Biotech Skills Lab

Protein Expression and Purification

miniPCR Biotech Skills Lab™:
Protein Expression and Purification

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Contents

At a glance

miniPCR Biotech Skills Labs™ help students envision their future in the biotechnology industry. Students will gain hands-on experience with essential biotech methods, hone skills like laboratory math, and explore new career paths.

Overview

In this activity, students express a mixture of fluorescent proteins and then use affinity purification to isolate a target protein. Using the BioBits® cell-free protein expression technology and magnetic bead-based purification makes small-scale protein expression and purification classroom-friendly.

TECHNIQUES USED

Micropipetting
Cell-free protein synthesis
Affinity purification
Fluorescence visualization

BIOTECH APPLICATIONS

Protein expression
Protein purification

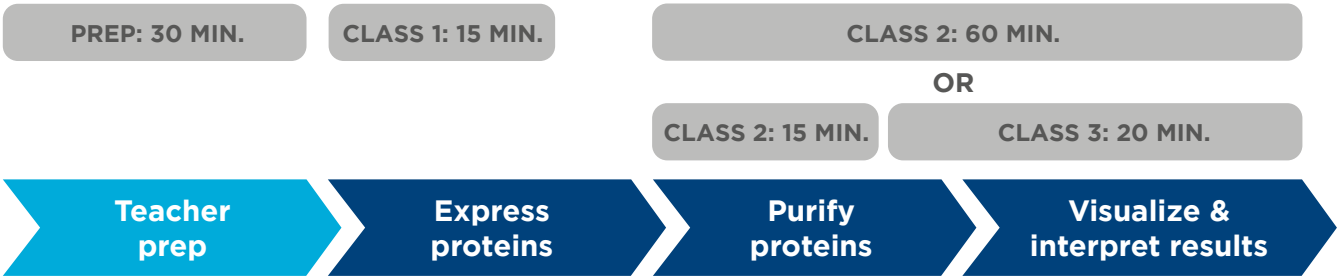
QUANTITATIVE SKILLS

Unit conversions
Yield calculations
Purity calculations

Required lab skills

- Students must be proficient in accurately pipetting liquids in the 5-20 µl range and using a micropipette to mix reagents.
- Instructional videos, worksheets, and free activities to help students build micropipetting skills can be found at https://www.minipcr.com/micropipetting/.

Planning your time



Refer to page 7 for details on teacher prep

This protocol offers flexibility in incubation times, allowing completion of the lab in two or three class periods. See the next page for details.

Technical support

If you have any questions about implementing this activity, contact support@minipcr.com.

Class time requirements

This protocol offers some flexibility in incubation times to help you manage the amount of class time needed.

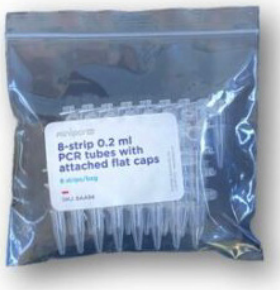
		Protocol steps	Time required
PROTEIN EXPRESSION	1	Add plasmid DNA to BioBits pellets	10 minutes
	2	Incubate at room temperature	Overnight
	STOP	Optional stopping point: After the initial overnight incubation at room temperature, you can store the fluorescent proteins in the refrigerator for up to one week before proceeding to the purification.	
PROTEIN PURIFICATION	3	Prepare magnetic beads	5 minutes
	4	Add protein to magnetic beads	5 minutes
	5	Bind protein of interest to magnetic beads	Minimum of 30 minutes at room temperature. Best results from overnight incubation in the refrigerator.
	STOP	Optional stopping point: After adding the protein to the beads, you can store your sample in the refrigerator for up to one week before completing the purification.	
	6	Wash magnetic beads	5 minutes
	7	Elute protein of interest	10 minutes
	8	Final observations	5 minutes

Materials needed

Supplied in kit (KT-1300-01)

- Materials are sufficient for eight lab groups to perform two purifications each.
- The kit contains two numbered bags that require different storage conditions, in addition to a bag of strip tubes that are necessary for the lab. Refer to the table below for details.
- Reagents must be used within six months of shipment.

	Provided	Required per group	Storage
Bag 1 contents (store in freezer)			
BioBits® pellets in PCR strip tubes	Four 8-tube strips to be split into strips of 4 tubes	1 strip of 4 tubes	Freezer (Keep BioBits pellets in the sealed pouch as long as possible)
GFP DNA	150 µl	15 µl	Freezer
RFP DNA	150 µl	15 µl	Freezer
Bag 2 contents (store in refrigerator)			
MagStrep XT Beads	100 µl	5 µl per purification	Refrigerator
XTW Wash Buffer	2 x 1,250 µl	150 µl per purification	Refrigerator
XTE Elution Buffer	500 µl	25 µl per purification	Refrigerator
Necessary reaction tubes			
0.2 ml 8-tube strips	Eight 8-tube strips to be split into strips of 4 tubes	1 strip of 4 tubes	Room temp.



Required equipment

AVAILABLE AT MINIPCR.COM

Item	Recommended quantity
Magnetic rack for PCR tubes *e.g.,* Pullit™	1 per group
P51™ Molecular Fluorescence Viewer	1 per group
Micropipettes and tips 2-20 µl adjustable 20-200 µl adjustable	 1 per group 1 per group

Other materials supplied by user

- Plastic tubes for dispensing samples
- Disposable laboratory gloves
- Protective eyewear
- Fine-tipped permanent marker
- Vortex mixer (optional)
- Microcentrifuge to spin down samples (optional)

Teacher prep

- Reagents are sufficient for eight student groups.
- Reagents can be aliquoted up to one week in advance. Store aliquoted reagents in the refrigerator until use.
- **Do not remove the BioBits® pellets from their packaging until the day of the lab** (see page 9).

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

The instructions in this guide have students purify a single protein expression mixture (Tube 4) to familiarize themselves with a purification workflow. The kit contains enough reagents for students to perform two purifications. If time permits, you can have your students repeat the purification procedure on the protein expression mixture in Tube 3.

Up to one week before the lab: Dispense reagents

Materials needed for this section

From Bag 1 of lab kit (stored in freezer):
- GFP DNA
- RFP DNA

From Bag 2 of lab kit (stored in the refrigerator):
- MagStrep XT Beads
- XTW Wash Buffer
- XTE Elution Buffer

Bag of strip tubes (stored at room temp.).

Equipment and materials supplied by user:
- Plastic tubes for dispensing reagents. 1.7 ml or 0.2 ml tubes can be used, but do not use the strip tubes provided with the kit
- 2-20 µl and 20-200 µl micropipettes and tips
- Fine-tipped permanent marker
- Scissors or razor

1. Thaw tubes containing GFP DNA and RFP DNA by placing them at room temperature.

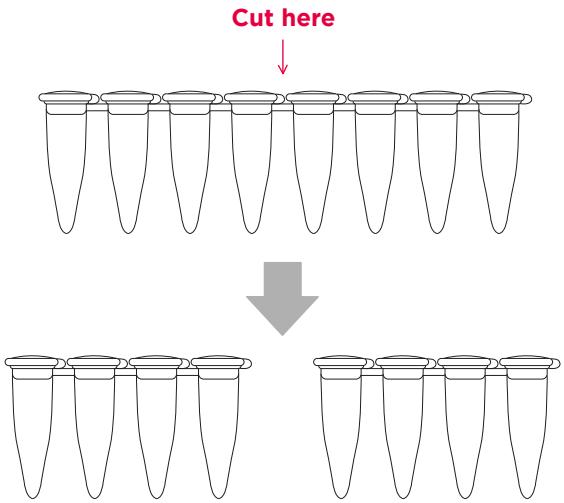
2. For each lab group, dispense the following reagents into labeled tubes. 1.7 ml or 0.2 ml tubes can be used, but do not use the strip tubes provided with the kit, as those are required in step 3.

   For class 1:
   - GFP DNA 15 µl
   - RFP DNA 15 µl

   For class 2:
   - XTW Wash Buffer 150 µl
   - XTE Elution Buffer 25 µl

3. Use a razor or scissors to cut each empty 8-tube strip into two strips of 4 tubes.

4. Label the first tube in each 4-tube strip “MB” for magnetic beads.



Distributing the magnetic beads
Because the beads settle in solution, it is essential to vortex or thoroughly mix the bead solution by pipetting up and down before dispensing it.

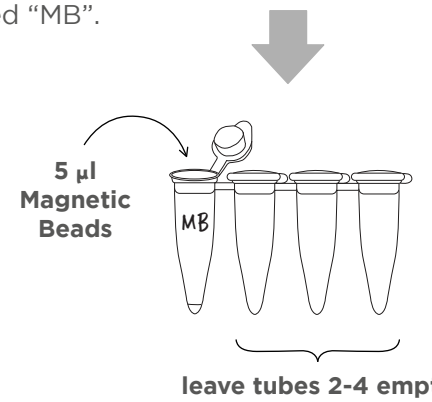
5. Add 5 µl of MagStrep XT Beads (well mixed) into the tube labeled “MB”.

6. Cap tube “MB”.

7. Leave the other three tubes in each strip empty for students to use for sample collection during the purification protocol.

8. Repeat steps 5-7 for each lab group.

9. If dispensing reagents more than 24 hours in advance, store all tubes in the refrigerator until use.



The day of the lab: Prepare BioBits pellets

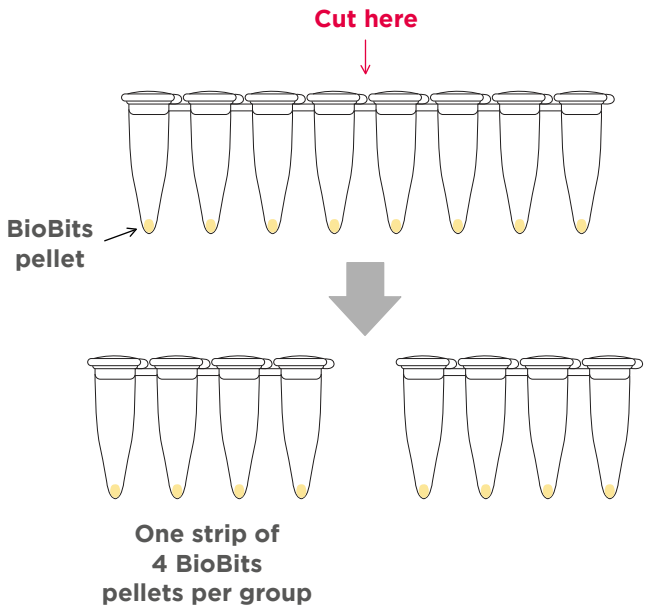
Materials needed for this section

From Bag 1 (stored in the freezer):
- BioBits pellets

Materials supplied by user:
- Fine-tipped permanent marker
- Scissors or razor

1. Remove the BioBits pellets from the airtight bag (do as close to the start of the lab as possible).

2. Use a razor or scissors to separate each strip of 8 BioBits pellets into two strips of 4 tubes.



3. Place any unused BioBits pellets in an airtight bag with the included desiccant card and store them in the freezer. Properly stored BioBits pellets will be stable for six months from the time of shipment.

At the start of class: Distribute reagents and supplies

For class 1, every lab group should have:

BioBits pellets	1 strip of 4 tubes
GFP DNA	15 µl
RFP DNA	15 µl
2-20 µl micropipette and tips	

For class 2, every lab group should have:

BioBits reactions from day 1	1 strip of 4 tubes
Wash Buffer	150 µl
Elution Buffer	25 µl
Magnetic Beads	5 µl (in the first tube of a 4-tube strip)
Empty PCR tubes (0.2 ml) for sample collection	3 (the remaining tubes in the 4-tube strip above)
2-20 µl micropipette and tips	
20-200 µl micropipette and tips	
Access to a P51 Molecular Fluorescence Viewer	
Access to a magnetic rack for PCR tubes (*e.g.* Pullit)	

The instructions in this guide have students purify a single protein expression mixture (Tube 4) to familiarize themselves with a purification workflow. The kit contains enough reagents for students to perform two purifications. If time permits, you can have your students repeat the purification procedure on the protein expression mixture in Tube 3. Distribute the same reagents and supplies listed in the table above for class 2.

Student guide

Background information

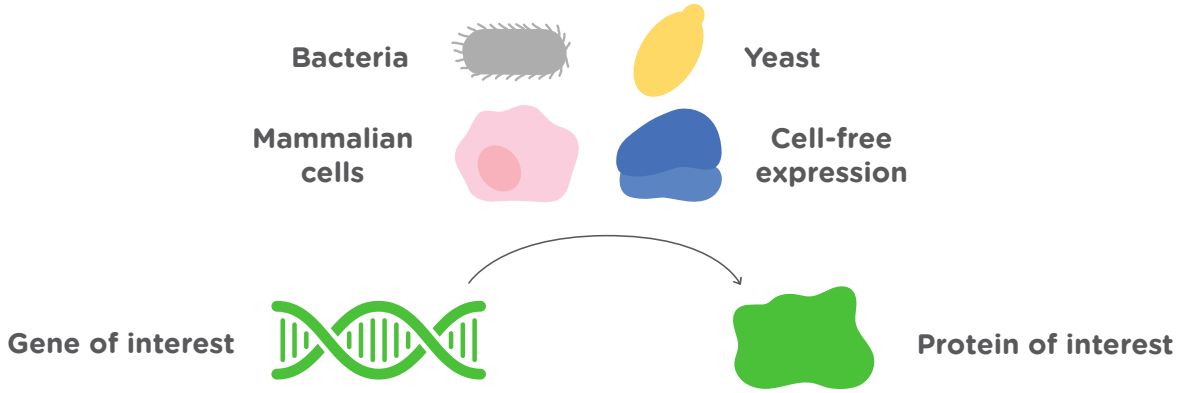
Protein expression

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.

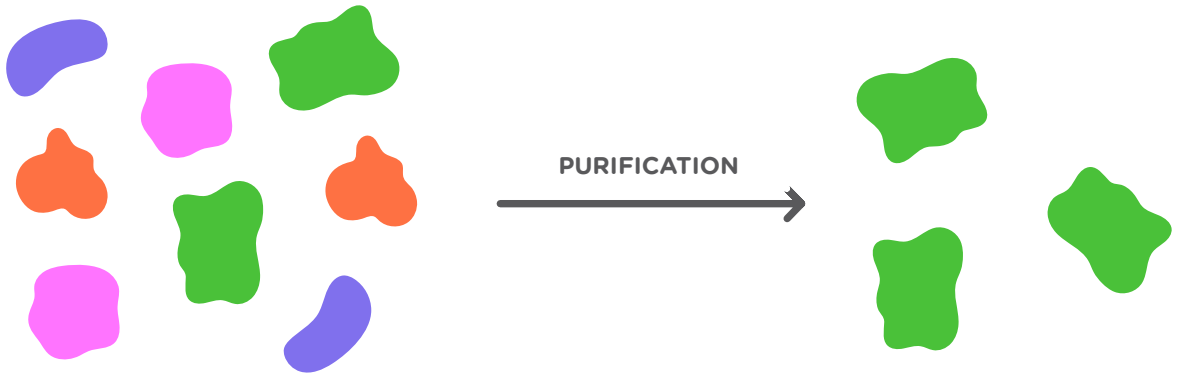
Check your understanding

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

Protein purification

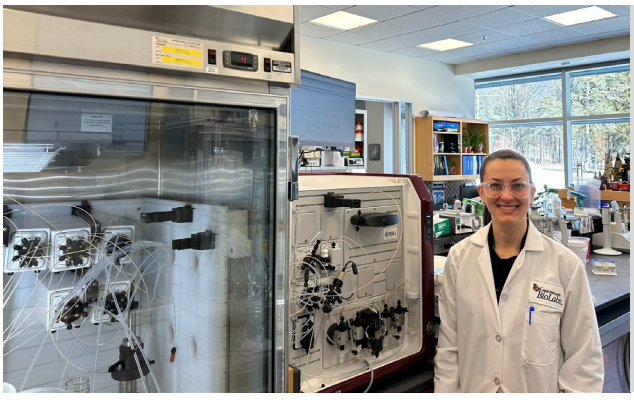
1

- 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.

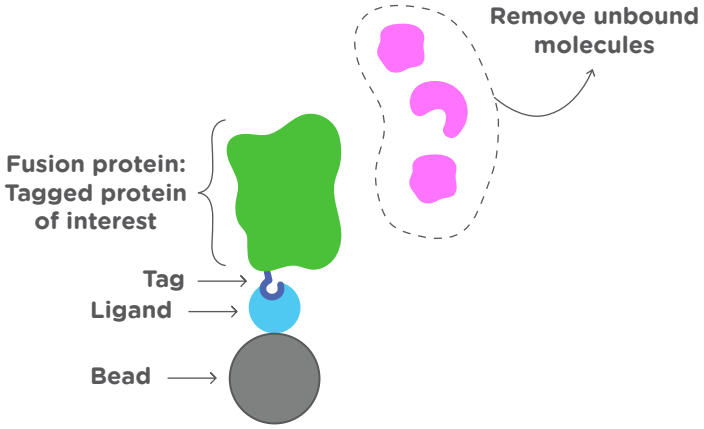
Affinity purification

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.

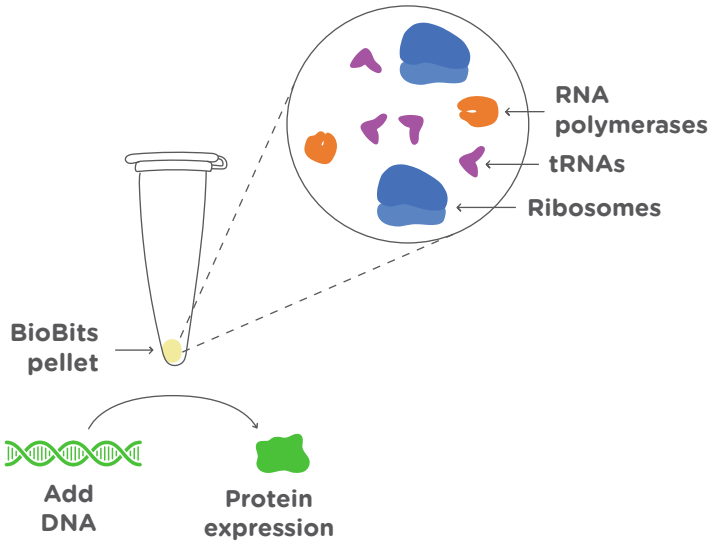
Expressing fluorescent proteins with cell-free technology

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

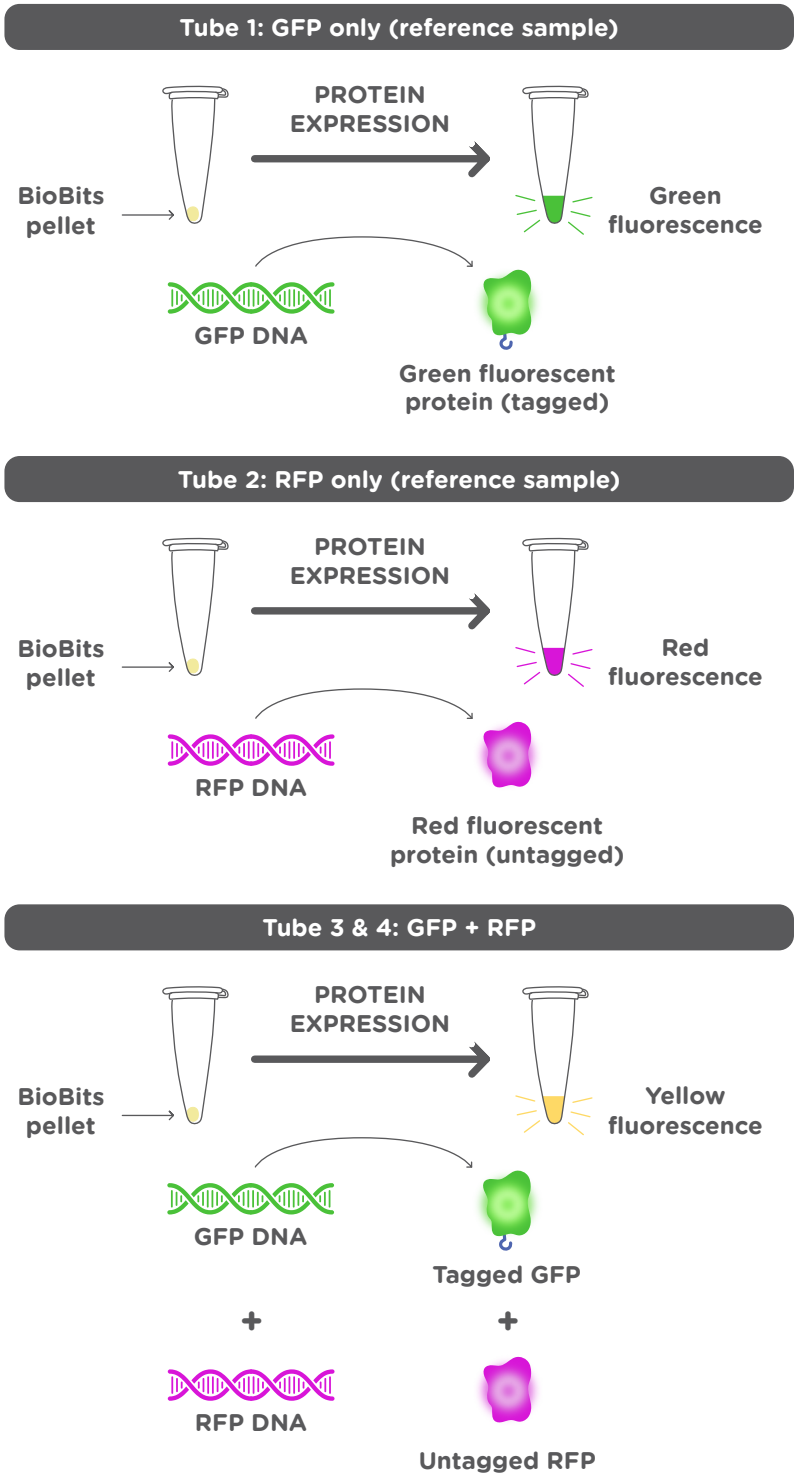
- You will add GFP DNA to the BioBits in tube 1.
- This will lead to the expression of green fluorescent protein.
- This tube will serve as a reference sample to demonstrate what GFP looks like on its own.

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.

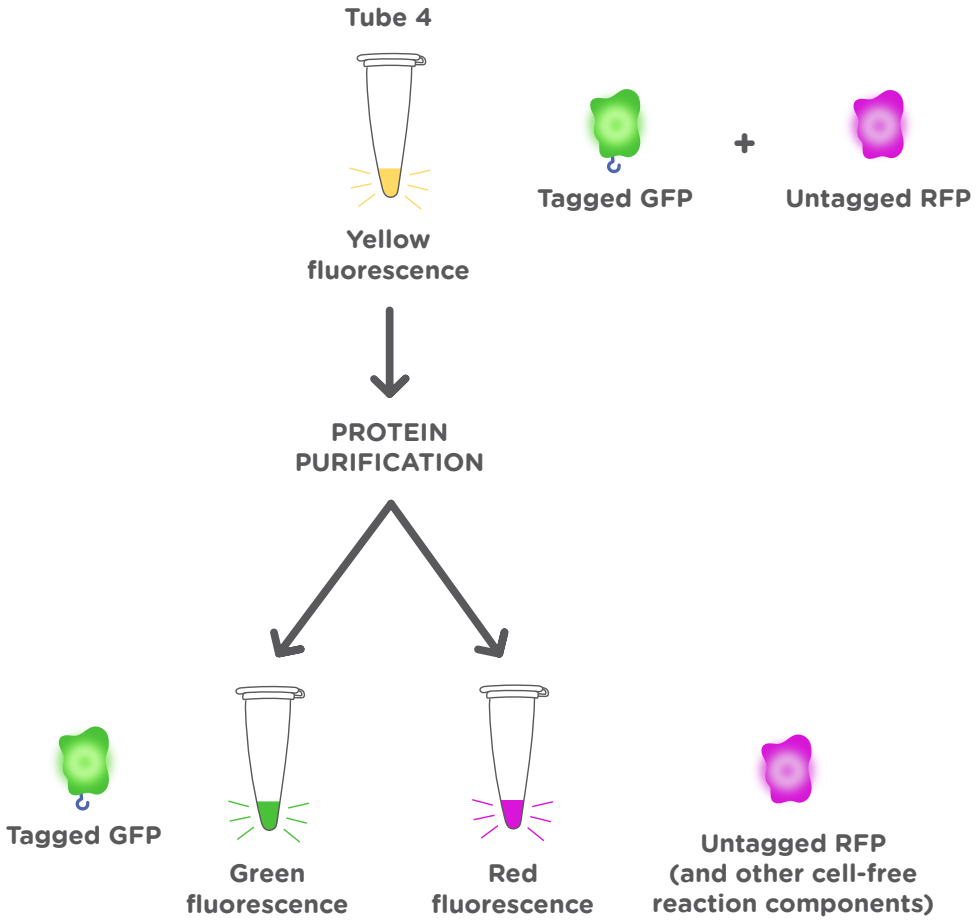


Color vision deficiency-friendly design: This lab guide uses magenta instead of red for the red fluorescent protein, making the diagrams more accessible for people with red-green color vision deficiency.

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.

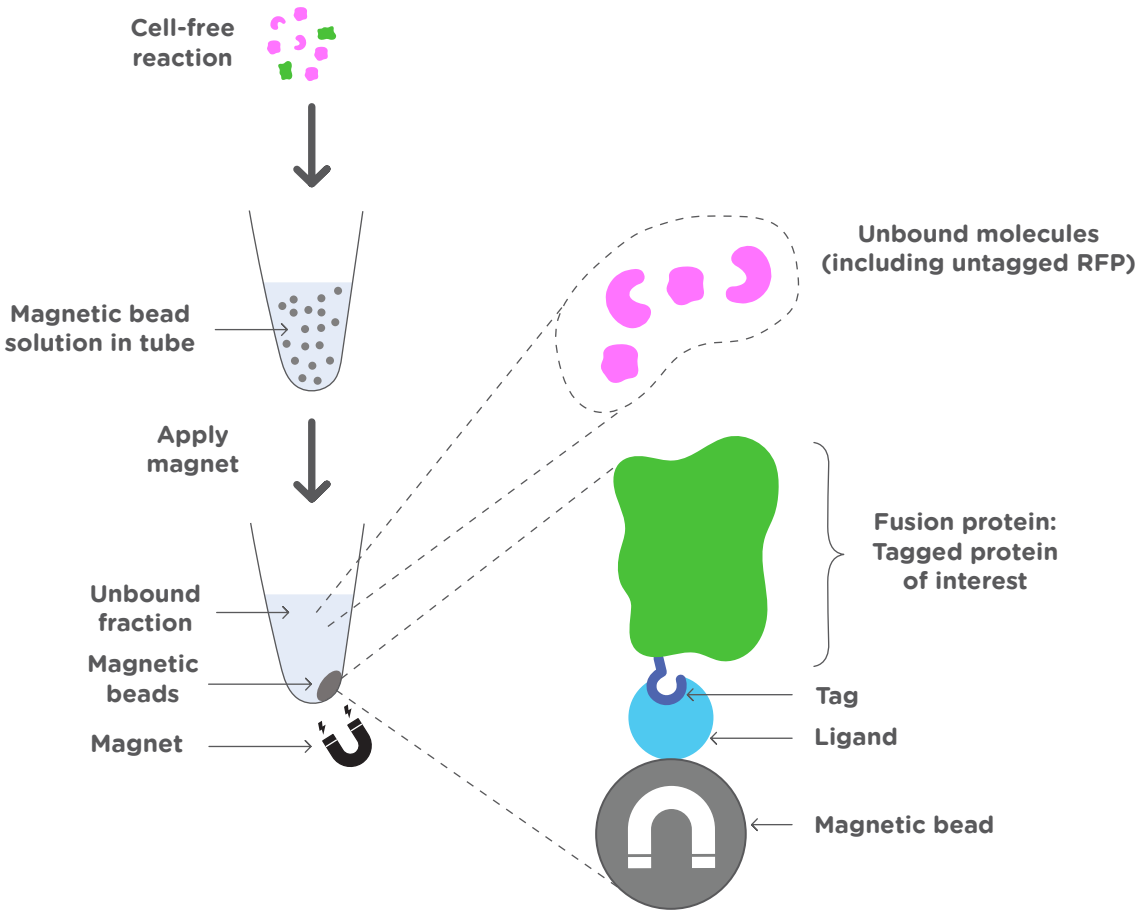
Affinity purification using magnetic beads

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.

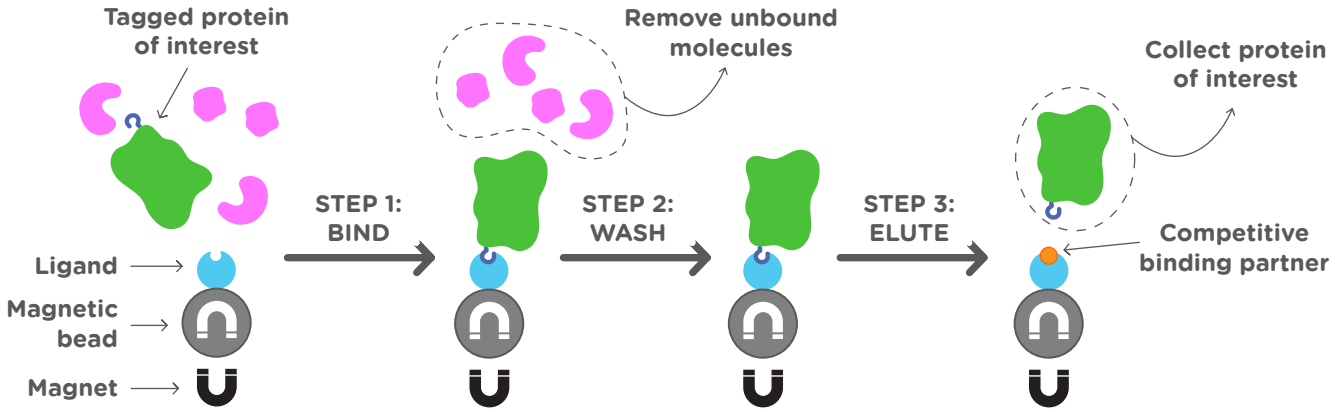
2

- 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.

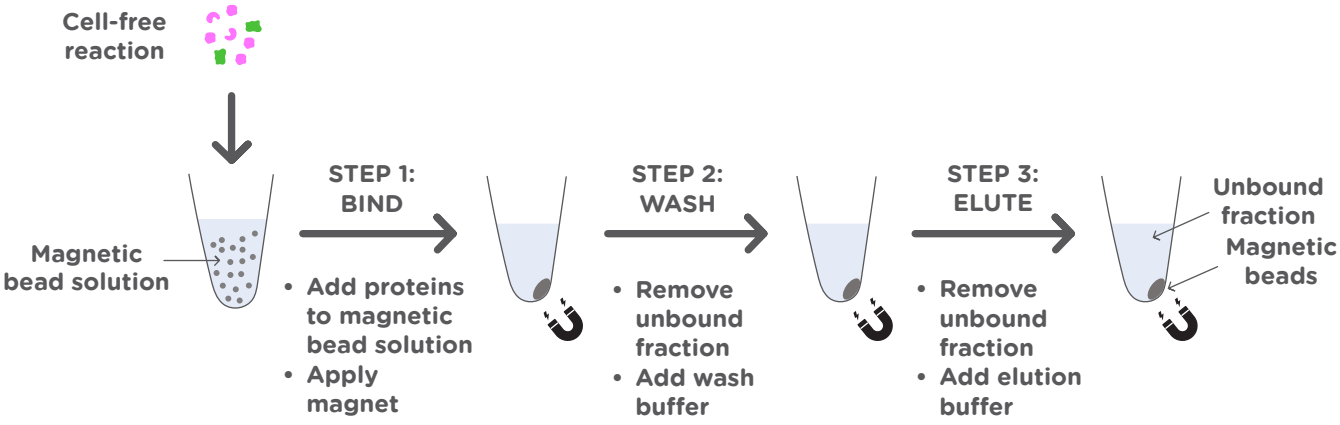
3

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.

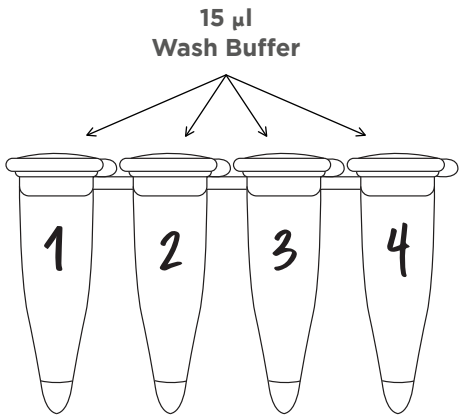
Part 2: Protein purification

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.

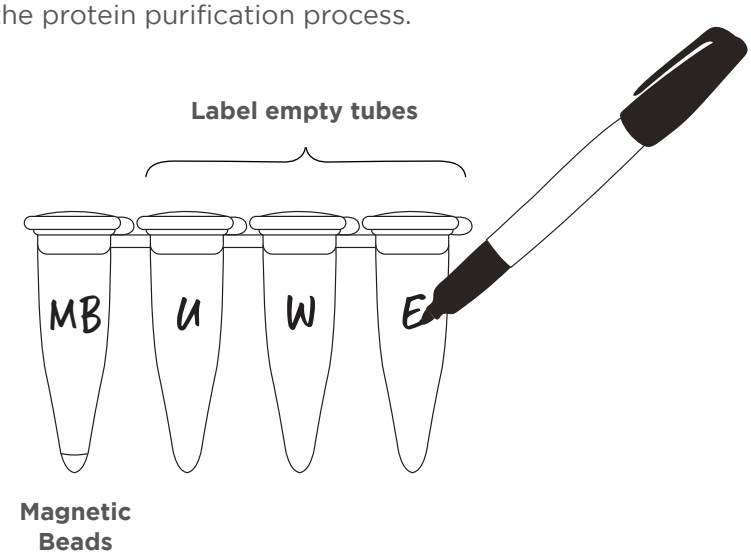


4. Set the BioBits reactions aside.

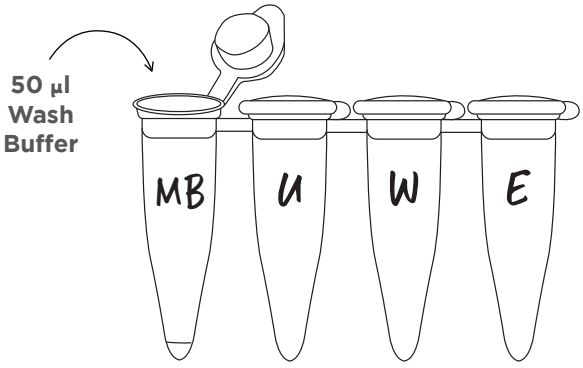
Prepare the magnetic beads

- 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.

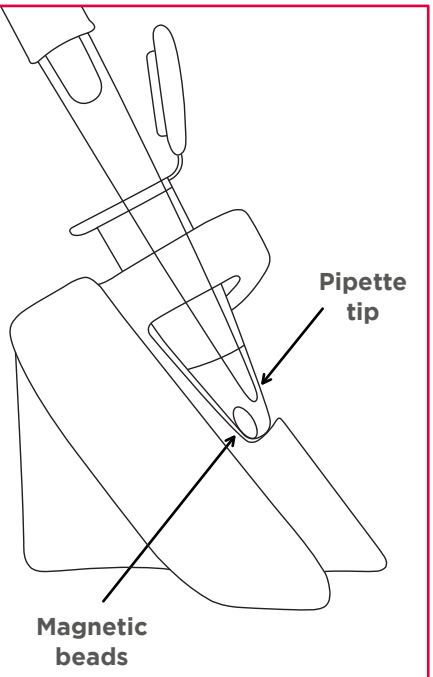
9. Place the strip of tubes in the magnetic rack and let the beads separate for 1 minute.

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.

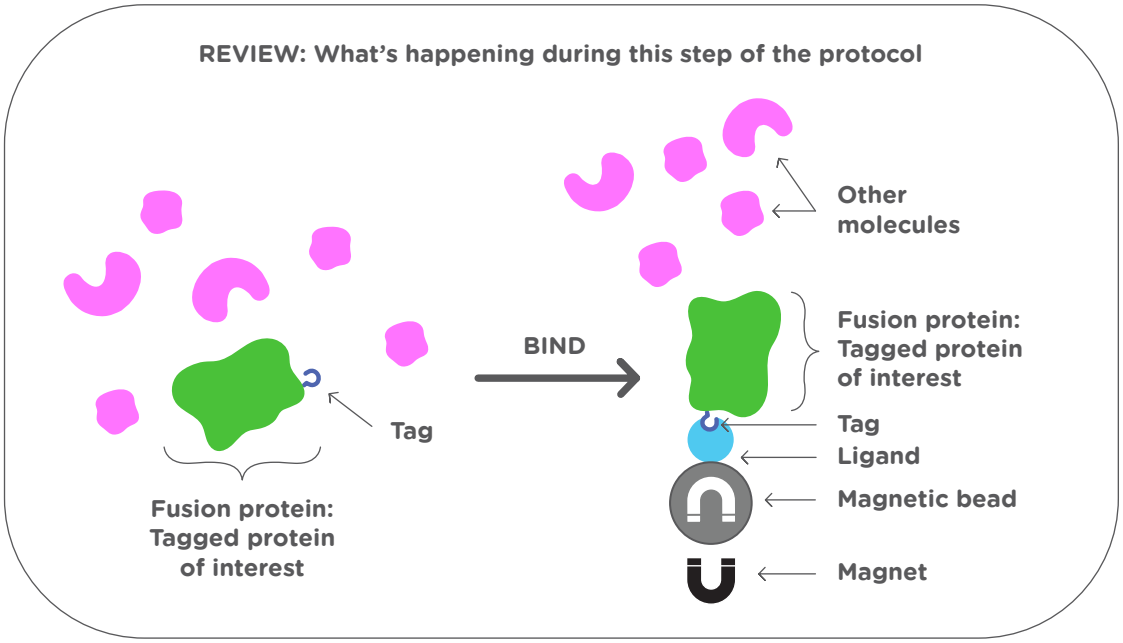
C. If you accidentally disturb the beads:
   - Pipette the solution back into the tube.
   - Leave the tube in the magnetic rack for 1 minute.
   - Try to remove the liquid again.



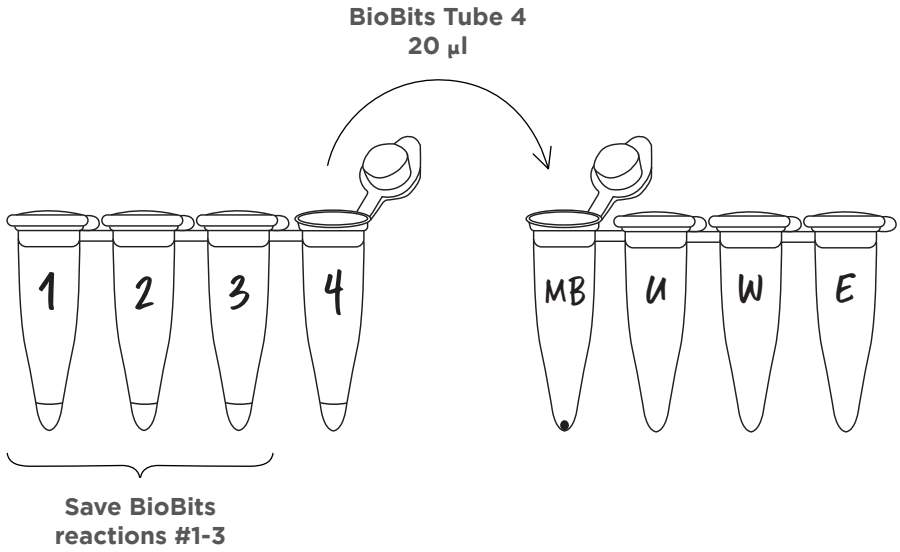
Video instructions
http://links.minipcr.com/PullitGuide

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.

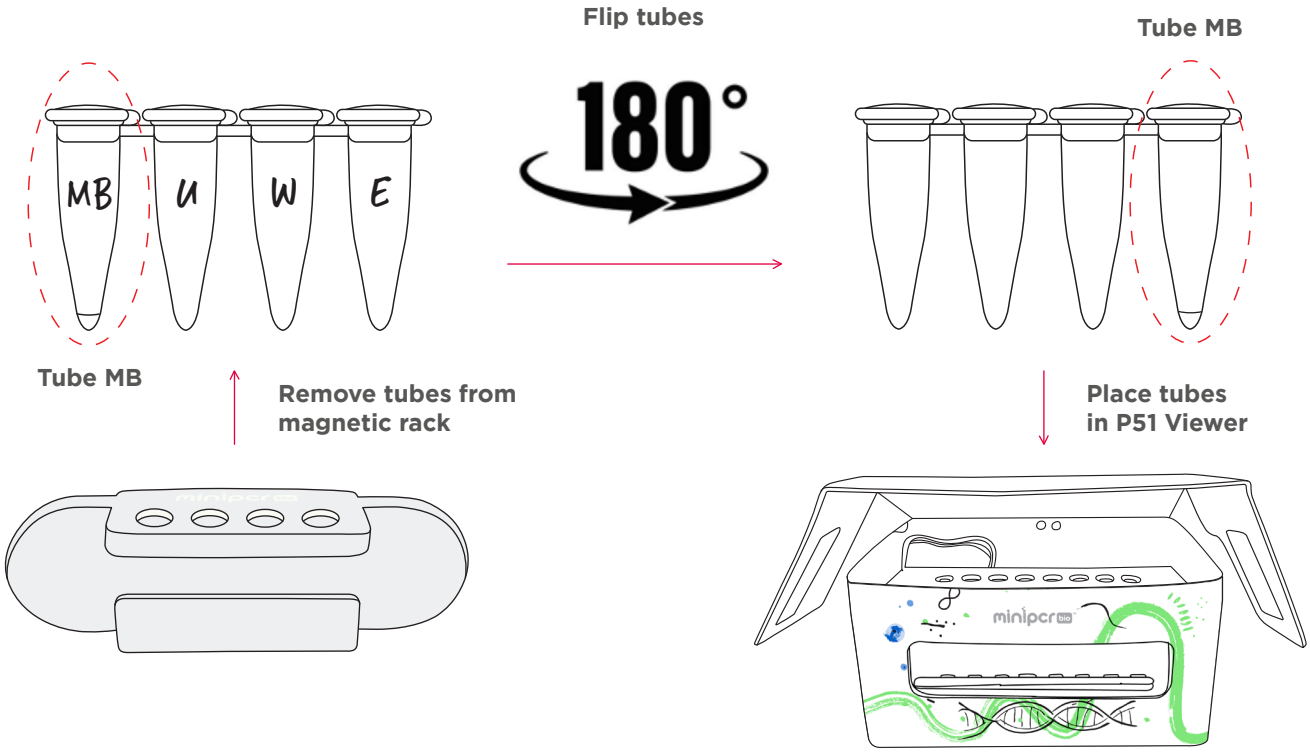
15. Incubate at room temperature for at least 30 minutes. Alternatively, incubate overnight in the refrigerator. Overnight incubation increases the purification yield.

    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.

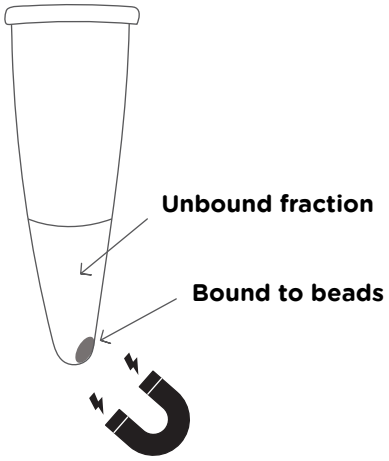
17. Turn on the P51's blue light.

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.

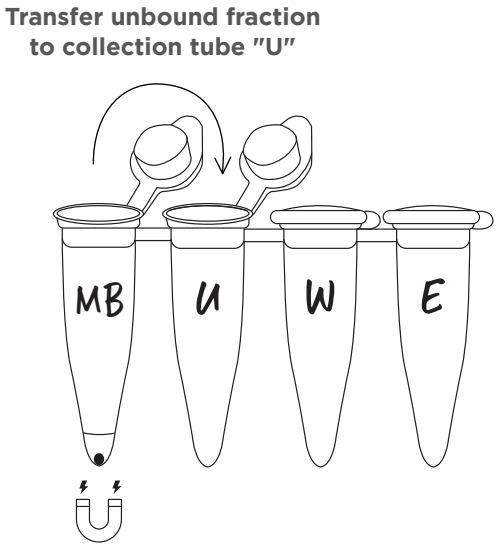


19. With the blue light on, observe tube MB through the viewing window. Record your observations in Table 2 below:

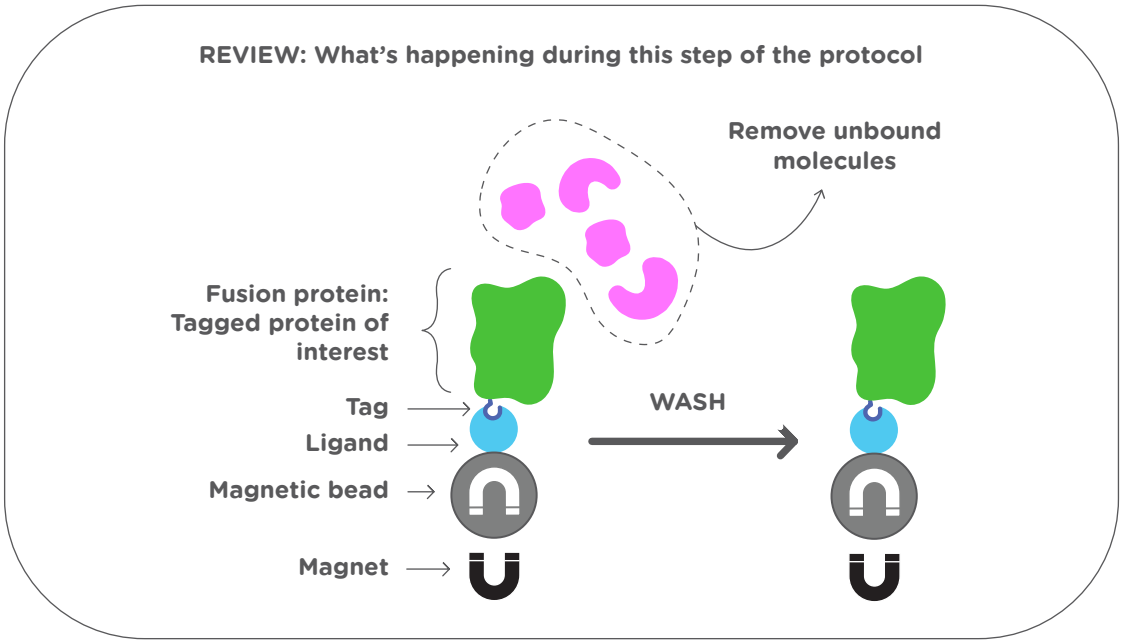
Table 2: Initial separation during purification		
	Unbound fraction	Bound to beads
Color of fluorescence (red/green/yellow)		



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.

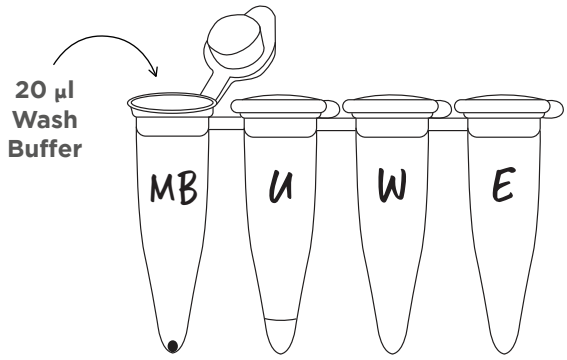


Wash the magnetic beads



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

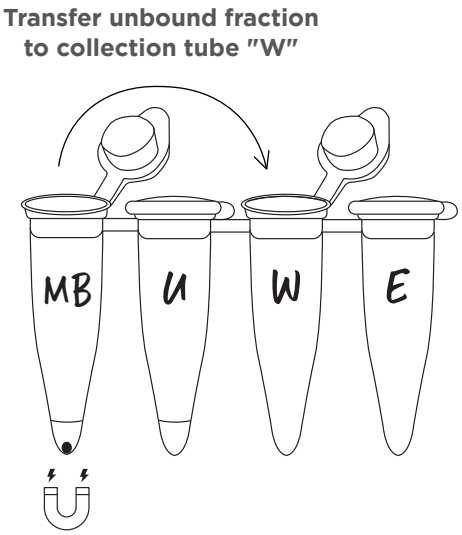
22. Add 20 µl of Wash Buffer to the magnetic beads in tube MB. There is no need to mix.



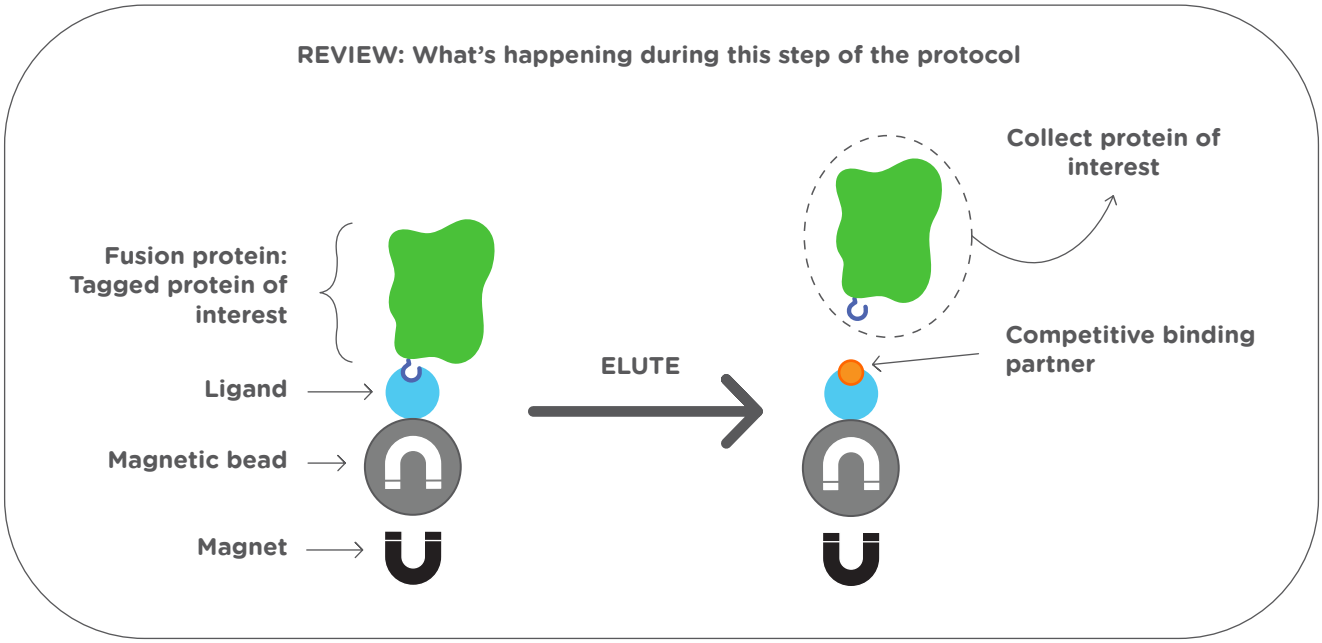
23. Cap the tube and make sure all the liquid is at the bottom.

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).

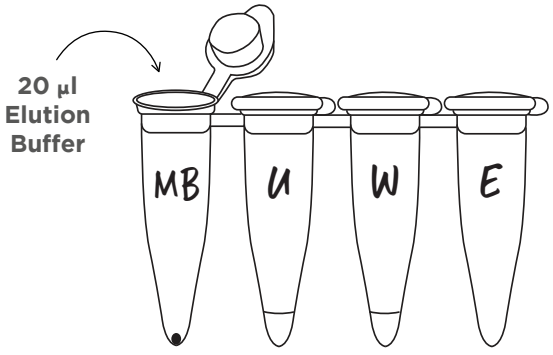


Elute the protein of interest



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

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



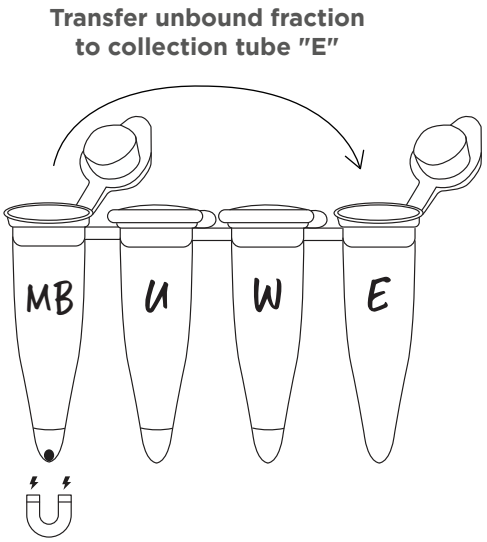
28. Thoroughly mix the reagents in tube MB by vortexing for 5 seconds or gently pipetting up and down five times.

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.

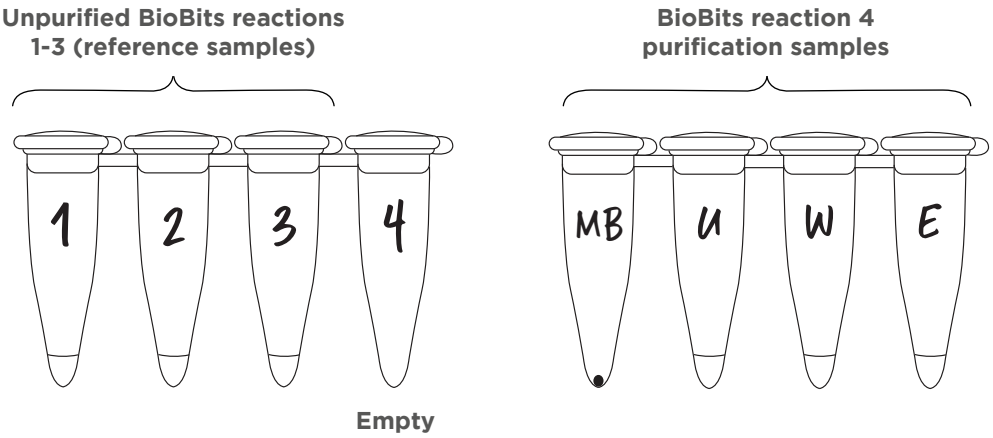
32. With the strip of tubes in the magnetic rack, insert your pipette tip in tube MB and carefully pipette the liquid off the magnetic beads. You should be able to remove approximately 20 µl. Transfer this volume to collection tube E ("E" for eluate).



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)					

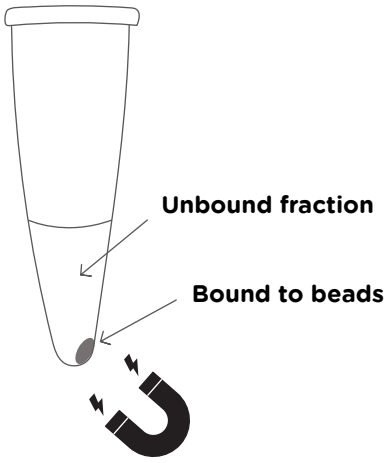
Post-lab analysis

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)		

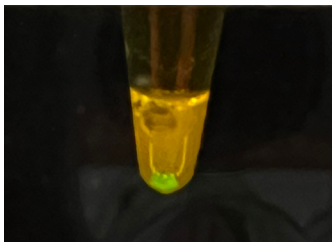


2. Which statement explains your observation?
   A. The GFP carries a tag because it is the protein of interest. This tag binds to the ligand coating the magnetic beads.
   B. The RFP carries a tag because it is the protein of interest. This tag binds to the ligand coating the magnetic beads.
   C. The 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.

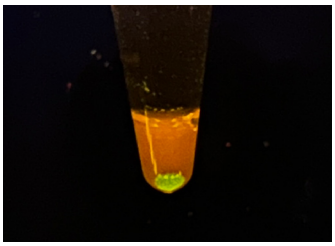
Critical thinking

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.

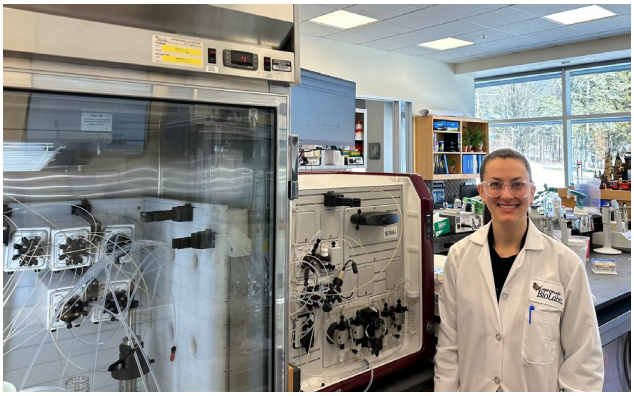
Score	4	3	2	1
CLAIM A statement that answers the original question/problem.	Makes a clear, accurate, and complete claim.	Makes an accurate and complete claim.	Makes an accurate but incomplete or vague claim.	Makes a claim that is inaccurate.
EVIDENCE Data from the experiment that supports the claim. 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

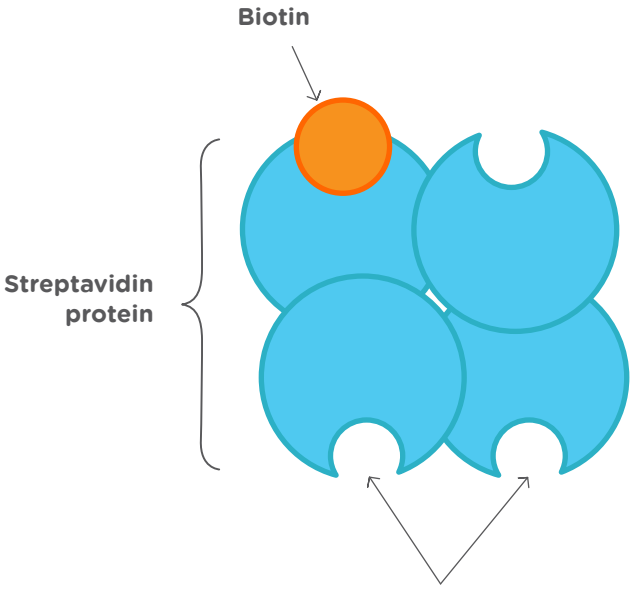
In this activity, you used affinity purification to isolate a tagged GFP fusion protein. This resource provides more information on the tag you used and its ligand.

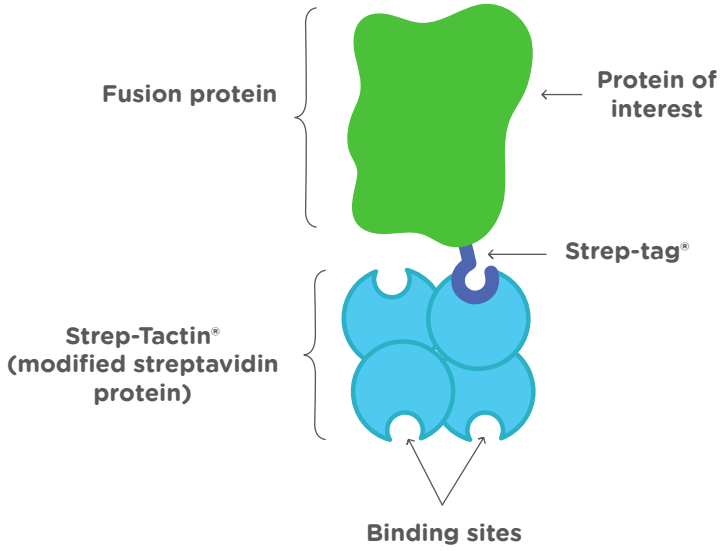
Streptavidin is a protein made of four identical subunits that has an extremely high binding affinity for a vitamin called biotin. Scientists realized that the high binding affinity between these naturally occurring molecules could be harnessed for biotech applications.

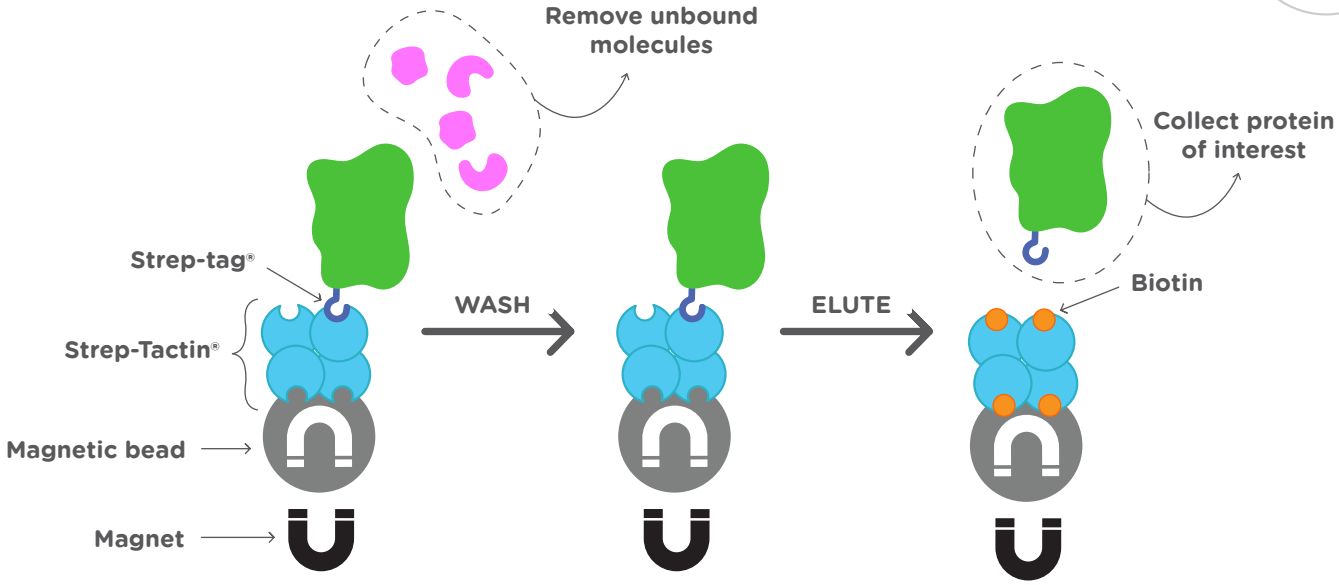
Scientists designed an eight-amino acid sequence that binds specifically to the biotin-binding domain of the streptavidin protein. This short sequence interacts with streptavidin through the binding sites that biotin normally occupies. The team that designed this sequence named it the Strep-tag®.

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.

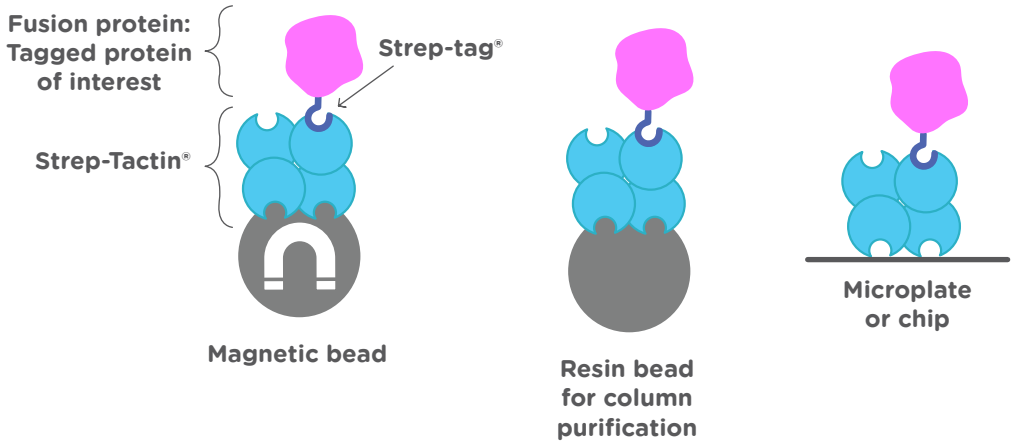






Fusion proteins with a Strep-tag® can be released from Strep-Tactin®XT by adding a competitive binding partner. In this activity, the elution buffer contained biotin, the natural ligand for the streptavidin protein. Because biotin will also bind to the Strep-Tactin®XT binding site, the tagged fusion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



Instructor guide

Expected results

Part 1: Protein expression

The photo below shows representative results after the overnight protein expression step.

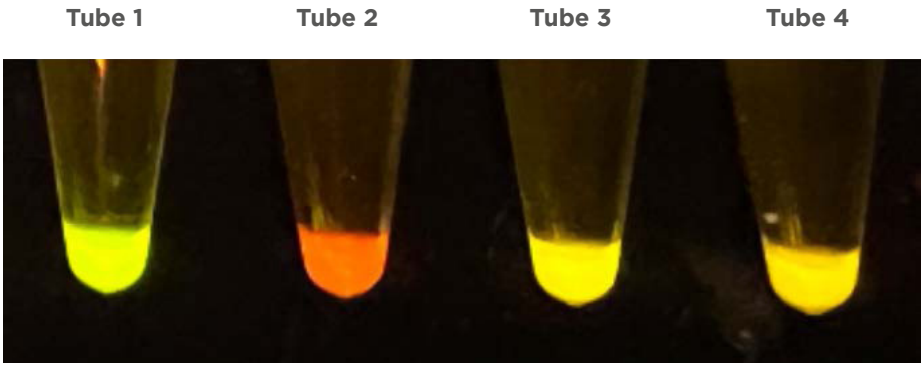


Table 1: Protein expression observations				
	Tube 1	Tube 2	Tube 3	Tube 4
DNA added	GFP	RFP	GFP + RFP	GFP + RFP
Color of fluorescence	Green	Red	Yellow	Yellow

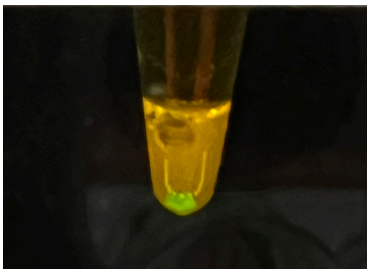
Explanation of results

- Tube 1: DNA encoding GFP was added to the BioBits pellets. Green fluorescence indicates the successful expression of the green fluorescent protein.
- Tube 2: DNA encoding RFP was added. Red fluorescence indicates the successful expression of the red fluorescent protein.
- Tubes 3 and 4: Both types of DNA, encoding GFP and RFP, were added. The combination of green and red fluorescence appears yellow. Yellow fluorescence thus indicates the successful expression of the green fluorescent protein and the red fluorescent protein.

Part 2: Protein purification
Initial separation during purification

The photos below show representative results after allowing the protein of interest to bind to the beads. Longer incubation times allow more GFP to bind to the beads.

30 min



Overnight

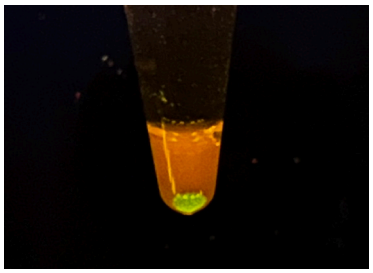
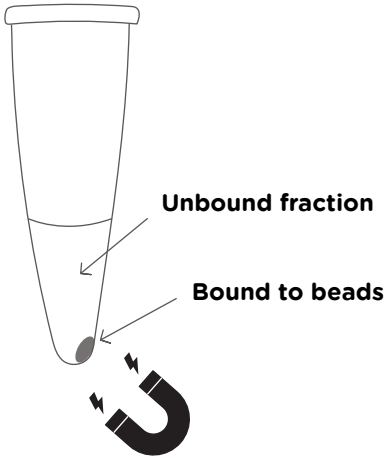


Table 2: Initial separation during purification		
	Unbound fraction	Bound to beads
Color of fluorescence	Red or orange	Green



Explanation of results

- GFP carries a tag and will bind to the magnetic beads.
- RFP does not carry a tag and will remain in the unbound fraction.
- If some GFP remains in the unbound fraction, the fluorescence will appear yellowish orange rather than red (see the 30 min. sample above).

Final protein purification observations

The photo below shows representative results. In this example, the binding was allowed to occur overnight, allowing most of the tagged GFP to bind to the beads before the wash step. After a shorter, 30-minute incubation, tube U may appear less red and more orange, due to the presence of unbound GFP in the unbound fraction.

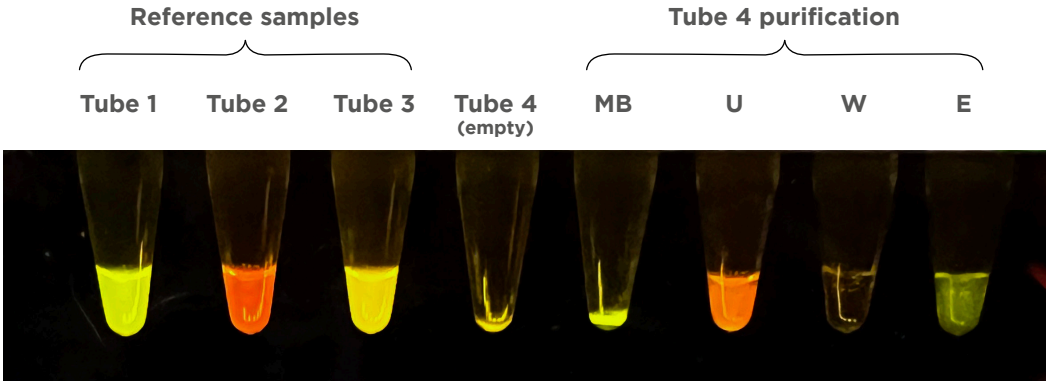


Table 3: Final protein purification observations

	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	Yellow	Green	Red	Very faint red	Green

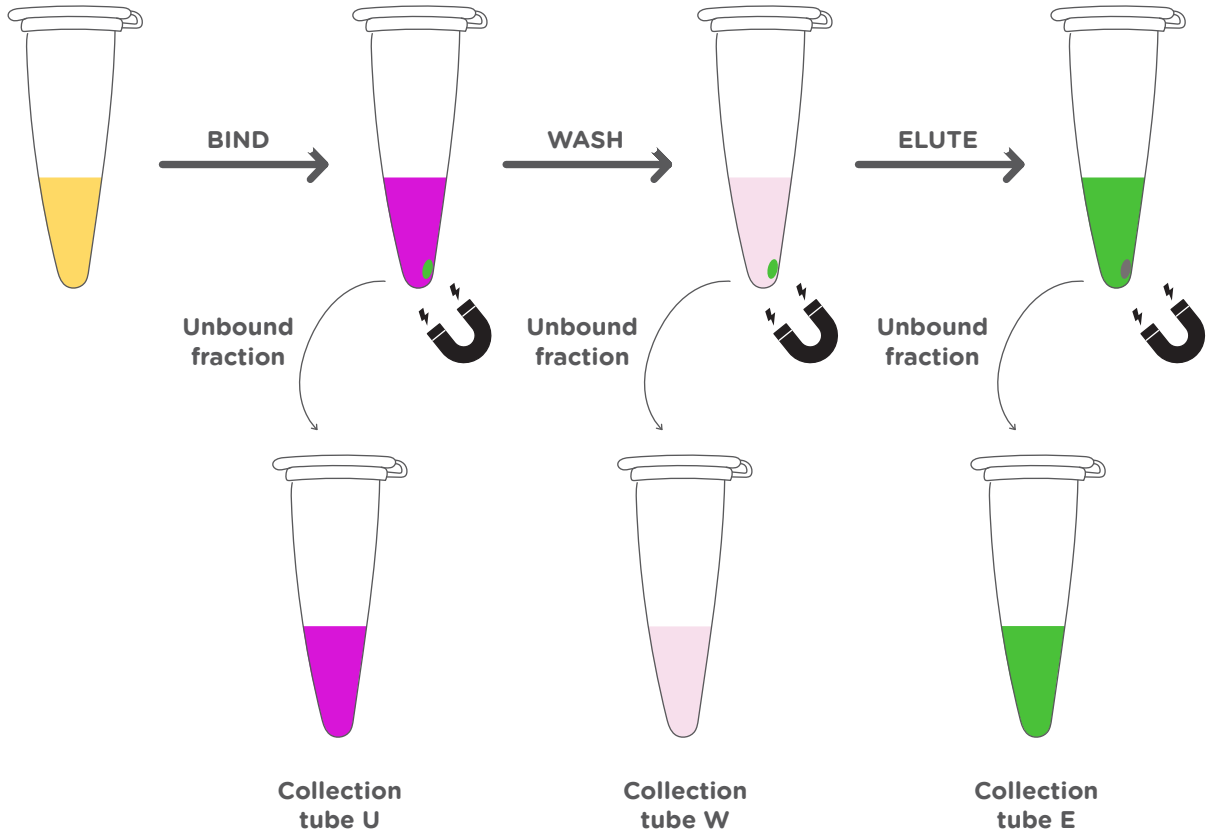
Refer to the next page for an explanation of the results.

For technical support, contact support@minipcr.com.

For answers to the student questions, email answers@minipcr.com.
Please include in the body of the email:

- The name of the lab.
- Your name, school, and job title.

The following diagram represents the expected purification progression:



Bind protein of interest:

- Tagged GFP binds to the magnetic beads.
- Untagged RFP remains in the unbound fraction and is collected in Tube U. Tube U should exhibit strong red fluorescence.

Wash:

- Tagged GFP remains bound to the magnetic beads.
- Most of the RFP was removed and transferred to Tube U in the previous step, but in this step, residual RFP is removed and collected in Tube W. Tube W should exhibit little to no red fluorescence.

Elute protein of interest:

- The Elution Buffer contains a competitive binding partner for the ligand on the beads.
- The addition of the Elution Buffer releases the tagged GFP from the beads into the unbound fraction, which is collected in Tube E (eluate). Tube E should exhibit green fluorescence.
- It is likely that a small amount of liquid will remain in tube MB with the magnetic beads. Because the tagged GFP is present in this liquid, the beads in tube MB may appear green.

Unexpected results and troubleshooting

Part 1: Protein expression

Little to no fluorescence following overnight rehydration of BioBits with DNA could be explained by the following:

- The P51 Fluorescence Viewer is not set up correctly.
  Ensure you are using the orange filter (not the yellow filter) for the P51 Fluorescence Viewer and that the protective film on the filter has been removed. When switched on, the P51 should emit bright blue light; if it doesn't, check the power source (either 9V battery or USB-C).
- The BioBits pellets were not properly rehydrated with DNA solution.
  Protein expression requires the addition of 5 µl of DNA solution to the BioBits pellets. Examine the tubes to ensure they contain the expected volume of liquid and that the BioBits pellets are dissolved.
- The reagents may be expired and/or have been stored incorrectly.
  The BioBits pellets and DNA samples can be stored in the freezer for up to six months after receipt. If the BioBits pellets bag is opened, the remaining pellets must be stored in the freezer in an airtight bag with the included orange desiccant card. Storage under different conditions or in excess of six months may impair performance.
- Protein expression reactions were exposed to direct bright light.
  Although it is acceptable to incubate the reactions under ambient light conditions (*i.e.,* regular indoor light), prolonged exposure to very bright light, such as direct sunlight, can cause the reaction components to degrade and/or the fluorescent proteins to bleach or degrade.

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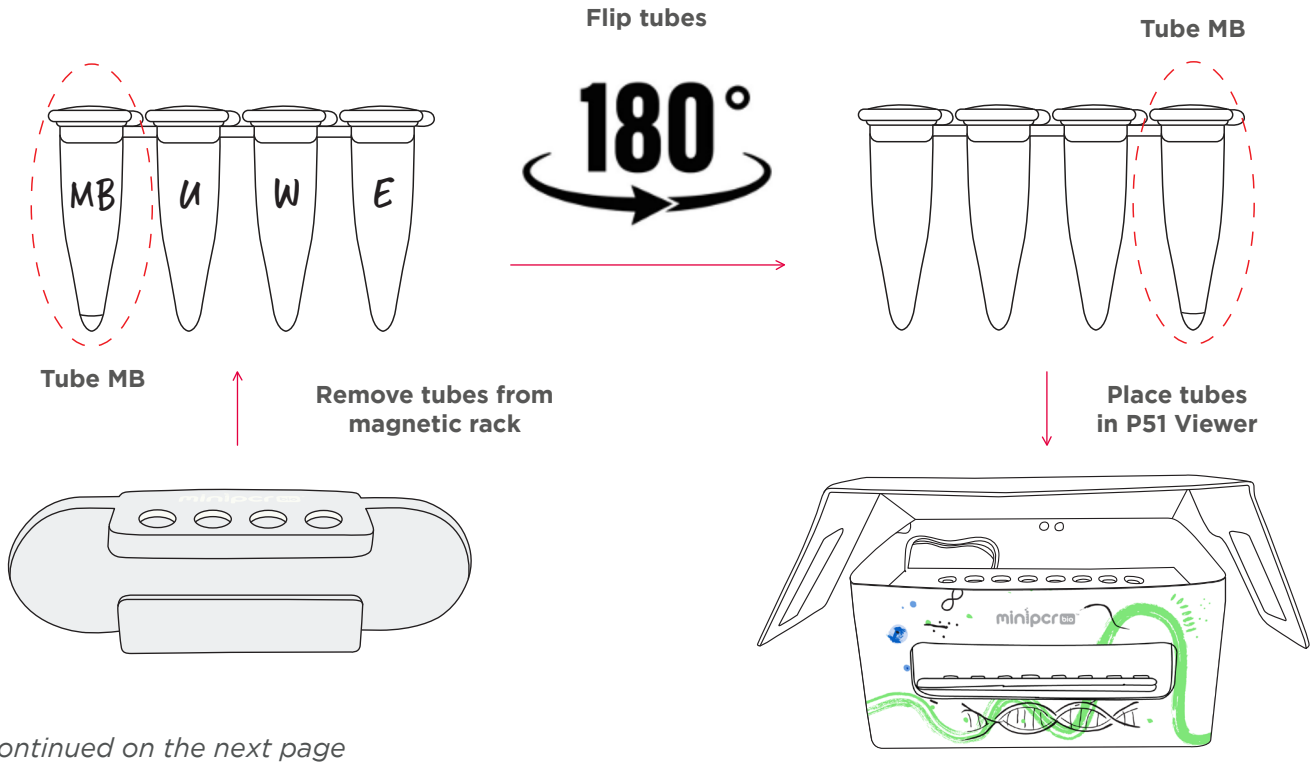
Part 2: Protein purification
Initial separation during purification

Little to no fluorescence after allowing the protein to bind to the beads could be explained by the following:

- The P51 Fluorescence Viewer is not set up correctly.
  Ensure you are using the orange filter (not the yellow filter) for the P51 Fluorescence Viewer and that the protective film on the filter has been removed. When switched on, the P51 should emit bright blue light; if it doesn't, check the power source (either 9V battery or USB-C).
- Protein expression reactions were exposed to direct bright light.
  Although it is acceptable to incubate the reactions under ambient light conditions (*i.e.*, regular indoor light), prolonged exposure to very bright direct light, such as direct sunlight, can cause the reaction components to degrade and/or the fluorescent proteins to bleach or degrade.

If the sample appears red without a green pellet visible:

- The tubes are not facing the correct direction.
  When the tubes are in the magnetic rack, the magnetic beads will collect along the back wall of the tube that is facing the rack magnets. When transferring them to the P51, the tubes must be flipped 180 degrees so the magnetic beads pellet faces the front viewing window.



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If the sample appears yellow:

- Lack of beads.
  Place the tubes in the magnetic rack and observe under normal light. You should be able to see the beads collecting on the back sidewall of the tube facing the magnet. If you don't see the beads, there are two possibilities:
  - The magnetic beads were not distributed properly to the students.
    While dispensing the magnetic beads, you must thoroughly mix the stock of magnetic beads either by vortex or pipetting up and down until the bead mixture is homogeneous. If the beads are not properly mixed, it is possible that students may have received little to no beads.
  - The magnetic beads were not handled properly by the students.
    Students may accidentally discard their magnetic beads if they fail to use a magnetic rack during the initial wash step of the beads. If students disturb the magnetic pellet with their pipette tip, they may accidentally discard their magnetic beads.
- The fluorescent proteins have not been incubated with the magnetic beads long enough.
  While 30 minutes is sufficient for enough GFP to bind to the beads to visualize separation, an overnight incubation in the refrigerator produces the most complete binding of GFP to the beads, resulting in better separation.
- The tubes were not moved quickly enough from the magnet to the blue light viewer.
  Once the tubes are removed from the magnet, the magnetic beads will begin to disperse in solution. If this happens, simply move the tubes back to the magnetic rack and repeat the magnetic separation.
- The Wash and Elution buffers were mixed up.
  If the Elution Buffer is accidentally used in the binding step, the tagged GFP will not bind the beads and will remain in the unbound fraction with the untagged RFP.
- The reagents may be expired and/or have been stored incorrectly.
  The magnetic beads and the buffers can be stored in the refrigerator for six months from receipt. If the reagents were stored longer or at the incorrect temperature, they may no longer be functional. In particular, the magnetic beads should not be stored in the freezer, as that degrades their function. Magnetic beads that have been frozen can still produce acceptable results, but they will not be as robust as properly stored magnetic beads.

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Final protein purification observations

Little to no fluorescence at the end of the purification protocol could be explained by the following:

- The P51 Fluorescence Viewer is not set up correctly.
  Ensure you are using the orange filter (not the yellow filter) for the P51 Fluorescence Viewer and that the protective film on the filter has been removed. When switched on, the P51 should emit bright blue light; if it doesn't, check the power source (either 9V battery or USB-C).

If Tube U is yellow, the following may have happened:

- Failure to bind the tagged GFP to the beads during the binding step.
  Refer to troubleshooting on the previous page.

If Tube W is green, the following may have happened:

- The Wash and Elution Buffers were mixed up.
  If the Elution Buffer is accidentally used in the wash step, the tagged GFP will be released from the beads and collected in tube W.

If Tube E is very faintly green or not green at all, the following may have happened:
(Note that Tube E is expected to be a dimmer green than reference Tube 1. Any visible green indicates that GFP was successfully purified.)

- Failure to bind the tagged GFP to the beads during the binding step.
  Refer to troubleshooting on the previous page.
- The Wash Buffer was left on the beads during the wash step for too long.
  The longer the wash step, the more GFP will be washed off the beads, and the dimmer the green in the E tube will be. This illustrates the purity vs. yield trade-off that often happens in purification.

Notes on lab design

This lab was created to make protein expression and purification more accessible to educational settings. The design of this lab has simplified certain elements to achieve this goal:

- Large-scale protein expression typically uses bacteria, yeast, or mammalian cells. Cell-free protein expression technology enables affordable small-scale protein expression in a classroom without culturing cells.
- Students purify fluorescent proteins to facilitate easy visualization of the purification process at every step. Typical protein purification requires additional analysis to verify the presence of the protein of interest, which is typically done at the end of the process.
- While tagging proteins is common in basic science research, it is less common in applied settings because the tag can interfere with protein function. Affinity purification in industrial settings often uses ligand/receptor interactions native to the protein of interest. For example, when producing monoclonal antibodies for therapeutic use, affinity purification using Protein A or G to capture the Fc region of the antibody is used. In other biotechnology applications where a fusion tag is used, it is often removed by proteolytic cleavage at the end of the process, so that the final purified protein resembles its natural form and function.
- Large-scale protein purification usually involves columns packed with beads. Using magnetic beads allows for affordable small-scale protein purification in a classroom setting.
- In biomanufacturing settings, multiple rounds of purification are typically performed to ensure very high purity of the final product.

Learning goals and skills developed

Student learning goals:

- Explain affinity purification
- Understand the role of each step in a protein purification workflow
- Analyze experimental results after each step of a protein purification workflow

Scientific inquiry skills

- Follow detailed experimental protocols
- Make a claim based on scientific evidence
- Use reasoning to justify a scientific claim

Biotechnology skills:

- Micropipetting
- Cell-free protein expression
- Affinity purification
- Fluorescence visualization
- Yield and purity calculations
- Unit conversion calculations

Standards alignment

Biotechnician Assistant Credentialing Exam (BACE)

KNOWLEDGE PORTION OF EXAM
General topics in biotechnology
• Discuss current techniques used in biotechnology and their applications • Describe careers in the biotechnology field
Technical skills/applications
• Discuss protein expression in model organisms • Discuss methods of molecule/protein isolation, purification, and quantification
PRACTICAL PORTION OF EXAM
Biotechnology skills
• Accurately measure liquids using micropipettes and serological pipets
Applied mathematics in biotechnology
• Use scientific notation, significant digits, and decimals correctly • Make conversions within the metric system, and use metric measurements
Laboratory equipment - demonstrate proper and safe use of:
• Micropipettes & serological pipets • Centrifuges • Vortexers
Research and scientific method
• Discuss good experimental design, including the proper use of controls • Analyze and interpret data, including the use of statistical analysis

Next Generation Science Standards (NGSS)

Science and Engineering Practices	Crosscutting Concepts
• Asking Questions and Defining Problems • Developing and Using Models • Planning and Carrying Out Investigations • Analyzing and Interpreting Data • Using Mathematics and Computational Thinking • Constructing Explanations and Designing Solutions • Engaging in Argument from Evidence • Obtaining, Evaluating, and Communicating Information	• Cause and Effect • Scale, Proportion, And Quantity • Systems and System Models • Structure and Function • Interdependence of Science, Engineering, and Technology • Influence of Engineering, Technology, and Science on Society and the Natural World