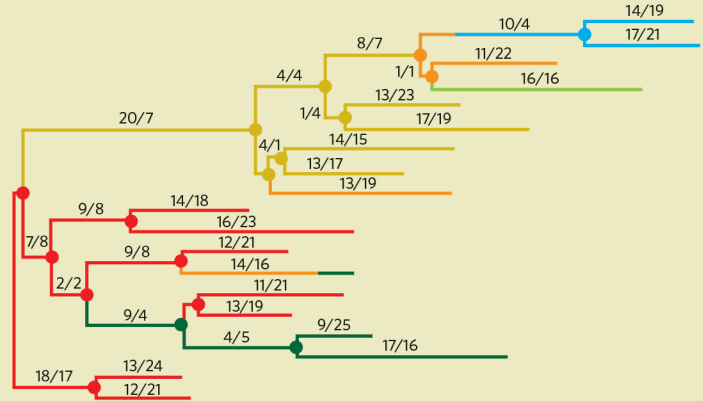


## ENSURING ACCURACY

One way to ensure that an ASR protein behaves like the true ancestor is to resurrect and test not only the best amino acid sequence generated by the algorithms, but a few proteins with the second-best guesses, or third-best guesses, and so on. If those alternative ancestors act like the best-guess version, then researchers figure the conclusions are probably robust. Recently, evolutionary synthetic biologist Eric Gaucher of Georgia State University tested ASR accuracy in a different way. He generated an entirely artificial phylogenetic tree, starting with red fluorescent protein and randomly mutating it to evolve 19 diversely colored fluorescent proteins. Then he used ASR to predict the ancestor of those 19 descendants, and compared the results to the true ancestors. The results were reassuring. Overall, the five different ASR algorithms he tried identified the ancestral sequence with about 97 percent accuracy (*Nat Commun*, 5:12847, 2016).



**EVOLVING PROTEINS:** The experimental evolution began with a red fluorescent protein gene (left). The 19 resulting proteins were sequenced, and the data were used to infer the sequences of the node proteins. (Colors represent protein fluorescence. The number of nonsynonymous and synonymous substitutions are shown along each branch. )