Gibson assembly of CRISPRainbow-6XsgRNAs

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

A. PCR amplification for individual sgRNAs

1) Primer sets:
   - sgRNA-Ex-F1: AATAATggtctcaTGACccttcacccgggctatttcc
   - sgRNA-In-R1: AATAATggtctcATTGCTGCCATTGCTCGAGGTGAGA
   - sgRNA-In-F2: AATAATggtctcaGCAccttcacccgggctatttcc
   - sgRNA-In-R2: AATAATggtctcAGTTCTGCCATTGCTCGAGGTGAGA
   - sgRNA-In-F3: AATAATggtctcaGAACccttcacccgggctatttcc
   - sgRNA-In-R3: AATAATggtctcAACTGTGCCATTGCTCGAGGTGAGA
   - sgRNA-In-F4: AATAATggtctcaCAGTccttcacccgggctatttcc
   - sgRNA-In-R4: AATAATggtctcAGTTCTGCCATTGCTCGAGGTGAGA
   - sgRNA-In-F5: AATAATggtctcaACACccttcacccgggctatttcc
   - sgRNA-In-R5: AATAATggtctcAGACATGCCATTGCTCGAGGTGAGA
   - sgRNA-In-F6: AATAATggtctcaTGTCccttcacccgggctatttcc
   - sgRNA-Ex-R1: AATAATggtctcAGAGTGCCATTGCTCGAGGTGAGA

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 Gibson assembly.
U6-sgRNA1-2XMS2
AATAATggctcaTGACcttcaccgagggccctatattccccatgattccttcataatttgctata
tacgatacaaggtgcttagagagataatttgaatttactgtaacacaaagatatta
gtacaaatatcgtgacgtagaagaatttaatattttctgtagtttgccagtttttaaattatg
ttttaaatggaactatcatatgtctaccgtaacctggaagtagattttcattttgtttt
atatctttgttagaaggacgaaccACCGNNNNNNNNNNNNNNNNNNNNNNNgttttagagctagcc
caacatgaggtcaccatgtctggagggcttagcaagttcataaataaggctagttcgggttac
acattggcccaagtaggacgatcaccatgtctgcagggccaaagttggcaccagtggtgtcttt
ttttgcaattctcgacctcgagacaattaagcAGAAtgagaccATTATT

U6-sgRNA1-2XPP7
AATAATggctcaGCAActtcaccgggagggctatattccccatgattccttcataatttgctata
tacgatacaaggtgcttagagagataatttgaatttactgtaacacaaagatatta
gtacaaatatcgtgacgtagaagaatttaatattttctgtagtttgccagtttttaaattatg
ttttaaatggaactatcatatgtctaccgtaacctggaagtagattttcattttgtttt
atatctttgttagaaggacgaaccACCGNNNNNNNNNNNNNNNNNNNNNNNgttttagagctagcc
gacgagcagcatatggcgtcgtctcgggtagcaagttcataaataaggctagttcgggttac
acattggcccaagtaggacgatcaccatgtctgcagggccaaagttggcaccagtggtgtcttt
agattctcgacctcgagacaattaagcAGAAtgagaccATTATT

U6-sgRNA1-2XboxB
AATAATggctcaGAACcttcaccgagggccctatattccccatgattccttcataatttgctata
tacgatacaaggtgcttagagagataatttgaatttactgtaacacaaagatatta
gtacaaatatcgtgacgtagaagaatttaatattttctgtagtttgccagtttttaaattatg
ttttaaatggaactatcatatgtctaccgtaacctggaagtagattttcattttgtttt
atatctttgttagaaggacgaaccACCGNNNNNNNNNNNNNNNNNNNNNNNgttttagagctagcc
cctgcgaagaagggccctagcaagttcataaataaggctagttcgggttacccggctgaa
acagggggcaagtaggacgacgcagtctggtctttttttgaattctcgacctcgagacaatatgcA
acAGTtgagaccATTATT

U6-sgRNA1-MS2-PP7
AATAATggctcaCAGTcttcaccgggagggccctatattccccatgattccttcataatttgctata
tacgatacaaggtgcttagagagataatttgaatttactgtaacacaaagatatta
gtacaaatatcgtgacgtagaagaattttctgtagtttgccagttttgaattttcattttgcttaaaattatg
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**U6-sgRNA1-PP7-boxB**

AATAATgtctcaACACcttcaccggagggcctatttccatgattctctcatatgtgtgcata ttcgataacagctgtagagagatgaatatttggacggtgaacacaaagatatggtatagatgatactttaagagaacagagtctcaactccttcaccgagggcctatttcccatgattctttttttttttaattctcgacctgacacaaatggcaACACtgaccccATTATT

**U6-sgRNA1-boxB-MS2**

AATAATgtctcaTGTCttttccaccggagggcctatttccatgattctctcatatgtgtgcata ttcgataacagctgtagagagatgaatatttggacggtgaacacaaagatatggtatagatgatactttaagagaacagagtctcaactccttcaccgagggcctatttcccatgattctttttttttttaattctcgacctgacacaaatggcaACTCtgaccccATTATT
B. Gibson assembly of pCRISPRainbow-6XsgRNAs

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

Reaction mix:

<table>
<thead>
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<th>Component</th>
<th>µl</th>
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<tr>
<td>pCRISPRainbow-DONOR1 (100 ng/µl)</td>
<td>1.0</td>
</tr>
<tr>
<td>10XSmartCut Buffer (NEB)</td>
<td>1.0</td>
</tr>
<tr>
<td>10 mM ATP (NEB)</td>
<td>1.0</td>
</tr>
<tr>
<td>BsaI (NEB)</td>
<td>0.6</td>
</tr>
<tr>
<td>T7 DNA ligase (NEB)</td>
<td>0.4</td>
</tr>
<tr>
<td>each sgRNA (PCR, 10ng/µl, 1µl each) total 6.0</td>
<td></td>
</tr>
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<td></td>
<td>10</td>
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</tbody>
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- 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: AACGCCAGCAACGCCGCGCT

*Notes: pCRISPRainbow-DONOR1 need CcdB Survival stains for growth.
CRISPRainbow-6XsgRNA

TGACccttcaccgagggccttatttccatgattctttcatatatgcgatacaaggtgtagtagtttgaatggactatcatcatgtttatattttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttt