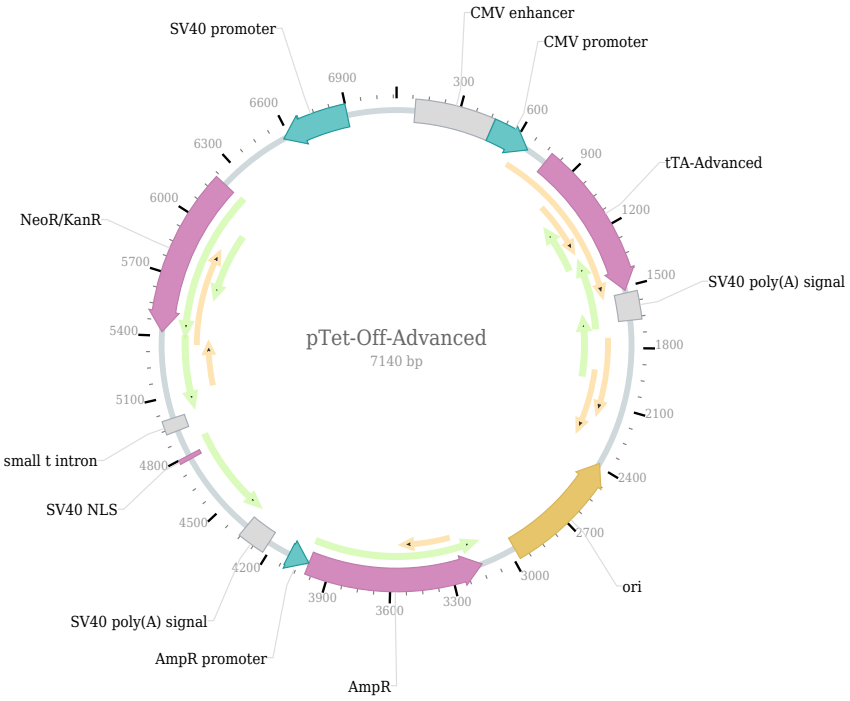
pTet-Off-Advanced

Vector ID	V011072
Antibiotic Resistance	Kanamycin
Length	7140 bp
Vector Type	Mammalian Expression Vectors

Product Information

Replication Origin	ori
Source/Author	Clontech
Selection Marker	Neomycin/G418(Geneticin)
Copy Number	High copy number
Promoter	SV40
Growth Strain(s)	DH10B
Comment	In the Tet-Off expression system, a tetracycline-controlled transactivator protein (tTA), which is composed of the Tet repressor DNA binding protein (TetR) from the Tc resistance operon of Escherichia coli transposon Tn10 fused to the strong transactivating domain(sequence: PTDALDDFDLDML) of VP16 from Herpes simplex virus, regulates expression of a target gene that is under transcriptional control of a tetracycline-responsive promoter element (TRE). The TRE is made up of Tet operator (tetO) sequence concatemers fused to a minimal promoter. In the absence of Tc or Dox, tTA binds to the TRE and activates transcription of the target gene. In the presence of Tc or Dox, tTA cannot bind to the TRE, and expression from the target gene remains inactive. The Tet-On system is based on a reverse tetracycline-controlled transactivator, rtTA. Like tTA, rtTA is a fusion protein comprised of the TetR repressor and the VP16 transactivation domain; however, a four amino acid change(E19G, A56P, D148E, H179R) in the tetR DNA binding moiety alters rtTA's binding characteristics such that it can only recognize the tetO sequences in the TRE of the target transgene in the presence of the Dox effector. Thus, in the Tet-On system, transcription of the TRE-regulated target gene is stimulated by rtTA only in the presence of Dox.

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 ℃.