

Gibson assembly of CRISPRainbow-6XsgRNAs

This protocol was used in Ma H *et al*, NBT, 2016. Hanhui Ma (20160427 @ UMMS).

A. PCR applification for individual sgRNAs

1) Primer sets:

sgRNA-Ex-F1: AATAATggtctcaTGACccttcaccgagggcctatttcc
sgRNA-In-R1: AATAATggtctcATTGCTGCCATTTGTCTCGAGGTCGAGA
sgRNA-In-F2: AATAATggtctcaGCAAccttcaccgagggcctatttcc
sgRNA-In-R2: AATAATggtctcAGTTCTGCCATTTGTCTCGAGGTCGAGA
sgRNA-In-F3: AATAATggtctcaGAACccttcaccgagggcctatttcc
sgRNA-In-R3: AATAATggtctcAACTGTGCCATTTGTCTCGAGGTCGAGA
sgRNA-In-F4: AATAATggtctcaCAGTccttcaccgagggcctatttcc
sgRNA-In-R4: AATAATggtctcAGTGTGCCATTTGTCTCGAGGTCGAGA
sgRNA-In-F5: AATAATggtctcaACACccttcaccgagggcctatttcc
sgRNA-In-R5: AATAATggtctcAGACATGCCATTTGTCTCGAGGTCGAGA
sgRNA-In-F6: AATAATggtctcaTGTCccttcaccgagggcctatttcc
sgRNA-Ex-R1: AATAATggtctcAGAGTTGCCATTTGTCTCGAGGTCGAGA

2) Templates from cloned individual sgRNA derived from:

pLH-sgRNA1-2XMS2
pLH-sgRNA1-2XPP7
pLH-sgRNA1-2XboxB
pLH-sgRNA1-MS2-PP7
pLH-sgRNA1-PP7-boxB
pLH-sgRNA1-boxB-MS2.

3) PCR reaction: Ta=55°C and Te=30Sec for 28 cycles.

4) Gel purification of ~500 bp fragments and diluted to 10 ng/μl.

5) Ready for Giblson assembly.

U6-sgRNA1-2XMS2

AATAATggtctcaTGACccttcaccgagggcctat t t t cccatgattccttcata t t t tgcata
tacgatacaaggctg t ttagagagataattggaattaatttgactgtaaacacaaagatatta
gtacaaaatacgtgacgtagaaagtaataatttcttg ggtagtttgcagttttaaaattatg
ttttaaaatggactatcatatgcttaccgtaacttgaaagtatttcgatttcttggctttat
atatcttgtggaaaggacgaaacACCGNNNNNNNNNNNNNNNNNNNNNNg t t t g a g a g c t a g g c
caacatgaggatcacccatgtctgcagggcctagcaagttcaaataaggctagtcgcgttatc
aacttg gccaacatgaggatcacccatgtctgcagggccaagtggcaccgagtcggtgcttt
ttttgaattctcgacctcgagacaaatggcaGCAAtgagaccATTATT

U6-sgRNA1-2XPP7

AATAATggtctcaGCAAccttcaccgagggcctat t t t cccatgattccttcata t t t tgcata
tacgatacaaggctg t ttagagagataattggaattaatttgactgtaaacacaaagatatta
gtacaaaatacgtgacgtagaaagtaataatttcttg ggtagtttgcagttttaaaattatg
ttttaaaatggactatcatatgcttaccgtaacttgaaagtatttcgatttcttggctttat
atatcttgtggaaaggacgaaacACCGNNNNNNNNNNNNNNNNNNNNNNg t t t g a g a g c t a c c g
gagcagacgatatggcgctcgctccggtagcaagttcaaataaggctagtcgcgttatcaactt
ggagcagacgatatggcgctcgctccaagtggcaccgagtcggtgctttttttgaattctcga
cctcgagacaaatggcaGAACtgagaccATTATT

U6-sgRNA1-2XboxB

AATAATggtctcaGAACccttcaccgagggcctat t t t cccatgattccttcata t t t tgcata
tacgatacaaggctg t ttagagagataattggaattaatttgactgtaaacacaaagatatta
gtacaaaatacgtgacgtagaaagtaataatttcttg ggtagtttgcagttttaaaattatg
ttttaaaatggactatcatatgcttaccgtaacttgaaagtatttcgatttcttggctttat
atatcttgtggaaaggacgaaacACCGNNNNNNNNNNNNNNNNNNNNNNg t t t g a g a g c t a g g g
ccctgaagaagggccctagcaagttcaaataaggctagtcgcgttatcaacttgggccttgaa
gaagggcccaagtggcaccgagtcggtgctttttttgaattctcgacctcgagacaaatggc
aCAGTtgagaccATTATT

U6-sgRNA1-MS2-PP7

AATAATggtctcaCAGTccttcaccgagggcctat t t t cccatgattccttcata t t t tgcata
tacgatacaaggctg t ttagagagataattggaattaatttgactgtaaacacaaagatatta
gtacaaaatacgtgacgtagaaagtaataatttcttg ggtagtttgcagttttaaaattatg

ttttaaaatggactatcatatgcttaccgtaacttgaaagtatttcgatttcttggctttat
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caacatgaggatcacccatgtctgcagggcctagcaagttcaaataaggctagtcggttacc
aacttgagcagacgatatggcgctcgctccaagtggcaccgagtcggtgctttttttgaatt
ctcgacctcgagacaaatggcaACACtgagaccATTATT

U6-sgRNA1-PP7-boxB

AATAATggtctcaACACccttcaccgagggcctatttcccatgattccttcataatttgcata
tacgatacaaggctgttagagagataattggaattaatttgactgtaaacacaaagatatta
gtacaaaatacgtgacgtagaaagtaataatttcttgggtagtttgcagttttaaaattatg
ttttaaaatggactatcatatgcttaccgtaacttgaaagtatttcgatttcttggctttat
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gagcagacgatatggcgctcgctccggtagcaagttcaaataaggctagtcggttaccactt
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gacaaatggcaTGTCtgagaccATTATT

U6-sgRNA1-boxB-MS2

AATAATggtctcaTGTCccttcaccgagggcctatttcccatgattccttcataatttgcata
tacgatacaaggctgttagagagataattggaattaatttgactgtaaacacaaagatatta
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ctcgagacaaatggcaACTCtgagaccATTATT

B. Gibson assembly of pCRISPRainbow-6XsgRNAs

Reagents:

- pCRISPRainbow-DONOR1 vectors*
- Enzymes and Buffer in reaction
- Stbl3 competent cells
- LB-Ampicillin plates

<u>Reaction mix:</u>	<u>μl</u>
pCRISPRainbow-DONOR1 (100 ng/μl)	1.0
10XSmartCut Buffer (NEB)	1.0
10 mM ATP (NEB)	1.0
BsaI (NEB)	0.6
T7 DNA ligase (NEB)	0.4
each sgRNA (PCR, 10ng/μl, 1μl each)	total 6.0
	10

- Incubate @ 37 °C for 60 min;
- Transform 5 μl of reaction mix into Stbl3 competent cells and spread on LB-Amp plates.

C. Minipreps and Sequencing

Reagents:

- QIAprep Spin Miniprep Kit
- Sequencing primers
DONOR1-Fseq: GCTACAACAAGGCAAGGCTTGACCGA
DONOR1-Rseq: AACGCCAGCAACGCGGCCTT

***Notes:** pCRISPRainbow-DONOR1 need CcdB Survival stains for growth.

CRISPRainbow-6XsgRNA

TGACccttcaccgagggcctat tttcccatgattccttcata ttttgcatatacgatacaaggctg
ttagagagataaattggaattaatttgactgtaaacacaaagatatttagtacaaaatacgtgacg
tagaaagtaataat tttcttgggtagtttgagtttttaaattatgtttttaaattggactatcat
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acACCGNNNNNNNNNNNNNNNNNNNNNNg tttgagagctaaggccaacatgaggatcacccatgtct
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ctgcagggccaagtggcaccgagtcggtgctttttttgaattctcgacctcgagacaaatggca
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