

**Additional file 1.**

Table 3: Changes observed in the T3Δ1.3A<sub>E</sub> adaptation and recombination test of compensatory evolution.

Bases affected (GenBank# AJ318471)	change	Element	Function	T3A <sub>E</sub>	R	1 <sup>st</sup> detected (hrs)
2405	G->A G204S	0.7	protein kinase	+(2/2)	(0/5)	
5659-6041	deleted and insertion <sup>1</sup>	φ1.05p, 1.05, φ1.1	promoter, ?, promoter	+(1/2)	-	0
6595-7635	engineered deletion	1.3	ligase	+(2/2)	-	
7766-7784	deleted	1.5	?	+(2/2)	-	10
7807	C->T R2C	1.6	?	+(2/2)	-	
8894	G->A G3S	2.5	ssDNA binding	+(1/2)	-	20
9773	T->G F63C	3	endonuclease	+(1/1)	-	0
10464	A->G N143S	3.5	lysozyme	+(1p/2)	*	
10733	G->A D22N	4A	primase	+(2/2)	-	20
12210	T->C L514S	4A	primase	+(1/1)	-**	
“	T->C L452S	4B	helicase	+(1/1)	-**	
“	T->C W41R	4.2	?	+(1/1)	-**	
14186	G->A A382T	5	DNA polymerase	(2/2)	+	
17150	G->A	RNAse III 6.5		+(2/2)	-	40
17493	A->C K4Q	6.7	adsorption	(1/1)	(1/2)	
22531	C->T A36V	11	tail A	+(2/2)	-**	0
24664	A->G Q545R	12	tail B	+(2/2)	-	20
32835	G->A A2T	17	tail fiber	+(1/1)	-	0
33039	T->C Y70H	17	“	+(1/1)	-	0
37606	G->A L4L	19.5	?	+(1/1)	+/-	
5665-6006	deleted	1.05, φ1.1	?, promoter	-(1/2)	-	0
6551	C->T	RNAse III 1.3		+/(1/2)	-	
11447	G->A V260M	4A	primase	+/(1p/1)	-	
“	G->A V198M	4B	helicase	+/(1p/1)	-	
15321	T->C W52R	5.3	?	+/(1/2)	-**	
15430	A->G H88R	5.3	?	+/(1p/2)	-**	

In the “T3A<sub>E</sub>” and “R” columns, a ratio in parentheses indicates the number of isolates in which the mutation was observed over the number of isolates sequenced, whereas a + or – sign indicates the presence or absence of the change in a consensus sequence of the population; +/- indicates multiple peaks in the sequence file. Mutations that were polymorphic in or absent from the consensus of T3Δ1.3A<sub>E</sub> are listed under the bold line. Column ‘R’ is for the recombinant test of compensatory evolution; a “-“ entry indicates that the change did not ascend in the test and thus was strictly compensatory except for the engineered ligase deletion. Insertion<sup>1</sup> is a duplication of T3 nucleotides 6515-6551 inserted just after base 5659; this

insertion contains part of the promoter and RNaseIII site upstream of 1.3 and should restore functionality of the  $\phi$ 1.05 promoter.

$\phi$  – promoter; ? – function unknown; \* = the mutation listed was absent in the recombinant, but a new mutation in the same codon had replaced it, so its status is ambiguous and is not considered strictly compensatory; -\*\* = sequence was obtained from a mix of 10 isolates from the population; p = multiple sequence peaks;