pLKO_TRC005 (TRC2 vector) was derived from pLKO_TRC001 with the following modifications:

(i) A KpnI restriction enzyme site was created next to AgeI, thereby two combination sites for shRNA cloning can be used, KpnI-EcoRI or AgeI-EcoRI.

(ii) A post-transcription regulation element from woodchuck hepatitis virus (WPRE) was inserted behind PAC gene (puromycin acetyltransferase gene).

**Location of Features (for other features, please refer to pLKO.1-puro):**

- Human U6 promoter: nt7004-7255
- Stuffer: nt7256-9124
- Human PGK promoter: nt4-508
- PAC (Puro; Puromycin acetyltransferase): nt529-1128
- WPRE (woodchuck post-transcription regulation element): nt1144-1732

**Note:**

DNA sequences within lentiviral genome including both 5’LTR and 3’LTR (not included the whole stuffer sequence) had been verified by TRC.