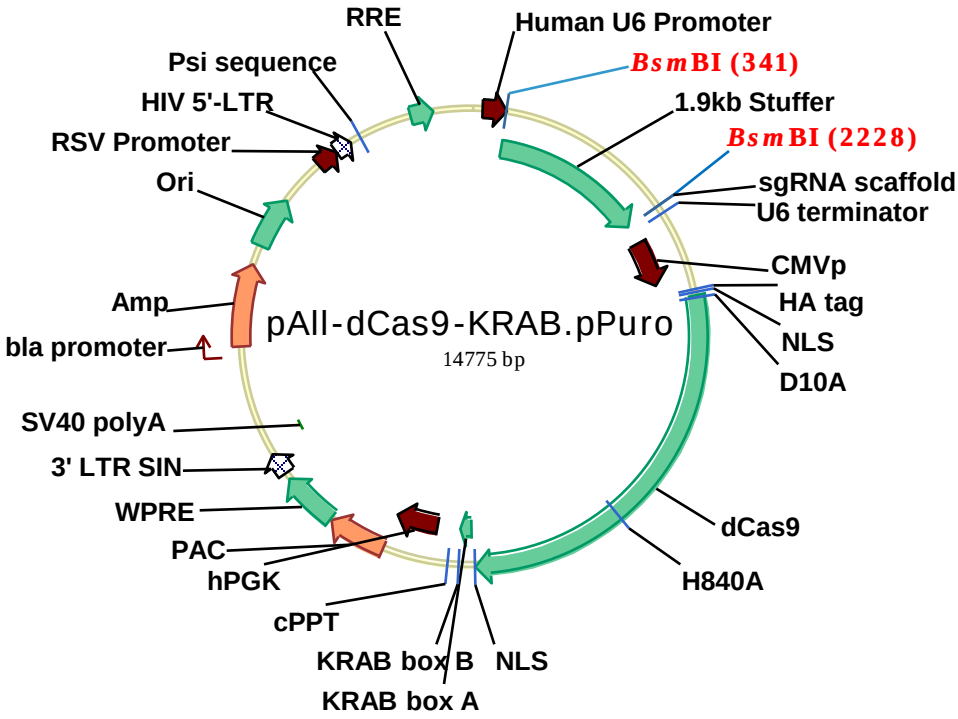

Description:

pAll-dCas9-KRAB.pPuro is an all-in-one CRISPR/Cas expression system:

1. The dCas9-KRAB fusion protein is expressed under the control of CMV promoter;
2. The catalytic inactive dCas9 protein is generated by two amino acid mutations at position D_{10}A (GAC to GCC) and position H_{840}A (CAT to GCG) in Cas9. The dCas9 protein is fused with a transcriptional repression domain KRAB;
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 generate 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
- dCas9 (D_{10}A; H_{840}A) : nt 3235-7335
- KRAB : nt 7369-7563
- cPPT : nt 7620-7737
- hPGK promoter : nt 7790-8296
- Puromycin (PAC) : nt 8308-8910
- WPRE : nt 8926-9514
- HIV 3'LTR : nt 9585-9820
- SV40 polyA : nt 9897-10027
- bla promoter : nt 10887-10985
- Amp : nt 10986-11843
- Ori : nt 12047-12587
- RSV promoter : nt 13072-13300
- HIV 5'LTR : nt 13301-13481
- Psi sequence : nt 13592-13636
- RRE : nt 14147-14388

Note:

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