Cloning Protocol for pH1v1 gRNA vector

Ranganathan V, Wahlin K, Maruotti J, Zack DJ. Expansion of the CRISPR-Cas9 genome targeting space through the use of H1 promoter-expressed guide RNA. Nat Commun. 2014 Aug 8;5:4516.
http://www.nature.com/ncomms/2014/140808/ncomms5516/full/ncomms5516.html

1) Identify 23bp CRISPR Targeting sequence in the form N20-NGG:

```
5'- NNNNNNNNNNNNNNNNNNNN NGG -3'
    ____________________ ___
        Target sequence   PAM
```

2) Design oligos to insert 20bp target sequence into H1 promoter vector via Gibson Assembly:

```
5'- NNNNNNNNNNNNNNNNNNNN -3'
    ||||||||||||||||||||
3'- nnnnnnnnnnnnnnnnnnnn -5'
```

*N corresponds to N20 target sequence;
n corresponds to complement sequence

Forward oligo; GAATCTTATAAGTTCTGTATGAGACCACTTTTTCCCNNNNNNNNNNNNNNNNNNNN
Reverse oligo; ACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAACnnnnnnnnnnnnnnnnnnnnG

3) PCR reaction to anneal and extend the two oligos as below:

```
GAATCTTATAAGTTCTGTATGAGACCACTTTTTCCCNNNNNNNNNNNNNNNNNNNN
                                    ||||||||||||||||||||
                                    GnnnnnnnnnnnnnnnnnnnnCAAAATCTCGATCTTTATCGTTCAATTTTATTCCGATCA
```

↓

```
GAATCTTATAAGTTCTGTATGAGACCACTTTTTCCCNNNNNNNNNNNNNNNNNNNNGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGT
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
CTTAGAATATTCAAGACATACTCTGGTGAAAAAGGGnnnnnnnnnnnnnnnnnnnnCAAAATCTCGATCTTTATCGTTCAATTTTATTCCGATCA
-----------------------------------                    ---------------------------------------
      overlap                                                                          overlap
```

4) Digest pH1v1 with AvrII

```
                                        Avr II
                                        ------
TGGGAATCTTATAAGTTCTGTATGAGACCACTTTTTCCCTAGGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAAC
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
ACCCTTAGAATATTCAAGACATACTCTGGTGAAAAAGGGATCCAAAATCTCGATCTTTATCGTTCAATTTTATTCCGATCAGGCAATAGTTG
```

↓

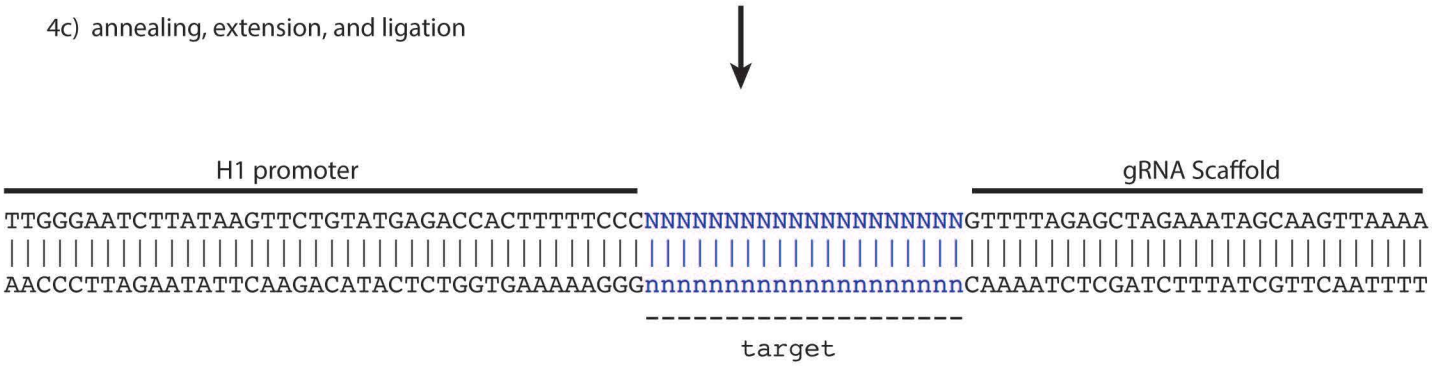
```
TGGGAATCTTATAAGTTCTGTATGAGACCACTTTTTCC     CTAGGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAAC
||||||||||||||||||||||||||||||||||||||     ||||||||||||||||||||||||||||||||||||||||||||||||||||
ACCCTTAGAATATTCAAGACATACTCTGGTGAAAAAGGGATC     CAAAATCTCGATCTTTATCGTTCAATTTTATTCCGATCAGGCAATAGTTG
```

4) Gibson Assembly

4a) 5'->3' vector resection:

4b) 5'->3' oligo resection:

4c) annealing, extension, and ligation



4) Sequence verify with M13R

H1 promoter sequence:
GGAATTCGAACGCTGACGTCATCAACCCGCTCCAAGGAATCGCGGGCCCAGTGTCACTAG
GCGGGAACACCCAGCGCGCGTGCGCCCTGGCAGGAAGATGGCTGTGAGGGACAGGGGAGT
GGCGCCCTGCAATATTTGCATGTCGCTATGTGTTCTGGGAAATCACCATAAACGTGAAAT
GTCTTTGGATTTGGGAATCTTATAAGTTCTGTATGAGACCACTTTTTCCC

gRNA Scaffold:
GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGT
GGCACCGAGTCGGTGCTTTTTTT