



Description:

p5w-dCas9-KRAB.pBsd was derived from pLAS5w.pBsd with the following modifications:

1. A 4.3 kb DNA fragment containing HA tag-NLS-dCas9-KRAB-NLS was cloned into *AfeI* and *EcoRI* sites;
2. The catalytic inactive dCas9 protein is generated by two amino acid mutations at position D₁₀A (GAC to GCC) and position H₈₄₀A (CAT to GCG) in Cas9;
3. The dCas9 protein is fused with a transcriptional repressor domain KRAB;
4. The dCas9-KRAB fusion protein is expressed under the control of human elongation factor 1 α (EF1 α) promoter.

Location of Features:

• EF1 α promoter	: nt 10-1268	• HIV 3'LTR	: nt 7686-7936
• HA tag	: nt 1474-1503	• SV40 polyA	: nt 7998-8128
• NLS	: nt 1504-1536; 5638-5670	• bla promoter	: nt 8988-9086
• dCas9 (D ₁₀ A; H ₈₄₀ A)	: nt 1537-5637	• Amp	: nt 9087-9944
• KRAB box	: nt 5671-5805; 5806-5865	• Ori	: nt 10148-10688
• cPPT	: nt 5910-6027	• RSV promoter:	nt 11173-11401
• hPGK promoter	: nt 6086-6586	• HIV 5'LTR	: nt 11402-11582
• Blasticidin (Bsd)	: nt 6598-7008	• Psi sequence	: nt 11693-11737
• WPRE	: nt 7027-7615	• RRE	: nt 12248-12489

Note:

1. EF1 α promoter sequencing primer (forward): 5'- TCAAGCCTCAGACAGTGGTTC -3'.