Biotech Skills Lab

Protein Expression and Purification

miniPCR Biotech Skills Lab™:
Protein Expression and Purification

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Student guide

Background information

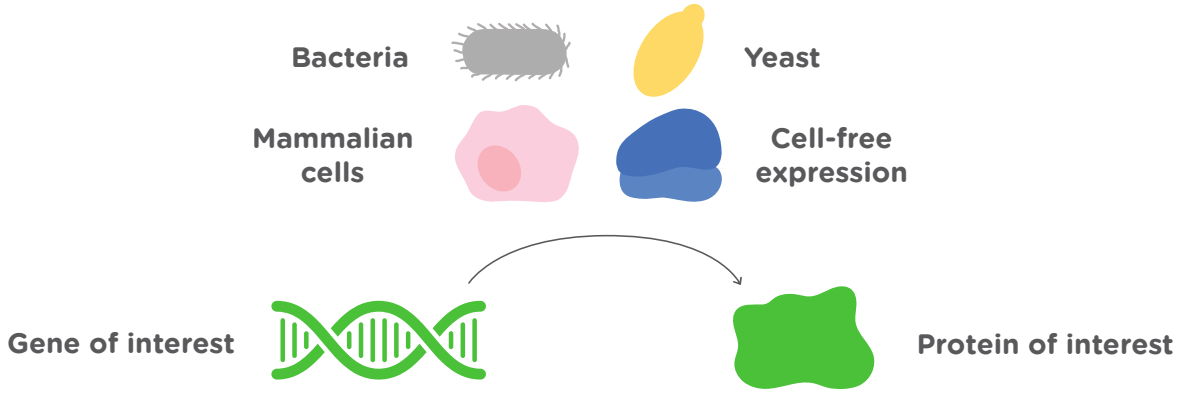
Protein expression

1

- You are probably familiar with proteins, the molecules that carry out most cellular functions.
- Scientists also engineer and manufacture proteins for wide-ranging applications, from stain-removing enzymes used in laundry detergents to protein-based medicines like insulin.

2

- Scientists typically use living cells like bacteria, yeast, or mammalian cells to produce proteins.
- In biotech, protein production is called **protein expression** because the cells are provided with DNA that is interpreted or "expressed" to produce the encoded protein.
- In this manner, cells are used like factories to make large quantities of whatever **protein of interest** the scientists need to produce.



3

- Because all organisms share the same genetic code, scientists can provide virtually any cell with the genetic instructions to make most proteins. For example, human proteins can be produced using bacterial, yeast, and mammalian cells.
- We can also make proteins using cellular components placed in a test tube. This technique is called **cell-free protein expression technology** because it enables protein production without the need for living cells.

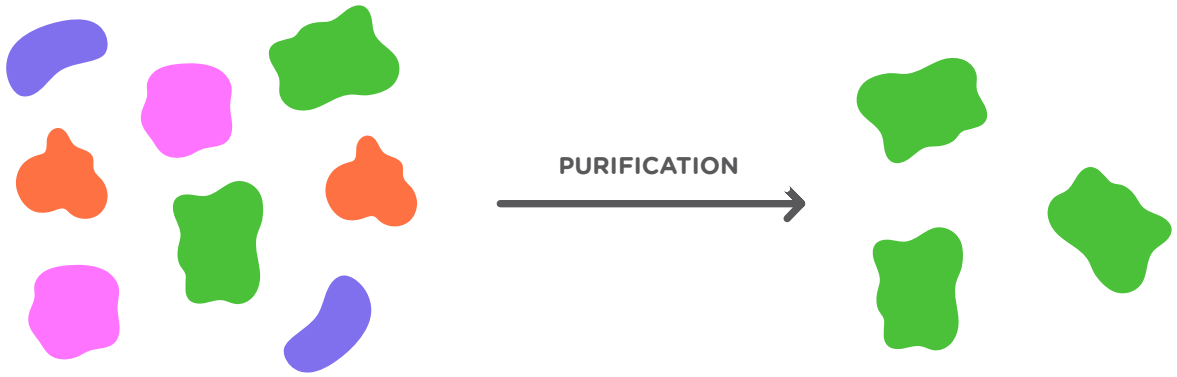
Check your understanding

Q1. True or False. Only bacterial cells can be used to express bacterial proteins.

Protein purification

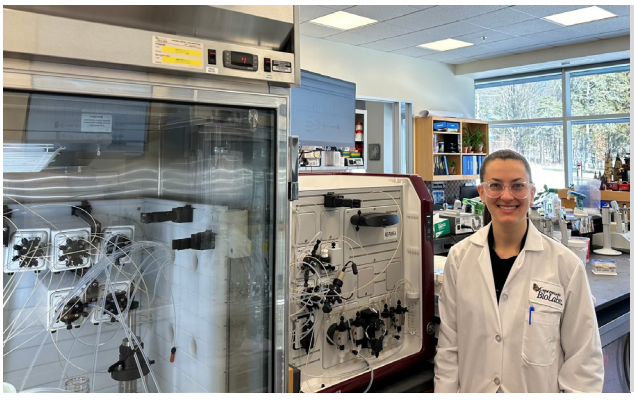
1

- After the protein of interest has been produced, it must be separated from other proteins and cellular components, such as lipids and nucleic acids.
- This process is called **protein purification**.



2

- There are many protein purification methods, but they all rely on separating molecules by their unique characteristics. Examples include separating molecules based on size, charge, or hydrophobicity.
- When proteins are produced for industrial or medical use, many rounds of purification are performed because the end product must be very pure.



Biotech careers

Daria McCallum works at New England Biolabs and has devoted many years to the purification of restriction endonuclease proteins. Learn more about Daria and her work in the *Biotech career profile* on page 40.

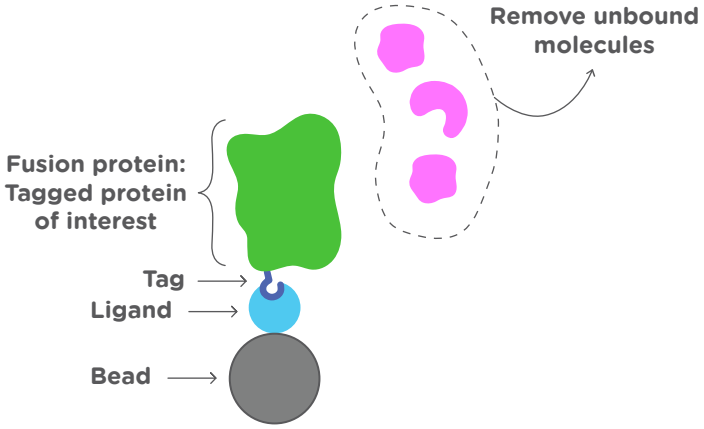
Affinity purification

1

- Today's activity will focus on **affinity purification**. This form of protein purification relies on beads coated with a **ligand** that specifically binds to, or has an affinity for, the protein of interest.
- When a mixture of molecules is applied to the beads, only the protein of interest binds to the ligand and is captured by the beads.

2

- The protein of interest you will purify includes a short **tag** made of a few amino acids, the building blocks of proteins.
- Scientists engineered the tag to bind with high affinity to the ligand present on the beads.
- The strong, specific interaction between the tag and the beads will allow you to isolate the protein of interest with relative ease.



3

- The tag is added by modifying the DNA that encodes the protein of interest.
- This modified protein is called a **fusion protein** because it combines sections encoded by two different genes, the original protein and the tag.
- Refer to page 42 to learn about the tag used in this activity.

Check your understanding

Q2. Which of the following is a benefit of using a tag and its ligand for protein purification?

A. It allows you to express the protein of interest in any cell type.
B. The tag and ligand bind each other with high affinity.
C. It helps to stabilize your protein of interest.
D. It is impossible to purify an untagged protein.

Today's lab

You will use cell-free protein expression to produce a protein of interest that carries a tag. Then, you will separate the tagged protein from everything else in the cell-free reaction using affinity purification.

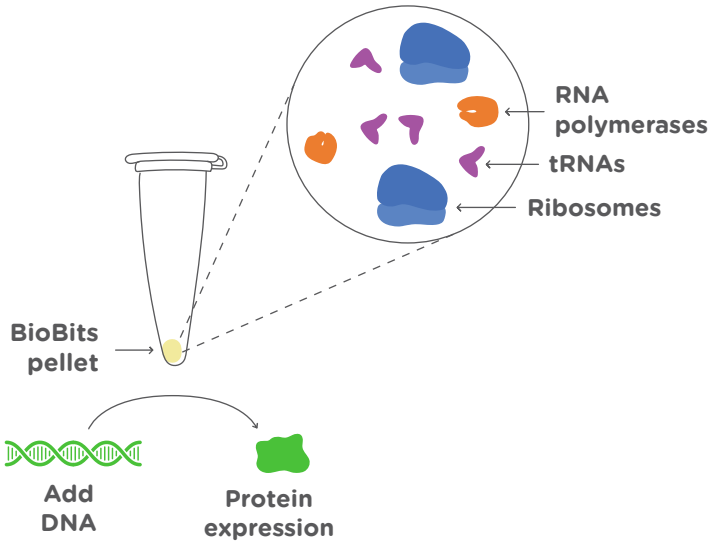
Expressing fluorescent proteins with cell-free technology

1

- In this activity, you will use cell-free protein expression technology called BioBits to produce proteins.
- **BioBits pellets** contain the enzymes and molecular machinery needed for protein expression.
- To trigger protein expression, you will add DNA to the BioBits pellets.

2

- You will use the BioBits pellets to express two proteins: (1) A tagged **green fluorescent protein (GFP)** that will serve as your protein of interest, and (2) an untagged **red fluorescent protein (RFP)**.
- You will use affinity purification to separate the tagged GFP from the untagged RFP and everything else in the reaction.
- Because you are using different colored fluorescent proteins, you will be able to track the purification process visually.



Check your understanding

Q3. Why are you expressing fluorescent proteins in this activity?

A. BioBits can only express fluorescent proteins.
B. Affinity purification only works for fluorescent proteins.
C. Fluorescent proteins are unstable.
D. Fluorescent proteins allow visual tracking of the purification process.

Your samples

1

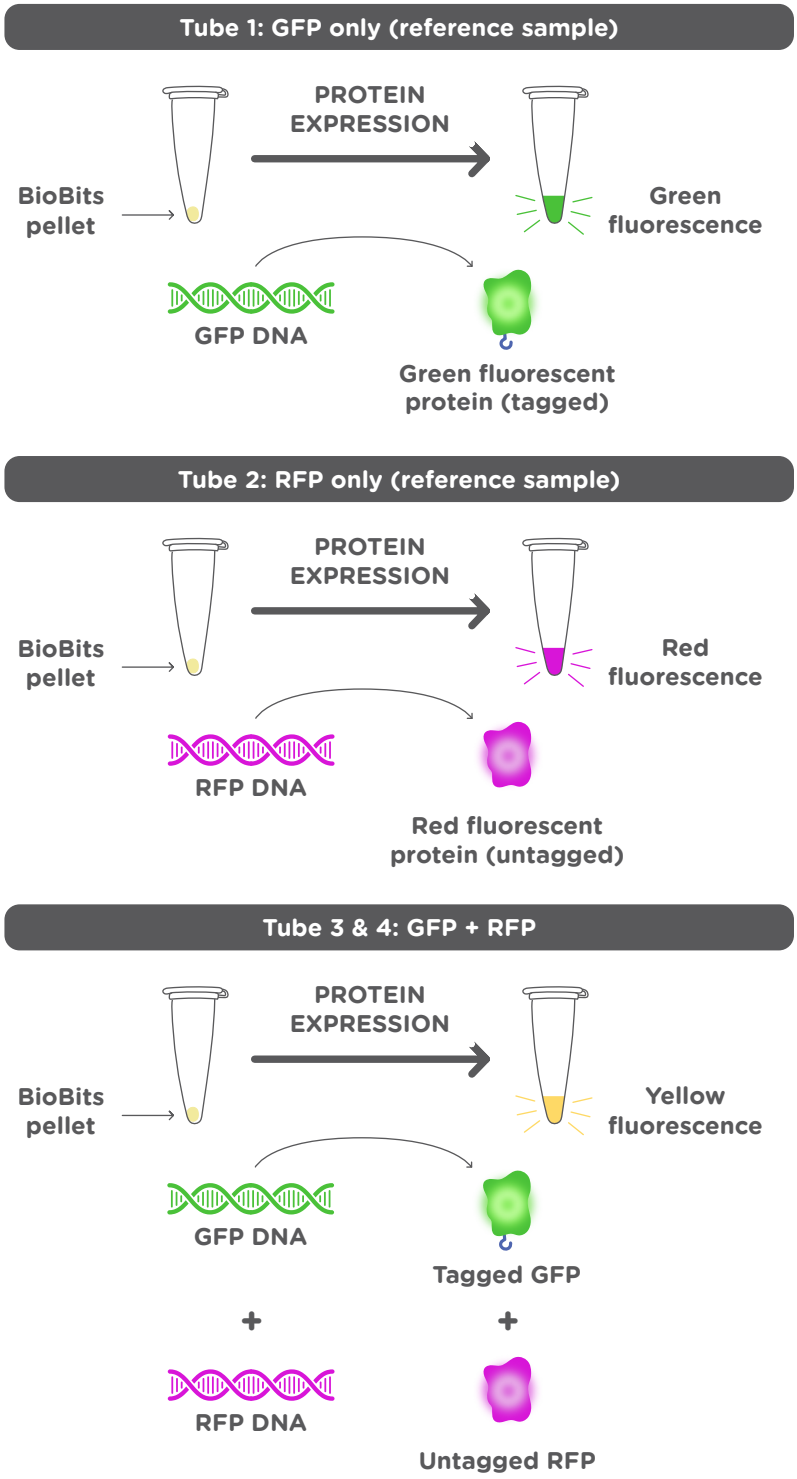
- You will add GFP DNA to the BioBits in tube 1.
- This will lead to the expression of green fluorescent protein.
- This tube will serve as a reference sample to demonstrate what GFP looks like on its own.

2

- You will add RFP DNA to the BioBits in tube 2.
- This will lead to the expression of red fluorescent protein.
- This tube will serve as a reference sample to demonstrate what RFP looks like on its own.

3

- You will add both GFP and RFP DNA to the BioBits in tubes 3 and 4.
- This will lead to the expression of both green and red fluorescent proteins.
- The combination of green and red fluorescence will appear yellow.

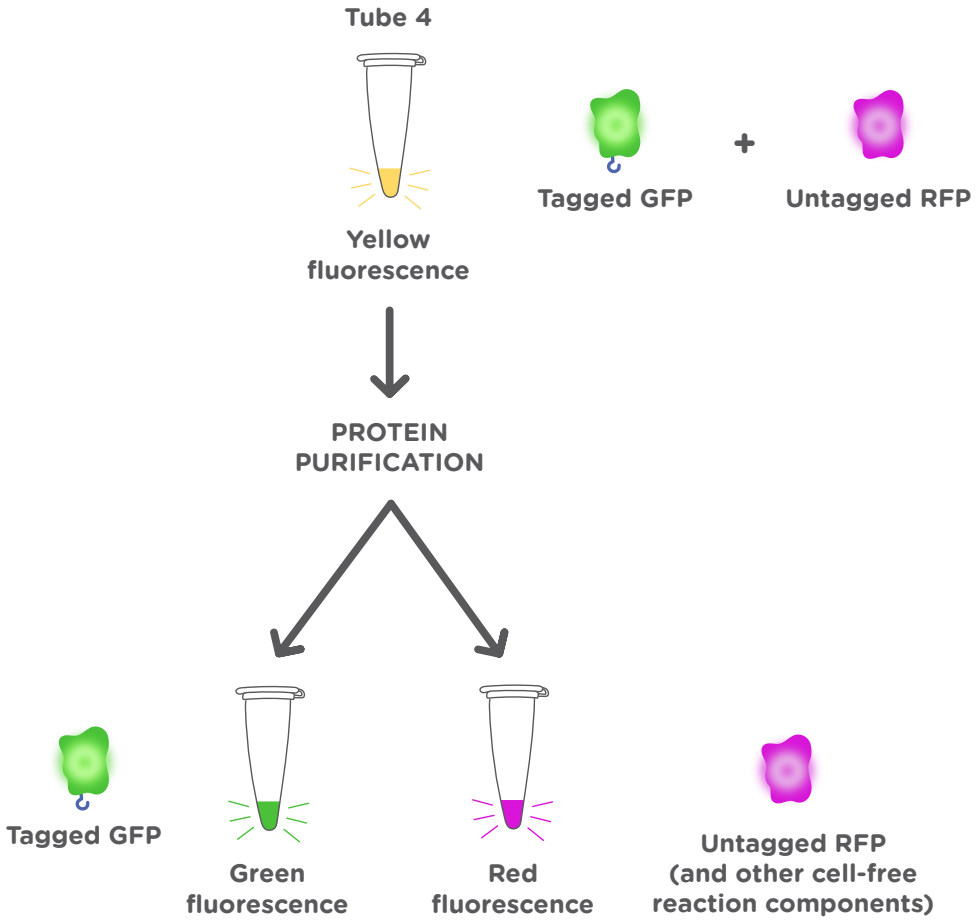


Color vision deficiency-friendly design: This lab guide uses magenta instead of red for the red fluorescent protein, making the diagrams more accessible for people with red-green color vision deficiency.

Purification goal

1

- You will purify the tagged GFP from the untagged RFP and the remaining cell-free components in tube 4.
- Because you are using fluorescent proteins, you can track the purification process visually.



2

- At the beginning of the purification, the mixture of GFP and RFP in tube 4 will appear yellow.
- At the end of the purification, you will have separated the GFP and RFP proteins from each other.

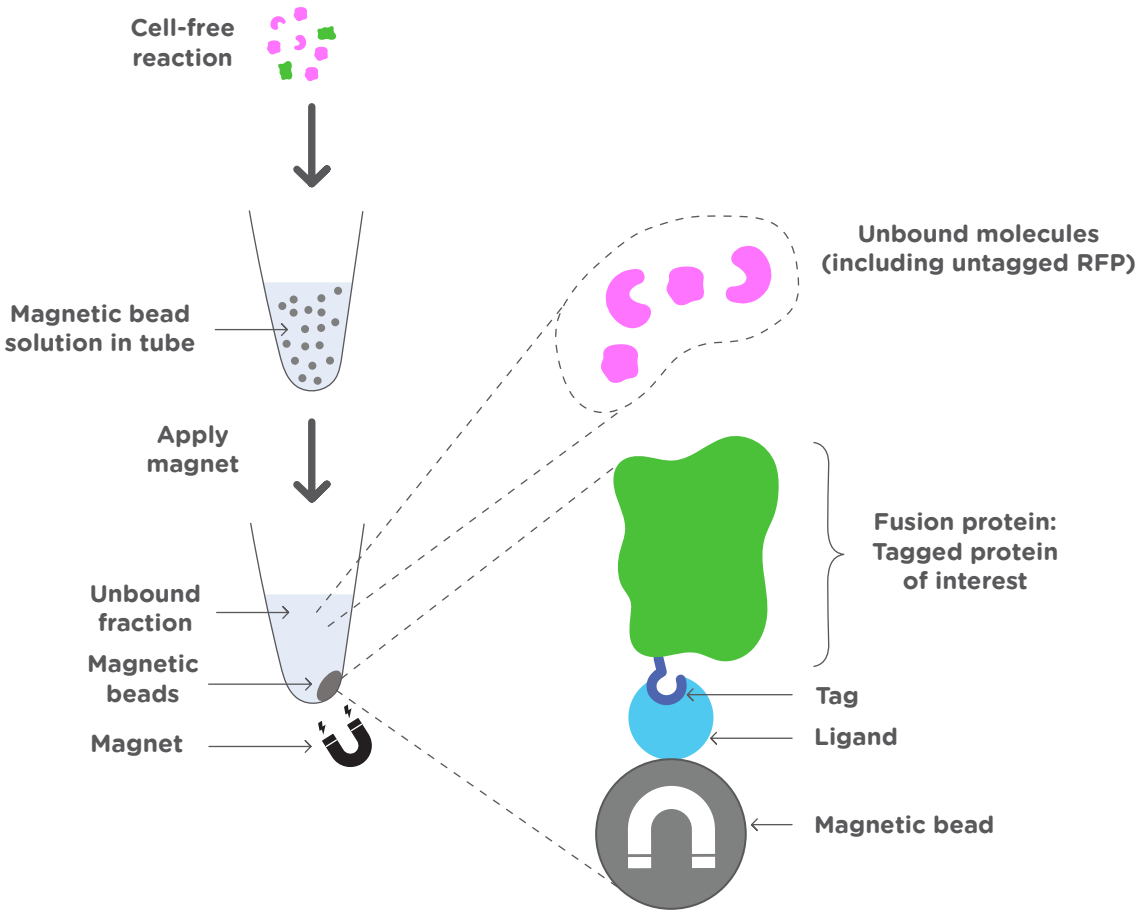
Affinity purification using magnetic beads

1

- You will use affinity purification to isolate the tagged GFP protein.
- The beads you will use are coated with the ligand that binds to the tag. The beads are also magnetic, and can be held in place by applying a strong magnet.

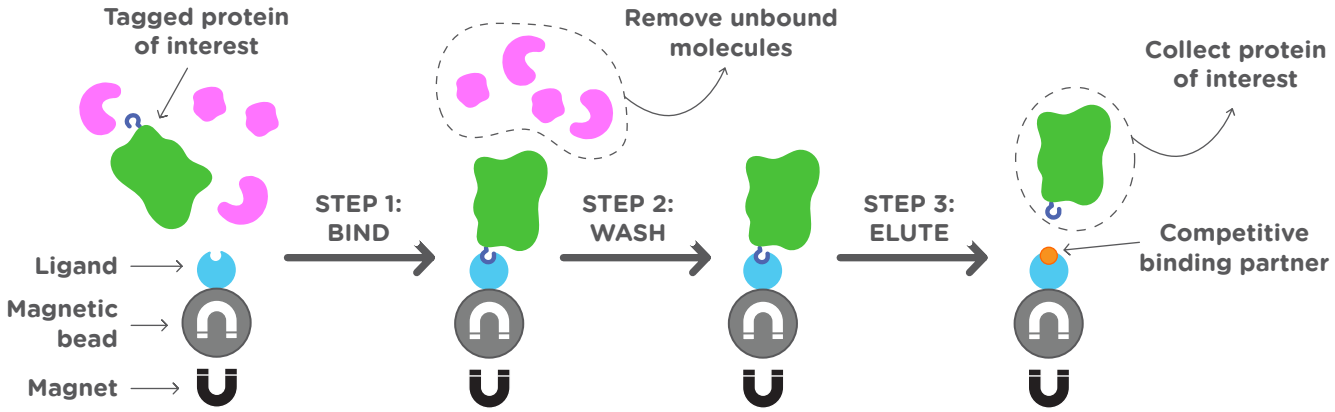
2

- The GFP used in this activity is tagged, so it will bind to the ligand coating the beads.
- Most other molecules will remain free-floating in the solution and can then be removed from the tube. In this activity, we will refer to this as the unbound **fraction**.
- In today's experiment, only the tagged GFP fusion protein will bind to the beads and stick to the magnet, allowing the removal of all other untagged molecules.



Purification workflow

To purify the tagged GFP protein, you will follow three basic steps. Many protein purification protocols follow these general steps:



1

Binding step

- You will add the protein expression mixture to the beads and allow the tag to bind to the ligand that coats the beads.
- Only proteins with the tag will bind the beads.

2

Wash step

- You will remove unbound molecules by pipetting off the unbound fraction.
- Then, you will add a wash buffer to help rinse off residual unbound molecules and pipette off the unbound fraction again.

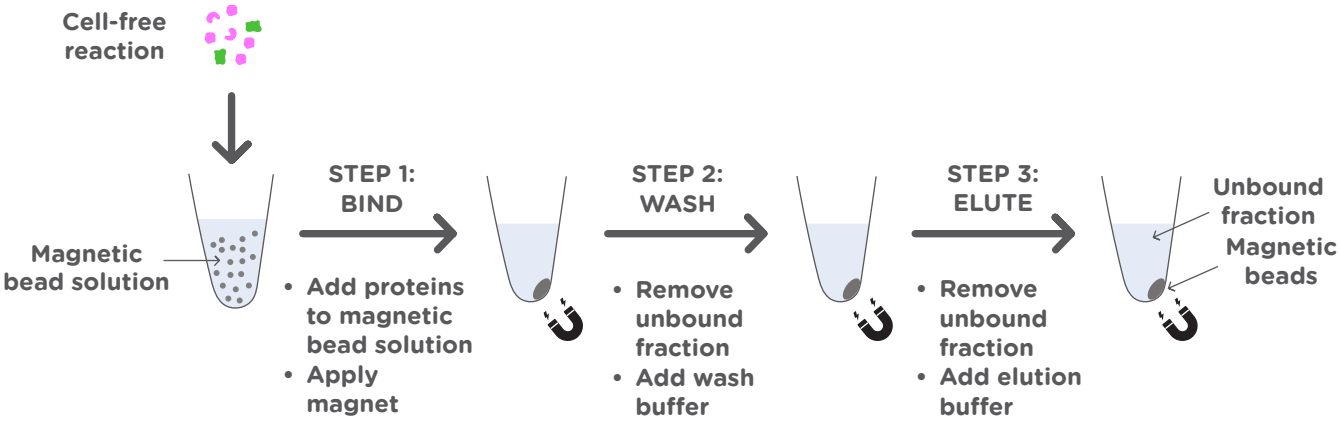
3

Elution step

- To release the tagged GFP fusion protein from the beads, you will add a competitive binding partner for the ligand.
- The competitive binding partner will bind the ligand and displace the tagged GFP fusion protein into the solution, which can then be collected.

Check your understanding

The diagram below outlines the three major steps of the purification protocol you will follow. Refer to the diagram to answer Q4-Q7. Remember that you will add a mixture of tagged GFP and untagged RFP from Tube 4 to the magnetic beads.



Q4. After step 1, the unbound fraction is expected to exhibit:

A. Green fluorescence
B. Red fluorescence
C. Yellow fluorescence
D. No fluorescence

Explain your reasoning:

Q5. After step 1, the magnetic beads are expected to exhibit:

A. Green fluorescence
B. Red fluorescence
C. Yellow fluorescence
D. No fluorescence

Explain your reasoning:

Q6. After step 3, the unbound fraction is expected to exhibit:

A. Green fluorescence
B. Red fluorescence
C. Yellow fluorescence
D. No fluorescence

Explain your reasoning:

Q7. After step 3, the magnetic beads are expected to exhibit:

A. Green fluorescence
B. Red fluorescence
C. Yellow fluorescence
D. No fluorescence

Explain your reasoning:

Glossary

Protein expression: The production of protein encoded by a gene. In biotechnology, scientists introduce DNA that codes for a specific protein into cells. These cells then produce the desired protein.

Protein of interest: The specific protein scientists are interested in studying or producing for applied use.

Cell-free protein expression technology: Protein production that occurs without living cells. The molecular machinery needed for protein synthesis is removed from living cells and used to make protein in a test tube. To learn more, visit https://dnadots.minipcr.com/dnadots/cell-free-technology.

Protein purification: The isolation of a protein of interest from a mixture. This activity focuses on a specific form of protein purification called affinity purification.

Affinity purification: A purification method that relies on the strong and specific interaction between a ligand and its binding partner. When a mixture of proteins is applied to the beads coated in the ligand, proteins that bind the ligand on the beads can be captured. The beads can be magnetic or packed into a column.

Ligand: A molecule that binds to a receptor or other biomolecule. In this activity, a ligand coats the surface of magnetic beads used for purification. Because the ligand binds the tag fused to the protein of interest, the beads capture the tagged protein of interest.

Tag: A short amino acid sequence added to a protein of interest to aid in purification. A tag is selected because it binds to a known ligand with high affinity. Scientists modify the DNA encoding the protein of interest to include DNA encoding the tag.

Fusion protein: A protein created by joining parts from two or more genes. When expressed, the protein will have the different parts fused together. In this activity, you will express a protein of interest with a tag fused to it to help with purification.

BioBits pellets: The specific cell-free protein expression technology used in this lab. BioBits pellets contain everything needed for transcription and translation in a test tube.

Green fluorescent protein (GFP): A fluorescent protein that emits green fluorescence after excitation with blue light. The GFP used in this activity was first derived from jellyfish and is tagged.

Red fluorescent protein (RFP): A fluorescent protein that emits red fluorescence after excitation with blue light. The RFP used in this activity was first derived from coral and is NOT tagged.

Fraction: During purification, a mixture is separated into parts, which are referred to as fractions. In this activity, molecules from the cell-free reaction can either be bound to the magnetic beads or unbound and floating in the solution.

Binding step: The first step in most protein purification protocols, where the protein of interest is captured. In this activity, a molecular mixture is added to the ligand-coated beads. The tag on the protein of interest binds with the ligand on the beads, eventually allowing it to be separated from everything else in the mixture.

Wash step: The second step in most protein purification protocols, where unwanted molecules are removed. In this activity, after allowing the protein of interest to bind to the beads, the unbound molecules are removed. Then, a wash buffer is added to remove residual unbound molecules. In some protein purification protocols, this step is repeated several times to increase purity.

Elution step: The last step in most protein purification protocols, where the target protein is released from the beads and collected. In this activity, a competitive binding partner that interacts with the ligand coating the beads is added. When the competitive binding partner binds the ligand coating the beads, the tagged protein of interest is released and can be collected.

Purity: A measure of the absence of contaminants in a sample. Purity is expressed as a percentage. For example, 95% purity means that 95% of the sample is the protein of interest, while 5% consists of other molecules.

Yield: A measure of how much protein of interest was obtained after purification. Yield is typically expressed in units of mass.

Biotech careers

Sarita Khanal Paudyal works at AbCellera on a team that expresses and purifies transmembrane proteins for use in the development of therapeutics. Learn more about Sarita and her work in the *Biotech career profile* on page 41.

Laboratory protocol

Protective gloves and eyewear should be worn for the entirety of this experiment.

Part 1: Set up BioBits reactions to express fluorescent proteins

1. Number your BioBits tubes 1-4.

2. Gently tap tubes on the table to collect pellets at the bottom.

3. Carefully remove the strip of lids, one cap at a time. If you pull the lids off with too much force, the BioBits pellets might fly out of the tube.

How to pipette when working with BioBits

- Touch your pipette tip to the side of the tube and dispense the liquid, then tap the tube on the tabletop to collect the liquid in the bottom of the tube.
- Do not touch your pipette tip to the BioBits pellet.
- Do not pipette up and down to mix.

4. Using a fresh pipette tip for each addition, add DNA to each BioBits pellet according to the table below:

	Tube 1	Tube 2	Tube 3	Tube 4
GFP DNA	5 µl GFP DNA	-	2.5 µl GFP DNA	2.5 µl GFP DNA
RFP DNA	-	5 µl RFP DNA	2.5 µl RFP DNA	2.5 µl RFP DNA

5. Close the caps on the tubes. When closed correctly, you should feel the caps “click” into place.

6. Make sure all the liquid and the BioBits pellets are at the bottom of the tube. If needed, spin the tube briefly in a microcentrifuge or shake the contents of the tube to the bottom with a flick of the wrist.

7. These are your BioBits reactions. Store the tubes at room temperature overnight. Avoid storing the tubes in direct sunlight (ambient indoor light is fine).

8. After the initial overnight incubation at room temperature, you can store the fluorescent proteins in the refrigerator for up to one week before proceeding to the purification.

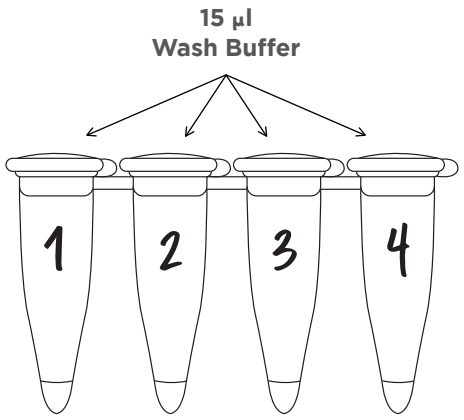
Part 2: Protein purification

1. The next day, observe your BioBits reactions in the P51 Viewer with the orange filter in place. Turn on the P51's blue light and observe the samples through the viewing window. Dimming the lights in the room can make it easier to observe the fluorescence.

2. Record the color of each reaction in Table 1 below:

Table 1: Protein expression observations				
	Tube 1	Tube 2	Tube 3	Tube 4
DNA added	GFP	RFP	GFP + RFP	GFP + RFP
Color of fluorescence (red/green/yellow)				

3. Using a fresh pipette tip for each sample, add 15 µl of Wash Buffer to each tube.

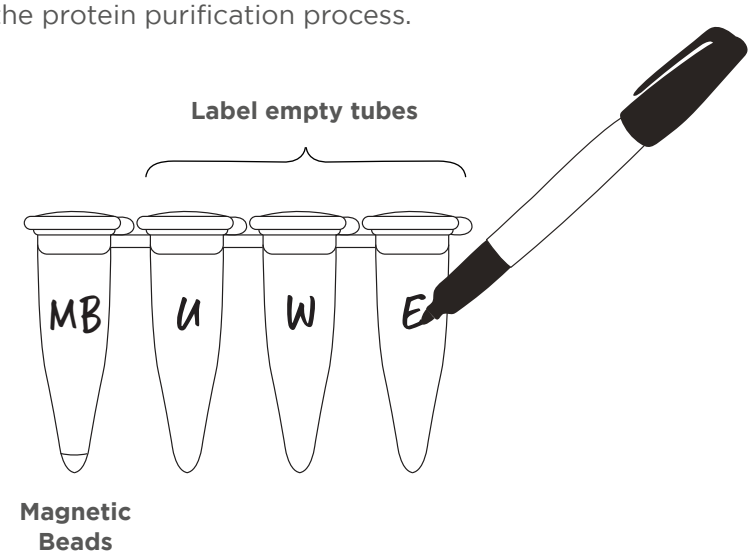


4. Set the BioBits reactions aside.

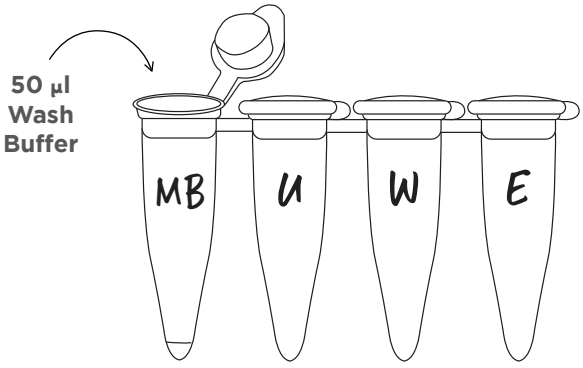
Prepare the magnetic beads

- You will receive a different strip of four tubes from your teacher.
- The first tube contains the magnetic beads, while the other three are empty.

5. Label the empty tubes U, W, and E. You will use these tubes to collect samples during different stages of the protein purification process.



6. Add 50 µl of Wash Buffer to tube MB that contains the magnetic beads.



7. Thoroughly mix the reagents in tube MB by vortexing for 5 seconds or gently pipetting up and down five times using only the first stop.
   - If using a vortexer, remember to close the tube lid before mixing.
   - If using a micropipette, remember to expel as much liquid as possible from the tip after mixing.

8. Cap tube MB and make sure all the liquid is collected at the bottom.

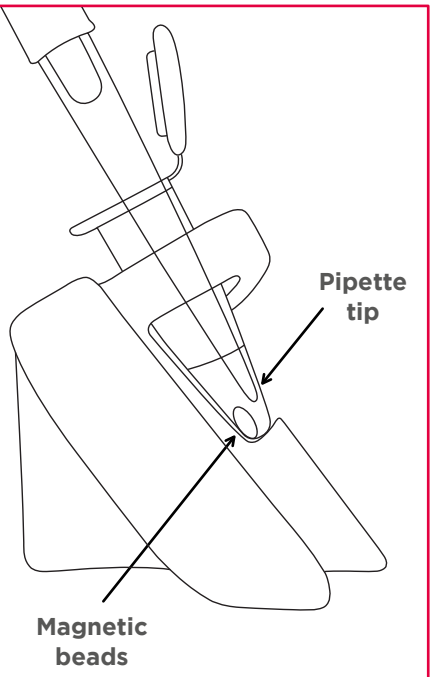
9. Place the strip of tubes in the magnetic rack and let the beads separate for 1 minute.

Tips on removing liquid from the magnetic beads

A. Note where the beads have collected:
   - The magnetic beads will collect on the side of the tube closest to the magnetic rack.
   - The beads may appear as a smear rather than a distinct pellet.
   - Keeping the magnetic rack at eye level while pipetting can make it easier to see the beads.

B. Remove the unbound fraction:
   - Place your pipette tip on the side of the tube opposite the magnetic rack in order to avoid disturbing the beads.
   - Release the plunger on your pipette slowly and remove as much liquid as possible without disturbing the beads. It is fine to leave a small amount of liquid.

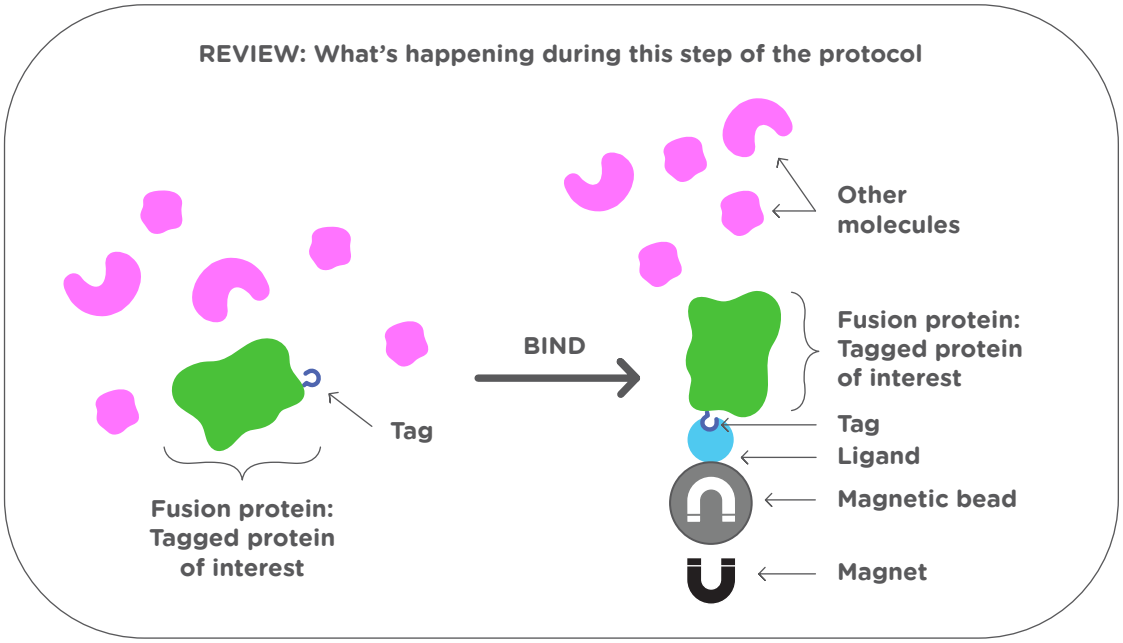
C. If you accidentally disturb the beads:
   - Pipette the solution back into the tube.
   - Leave the tube in the magnetic rack for 1 minute.
   - Try to remove the liquid again.



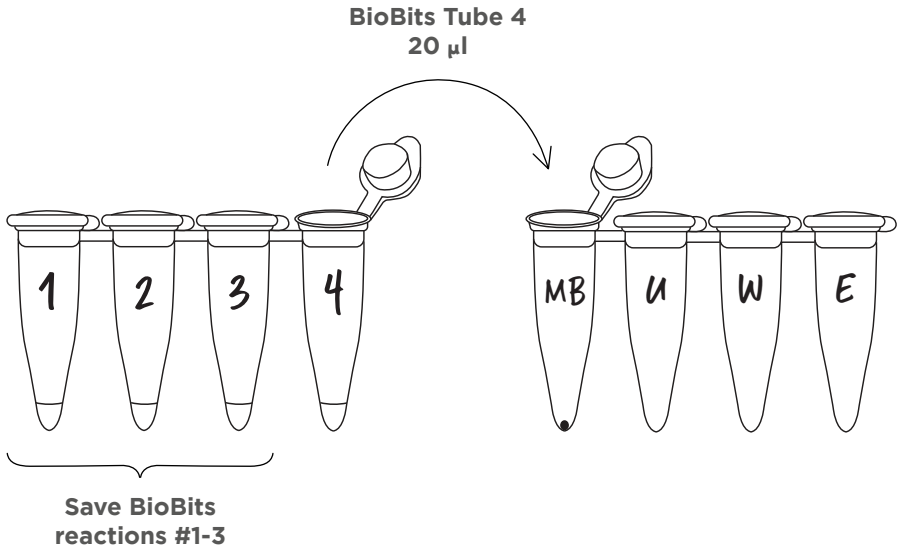
Video instructions
http://links.minipcr.com/PullitGuide

10. With the strip of tubes in the magnetic rack, carefully pipette the liquid off the magnetic beads in tube MB. The beads may be difficult to see during this first step, but they will be on the wall of the tube closest to the magnetic rack. You should be able to remove ~ 55 µl of Wash Buffer, which can be discarded.

Bind protein of interest to the magnetic beads



11. Transfer all 20 µl of your tube 4 BioBits reaction to the magnetic beads in tube MB. Store the remaining BioBits reactions in the refrigerator. You will use these tubes as reference samples when interpreting your purification results.



12. Thoroughly mix the reagents in tube MB by vortexing for 5 seconds or gently pipetting up and down five times.

13. Cap tube MB and make sure the liquid is collected at the bottom.

14. Place the strip of tubes in a non-magnetic tube rack or leave them lying flat.

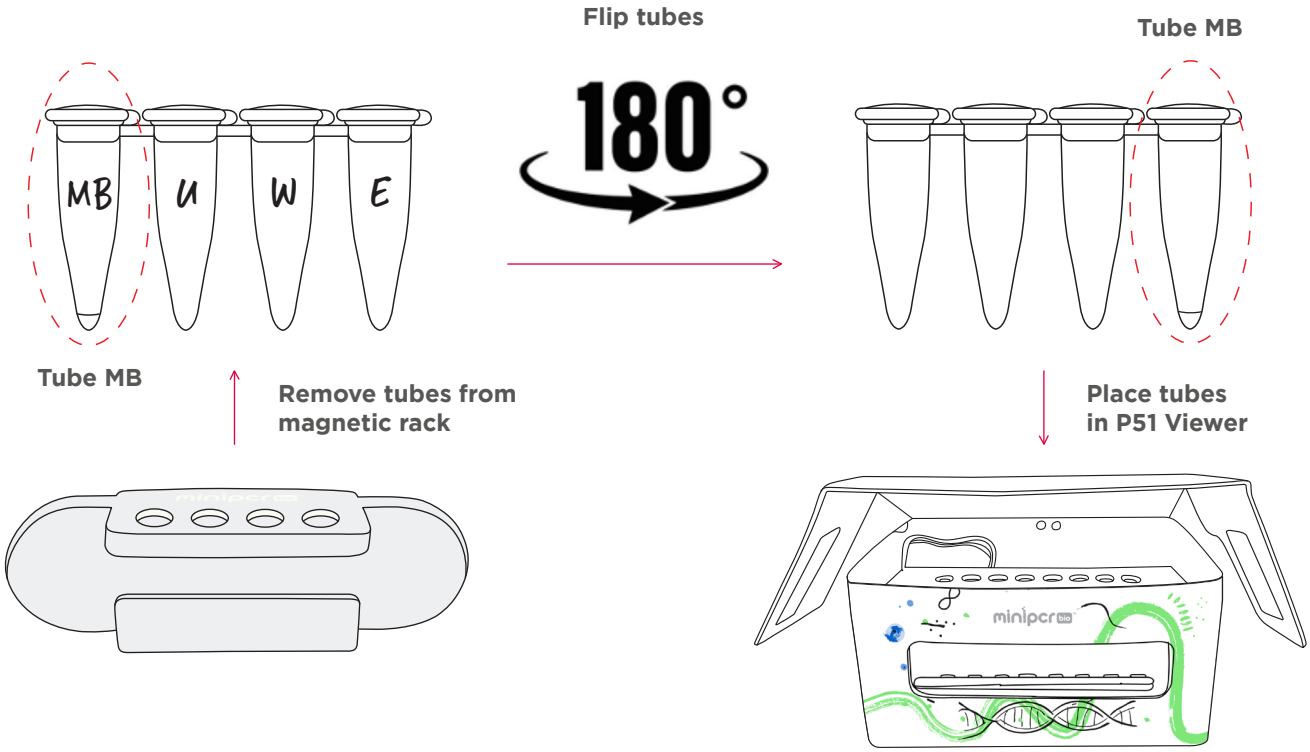
15. Incubate at room temperature for at least 30 minutes. Alternatively, incubate overnight in the refrigerator. Overnight incubation increases the purification yield.

    Optional stopping point: You can leave the strip of tubes in the refrigerator for up to one week before proceeding to step 16.

16. Place the strip of tubes in the magnetic rack and let the beads separate for 1 minute.

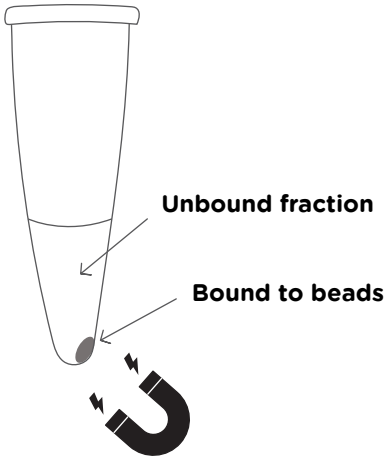
17. Turn on the P51's blue light.

18. Quickly transfer the strip of tubes from the magnetic rack to the P51 Viewer, rotating the tubes 180° so the backs of the tubes where the beads accumulated are now facing the front (see diagram below).
    - Rotating the tubes in this way will make it easier to observe the beads.
    - If the beads dislodge, transfer the strip of tubes back to the magnetic rack so that the beads can collect again along the back wall before moving the tubes back to the P51 Viewer.

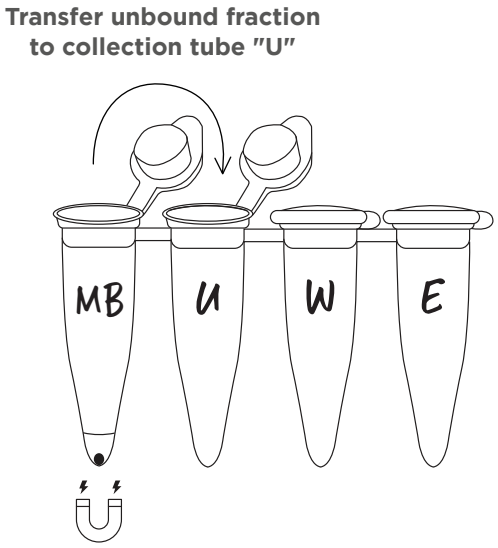


19. With the blue light on, observe tube MB through the viewing window. Record your observations in Table 2 below:

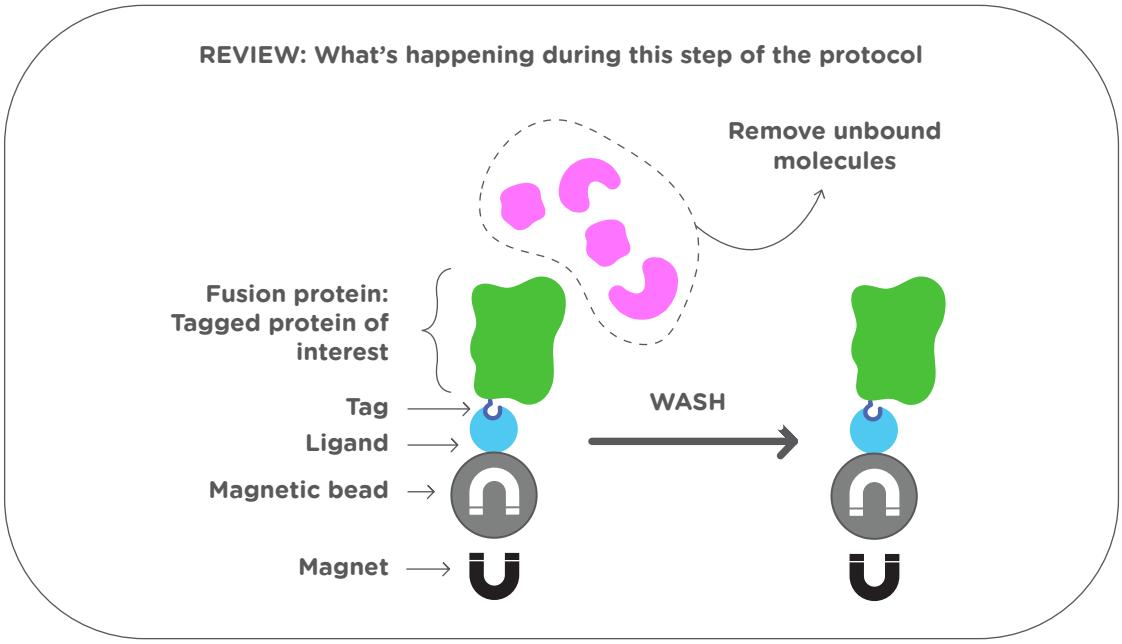
Table 2: Initial separation during purification		
	Unbound fraction	Bound to beads
Color of fluorescence (red/green/yellow)		



20. Place the strip of tubes back in the magnetic rack and let the beads separate for 1 minute. With the strip of tubes in the magnetic rack, insert your pipette tip in tube MB and carefully pipette the liquid off the magnetic beads. You should be able to remove approximately 20 µl. Transfer this volume to collection tube U.

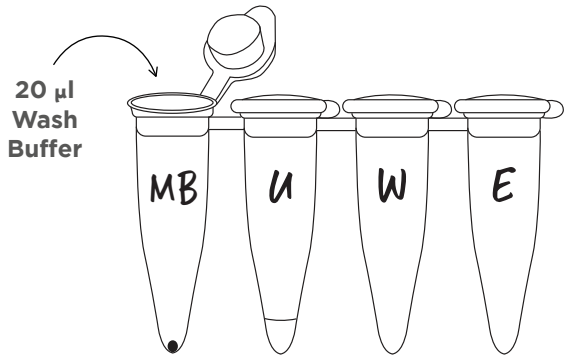


Wash the magnetic beads



21. Remove the strip of tubes from the magnetic rack.

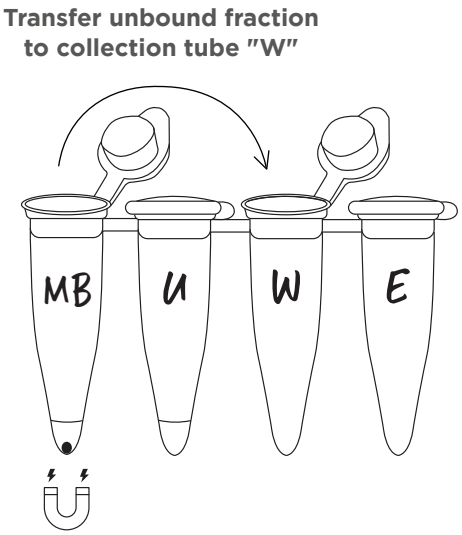
22. Add 20 µl of Wash Buffer to the magnetic beads in tube MB. There is no need to mix.



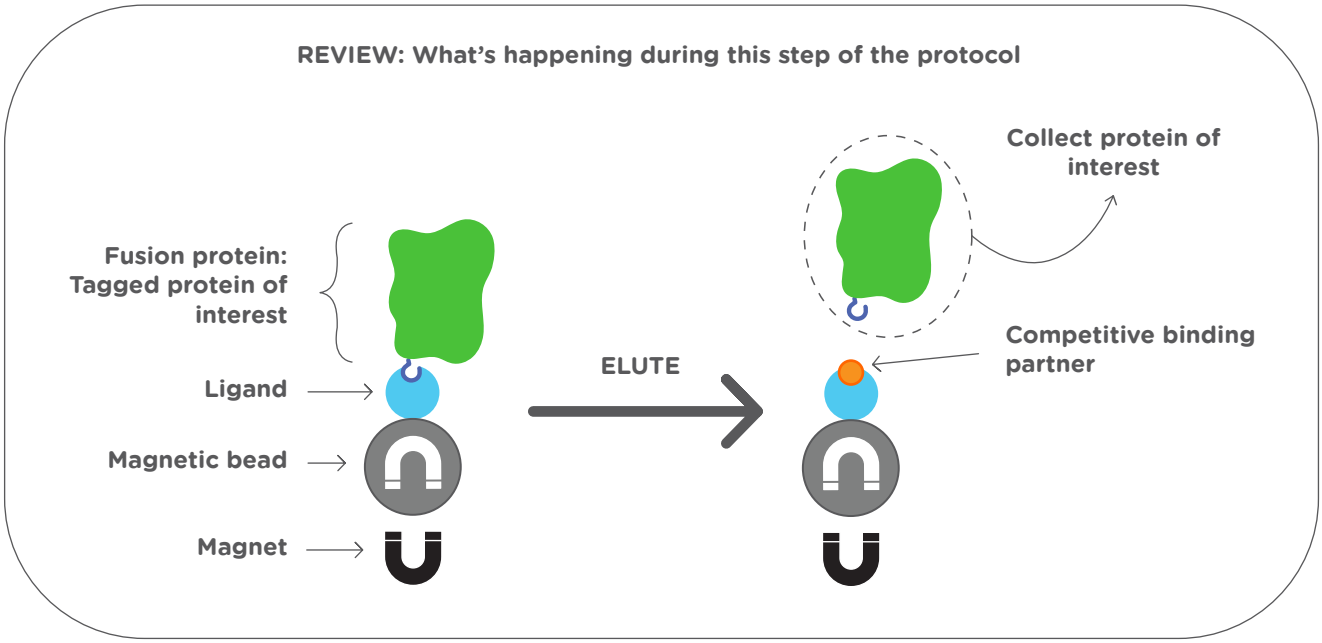
23. Cap the tube and make sure all the liquid is at the bottom.

24. Place the strip of tubes in the magnetic rack and let the beads separate for 1 minute.

25. With the strip of tubes in the magnetic rack, insert your pipette tip in tube MB and carefully pipette the liquid off the magnetic beads. You should be able to remove approximately 20 µl. Transfer this volume to collection tube W (“W” for wash).

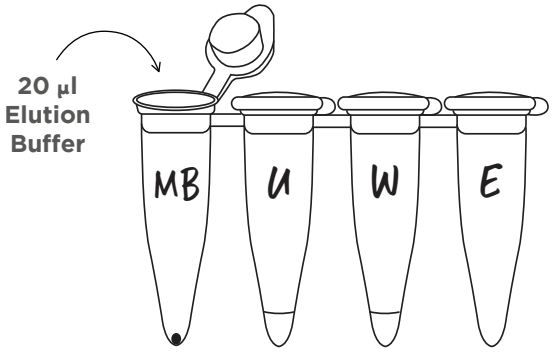


Elute the protein of interest



26. Remove the strip of tubes from the magnetic rack.

27. Add 20 µl of Elution Buffer to the magnetic beads in tube MB.



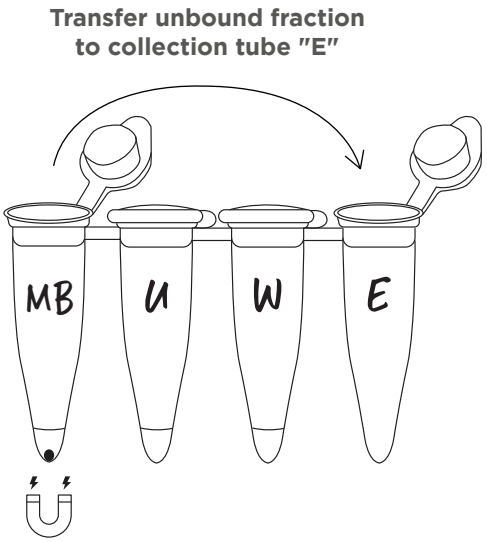
28. Thoroughly mix the reagents in tube MB by vortexing for 5 seconds or gently pipetting up and down five times.

29. Cap tube MB and make sure all the liquid is at the bottom of the tube.

30. Place the tubes in a non-magnetic tube rack or leave them lying flat. Incubate at room temperature for 5 minutes.

31. Place the strip of tubes in the magnetic rack and let the beads separate for 1 minute.

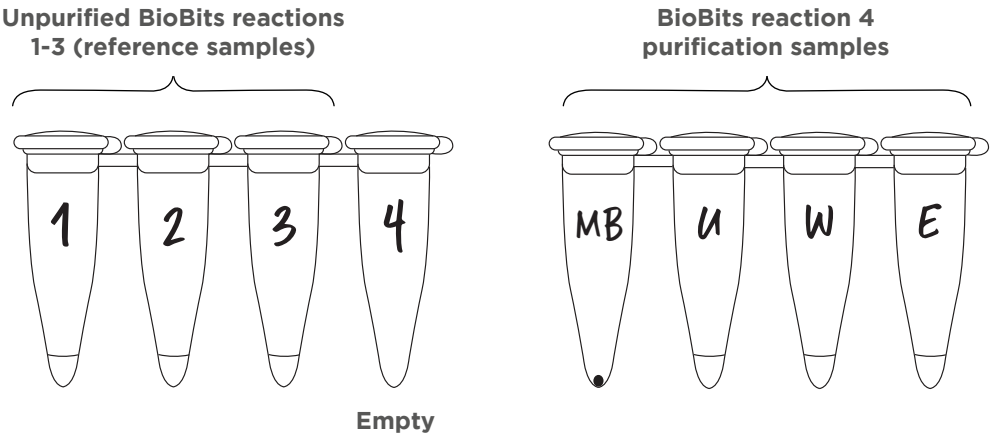
32. With the strip of tubes in the magnetic rack, insert your pipette tip in tube MB and carefully pipette the liquid off the magnetic beads. You should be able to remove approximately 20 µl. Transfer this volume to collection tube E ("E" for eluate).



33. Leave the beads in the tube MB for observation.

Final observations

34. Place both the unpurified BioBits reactions and your purification samples in the P51 Viewer with the orange filter in place. Turn on the P51's blue light and observe the samples through the viewing window. Dimming the lights in the room can make it easier to observe the fluorescence.



35. Recall that BioBits reactions 3 and 4 both contained a mix of GFP and RFP. Compare the unpurified BioBits reaction 3 to your purification fractions in the table below.

Table 3: Final protein purification observations

	Tube 3: GFP + RFP	Tube 4: GFP + RFP purification fractions			
	Unpurified reference	Beads Tube MB	Collection Tube U	Collection Tube W	Collection Tube E
Color of fluorescence (red/green/yellow)					

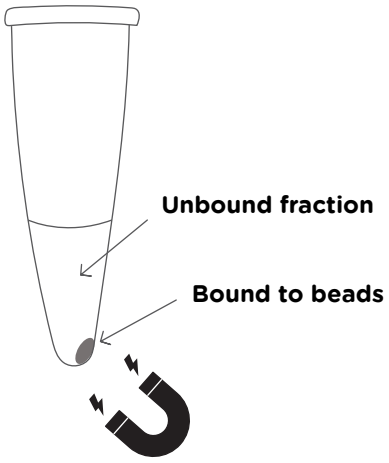
Post-lab analysis

Interpreting results

You started with a mix of tagged GFP and untagged RFP, then followed a protocol to purify the GFP.

1. Go back to Table 2 on page 29, where you observed the unbound vs. bound fractions when the magnet was first applied. Record your observations again in the table below.

Table 2: Initial separation during purification		
	Unbound fraction	Bound to beads
Color of fluorescence (red/green/yellow)		

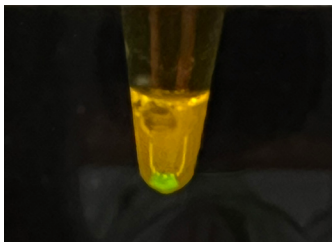


2. Which statement explains your observation?
   A. The GFP carries a tag because it is the protein of interest. This tag binds to the ligand coating the magnetic beads.
   B. The RFP carries a tag because it is the protein of interest. This tag binds to the ligand coating the magnetic beads.
   C. The GFP carries a tag because it is not the protein of interest. This tag binds to the ligand coating the magnetic beads.
   D. The RFP carries a tag because it is not the protein of interest. This tag binds to the ligand coating the magnetic beads.

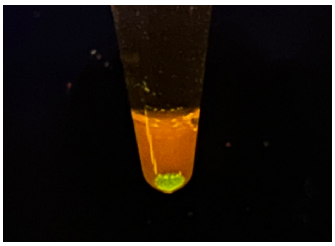
Critical thinking

3. The photo below compares the initial separation of the GFP and RFP after allowing the tagged GFP to bind to the beads for different amounts of time. The image on the left was taken after incubating the protein mixture with the beads for only 30 minutes, while the results on the right were obtained after an overnight incubation. Notice that after a 30-minute incubation, the unbound fraction is more orange, whereas after an overnight incubation, it is more red. What could explain this observation?

30 min



Overnight



4. Imagine your experimental goal changed so your protein of interest was now RFP. How would you change your experimental design to address this?

5. **Purity** is a measure of the absence of contaminants in a sample. **Yield** is a measure of the amount of your desired product. Adding any purification steps will increase the purity of your desired product but decrease the yield. Imagine you work on a team that produces protein-based medicines for human use. Would you prioritize purity or yield? Explain your reasoning.

Quantitative skills

6. Imagine that you work in a biotechnology lab that performs larger-scale protein expression using bacterial cultures. You have expressed and purified a different protein, Enzyme A.
   A. At the end of the protein purification protocol, you have 80 ml of solution. You determine the total protein concentration in the solution is 0.52 mg/ml. Use this information to calculate the total amount of protein in milligrams. Show your work.

   B. Your labmate performed further analysis to determine the concentration of Enzyme A in your 80 ml solution, but they reported the results to you as 312 µg/ml. Use this information to calculate the amount of Enzyme A in milligrams. Note: There are 1,000 micrograms in 1 milligram. Show your work.

   C. Use the following formula to calculate the purity of your sample. Show your work.

$$\text{Purity} = \frac{\text{target protein}}{\text{total protein}}$$

CER table

Fill in the table based on your results from the lab. Use the rubric on the next page to help your answers.

Question:

Which protein, GFP or RFP, bound to the ligand on the beads?

Claim

Make a clear statement that answers the above question.

Evidence

Provide data from the lab that supports your claim.

Reasoning

Explain clearly why the data you presented supports your claim. Include the underlying scientific principles that link your evidence to your claim.

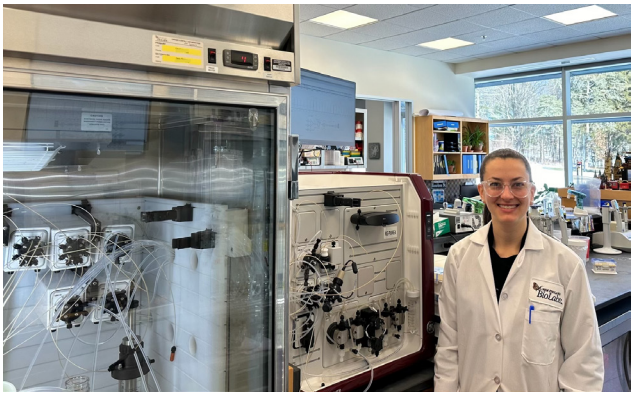
Score	4	3	2	1
CLAIM A statement that answers the original question/problem.	Makes a clear, accurate, and complete claim.	Makes an accurate and complete claim.	Makes an accurate but incomplete or vague claim.	Makes a claim that is inaccurate.
EVIDENCE Data from the experiment that supports the claim. Data must be relevant and sufficient to support the claim.	All of the evidence presented is hightly relevant and clearly sufficient to support the claim.	Provides evidence that is relevant and sufficient to support the claim.	Provides relevant but insufficient evidence to support the claim. May include some non-relevant evidence.	Only provides evidence that does not support claim.
REASONING Explain why your evidence supports your claim. This must include scientific principles/ knowledge that you have about the topic to show why the data counts as evidence.	Provides reasoning that clearly links the evidence to the claim. Relevant scientific principles are well integrated in the reasoning.	Provides reasoning that links the evidence to the claim. Relevant scientific principles are discussed.	Provides reasoning that links the evidence to the claim, but does not include relevant scientific principles or uses them incorrectly.	Provides reasoning that does not link the evidence to the claim. Does not include relevant scientific principles or uses them incorrectly.

We recommend that teachers use the following scale when assessing this assignment using the rubric. Teachers should feel free to adjust this scale to their expectations.

Rubric score	3	4	5	6	7	8	9	10	11	12
Equivalent	55	60	65	70	75	80	85	90	95	100

Biotech career profile

Daria McCallum, New England Biolabs



New England Biolabs is a world leader in the discovery and production of enzymes for molecular biology applications. Daria has played several roles at New England Biolabs, including more than six years working on protein production with a focus on purifying restriction endonucleases.

What education or training did you complete to pursue your career?
I earned a Bachelor of Science degree in Biology from Pennsylvania State University and completed several internships at New England Biolabs as a high school student and while I was in college. I can't overstate how pivotal internships have been in my professional and career development. My top piece of advice for students interested in a biotech career is to get an internship in a lab. No class I took (labs included) remotely compared to the learning experience of my internships.

What does your job involve?
My team determines the ideal conditions for the purification of individual proteins, studies enzyme biochemistry, helps meet specific customer requirements, and troubleshoots product issues.

What do you love about your job?
I love that every day is different. No two proteins I work on follow the same path. This motivates me to be engaged and excited about work, and I am constantly learning, changing my ways of thinking, and challenging myself.

Is there other advice you have for biotech students?
Don't be afraid to make mistakes. We're all human! I've made every mistake in the book. To name just a few, I've messed up basic dilution calculations, knocked over bottles of purified enzyme, and set up reactions with missing components. Mistakes are valuable learning experiences! Ask your supervisor, mentor, or colleagues questions to learn from your mistakes. Can you help me determine where my error occurred? Can I shadow you next time you go through this procedure? Do you have any tips or tricks that help you with this protocol?

Biotech career profile

Sarita Khanal Paudyal, AbCellera

AbCellera develops antibody-based medicines. Sarita currently works on a team that expresses and purifies transmembrane proteins for use in the development of therapeutics.

Has your career path taken any unexpected turns?
I have a degree in business from my home country, Nepal, but I moved to South Korea with my family and lived there for several years. I learned Korean and worked as a language teacher and translator. Eventually, my family moved to a small island off the coast of South Korea. I got a job as a research technician at the Citrus Research Institute. This was my introduction to working in a lab environment.

What education or training did you complete to pursue your biotech career?
When my family moved to the United States, I wanted to get into the biotech industry because of all the job opportunities. I earned a certificate in Biotechnology and Compliance from Quincy College in Massachusetts and have been working in the biotech industry in the Boston area ever since.

What does your current job involve?
My main job responsibility is to purify proteins! I work for a company with expertise in the expression and purification of transmembrane proteins. Transmembrane proteins, like ion channels and G protein-coupled receptors, are involved in many diseases. Being able to express and purify these proteins is essential to developing antibody-based therapies for diseases that involve these proteins.

What advice would you give to someone interested in pursuing a career in biotech?
I was scared to change my career path to a different field, but I am so glad I did. There are lots of great jobs in biotech, and working in this field is exciting. It doesn't matter what you studied before or what type of job you have now—there are many ways to receive training in the biotech field.

Strep-tag® technology

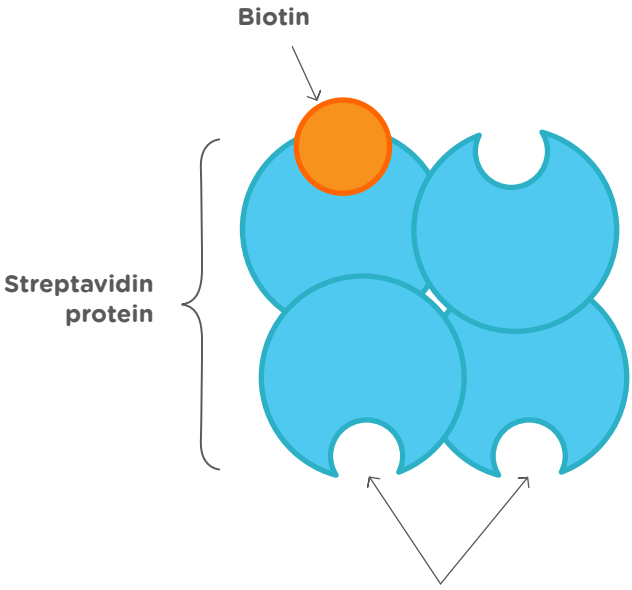
In this activity, you used affinity purification to isolate a tagged GFP fusion protein. This resource provides more information on the tag you used and its ligand.

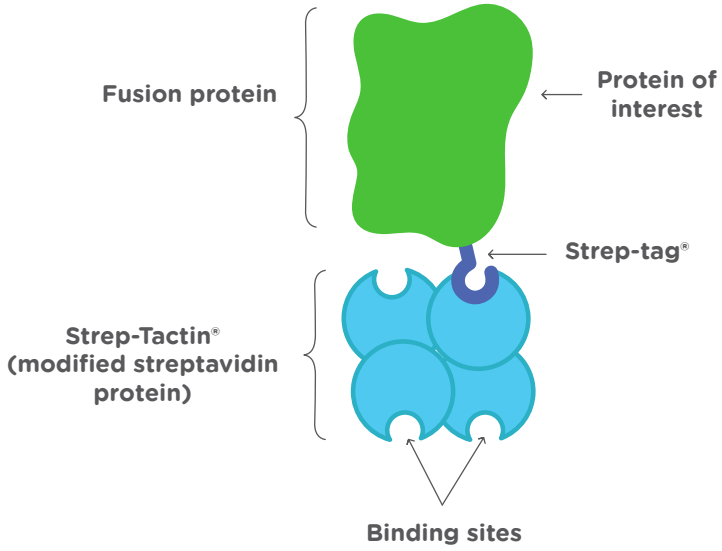
Streptavidin is a protein made of four identical subunits that has an extremely high binding affinity for a vitamin called biotin. Scientists realized that the high binding affinity between these naturally occurring molecules could be harnessed for biotech applications.

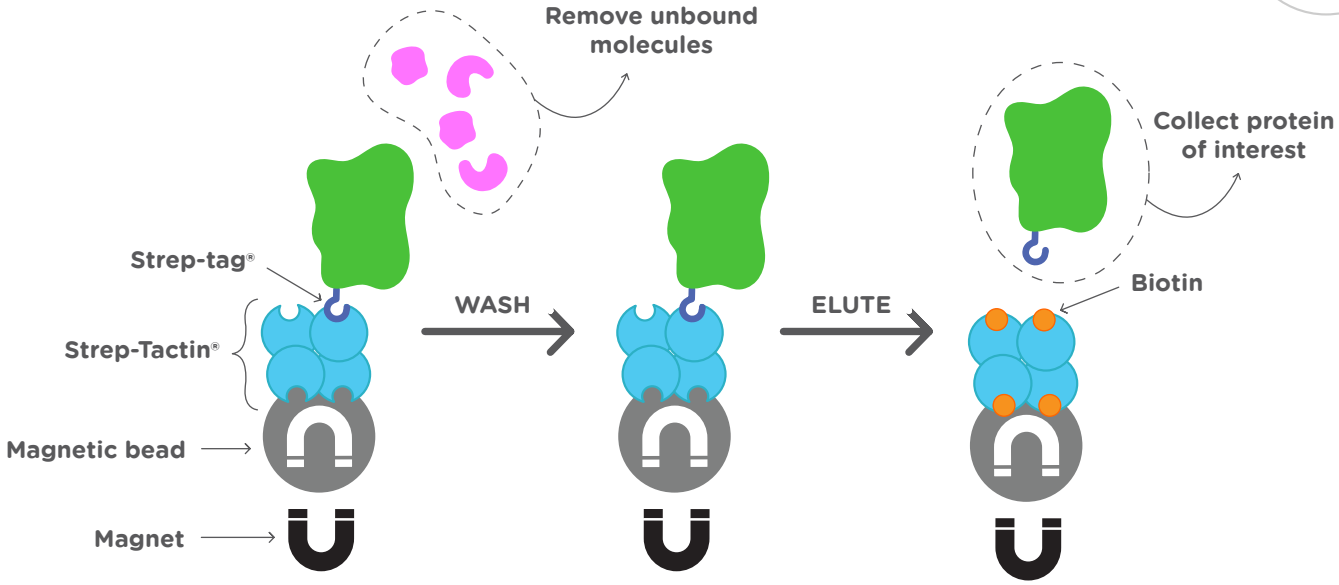
Scientists designed an eight-amino acid sequence that binds specifically to the biotin-binding domain of the streptavidin protein. This short sequence interacts with streptavidin through the binding sites that biotin normally occupies. The team that designed this sequence named it the Strep-tag®.

Next, scientists also modified the streptavidin protein to have an even higher binding affinity for the Strep-tag®. In this activity, you are using a version of the streptavidin protein created by IBA Lifesciences called Strep-Tactin®XT.

You can use the interaction between the Strep-tag® and the Strep-Tactin®XT protein to capture or detect molecules of interest. Strep-Tactin®XT can be used to selectively capture fusion proteins with a Strep-tag® from a complex mixture of other proteins and molecules.







Fusion proteins with a Strep-tag® can be released from Strep-Tactin®XT by adding a competitive binding partner. In this activity, the elution buffer contained biotin, the natural ligand for the streptavidin protein. Because biotin will also bind to the Strep-Tactin®XT binding site, the tagged fusion proteins will be released and can be collected.

Strep-tag® technology is powerful because it can be used in many different biotech assays and with most proteins of interest. In this activity, you used magnetic beads coated with Strep-Tactin®XT to purify tagged GFP. But Strep-Tactin®XT can also be attached to a resin for column purification or a solid surface like a microplate.