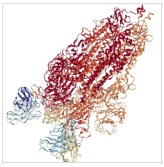

Oct 30, 2020

BSCI:414--Lab 9: Cloning the SARS-CoV-2 Spike Protein into a E. coli Protein Expression Vector

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1 *Works for me* This protocol is published without a DOI.

USG Fall 2020 BSCI:414

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ABSTRACT

The SARS-CoV-2 spike protein does not express solubly in E. coli cells. This may be due to E. coli's inability to glycosylate or create disulfide bonds. The spike protein can be cloned into a vector containing the periplasmic localization peptide (pelB) like Novagen's pET-26 so the protein is transported to the oxidizing environment of the periplasm. Disulfide bonds may also be favored by expressing in modified E. coli cells like NEB's SHuffle strain. Glycosylation may be improved by expressing in a modified E. coli cell line capable of N-linked glycosylation like CLM24.

PROTOCOL CITATION

Harley King 2020. BSCI:414--Lab 9: Cloning the SARS-CoV-2 Spike Protein into a E. coli Protein Expression Vector. **protocols.io**
https://protocols.io/view/bsci-414-lab-9-cloning-the-sars-cov-2-spike-protei-bn9pmh5n

CREATED

Oct 30, 2020

LAST MODIFIED

Oct 30, 2020

PROTOCOL INTEGER ID

44047

ABSTRACT

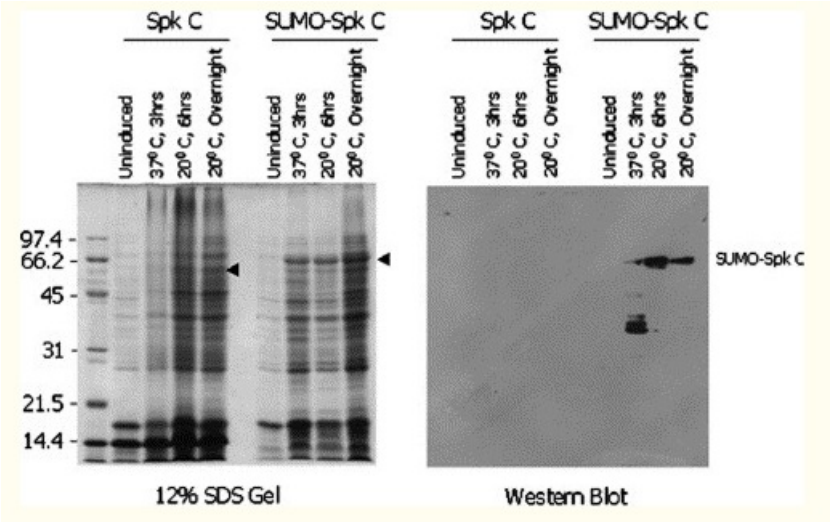
The SARS-CoV-2 spike protein does not express solubly in E. coli cells. This may be due to E. coli's inability to glycosylate or create disulfide bonds. The spike protein can be cloned into a vector containing the periplasmic localization peptide (pelB) like Novagen's pET-26 so the protein is transported to the oxidizing environment of the periplasm. Disulfide bonds may also be favored by expressing in modified E. coli cells like NEB's SHuffle strain. Glycosylation may be improved by expressing in a modified E. coli cell line capable of N-linked glycosylation like CLM24.

Explore the Spike Protein

1 Explore the structure of the SARS-CoV-2 spike protein using Cov3D.

Explore the spike protein using this link: https://cov3d.ibbr.umd.edu/viewer7k90

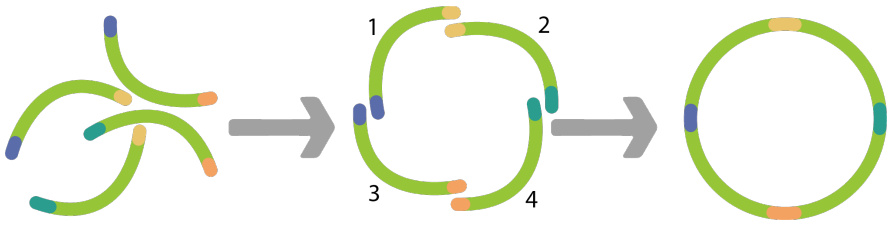
2 Read abstract of Zuo et al. concerning previous attempts at expressing SARS-CoV spike protein fused with SUMO tag.



C-terminus of SARS-CoV spike protein expressed more solubly with SUMO tag.

Discuss Gibson Cloning

3



Gibson cloning uses three enzymes and overlapping, complimentary bases to ligate fragments into vectors.

Clone spike -sumo into pET28.

4 Using a Gibson cloning strategy in Benchling, clone SARS-CoV-2 spike-SUMO into pET28. Open three plasmids in Benchling. Cleave all other tabs.

1. SumoPro
2. pET-28 Vector
3. SARS-CoV2-Spike-delta21