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

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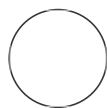
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## Plasmid Construction Protocol

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### 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.