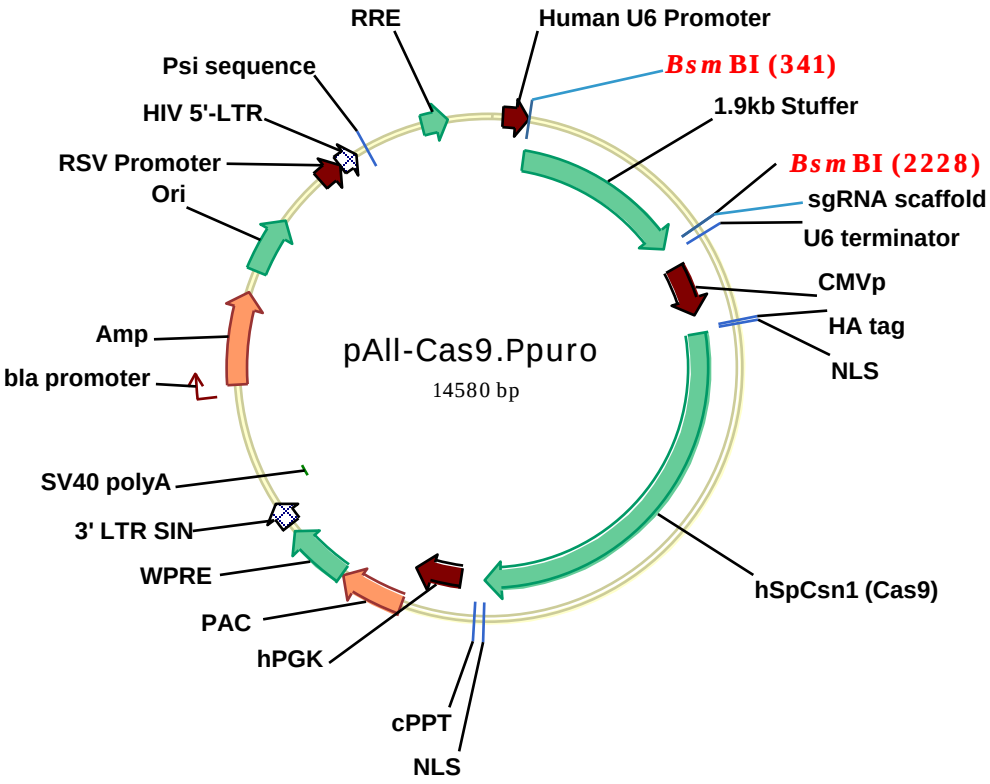

Description:

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

1. The Cas9 protein is expressed under the control of CMV promoter;
2. The Cas9 protein is an RNA-guided DNA endonuclease that cleaves the target DNA and generates site-directed double strand breaks;
3. The expression of sgRNA is controlled by human U6 promoter;
4. The plasmid can be digested by *BsmB*I, which will remove the 1.9 kb stuffer and genertae sticky ends for cloning of sgRNA oligos (please see Protocol for sgRNA construction).

Location of Features:

- U6 promoter : nt 96-344
- Stuffer : nt 345-2227
- sgRNA scaffold : nt 2228-2313
- U6 terminator : nt 2314-2319
- CMV promoter : nt 2464-3032
- HA tag : nt 3172-3201
- NLS : nt 3202-3234; nt 7336-7368
- Cas9 (hspCsn1) : nt 3235-7335
- cPPT : nt 7425-7542
- hPGK promoter : nt 7595-8101
- Puromycin (PAC) : nt 8113-8715
- WPRE : nt 8731-9319
- HIV 3'LTR : nt 9390-9625
- SV40 polyA : nt 9702-9832
- bla promoter : nt 10692-10790
- Amp : nt 10791-11648
- Ori : nt 11852-12392
- RSV promoter : nt 12877-13105
- HIV 5'LTR : nt 13106-13286
- Psi sequence : nt 13397-13441
- RRE : nt 13952-14193

Note:

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