

**Extended Data Table 2. Oligonucleotide Primers for HCMV BAC recombineering procedures.**

Primer Name	Purpose	PCR template	BAC targeted for modification	Sequence, 5'-3'
UL141 repair Kan Fw	Repair UL141 frameshift in HCMV strain TB40/E	I-SceI-AphAI (KanR) sequence (PMID: 16526409)	TB40-BAC4 (PMID: 18198366) or derivatives thereof	GTCGCCGGCGCCGGTGTGG TCGCCGAGGGAGAGCAAGTTA CCATCCCCTGCACGGTCATAG GGATAACAGGGTAATCGATTT
UL141 repair Kan Rv				CCATGGGCCAGGAGTGTGTCA TGACCGTGCAGGGGATGGTAA CTTGCTCTCCCTCGGCGAGCC AGTGTTACAACCAATTAACC
UL141_FLAG_Kan_Fw	Add FLAG epitope tag to the C-terminus of UL141	I-SceI-AphAI (KanR) sequence (PMID: 16526409)	TB40-BAC4 (PMID: 18198366), TR3 (PMID: 31848362) or derivatives thereof	GACTTACGATAGTTACCCCGGT GTTAAAAAGATGAAGAGGGAC TACAAGGATGACGACGATAAGT GAGAACATAGGGATAACAGGG TAATCGATTT
UL141_FLAG_Kan_Rv				TTTTTTAACATGTTATTTTTTTTATTT TATGCGTGTTCCTCACTTATCGTCG TCATCCTTGTAGTCCCTCTTCAGC CAGTGTTACAACCAATTAACC
Xfer_UL141_FLAG_Fw	Replace UL131-UL128 with UL141-FLAG CDS	TR3-UL141-FLAG-Kan-integrate (intermediate in BAC recombineering)	AD169rv (Hobom et al., 2006, PMID: 10933677)	ATGATGTCTCATAATAAAGCTTTCT TTCTCAGTCTGCAACAGCGTCTC TGCGAAAAAGG
Xfer_UL141_FLAG_Rv				CGACAGAAATCTCAAACCGCGTAT TTCGGACAAAACACACCAACAC GCCATTCAATCC
EGFP_in_Kan_Fw	To produce an I-SceI-AphAI-disrupted eGFP locus in a GFP+ TB40-BAC4 derivative to enable transfer of the eGFP locus to the TR3 BAC	I-SceI-AphAI (KanR) sequence (PMID: 16526409)	TB40E_5: a TB40-BAC-4 derivative expressing eGFP (PMID: 22241980)	GGCAACTACAAGACCCGCGCCG AGGTGAAGTTCGAGGGCGACAC CCTGGTGAACCGCATCTAGGGAT AACAGGGTAATCGATTT
EGFP_in_Kan_Rv				AAGTCGATGCCCTTCAGCTCGAT GCGGTTACCAGGGTGTGCCCC TCGAACTTACCTCGGCCAGTGT TACAACCAATTAACC
Us34CT_Fw	Transplant the AphAI-disrupted eGFP expression cassette from TB40-BAC4 GFP to TR3	TB40E_5_eGFP-I-SceI-AphAI, a TB40-BAC-4 derivative expressing eGFP (PMID: 22241980) in which the eGFP locus is disrupted by an I-SceI-AphAI sequence that can later be removed via en passant BAC mutagenesis	TR3 (PMID: 31848362)	TGTTCCCTCCTTGAACCGC
TRS1/Us34				CTCCGCATCCCACCATTCTT