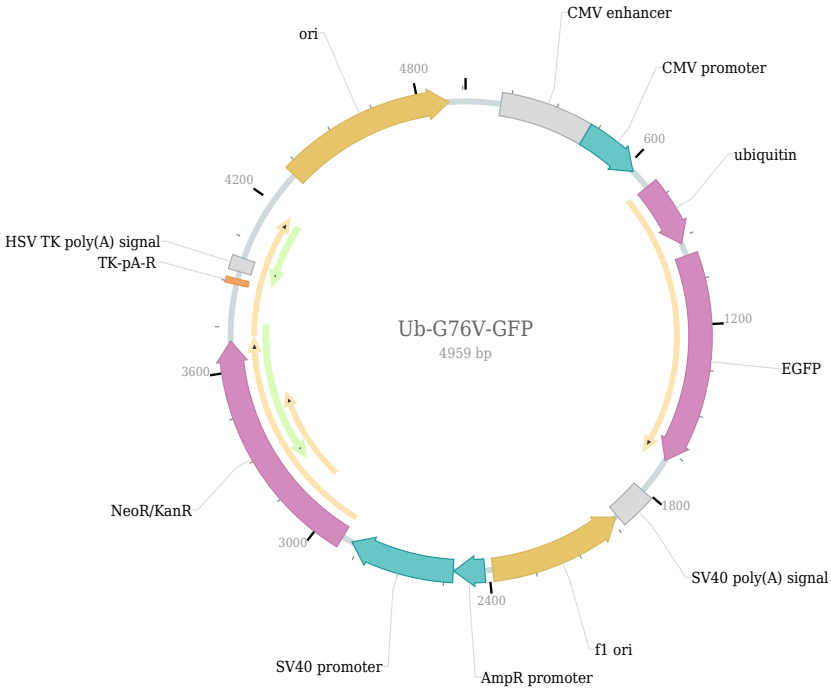
Ub-G76V-GFP

载体编号 Vector ID	V000830
抗性 Antibiotic Resistance	Kanamycin
长度 Length	4959 bp
载体类型 Vector Type	Mammalian Expression

产品信息

复制子 Replication Origin	ori
筛选标记 Selection Marker	Neomycin (select with G418)
拷贝数 Copy Number	High Copy
克隆方法 Cloning Method	Restriction Enzyme
5'引物 5' Primer	EGFP-N (CGTCGCCGTCCAGCTCGACCAG)
培养菌株 Growth Strain(s)	DH10B
备注 Comment	The UbG76V-GFP reporter construct, first reported by Dantuma et al. in Nature Biotechnology in 2000, represents the world's first fluorescent plasmid tool capable of quantitatively measuring the activity of the ubiquitin-proteasome system (UPS) in living intact cells. Wild-type ubiquitin contains a glycine residue at position 76 (Gly76) on its C-terminus, which serves as the canonical cleavage site recognized by deubiquitinating enzymes (DUBs). When this glycine is mutated to valine, generating the G76V variant, two critical functional alterations occur. First, DUBs lose the ability to cleave the N-terminal fused ubiquitin tag from the GFP moiety, so the fusion protein permanently retains a ubiquitin-derived degradation signal. Second, lysine residues within the mutant ubiquitin segment are continuously modified with K48-linked polyubiquitin chains by cellular E3 ubiquitin ligases. These polyubiquitin conjugates are readily recognized by the 19S regulatory complex of the proteasome, which mediates substrate unfolding and rapid translocation into the 20S catalytic core for complete proteolytic degradation.

质粒图谱



复溶方法

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 ℃.