

Supplemental Tables

Table S1. Genome Amplification Break Points in Replicates of Vaccinia Virus near K3L, Related to Figure 2

breakpoint	K4L break	K2L break	A*	B*	C*	parent***
1	31,725	30,296	1,757	2	3	0
2	31,573	28,284**	0	338	1	0
3	30,837	30,284	6	2,444	1,938	0
4	30,840	30,287	5	2,269	202	0
total aligned reads:			4.5E+06	5.9E+06	3.7E+06	1.7E+06

break = breakpoint coordinate in reference genome (K2L ORF 29204..30313, K4L ORF 30681..31955)

* number of sequencing reads with breakpoint (multiplexed sequencing run)

** breakpoint occurs upstream of K2L ORF in K1L

*** parent strain was sequenced separately from the passage 10 replicates

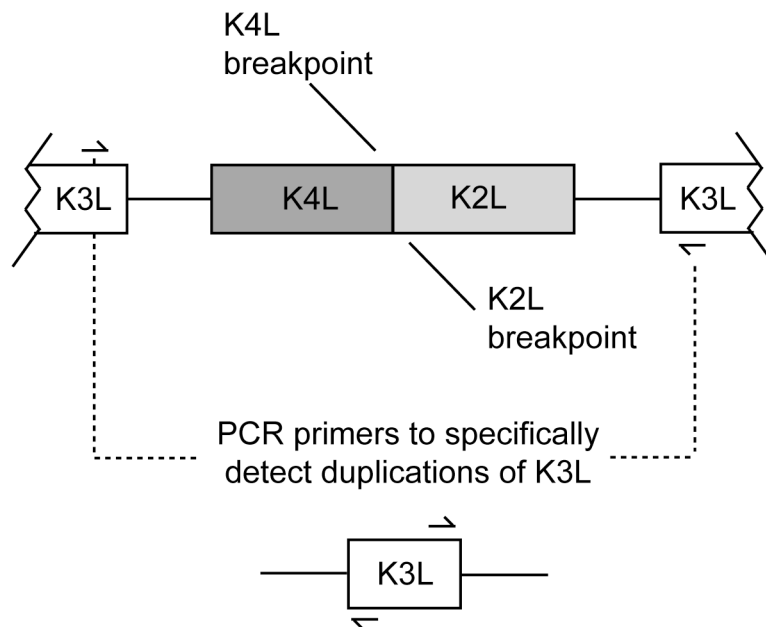


Table S2. Polymorphisms in Vaccinia Virus Populations from Experimental Evolution, Related to Figure 2

gene	variant	sub.	parent*	A*	B*	C*
K3L	H47R	T to C	0/917 (0%)	148/5510 (3%)	4/7627 -	541/4687 (12%)
E9L	E495G	T to C	0/825 (0%)	9/1818 -	11/2383 -	923/1475 (63%)
H2R	syn	C to T	0/631 (0%)	12/1693 -	905/2338 (39%)	11/1368 -

syn = synonymous substitution

* Showing a ratio of reads with the polymorphism to total reads. A 1% frequency cut-off was used to account for potential barcode misassignment in the multiplexed sequencing run with replicates A-C.

Table S3. Duplication-Specific Primers at Locations across Vaccinia Genomes, Related to Figure 5

ORF	Forward primer	Coord.*	Reverse primer	Coord.*
C20L**	GAT AGG TTC GAC ATT CAG AAA GAC G	6,874- 6,850	GTT GAA GGT AAG TGT GTA ACC GC	7,093- 7,115
C3L	CTC CAG GAA ATA CAT GGA AGC CG	21,806- 21,784	CGC ATC CTA TTC CCA ACA ATG TC	22,503- 22,525
K3L	GGG ATA AAC TGG TAG GGA AAA CTG TAA AAG	30,466- 30,437	CAG AGT GAG GAT AGT CAA AAA GAT AAA TGT ATA G	30,521- 30,554
F13L	GAG ATT GGG TAT CTA GCC ACA GTA	44,001- 43,978	TTC TAC CAG CCT ACA TTT TGC TCC	45,029- 45,052
I7L	CGT ATG GAA AAA TGG ACC AAA AAG TC	68,886- 68,861	CAT CTA TAT TGC TAC ATA ATC CAG CTA GTG	69,963- 69,992
J2R	GAT ATG TAT CAA TCG GTG TGT AGA AAG TG	84,344- 84,372	CTG TAC TTT TAC CTG AAA ACA TGG GG	83,912- 83,887
D5R	GCC AAT GTT AGT GAT GAA TAT CTG CA	103,718- 103,743	CCT CGA TAA TAA AAT CTT GAA TAG CCG T	101,696- 101,669
A4L	GGG CAA TAG ATC AAA TTC TAC AAC TAG T	116,480- 116,453	GGT GTA GAG GAT TCT GCC AGC	117,221- 117,241
A29L	AAG CAT TGT CTG ATG CGT GTA A	139,824- 139,803	GTT CGA GTT CAA CGA CGA TTG A	140,644- 140,665
A52R	GGA GAA ATG AGA AAC TGT TTT CTA GAT GG	158,199- 158,227	TTT CCC TCC TAG TAG TCA CCG	157,820- 157,800
B20R	GAA ACA CAA GAT CAA AGT ACA CAA CAC A	179,645- 179,672	TCG GTG AGA TAC AAA TAC CTA GAT AGT C	179,343- 179,316

* 'L' ORFs coordinate numbers based on complement sequence, Copenhagen strain

** same for B26R (coordinates 184,864-184,888; 184,645-184,623)

Table S4. Sequenced Duplications in Vaccinia Strains, Related to Figure 5

ORF (strain)	'right' break*	'left' break*	Fully duplicated ORFs
C20L (WR)	7,345	6,659	C20L
C3L (WR)	22,675	21,741	C3L
K3L (WR)	30,738	30,261	K3L
K3L (WR)	30,587	30,399	-
B20R (VC2)	179,726	179,121	B20R
B20R (Δ E3L)	179,905	179,025	B20R
B26R** (WR)	185,174	184,579	B26R

* genomic coordinates from vaccinia Copenhagen reference (Genbank M35027)

** same as C20L region in reverse orientation

Table S5. Nonrecurring Genomic Break Points Consistent with Duplications, Related to Figure 5

virus strain	'right' break*	'left' break*	inferred duplicated ORFs
ΔE3L parent	156,405	143,153	A35R - A49R
ΔE3L parent	162,444	22,338	large genomic block
A	31,413	30,525	-**
A	67,627	66,482	-***
A	104,930	34,700	large genomic block
A	107,526	107,330	-
B	29,837	22,298	C1L, N1L, N2L, M1L, M2L, K1L
B	47,387	24,975	N2L, M1L, M2L, K1L-K7L, F1L-F17L**
B	47,467	25,066	N2L, M1L, M2L, K1L-K7L, F1L-F17L**
B	55,156	55,104	-
B	141,790	141,697	-
B	169,683	168,828	B7R
C	24,505	23,524	-
C	90,475	87,550	'H orf A'***
C	104,395	104,298	-
C	167,515	89,039	large genomic block
C	171,274	100,726	large genomic block

* genomic coordinates from vaccinia Copenhagen reference (Genbank M35027)

** includes K3L

*** confirmed by PCR and sequencing

Table S6. Vaccinia Virus Genome Comparison between Δ E3L and Copenhagen Reference Strain (Genbank M35027), Related to Figure 2

Coordinate (bp)	Copenhagen nucleotide	ΔE3L nucleotide	Variant freq (%)	Gene	Variant
16,033	A	G	98.6	intergenic	-
23,443	C	T	99.8	C2L	syn
24,256	G	C	100.0	C1L	syn
25,525	G	C	100.0	N2L	P121R
35,080-1	CG	GC	99.3	F3L	T313S
44,312	A	G	99.6	F13L	syn
46,742-3	CG	GC	99.2	F16L	R10A
77,258	A	T	99.9	G7L	V348E
81,834	G	A	99.6	L3L	L1384F
85,139	T	C	99.8	J3R	V209A
104,656	C	T	99.5	D6R	T180M
114,376	-	C	88.4	intergenic	-
145,839	T	A	97.2	intergenic	-
148,904	A	T	99.7	A41L	syn
152,699	-	C	81.2	A46R	frameshift

syn = synonymous substitution