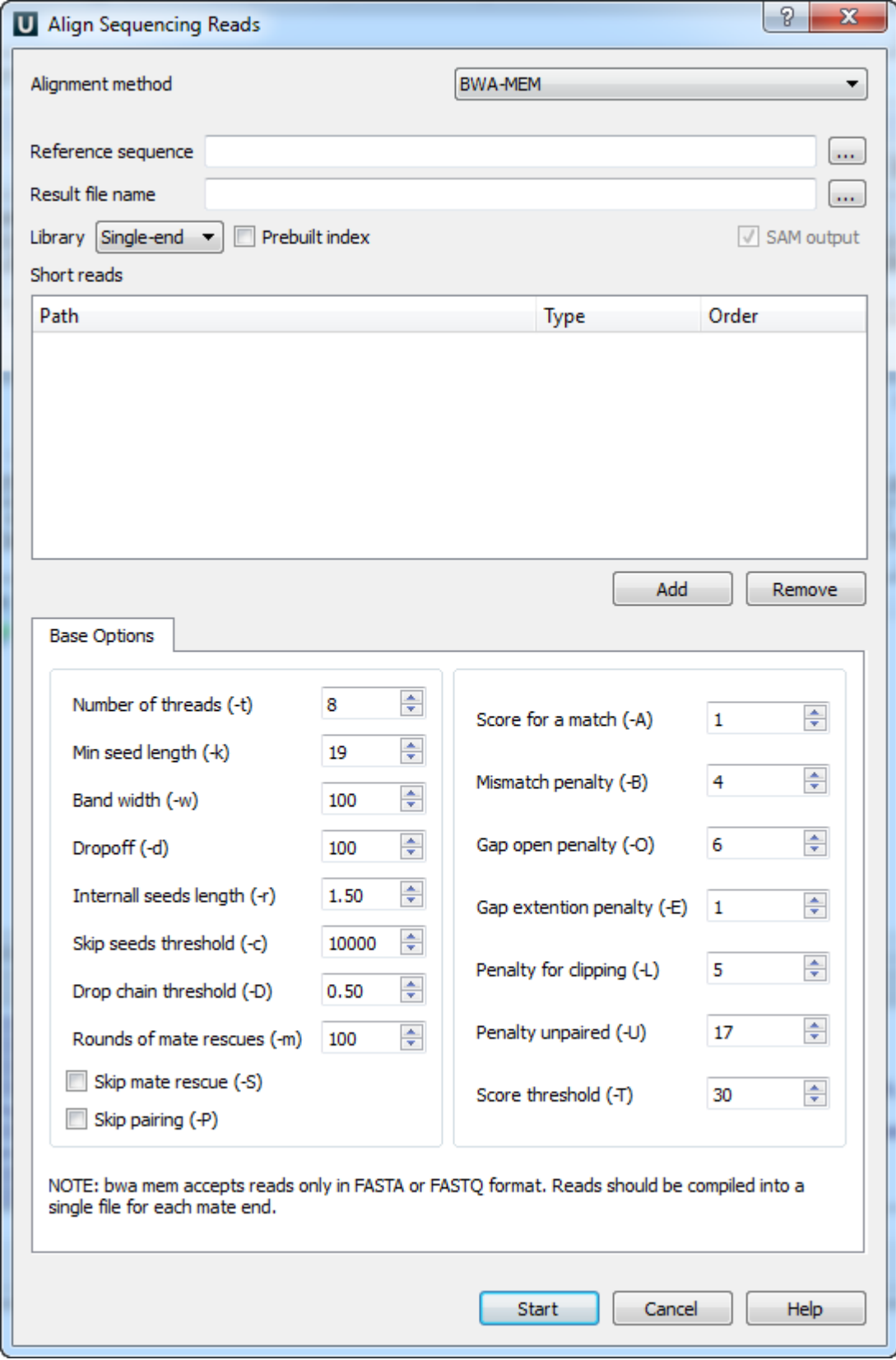


Aligning Short Reads with BWA-MEM

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 *BWA-MEM*. The dialog looks as follows:



The **Align Sequencing Reads** dialog box is shown. It features a title bar with a question mark and a close button. The main area contains several sections: 'Alignment method' with a dropdown set to 'BWA-MEM'; 'Reference sequence' and 'Result file name' with text boxes and browse buttons; 'Library' with a 'Single-end' dropdown and a 'Prebuilt index' checkbox; a checked 'SAM output' checkbox; a 'Short reads' section with a table (Path, Type, Order) and 'Add'/'Remove' buttons; and a 'Base Options' section with two columns of settings. At the bottom, there is a 'NOTE' and 'Start', 'Cancel', and 'Help' buttons.

Alignment method: BWA-MEM

Reference sequence: ...

Result file name: ...

Library: Single-end ☐ Prebuilt index ☒ SAM output

Short reads

Path	Type	Order
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Base Options

Number of threads (-t)	8	Score for a match (-A)	1
Min seed length (-k)	19	Mismatch penalty (-B)	4
Band width (-w)	100	Gap open penalty (-O)	6
Dropoff (-d)	100	Gap extension penalty (-E)	1
Internall seeds length (-r)	1.50	Penalty for clipping (-L)	5
Skip seeds threshold (-c)	10000	Penalty unpaired (-U)	17
Drop chain threshold (-D)	0.50	Score threshold (-T)	30
Rounds of mate rescues (-m)	100		
☐ Skip mate rescue (-S)			
☐ Skip pairing (-P)			

NOTE: bwa mem accepts reads only in FASTA or FASTQ format. Reads should be compiled into a single file for each mate end.

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.

Prebuilt index — check this box to use an index file instead of a source reference sequence. Also you can [build it manually](#).

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

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.

Number of threads (-t) — number of threads.

Min seed length (-k) — minimum seed length. Matches shorter than *INT* will be missed. The alignment speed is usually insensitive to this value unless it significantly deviates 20.

Band width (-w) — band width. Essentially, gaps longer than *INT* will not be found. Note that the maximum gap length is also affected by the scoring matrix and the hit length, not solely determined by this option.

Dropoff (-d) — off-diagonal X-dropoff (Z-dropoff). Stop extension when the difference between the best and the current extension score is above $|i-j| * A + INT$, where i and j are the current positions of the query and reference, respectively, and A is the matching score. Z-dropoff is similar to BLAST's X-dropoff except that it doesn't penalize gaps in one of the sequences in the alignment. Z-dropoff not only avoids unnecessary extension, but also reduces poor alignments inside a long good alignment.

Internall seeds length (-r) - trigger re-seeding for a MEM longer than $minSeedLen * FLOAT$. This is a key heuristic parameter for tuning the performance. Larger value yields fewer seeds, which leads to faster alignment speed but lower accuracy.

Skip seeds threshold (-c) - discard a MEM if it has more than *INT* occurence in the genome. This is an insensitive parameter.

Drop chain threshold (-D) - drop chains shorter than *FLOAT* fraction of the longest overlapping chain.

Rounds of mate rescues (-m) - perform at most *INT* rounds of mate rescues for each read.

Skip mate rescue (-S) - skip mate rescue.

Skip pairing (-P) - in the paired-end mode, perform SW to rescue missing hits only but do not try to find hits that fit a proper pair.

Score for a match (-A) - matching score.

Mismatch penalty (-B) - mismatch penalty. The sequence error rate is approximately: $\{.75 * \exp[-\log(4) * B/A]\}$.

Gap open penalty (-O) - gap open penalty.

Gap extention penalty (-E) - gap extension penalty. A gap of length k costs $O + k * E$ (i.e. *Gap open penalty* is for opening a zero-length gap).

Penalty for clipping (-L) - clipping penalty. When performing SW extension, BWA-MEM keeps track of the best score reaching the end of query. If this score is larger than the best SW score minus the clipping penalty, clipping will not be applied. Note that in this case, the SAM AS tag reports the best SW score; clipping penalty is not deducted.

Penalty unpaired (-U) - penalty for an unpaired read pair. BWA-MEM scores an unpaired read pair as $scoreRead1 + scoreRead2 - INT$ and scores a paired as $scoreRead1 + scoreRead2 - insertPenalty$. It compares these two scores to determine whether we should force pairing.

Score threshold (-T) - don't output alignment with score lower than score threshold. This option only affects output.

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