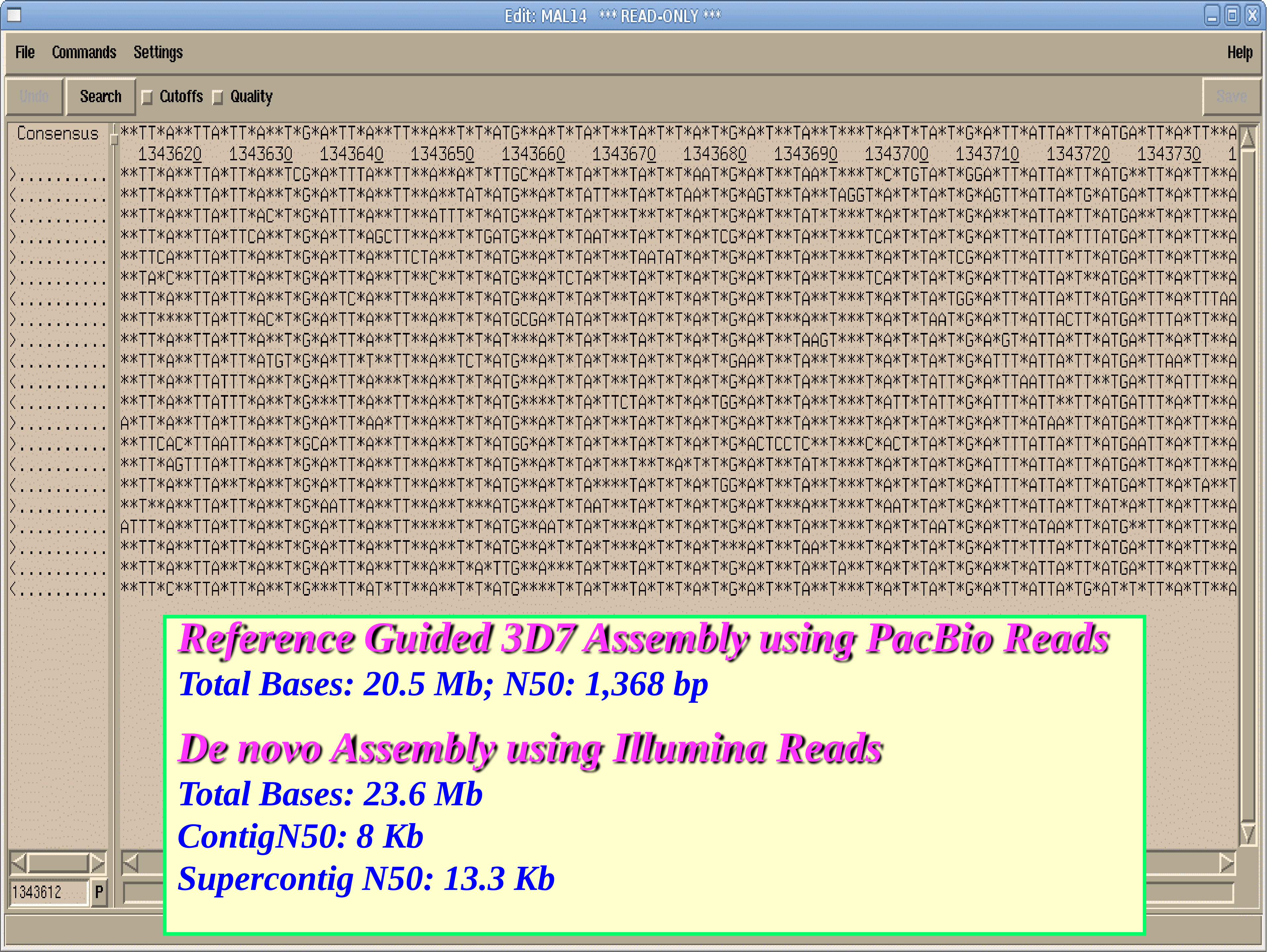

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Reference Guided 3D7 Assembly using PacBio Reads

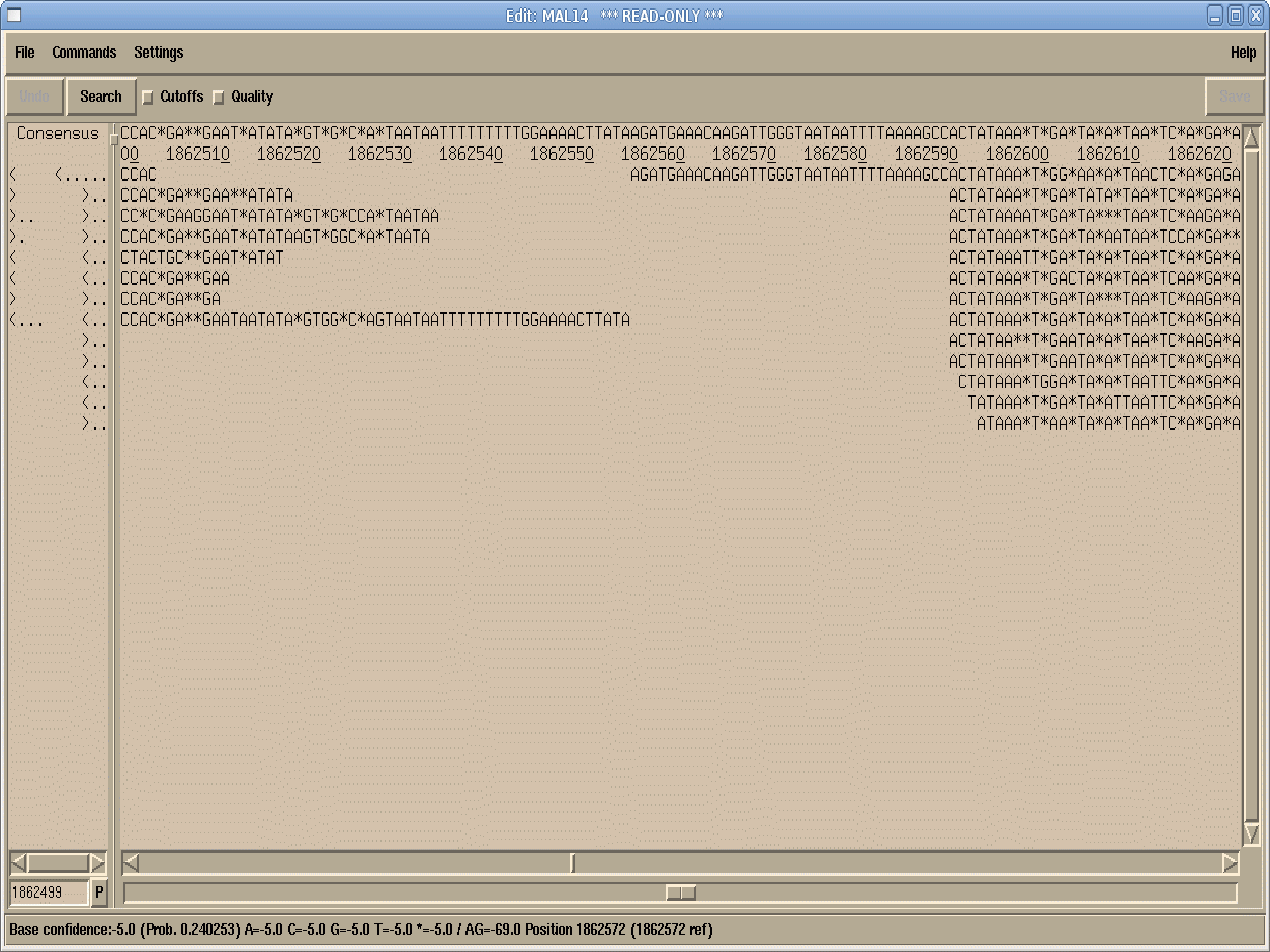
Total Bases: 20.5 Mb; N50: 1,368 bp

De novo Assembly using Illumina Reads

Total Bases: 23.6 Mb

ContigN50: 8 Kb

Supercontig N50: 13.3 Kb



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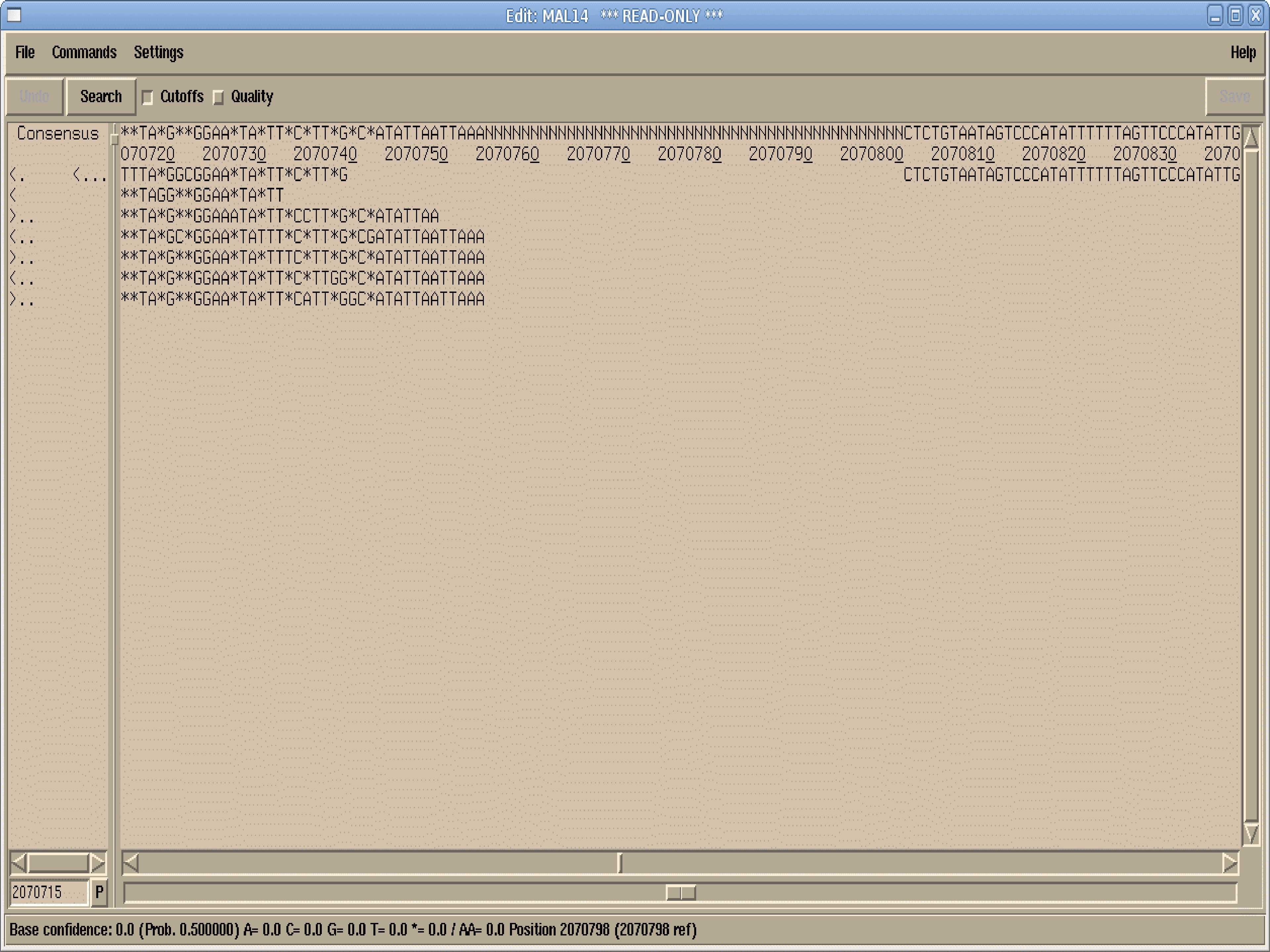
1862499

Base confidence:-5.0 (Prob. 0.240253) A=-5.0 C=-5.0 G=-5.0 T=-5.0 *=-5.0 / AG=-69.0 Position 1862572 (1862572 ref)



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<           **TAGG**GGAA*TA*TT
>..         **TA*G**GGAAATA*TT*CCTT*G*C*ATATTAA
<..         **TA*GC*GGAA*TATTT*C*TT*G*CGATATTAATTAAA
>..         **TA*G**GGAA*TA*TTTC*TT*G*C*ATATTAATTAAA
<..         **TA*G**GGAA*TA*TT*C*TTGG*C*ATATTAATTAAA
>..         **TA*G**GGAA*TA*TT*CATT*GGC*ATATTAATTAAA
```

```
2070715
```

```
Base confidence: 0.0 (Prob. 0.500000) A= 0.0 C= 0.0 G= 0.0 T= 0.0 *= 0.0 / AA= 0.0 Position 2070798 (2070798 ref)
```

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