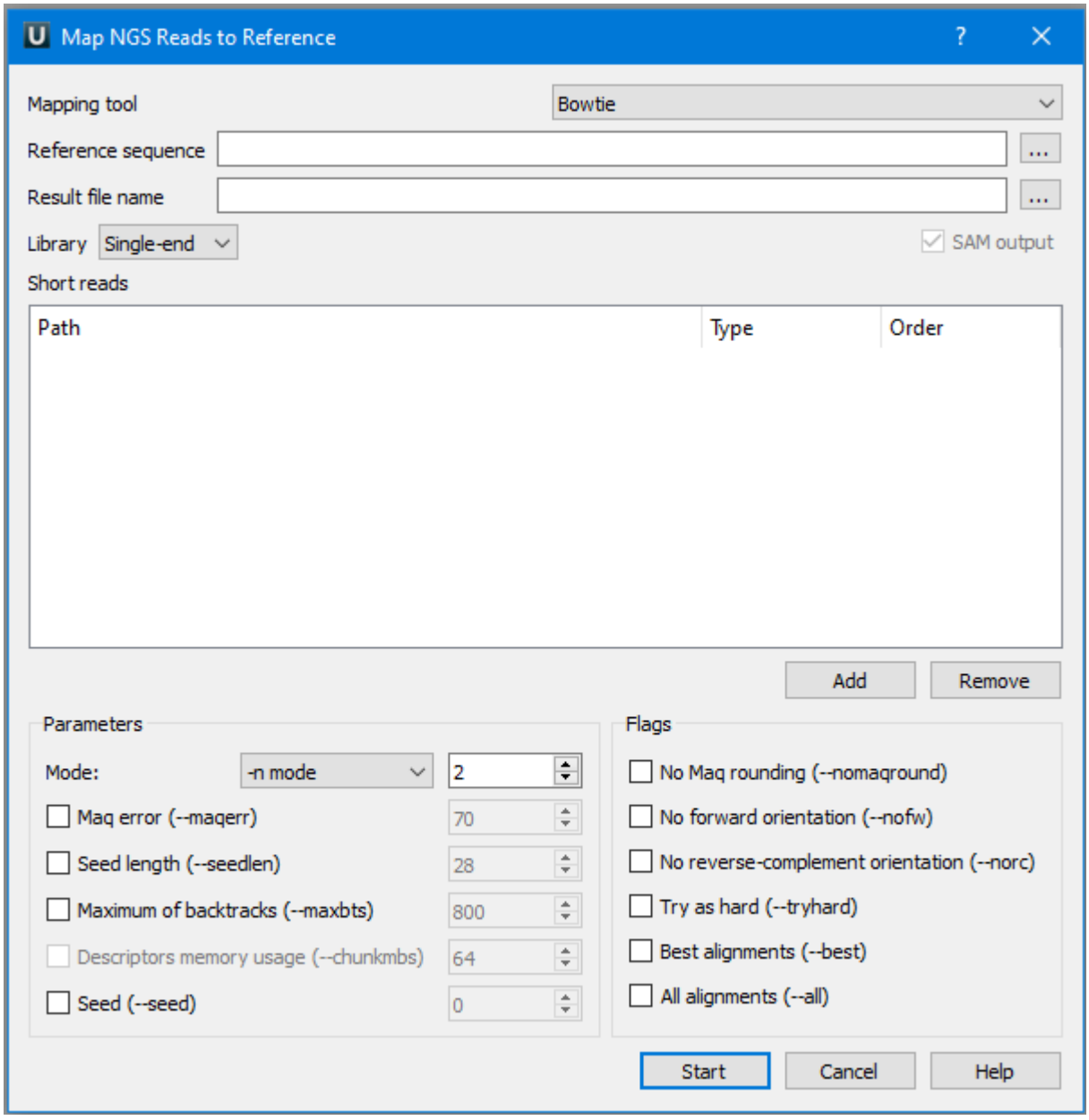
Bowtie Aligning Short Reads

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



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.

⚠ Short reads length for *Bowtie* can't be more than 1024.

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

Select one of the following alignment modes:

The *-n* alignment mode:

When the *-n mode* is selected, *Bowtie* determines which alignments are valid according to the following policy. Alignments may have no more than N mismatches (where N is a number 0-3) in the first L bases (where L is a number 5 or greater, set with *Seed length*) on the high-quality (left) end of the read. The sum of the Phred quality values at all mismatched positions (not just in the seed) may not exceed E (set with *Maq error*). Where qualities are unavailable (e.g. if the reads are from a FASTA file), the Phred quality defaults to 40.

The *-v* alignment mode:

In *-v mode*, alignments may have no more than V mismatches, where V may be a number from 0 through 3. Quality values are ignored. The *-v mode* is mutually exclusive with the *-n mode*.

The following parameters are available:

Maq error (–maqerr) — maximum permitted total of quality values at all mismatched read positions throughout the entire alignment, not just in the "seed". The default is 70. By default, *Bowtie* rounds quality values to the nearest 10 and saturates at 30. Note that the rounding can be disabled with *No Maq rounding*.

Seed Length (–seedlen) — the number of bases on the high-quality end of the read to which the *-n* applies. The lowest permitted setting is 5 and the default is 28.

Maximum of backtracks (-maxbts) — the maximum number of backtracks (default: 125 without *Best*, 800 with *Best*). A "backtrack" is the introduction of a speculative substitution into the alignment.

Descriptors memory usage (–chunkmbs) — the number of megabytes of memory a given thread is given to store path descriptors in the *Best* flag. Default: 64. This parameter is available if the *Best* flag is checked.

Seed (–seed) — pseudo-random number generator.

The following flags are available:

No Maq rounding (–nomaqround) — Maq (Mapping and Assembly with Quality) accepts quality values in the Phred quality scale, but internally rounds values to the nearest 10, with a maximum of 30. By default, *Bowtie* also rounds this way. *No Maq rounding* prevents this rounding in *Bowtie*.

No forward orientation (–nofw) — do not attempt to align against the forward reference strand.

No reverse-complement orientation (–norc) — do not attempt to align against the reverse-complement reference strand.

Try as hard (–tryhard) — try as hard as possible to find valid alignments when they exist, including paired-end alignments.

Best alignments (–best) — make *Bowtie* guarantee that reported singleton alignments are "best" in terms of stratum (i.e. number of mismatches, or mismatches in the seed for the case of *-n mode*) and in terms of the quality values at the mismatched position(s).

All alignments (–all) — report all valid alignments per read or pair. Validity of alignments is determined by the alignment policy (combined effects of *-n mode*, *-v mode*, *Seed length*, and *Maq error*).

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