


# Allowing multi-nucleotide substitutions

- Some of the methods (e.g. BUSTED, aBSREL, RELAX) can extend substitution models to allow instantaneous double- and triple-“hits” (e.g. ACC to AGG)
- Sometimes multi-nucleotide changes along short branches at a single site can drive selection signal (possible false positives?)
- HyPhy includes a simple standard analysis for estimating alignment-wide multiple-hit rates.

## JOURNAL ARTICLE

### Evolutionary Shortcuts via Multinucleotide Substitutions and Their Impact on Natural Selection Analyses

Alexander G Lucaci, Jordan D Zehr, David Enard, Joseph W Thornton, Sergei L Kosakovsky Pond 

*Molecular Biology and Evolution*, Volume 40, Issue 7, July 2023, msad150,  
<https://doi.org/10.1093/molbev/msad150>

**Published:** 03 July 2023

### Extra base hits: Widespread empirical support for instantaneous multiple-nucleotide changes

Alexander G. Lucaci , Sadie R. Wisotsky , Stephen D. Shank, Steven Weaver, Sergei L. Kosakovsky Pond 

Published: March 12, 2021 • <https://doi.org/10.1371/journal.pone.0248337>

[See the preprint](#)

# Examining multinucleotide substitutions

## Site Substitutions



### Evidence Ratio Thresholds

Use the sliders or input to specify the evidence ratio range of each respective model. For example, if three-hit substitutions with 3H+ support are defined as those occurring at sites with  $ER(3H+:2H) > 5$ , set 3H+ range to a minimum of 5 but leave all other settings to their respective maximum ranges.

Use the **# of nucleotide changes** slider to specify the minimum number of substitutions to display.

