Oct 09, 2024 Version 1

Designing sgRNA Oligos and Inserting Guides into the GEARBOCS Vector V.1

Forked from a private protocol

In 1 collection

DOI
https://dx.doi.org/10.17504/protocols.io.5jyl8271rl2w/v1

Luke Bradley[1]

[1]Duke University

Eroglu_Lab

Luke Bradley
Duke University

DOI: https://dx.doi.org/10.17504/protocols.io.5jyl8271rl2w/v1

Protocol Citation: Luke Bradley 2024. Designing sgRNA Oligos and Inserting Guides into the GEARBOCS Vector . **protocols.io** https://dx.doi.org/10.17504/protocols.io.5jyl8271rl2w/v1

Protocol status: Working
We use this protocol and it's working

Created: September 27, 2024

Last Modified: October 09, 2024

Protocol Integer ID: 108552

Keywords: designing sgrna oligo, sgrnas for gene, designing sgrna, specific genetic manipulation in crispr, sgrna oligo, gearbocs vector backbone, gearbocs vector, inserting guides into the gearbocs vector, crispr, cas9 mice, gearbocs system, specific genetic manipulation, gene

Funders Acknowledgements:
ASAP
Grant ID: 383000083

Abstract

This protocol describes designing sgRNAs for genes of interest using the GEARBOCS vector backbone. This method allows researchers to utilize the GEARBOCS system for astrocyte-specific genetic manipulation in CRISPR/Cas9 mice for in vivo assays

Designing sgRNA oligos

1 Use **UCSC** to get gene's exons and introns. Make sure the area you are targeting is in all isoforms.

2 Before designing your own guides, check the literature for guides that you can use.

3 Second, try **CRISPick** with the exon you want to target.
- Reference Genome: Mouse GRCm38 (NCBI RefSeq v.108.20200622)
- Mechanism: CRISPRko
- Enzyme: SpyoCas9(NGG) and **Hsu (2013)** tracrRNA
- Target(s): Bulk/Advanced targets – enter up to 2000 bp
- CRISPick Quota**:** 10

4 Make sure these aren't targeting helical structures or motifs by searching uniprot (https://www.uniprot.org/uniprotkb/P55088/entry).

5 **Ordering oligos for sgRNA annealing:**

6 Add the following bolded sequences to 20bp sgRNAs to add restriction enzyme recognition sites. We have written a script to add the overhangs for you **here**. It requires you to enter your gRNA names and sequences and click run on that website.

7 Example:

8 Aqp4 gRNA FW: **CACCG** ATTGTCTTCCGTATGACTAGAGG

9 Aqp4 gRNA RV: **AAAC** CCTCTAGTCATACGGAAGACAAT **C**

10 ^non-bolded sequence to add the SapI restriction enzyme recognition sites

Insert guides into GEARBOCS vector

11 **GEARBOCS Vector digestion:**

12 Set up the digestion reaction with the following components in a 0.6µL tube

1. GEARBOCS Vector → 2ug
2. SapI Enzyme → 1uL
3. NEB rCutSmart buffer → 5uL
4. UltraPure H2O → XuL (Volume will vary based on volume of vector)
5. Total Volume = 50uL

13 Incubate @37˚C for 1 hour and run the sample in 1% agarose gel.

14 Elute the linearized vector in 25µL water using Qiagen Gel purification kit.

15 Use the eluted sample for ligation or preserve in -20˚C

16 **sgRNA Oligo Annealing:**

17 Set up the annealing reaction with the following components in a 0.2µL tube.

1. UltraPure Water → 7µl
2. Forward Primer, 100uM → 1µl
3. Forward Primer, 100uM → 1µl
4. NEB Buffer 2.1 → 1µl
5. Total = 10µL

18 Heat to 95'C for 5 min (use heat block). Cool to room temp over 1-2 hours

19 Once the annealing is completed, add 90µL water to the tube and mix well

20 Use the annealed sample for ligation or preserve the rest in -20˚C

21 **Ligation:**

22 Take 6µL of annealed sample for the ligation into pUGC Vector and keep the reaction as below

1. Insert- Annealed Oligo → 6uL
2. GEARBOCS-SapI digested → 2µl
3. T4 Ligase Buffer → 1µl
4. T4 Ligase → 1µl
5. Total → 10uL

23 Incubate at 4°C overnight and transform 4 µL into Stbl3 cells the next day. Plate 50 uL of S.O.C. medium + cells.

24 Pick clones for minicultures.

25 Save 0.5-1 mL for glycerol stock and mini-prep the rest for sequencing with the U6 primer.