

# Alignment with bowtie2

Tools

bowtie2

Alignment

Bowtie2 - map reads against reference genome

Bowtie2 - map reads against reference genome (Galaxy Version 2.2.6.2) Options

Is this single or paired library

Paired-end

FASTQ file #1

18: HG0101\_clean\_1.fastq

FASTQ file #2

19: HG0101\_clean\_2.fastq

Write unaligned reads (in fastq format) to separate file(s)

Yes No

Write aligned reads (in fastq format) to separate file(s)

Yes No

Do you want to set paired-end options?

No

Will you select a reference genome from your history or use a built-in index?

Use a genome from the history and build index

GRCh37\_region1.fasta

150 000nt, chr20

31: Bowtie2 on data 1, data 19, and data 18: aligned reads (sorted BAM)

31: HG0101\_bowtie2.bam

Save the bowtie2 mapping statistics to the history

Yes No

# 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

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	...
read1	4	*	0	0	*	...
read3	4	*	0	0	*	...
QNAME	FLAG	RNAME	POS	MAPQ	CIGAR	



## 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
```

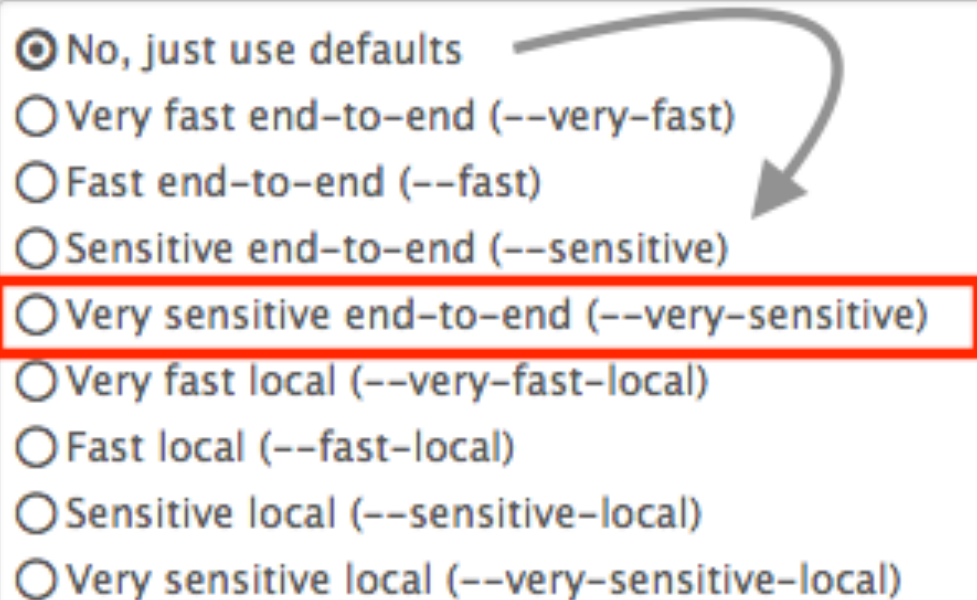
```
      |73120      |73130      |73140      |73150      |73160      |73170      |73180
GATGAAAGGAGTACTCAGACACAGATATTCAGTGAGAGAGCAGGCTAGGGGACTGCCAGCACTAGGGGCCGAAGAGA
|||||:|||||.|||||
GATGAAAGGAGTACTCAGACACAGATATCCAGTGAGAGAGCAGGATAGGGGACTGCCAGCACTAGGGGCCGAAGAGA
      |10       |20       |30       |40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