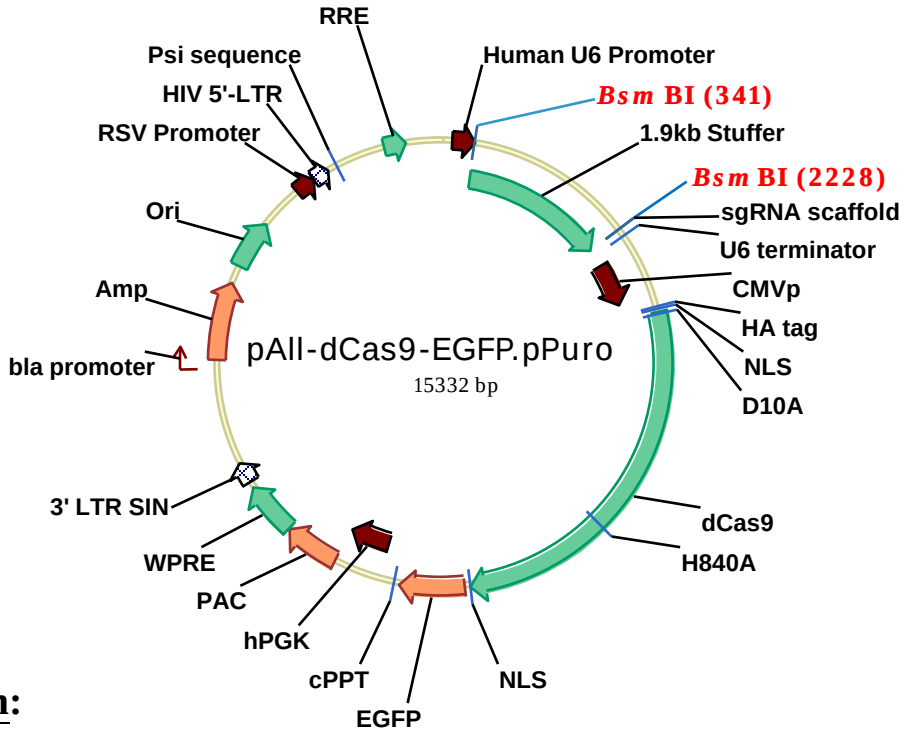

Description:

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

1. The dCas9-EGFP 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 enhanced green fluorescent protein (EGFP);
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
- dCas9 ($D_{10}A$; $H_{840}A$) : nt 3235-7335
- EGFP : nt 7396-8115
- cPPT : nt 8177-8294
- hPGK promoter : nt 8347-8853
- Puromycin (PAC) : nt 8865-9467
- WPRE : nt 9483-10071
- HIV 3'LTR : nt 10142-10377
- SV40 polyA : nt 10454-10584
- bla promoter : nt 11444-11542
- Amp : nt 11543-12400
- Ori : nt 12604-13144
- RSV promoter : nt 13629-13857
- HIV 5'LTR : nt 13858-14038
- Psi sequence : nt 14149-14193
- RRE : nt 14704-14945

Note:

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