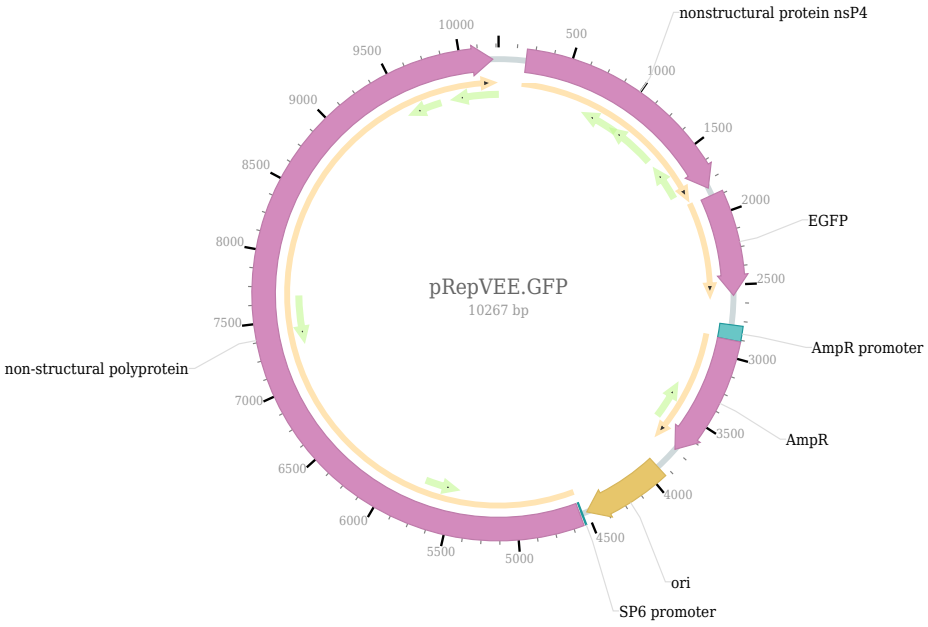
pRepVEE.GFP

Vector ID	V003760
Antibiotic Resistance	Ampicillin
Length	10267 bp
Vector Type	Cloning vector

Product Information

Replication Origin	ori
Source/Author	Kim YG, Baltabekova AZ, Shustov AV.
Growth Strain(s)	Top10
Growth Temperature	37℃
Comment	The VEE-specific part corresponds to the strain TC-83 (Genbank No L01443) with one mutation (T3865->A, an encoded amino acid changes from Gln739 to Leu in a protein nsp2). This mutation was intentionally introduced into the replicon to make its replication noncytopathic. The cDNA copy was cloned in a plasmid pRepVEE.GFP wherein it is placed downstream of a promoter of SP6 RNA polymerase. The SP6 promoter allows synthesizing of the replicon RNA using an in vitro transcription. A unique MluI site is engineered downstream of the replicon's 3'-end; this site is used to linearize the plasmid DNA before a run-off transcription. In the replicon RepVEE.GFP a GFP gene is engineered downstream of the first viral subgenomic promoter.

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 ℃.