Jul 08, 2022

Plasmid construction

Forked from Plasmid construction

Adam Yokom[1]

[1]University of California, Berkeley

1 *Works for me* Share

This protocol is published without a DOI.

Adam Yokom

DISCLAIMER

DISCLAIMER – FOR INFORMATIONAL PURPOSES ONLY; USE AT YOUR OWN RISK

The protocol content here is for informational purposes only and does not constitute legal, medical, clinical, or safety advice, or otherwise; content added to protocols.io is not peer reviewed and may not have undergone a formal approval of any kind. Information presented in this protocol should not substitute for independent professional judgment, advice, diagnosis, or treatment. Any action you take or refrain from taking using or relying upon the information presented here is strictly at your own risk. You agree that neither the Company nor any of the authors, contributors, administrators, or anyone else associated with protocols.io, can be held responsible for your use of the information contained in or linked to this protocol or any of our Sites/Apps and Services.

ABSTRACT

plasmid construction

PROTOCOL CITATION

Adam Yokom 2022. Plasmid construction. **protocols.io** https://protocols.io/view/plasmid-construction-cczbsx2n

FORK NOTE

FORK FROM

Forked from Plasmid construction, Chunmei Chang

KEYWORDS

ASAPCRN

CREATED

Jul 08, 2022

LAST MODIFIED

Jul 08, 2022

PROTOCOL INTEGER ID

66307

MATERIALS TEXT

Q5® High-Fidelity DNA Polymerases (NEB)
restriction enzymes (NEB)
quick CIP (NEB)
T4 ligase (NEB)
Gel extraction kit (Bio Basic)

1 Amplify the insert gene fragment by PCR with primers including 21 nt of overlapping sequence with the target gene.

2 Linearize the backbone with restriction enzymes (NEB).

3 Treat linearized backbone with quick CIP (NEB).

4 Run PCR products and linearized backbone in an agarose gel to confirm the size.

5 Purify the DNA from gel using a Gel extraction kit (Bio Basic).

6 Ligate the linearized backbone and the insert with the T4 ligase (NEB).

7 Transform the ligation products into home made competent cells.

8 Perform colony PCR to screen for colons that with inserted gene.

9 Sequence to verify that the inserted gene is correct.