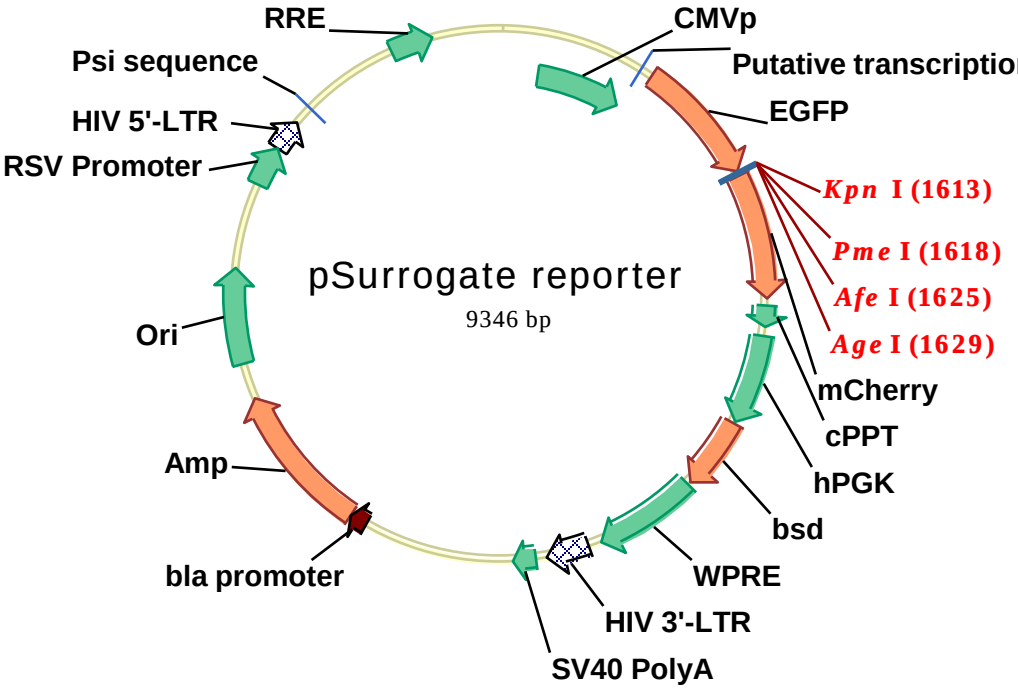

Description:

pSurrogate reporter is a dual fluorescent lentivector, which is composed of two fluorescent genes (EGFP and mCherry) linked by a multiple cloning site (MCS).

1. The MCS contains *Kpn*I-*Pme*I-*Afe*I-*Age*I, which allows cloning of any sgRNA-targeting sequence;
2. The plasmid can be digested by *Kpn*I *and Age*I, which will reveal sticky ends for cloning of annealed sgRNA-targeting oligos (please see Protocol for surrogate construction).
3. pSurrogate reporter is a useful tool to validate the activity of a sgRNA, and can also be used to visualize and enrich the cells with gene editing event.

Location of Features:

- CMV promoter : nt 234-802
- EGFP : nt 885-1607
- MCS : nt 1608-1633
- mCherry : nt 1634-2344
- cPPT : nt 2386-2503
- hPGK promoter : nt 2556-3062
- Blasticidin (Bsd) : nt 3074-3484
- WPRE : nt 3503-4091
- HIV 3'LTR : nt 4162-4406
- SV40 polyA : nt 4468-4598
- bla promoter : nt 5458-5556
- Amp : nt 5557-6414
- Ori : nt 6618-7158
- RSV promoter : nt 7643-7871
- HIV 5'LTR : nt 7872-8052
- Psi sequence : nt 8163-8207
- RRE : nt 8718-8959

Note:

Please be sure that the mCherry CDS is out of frame relative to the open reading frame (ORF) of EGFP after cloning. Also, avoid potential in-frame STOP codon formation that may be generated after indel reaction in the spacer region of EGFP and mCherry genes.