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pCEP4SIVTat prokaryotic/eukaryotic vector description

SIVmne mRNA coding for Tat was amplified from *M. fascicularis* PBMC using RT-PCR with specific primers beginning at the AUG initiation codon and ending at the UAG stop codon. The SIV *tat* gene is 396 bases in length, comprised of exon 1 (1-296) and exon 2 (297-396). The complete spliced Tat nucleotide DNA sequence is given below:

>SIVtat from SIVmne R. Grant UWNPRC Seattle WA
ATGGAGACACCCTTGAGGGAGCAGGAGAACTCATTAAAATCCTCCAACGGGCGCTCTTCATGC
ACTTCAGAGGCGGCTGCAACCACTCTAGAATCGGCCAATCTGGAGGAGGAAATCCTCTCTCAA
CTATACCGACCTCTAGAAGCATGCTATAACACGTGCTATTGCAAAAAGTGTTGCTACCATTGCCAGT
TTTGTTTTCTTAAAAAGGGCTTGGGGATATGTTATGAGCAGTCACGCAGAAAAAGAAGAACTCCGA
AGAAGGCTAAGGTTAATACATCTTCTGCATCAAACAACAGACCCATATCTGACAGGACCAGGCA
CTGCCAACCAAAGAAGGAACAGAAGGAGACGGTGGAGGCAGCGGTGGCAACAGCTCCTGG
CCTTGGCAGATAG

The complete SIV Tat gene PCR product was cloned into pCRII (Invitrogen) and then subcloned into pCEP4 (Invitrogen) between the XhoI and BamHI sites as illustrated.

-----pCEP4 CMV IE promoter —XhoI – <u>ATG Tat----------Tat TAG</u> – BamHI –pCEP4-----

The pCEP4-SIVTat plasmid was used to transform *E. coli* JM109 and positive clones were selected by growth on ampicillin and were confirmed by DNA sequencing.

pCEP4SIVTat eukaryotic expression vector propagation

1) Remove some of the frozen stock culture using a sterile loop and place in desired volume of LB media containing 100 μg/ml ampicillin. (Note: If stock culture is kept frozen it may be used many times)
2) Incubate liquid culture at 37°C overnight with vigorous shaking.
3) Proceed with plasmid purification or subculture for future use.