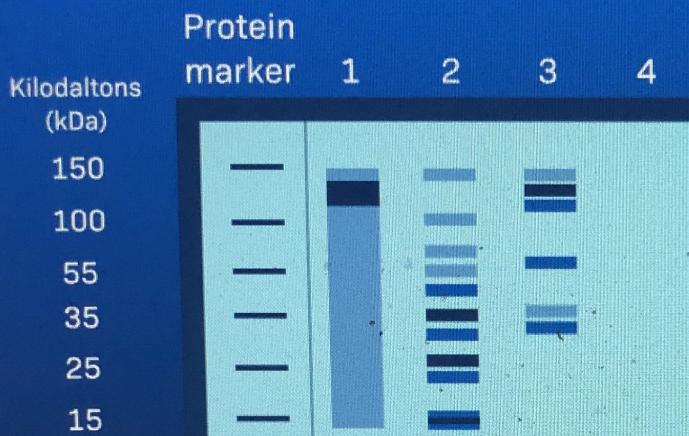


cellular impurities having come out in the flow through and wash.

1. What is the nature of the prominent protein band in lane 1?



Protein marker = Proteins of known sizes
1 = Your Whole cell extract
2 = Your Flow through
3 = Your Wash
4 = Your Eluate

A It is overexpressed ACE2 protein.

It is the overexpressed viral S protein.

Feedback: Correct. It is very likely that the dark band standing out from the others is S since it is overexpressed and has the correct molecular weight of ~125 kDa. For a definite confirmation, a Western blot would be necessary.

C The band is a contamination of the culture.

Attempts left: 2

Submit answer

Next section

Attempts left: 2

Submit answer

2. What is the purpose of testing the flow through and wash along with the product?



Both serve as a control to follow which proteins remain bound to the column.

Feedback: Correct.

B

It helps to identify the proteins present in the sample.

C

Both are a negative control that will not contain any proteins of interest.

Attempts left: 2

Submit answer

3. What might be the reason that the S protein is not visible in lane 4?

Kilodaltons
(kDa)

Protein
marker 1 2 3 4

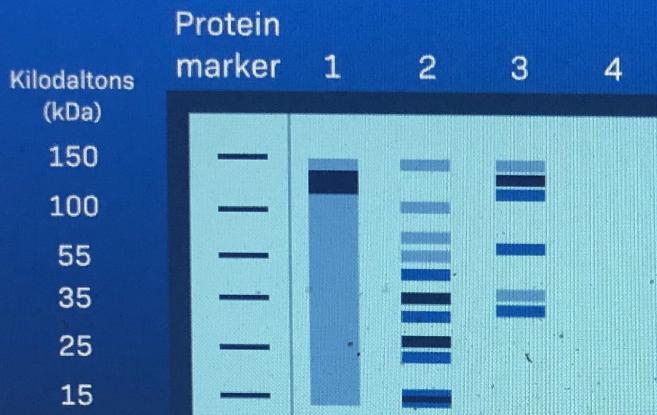
Protein marker → Proteins

Next section





3. What might be the reason that the S protein is not visible in lane 4?



Protein marker = Proteins of known sizes

1 = Your Whole cell extract

2 = Your Flow through

3 = Your Wash

4 = Your Eluate

- A S did not bind to the column and ended up in the flow through.

- The S protein initially bound to the column but was then removed in the washing step.

Feedback: Correct. A band the size of S at 125 kDa is clearly visible in lane 3. Since it is not visible in the flow through in lane 2, it initially bound weakly but was removed in the washing step.

- C It got stuck on the column and was not eluted.

Attempts left: 2

Submit answer

Next section

Attempts left: 2

Submit answer



4. Based on these results, were you successful in purifying the S protein?

A Yes.

✓ No.

Feedback: Correct. While there was evidence that your cells produced the S protein, there is no band in your eluate. You were not able to enrich and purify it.

Attempts left: 1

Submit answer



Before doing any experiments, it is hard to predict which S protein truncation will be the easiest to purify and which will be the best at causing the desired immune response. Since we don't know which truncation of the protein will work best as a vaccine, your labmates created a number of different S protein truncations to test.

While you were purifying the S protein (the spike protein minus the transmembrane domain), your colleagues were expressing two other recombinant proteins, called S1 (70 kDa) and S2 (55 kDa). S1 is the N-terminal part of the S protein containing the domain important for binding to the human

Next section



5. Based on the gel results, was purification for either S1 or S2 successful?



The purification for both proteins was successful. Only single bands for S1 and S2 are visible in the eluate.

Feedback: Correct.

B

Neither purification worked. Compared to the whole cell extract, too many proteins were lost.

C

Only the purification for S1 worked. The number of proteins present in the whole cell extract was reduced in the purification process to a single protein.

Attempts left: 2

Submit answer

Your lab has finished purifying two promising targets for vaccine development, the S1 and S2 truncations of the recombinant S protein of SARS-CoV-2.

Of course, vaccine development does not end with the purified protein. The next

Next section



periods and need to be kept at -20°C. After completing the restriction digest and ligation steps, you run a DNA gel to verify your results. Unfortunately, you do not see multiple bands indicating DNA fragments, casting doubt on whether the restriction digest worked. As you brainstorm possible errors in your process, you remember that the enzymes you used were left at room temperature overnight by accident.

1. What could you do to see if this was the source of error?

A

Return the restriction enzyme to the freezer before redoing the restriction digest.

B

To compensate for any loss in effectiveness, simply add more than the usual amount of enzyme to the reaction.



Take out fresh restriction enzymes from the freezer and repeat the restriction digest.

Feedback: Repeating the experiment, changing one component at a time will help you narrow down if a particular component isn't working.

Attempts left: 2

Submit answer

Thinking about plating your transformed yeast on selective media:

Next section



brainstorm possible errors in your process, your lab mates suggest you check the protocol and wait another day to see if your growth is delayed.



LA
HO
CO

2. What common error did they think you made? Select all that apply:



The plasmid was not added to the yeast and transformation did not occur.

Feedback: Following all steps in the procedure in the correct sequence is important. Check your lab notebook where you record any changes to the procedure, observations, or additional notes. If this was the mistake, waiting another day will not result in any change. Without the plasmid that carries an antibiotic resistance gene, the yeast will not survive in the selective media that contains the antibiotic.



The incubator temperature was set too low to promote adequate growth.

Feedback: This is a common mistake. If the temperature is too low, often the yeast will continue to grow but at a slower rate.



The concentration of the selective agent was too low and will take longer to work.

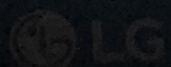
Attempts left: 2

Submit answer

Next section



54°



growth.



3. What should your next step be?



Repeat the procedure by inoculating a fresh liquid culture with a new yeast colony, and in addition also inoculate another liquid culture with a yeast strain you know grows well. Make a third sample of liquid media that isn't inoculated with any yeast and incubate all three samples overnight. The next day, you can compare your transformed culture with the control strain culture and the uninoculated media to look for differences in the amount of growth.

Feedback: This is a good next step. Comparing your results to a control strain will help you determine where the error is.

B

Prepare new liquid media as you may not have added the appropriate nutrients for growth.

C

Choose a different transformed colony and add to your existing liquid culture so as to not waste materials.

+ Add te

Attempts left: 2

Submit answer

Thinking about cell lysis:

Next section



4. What does it mean that there are no soluble proteins?

A

Cell lysis worked but the cell has no soluble proteins.



Cell lysis did not work as the soluble proteins inside the cell should be in the cell lysate.

Feedback: If the cells are lysed, or broken open, we expect to find soluble proteins that would normally be only inside the cell, outside in the solution of cell lysate. Cells have many soluble proteins. No soluble proteins indicates your lysis did not work.

Attempts left: 2

Submit answer

5. What should you do next? Select all that apply:

A

Cell lysis worked so the next step is to purify the protein we are interested in.



If you have tried lysing for 5 minutes, repeat the lysis step for longer.

Feedback: This could work. If you verify that the soluble proteins are not present in the supernatant then a next step could be to resuspend the cell pellet and try a different method of lysis.

Next section





5. What should you do next? Select all that apply:

A

Cell lysis worked so the next step is to purify the protein we are interested in.



If you have tried lysing for 5 minutes, repeat the lysis step for longer.

Feedback: This could work. If you verify that the soluble proteins are not present in the supernatant then a next step could be to resuspend the cell pellet and try a different method of lysis.



If you have been repeating the lysis step for 30 minutes you should try something else.

Feedback: This is a good idea because trying to lyse a cell for too long can cause the proteins to denature. There are many different options for cell lysis such as sonication (applying sound energy) or different detergents to break open yeast cells and solubilize proteins. Changing one variable at a time can help to determine what part of the technique is not effective.

Attempts left: 2

Submit answer

Thinking about protein purification:

Next section



Thinking about protein purification:

You finish the protein purification and examine the eluate on a protein gel. Unfortunately, you don't see any protein bands in the eluate lane.



6. What should you do next?



You should check your control samples from your purification process, including the flow through and wash steps. This helps you determine at what stage you lost the protein of interest.

Feedback: This is a good next step. If the protein was expressed in cells at all, it has to be somewhere. With the wrong buffer, it is possible that it came off the purification column too early and ended up in the flow through or wash fractions.

B

You should try a different column, the problem may be that the protein is not binding to the column.

C

Repeat the experiment with a different protein target since this one was unable to be purified.

Attempts left: 2

Submit answer

Next section



Your lab has successfully purified two recombinant spike protein truncations, S1 and S2, that serve as promising targets for vaccine development. From here, you'll continue only with the S1 truncation, which contains the important ACE2 receptor-binding domain. You will now consider the next experimental steps to arrive at a successful vaccine.

1. To test whether the vaccine is safe and effective for humans, what's the next step?



You need to study the effects of adding the purified protein to an organism to determine how their immune system responds and if it produces antibodies against the purified protein.

Feedback: Correct.

B

You need to study the effects of adding the purified protein to tissue culture, to determine how individual cells respond to the protein.

C

You need to study the effects of adding the purified protein to the virus, to determine if it can stop virus binding to cells.

Attempts left: 2

Check answer

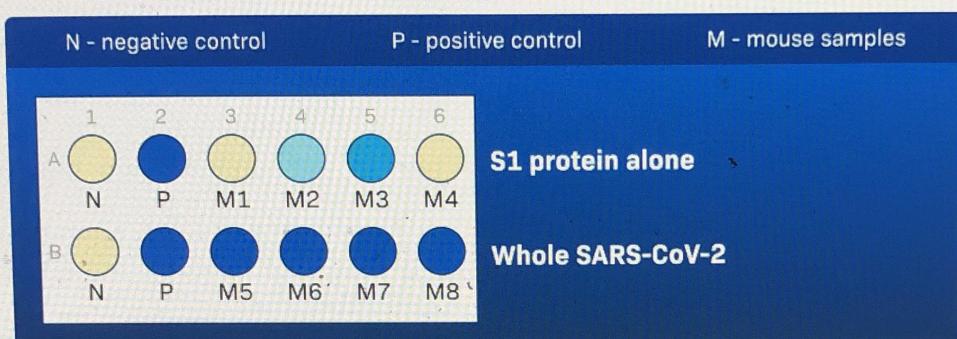
The development of the vaccine does not end with the purified protein. The next

End simulation





2. Four mice (M1, M2, M3 and M4) were injected with the recombinant S1 protein alone and four mice (M5, M6, M7, and M8) were injected with the whole, attenuated (weakened) virus. The ELISA analyzing the antibodies generated by these mice is shown below. What can you determine about the immune response of the mice that were injected either with S1 alone or with the whole virus?



The resulting well colors indicate that all the mice injected with the whole virus generated antibodies against S1 and some of the mice injected with S1 alone produced antibodies against S1.

Feedback: Correct.



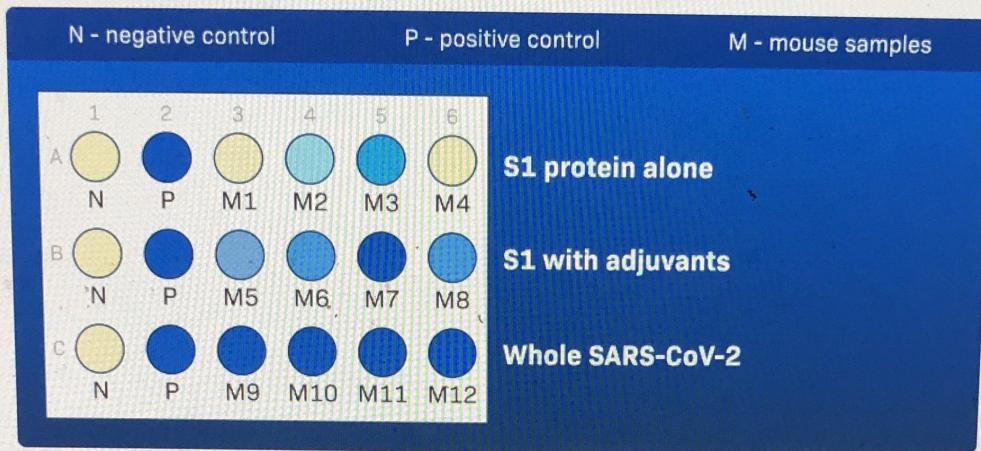
B The ELISA color differences between mice injected with S1 or the whole virus indicate that only the whole virus triggered antibody production.



C The difference in the appearance of blue color indicates that the mice generated antibodies only against S1 but not the whole virus.

End simulation

3. If the viral purified proteins alone fail to trigger a response, adjuvants can be added to the vaccine. Adjuvants are designed to enhance the immune response to produce more antibodies against a certain antigen. Adjuvants were added to the S1 sample when injected into four mice (M5-8 in this experiment). What do the results show?



A The color change indicates that the mice produced more antibody against the S1 protein alone, which resulted in more wells of a yellow color.

✓ The mice produced more antibody against S1 with added adjuvant than with S1 alone, which results in a stronger signal (a darker blue) in the wells.

Feedback: Correct.

C The mice produced fewer antibodies against S1 with adjuvant, resulting in wells with a more intense blue color.

End simulation



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