

BUSTED analysis summary

- **West Nile Virus NS3 protein**

- No statistical support for selection; ML point estimate allocates a small proportion of sites (~1%) to the selected group ($dN/dS \sim 2$)
- The rest of the gene is very strongly conserved ($dN/dS = 0.004$)

- **HIV-1 transmission pair**

- Very strong evidence of strong episodic diversification ($dN/dS \sim 100$) on a small proportion of sites (2%)
- The rest of the gene evolves with weak purifying selection ($dN/dS = 0.6-0.7$)

- **SARS-CoV-2 spike**

- Evidence of episodic diversification ($dN/dS \sim 15$) on a small proportion of sites (~2%)
- Most of the rest of the gene evolves with purifying selection ($dN/dS = 0.2$)

Substitution models in the BUSTED framework

Table 1. Substitution Models Considered in this Paper.

| Model | Reference | Nonsynonymous Rates | Synonymous Rates | Multinucleotide Substitutions | Number of Parameters |
|--------|--|--|--|--|-----------------------------|
| BUSTED | Murrell et al. (2015) | Random effects branch–site modeled by a $K(=3)$ -bin discrete distribution | None | None | $B + 13 + 2 \times K$ |
| +S | Wisotsky et al. (2020) | Random branch–site effects modeled by a $K(=3)$ -bin general discrete distribution | Random site effects modeled by an $L(=3)$ -bin unit mean general discrete distribution | None | $B + 11 + 2 \times (K + L)$ |
| +MH | Lucaci et al. (2021) | Random branch–site effects modeled by a $K(=3)$ -bin general discrete distribution | None | Alignment-wide double- (δ) and triple- (ψ) nucleotide substitution rates | $B + 15 + 2 \times K$ |
| +S+MH | This paper | Random branch–site effects modeled by a $K(=3)$ -bin general discrete distribution | Random site effects modeled by an $L(=3)$ -bin unit mean general discrete distribution | Alignment-wide double- (δ) and triple- (ψ) nucleotide substitution rates | $B + 13 + 2 \times (K + L)$ |

NOTE.— B , the number of branches in the phylogenetic tree. K and L are user-tunable parameters, set to 3 each by default.

Lucaci AG, Zehr JD, Enard D, Thornton JW, Kosakovsky Pond SL. Evolutionary Shortcuts via Multinucleotide Substitutions and Their Impact on Natural Selection Analyses. Mol Biol Evol. 2023 Jul 5;40(7):msad150. doi: 10.1093/molbev/msad150. PMID: 37395787; PMCID: PMC10336034