

We test how IQ-TREE (Minh et al. IQ-TREE 2: New Models and Efficient Methods for Phylogenetic Inference in the Genomic Era. *Molecular Biology and Evolution* 37, 2020) recovers the branch lengths from alignments simulated by GenPhylo. Using as input a tree topology (without specifying branch lengths) and an alignment generated with our simulator, we employed their 12.12 model to estimate the branch lengths. This is the most general non-reversible continuous-time DNA model (with no restrictions on the rate matrix).

In order to analyze the difference on branch length estimations performed by IQ-TREE, we focused on the particular case of quartets with all branches with the same length. We took into account different parameters, such as the branch lengths (0.1 and 0.5), the alignment length (1000 and 10000) and the type of branch (internal or external). The metric used is the absolute error between the branch length used to simulate the alignments and the one estimated by IQ-TREE. The two plots that show the results.

We observe that IQ-TREE accurately estimates branch lengths on large alignments generated with a short branch length of 0.1, and performs a bit worse for shorter alignments or larger branch lengths. This gives us confidence in the proper functioning of our alignment generator. It is natural to achieve better results when working with large alignments, as shorter sequences accumulate more sampling error when we generate them. 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. Also, we must bear in mind that IQ-TREE is using estimating a continuous-time model, while the alignments were generated under the general Markov model.

