



Description:

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

1. A 4.9 kb DNA fragment containing HA tag-NLS-dCas9-EGFP-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 enhanced green fluorescent protein (EGFP);
4. The dCas9-EGFP 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 8243-8493
• HA tag	: nt 1474-1503	• SV40 polyA	: nt 8555-8685
• NLS	: nt 1504-1536; 5638-5670	• bla promoter	: nt 9545-9643
• dCas9 (D ₁₀ A; H ₈₄₀ A)	: nt 1537-5637	• Amp	: nt 9644-10501
• EGFP	: nt 5698-6417	• Ori	: nt 10705-11245
• cPPT	: nt 6467-6584	• RSV promoter:	nt 11730-11958
• hPGK promoter	: nt 6643-7143	• HIV 5'LTR	: nt 11959-12139
• Blasticidin (Bsd)	: nt 7155-7565	• Psi sequence	: nt 12250-12294
• WPRE	: nt 7584-8172	• RRE	: nt 12805-13046

Note:

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