1. Positive Control

A positive control is a sample in an experiment that produces a known result to compare with the test sample after the same treatment. It is used to control for unknown variables and confirms that all your reagents work.

In this case, the positive control is a cell line expressing wild-type CCR5.

Which of the following do you think will be a good positive control for this experiment? Click on a cell line to select it.

A T Helper cell line

B Macrophage cell line without CD4

C Edited macrophage

✓ Unedited wild-type macrophage

Correct. Wild-type macrophages carry the undisrupted CCR5 gene and express the co-receptor on the cell surface at normal levels. This serves as a standard to determine the CCR5 protein level in CRISPR-edited macrophages.

2. Negative Control

The negative control is the opposite of the positive control: the positive control produces a known result, whereas the negative control is designed to produce no response after the same treatment.

In our example, a negative control would be a cell line not expressing CCR5. Accordingly, the protein gel should not show a CCR5 band. The negative control confirms that no contaminating or artefact band mimics CCR5 presence, even though there is no actual CCR5 protein present in the cells.

Which of the following cell lines will provide an effective negative control for this experiment? Expression levels of the protein of interest are shown next to each cell type as bars: the longer the bar, the higher the expression level of CCR5.

A Bone marrow and immune system 

B Muscle 

C Lung 

D Liver and gallbladder 

✓ Pancreas 

Identify controls and samples

Below are four different cell cultures: your two CRISPR-edited cell lines as well as the wild type macrophage and the pancreas cells. Drag and drop each cell culture into the correct box below as either the positive control, the negative control, or your samples of interest. Use each culture once.

drag


wild type cells


pancreas cells


edit 4 cells


edit 7 cells

Positive control	Negative control	Sample of interest
Drag and drop here	Drag and drop here	
 wild type cells	 pancreas cells	 edit 4 cells  edit 7 cells

Predict expected outcome

Shown below are representations of four possible outcomes when running a protein gel. Identify which gel has run long enough and is ready for analysis by clicking on it. Visible on the gels are only the pre-stained protein ladder and the blue dye front used in SDS sample buffer. The proteins from the cellular extract are invisible at this stage.

The gel has run all the way to the end, and the proteins are optimally separated for analysis.

Submit answer

3. Next step

After disrupting the CCR5 gene in the two macrophages cell lines 4 and 7 (now called edit 4 and edit 7), what will be your next experimental step to verify that these cell lines are now HIV-resistant macrophages?

A To test how well HIV infection of macrophages is inhibited.

B To test for the presence of HIV RNA in your macrophage cell lines.

C To design a second set of gRNAs to disrupt gp120.

✓ To test the protein expression of CCR5.

Correct.

Attempts left: 1

Submit answer

1. What is the purpose of adding SDS to the cell cultures?

A SDS solubilizes membrane proteins.

B SDS breaks down cell membranes and releases the cell content.

C SDS destroys protein.

D SDS unfolds proteins and covers them with negative charge. Negatively charged proteins can be separated in a protein gel according to mass after applying an electrical current.

✓ All except C.

Correct. All except C.

1. How should you micropipette samples into the wells?

A Quickly, so the proteins go down in the well.

✓ Slowly, to prevent it spilling over into the next well.

Correct.

C Slowly, but move the plunger up and down to mix.

1. How do you know if the gel has run for long enough?

A The dye front is missing.

B The dye front is at the top across the wells.

✓ The dye front is at the bottom of the gel.

Correct.

1. Protein samples are separated using the following technique:

✓ SDS polyacrylamide gel electrophoresis.

B Nitrocellulose membrane transfer.

C Agarose gel electrophoresis.

Attempts left: 1

Submit answer

2. Using SDS imparts an overall _____ charge to the proteins.

A positive

✓ negative

C neutral

Attempts left: 1

Submit answer

3. In a PAGE gel, proteins are separated only based on their _____?

A hydrophobicity

✓ molecular weight

C charge

Attempts left: 1

Submit answer

You did not allow for enough time when running the gel.

B The temperature of the gel during the run was too low.

C You forgot to turn on the power source.

Attempts left: 1

Submit answer

5. After running your protein gel, you can inspect the gel for protein bands and determine if your previous gene editing step worked. Is this statement true?

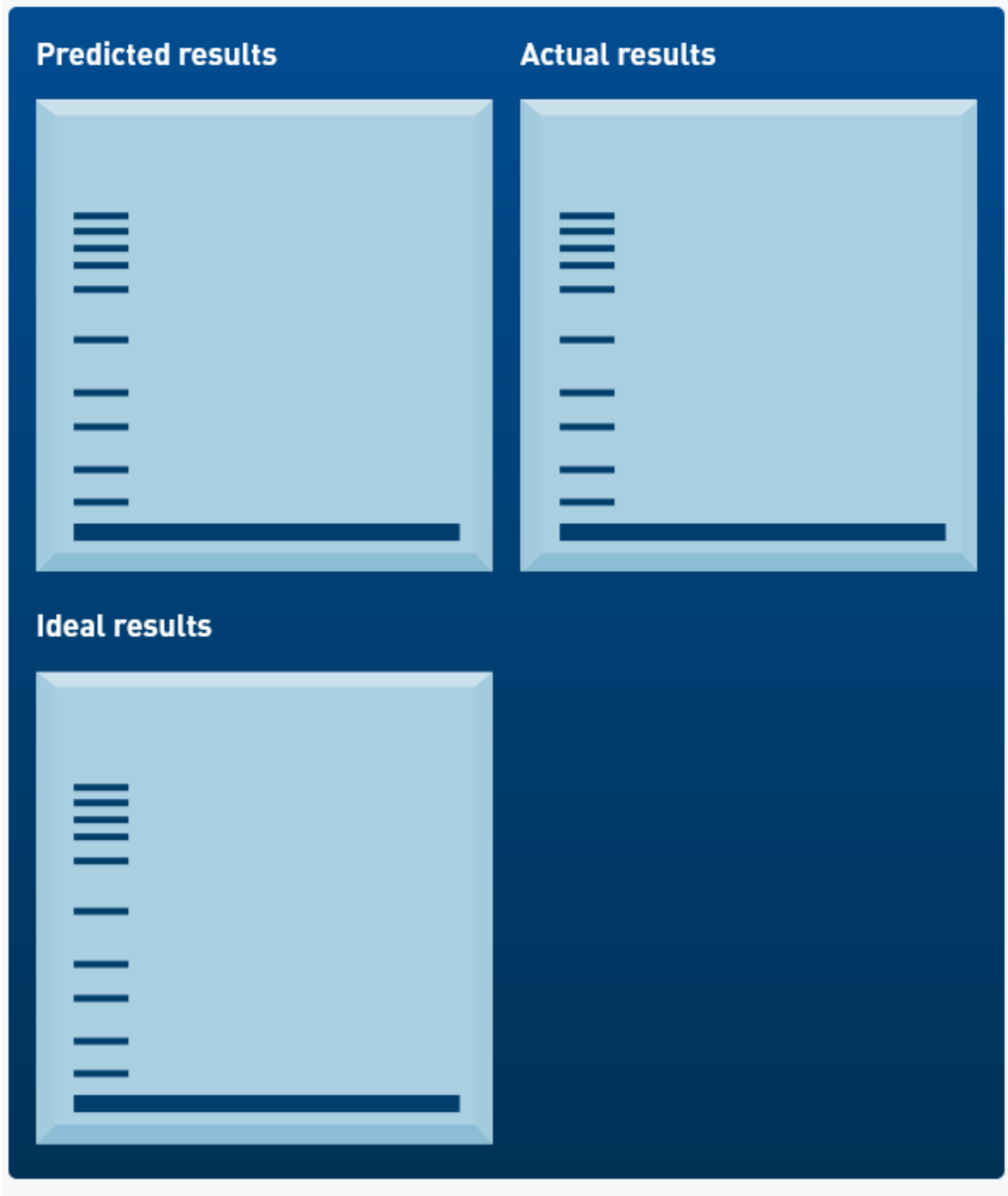
A Yes, running a protein gel is the final step of the experiment.

No, you need to visualize the protein bands first, preferably by Western blot.

C No, the only way to determine if the experiment worked is to infect the edited cell lines with HIV.

Attempts left: 2

Submit answer


Predicted results
Actual results
Ideal results