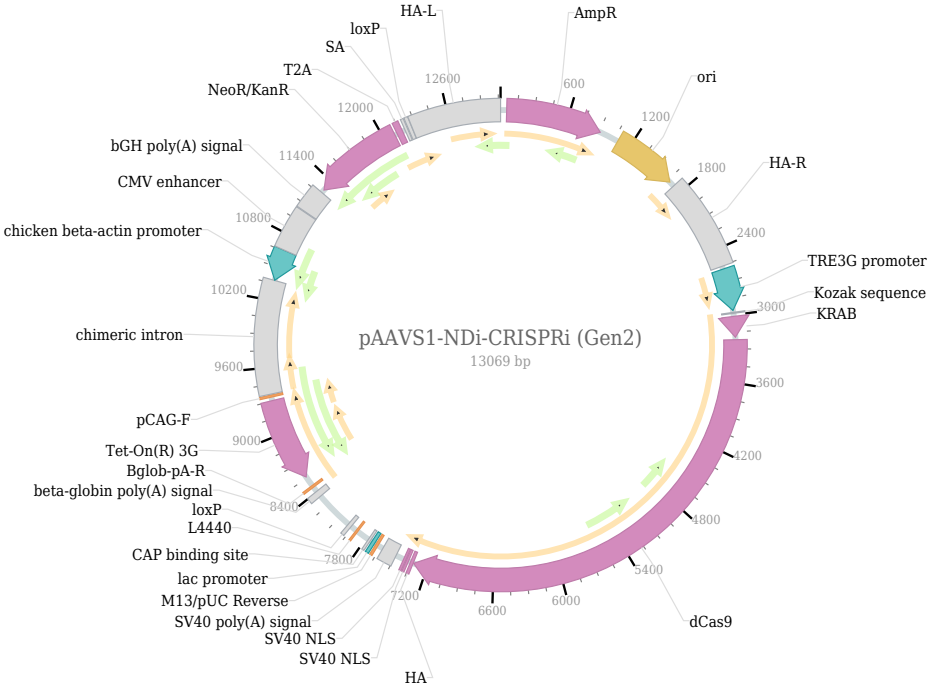
pAAVS1-NDi-CRISPRi (Gen2)

Vector ID	V000627
Antibiotic Resistance	Ampicillin
Length	13069 bp
Vector Type	Mammalian Expression, CRISPR

Product Information

Replication Origin	ori
Selection Marker	Neomycin (select with G418)
Copy Number	High Copy
Promoter	TRE3G
Cloning Method	Restriction Enzyme
5' Primer	CTCTAGAGCCTCTGCTAACC
3' Primer	CAGAGGGAAAAAGATCTCAGT
Growth Strain(s)	stbl3
Growth Temperature	37℃
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.

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 ℃.