SEP 30, 2023

Protocol Citation: Xuefeng Ren, Annan SI Cook 2023. Plasmid Construction Protocol. **protocols.io** https://protocols.io/view/plasmid-construction-protocol-c2n7ydhn

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

Created: Sep 25, 2023

Last Modified: Sep 30, 2023

PROTOCOL integer ID: 88511

Plasmid Construction Protocol

Xuefeng Ren[1,2], Annan SI Cook[1,2]

[1]University of California, Berkeley; [2]Aligning Science Across Parkinson's

Annan SI Cook

ABSTRACT

This protocol details plasmid construction in general terms for the insertion of genes into the pCAG mammalian expression vector.

ATTACHMENTS

852-2204.pdf

MATERIALS

Materials

- DNA sequences encoding PI3KC3-C1 subunits and VPS15 mutants (codon-optimized and synthesized)
- pCAG vector
- Restriction enzymes (e.g., New England Biolabs enzymes)
- Gibson assembly kit
- Sanger sequencing service

Design Plasmid Constructs

1 This protocol uses the pCAG vector and NEB restriction enzymes and Gibson Assembly kits.

2 Obtain the DNA sequences for the genes of interest from your preferred DNA synthesis vendor.

Digestion or Gibson Assembly

3 Decide whether to use restriction digestion or Gibson assembly for subcloning.

4 **Restriction Digestion**:

4.1 Digest both the pCAG vector and the DNA fragments (genes/tags) using appropriate restriction enzymes.

4.2 Purify the digested fragments using a DNA purification kit.

5 **Gibson Assembly:**
Follow the manufacturer's protocol for the Gibson assembly kit to assemble the DNA fragments into the pCAG vector.

Ligation

6 If using restriction digestion, perform a ligation reaction to insert the digested DNA fragments into the linearized pCAG vector.

7 Use a DNA ligation kit and follow the manufacturer's instructions.

Transformation

8 Transform the ligated plasmids into competent *E. coli* cells.

9 Plate the transformed cells on selective agar plates containing the appropriate antibiotic for plasmid selection.

Bacterial Culture

10 Incubate the plates Overnight at an appropriate temperature for *E. coli* growth (e.g., 37 °C).

Plasmid Isolation

11 Pick colonies that have grown on the plates and inoculate them into liquid culture with the same antibiotic.

12 Grow the cultures to obtain a sufficient amount of plasmid DNA.

13 Isolate the plasmid DNA using a plasmid purification kit.

14 Sanger sequence the results.