A rapid sgRNA cloning protocol for Multicolor CRISPR (V0315)

This protocol was adapted from Ma H *et al*, PNAS, 2015. Hanhui Ma (Thoru Pedeson's lab @ UMMS).

A. Design sgRNA oligos

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Fwd (all): ACCGNNNNNNNNNNNNNNNNNNN
Rev (Sp):  AAACNNNNNNNNNNNNNNNNNNN
Rev (Nm):  CAACNNNNNNNNNNNNNNNNNNN
Rev (St):  AGACNNNNNNNNNNNNNNNNNNN
PAM Choice:
Sp dCas9: NGG
Nm dCas9: NNNNGATT
St dCas9: NNAGAAW
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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-sgRNA vectors*
- Enzymes and Buffer in reaction
- Stbl3 competent cells
- LB- Ampicillin plates

Reaction mix:	μl
pLH-sgRNA 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-sgRNA vectors:**

pLH-sgRNA2 for Sp dCas9.

pLH-nmsgRNA1.1 for Nm dCas9.

pLH-st1sgRNA1.1, pLH-st1sgRNA2.1 and pLH-st1sgRNA3.1 for St1 dCas9.

All these vector plasmids are available @ Addgene and they require CcdB Survival cells for growth.