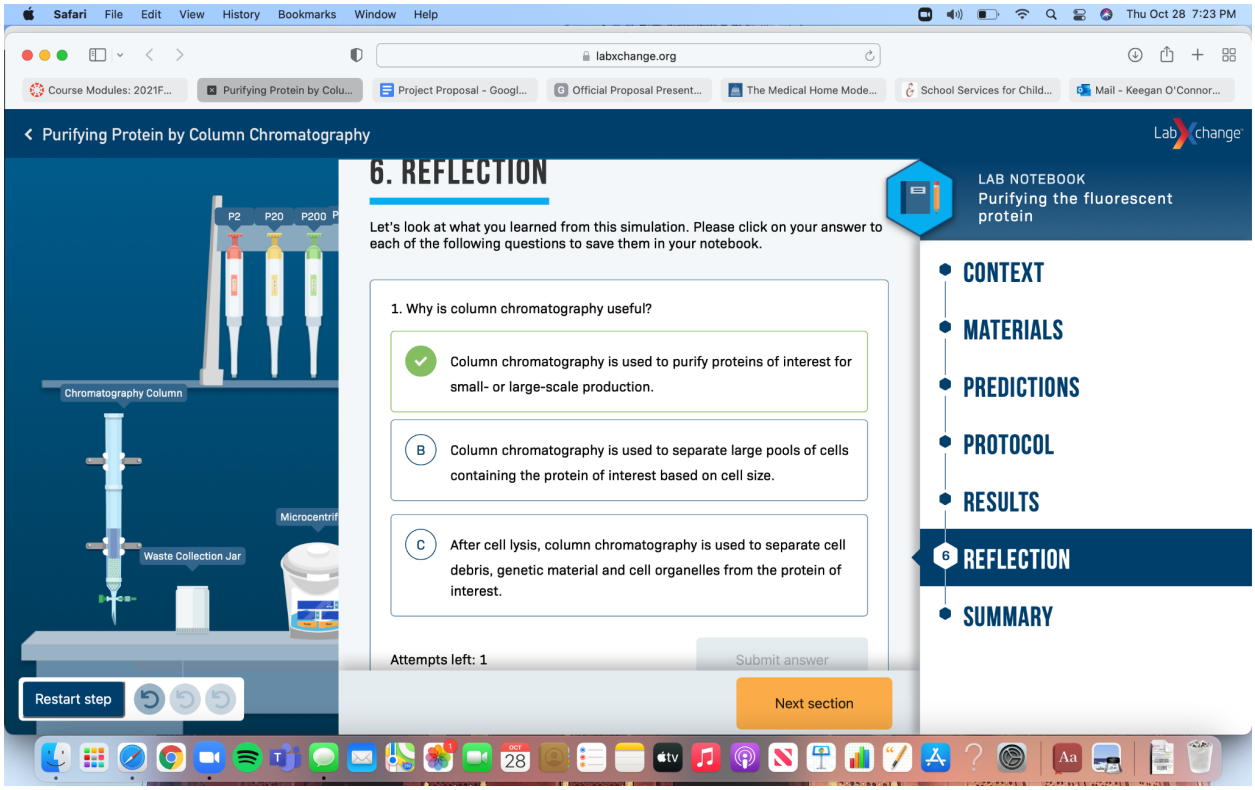
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6. REFLECTION
Let's look at what you learned from this simulation. Please click on your answer to each of the following questions to save them in your notebook.
1. Why is column chromatography useful?
Column chromatography is used to purify proteins of interest for small- or large-scale production.
B
Column chromatography is used to separate large pools of cells containing the protein of interest based on cell size.
C
After cell lysis, column chromatography is used to separate cell debris, genetic material and cell organelles from the protein of interest.
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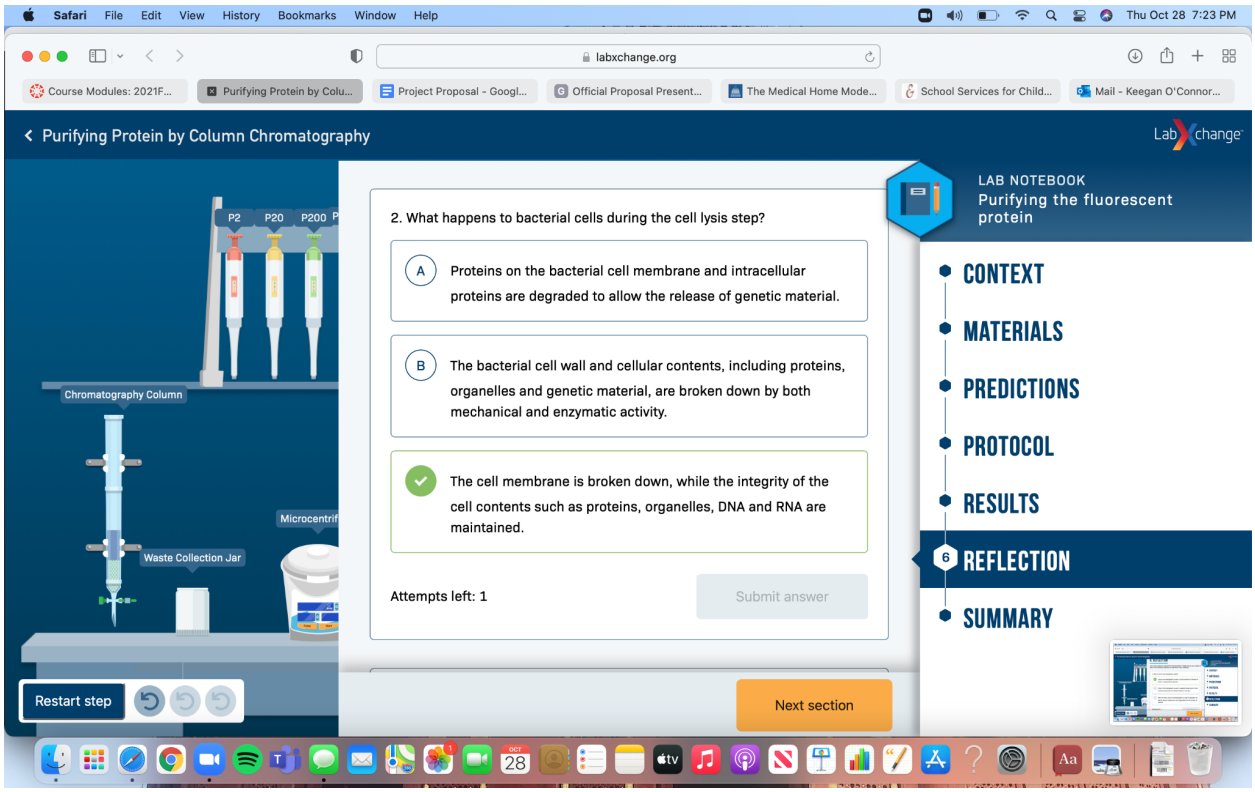
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2. What happens to bacterial cells during the cell lysis step?
A
Proteins on the bacterial cell membrane and intracellular proteins are degraded to allow the release of genetic material.
B
The bacterial cell wall and cellular contents, including proteins, organelles and genetic material, are broken down by both mechanical and enzymatic activity.
The cell membrane is broken down, while the integrity of the cell contents such as proteins, organelles, DNA and RNA are maintained.
Attempts left: 1
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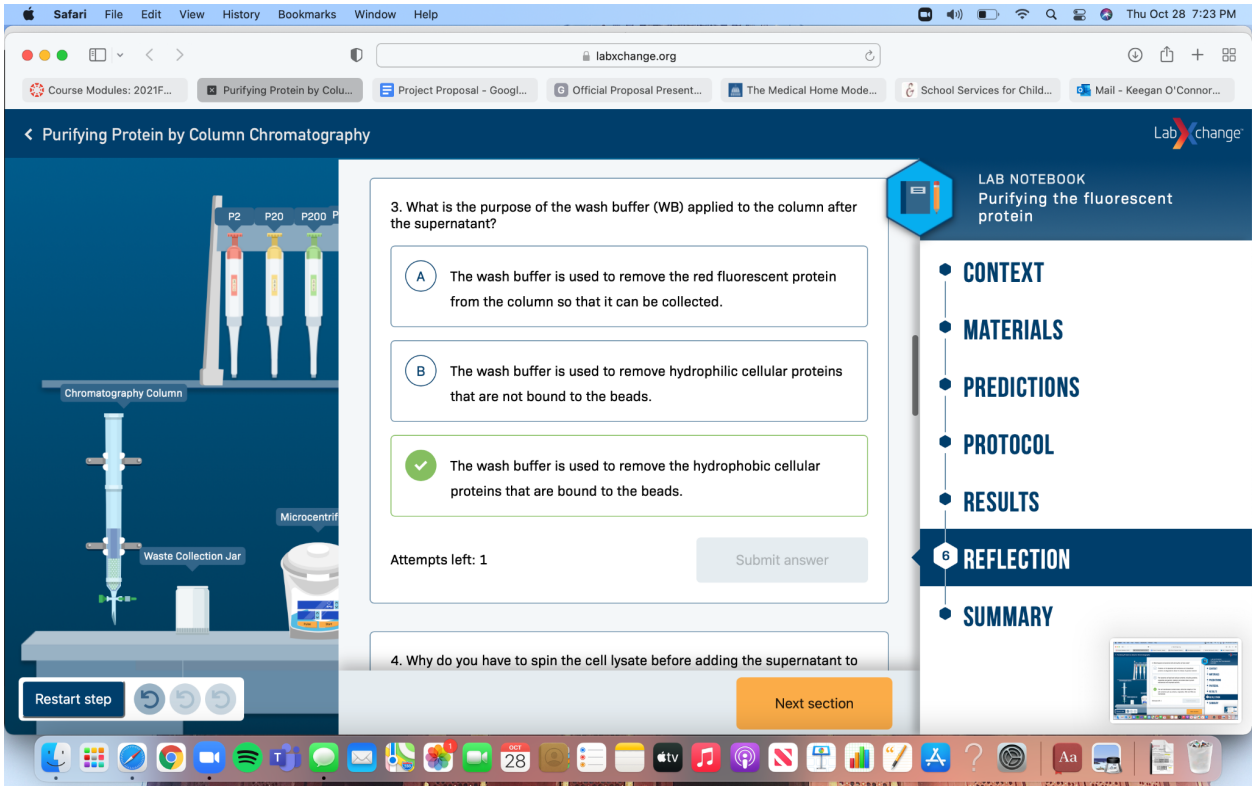
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3. What is the purpose of the wash buffer (WB) applied to the column after the supernatant?
A
The wash buffer is used to remove the red fluorescent protein from the column so that it can be collected.
B
The wash buffer is used to remove hydrophilic cellular proteins that are not bound to the beads.
The wash buffer is used to remove the hydrophobic cellular proteins that are bound to the beads.
Attempts left: 1
Submit answer
4. Why do you have to spin the cell lysate before adding the supernatant to
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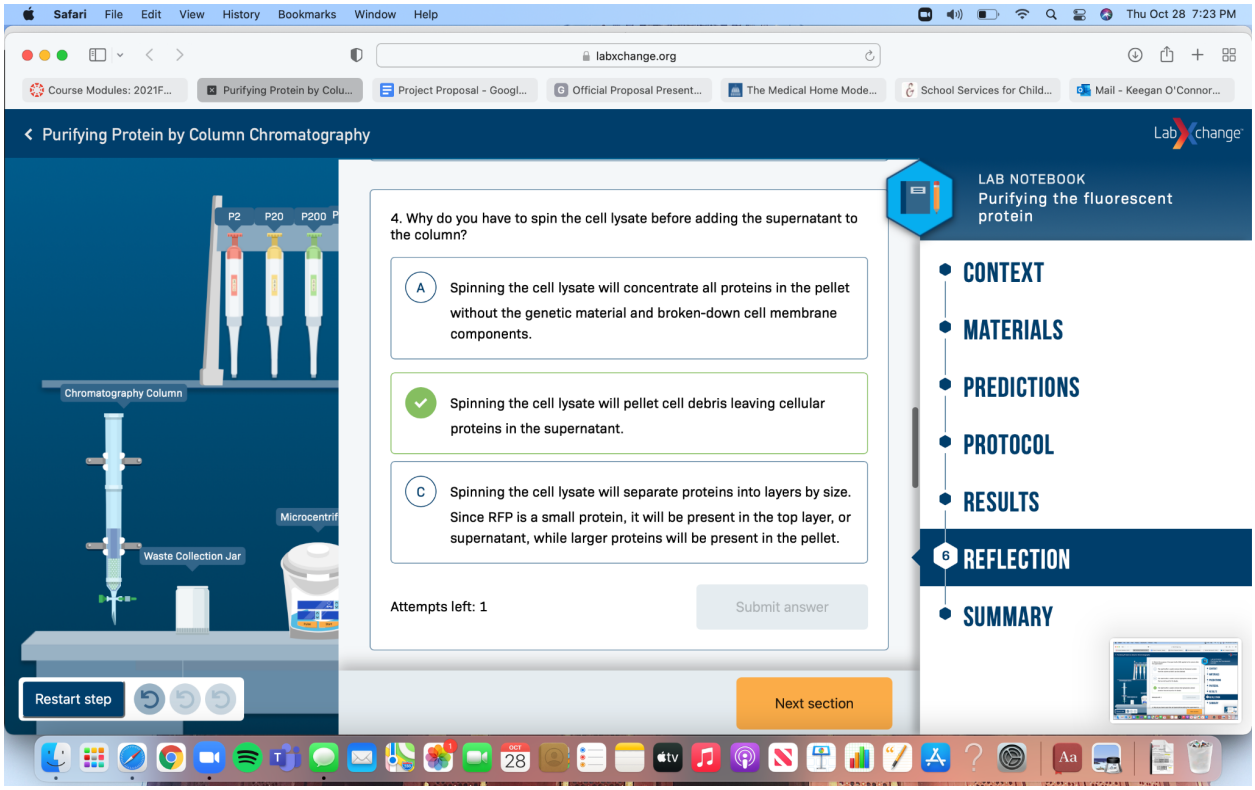
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4. Why do you have to spin the cell lysate before adding the supernatant to the column?
A
Spinning the cell lysate will concentrate all proteins in the pellet without the genetic material and broken-down cell membrane components.
Spinning the cell lysate will pellet cell debris leaving cellular proteins in the supernatant.
C
Spinning the cell lysate will separate proteins into layers by size. Since RFP is a small protein, it will be present in the top layer, or supernatant, while larger proteins will be present in the pellet.
Attempts left: 1
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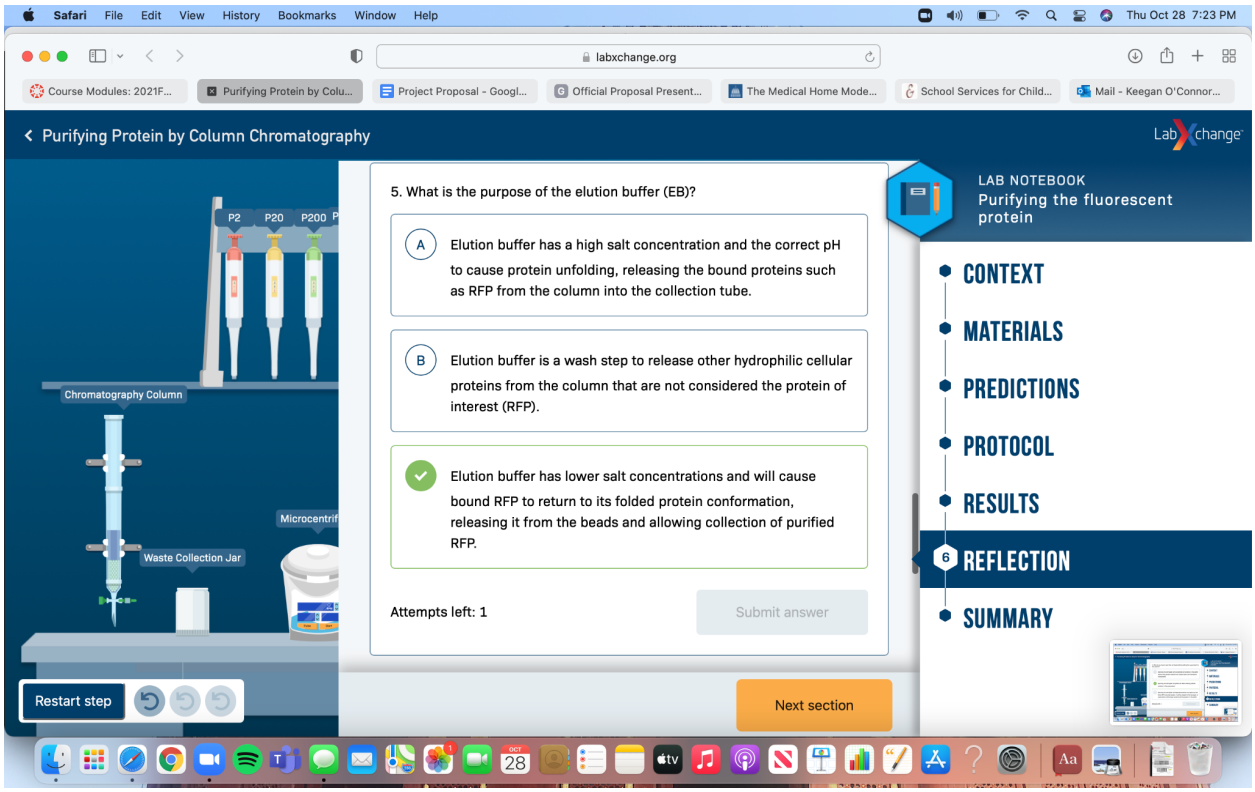
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5. What is the purpose of the elution buffer (EB)?
A
Elution buffer has a high salt concentration and the correct pH to cause protein unfolding, releasing the bound proteins such as RFP from the column into the collection tube.
B
Elution buffer is a wash step to release other hydrophilic cellular proteins from the column that are not considered the protein of interest (RFP).
Elution buffer has lower salt concentrations and will cause bound RFP to return to its folded protein conformation, releasing it from the beads and allowing collection of purified RFP.
Attempts left: 1
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