

Table S1. Peptide number and amino acid coverage for MVA/HIV62 vector inserts.

RF	Total and (ARF only) OLPs	Large Pool Name	Total OLPs per Large Pool	Total OLPs per Subpool	OLP Coverage per RF	
gag region						
FORWARD	334 (211)	F1	123	123	100.0%	
		F2	40	10	68.5%	
				10		
				10		
		F3	44	9	85.6%	
				9		
				10		
				9		
				7		
REVERSE		334 (211)	R1	39	10	84.4%
					10	
					9	
	R2		55	11	71.5%	
				11		
				11		
				11		
				11		
	R3		33	11	70.2%	
11						
pol region						
FORWARD	678 (430)		F1	248	248	100%
		F2	71	8	99.4%	
				8		
				10		
				7		
				8		
				9		
				10		
				11		
		F3	98	10	100.0%	
				10		
				10		
				10		
				9		
				11		
				10		
				10		
REVERSE		678 (430)	R1	85	8	95.8%
					10	
					10	
					10	
					10	
					12	
					12	
	11					
	R2		91	10	94.7%	
				10		
				10		
				10		
				10		
				10		
				10		
				10		
	R3		85	11	95.9%	
10						
10						
10						
11						
11						
11						
11						

Overlapping peptides of 8 to 18 amino acids in length were pooled for each of the six reading frames (RF) of the gag and PR-RT MVA/HIV62 vector inserts. To further map responses, smaller subpools were prepared from individual coverage was assessed for each reading frame using the number of residues tested that passed quality control standards and the total number of coding residues (i.e. gag frame.

Sequence	RF1 Coverage	RF2 Coverage
1	ATGTTAAAGTCTGAAAGAGGAAATGTCGAGATGAG	ATGTTAAAGTCTGAAAGAGGAAATGTCGAGATGAG
2	ATGTTAAAGTCTGAAAGAGGAAATGTCGAGATGAG	ATGTTAAAGTCTGAAAGAGGAAATGTCGAGATGAG
3	ATGTTAAAGTCTGAAAGAGGAAATGTCGAGATGAG	ATGTTAAAGTCTGAAAGAGGAAATGTCGAGATGAG
4	ATGTTAAAGTCTGAAAGAGGAAATGTCGAGATGAG	ATGTTAAAGTCTGAAAGAGGAAATGTCGAGATGAG
5	ATGTTAAAGTCTGAAAGAGGAAATGTCGAGATGAG	ATGTTAAAGTCTGAAAGAGGAAATGTCGAGATGAG
6	ATGTTAAAGTCTGAAAGAGGAAATGTCGAGATGAG	ATGTTAAAGTCTGAAAGAGGAAATGTCGAGATGAG
7	ATGTTAAAGTCTGAAAGAGGAAATGTCGAGATGAG	ATGTTAAAGTCTGAAAGAGGAAATGTCGAGATGAG
8	ATGTTAAAGTCTGAAAGAGGAAATGTCGAGATGAG	ATGTTAAAGTCTGAAAGAGGAAATGTCGAGATGAG
9	ATGTTAAAGTCTGAAAGAGGAAATGTCGAGATGAG	ATGTTAAAGTCTGAAAGAGGAAATGTCGAGATGAG
10	ATGTTAAAGTCTGAAAGAGGAAATGTCGAGATGAG	ATGTTAAAGTCTGAAAGAGGAAATGTCGAGATGAG
11	ATGTTAAAGTCTGAAAGAGGAAATGTCGAGATGAG	ATGTTAAAGTCTGAAAGAGGAAATGTCGAGATGAG
12	ATGTTAAAGTCTGAAAGAGGAAATGTCGAGATGAG	ATGTTAAAGTCTGAAAGAGGAAATGTCGAGATGAG
13	ATGTTAAAGTCTGAAAGAGGAAATGTCGAGATGAG	ATGTTAAAGTCTGAAAGAGGAAATGTCGAGATGAG
14	ATGTTAAAGTCTGAAAGAGGAAATGTCGAGATGAG	ATGTTAAAGTCTGAAAGAGGAAATGTCGAGATGAG
15	ATGTTAAAGTCTGAAAGAGGAAATGTCGAGATGAG	ATGTTAAAGTCTGAAAGAGGAAATGTCGAGATGAG
16	ATGTTAAAGTCTGAAAGAGGAAATGTCGAGATGAG	ATGTTAAAGTCTGAAAGAGGAAATGTCGAGATGAG
17	ATGTTAAAGTCTGAAAGAGGAAATGTCGAGATGAG	ATGTTAAAGTCTGAAAGAGGAAATGTCGAGATGAG
18	ATGTTAAAGTCTGAAAGAGGAAATGTCGAGATGAG	ATGTTAAAGTCTGAAAGAGGAAATGTCGAGATGAG

Sequence	TFV Consensus	Noncodon-optimized MRKAd5
<i>Sense</i>	ATTTAAAAATCTGAAAACAGGAAAATATGCAAGAATGAG	ATTTAAAAATCTGAAAACAGGAAAATATGCAAGAAAGAG
F1 (protein)	F K N L K T G K Y A R M R	F K N L K T G K Y A R T R
F2	H L K I * K Q E N M Q E *	H L K I * K Q E N M Q E R
F3	I * K S E N R K I C K N E	I * K S E N R K I C K N E
<i>Antisense</i>	TAAATTTTGTAGACTTTTGTCTTTTATACGTTCTTACTC	TAAATTTTGTAGACTTTTGTCTTTTATACGTTCTTCTC
R1	K F I Q F C S F I C S H P	K F I Q F C S F I C S R P
R2 (CE)	N L < F R F V P F Y A L I L	N L < F R F V P F Y A L V L
R3	M * F D S F L F I H L F S	M * F D S F L F I H L F S
Sequence	Noncodon-optimized MVA/HIV62	Codon-optimized MRKAd5
<i>Sense</i>	ATTTAAAAATCTGAAAACAGGAAAATATGCAAGAATGAG	CTTCAAGAACTGGAAGACTGGCAAGTATGCCAGGATGAG
F1 (protein)	F K N L K T G K Y A R M R	F K N L K T G K Y A R M R
F2	H L K I * K Q E N M Q E *	P S R T * R L A S M P G *
F3	I * K S E N R K I C K N E	L Q E P E D W Q V C Q D E
<i>Antisense</i>	TAAATTTTGTAGACTTTTGTCTTTTATACGTTCTTACTC	GAAGTTCTTGACTTCTGACCGTTCATACGGTCTTACTC
R1	K F I Q F C S F I C S H P	E L V Q L S A L I G P H P
R2 (CE)	N L < F R F V P F Y A L I L	K L < F R F V P L Y A L I L
R3	M * F D S F L F I H L F S	R * S G S S Q C T H W S S

Figure S1. Decreased conservation of a CE encoded by reverse frame 2 of a codon-optimized vaccine vector. Conservation of a previously recognized CE (AF9; arrow) encoded by reverse frame 2 of the *pol* region was evaluated using a consensus sequence (top, left quadrant) of 12 acutely-infected individuals' TFV sequences. Differences in nucleotides and amino acids are highlighted with respect to the consensus sequence for a noncodon-optimized vector insert (MVA/HIV62; bottom, left quadrant), a codon-optimized vector insert (MRKAd5; bottom, right quadrant) and the *wild type* sequence (LAV-1, i.e. noncodon-optimized MRKAd5) from which it was derived. Reading frames (forward, F; reverse, R) refer to translation of codons beginning at base 1, 2, or 3 of the 5' terminus in the sense or antisense transcript, respectively.