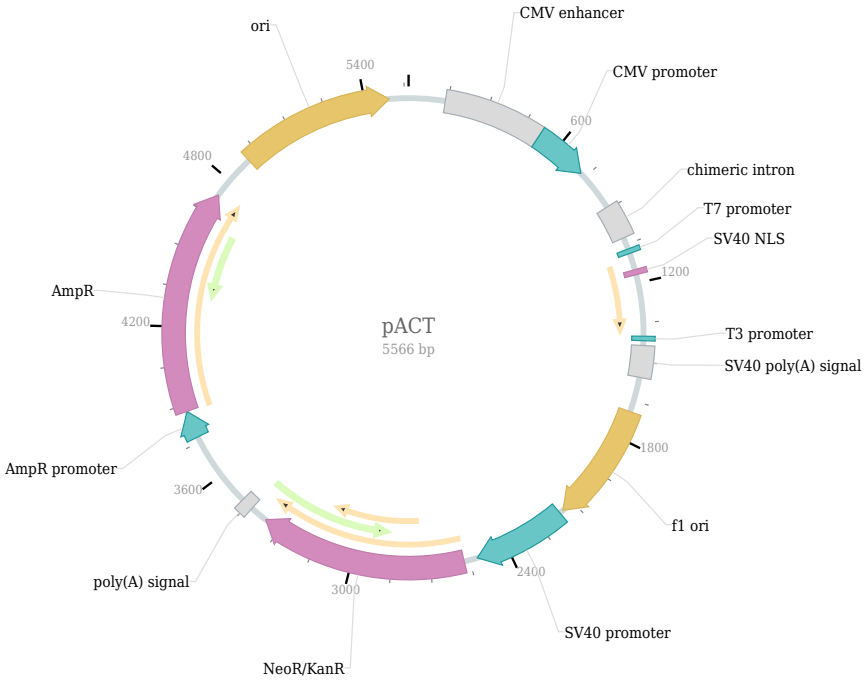
pACT

Vector ID	V006726
Antibiotic Resistance	Ampicillin
Length	5566 bp
Vector Type	Mammalian Two-Hybrid System

Product Information

Replication Origin	ori
Selection Marker	Neomycin
Promoter	CMV
Comment	The pACT Vector is a high-copy plasmid in which the human cytomegalovirus (CMV) immediate early promoter drives expression of the herpes virus VP16 activation domain (amino acids 411–456). A chimeric intron is located 5′ of the gene segment, and a multiple cloning region is located 3′ of the gene segment for insertion of cDNA clones of interest (Figures 2 and 3). The presence of this chimeric intron, in context with the CMV promoter and polyadenylation signal, can result in increased protein expression of cDNA genes linked to these elements (6). The stop codons and SV40 late polyadenylation region, demonstrated to be efficient for mRNA production (7), are at the 3′ end of the fusion gene. The fusion gene region is flanked by T7 and T3 RNA polymerase promoters for the synthesis of sense and antisense RNA products. The T7 promoter allows the construct to be translated using the TNT T7 Quick Coupled Transcription/Translation System (Cat.# L1170). Also located on this vector is the neomycin phosphotransferase gene (from Tn5) driven by the SV40 early promoter and followed by a synthetic polyadenylation cassette. This gene confers resistance to the neomycin analog, G418 (Geneticin; 8). The plasmid backbone contains an f1 origin of replication for the production of single-stranded DNA (ssDNA) and the β-lactamase (Ampr) gene for selection of the vector DNA in E. coli.

Vector Map



Plasmid Resuspension Protocol

1. Centrifuge at 5,000×g for 5 min.
2. Carefully open the tube and add sterile water to dissolve the DNA: add 20 µl for 5 µg plasmid, and 100 µl for 100 µg plasmid.
3. Close the tube and incubate for 10 minutes at room temperature.
4. Briefly vortex the tube and then do a quick spin to concentrate the liquid at the bottom. Speed is less than 5000×g.
5. Store the plasmid at -20 ℃.