

### 1. Identify a targetable 20-bp sequence

Genome sequence (*white* locus)

```

... GGCCTATCCGGGCGAACTTTTGGCCCTGATGGGCAGTTCCGGTGC CCGAAAAGACGACCCTGCTGAATGCCCTT...
      |||
... CCGGATAGGCCGCTTGA AAACCGGCAC TACCCGTC AAGGCCACG GCCTTTCTGCTGGGACGACTTACGGGAA...
    
```

PAM

### 2. Design oligonucleotides

w-ex3-3-F: CTTCGTGATGGGCAGTTCCGGTGC

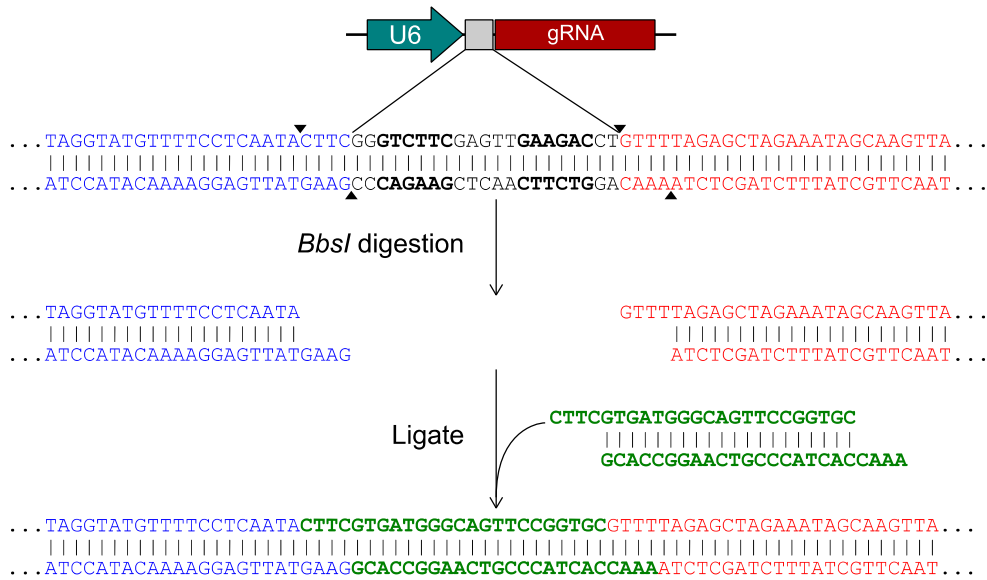
w-ex3-3-R: AAACGCACCGGAACTGCCCATCAC

### 3. Anneal oligonucleotides

```

CTTCGTGATGGGCAGTTCCGGTGC
|||
GCACCGGAACTGCCCATCACAAA
    
```

### 4. Digest vector and clone annealed oligos



**Figure S2** Overview of the cloning strategy.