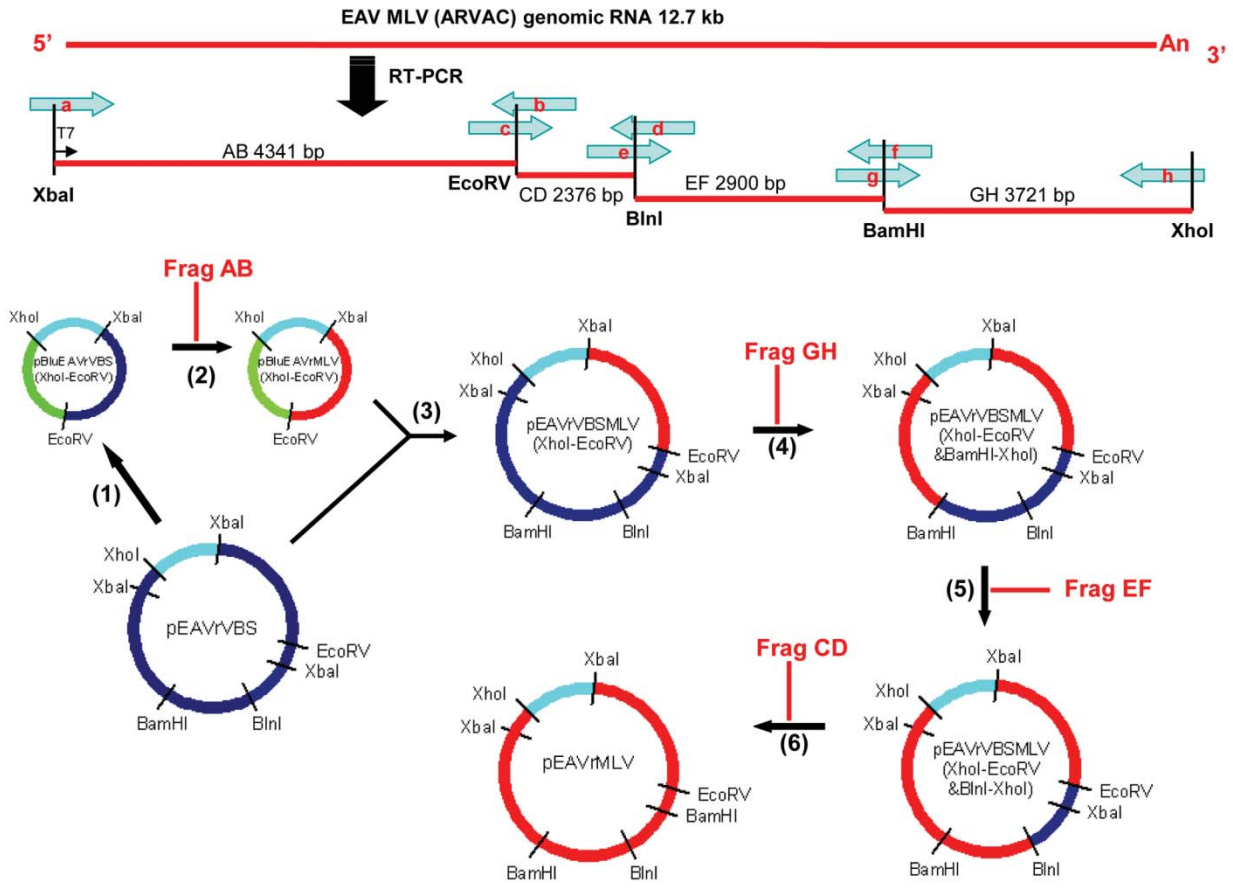

Supplementary Figure S1. Flow diagram showing the strategy of construction of a full-length cDNA clone of the MLV vaccine strain of EAV. The virulent infectious cDNA clone pEAVrVBS (1) was used as the backbone to construct the MLV vaccine clone. Briefly, four overlapping fragments (AB, CD, EF, and GH) that encompass the entire genome of the MLV vaccine strain (ARVAC®) of EAV were RT-PCR amplified using four pairs of oligonucleotide primers (a&b, c&d, e&f, and g&h). Step 1: A shuttle vector pBluEAVrVBS(XhoI-EcoRV) was constructed by replacing the fragment XhoI-EcoRV of the plasmid pBlu2SKP (Stratagene) with the fragment XhoI-EcoRV of the full-length clone pEAVrVBS; Step 2: The fragment AB was digested with restriction enzymes XbaI and EcoRV and then cloned into the shuttle vector pBluEAVrVBS(XhoI-EcoRV) which was also cut with the same restriction enzymes, to obtain the recombinant plasmid pBluMLV(XhoI-EcoRV); Step 3: The plasmid pBluMLV(XhoI-EcoRV) was digested with XhoI and EcoRV and then cloned into the full-length clone pEAVrVBS to obtain the recombinant plasmid pEAVrVBSMLV(XhoI-EcoRV); Step 4: The fragment GH was digested with BamHI and XhoI and then cloned into the plasmid pEAVrVBSMLV(XhoI-EcoRV) to obtain the recombinant plasmid pEAVrVBSMLV(XhoI-EcoRV&BamHI-XhoI); Step 5: The fragment EF was digested with BlnI and BamHI and then cloned into the plasmid pEAVrVBSMLV(XhoI-EcoRV&BamHI-XhoI) to obtain the recombinant plasmid pEAVrVBSMLV(XhoI-EcoRV&BlnI-XhoI); Step 6: The fragment CD was digested with EcoRV and BlnI and then cloned into the plasmid pEAVrVBSMLV(XhoI-EcoRV&BlnI-XhoI) to obtain the full-length clone pEAVrMLV.

1. **Balasuriya, U. B., E. J. Snijder, H. W. Heidner, J. Zhang, J. C. Zevenhoven-Dobbe, J. D. Boone, W. H. McCollum, P. J. Timoney, and N. J. MacLachlan.** 2007. Development and characterization of an infectious cDNA clone of the virulent Bucyrus strain of equine arteritis virus. J Gen Virol **88:**918-24.

Supplementary Table S1. Oligonucleotide primers used to amplify the genome of the MLV vaccine strain of EAV

Fragment	Primers for reverse transcription	Primers for PCR amplification	Amplicon size (bp)
Frag AB (XbaI-EcoRV)	4600N: 5'GTCATCATCAGTGAGGGCAG3'	primer a: 5'CAACGCGAGCTCTCTAGATTAATACGACTC ACTATAGCTCGAAGTGTGTATGGTG3'	4341
Frag CD (EcoRV-BlnI)	6731N: 5'CCCCCGCGTTTGGTGAATGC3'	primer c: 5'TGCTTGTTCCATCTGGTCTG3' primer d: 5'TCTCCAGGTCTGTTTCAAGG3'	2376
Frag EF (BlnI-BamHI)	9614N: 5'ACTTCTGTTGAGCTGAGGAG3'	primer e: 5'ATTAGGAGCATTCTGGGCACC3' primer f: 5'ACGCGACTCAGTGTCTCAGG3'	2900
Frag GH (BamHI-XhoI)	12750N1: 5'GTCTTCAAGAATTAATTCCCCTCGAGTT TTTTTTTTTTTTTTTTTTGGTTCCTGGGTGGCTAATAAC3'	primer g: 5'TATTCTCGTCCGGTAGGTTCG3' primer h: 5'GTCTTCAAGAATTAATTCCCCTCG3'	3721