

SUPPLEMENTARY

Latent Macrophage and Immature B Cell Lines Generated with Hygromycin-Resistant Murine Gammaherpesvirus 68 Genome Expresses Modest Levels of Viral miRNAs

M. Kara

Table S1. Ligation reaction set up

Ligase Reaction	
10X T4 DNA Ligase buffer	10 µl
DNA	10 µl
Ligase	2 µl
H ₂ O	78 µl

Table S2. Primers used for DC-PCR for MHV68. All primers are listed to 5' to 3'.

Primer	Sequence	MHV68 Genomic location	Distance to closest BamHI site
DCTR Bam Lout	CTCTCAACTAACACTAACAGAGGATTT	16671 +	536
DCTR Bam Rin	TAGCGCCACCATGGTGGTAAACAA	16988 +	219
DCTR Bam Lin	GGCTTTGTGGTCGTTACACCTC	116851 -	88
DCTR Bam Rout	ATGTCTACACCTACATGCCCGCATC	117108 -	345
DC64 Bam Lout	CAACCACAGAATATAACACCCATCTACTG	90319 +	484
DC64 Bam Lin	AACCAGTCCCCAACTGAAAGAACG	90633 +	170
DC64 Bam Rin	ATTGCTGTAAGCATGTAATTAATA	77206 -	100
DC64 Bam Rout	TGATTTTGTGCTGGAATTGCACCTG	77426 -	320

BamHI cuts the viral genome (WUMS reference genome NC_001826.2) at nucleotide position 17207 and 116763 which are nearest to the primer binding sites. When BamHI digested ends are ligated, the expected PCR product sizes are 881 bp for TR 1st PCR, 307 bp for the 2nd round PCR. For ORF64, the expected size is 804 bp for the 1st round and 270 bp for the 2nd round PCR.

Table S3. PCR parameters used for DC-PCR.

PCR parameters	
1. Initial denaturation	94 °C 5 minutes
35 cycles	2. Denaturation 94 °C 5 minutes
	3. Annealing 58 °C 1 minute for 1 st round
	56 °C 1 minute for 2 nd round
	4. Extension 72 °C 1 minute
5. Final extension	72 °C 7 minutes

Table S4. Sequences for TaqMan primers and probes.

miRNA name	Sequence	Assay ID
snoRNA234	CUUUUGGAACUGAAUCUAAGUGAUUU AACAAAAAUUCGUCACUACCACUGAGA	001234
mgHV-miR-M1-1-3p	UAGAAAUGGCCGUACUCCUUU	005838_mat
mgHV-miR-M1-5-5p	AGAGUUGAGAUCGGGUCGUCUC	008478_mat
mgHV-miR-M1-6-3p	UGAAACUGUGUGAGGUGGUUUU	006180_mat
mgHV-miR-M1-8-5p	CACUCACUGGGGGUUUGGUCAGG	006796_mat
Mmu-miR-21-5p	UAGCUUAUCAGACUGAUGUUGA	000397
Mmu-miR 146a-5p	UGAGAACUGAAUCCAUGGGUU	000468

Table S5. Primers to generate M3 probe with in vitro transcription. Italic portion of M3 T7 FWD primer is the T7 promoter sequence. Underlined bold 'G' is the first nucleotide of IVT product.

Primer	Sequence	Genomic location to WUMS strain
M3 T7 FWD	<i>TAATACGACTCACTATAG</i> <u>G</u> GGAGAATGCCAGCACACTCAGTCAC	7058
M3 REV	CAGAAAGTGTGTGTGTTGAGGTG	7347