

Bowtie 2 Aligning Short Reads

When you select the *Tools > Align to reference > Align short reads* item in the main menu, the *Align Sequencing Reads* dialog appears. Set value of the *Align short reads method* parameter to *Bowtie 2*. The dialog looks as follows:

Align Sequencing Reads

Alignment method: **Bowtie2**

Reference sequence:

Result file name:

Library: **Single-end** ☐ Prebuilt index ☒ SAM output

Short reads

Path	Type	Order
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Parameters

Mode: **--end-to-end**

Number of mismatches:

☐ Seed length (--L):

☐ Add columns to allow gaps (--dpad):

☐ Disallow gaps (--gbar):

☐ Seed (--seed):

Flags

☐ No unpaired alignments (--no-mixed)

☐ No discordant alignments (--no-discordant)

☐ No forward orientation (--nofw)

☐ No reverse-complement orientation (--norc)

☐ No overlapping mates (--no-overlap)

☐ No mates containing one another (--no-contain)

Buttons: Add, Remove, Start, Cancel, Help

There are the following parameters:

Reference sequence — DNA sequence to align short reads to. This parameter is required.

Result file name — file in SAM format to write the result of the alignment into. This parameter is required.

Library - single-end or paired-end reads.

Prebuilt index — check this box to use an index file instead of a source reference sequence. The index is a set of 6 files with suffixes .1.ebwt, .2.ebwt, .3.ebwt, .4.ebwt, .rev.1.ebwt, and .rev.2.ebwt. The index is created during the alignment. Also you can [build it manually](#).

SAM output — always save the output file in the SAM format (the option is disabled for *Bowtie*).

Short reads — each added short read is a small DNA sequence file. At least one read should be added.

You can also configure other parameters. They are the same as in the original *Bowtie 2* (you can read detailed description of the parameters on the [Bowtie 2 manual page](#)).

Select one of the following alignment modes:

The `--end-to-end` alignment mode:

By default, Bowtie 2 performs end-to-end read alignment. That is, it searches for alignments involving all of the read characters. This is also called an "untrimmed" or "unclipped" alignment.

When the `--local` option is specified, Bowtie 2 performs local read alignment. In this mode, Bowtie 2 might "trim" or "clip" some read characters from one or both ends of the alignment if doing so maximizes the alignment score.

The following parameters are available:

Number of mismatches (`--N`) — sets the number of mismatches to allowed in a seed alignment during multiseed alignment. Can be set to 0 or 1. Setting this higher makes alignment slower (often much slower) but increases sensitivity.

Seed length (`--L`) — Sets the length of the seed substrings to align during multiseed alignment. Smaller values make alignment slower but more sensitive.

Add columns to allow gaps (`--dpad`) — "Pads" dynamic programming problems by `<int>` columns on either side to allow gaps.

Disallow gaps (`--gbar`) — disallow gaps within `<int>` positions of the beginning or end of the read.

Seed (`--seed`) — use `<int>` as the seed for pseudo-random number generator.

The following flags are available:

No unpaired alignments (`--no-mixed`) — by default, when bowtie2 cannot find a concordant or discordant alignment for a pair, it then tries to find alignments for the individual mates. This option disables that behavior.

No discordant alignments (`--no-discordant`) — by default, bowtie2 looks for discordant alignments if it cannot find any concordant alignments. A discordant alignment is an alignment where both mates align uniquely, but that does not satisfy the paired-end constraints. This option disables that behavior.

No forward orientation (`--nofw`) — if `--nofw` is specified, bowtie2 will not attempt to align unpaired reads to the forward (Watson) reference strand.

No reverse-complement orientation (`--norc`) — if `--norc` is specified, bowtie2 will not attempt to align unpaired reads against the reverse-complement (Crick) reference strand.

No overlapping mates (`--no-overlap`) — if one mate alignment overlaps the other at all, consider that to be non-concordant.

No mates containing one another (`--no-contain`) — if one mate alignment contains the other, consider that to be non-concordant.

Select the required parameters and press the *Start* button.