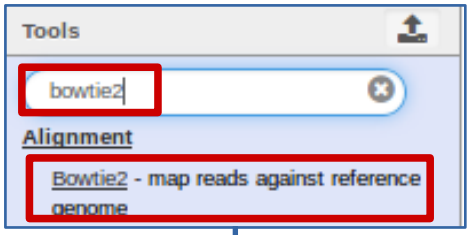
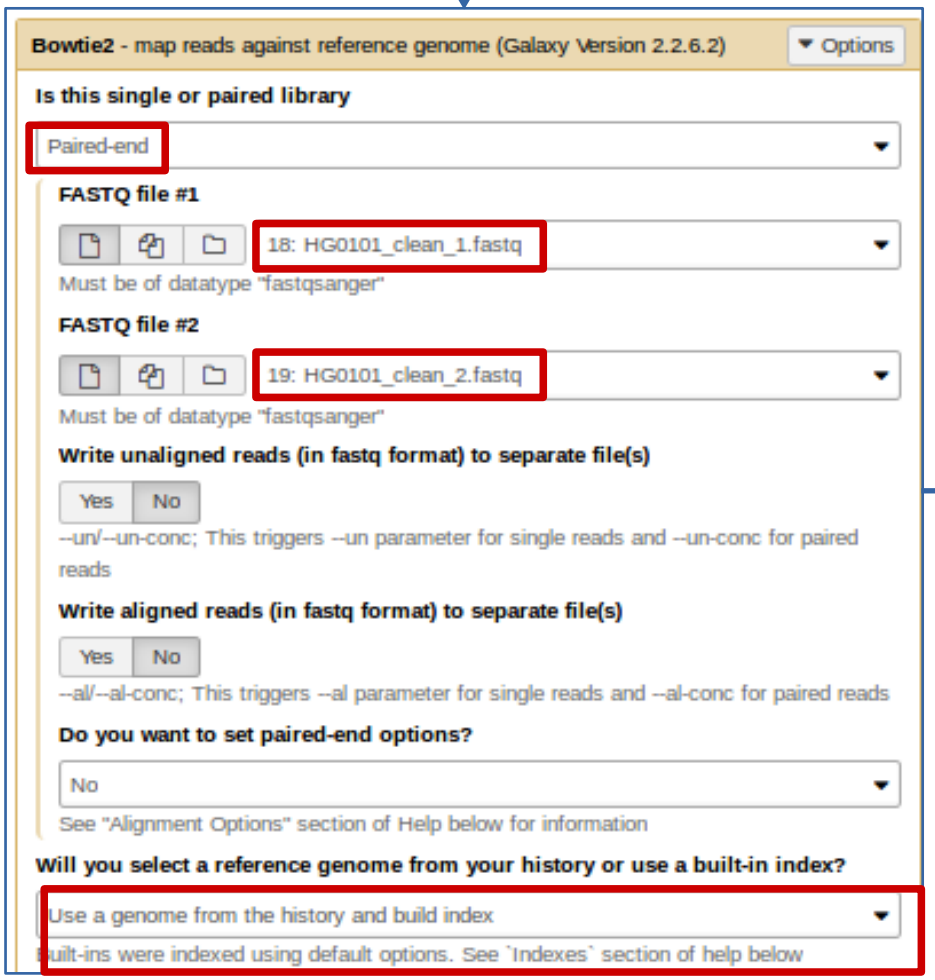
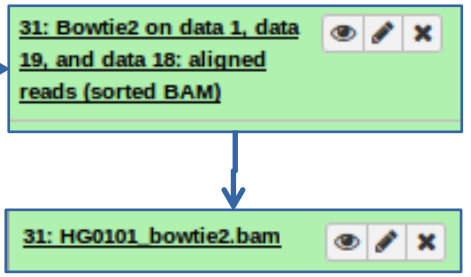
Alignment with bowtie2

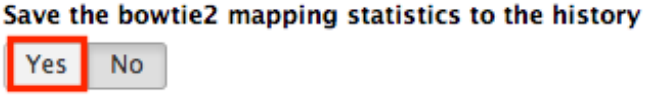




GRCh37_region1.fasta

150 000nt, chr20





Bowtie2: Trying presets

Do you want to use presets?

- ⊘ No, just use defaults sensitive end-to-end
- ● Very fast end-to-end (--very-fast)
- ○ Fast end-to-end (--fast)
- ○ Sensitive end-to-end (--sensitive)
- ● Very sensitive end-to-end (--very-sensitive)
- ● Very fast local (--very-fast-local)
- ○ Fast local (--fast-local)
- ○ Sensitive local (--sensitive-local)
- ○ Very sensitive local (--very-sensitive-local)

Allow selecting among several preset parameter settings. Choosing between these will result in dramatic changes in runtime. See help below to understand effects of these presets.

Very fast end-to-end

```
4065 reads; of these:
  4065 (100.00%) were paired; of these:
          26 (0.64%) aligned concordantly 0 times
          4002 (98.45%) aligned concordantly exactly 1 time
           37 (0.91%) aligned concordantly >1 times
----
26 pairs aligned concordantly 0 times; of these:
          4 (15.38%) aligned discordantly 1 time
----
22 pairs aligned 0 times concordantly or discordantly; of these:
          44 mates make up the pairs; of these:
          23 (52.27%) aligned 0 times
          19 (43.18%) aligned exactly 1 time
          2 (4.55%) aligned >1 times
99.72% overall alignment rate
```

Very sensitive end-to-end

4065 reads; of these:
  4065 (100.00%) were paired; of these:
        25 (0.62%) aligned concordantly 0 times
        4000 (98.40%) aligned concordantly exactly 1 time
        40 (0.98%) aligned concordantly >1 times
 ----
25 pairs aligned concordantly 0 times; of these:
        4 (16.00%) aligned discordantly 1 time
 ---- 21 pairs aligned 0 times concordantly or discordantly; of these:
        42 mates make up the pairs; of these:
        18 (42.86%) aligned 0 times
        18 (42.86%) aligned exactly 1 time
        6 (14.29%) aligned >1 times
99.78% overall alignment rate

Very fast local

```
4065 reads; of these:
  4065 (100.00%) were paired; of these:
        78 (1.92%) aligned concordantly 0 times
        3858 (94.91%) aligned concordantly exactly 1 time
        129 (3.17%) aligned concordantly >1 times
        ----
        78 pairs aligned concordantly 0 times; of these:
          3 (3.85%) aligned discordantly 1 time
        ----
        75 pairs aligned 0 times concordantly or discordantly; of these:
        150 mates make up the pairs; of these:
        79 (52.67%) aligned 0 times
        60 (40.00%) aligned exactly 1 time
        11 (7.33%) aligned >1 times
99.03% overall alignment rate
```

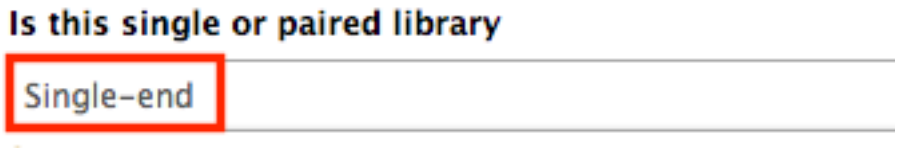
Bowtie2: Playing with advanced options

Read file: tweak_single_end.fq

Three reads, single-end

Same reference

Default parameters



How many alignments do you find?

Mapping statistics

3 reads; of these:

3 (100.00%) were unpaired; of these:

2 (66.67%) aligned 0 times

0 (0.00%) aligned exactly 1 time

1 (33.33%) aligned >1 times

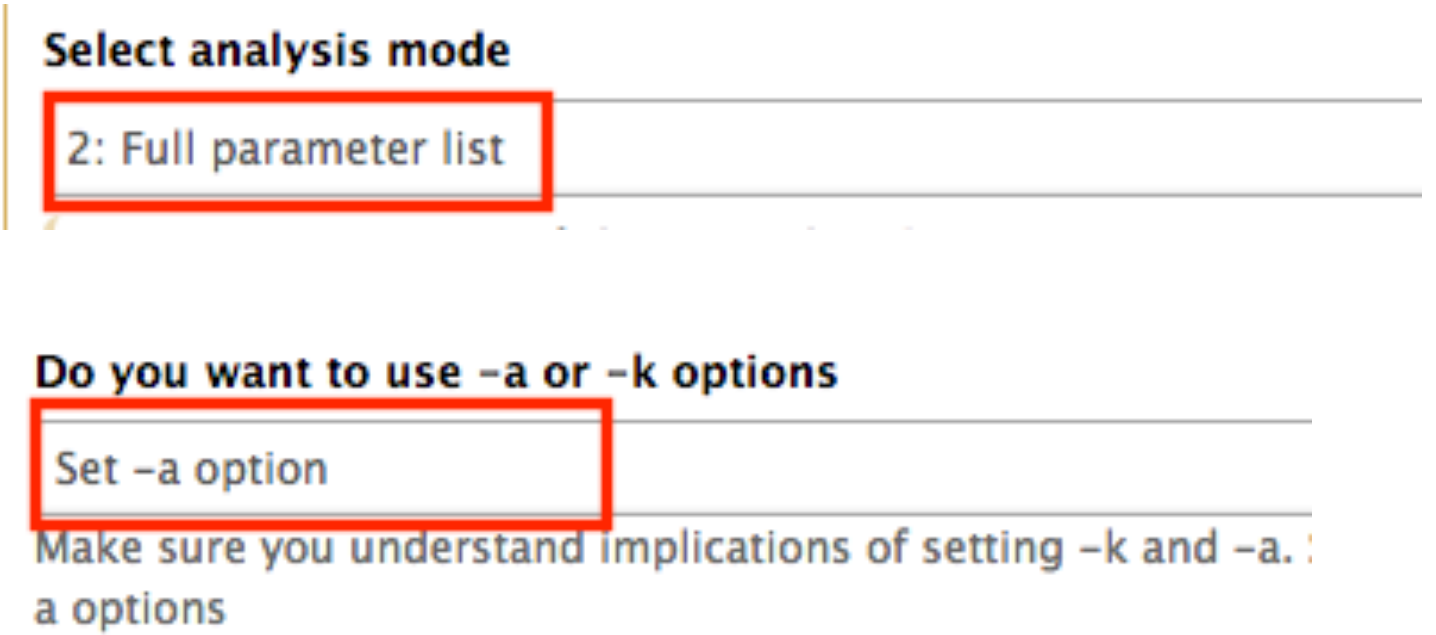
33.33% overall alignment rate

SAM file (first columns)

read2	0	chr20	73116	1	77M	…
read1	4	*	0	0	*	…
read3	4	*	0	0	*	…

QNAME FLAG RNAME POS MAPQ CIGAR

Option -a (all alignments)



SAM file (first columns)

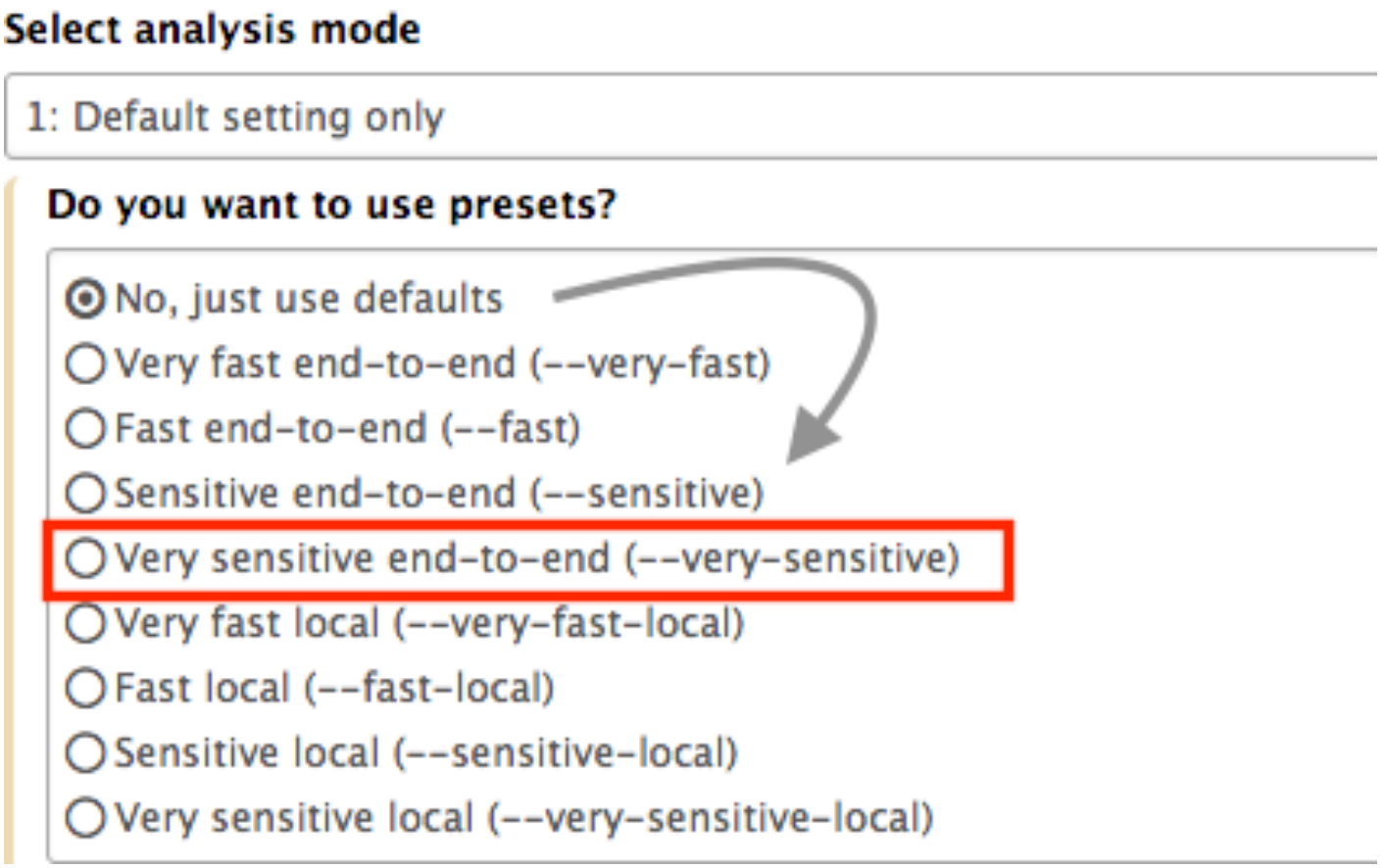
read2	256	chr20	68543	1	77M	…
read2	0	chr20	73116	1	77M	…
read1	4	*	0	0	*	…
read3	4	*	0	0	*	…

Read 2 : 77 nt, two alignments

```
      |68550    |68560    |68570    |68580    |68590    |68600    |68610
GATGAAAGGAGTACTCAGATACAGATATCCAGTGAAAGAGCAGGATAGGGGACTGCCAGCACTAGGGGCCGAAGAGA
||||||||||||||||||:|||||||||||||||:|||||||||||||||||||||||||||||||||||||||||
GATGAAAGGAGTACTCAGACACAGATATCCAGTGAGAGAGCAGGATAGGGGACTGCCAGCACTAGGGGCCGAAGAGA
         |10       |20       |30       |40       |50       |60       |70
```

```
    |73120    |73130    |73140    |73150    |73160    |73170    |73180
GATGAAAGGAGTACTCAGACACAGATATTCAGTGAGAGAGCAGGCTAGGGGACTGCCAGCACTAGGGGCCGAAGAGA
|||||||||||||||||||||||||:|||||||||||||||.||||||||||||||||||||||||||||||||||
GATGAAAGGAGTACTCAGACACAGATATCCAGTGAGAGAGCAGGATAGGGGACTGCCAGCACTAGGGGCCGAAGAGA
         |10       |20       |30       |40       |50       |60       |70
```

Sensitivity



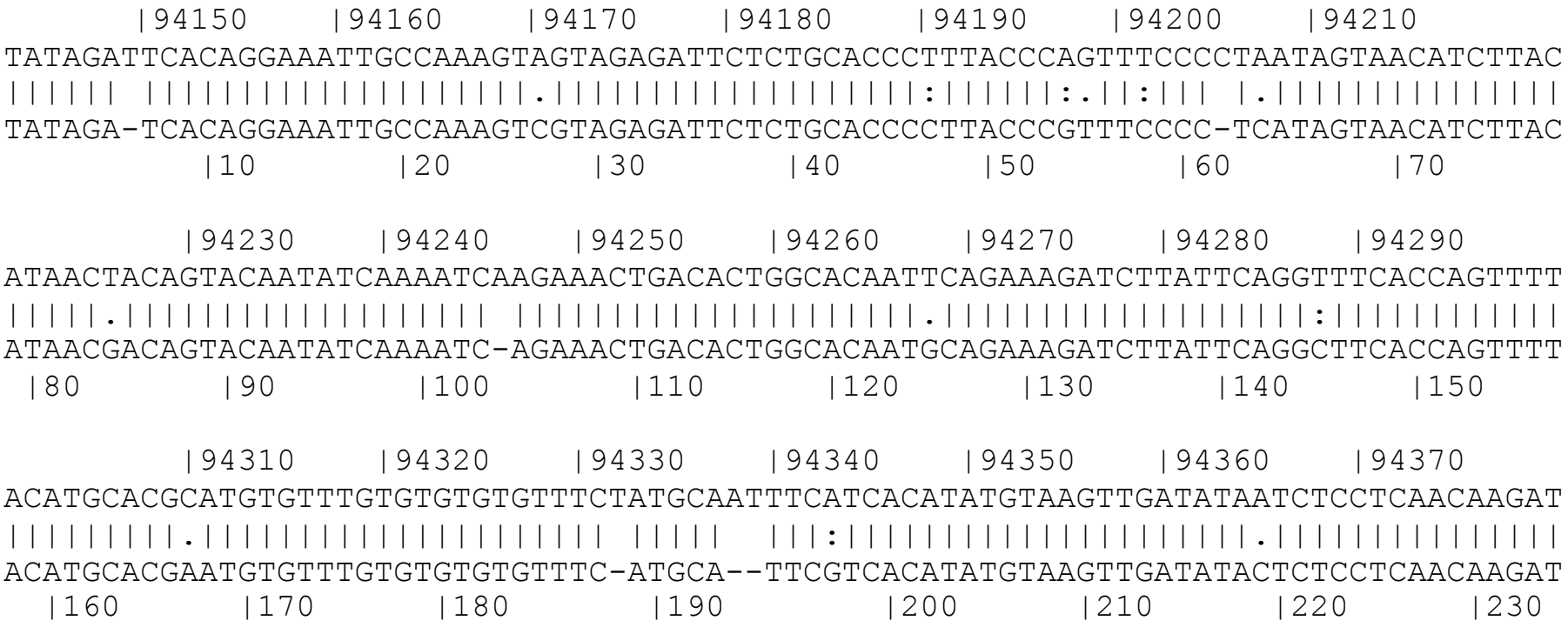
Mapping statistics

3 reads; of these:
    3 (100.00%) were unpaired; of these:
        1 (33.33%) aligned 0 times
        1 (33.33%) aligned exactly
        1 time
        1 (33.33%) aligned >1 times
66.67% overall alignment rate

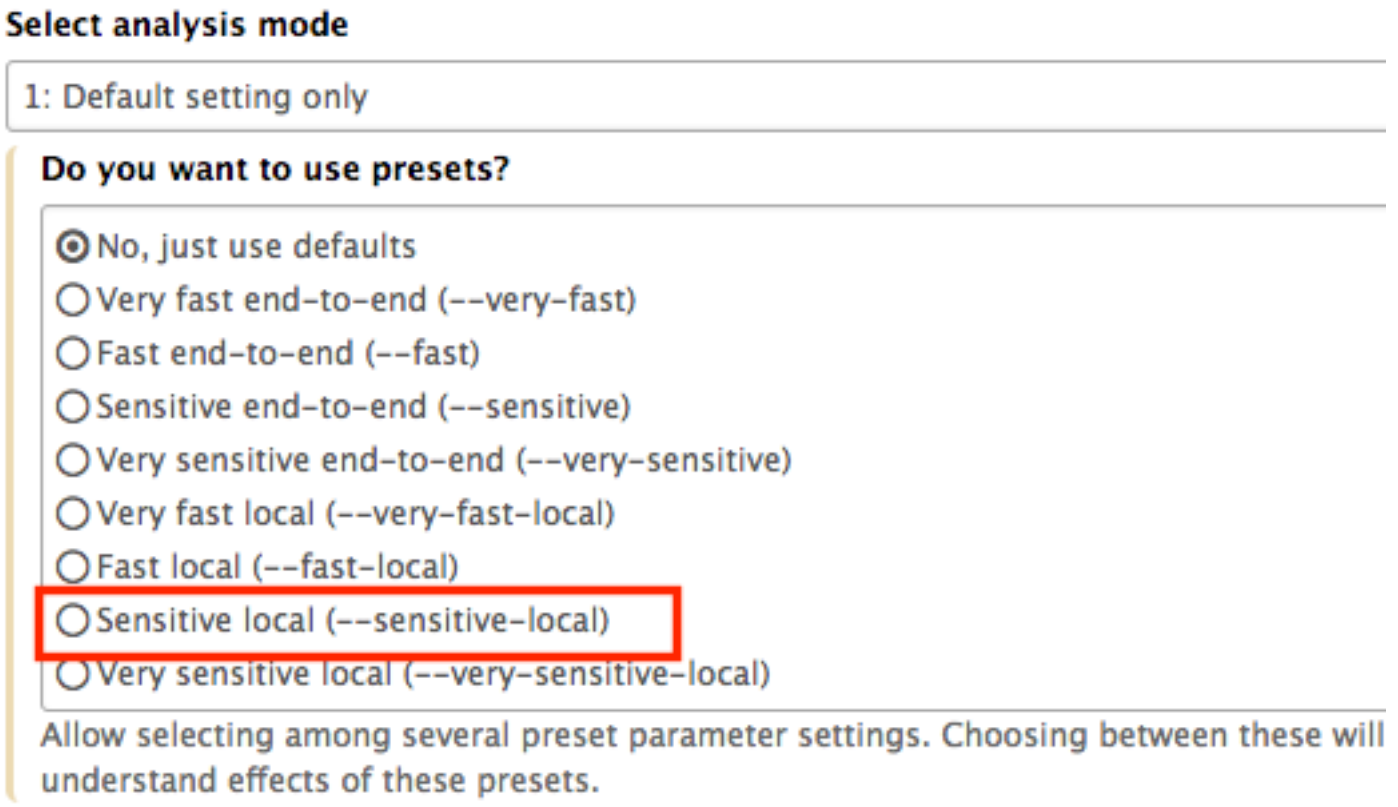
SAM file (first columns)

```
read2   0    chr20   68543     1  77M
read3   0    chr20   94141    23  50M1D85M1D5M2D41M
read1   4      *       0       0   *
```

Read 3 : 234 nt


|94150 |94160 |94170 |94180 |94190 |94200 |94210
TATAGATTCACAGGAAATTGCCAAAGTAGTAGAGATTCTCTGCACCCTTTACCCAGTTTCCCCTAATAGTAACATCTTAC
TATAGA-TCACAGGAAATTGCCAAAGTCGTAGAGATTCTCTGCACCCCTTACCCGTTTCCCC-TCATAGTAACATCTTAC
|10 |20 |30 |40 |50 |60 |70
|94230 |94240 |94250 |94260 |94270 |94280 |94290
ATAACTACAGTACAATATCAAAATCAAGAAACTGACACTGGCACAATTCAGAAAGATCTTATTCAGGTTTCACCAGTTTT
ATAACGACAGTACAATATCAAAATC-AGAAACTGACACTGGCACAATGCAGAAAGATCTTATTCAGGCTTCACCAGTTTT
|80 |90 |100 |110 |120 |130 |140 |150
|94310 |94320 |94330 |94340 |94350 |94360 |94370
ACATGCACGCATGTGTTTGTGTGTGTGTTTCTATGCAATTTCATCACATATGTAAGTTGATATAATCTCCTCAACAAGAT
ACATGCACGAATGTGTTTGTGTGTGTGTTTC-ATGCA--TTCGTCACATATGTAAGTTGATATACTCTCCTCAACAAGAT
|160 |170 |180 |190 |200 |210 |220 |230

End-to-end mode versus local mode



Mapping statistics

3 reads; of these:

3 (100.00%) were unpaired; of these:

0 (0.00%) aligned 0 times

2 (66.67%) aligned exactly 1 time

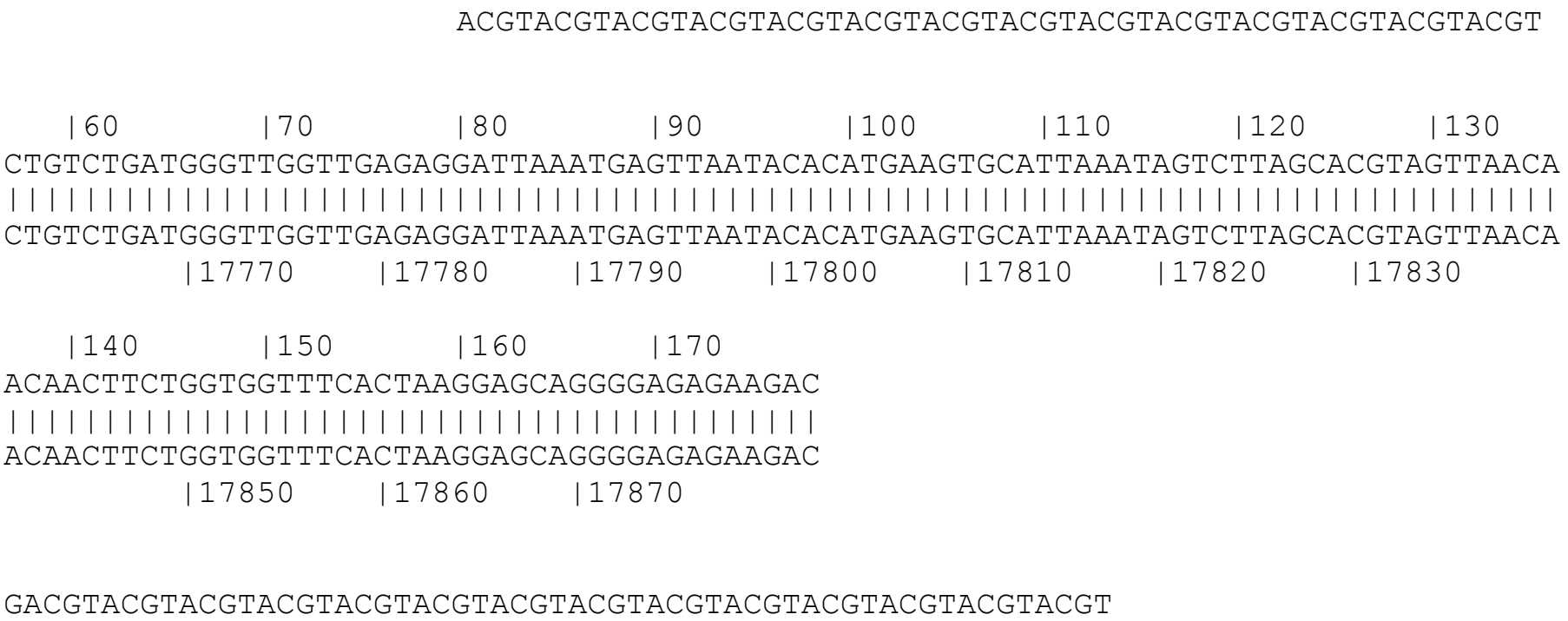
1 (33.33%) aligned >1 times

100.00% overall alignment rate

SAM file (first columns)

```
read1  0  chr20  17761  28  56S122M54S
read2  0  chr20  68543   1  77M
read3  0  chr20  94141  44  6M1D47M1D50M1D85M1D5M2D41M
```

Read 1 : 232 nt


ACGTACGTACGTACGTACGTACGTACGTACGTACGTACGTACGTACGTACGT
|60 |70 |80 |90 |100 |110 |120 |130
CTGTCTGATGGGTTGGTTGAGAGGATTAAATGAGTTAATACACATGAAGTGCATTAAATAGTCTTAGCACGTAGTTAACA
CTGTCTGATGGGTTGGTTGAGAGGATTAAATGAGTTAATACACATGAAGTGCATTAAATAGTCTTAGCACGTAGTTAACA
|17770 |17780 |17790 |17800 |17810 |17820 |17830
|140 |150 |160 |170
ACAACTTCTGGTGGTTTCACTAAGGAGCAGGGGAGAGAAGAC
ACAACTTCTGGTGGTTTCACTAAGGAGCAGGGGAGAGAAGAC
|17850 |17860 |17870
GACGTACGTACGTACGTACGTACGTACGTACGTACGTACGTACGTACGTACGT

Concordant/discordant (paired reads)

concordant_discordant1.fq

concordant_discordant2.fq

Read 1 (2x180nt): 19861 + / 20161 +

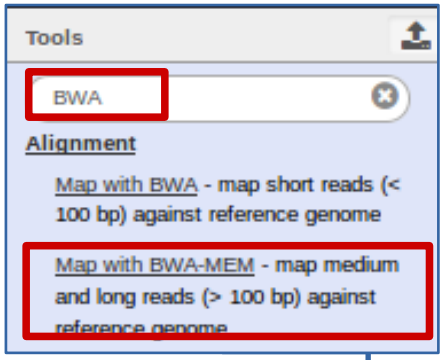
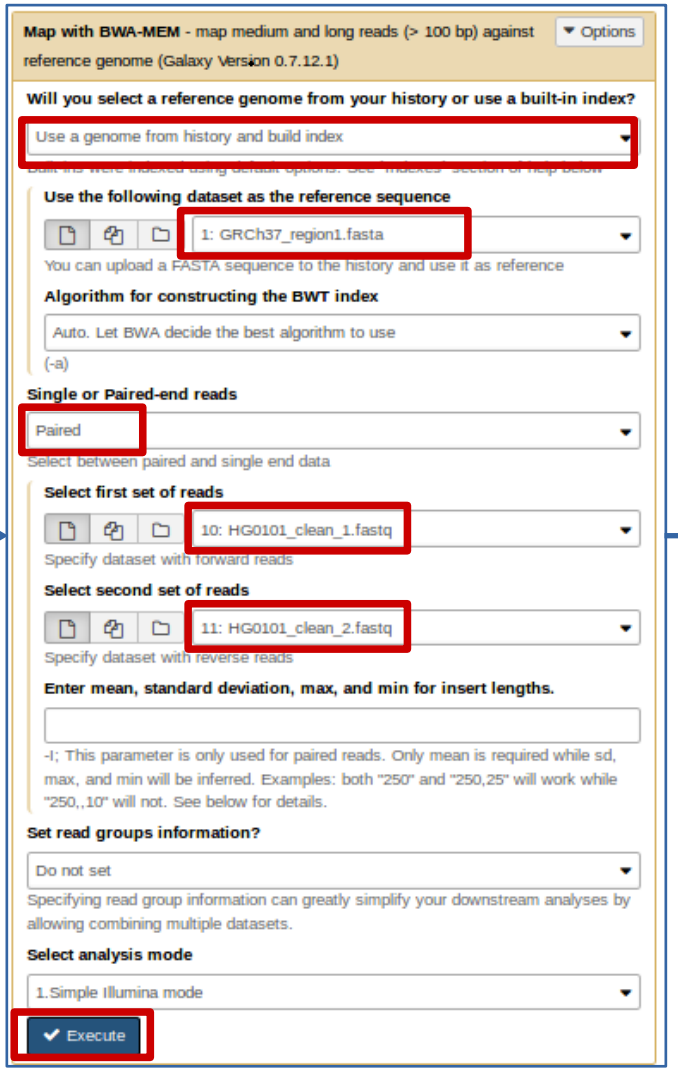
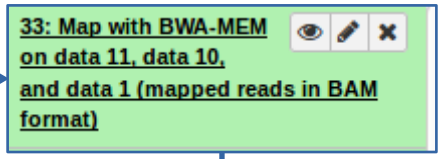
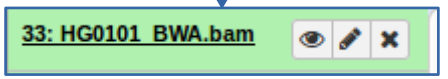
Read 2 (2x180nt): 29341 + / 29641 -

Read 3 (2x180nt): 42301 + / 50401 -

Read4 (2x180nt): / 149341 +

How many alignments do you find ?
Concordant ? Discordant ? Why ?

4 reads; of these:
  4 (100.00%) were paired; of these:
    3 (75.00%) aligned concordantly 0 times
    1 (25.00%) aligned concordantly exactly 1 time
    0 (0.00%) aligned concordantly >1 times
  ----
   3 pairs aligned concordantly 0 times; of these:
     2 (66.67%) aligned discordantly 1 time
  ----
    1 pairs aligned 0 times concordantly or discordantly; of these:
     2 mates make up the pairs; of these:
       1 (50.00%) aligned 0 times
       1 (0.00%) aligned exactly 1 time
       0 (50.00%) aligned >1 times
87.50% overall alignment rate

read1	65	chr20	19861	42	180M
read1	129	chr20	20161	42	180M
read2	99	chr20	29341	42	180M
read2	147	chr20	29641	42	180M
read3	97	chr20	42301	42	180M
read3	145	chr20	50401	42	180M
read4	137	chr20	149341	42	180M
read4	69	chr20	149341	0	*

Alignment with BWA