

	K214E	1									
	N215D	1									
	C499F	-2									
	G521R	-2									
	N537I	-3									

a. Amino acid substitutions detected by whole-genome sequencing of eight T7- evolved mutants relative to the parental T7 genome. Mutations are listed by protein, with corresponding residue substitutions.

b. BLOSUM (BLOcks SUBstitution Matrix) scores indicating the physicochemical similarity between substituted amino acids. Background cell colours are coded according to BLOSUM score, with positive scores (blue shades) indicating conservative substitutions, near-zero scores (neutral shades) indicating moderate changes, and negative scores (red shades) indicating non-conservative substitutions with greater physicochemical divergence.

c. Amino acids substitutions present in T7 WT relative to the database reference sequences (AY264774) are highlighted with gray background; positions that reverted to the reference sequence are shown with a white background.

d. Mutant names or point mutations derived from the positive-selection evolution process are highlighted with a blue background, whereas those derived from the combined positive and negative selection process are highlighted with a purple background.