

**Supplemental Table S1: Details of transposon gene interruptions into the MeHV-1 infectious clone pMeHV1-C18.**

The clone name, gene designation and nucleotide position of the transposon insertion are detailed. Insertion locations were mapped to the MeHV-1 reference genome sequence [Genbank: NC\_002641.1]. The orientation of the transposon insertion into the parent iBAC genome, either forward (+) or reverse (-) in respect to the MeHV-1 reference genome sequence, was determined by DNA sequencing. Insertions within repeat regions of Tn5 insertion clones were further characterised by restriction enzyme analysis with *SbfI* and *FseI*.

<b>Gene/ element</b>	<b>Clone</b>	<b>Nucleotide position (+/-)</b>
<i>vLip</i> (HVT005)	MuAΔ38	7047-7051 (+)
<i>LORF2</i> (HVT007)	MuAΔ30	9327-9331 (+)
<i>UL6</i>	MuAΔ75	17747-17751 (+)
	MuAΔ73	17873-17877 (+)
<i>UL8</i>	MuAΔ58	20160-20164 (-)
<i>UL9</i>	Tn5Δ2	22352-22360 (+)
	MuAΔ29	23883-23887 (-)
<i>UL10</i>	MuAΔ28	25857-25861 (+)
	MuAΔ48	26030-26034 (+)
Intergenic	MuAΔ74	26188-26192 (+)
<i>UL13</i>	MuAΔ69	28881-28885 (+)
<i>UL17</i>	MuAΔ27	32633-32637 (+)
	Tn5Δ12	33504-33512 (-)
	MuAΔ32	34287-34291 (-)
<i>UL19</i>	MuAΔ66	40885-40889 (+)
<i>UL21</i>	MuAΔ37	42375-42379 (+)
	Tn5Δ14	42635-42643 (-)
	MuAΔ41	43080-43084 (-)
<i>UL26</i>	MuAΔ53	50761-50765 (-)
<i>UL26.5</i>	MuAΔ31	51870-51874 (-)
<i>UL27</i>	MuAΔ43	53097-53101 (-)
	MuAΔ64	54786-54790 (+)
	MuAΔ33	55039-55043 (-)
<i>UL29</i>	Tn5Δ7	58098-58106 (+)
	MuAΔ26	59940-59944 (-)
Intergenic	MuAΔ40	68440-68444 (+)
<i>UL36</i>	MuAΔ81	72199-72203 (+)
	MuAΔ52	72288-72292 (-)
	MuAΔ79	78147-78151 (-)
<i>UL37</i>	Tn5Δ6	80288-80296 (+)
<i>UL39</i>	Tn5Δ23	86217-86225 (-)
<i>UL40</i>	Tn5Δ18	87217-87225 (+)
Intergenic	MuAΔ35	89528-89532 (-)
<i>UL42</i>	MuAΔ42	90563-90567 (+)
<i>UL47</i>	MuAΔ80	98798-98802 (-)
Intergenic	MuAΔ61	99684-99688 (+)
<i>UL48</i>	MuAΔ71	99923-99927 (+)

	MuAΔ72	100650-100654 (-)
<i>UL52</i>	Tn5Δ17 MuAΔ57 MuAΔ67	105088-105096 (+) 105362-105366 (-) 105424-105428 (-)
<i>UL53</i>	MuAΔ68	108699-108703 (-)
Intergenic	MuAΔ60 MuAΔ77	108914-108918 (-) 111349-111353 (-)
<i>LORF4A</i> ( <i>HVT067</i> )	Tn5Δ10	113752-113760 (-)
<i>LORF5</i> ( <i>HVT069</i> )	MuAΔ59 MuAΔ82	115165-115169 (+) 115602-115606 (-)
Intergenic	MuAΔ63  MuAΔ54  MuAΔ55	3898-3902 or 119785-119789 (ND) 1933-1937 or 121750-121754 (ND) 124295-124299 or 158297-158301 (ND)
<i>vNR13</i> ( <i>HVT079</i> / <i>HVT096</i> )	MuAΔ46	124989-124993 or 157603-157607
<i>icp4</i> ( <i>HVT080</i> / <i>HVT095</i> )	MuAΔ34  MuAΔ78  MuAΔ47  Tn5Δ19 MuAΔ83  Tn5Δ22 Tn5Δ4 Tn5Δ1 Tn5Δ3 Tn5Δ11 Tn5Δ8	128905-128909 or 153687-153691 (ND) 129979-129983 or 152612-152617 (ND) 130476-130480 or 152116-152120 (ND) 130947-130955 (+) 131154-131158 or 151438-151442 (ND) 132257-132365 (-) 149786-149794 (+) 151300-151308 (-) 153044-153052 (-) 154273-154281 (+) 154730-154738 (+)
Intergenic	MuAΔ56  MuAΔ25  MuAΔ51  MuAΔ44  MuAΔ50	133260-133264 or 149332-149336 (ND) 134298-134302 or 148294-148298 (ND) 134546-134550 or 148046-148050 (ND) 135789-135793 or 146803-146807 (ND) 135863-135867 or 146729-146733 (ND)
<i>US3</i>	MuAΔ70 MuAΔ45	141220-141224 (+) 141654-141658 (+)
<i>US6</i>	MuAΔ49	143203-143207 (-)
<i>US8</i>	Tn5Δ13 Tn5Δ21	145780-145788 (-) 145937-145945 (+)

	MuAΔ76	136053-135057 or 146539-146543 (ND)
Intergenic	Tn5Δ24	156371-156379 (-)
a-like sequences	Tn5Δ16 Tn5Δ20	Not determined
	MuAΔ65 MuAΔ84	BAC vector sequences

Supplemental Table S2: **Oligonucleotides used in this study.**

Oligonucleotide	Sequence (5' → 3')
<KAN2> FP1	ACCTACAACAAAGCTCTCATCAACC
KanMEFwd	CTCCTTCATTACAGAAACGGC
<KAN2> RP1	GCAATGTAACATCAGAGATTTTGAG
KanMERev	ACAATCGATAGATTGTGCGCAC
HVT27Fwd	CGAAAGCGGATAACCAGAAC
HVTFrag5Rev	AAATCGCCATTTTACGCATC
KanRMfeIFwd	TGAGTTAACGTCGGTTGATGAGAGCTTTGTTGTAGGTG
GFPseq	CGTCGCCGTCCAGCTCGACCAG
ECGFPH Fwd	G TTCCTAAGCTTTTGCTGGCCTTTTGCTCACATGT

Supplemental Figure S1: **Primary chicken embryo fibroblasts (CEFs) were used as a negative control**

Primary chicken embryo fibroblasts (CEFs) were monitored as a negative control to assess the replication capacity of transposition mutants in cell culture. Day 7 of passage 1 a: brightfield ×100; and b: fluorescent microscopy ×100

### Uninfected

