



Description:

pAll-Cas9 D₁₀A.pPuro is an all-in-one CRISPR/Cas expression system:

1. The Cas9 D₁₀A protein is expressed under the control of CMV promoter;
2. The Cas9 D₁₀A nickase protein is generated by one amino acid mutation at position D₁₀A (GAC to GCC) in Cas9;
3. The expression of sgRNA is controlled by human U6 promoter;
4. The plasmid can be digested by *BsmBI*, which will remove the 1.9 kb stuffer and generate sticky ends for cloning of sgRNA oligos (please see [Protocol for sgRNA construction](#)).

Location of Features:

• U6 promoter	: nt 96-344	• Puromycin (PAC)	: nt 8113-8715
• Stuffer	: nt 345-2227	• WPRE	: nt 8731-9319
• sgRNA scaffold	: nt 2228-2313	• HIV 3'LTR	: nt 9390-9625
• U6 terminator	: nt 2314-2319	• SV40 polyA	: nt 9702-9832
• CMV promoter	: nt 2464-3032	• bla promoter	: nt 10692-10790
• HA tag	: nt 3172-3201	• Amp	: nt 10791-11648
• NLS	: nt 3202-3234; nt 7336-7368	• Ori	: nt 11852-12392
• Cas9 (D ₁₀ A)	: nt 3235-7335	• RSV promoter	: nt 12877-13105
• cPPT	: nt 7425-7542	• HIV 5'LTR	: nt 13106-13286
• hPGK promoter	: nt 7595-8101	• Psi sequence	: nt 13397-13441
		• RRE	: nt 13952-14193

Note:

U6 promoter sequencing primer (forward): 5'- TACAAAATACGTGACGTAG-3'.