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# BSCI:414--Lab 11:Confirming Cloning of spike(RBD)-SUMO/pET28

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

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ABSTRACT

We've amplified our target genes (SARS-CoV-2 spike and spike (RBD) and SUMO) with primers. Then we purified and performed Gibson Assembly. After transformation we tested a few colonies and found that they were positive. We sent the purified plasmid away for sequencing and now we wish to confirm if we correctly cloned.

PROTOCOL CITATION

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ABSTRACT

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## Watch Cloning Video

1 Review the Benchling notebook regarding Gibson Assembly for cloning the spike-SUMO and RBD genes into pET28.
https://benchling.com/s/etr-kXqsFUhKxGCbyUGyCx9V

**Creating Spike-related Plasmids for Heterologous E. coli Expression · Benchling.pdf**

## Create Alignment with Chromatograms

2 Open the "F20_spike(RBD)-SUMO/pET28" plasmid from our BSCI:414 plasmids folder. Make a copy into a new folder under "BSCI:414 Lab 11".

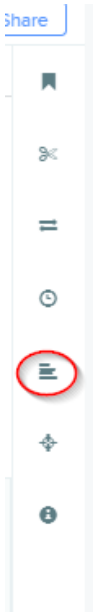
3 Download the 4 sequence chromatograms from our class folder onto your computer.
- BSCI414>Files>Lab Results>Lab 11 spike cloning>RBD_pET28 chromatograms

These chromatograms represent sequence using single primers: T7_promoter and T7_terminator.

| Primer | Primer Sequence |
|---|---|
| T7_promoter | TAATACGACTCACTATAGGG |
| T7_terminator | gctagttattgctcagcgg |

Sequencing primers

4 Select the "Alignments" icon on the right side.




Benchling "Alignments" icon.

5 Add the chromatograms and select "Align".

Are there differences?

What kind of differences?

## Which Cell Line Should Be Transformed?

6 Next steps: transformation but into which cell lines?