Cloning Strategy

A subgenomic fragment (corresponding to nucleotides 1549 to 4589 of the EBOV genome) of the full-length cDNA clone plasmid pAmp-rgEBOV was subcloned into the pKan vector using DrdI and NruI (pKan_rgZ1.2-DrdI-NruI). In this plasmid a second NP-VP35 non-coding region with two BspMI sites for later insertion of reporter open reading frames was introduced by amplifying most of the plasmid (including the origin of replication and the kanamycin resistance gene) using the forward primer CACATCCGCTCTCGAGGTGAC, which binds at an internal XhoI site in the VP35 open reading frame, and the reverse primer GTTACTCGAGACCTGCTTGTCATCTTGTTAGACCAGCTTTTC, which binds at the beginning of the VP35 ORF. This fragment was ligated after XhoI digestion to a PCR fragment spanning the NP-VP35 non-coding region as well as part of the VP35 open reading frame up to the XhoI site in VP35 using the forward primer GAATCTCGAGACCTGCTCAGTGAATGAGCATGGAACAATGG and the reverse primer GTCACCTCGAGAGCGGATGTG (pKan_rgZ1.2-DrdI-NruI-iORF). The open reading frames for eGPF (Genbank accession number U76561.1) or luc2 (Genbank accession number AY738222.1) were amplified using the primers CGAATTCTACCTGCGTCAGATGGTGAGCAAGGGCGAGG and CGAATTCACCTGCTCGTTTCACTTGTACAGCTCGTCCATGCC, or CGACGAATTCGTCTCGGATGGAAGATGCCAAAAACATTAAGAAG and CATATACTCGAGCGTCTCATTCACACGGCGATCTTGCCG, respectively, and cloned into pKan_rgZ1.2-DrdI-NruI-iORF using BspMi and BsmBI, resulting in pKan_rgZ1.2-DrdI-NruI-ieGPF and pKan_rgZ1.2-DrdI-NruI-iluc2. The subgenomic fragments were then cloned back into pAmp-rgEBOV via DrdI and NruI.