

## =====Digestion=====

pLKO	2	ug
Agel	0.25	uL
EcoRI	0.25	uL
Cutsmart buffer	2	uL
Water	Add to 20	uL

37 Celsius for 4 hour

## =====Anneal=====

Forward Primer (100 uM)	1	uL
Reverse Primer (100 uM)	1	uL
PNK kinase	0.5	uL
T4 ligation buffer	1	uL
Water	6.5	uL

37 Celsius for 30 min

95 Celsius for 5 min

94 Celsius for 1 min (-1 Celsius/Cycle, 70 Cycle)

4 Celius Forever

## =====Ligation=====

Annealed Product	1	uL
Backbone	20	ng
T4 ligase	1	uL
T4 ligation buffer	1	uL
Water	Add to 10	uL

25 Celsius for 2 hour

## =====Transformation=====

Add ~100 uL bacteria to the ligation product

0 Celsius for 20 min

42 Celsius for 90 sec

0 Celsius for 5 min