pLAS1.3xLacO 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 inserted into shRNA cloning sites, BfuAI and BfuAI/EcoRI. 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 EcoRI and BfuAI;
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 acetyltransferase) : nt519-1115
- GSG-F2A : nt 1122-1220
- LacI (Lac repressor) : 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 BamHI and ShfI restriction enzymes.