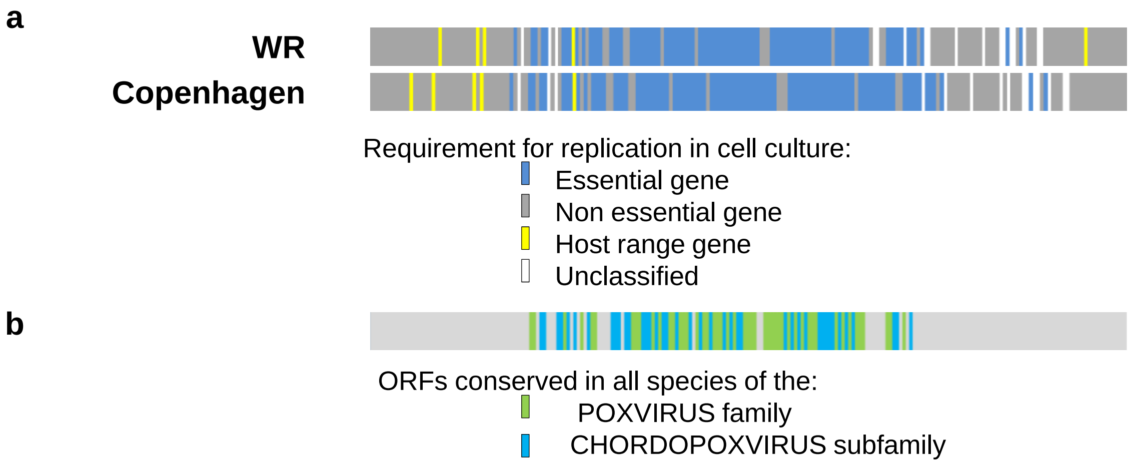

Figure S1: Distribution of essential and conserved genes

(a) Maps of the VACV WR and VACV Copenhagen genomes. Each of the annotated ORFs of VACV Copenhagen, or VACV WR are represented by a single thin vertical line. Genes were classified according to whether they are required for virus replication in cell culture on the basis of published reports of VACV strains with a deletion or disruption of one or more genes. The 223 ORFs shown for VACV WR include the 218 ORFs present in the original genome annotation (NC_006998) plus VACWR53.5 (*F14.5L*), VACWR69.5 (*O3L*), VACWR153.5 (*A30.5L*), VACWR181.5, and VACWR204.5. The 207 ORFs shown for VACV Copenhagen include the 198 major ORFs from the original annotation (Goebel et al., 1990), *G5.5R*, *A2.5L*, *A14.5L*, the orthologue of VACWR169, as well as the five additional ORFs listed for WR above. (b) Map of the VACV WR genome showing distribution of genes conserved across all poxviruses (green) and the chordopoxvirus subfamily (blue), according to analysis by Lefkowitz et al. (2006) Virus Res. 117:105-8.

Table S1: Vaccinia virus genes essential for growth in cell culture

Supplementary Table 1: Vaccinia virus genes essential for growth in cell culture

Function	Gene name [1]	Reference
Production of mature virions (MV). Includes factors required for genome replication and gene expression	VACWR043 (F4L)[2]	(Gammon et al., 2010)
	VACWR049 (F10L)	(Traktman et al., 1995; Szajner, Weisberg, and Moss, 2004; Punjabi and Traktman, 2005)
	VACWR056 (F17R)	(Zhang and Moss, 1991)
	VACWR057 (E1L)[3]	(Gershon et al., 1991)
	VACWR060 (E4L)[3]	(Ahn et al., 1990)
	VACWR062 (E6R)	(Resch, Weisberg, and Moss, 2009; Boyd et al., 2010)
	VACWR064 (E8R)	(Kato, Condit, and Moussatche, 2007)
	VACWR065 (E9L)[3]	(Earl, Jones, and Moss, 1986)
	VACWR066 (E10R)	(Senkevich, Weisberg, and Moss, 2000)
	VACWR070 (I1L)	(Klemperer et al., 1997; DeMasi et al., 2001)
	VACWR072 (I3L)	(Rochester and Traktman, 1998; Greseth et al., 2012)
	VACWR075 (I6L)	(Grubisha and Traktman, 2003)
	VACWR076 (I7L)	(Kane and Shuman, 1993; Ansarah-Sobrinho and Moss, 2004a; Byrd and Hruby, 2005)
	VACWR077 (I8R)	(Bayliss and Smith, 1996)
	VACWR078 (G1L)	(Ansarah-Sobrinho and Moss, 2004b; Hedengren-Olcott et al., 2004)
	VACWR080 (G2R)	(Black and Condit, 1996; Cresawn et al., 2007)
	VACWR081 (G4L)	(White, Weisberg, and Moss, 2000)
	VACWR082 (G5R)	(da Fonseca et al., 2004; Senkevich, Koonin, and Moss, 2009)
	VACWR083 (G5.5R)[3]	(Amegadzie, Ahn, and Moss, 1992)
	VACWR085 (G7L)	(Szajner et al., 2003)
	VACWR086 (G8R)	(Zhang, Keck, and Moss, 1992)
	VACWR089 (L2R)	(Maruri-Avidal et al., 2011)
	VACWR090 (L3L)	(Resch and Moss, 2005)
	VACWR091 (L4R)	(Wilcock and Smith, 1996; Jesus, Moussatche, and Condit, 2014)
	VACWR093 (J1R)	(Chiu and Chang, 2002)
	VACWR095 (J3R)	(Latner et al., 2000; Xiang et al., 2000)
	VACWR096 (J4R)	(Thompson, Hooda-Dhingra, and Condit, 1989)
	VACWR098 (J6R)	(Thompson, Hooda-Dhingra, and Condit, 1989)
	VACWR099 (H1L)	(Liu, Lemon, and Traktman, 1995)
	VACWR101 (H3L)[2]	(da Fonseca et al., 2000)
	VACWR102 (H4L)	(Kane and Shuman, 1992; Zhang, Ahn, and Moss, 1994)
	VACWR103 (H5R)	(DeMasi and Traktman, 2000; Cresawn and Condit, 2007; D'Costa et al., 2010; Boyle, Greseth, and Traktman, 2015)

Table S1: Vaccinia virus genes essential for growth in cell culture

Function	Gene name [1]	Reference
	VACWR104 (H6R)[3]	(da Fonseca and Moss, 2003)
	VACWR105 (H7R)	(Satheshkumar, Weisberg, and Moss, 2009; Meng et al., 2013)
	VACWR106 (D1R)	(Hassett et al., 1997; Shatzer, Kato, and Condit, 2008)
	VACWR107 (D2L)	(Dyster and Niles, 1991; Szajner et al., 2004)
	VACWR108 (D3R)	(Dyster and Niles, 1991; Szajner et al., 2004)
	VACWR109 (D4R)[3]	(Stuart et al., 1993; Millns, Carpenter, and DeLange, 1994)
	VACWR110 (D5R)	(Evans and Traktman, 1992; Boyle, Arps, and Traktman, 2007; Kilcher et al., 2014)
	VACWR111 (D6R)	(Li, Pennington, and Broyles, 1994; Hu et al., 1996; Hagen et al., 2014)
Production of mature virions (MV). Includes factors required for genome replication and gene expression	VACWR112 (D7R)[3]	(Seto et al., 1987; Ahn, Jones, and Moss, 1990)
	VACWR116 (D11L)[3]	(Christen et al., 1998)
	VACWR117 (D12L)	(Niles et al., 1989)
	VACWR118 (D13L)	(Zhang and Moss, 1992)
	VACWR119 (A1L)[3]	(Keck, Baldick, and Moss, 1990)
	VACWR120 (A2L)[3]	(Keck, Baldick, and Moss, 1990; Hubbs and Wright, 1996)
	VACWR121 (A2.5L)	(Senkevich et al., 2002)
	VACWR122 (A3L)	(Kato et al., 2004b; Jesus et al., 2015)
	VACWR123 (A4L)	(Williams et al., 1999)
	VACWR124 (A5R)[3]	(Ahn et al., 1992)
	VACWR125 (A6L)	(Meng et al., 2007)
	VACWR126 (A7L)	(Hu et al., 1998) (Hagen et al., 2014)
	VACWR127 (A8R)	(Warren, Cotter, and Moss, 2012)
	VACWR128 (A9L)	(Yeh, Moss, and Wolffe, 2000)
	VACWR129 (A10L)	(Heljasvaara et al., 2001; Rodriguez et al., 2006)
	VACWR130 (A11R)	(Resch, Weisberg, and Moss, 2005)
	VACWR131 (A12L)	(Yang and Hruby, 2007)
	VACWR132 (A13L)	(Unger and Traktman, 2004)
	VACWR133 (A14L)	(Rodriguez et al., 1998; Traktman et al., 2000)
	VACWR135 (A15L)	(Szajner et al., 2004)

Table S1: Vaccinia virus genes essential for growth in cell culture

Function	Gene name [1]	Reference
	VACWR137 (A17L)	(Rodriguez, Esteban, and Rodriguez, 1995; Wolffe et al., 1996)
	VACWR138 (A18R)	(Bayliss and Condit, 1993; Simpson and Condit, 1994)
	VACWR139 (A19L)	(Satheshkumar, Weisberg, and Moss, 2013)
	VACWR141 (A20R)	(Ishii and Moss, 2001; Punjabi et al., 2001)
	VACWR142 (A22R)	(Garcia and Moss, 2001)
	VACWR143 (A23R)	(Warren, Cotter, and Moss, 2012)
	VACWR144 (A24R)	(Hooda-Dhingra et al., 1990; Condit et al., 1991)
	VACWR152 (A29L)[3]	(Amegadzie, Ahn, and Moss, 1991)
	VACWR153 (A30L)	(Szajner et al., 2001)
	VACWR153.5 (A30.5L)	(Maruri-Avidal, Weisberg, and Moss, 2013)
	VACWR155 (A32L)	(Cassetti et al., 1998)
	VACWR183 (B1R)	(Rempel and Traktman, 1992)
WV-, EV-, or actin tail- related	VACWR051 (F12L)	(Zhang, Wilcock, and Smith, 2000)
	VACWR052 (F13L)	(Blasco and Moss, 1991)
	VACWR058 (E2L)	(Domi, Weisberg, and Moss, 2008; Dodding et al., 2009)
	VACWR150 (A27L)	(Rodriguez and Smith, 1990; Ward, 2005)
	VACWR156 (A33R)	(Roper et al., 1998)
	VACWR157 (A34R)	(Duncan and Smith, 1992a; McIntosh and Smith, 1996; Wolffe et al., 1997)
	VACWR159 (A36R)	(Parkinson and Smith, 1994)
	VACWR187 (B5R)	(Engelstad and Smith, 1993; Wolffe, Isaacs, and Moss, 1993)
Entry or infectivity	VACWR048 (F9L)	(Brown, Senkevich, and Moss, 2006)
	VACWR067 (E11L)	(Wang and Shuman, 1996)
	VACWR069.5 (O3L)	(Satheshkumar and Moss, 2009)
	VACWR071 (I2L)	(Nichols et al., 2008)
	VACWR079 (G3L)	(Izmailyan et al., 2006)
	VACWR087 (G9R)	(Ojeda, Domi, and Moss, 2006)

Table S1: Vaccinia virus genes essential for growth in cell culture

Function	Gene name [1]	Reference
	VACWR088 (L1R)	(Ravanello and Hruby, 1994; Bisht, Weisberg, and Moss, 2008)
	VACWR092 (L5R)	(Townsley, Senkevich, and Moss, 2005a)
	VACWR097 (J5L)	(Zajac, Spehner, and Drillien, 1995; Wolfe, Ojeda, and Moss, 2012)
	VACWR100 (H2R)	(Senkevich and Moss, 2005)
	VACWR136 (A16L)	(Ojeda, Senkevich, and Moss, 2006)
	VACWR140 (A21L)	(Townsley, Senkevich, and Moss, 2005b)
	VACWR151 (A28L)	(Senkevich, Ward, and Moss, 2004a; Senkevich, Ward, and Moss, 2004b)

[1] Gene numbers for VACV WR are given first followed by name according to the Copenhagen HindIII fragment nomenclature.
[2] Not considered essential in publications, but are included here because deletions have defects greater than 10-fold.
[3] Included on the basis of described function as shown by in vitro assays, but not deletion.

Supplementary Table 2: Vaccinia virus genes non-essential for growth in cell culture

Gene[1]	Parent strain [2]	Mutation strategy[3]	Phenotype of knockout virus			Reference
			Cell line[2]	**Replication[4]**	**Plaque**	
VACWR001/218 C23L/B29R Chemokine binding protein*	Lister	INS (lacZ)	CV-1	viable	?	(Patel et al., 1990)
	Praha TK-	INS (lacZ)	unk.	viable	?	(Gabriel et al., 2012)
VACWR009/210 C11R VACV growth factor (VGF)	WR	INS (lacZ)	BS-C-1	MSC =	10% smaller	(Buller et al., 1988a)
			BS-C-1 (Q)	MSC slightly reduced	30% smaller	
			Swiss 3T3	MSC slightly reduced	?	
			Swiss 3T3 (Q)	MSC: reduced		
			HeLa	viable		
			A431	viable	Larger foci & cell clearance	(Buller et al., 1988b)
			BS-C-1	3x decrease (sup 24 h.p.i.)	small plaque	(Postigo et al., 2009)
	NYC BH	DIS (lacZ)	BSC40	SSC=	No cell clearanceo r surround-ing circle of raised cells.	(Lee et al., 1992)
VACWR010/209 (WR-C16L) C10L	WR	INS (gpt/ eGFP) marker-less	BS-C-1	M&SSC=	smaller	(Fahy et al., 2008)
			RK13			
VACWR013 (WR-C12L) no homologue	WR	TDS (gpt)	BS-C-1	MSC =	=	(Symons et al., 2002a)

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[1] Gene number for VACVWR and gene name in Copenhagen HindIII-fragment nomenclature (italics) are shown. In cases where the WR gene is referred to in the literature by an alternate HindIII-based name to the homologue from Copenhagen this is indicated in brackets eg. *WR-C16L* is *C10L* in Copenhagen. A short description of the gene product is also provided.
[2] Parent strain of virus and cell line: **unk.** unnamed VACV strain or cell line was used; **(Q)**: cells were in quiescent or resting state; **(P)**: primary cells. **COP.** VACV Copenhagen
[3] **INS**: deletion of part of the reading frame during insertion of the foreign marker gene named in brackets; **DIS**: insertion of a foreign marker gene without deletion of any of the VACV ORF. **f/s:** frameshift mutations leading to truncation of the protein, position of stop codon is indicated in brackets; **ind**: replacement of the endogenous copy of the gene with an IPTG or tetracycline (tet) inducible copy. vT7lacOI is a WR-derived VACV strain that allows IPTG-dependent control of gene expression. vT7lacOI expresses T7 RNA polymerase under control of the lac operator and the lac repressor. The gene of interest is placed downstream of a T7 promoter and lac operator.
Marker genes: ***gpt****: E. coli* guanine xanthine phosphoribosyl transferase gene; **CAT**:chloramphenicol acetyltransferase; ***lacZ***: Gene encoding *E.coli* β-galactosidase (substrate: X-gal), **gus:** gene encoding β-glucoronidase (chromogenic substrate XGluc). ***hyg*****:** hygromycin resistance gene; **GFP**: green fluorescent protein; **Cherry**: mCherry red fluorescent protein; **neo**: neomycin resistance gene; **markerless**: marker genes were removed in an additional step.
The name of the virus strain produced is shown in brackets if used to produce virus strains shown later in this table.
[4] **MSC**: multiple step growth curve (m.o.i.≤0.1); **SSC:** single step growth curve (m.o.i.≥1); **MS (X)**: low m.o.i. infection, virus titre was measured at X h.p.i.; **sup**: virus present in supernatant; **viable**: virus isolated or grown in this cell line, no further test of replication. =: growth/plaque similar to wild type, parent, or revertant; **TEM**: transmission electron microscope sections indicate no change in distribution or number of immature or mature virions. **?**: no data presented

Table S2: Vaccinia virus genes non-essential for growth in cell culture

Gene[1]	Parent strain [2]	Mutation strategy[3]	Phenotype of knockout virus			Reference
			Cell line[2]	Replication[4]	Plaque	
IL-18 inhibitor	Tiantan	INS (lacZ)	CEF (P)	viable	?	(Dai et al., 2008)
			HeLa	MS(24) =		
			TK143			
VACWR022 C6L	WR	TDS (gpt)	BS-C-1	M&SSC =	=	(Unterholzner et al., 2011)
			RK13	viable		
			TK143			
VACWR024 C4L	WR	TDS (gpt)	BS-C-1	M&SSC =	=	(Ember et al., 2012)
			RK13	viable		
VACWR025 C3L Complement control protein (VCP)	WR	INS (gpt) (=vSIGK1 & vSIGK3)	CV-1	viable	?	(Kotwal et al., 1990)
		deletion of gpt & C3L ORF from vSIGK3	BS-C-1	M&SSC =	?	(Girgis et al., 2011)
VACWR026 C2L BTB-kelch protein	WR	TDS (gpt)	BS-C-1	M&SSC =	Indistinct border	(Pires de Miranda et al., 2003)
VACWR028 N1L	WR	TDS (gpt)	BS-C-1	MSC =	=	(Bartlett et al., 2002)
	WR	INS (lacZ)	BS-C-1	viable	?	(Kotwal, Hügin, and Moss, 1989)
			HeLa			
	WR	INS (Cherry)	BS-C-1	viable	=	(Postigo and Way, 2012)
VACWR029 N2L	WR	TDS (gpt)	BS-C-1	M&SSC =	=	(Ferguson et al., 2013)
VACWR031 M2L	NYC BH	INS (lacZ) (K1L & M2L) K1L restored	BSC40	=	=	(Smith et al., 1993)
			CV-1			
			RK13			
	WR	INS (neo/gus)	BS-C-1	viable	?	(Hinthong, Jin, and Shisler, 2008)
VACWR033 K2L Serpin (SPI-3) Fusion inhibitor	WR	TDS (gpt)	CV-1	viable	Cell fusion	(Law and Smith, 1992)
			BS-C-1			
			D980R			
			BHK-21		No cell fusion. CPE =	
			RK13			
			TK-143			
			Vero			
	WR	INS (gpt)	CV-1	viable	Cell fusion	(Turner and Moyer, 1992)
	WR	DIS (hyg)	CV-1	viable	Cell fusion	(Zhou et al., 1992)
VACWR035 K4L Nicking-joining enzyme	IHD-J	INS (gpt)	BS-C-1	?	wt	(Blasco and Moss, 1991);
			RK13	= (unk. m.o.i.)	?	
	WR	INS (neo/gus)	BS-C-1	viable	=	(Eckert et al., 2005)
VACWR039 K7R	WR	TDS (gpt)	BS-C-1	SSGC =	=	(Benfield et al., 2013)
		f/s (a.a. 1)	BS-C-1	viable	?	
VACWR040 F1L	WR	INS (gpt)	HeLa	?	?	(Postigo et al., 2006)

Table S2: Vaccinia virus genes non-essential for growth in cell culture

Gene[1]	Parent strain [2]	Mutation strategy[3]	Phenotype of knockout virus			Reference
			Cell line[2]	Replication[4]	Plaque	
Apoptosis inhibitor	WR/ VGF-	INS (gpt)	BS-C-1	= (24 h.p.i. sup.)	=	(Postigo et al., 2009)
	COP.	INS (GFP)	CV-1	SSC =	=	(Wasilenko et al., 2005)
VACWR041 F2L dUTPase	WR	INS (RFP)	BS-C-1	SSC similar	=	(De Silva and Moss, 2008)
			HFF	M&SSC= wt	?	
			HFF (Q)	M&SSC= wt		
	WRΔ D4R	INS (RFP) marker-less also produced	BS-C-1	SSC similar	=	
			HFF	M&SSC =	?	
			HFF (Q)	SSC = MSC reduced		
	WR	INS (GFP)	HFF	MSC slightly reduced (2.5x)	=	(Prichard et al., 2008)
VACWR042 F3L BTB/kelch protein	WR	TDS (gpt)	BS-C-1	M&SSC =	=	(Froggatt, Smith, and Beard, 2007)
			RK13	viable		
			CV-1			
			TK-143			
VACWR044 F5L	WR	TDS (GFP/bsd)	BS-C-1	M&SSC =	small plaque	This thesis Chapter 5
VACWR046 F7L	VV-WR-L929 (TK-)	INS (luciferase+ HSVtk)	TK-143B	SSC =	?	(Coupar, Oke, and Andrew, 2000)
			CV-1	viable		
	NYC BH	DIS (lacZ)	BSC40	SSC =	=	(Lee et al., 1992)
VACWR047 F8L	WR	INS (gpt)	HeLa	viable	=	(Higley and Way, 1997)
			RK13	viable	?	
VACWR050 F11L	WR	Stop a.a. 182 with TDS (gpt)	BSC40	viable	?	(Kato et al., 2004a)
	WR	Stop a.a. 15 or 75 with TDS (gpt)	BSC40	SSC =	smaller	(Morales et al., 2008)
	WRΔ F12L	INS (gpt), restoration of F12L	BS-C-1	viable	smaller	(Cordeiro et al., 2009)
			HeLa	slightly reduced (8 h.p.i. 2.5x sup)	?	
VACWR053.5 F14.5L	vT7lacO I	ind (IPTG)	BS-C-40	M&SSC =	=	(Izmailyan and Chang, 2008)
			BSC1	viable	cell adhesion altered when over-expressed	
			RK13	?		
	WR	INS (gpt)	BSC40????	=	=	
VACWR055 F16L	WR	INS (GFP)	BS-C-1	SSC =	=	(Senkevich, Koonin, and Moss, 2011)
			RK13	SSC =		
			HeLa	SSC =		
			A543	viable		
			CEF	viable		
			HFF (P)	SSC = (Q)		

Table S2: Vaccinia virus genes non-essential for growth in cell culture

Gene[1]	Parent strain [2]	Mutation strategy[3]	Phenotype of knockout virus			Reference
			Cell line[2]	Replication[4]	Plaque	
VACWR061 E5R	Dairen	INS (gpt)	CV-1	viable	=	(Douglass and Dumbell, 1996)
			rabbit kidney			
			human fibroblast		=	
			chorioallantoic membrane		= (pock)	
VACWR063 E7R	WR and LIVP?	INS (lacZ)	?	?	?	(Chernos et al., 1993) Paper in Russian
VACWR068 O1L	CVA (BAC)	INS (kan or rpsL/neo) markerless (BAC)	293A	M&SSC =	smaller; less mono-layer disruption	(Schweneker et al., 2012)
			293A (Q)	MSC CA = sup. reduced	?	
			HeLa	?	smaller	
			143B			
			CV-1			
VACWR069 O2L glutaredoxin	WR	INS (gpt)	BSC-40	SS(12h) =	=	(Rajagopal et al., 1995)
			BS-C-1	viable		
VACWR073 I4L Large subunit ribonucleotide reductase	unk. (TK-variant also tested)	λ disruption	BSC-40	SSC =	=	(Child et al., 1990)
			A549	SSC =	?	
			A549 (A)	SSC =		
			BSC-40	SS(12h) =	=	(Rajagopal et al., 1995)
VACWR074 I5L Component of viral membrane	WR	INS (GFP)	BS-C-1	yield similar	=	(Sood, Ward, and Moss, 2008)
			HuTK-	viable		
			RK13			
			BHK-21			
			HeLa			
			CV-1			
			human epidermal keratinocytes (P)			
		f/s (stop a.a. 15 or 79)	BS-C-1	SSC =	=	
		ind (tet)	BSC-40	SSC =	=	(Unger et al., 2008)
			HFF (P)		?	
		TDS (neo)	BSC-40	SSC =	=	
			HFF (P)		?	
VACWR084 G6R Virion protein NlpC/P60 superfamily member	WR	EGFP insertion (=vΔG6)	BS-C-1	MSC =	=	(Senkevich et al., 2008)
			HeLa S3	viable		
			RK13			
			MRC-5			
			FRhL			
			HFF (P)			

Table S2: Vaccinia virus genes non-essential for growth in cell culture

Gene[1]	Parent strain [2]	Mutation strategy[3]	Phenotype of knockout virus			Reference
			Cell line[2]	Replication[4]	Plaque	
			HEKn (P)			
			CEF (P)	3-5x fewer plaques	small (due to EGFP)	
	WR (vΔG6)	f/s (stop a.a. 24)	CEF (P)	= WR	= WR	
VACWR094 J2R Thymidine kinase	NYC BH	INS (lacZ)	BSC40	SSC =	?	(Lee et al., 1992)
	unk.	DIS (neo)	BSC40	SSC =	=	(Child et al., 1990)
			A549		?	
			A549 (serum starved)		?	
	WR	27.4 kb λ DIS	CV-1	SSC =	=	(Smith and Moss, 1983)
			TK-143	viable		
VACWR113 D8L Virion protein	WR	f/s (stop a.a. 249)	BSC40	SSC =	?	(Niles and Seto, 1988)
	VVLUC (WR TK-)	DIS (lacZ)	BSC40	sup= m.o.i.=0.5	?	(Rodriguez, Rodriguez, and Esteban, 1992)
			HeLa			
VACWR114 D9R mRNA-decapping enzyme	WR	INS (neo/gus)	BS-C-1	viable	?	(Dvoracek and Shors, 2003)
	WR	INS (eGFP)	BS-C-1	SSC =	slightly smaller	(Parrish and Moss, 2006)
			HeLa S3	viable	n/a	
VACWR115 D10R mRNA-decapping enzyme	WR	INS (eGFP) (ΔD10)	BS-C-1	SSC =	slightly smaller	(Parrish and Moss, 2006)
			HeLa S3	increased particle: pfu	n/a	
			BS-C-1	SSC =	slightly smaller	(Liu et al., 2014)
			MEF	SSC =	=	
	ΔD10	D10R with 2 stop codons replaces eGFP	BS-C-1	SSC =	=	
			MEF			
VACWR134 A14.5L Virion membrane protein	WR	INS (neo/gus)	BS-C-1	M&SSC = (CA+sup)	=	(Betakova, Wolffe, and Moss, 2000)
			CV-1	viable		
			huTK-143B			
			A549			
			RK13			
			BHK-21			
VACWR148 no orthologue ATI gene fragment (1/4)	WR	INS (lacZ/cat)	hu143b	viable	?	(Patel et al., 1988)
		INS (luc-gpt)	HeLa	SSC =	?	(Chang et al., 2010)
VACWR149	WR	DIS (gpt)	HeLa-S3	viable	?	

Table S2: Vaccinia virus genes non-essential for growth in cell culture

Gene[1]	Parent strain [2]	Mutation strategy[3]	Phenotype of knockout virus			Reference
			Cell line[2]	Replication[4]	Plaque	
(5' end of A26L) p4c IMV protein laminin binding protein			hu143B			(McKelvey et al., 2002)
	WR32-7/Ind14 K	Repair of A26L truncation	BSC40	SSC =	=	(Chiu et al., 2007)
	WR	INS (luc-gpt)	HeLa	SSC =	?	(Chang et al., 2010)
VACWR158 A35R	WR	INS (gpt)	HeLa	viable	=	(Roper, 2006)
			BS-C-1	SSC =		
			RK13	viable		
			CV-1			
			huTK-			
			A549			
			BHK-21			
			MRC-5			
VACWR162 A38L	WR	INS (gpt)	BS-C-1	SSC =	slightly smaller	(Parkinson, Sanderson, and Smith, 1995)
			CV-1	viable		
			HeLa D980R			
			TK-143B			
			Vero			
			RK13	$CsCl_2$ =		
VACWR163/VACWR164 A39R	COP.	TDS (gpt)	BS-C-1	MSC =	=	(Gardner et al., 2001)
	WR	Insertion of COP A39R				
VACWR165 A40R	WR	INS (gpt)	BS-C-1	viable	=	(Wilcock et al., 1999)
			CV-1	MSC =		
			RK13	MS = $CsCl_2$ =	?	
VACWR166 A41L	WR	TDS (gpt)	BS-C-1	MS =	=	(Ng et al., 2001)
			Hela D980R	viable	?	
			CV-1			
VACWR167 A42R profilin homologue	WR	INS (gpt)	BS-C-1	= unk. m.o.i.	=	(Blasco, Cole, and Moss, 1991)
			RK13		?	
	IHD-J	INS (gpt)	BS-C-1		=	
			RK-13	= unk. m.o.i TEM =	?	
VACWR168 A43R non-virion type I membrane glycoprotein	WR	INS (gpt)	CV-1	MSC =	=	(Duncan and Smith, 1992c)
			RK13			
			B-SC-1			
			BHK-21			
			TK-143			
	WR	INS (GFP)	BS-C-1	SSC =	=	(Sood and Moss, 2010)
			BHK-21	viable		
			CV-1			
			HeLa			
			HuTK-			
			RK13			

Table S2: Vaccinia virus genes non-essential for growth in cell culture

Gene[1]	Parent strain [2]	Mutation strategy[3]	Phenotype of knockout virus: Cell line[2]	Phenotype of knockout virus: Replication[4]	Phenotype of knockout virus: Plaque	Reference
			A549			
			human epidermal keratinocyte (P)			
	IHD-J	INS (GFP)	BS-C-1	viable	comet =	
VACWR170 A44L 3-β-hydroxysteroid dehydrogenase homologue	WR	INS (gpt)	CV-1	SSC: 2-3x reduction MSC =	=	(Moore and Smith, 1992)
			BS-C-1	SSC : 2-3x reduction	?	
	Praha P13	INS (gpt)	CV-1	viable	?	(Sroller et al., 1998)
	Praha P20	INS (gpt)	CV-1			
	WR	INS (gpt)	CV-1			
	DRYVAX Wyeth derived virus (DD50)	INS (gpt)	CV-1			
VACWR171 A45R Virion core protein Superoxide dismutase homologue (inactive)	WR	TDS (gpt)	BS-C-1	M&SSC =	=	(Almazan, Tscharke, and Smith, 2001)
			CV-1	=	?	
			RK13			
			HeLa			
			HeLa D980R			
			P338D1	M&SSC		
			U937			
			murine resident peritoneal macrophages (P)	no virus production (M or S)		
			bone marrow-derived macrophages (P)	no virus production (M or S)		
VACWR172 A46R inhibitor of TLR signalling	WR	TDS (gpt)	unk.	viable	?	(Stack et al., 2005)
VACWR173 A47L unknown function	WR	TDS (GFP/bsd)	BS-C-1	MSC =	=	(Yuen et al., 2010)
			BHK-21		?	
			DC2.4			
VACWR174 A48R Thymidylate kinase	WR	INS (gpt)	CV-1	viable	?	(Hughes et al., 1991)
	vHBs4 (TK-)		CV-1		?	
VACWR175 A49R	WR	TDS (gpt)	CV-1	M&SSC =	?	(Mansur et al., 2013)
			BS-C-1	viable	=	
VACWR176 A50R DNA ligase	WR	INS (gpt)	CV-1	SSC =	=	(Kerr and Smith, 1991)
			TK-143	viable		
			RK13			
			rat EF (P)	SSC =	?	

Table S2: Vaccinia virus genes non-essential for growth in cell culture

Gene[1]	Parent strain [2]	Mutation strategy[3]	Phenotype of knockout virus			Reference
			Cell line[2]	Replication[4]	Plaque	
		INS (gpt) markerless	GM8505	=	=	
	WR	INS (lacZ) (=611-3)	BSC-40	viable	?	(DeLange et al., 1995)
		Deletion 1 kb =ΔL29 (drug selection provided by deletion of gene)	BSC-40	viable	small plaque	
		(used 611-3 &ΔL29)	SIRC	SSC =	?	(Parks et al., 1998)
			BSC-40	SSC reduced 4x (72 h.p.i.)	small plaque	
	WR	INS (GFP)	BS-C-1	SSC =	?	(Paran et al., 2009)
			BHK-21			
			RK13			
			HeLa	SSC reduced 50%		
			HFF (Q)	M&SSC reduced		
			HFF	=		
		Premature stop (75 a.a.)	BS-C-1	SSC =		
			BHK-21			
VACWR178 A52R inhibitor of TLR signalling, NFκB activation	unk.	TDS (gpt)	unk.	=	=	(Harte et al., 2003)
VACWR179 (fragment) A53R (fragment) TNF receptor homologue (CrmC)	Tiantan	INS (lacZ)	CEF (P)	viable	?	(Dai et al., 2008)
			HeLa	MS(24) =		
			TK143			
	USSR	TDS (gpt)	BS-C-1	SSC =	=	(Reading, Khanna, and Smith, 2002)
VACWR180 A55R BTB/kelch protein	WR	TDS (gpt)	BS-C-1	M&SSC =	Indistinct border	(Beard, Froggatt, and Smith, 2006)
VACWR181 A56R haemagglutinin	IHD-J	Spontaneous f/s (=IHD-W)	HeLa	SSC=IHD-J	Cell fusion	(Ichihashi and Dales, 1971) Sequencing (Brown, Bloom, and Moyer, 1991)
			L_2	SSC=IHD-J	?	
			CEF	SSC=IHD-J		
	NYCBH	INS (lacZ)	BSC40	SSC =	Cell fusion	(Lee et al., 1992)
VACWR186 B4R	COP.	INS (YFP/gpt)	BGMK	M&SSC =	30-60% smaller	(Burles et al., 2014)
	vP811		BGMK	SSC = MSC: 5-10x reduced	50-70% smaller	
VACWR189	WR	TDS (gpt)	BS-C-1	M&SSC =	=	

Table S2: Vaccinia virus genes non-essential for growth in cell culture

Gene[1]	Parent strain [2]	Mutation strategy[3]	Phenotype of knockout virus			Reference
			Cell line[2]	Replication[4]	Plaque	
B7R			HeLa D980R	viable	?	(Price et al., 2000)
			Jurkat E6.1	SSC =		
			THP-1			
VACWR190 B8R IFN-γ viroceptor	WR	TDS (gpt)	BS-C-1	MSC =	=	(Symons et al., 2002b)
	WR	INS (lacZ)	BS-C-1	viable	=	(Verardi et al., 2001)
			A549	M&SSC =	?	
			L929			
	v50 (TK-)		BS-C-1	viable	=	
			A549	M&SSC =	?	
			L929			
VACWR191 B9R	WR	TDS (gpt)	BS-C-1	viable	=	(Price, Tscharke, and Smith, 2002)
			TK-143			
			RK13	M&SSC =		
			HeLa D980R	viable	?	
VACWR194 B12R Ser/Thr kinase homologue (nonfunctional)	WR	TDS (gpt)	D98R	viable	?	(Banham and Smith, 1993)
			BS-C-1	MSC =		
VACWR195 B13R /B14R (fragments) Serpin (SPI-2) crmA homologue, Intracellular inhibitor of IL-1β converting enzyme (caspase 1) and granzyme B	WR	INS (lacZ)with TDS (gpt)	BS-C-1	MSC =	=	(Legrand et al., 2004)
			L929		?	
			A549		=	
	v50 (TK-)		BS-C-1			
			L929		?	
			A549		=	
	WR	TDS (gpt)	BS-C-1	M&SSC =	?	(Kettle et al., 1995)
	WR	DIS (hyg)	CV-1	viable	no cell fusion	(Zhou et al., 1992)
	WR	INS (gpt)	BS-C-1	viable	=	(Shisler, Isaacs, and Moss, 1999)
			A549		=	
			Human keratinocyte (P)		=	
VACWR196 (WR-B14R) B15R	WR	TDS (gpt)	CV-1	M&SSC =	40% smaller	(Chen, Jacobs, and Smith, 2006)
			BS-C-1		20% smaller	
			RK13	viable	=	
VACWR197 (WR-B15R) 270/B16R (fragments) IL-1β binding protein	WR	TDS (gpt)	D98	viable	?	(Alcami and Smith, 1992)
	unk.	INS (gpt)	unk.	viable	?	(Spriggs et al., 1992)
VACWR200 (WR-B18R) B19R IFN-α/β viroceptor	WR	TDS (gpt)	D98	viable	?	(Alcami and Smith, 1992)
	unk.	TDS (gpt)	unk.	viable	?	(Spriggs et al., 1992)

Table S2: Vaccinia virus genes non-essential for growth in cell culture

* The chemokine binding protein is not secreted by either Copenhagen or WR. The annotated ORFs *C23L/B29R* in Copenhagen and VACWR001/VACWR218 of WR initiate translation from a downstream methionine in the same reading frame as the chemokine binding protein expressed from CPXV and other VACV strains (35 kDa protein). A truncated protein of 7.5 kDa is produced by WR, out of frame with the CPXV homologues, consistent with translation initiating from the same methionine as the CPXV homologues, although this ORF is not annotated in WR or Copenhagen.

VV-WR-L929: L929 adapted WR, the strain used in this case also has the thymidine kinase locus disrupted by a foreign gene.

WR32-7/Ind14K : was produced from a variant of WR with an 8 MDa deletion at the left end of the genome and possibly other unidentified changes. An inducible copy of the A27L gene was inserted along with the lacI repressor gene into the TK locus. The natural A27L gene is disrupted by gpt (Paez, Dallo, and Esteban, 1987; Rodriguez and Smith, 1990; Sanderson, Hollinshead, and Smith, 2000).

VVLUC (WR-derivative); v50 (WR-derivative); vHBs4: The thymidine kinase locus in these strains is disrupted.

Supplementary Table 3: Genes assumed to be non-essential on the basis of viruses with large deletions

Gene Name WR	Gene Name Copenhagen	Virus
VACWR002/VACWR217	pseudogene	vP811, vP759, vP457, vSSK2, vGS100
VACWR003/VACWR216 [1, 2]	no orthologue	vP457, vSSK2, vGS100
VACWR004/VACWR215	C22L/B28R	vP811, vP759, vP457, vSSK2, vGS100
VACWR005/VACWR214	pseudogene	vP811, vP457, vSSK2, vGS100
VACWR006/VACWR213	C21L/B27R	vP811, vP457, vSSK2, vGS100
VACWR007/VACWR212 [2]	no orthologue	vP457, vSSK2, vGS100
pseudogene	C20L/B26R	vP811, vP457
VACWR008/VACWR211	C19L/B25R	vP811, vP457
no orthologue	C18L/B24R	vP811
no orthologue	C17L/B23R	vP811
no orthologue	C16L/B22R	vP811
no orthologue	C15L/B21R	vP811
VACWR011/VACWR208 [2]	no orthologue	vP457, vSSK2, vGS100
VACWR012/VACWR207 [2]	no orthologue	vP457, vSSK2, vGS100
VACWR014	no orthologue	vP457
VACWR015	no orthologue	vP457
VACWR016	no orthologue	vP457
VACWR017	no orthologue	vP457
VACWR018	no orthologue	vP457
VACWR019	C9L	vP811, vP457
VACWR020	C8L	vP811, vP457
VACWR023	C5L	vP811, vP457
VACWR027	C1L	vP811, vP457
VACWR030	M1L	vP811, vP457
VACWR036	pseudogene	vP811
VACWR037	K5L	vP811
VACWR038	K6L	vP811
VACWR145 [1]	A25L	vP811, vP759
VACWR198	B17L	vP759, vSSK2
VACWR199	B18R	vP759, vSSK2
VACWR201	pseudogene	vP759, vSSK2
VACWR202/203	B20R	vP759, vSSK2
VACWR204	no orthologue	vSSK2
VACWR204.5	264 (at left)	vP811, vSSK2
VACWR206	C13L/C14L	vP811, vSSK2, vGS100

[1] Not expressed (Yang et al., 2011)
[2] Single copy remains in vP457, vSSK2, vGS100.

Table S4: Vaccinia virus genes with no published deletions

Supplementary Table 4: Vaccinia virus genes with no published deletions

Gene Name WR	Gene name Copenhagen	Notes
VACWR045	F6L	Found in all orthopoxviruses sequenced prior to 2004 (Gubser et al., 2004)
VACWR053	F14L	
VACWR054	F15L	Found in most chordopoxviruses (Upton et al., 2003; Delhon et al., 2004)
VACWR146	no orthologue	Gene fragment, no promoter (poxvirus.org)
VACWR147	no orthologue	Gene fragment, no promoter (poxvirus.org)
VACWR154	A31R	Found in mature virions (Chung et al., 2006)
VACWR160	A37R	
VACWR161	pseudogene	Gene fragment, no promoter (poxvirus.org)
VACWR169	268	
VACWR177	A51R	
pseudogene	A54L	Gene fragment, no promoter (poxvirus.org)
VACWR181.5	269	
VACWR182	A57R	Gene fragment, no promoter (poxvirus.org)
VACWR184	B2R	
VACWR185	B3R	Gene fragment, no promoter (poxvirus.org)
VACWR188	B6R	
VACWR192	B10R	Gene fragment, no promoter (poxvirus.org)
VACWR193	B11R	

Table S5: Host range genes of vaccinia virus

Supplementary Table 5: Host range genes of vaccinia virus

Deleted Gene(s)	Parent strain	Cell lines				Reference
		Normal replication		Severe replication defect		
VACWR021 C7L	unk.	HeLa MRC-5 HEp2 RK-13	BHK-21 CEF BRL	Dede NRK		(Oguiura, Spehner, and Drillien, 1993)
	COP. (TK-)	MRC-5 Vero LLC- PK1	RK13			(Perkus et al., 1990)
VACWR032 K1L		Dede HeLa HEp2 MRC-5	BRL BHK-21 CEF	RK-13 NRK		(Oguiura, Spehner, and Drillien, 1993)
	COP. (TK-)	MRC-5 Vero	LLC-PK1	RK-13		(Perkus et al., 1990)
ΔC7LΔK1L double	WR (TK-)	Huh7 MCF-7 Vero P815		A431 HT-3 Ca Ski SKOV-3	RK13 HeLa NIH/3T3 LA-4	(Meng and Xiang, 2006; Meng, Chao, and Xiang, 2008; Meng et al., 2009; Meng et al., 2012)
	WR	BHK-21 CEF BRL		HEp2 RK-13 NRK	MRC-5 Dede HeLa	(Oguiura, Spehner, and Drillien, 1993)
	COP. (TK-)	Vero		MRC-5 LLC-PK1 RK-13	WISH HeLa Detroit	(Perkus et al., 1990)
VACWR034 K3L	COP.	L-929 HeLa (5x)		BHK-21		(Langland and Jacobs, 2002)
	WR	CV-1				(Rice et al., 2011)
VACWR059 E3L	COP.	BHK-21		HeLa		(Langland and Jacobs, 2002)
		CEF (P) RK-13		Vero HeLa	L929	(Beattie et al., 1996)
				BSC-40		(Xiang et al., 2002)
	WR	BHK-21		Vero HeLa	U20S	(Simpson-Holley et al., 2011)
	WR	BHK-21		PK15		(Rice et al., 2011)
VACWR205 (WR-B22R or WR-B24R) C12L Serpin (SPI-1)	WR	BS-C-1				(Kettle et al., 1995)
	WR	BS-C-1	L929	A549		(Legrand et al., 2004)
	WR (TK-)	BS-C-1 L929	A549 (10x)			
	WR	PK15 BS-C-1	HaCaT	A549 human keratinocyte (P)		(Shisler, Isaacs, and Moss, 1999)
	WR	CV-1				(Zhou et al., 1992)

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Supplementary Table 6: Oligodeoxynucleotide primers used for InFusion Cloning

Name	Sequence[1]
pSSGbpro	**CATGCTCGAGCGGCC**GC CGCTTACAATTTCCTGATGC
811homLfwdBsp	GGGATTTTGGTCATGA TGTCTGGTAACCTAGATGGGCA
811homLfwdNot	ATCACACTGGCGGCCGC TGTCTGGTAACCTAGATGGGCA
811homLrevBsp	TTTGATAATCTCATGA TGAAGGTGCAACCGATGATACT
F5LrightXba	AATTGGGCCCTCTAG ACGGATTTGACTGTGTGGAGA
C7LleftXba	CGAGCATGCATCTAG ATCGAAGACATGGTTACTCCT
811F5INF	**ACTATGGAATAAGCC** TCAGTTATCTATATGCCTGTACTTG
C7Lright	*GGCTTATTCCATAGT*AGCTTGTG
811rightfwd	AATTGGGCCCTCTAG AGACAATCATCTTGGAGCAACAG
811rightrev	**GGCCGCTCGAGCATG** CATAGTAATCGATATTGGTCGTGTAGC
811homLrev	*TGAAGGTGCAACCGA*TGATACT
mCherryrev	TCGGTTGCACCTTCA tta CTTGTACAGCTCGTCCATGC[2]
ITRrhomfwd	AATTGGGCCCTCTAGA GACTATCGGCGTACTATCCA

Name	Sequence[1]
ITRrhomrev	**GGCCGCTCGAGCATG** TTGATTCTAATATAATCTTGCACAA
WRRUfwd	***TTGTGCAAGATTATATTAGAATCAA***[3]
mCherryRU	TATAATCTTGCACAAtta CTTGTACAGCTCGTCCATGC[2]
WRRUrev	ATCACACTGGCGGCCGC CCGGAAGGGACTATATGACTAAC
WRRUfwdNotI	**CATGCTCGAGCGGCCGC** TTGTGCAAGATTATATTAGAATCAA
WRC10Lleft	GAGCGGCCGCACCGG GTTCTCGTGATTCGTCAAAC
WRC11Rright	TTGCACCTTCACCGG ACTCCGTGTTTATGATCATTTT
WRC10Lright	TTGCACCTTCACCGG AATGCTAAGTATGCGATGTATCT
WRC11Rleft	GAGCGGCCGCACCGG AGATACATCGCATACTTAGCATT
WR198right	**GGCCGCTCGAGCATG** ATGCTTTGTGGTAAAAGTCCTC
WR198left	ACTATGCATGCTCGAG GGCGTTTACTATTCCTAGCATC
WR202right	CAAGCGGCCGCTCGAG GCCATCATTATGTTCTTGCC
WR201fwd	**CATGCTCGAGCGGCCGC** GTATCTCACCGATAGAGAACAT
WR206right	***TTGATTCTAATATAATCTTGCACAA***[3]
811homLrevAce	**GAGCGGCCGC*ACCGG*** *T*GAAGGTGCAACCGATGATACT
ITRrhomRevAce	**CCGGTGCGGCCGCTC** TTGATTCTAATATAATCTTGCACAA
WRC11Rinf	**CATGCTCGAGCGGCCGC** AGATACATCGCATACTTAGCATT

[1]InFusion tails are complementary to vector (underlined) or a neighbouring insert (bold).
[2]mCherryRU and mCherryrev restore the stop codon (in red) to the mCherry gene when mCherry-bsdR fusion gene from pSSmCB is used as template.
[3]Bold italics are required for priming but are also complementary to a neighbouring insert: WR206right and WRRUfwd are complementary**.**

Supplementary Table 7: InFusion cloning reactions

Use of plasmid	Plasmid produced[1]	Parental plasmid (Restriction enzyme)	Template DNA for insert	Forward primer for insert	Reverse primer for insert
To delete WR001-WR043 (ΔLeft)	pSSGb::811homL	pSSGb (BspHI)	WR DNA	*811homLfwdBsp*	*811homLrevBsp*
	pBII::811homRC7L	pCR-Blunt II (XbaI)	WR DNA	*811F5INF*	*F5LrightXba*
			WR DNA	*C7LleftXba*	*C7Lright*
	pBII::811C7LGb	pBII::811homRC7L (NotI)	pSSGb::811homL	*811homLfwdNot*	*pSSGbpro*
To delete WR195-WR218 (ΔRight)	**pBII::811rightmC**	pCR-Blunt II (NotI and XbaI)	WR DNA	*811homLfwdNot*	*811homLrev*
			WR DNA	*811rightfwd*	*811rightrev*
			pSSmCB	*pSSGbpro*	*mCherryrev*
To delete WR195-WR206 (ΔUniqueR)	**pBII::UniqueRmC**	pCR-Blunt II (NotI and XbaI)	WR DNA	*811rightfwd*	*811rightrev*
			pSSmCB	*pSSGbpro*	*mCherryRU*
			WR DNA	*WRRUfwd*	*WRRUrev*
To delete WR207-WR218 (ΔITRr)	**pBII::ITRmC**	pCR-Blunt II (NotI and XbaI)	WR DNA	*811homLfwdNot*	*811homLrev*
			pSSmCB	*pSSGbpro*	*mCherryrev*
			WR DNA	*ITRrhomfwd*	*ITRrhomrev*
To restore C11R, C10L, or C11R and C10L to WRΔLΔITRr	pBII::ITRH	pCR-Blunt II (NotI and XbaI)	WR DNA	*811homLfwdNot*	*811homLrevAce*
			WR DNA	*ITRrhomfwd*	*ITRrhomRevAce*
To restore C11R and C10L	**pBII::ITRC11RC10L**	pBII::ITRH (AgeI)	WR DNA	*WRC10Lleft*	*WRC11Rright*
To restore C10L	**pBII::ITRC10L**	pBII::ITRH (AgeI)	WR DNA	*WRC10Lleft*	*WRC10Lright*
To restore C11R	**pBII::ITRC11R**	pBII::ITRH (AgeI)	WR DNA	*WRC11Rleft*	*WRC11Rright*
To restore WR195-WR198 to WRΔLΔUniqueR	**pBII::WR195-198**	pCR-Blunt II (NotI and XbaI)	WR DNA	*811rightfwd*	*WR198right*
			WR DNA	*WRRUfwdNotI*	*WRRUrev*
To restore WR198-WR202 to WRΔLΔUniqueR	pBII::UniqueH	pCR-Blunt II (NotI and XbaI)	WR DNA	*811rightfwd*	*811rightrev*
			WR DNA	*WRRUfwdNotI*	*WRRUrev*
	pBII::RUWR198-202	pBII::UniqueH (XhoI)	WR DNA	*WR198left*	*WR202right*
To restore WR201-WR206 to WRΔLΔUniqueR	**pBII::WR201-206**	pCR-Blunt II (NotI and XbaI)	WR DNA	*WRRUfwd*	*WRRUrev*
			WR DNA	*WR201fwd*	*WR206right*
			WR DNA	*811rightfwd*	*811rightrev*
To restore C11R to WRΔLR	**pBII::LRC11R**	pCR-Blunt II (NotI and XbaI)	WR DNA	*811homLfwdNot*	*811homLrev*
			WR DNA	*811rightfwd*	*811rightrev*
			WR DNA	*WRC11Rinf*	*WRC11Rright*

[1] Plasmids shown in bold were used in infection-transfection experiments to produce recombinant viruses.