Lentiviral vector digestion, oligo annealing and cloning into digested vector

1: Digest of the lentiviral CRISPR plasmid with BsmB1 (55℃, 2H)

pLenti-Guide-Puro or pLKO5.sgRNA.EFS.tRFP657	2ul (1000ng/ul)
10×3.1 buffer	5ul
BsmB1	1ul
Water	42ul
Total	50ul

Loading(20-30mins) and Gel extraction

2: Anneal each pair of oligos

100uM Forward oligo	1ul
100uM Revers oligo	1ul
10×T buffer	5ul
Water	43ul
Total	50ul

Annealing reaction in a thermocycler using the following parameters:

98℃ 5min

70℃ 10min

Room temperature 1houre (at least) * to take out tube together with Heat block.

Anneal product diluted x10. (to 500ul) by MQ.

3: Ligation (16℃,1H)

Oligo	5ul
Vector	3ul
Oligo-Vector mixture	2ul
Ligation mix	2ul
Total	4ul

4: Transformation~5: Colony pick up and miniprep

Same as usual (antibiotic: Ampicillin, E. coli: Dh5a, miniprep 25 ul TE dilution)

6: Confirmation of the product (Cut Check: 37°C, 1H)

In order to save restriction enzymes, **0.3ul/sample of enzyme** is enough for cut check. Since 0.3ul is difficult to pick, it is recommended to make pre-mix (enzyme + buffer + MQ) at first.

pLKO5.sgRNA.EFS.tRFP657 products	1ul
BmgB1	0.3ul
stu1	0.3ul
10×3.1 buffer	1ul
MQ	7.4ul
TOTAL	10ul

pLenti-Guide-Puro products	1ul
Xho1	0.3ul
10×H buffer	1ul
MQ	7.6ul
TOTAL	10ul

Loading(20-30mins)

If **pLKO5.sgRNA.EFS.tRFP657 product** is successfully made, there should be 2 bands around 7000bp and 1000bp.
If failed, there will be 2 bands around 9000bp and 1000bp.

If pLenti-Guide-Puro product is successfully made, there should be 1 single band around 7000bp,
If failed, there will be 2 bands around 5000bp and 2500bp.