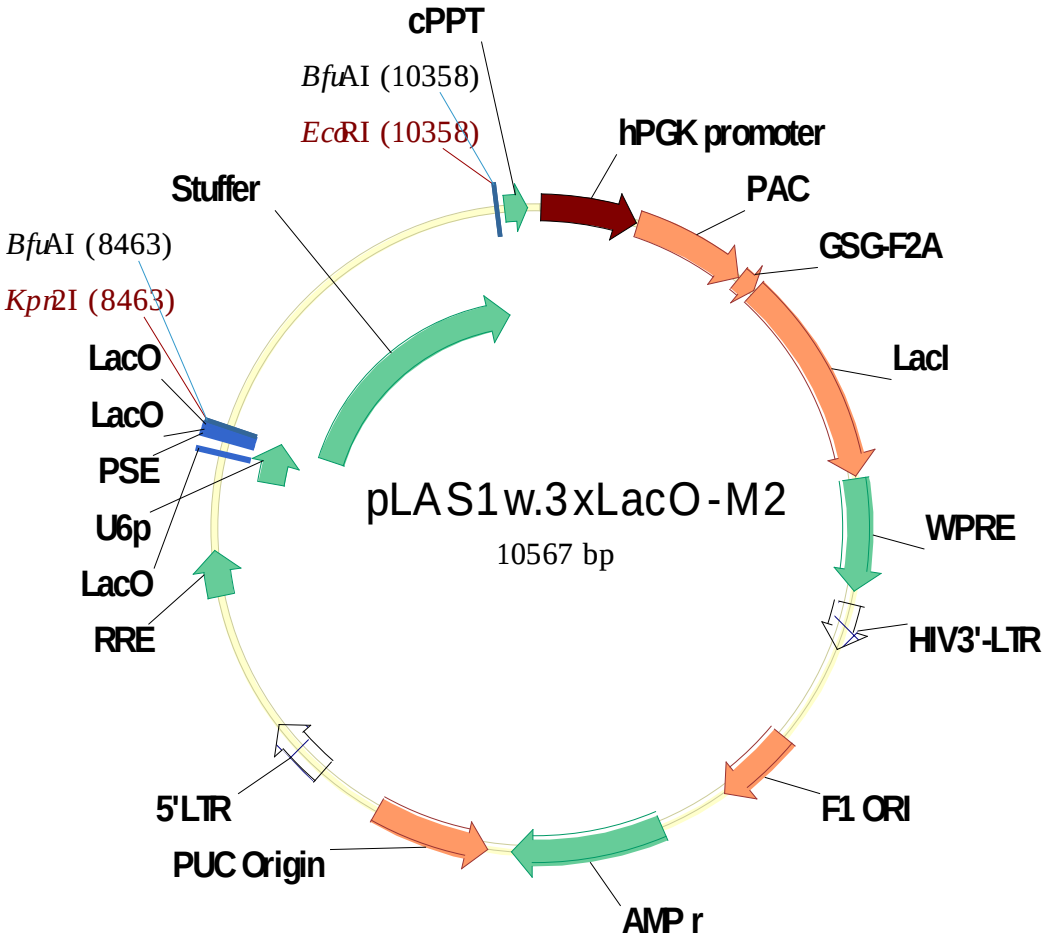

Description:

pLAS1w.3xLacO-M2 was derived from pLKO_TRC005 with the following modifications:

1. Three portions of sequences on U6p promoter were replaced with three copies of LacI operator sequence which are labeled with LacO on the map;
2. A 1.9 kb fragment of stuffer sequence was mutated from pLAS1w.3xLacO, stuffer have shRNA cloning sites, *Bfu*AI/*Kpn*2I and *Bfu*AI/*Eco*RI. This allows one to monitor the cutting efficiency of the vector by observing the release of the 1.9 kb fragment from the vector after digesting it with *Eco*RI and *Bfu*AI/*Kpn*2I;
3. ORF of *LacI* repressor was introduced into the downstream of *PAC* gene by fusing it with GSG-F2A sequence to link *PAC* ORF. This fusion results in maintaining the same reading frame from *PAC* to *LacI*.

Location of Features:

- PAC (Puromycin acetyltranferase) : nt519-1115
- GSG-F2A : nt 1122-1220
- LacI (Lac Inhibitor) : nt1227-2345
- LacO (Lac Operon) : nt 8322- 8342; 8417-8436, 8443-8462.

Note:

1. Primer for sequencing shRNA: 5'-ATTTCTTGGGTAGTTTGCAG-3'

2. PAC gene can be replaced with other selection marker by utilizing *Bam*HI and *Sbf*I restriction enzymes.