

Bowtie2: Playing with advanced options

Read file: `tweak_single_end.fq`

Three reads, single-end

Same reference

Default parameters

Is this single or paired library

Single-end

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	...
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read1	4	*	0	0	*	...
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read3	4	*	0	0	*	...
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QNAME	FLAG	RNAME	POS	MAPQ	CIGAR	
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Option -a (all alignments)

Select analysis mode

2: Full parameter list

Do you want to use -a or -k options

Set -a option

Make sure you understand implications of setting -k and -a. :
a options

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
```

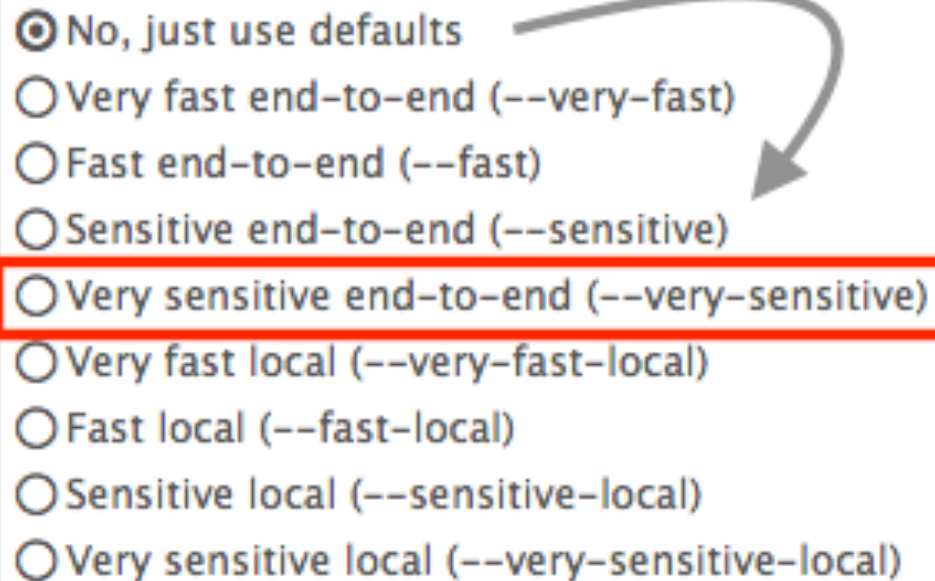
```
      |73120      |73130      |73140      |73150      |73160      |73170      |73180
GATGAAAGGAGTACTCAGACACAGATATTCAGTGAGAGAGCAGGCTAGGGGACTGCCAGCACTAGGGGCCGAAGAGA
|||||:|||||.|||||
GATGAAAGGAGTACTCAGACACAGATATCCAGTGAGAGAGCAGGATAGGGGACTGCCAGCACTAGGGGCCGAAGAGA
      |10       |20       |30       |40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

Sensitivity

Select analysis mode

1: Default setting only

Do you want to use presets?

- No, just use defaults
 - 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)
- 

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-TCACAGGAAATTGCCAAAGTCGTAGAGATTCTCTGCACCCTTACCCGTTTCCCC-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
ACATGCACGCATGTGTTTGTGTGTGTGTTTCTATGCAATTCATCACATATGTAAGTTGATATAATCTCCTCAACAAGAT
|||||.|||||:|:|.|||||
ACATGCACGAATGTGTTTGTGTGTGTGTTT-ATGCA--TTCGTCACATATGTAAGTTGATATACTCCTCAACAAGAT
|160     |170     |180     |190     |200     |210     |220     |230
```

End-to-end mode versus local mode

Select analysis mode

1: Default setting only

Do you want to use presets?

- No, just use defaults
- 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 understand effects of these presets.

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

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	*