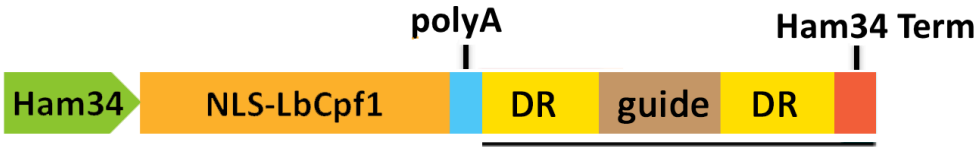
Appendix S2. Guidelines for vector construction

- - - - - - - - - - - - PART A. Using pSTU-1 to express a single guide RNA: - - - - - - - - - - - -



1. The final construct should look like the diagram shown above (the *nptII* expression cassette is not shown).

2. The starting plasmid, pSTU-1, contains the following:

LbCpf1-stopcodon-PolyA-BsaI site-random sequence-BsaI site-DR

3. To express a guide RNA of interest, the "random sequence" stuffer fragment is removed by *Bsa*I digestion and replaced with a double-stranded dsDNA containing cohesive ends that will anneal with the *Bsa*I sequences. This enzyme has the following recognition site:

```
5'...G G T C T C (N)1↓...3'
3'...C C A G A G (N)5↑...5'
```

4. Only the DR close to the *Ham34* terminator is in the vector. Therefore, when designing oligonucleotides for cloning, a 5' DR must be included. The 5' DR and sgRNA are to be cloned in the *Bsa*I site.

5. The next steps show how to clone this 23-nt guide RNA, which uses a TTTA PAM: TTTAATCAGTGCTCGACGGACTCCGGC (note that the PAM is not included in the sgRNA itself).

6. Two oligonucleotides should be annealed and ligated into *Bsa*I-digested pSTU-1. When annealed, they should look like this:

```
5'-AAAATAATTTCTACTAAGTGTAGATATCAGTGCTCGACGGACTCCGGC
3'-     ATTAAAGATGATTCACATCTATAGTCACGAGCTGCCTGAGGCCGATTA
```

Blue: sticky ends for cloning into *Bsa*I. Red: the 5' DR. Brown: the 23-mer sgRNA

and the two oligonucleotides would be:

```
Seq1F:  5'-AAAATAATTTCTACTAAGTGTAGATATCAGTGCTCGACGGACTCCGGC
Seq1R:  5'-ATTAGCCGGAGTCCGTCGAGCACTGATATCTACACTTAGTAGAAATTA
```

7. If instead the goal was to express a sgRNA containing a 20-nt match to the genome sequence with 4 T's at the 3' end, the annealed oligos should look like this:

```
5'-AAAATAATTTCTACTAAGTGTAGATATCAGTGCTCGACGGACTCCTTTT
3'-    ATTAAAGATGATTCACATCTATAGTCACGAGCTGCCTGAGGAAAAATTA
```

- - - - - - - - - - - - PART B. Using pSTU-1 to express an array of guide RNAs: - - - - - - - - - - - -

1. The final construct should look like the diagram shown above. It may also be possible to make much larger arrays, *e.g.* multiplexing three or more sgRNAs.

2. This example is for making an array of two 23-nt guide RNAs, each with a TTTV PAM

Target 1: TTTAATCAGTGCTCGACGGACTCCGGC

Target 2: TTTCGTGCTCTGTTCGCTGCCACCGTT

3. Only the DR close to the *Ham34* terminator is in the vector. Therefore, when designing oligonucleotides for cloning, a 5' DR must be included. The 5' DR, middle DR, and the two sgRNA regions are to be cloned in the *Bsa*I site.

4. Two oligonucleotides should be annealed and ligated into *Bsa*I-digested pSTU-1. When annealed, they should look like this:

```
5'-AAAATAATTTCTACTAAGTGTAGATATCAGTGCTCGACGGACTCCGGCTAATTTCTACTAAGTGTAGATGTGCTCTGTTCGCTGCCACCGTT
3'-    ATTAAAGATGATTCACATCTATAGTCACGAGCTGCCTGAGGCCGATTAAAGATGATTCACATCTACACGAGACAAGCGACGGTGGCAAATTA
```

Blue: for cloning into *Bsa*I. Red: the two DR's. Brown: 23-mer sgRNA for target 1. Purple: 23-mer for target 2.

and the two oligonucleotides would be:

```
Seq2F:5'-AAAATAATTTCTACTAAGTGTAGATATCAGTGCTCGACGGACTCCGGCTAATTTCTACTAAGTGTAGATGTGCTCTGTTCGCTGCCACCGTT
Seq2R:5'-ATTAAACGGTGGCAGCGAACAGAGCACATCTACACTTAGTAGAAATTAGCCGGAGTCCGTCGAGCACTGATATCTACACTTAGTAGAAATTA
```