



Description:

p5w-Cas9.D₁₀A pBsd was derived from pLAS5w.pBsd with the following modifications:

1. A 4.2 kb DNA fragment containing HA tag-NLS-Cas9 D₁₀A-NLS was cloned into *Afe*I and *Eco*RI sites;
2. The Cas9 D₁₀A protein is generated by one amino acid mutation at position D₁₀A (GAC to GCC) in Cas9, and functions as an RNA-guided DNA nickase;
3. The Cas9 D₁₀A protein is expressed under the control of human elongation factor 1 α (EF1 α) promoter.

Location of Features:

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|-------------------------|---------------------------|-----------------|------------------|
| • EF1 α promoter | : nt 10-1268 | • HIV 3'LTR | : nt 7491-7741 |
| • HA tag | : nt 1474-1503 | • SV40 polyA | : nt 7803-7933 |
| • NLS | : nt 1504-1536; 5638-5670 | • bla promoter | : nt 8793-8891 |
| • Cas9 (hSpCsn1) | : nt 1537-5637 | • Amp | : nt 8892-9749 |
| • cPPT | : nt 5715-5832 | • Ori | : nt 9953-10493 |
| • hPGK promoter | : nt 5891-6391 | • RSV promoter: | nt 10978-11206 |
| • Blasticidin (Bsd) | : nt 6403-6813 | • HIV 5'LTR | : nt 11207-11387 |
| • WPRE | : nt 6832-7420 | • Psi sequence | : nt 11498-11542 |
| | | • RRE | : nt 12053-12294 |

Note:

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