



**Basis for each recommendation:**

Accuracy can often be improved considerably by including closely related sequences, which can then be removed post-alignment. The additional sequences act as "stepping stones" between distant sequences.

Alignment of amino acid sequences is more accurate than nucleotide sequences, which is why aligning their translations is preferable. AlignTranslation works by translating, aligning, and then reverse translating.

Staggering the alignment decreases false positive homologies by adding gaps.

Chained guide trees offer reasonable accuracy and faster speed when aligning thousands of sequences.

Iteration and refinement steps are unnecessary when all the sequences are very similar or when a chained guide tree is being used.

Restricting the alignment space increases speed without affecting accuracy unless aligning fragments.