Oct 09, 2024

Designing sgRNA Oligos and Inserting Guides into the GEARBOCS Vector

Forked from Designing sgRNA Oligos and Inserting Guides into the GEARBOCS Vector

In 1 collection

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

Luke Bradley[1]

[1]Duke University

ASAP Collaborative Rese… Eroglu_Lab

Luke Bradley
Duke University

DOI: 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

Funders Acknowledgement:
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.