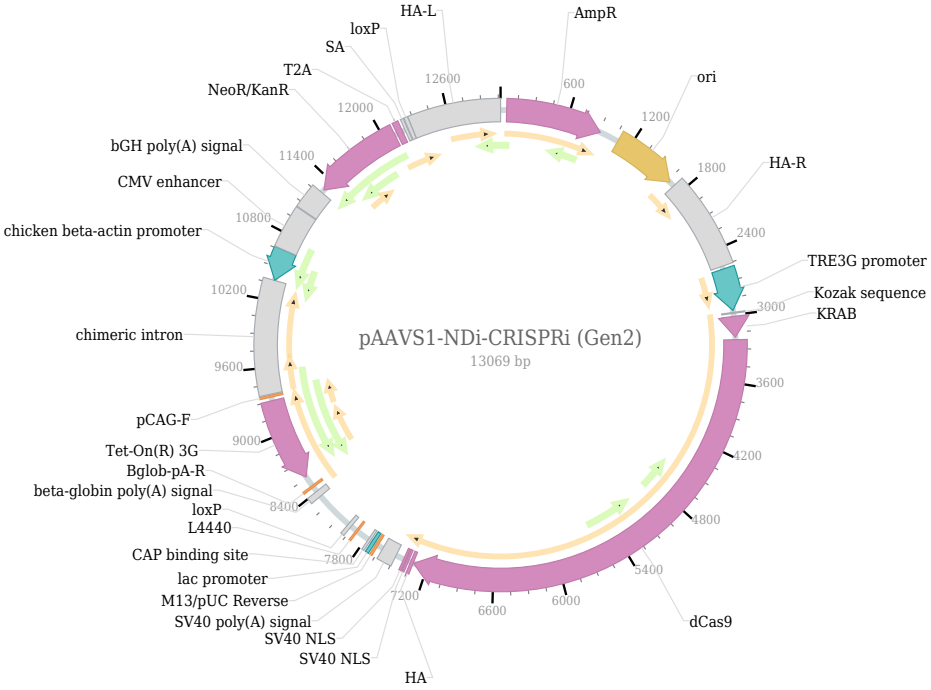
pAAVS1-NDi-CRISPRi (Gen2)

载体编号 Vector ID	V000627
抗性 Antibiotic Resistance	Ampicillin
长度 Length	13069 bp
载体类型 Vector Type	Mammalian Expression, CRISPR

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
启动子 Promoter	TRE3G
克隆方法 Cloning Method	Restriction Enzyme
5' 引物 5' Primer	CTCTAGAGCCTCTGCTAACC
3' 引物 3' Primer	CAGAGGGAAAAAGATCTCAGT
培养菌株 Growth Strain(s)	stbl3
培养温度 Growth Temperature	37℃
备注 Comment	pAAVS1-NDi-CRISPRi (Gen2) is a doxycycline-inducible safe-harbor knock-in vector developed for human iPSCs and their derivatives, integrating into the AAVS1 locus via TALEN-mediated homologous recombination. Its core expression cassette TRE3G-dCas9-KRAB-P2A-mCherry enables reversible, homogeneous transcriptional repression without permanent genomic DNA breaks, relying on Tet-On 3G rtTA constitutively driven by the CAG promoter. Without doxycycline, the system exhibits zero leaky dCas9-KRAB expression, while 48-hour doxycycline treatment triggers uniform target gene silencing up to 90–99% at transcription start sites across all cell populations. Gen2 improves upon Gen1 with enhanced TRE3G induction strength, brighter mCherry fluorescence for easy reporter quantification, higher homologous integration efficiency, and reduced long-term cellular toxicity after sustained doxycycline culture. This vector supports tunable gene knockdown via gRNA positioning or doxycycline titration, plus allele-specific silencing using SNP-altered PAM sequences to model human haploinsufficiency disorders. It works efficiently in multiple cell types including pluripotent stem cells, cardiac progenitors, cardiomyocytes and T lymphocytes, with minimal off-target transcriptional changes validated by RNA-seq.

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 ℃.