To verify the precision of our alignment simulator, we used IQ-TREE, a phylogenetic software for maximum likelihood analysis described in *Trifinopoulos J, Nguyen LT, von Haeseler A, Minh BQ. W-IQ-TREE: a fast online phylogenetic tool for maximum likelihood analysis. Nucleic Acids Res. 2016;44(W1):W232-W235. doi:10.1093/nar/gkw256.* Using as inputs a tree topology (without specifying branch lengths) and an alignment generated with our simulator and corresponding to the input tree, we employed their 12.12 model to estimate the branch lengths. This is the most general non-reversible DNA model and is equivalent to the unrestricted one.

When analyzing whether the branch length estimations performed by IQ-TREE were close to the ones used as input in our simulator, we focused on the particular case of quartets. We took into account different parameters, such as the branch length (0.1 and 0.5), the alignment length (1000 and 10000) and the type of branch (internal, the branch that joins the two internal nodes, or external, a branch that joins one internal node with one leaf). The metric used is the absolute error between the branch length used to simulate the alignments and the one estimated by IQ-TREE. We include in the Appendix two plots that show the obtained results.

In particular, we observed that IQ-TREE accurately estimates branch lengths with large alignments generated by our simulator given a pre-set number of substitutions (particularly with a branch length of 0.1). This gives us confidence in the proper functioning of our alignment generator. It is logical to achieve better results when working with large alignments, as shorter sequences accumulate more sampling error when we generate them (that is, alignments of length 10000 capture the simulated transition probabilities much better than alignments of length 1000 do). Regarding branch lengths, we also expected to obtain better results with shorter branches, since the paralinear distance formula used to define the branch lengths works very well for short branches but loses precision with larger ones.