Expression of SARS-CoV-2 Spike Proteins Using Drosophila S2 Cells

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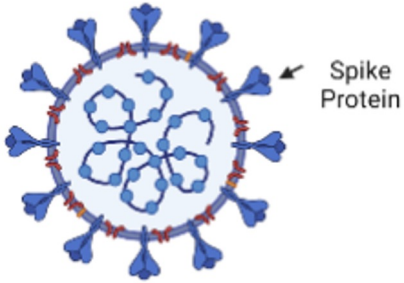
Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)



- Member of Coronaviridae family
- Positive-sense single-stranded RNA virus
- Distantly related to SARS-CoV-1, virus that caused the 2002–2004 SARS outbreak
- Caused 185 million confirmed cases with over 4 million deaths worldwide
  - Over 33 million cases and 606,000 fatalities were reported in the U.S.
- Infection results in COVID-19 disease
  - High fever, chills, cough, shortness of breath, and difficulty in breathing
- Transmission occurs through aerosols and direct or indirect contact
- Spike glycoprotein on outer surface of virus
  - Mediates entry into host cell through binding of the receptor binding domain (RBD) to human angiotensin-converting enzyme 2 (hACE2)
  - Target for neutralizing antibodies and T-cell mediated responses which can facilitate viral clearance
  - Lead antigen for all current vaccines and an important analyte for diagnostic tests

Recombinant proteins: Proteins expressed by host cells transfected with foreign genes

Cell Expression Systems

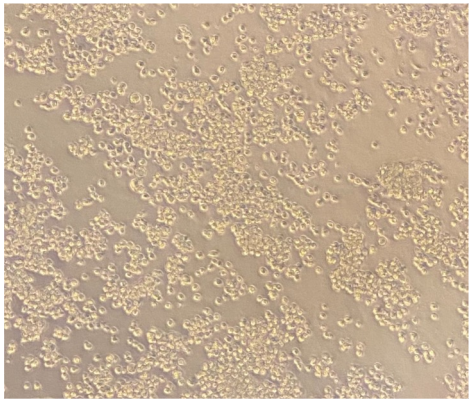
- Mammalian
- Insect
- Bacteria
- Yeast
- Algae

Advantages

- Post-translational modification
- Less stringent culturing conditions
- Harbor fewer mammalian pathogens
- Require fewer purification steps
- Grows to high density
- Produce high yields
- Scalable
- Perfusable

Disadvantages

- Slightly different post translational modification than mammalian cell lines
- Not as widely used



Hypothesis

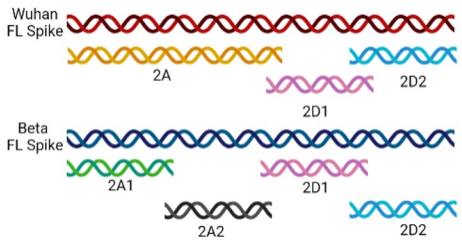
SARS-CoV-2 spike antigens derived from Drosophila melanogaster S2 insect cells are highly native-like and reactive to COVID-19 convalescent sera and spike-specific monoclonal antibodies (mAbs)

Purpose

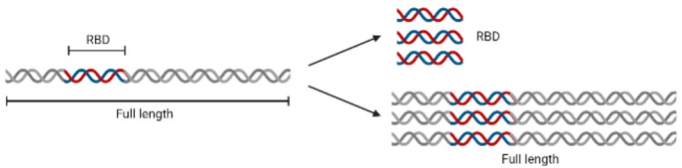
Establish a stable expression system for high-quality SARS-CoV-2 antigens that can be used for diagnostic tests and preventative countermeasures.

Methods

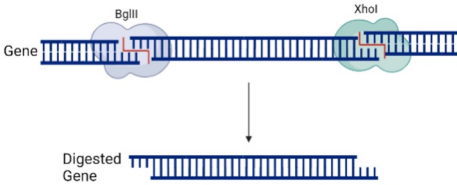
Gene assembly of synthetic DNA fragments using HIFI assembly kit



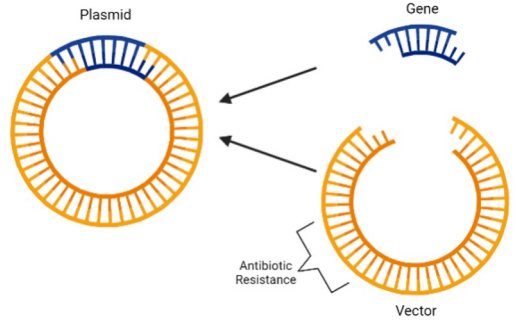
PCR Amplification of the Full Length (FL) and Receptor Binding Domain (RBD)



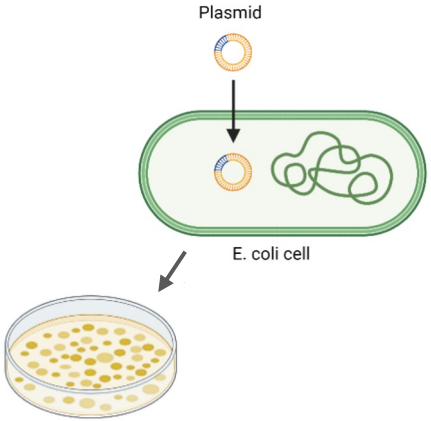
Restriction Digest BglII and XhoI



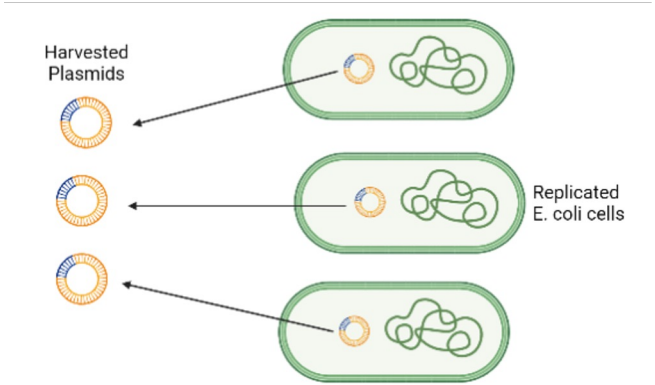
Ligation



Transformation

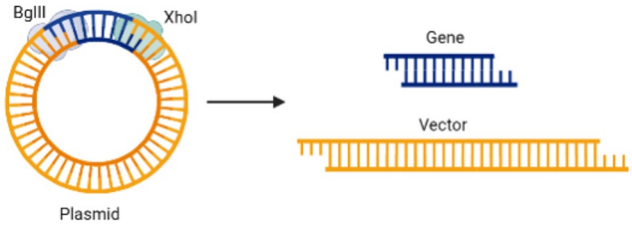


Mini prep

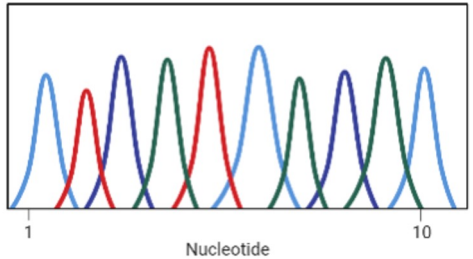


Methods

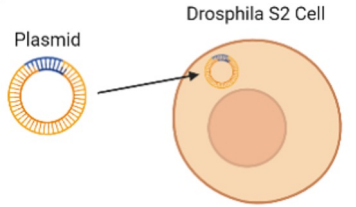
Screen colonies with restriction digest using BglII and XhoI



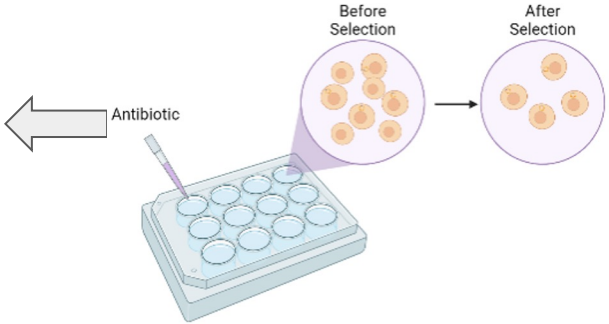
Sequencing



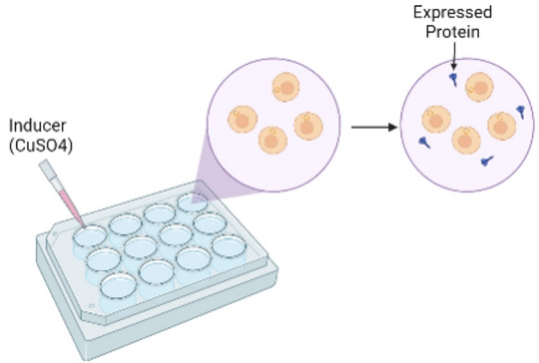
Transfection



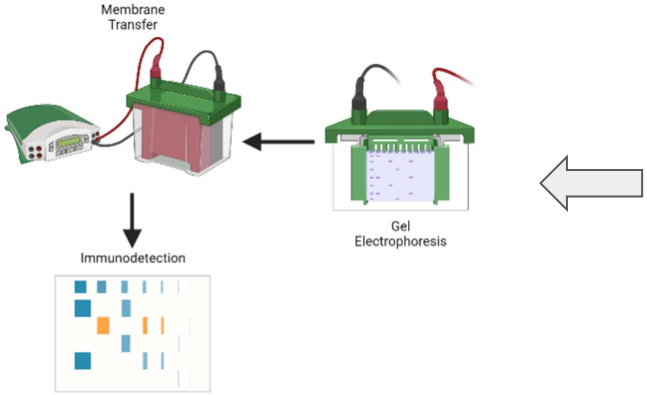
Antibiotic Selection/ Generate transformed cell lines



Induction/ Expression

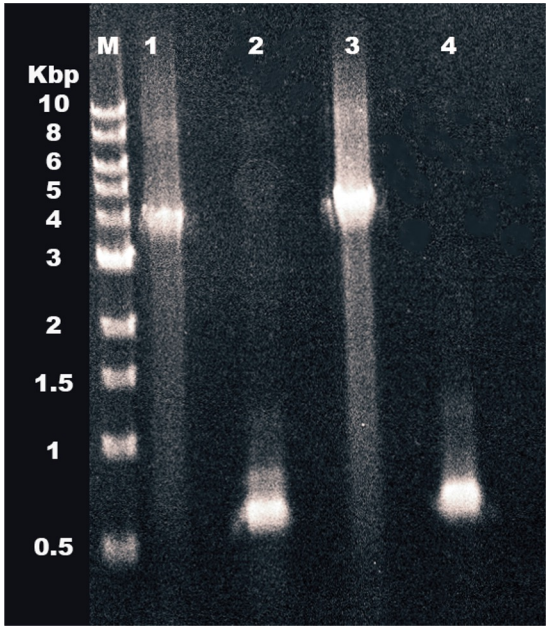


Western Blotting with CR3022 mAb and COVID-19 convalescent human



Results

PCR Amplification of Genes



Spike (~3.6 kbp)

RBD (~700 bp)

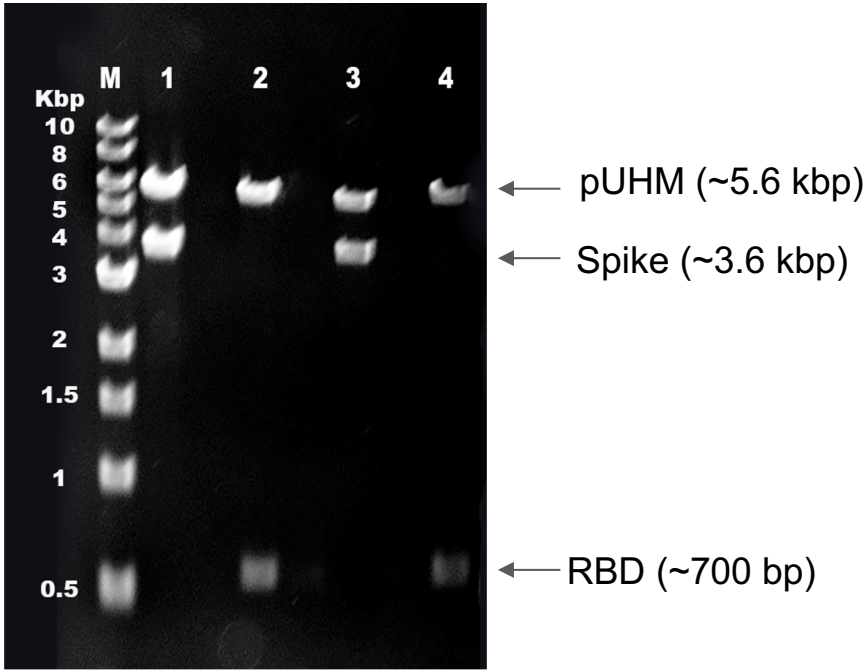
M: Standard

1. SARS-CoV-2 Spike (Wuhan, Hu1, Lineage A)
2. SARS-CoV-2 RBD (Wuhan, Hu1, Lineage A)
3. SARS-CoV-2 Spike (Beta, Lineage B.1.351)
4. SARS-CoV-2 RBD (Beta, Lineage B.1.351)

PCR genes were amplified using primers targeting the full-length Spike and RBD portions of the synthetic gene. 10 µL of each reaction was loaded into a 1% agarose gel and subjected to gel electrophoresis

Results

Restriction enzyme digest of ligated pUHM plasmid containing each gene



M: Standard

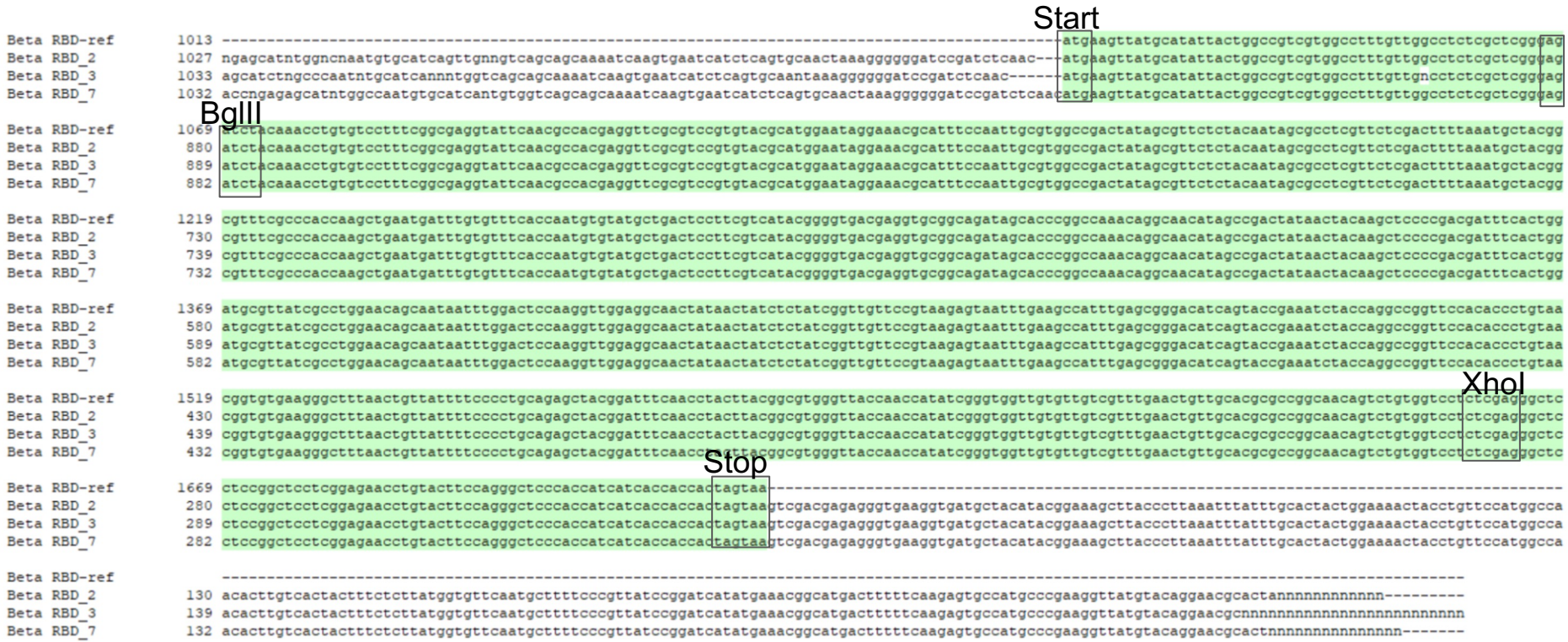
1. pUHM: SARS-CoV-2 Spike (Wuhan, Hu1, Lineage A)
2. pUHM: SARS-CoV-2 RBD (Wuhan, Hu1, Lineage A)
3. pUHM: SARS-CoV-2 Spike (Beta, Lineage B.1.351)
4. pUHM: SARS-CoV-2 RBD (Beta, Lineage B.1.351)

Plasmids were digested with BglII and XhoI overnight at 37 °C

10 µL of each reaction was loaded into a 1% agarose gel and subjected to gel electrophoresis

Results

Sequencing result of ligating the SARS-CoV-2 RBD into the pUHMS2v plasmid

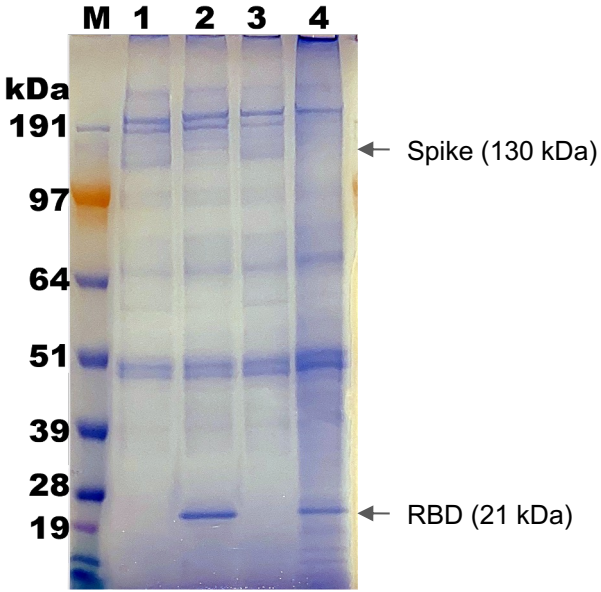


Highlighted regions show that the gene matches the ref gene/plasmid and is inframe with the start codon, restriction sites and stop codons

Results

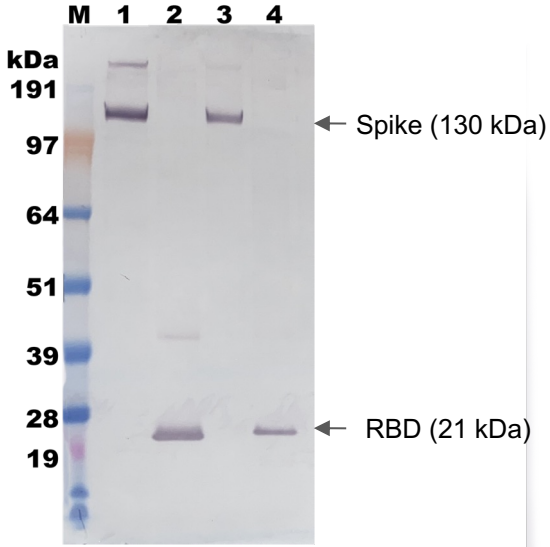
Protein Expression from Drosophila S2 cells

Coomassie stain

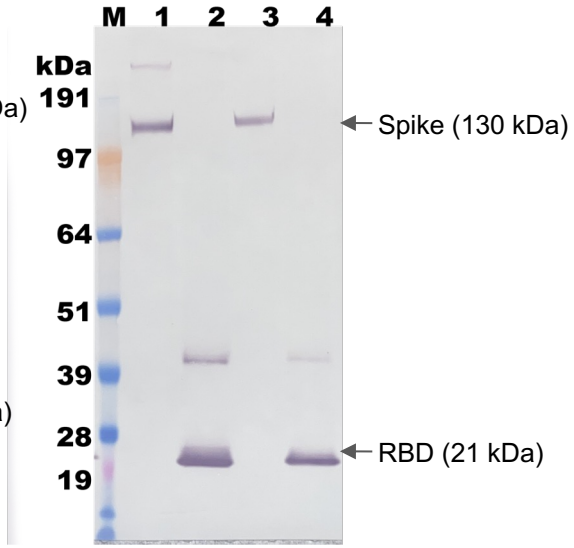


Western Blot

Human sera



CR3022 mAb



M: Standard

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2. SARS-CoV-2 RBD (Wuhan, Hu1, Lineage A)
3. SARS-CoV-2 Spike (Beta, Lineage B.1.351)
4. SARS-CoV-2 RBD (Beta, Lineage B.1.351)

Western blots were probed with COVID-19 convalescent human (1:500) and CR3022 mAb (1:1000) and detected using an anti-human IgG conjugated with alkaline phosphatase.

Conclusion

- FL spike and RBD antigens of two SARS-CoV-2 strains produced from Drosophila S2 cells are correctly folded and reactive to COVID-19 convalescent human and to the SARS-CoV mAb CR3022.

- These antigens are candidates to be used in diagnostic antigen tests and preventative countermeasures, such as vaccines
  - Studies to evaluate the reactivity of the antigens in the field to determine seroprevalence are ongoing
  - Studies to determine immunogenicity of these antigens in mice are ongoing

Acknowledgements

Mentors: Dr. Albert To, Dr. Axel Lehrer

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