Supplementary Materials **by**

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TRANSLATIONAL EFFICIENCY OF BVDV-IRES AND EMCV-IRES FOR CYTOPLASMIC EXPRESSION OF T7 RNA POLYMERASE-BASED PLASMIDS IN MAMMALIAN CELL LINES-A COMPARATIVE STUDY

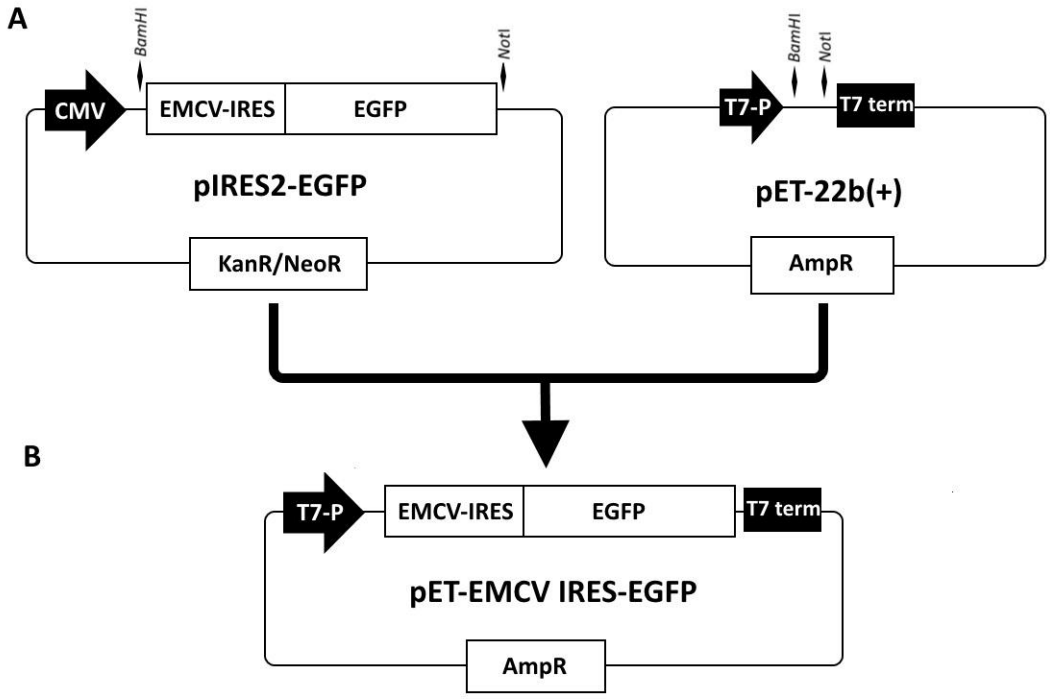

Figure 1sup) Shematic diagram of cloning steps for construction of pET-EMCV IRES-EGFP. A) EMCV IRES-EGFP cassete was cut from pIRES2-EGFP plasmid (Clontech Laboratories, Mountain View, CA, USA) with *BamHI-NotI* double digestion. B) the digested fragment (the DNA fragment corresponding to IRES-EGFP) was gel purified with gene jet kit (Thermo scientific, USA) and sub-cloned into the same site of pET-22b (Novagen, Germany). T7-P and T7 term denote to T7 promoter and termination sequences, respectively. KanR, NeoR and AmpR denote to kanamyci, Neomycin and Ampicilin resistance, respectively.

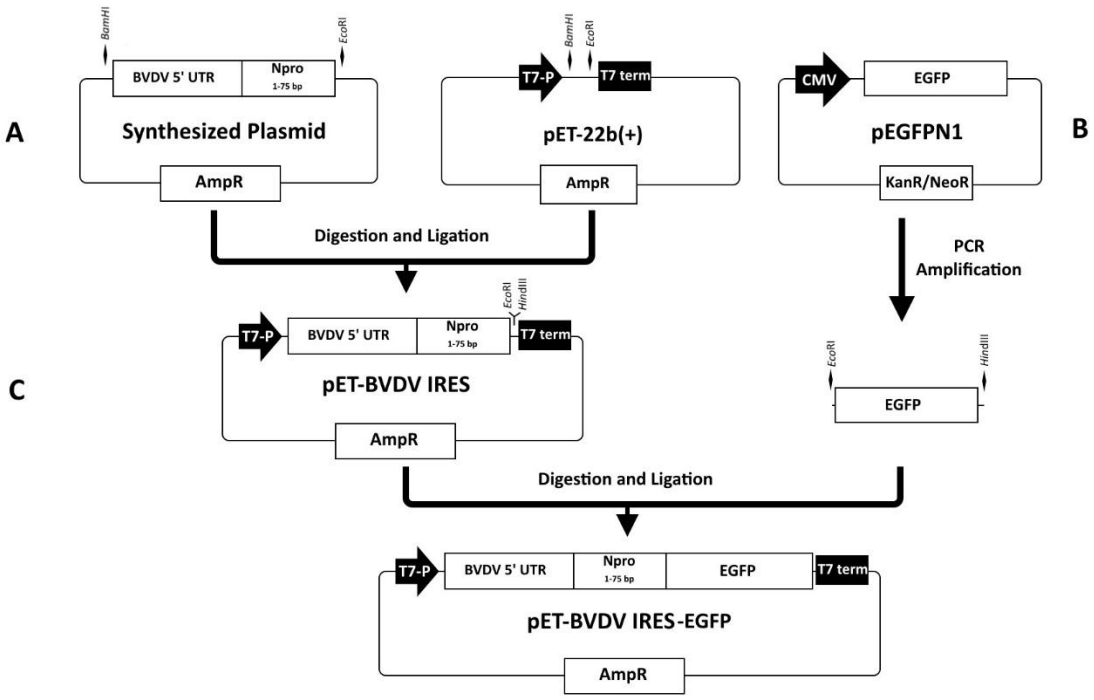

Figure 2sup) shematic diagram of the cloning strategies for construction of pET-BVDV IRES-EGFP. A) BVDV 5`UTR- Npro (5'UTR sequence from Bovine Viral Diarrhea virus genome including the first 75 nucleotide from the N-terminus of Npro gene of BVDV coding region) was synthesized (Biomatik, Canada**)** . The synthesized sequence was double from the plasmid with *BamHI- EcoRI* double digestion and inserted into the same sites of pET-22b(+), downstream of T7 promoter to construct, pET-BVDV IRES vector. B) EGFP gene was amplified with PCR using pEGFPN1 as template. C) To obtain the final pET-BVDV IRES-EGFP reporter construct, the coding sequence of Enhanced Green Fluorescence Protein (EGFP), EGFP gene was was amplified with EGFP-F and EGFP-R primers (Table 1) using pEGFPN1 plasmid (Clontech Laboratories, Mountain View, CA, USA) as template for PCR. The PCR product was double digested with *EcoR*I-*Hin*dIII restriction enzymes and cloned into the same sites of the pET-BVDV IRES to construct final pET-BVDV IRES-EGFP vector. T7-P andT7 term denote to T7 promoter and termination sequences, respectively. KanR, NeoR and AmpR denote to kanamyci, Neomycin and Ampicilin resistance, respectively. UTR denotes to the ¨Untranslated region encoding IRES.

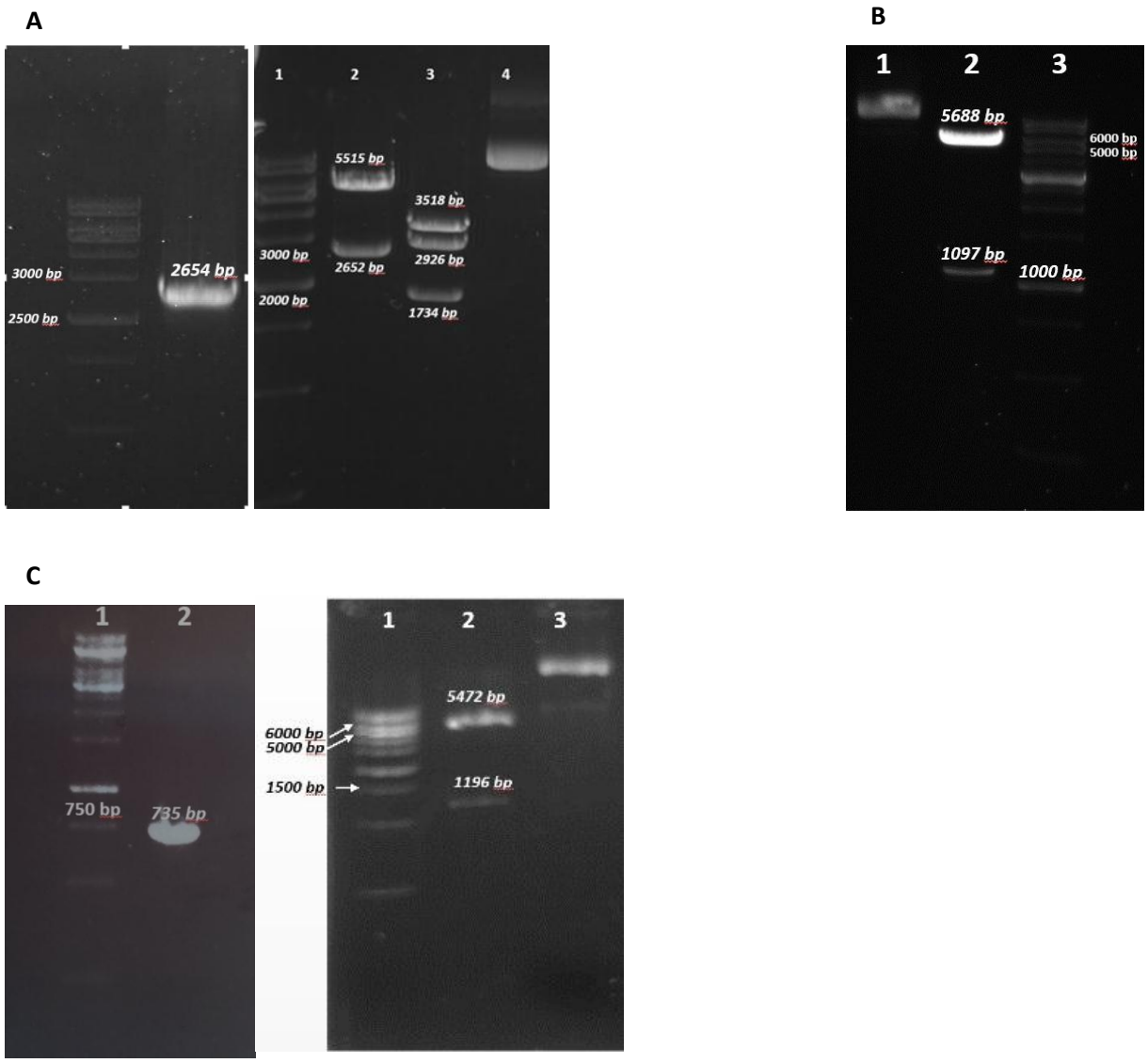

Figure 3sup) Restriction analysis of the constructed plasmids. A) PCR product gele electrophoresis in left: lane1: 1kb DNA marker. Lane 2: amplicon corresponding to T7 RNA polymerase gene. Cloning confirmation of T7 RNA polymerase gene in pCDNA3.1\Hygro(+) in right:Lane1: 1 kb ladder. Lane 2: pHygro-T7 digestion with *NdeI*- HindIII. Lane 3: pHygro-T7 digestion with *NdeI*. Lane 4: undigested pHygro-T7. B) confirmation of EMCV IRES-EGFP fargment subcloning in pET-22b(+). Lane 1: undigested pET-EMCV IRES-EGFP. Lane2: pET-EMCV IRES-EGFP double digested with XhoI- HindIII. Lane 3: 1 kb DNA ladder. C) PCR product gele electrophoresis in left: lane1: 1kb DNA marker. Lane 2: amplicon corresponding to EGFP gene. Confirmation of pET-BVDV IRES-EGFP with *BamHI- HindIII* double digestion in right. Lane1: 1kb DNA ladder. Lane 2: digested pET-BVDV IRES-EGFP. Lane 3: undigested plasmid. As shown in Figure 3S.B and 3S.C, the expected 1097 bp bands (for digestion of pET-EMCV IRES- EGFP) and 1196bp and 5472 bp bands (for digestion of pET-BVDV IRES-EGFP) confirmed the correct insertion of the cloned fragments in two constructed reporter vector.