A rapid sgRNA cloning protocol for CRISPRainbow (V20160427)

This protocol was adapted from Ma H *et al*, NBT, 2016. Hanhui Ma @ UMMS.

A. Design sgRNA oligos

`Fwd: ACCGNNNNNNNNNNNNNNNNNNN`

`Rev: AAACNNNNNNNNNNNNNNNNNNN`

B. Annealing oligos

Reagents:

- Annealing Buffer

	Stock	ml
100 mM Tris-HCl pH8.0	1 M	0.25
50 mM NaCl	5 M	0.25
1 mM EDTA	0.5 M	0.05
ddH_2O		24.5
Total		25

- 100 μM oligos

Annealing:	μl
Annealing buffer	40
Fwd oligo (100 μM)	5
Rev oligo (100 μM)	5
Total	50

- Incubate @ 95 °C for 3 min and slowly cool down to room temperature;
- Dilute 5 μl of annealed oligos to 245 μl water and final concentration is 200 nM (about 3ng/ul, ready for use).

C. Cloning annealed oligos into vectors

Reagents:

- pLH-sgRNA1 vectors*
- Enzymes and Buffer in reaction
- Stbl3 competent cells
- LB-Ampicillin plates

Reaction mix:	μl
pLH-sgRNA1 vector (100 ng/μl)	1
10XSmartCut Buffer (NEB)	1
10 mM ATP (NEB)	1
BbsI (NEB)	0.5
T7 DNA ligase (NEB)	0.3
ddH_2O	5.2
Annealed oligos (200 nM)	1
	10

- Incubate @ 37 °C for 15 min;
- Transform 5 μl of reaction mix into Stbl3 competent cells and spread on LB-Amp plates.

D. Minipreps and Sequencing

Reagents:

- QIAprep Spin Miniprep Kit
- Sequencing primer pLKO.1-Rseq: CTATTCTTTCCCCTGCACTGTACCC

* **Notes of pLH-sgRNA1 vectors:**

sgRNA1 carried a pair of mutations described as sgRNAplus in Supplementary Figure 1, Ma H *et al*, NBT, 2016.

All the sgRNA vector plasmids require CcdB Survival cells for growth.