October 2023

Gene Regulation of the Red Fluorescent Protein in the PARA-R Plasmid

By Anthony Fernandes, ABE Greater Los Angeles Area

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Greater Los Angeles

Master Teacher Fellowship

Gene Regulation of the Red Fluorescent Protein in the PARA-R Plasmid

TIME FRAME: 11 hours

SUGGESTED AGE RANGE: High School Grades 9 to 10

SUGGESTED COURSE OR CONTENT AREA: Biology, AP Biology, Biotechnology

CONNECTION DESCRIPTIONS:

- Molecular modeling
- Connections/tie-ins between ABE and Genetics
- Integrating inquiry
- Data analysis/Data literacy
- Project or problem-based learning

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PROGRAM SITE: Pasadena, Greater Los Angeles Area

Lesson 1 (1 hour)

Gene regulation: Introduction to the Operon

1. **Overview**: In this lesson, the concept of the operon as a genetic regulatory unit in prokaryotes is introduced.
2. **Learning Goals**
    a. Students will identify the fundamental structural and functional components of a standard operon.
    b. Inducible and repressible systems will be compared and contrasted.
3. **Assessed Outcome**
    a. Complete the reading comprehension guide **Structural Components of the Prokaryotic Operon** Document
4. **Key Vocabulary**: Regulatory Genes, Structural Genes, Operon, Operator, Promoter, Activator, Inducer, Repressor: constitutive, inducible, repressible
5. Materials and LabXchange Pathway(s): Gene Regulation: Operon Theory
6. Teacher Preparation: Review LabXchange text and fundamental concepts of the operon
7. Lab Safety Considerations: Not Applicable
8. Sequence of Activities

Activity Description	*Time*	*Materials*
1. Students will read the **Learning Objectives, Prokaryotic Gene regulation, and Regulation by Repression in the 11.7 Gene Regulation: Operon Theory**	20 min	Labxchange article: Operon Theory
2. Students will complete the Exercise **Structural Components of the Prokaryotic Operon**	20 min	See Appendix A for **Structural Components of the Prokaryotic Operon** Document
3. Group and Class Discussion comparing & contrasting inducible and repressible systems	20 min	**Structural Components of the Prokaryotic Operon** Document

Lesson 2 (2 hours)

Gene Regulation: Tryptophan Operon: a Repressible System

1. **Overview**: The tryptophan (trp) operon is a useful model to introduce a repressible system. The transcription of the structural genes of this operon result in the synthesis of tryptophan. The genes are expressed when tryptophan levels are low. As the concentration of tryptophan increases, transcription ceases and consequently expression is halted as the need for tryptophan is reduced. This form of negative control is accomplished when two molecules of tryptophan bind to the repressor molecule and ultimately to the operator preventing the RNA polymerase from binding to the promoter region of the trp operon.
2. **Learning Goals**:
    a. Identify the structural components of the trp operon.
    b. Describe the conditions that activate and inhibit this operon.
3. **Assessed Outcome**
    a. Complete the **tryptophan operon** and model of the **trp operon**
4. **Key Vocabulary** structural genes operon operator promoter tryptophan repressor
5. **Materials and LabXchange Pathway(s)** Gene Regulation: Operon Theory
   Modeling Operons
6. **Teacher Preparation**: Review Tryptophan Operon
7. **Lab Safety Considerations** Not applicable
8. **Sequence of Activities**

Activity Description	*Time*	*Materials*
1. Read the text in LabXchange	20 min	Gene Regulation: Operon Theory
2. Complete the Reading Guide Tryptophan Operon	20 min	See Appendix B **The Tryptophan Operon**
3. Perform the activity in **Modeling Prokaryotic Operons (Carolina)** for the Trp Operon Modeling	1 hour	**Modeling Prokayotic Operons** From Carolina Biological
4. Presentations. Have students present models with an emphasis on activated and repressed states	30 min	

Lesson 3 (2–3 hours)

Gene Regulation: Inducible Operons: The Lactose & Arabinose Operons

1. **Overview:** Inducible operons are inactive to the binding of the repressor protein to the operator. When an inducer binds to the repressor, a conformational change occurs in the repressor resulting in the removal of this molecule from the operator. For the lactose (lac) operon, this inducer is allolactose (an isomer of lactose) whereas in the arabinose operon, the inducer is the sugar arabinose. In both cases, the presence of the inducer turns on the gene. However, there is a second operon that inhibits the operon due to the presence of high glucose levels. This modulation is called catabolite activation. For transcription of these operons, the CRP cAMP complex must bind to the. When glucose levels are high, the CRP cAMP complex is removed and transcription of the operon structural genes is reduced or halted. Thus, two conditions must be present for the operon to be active. For the lac operon, allolactose levels must be high and glucose levels low; similarly, for the arabinose operon, arabinose levels must be high and glucose levels low.
2. **Learning Goals**:
    a. Identify the structural components of the lac and arabinose operons
    b. Describe the conditions that activate and inhibit these operons
3. **Assessed Outcome:**
    a. Complete the **Lactose and Arabinose Operons** Reading Guide
    b. Create the Lactose Model (Modeling Prokaryotic Organism)
4. **Key Vocabulary:** arabinose operon lactose operon lactose Arabinose repressor catabolite activator protein Cyclic AMPpromoter operator
5. Materials and LabXchange Pathway(s): The Lac Operon: An inducible Operon, Arabinose Operon, Modeling Prokayotic Operons
6. **Teacher Preparation** Review the lactose and arabinose operons
7. **Lab Safety Considerations** Not applicable
8. **Sequence of Activities**

Activity Description	*Time*	*Materials*
1. Read the text in LabXchange **The Lac Operon: An Inducible Operon** and the text **Arabinose Operon** from the Biology Reader	20 min	The Lac Operon: An inducible Operon Arabinose Operon
2. Complete the Reading **Guide Lactose & Arabinose Operons** and	20 min	**The Lactose & Arabinose Operons**

Activity Description	*Time*	*Materials*
		See Appendix C
3. Perform the activity in **Modeling Prokaryotic Operons (Carolina)** for the Trp Operon Modeling	1 hour	**Modeling Prokayotic Operons** From Carolina Biological
4. Presentations. Have students present models with an emphasis on activated and repressed states	30 min	

Lesson 4 (6 hours)

Gene Regulation: A Scientific Inquiry into the Gene Regulation of the RFP (Red Fluorescent Protein) of the PARA-R plasmid

1. **Overview**: The transformation lab of the ABE *Foundations of Biotech* labs demonstrates that the expression of the *rfp* gene (red fluorescent protein) is dependent upon the presence of the arabinose. The LB/amp/ara (Luria Bertani, ampicillin, arabinose agar) plates are used to select for PARA-R plasmids that contain the ampicillin resistant and *rfp* genes. The *rfp* gene has been linked to the pBAD promoter which is naturally found in the arabinose operon. The pBAD contains two operator sequences: one binds to the ARA C repressor protein and the second operator binds to the CRP camp activator protein. Arabinose removes the ARA C protein and low glucose levels result in the binding of CAP cAMP complex to the CAP site. In this lesson, students will explore the conditions that activate and repress the expression of the *rfp* gene.
2. **Learning Goals**:
    a. Conduct research on what sugars will induce and repress the pBAD promoter. Possible suggestions are **arabinose, xylose, glucose, IPTG, lactose,** & **sucrose**
    b. Design an experiment to test various percent concentrations of these sugars on the growth of *E. coli* that have been transformed with PARA-R plasmid. Possible suggestions are 1% and 10 % solutions of the above listed sugars.
    c. Engage in a discussion with classmates and the instructor on analysis of the results from this experiment
3. **Assessed Outcome**: Construct a lab report or a presentation on a trifold board which include **introduction, Materials & Methods, Results, and Discussion**
4. **Key Vocabulary:** arabinose lactose sucrose glucose
   IPTG (Isopropyl ß-D-1-thiogalactopyranoside) xylose
   LB broth ampicillin autoclave agar
5. **Materials and LabXchange** Pathway(s) Refer to Appendices D, E, F, G
6. **Teacher Preparation**: Review protocols and prepare agar plates if not prepared by students. Review MSDS sheets for reagents involved
7. **Lab Safety Considerations**: Review MSDS sheets for all reagents and chemicals involved. Examples: **arabinose, xylose, glucose, IPTG, lactose,** & **sucrose, ampicillin.** Review the proper handling and disposal of E. coli.

8. Sequence of Activities

Activity Description	*Time*	*Materials*
1. Conduct research on sugars that could induce or repress the expression of the *rfp* protein	15–60 min	Online Resources, textbooks, • AraC protein, regulation of the L-arabinose operon in Escherichia coli, and the light switch mechanism of AraC action • Positive Transcription Control: The Glucose Effect • Regulation of Arabinose and Xylose Metabolism in Escherichia coli • arabinose operon
2. Discussion on the results of the literature research	15–60 min	Class Discussion led by instructor. Use whiteboard, Google docs, etc. to facilitate discussion
3. State a hypothesis with respect to the various sugars and design an experiment to test the hypothesis. Students may pour agar plates or teacher may do this in advance based on students' agar recipes.	30–60 min	LB agar, ampicillin, 1 % arabinose See Appendix D for agar preparation, Appendix E for sterile filtration, and Appendix F for transformation and plate streaking
4 Perform the experiment dispensing 100 µL of competent *E. coli* cells that have been transformed with the PARA-R plasmid.	30 min	P-200 micro pipettor Prepared agar plates with various sugars
5. Incubate the agar plates overnight in an incubator set at 37°C.	Overnight incubation	Incubator set @ 37°C
6. Record the results of the bacterial colonies with respect to expression of the red pigment.	15–30 min	Lab notebook, gloves, disinfectant
7. Engage in a class discussion on an analysis of the results- which sugars resulted in the repression of the gene and which resulted in the expression of the gene.	30–60 min	Whiteboards, poster paper

Activity Description	*Time*	*Materials*
8. Complete the **An Investigative Study on the Effects of Various Sugars on the Expression of the RFP Gene of Para-R Plasmid.**	30 min	Appendix G **An Investigative Study on the Effects of Various Sugars on the Expression of the RFP Gene of Para-R Plasmid**
9. Optional Activity Draw diagrams pBAD operon loop in the repressed state and with loop unfolded in the repressed state.	30–45 min	Poster board, markers, construction paper

Acknowledgments

First and foremost, I wish to thank the contributions and assistance from Dr. Wendie Johnston, director of Pasadena Bio Collaborative and her dedicated research team: Lia Meza, Isabella Ajemian, Lauren Hsu, Taylor Waimrin, Mark Kugel, Max Shen, and Jocelyne Ruiz, Lauren Hsu, Amanda Zhou, Diana Mena, and Danielle Brillantes. Dr. Raj Perera of Poseidon, LLC generously donated the sugars used in this experiment and offered suggestions on the effects of IPTG. When I first suggested my ideas, Dr. Johnston said this is "going to be fun" and generously offered the services of her facility and her student summer interns. Renee Bixby, Pasadena distribution site coordinator, also gladly assisted me with *E. coli* that had been transformed with para-R plasmid.

I also want to thank Shahira Badran, Amgen Biotech Experience program site director – Greater LA Area, LA Promise Fund, and to Marty Ikkanda and Karin Steinhauer of Pierce College, for listening to my ideas. Lastly, I want to thank the Master Teacher Fellowship Program directors for the support and guidance: Courtney Arthur, Dr. Candice Johnson, and Jessica Juliuson. I also want to extend a big Thank You to all of the MFT 2023 cohort colleagues: Ingeborg van der Neut, Julie Pankowicz, Teresa Harris, Sydney Johnson, Susan Senior, Chris Oskam, Aysegul Altun, Sarah Sanford, and Colleen Simon-O'Neill.

Appendix A

Structural Components of the Prokaryotic Operon

Directions: Read the text **Gene Regulation: Operon Theory** in Labxchange and describe the function of the following operon terms:

Regulatory Genes:

Structural Genes:

Operon:

Operator:

Promoter:

Activator:

Inducer:

Repressor:

Question: Discuss the similarities and differences among **constitutive, inducible, and repressible operons**

Appendix B
The Tryptophan Operon

Read the text **Gene Regulation: Operon Theory** in Labxchange and describe the function of the following operon terms:

1. Describe the regulatory genes of the trp operon.

2. What is the biosynthetic outcome of the structural genes of the trp operon?

3. Under what conditions is transcription initiated? Under what conditions is transcription repressed?

4. Explain how the mechanism of activation and repression of this operon works with a focus on the roles of tryptophan and the repressor.

Appendix C

The Lactose & Arabinose Operons

Read **Gene Regulation: Operon Theory** in Labxchange and answer the following questions:

1. Identify the inducer molecules for the lactose and arabinose operons respectively, and indicate the role these molecules play in the transcription of these operons.

2. A second site is necessary for transcription to occur. The CAP site, which binds the Catabolite Activator Protein, enhances transcription. This protein binds to cyclic AMP (cAMP), and the resulting complex binds to the site in the promoter. Cyclic AMP levels are high when this sugar is low. What sugar is it?

3. The structural genes of these two operons code for enzymes involved in the digestion of certain sugars. Name the two sugars that are digested in the lactose and arabinose operons respectively.

4. Explain why transcription of these operons is inactivated when glucose levels are high? What is the evolutionary advantage of this?

Appendix D

Preparation of Agar plates (grid 1)

1. Dissolve 40 g of LB agar media in 1000 mL of DI water.
2. Autoclave at 121°C for 30 minutes (this is preferred to adding sugars prior to autoclaving to avoid the charring of sugars)
3. Sterile filter the sugar solutions
4. Cool the agar to 55°C and add the appropriate sugar solution and 1ml of ampicillin (100 ug/mL)
5. Pour approximately 20 mL of agar per plate.

Agar Plates	**Arabinose**	**Glucose**	**Xylose**	**IPTG (Isopropyl ß-D-1-thiogalactopyranoside**	**Lactose**
1 (Control) LB/amp/ara	1g/1000mL 0.1 % solution	NA	NA	NA	NA
2 LB/amp/ara/glu	1g/1000 mL 0.1 % solution	1g/1000mL 0.1 % solution	NA	NA	NA
3 LB/amp/ara/xyl	1g/1000mL 0.1 % solution	NA	1g/1000mL 0.1 % solution	NA	NA
4 LB/amp/ara/IGTP	1g/1000mL 0.1 % solution	NA	NA	1g/1000mL 0.1 % solution	NA
5 LB/amp/ara/lac	1g/1000mL 0.1 % solution	NA	NA	NA	1g/100mL 0.1 % solution
6 LB/amp/ara/glu	1g/1000mL 0.1 % solution	10g/1000mL 1.0 % solution	NA	NA	NA
7 LB/amp/ara/xyl	1g/1000mL 0.1 % solution	NA	10g/1000mL 1.0 % solution	NA	NA
8 LB/amp/ara/IGTP	1g/1000mL 0.1 % solution	NA	NA	10g/1000mL 1.0 % solution	NA
9 LB/amp/ara/lac	1g/1000mL 0.1 % solution	NA	NA	NA	10g/1000mL 1.0 % solution

Store the plates at 4°C.

Alternate Preparation of Agar plates (grid 2)

(Modified by Wendie Johnston PhD Lab Director of Pasadena Bio Collaborative & Raj Perera Phd, Poseidon LLC)

In this grid, two approaches were used, the agar poured in plates 1 to 3, 7 to 9 had the sugars autoclaved; plates 4 to 6, 10 to 12 had the sugars sterile filtered.

	Plates - striped red/green/dark blue	**Ampicillin**	**Glucose (add light blue stripe)**	**Sucrose (add 2nd green stripe)**	**Lactose (add orange stripe)**
Control	LB-(amp)-ara	added after autoclaved (55C)	0	0	0
#1	LB-(amp)-ara	added after autoclaved (55C)	0.1% autoclaved		
#2	LB-(amp)-ara	added after autoclaved (55C)		0.1% autoclaved	
#3	LB-(amp)-ara	added after autoclaved (55C)			0.1% autoclaved
#4	LB-(amp)-ara	added after autoclaved (55C)	0.1% sterile filter added after autoclave		
#5	LB-(amp)-ara	added after autoclaved (55C)		0.1% sterile filter added after autoclave	
#6	LB-(amp)-ara	added after autoclaved (55C)			0.1% sterile filter added after autoclave
#7	LB-(amp)-ara	added after autoclaved (55C)	1.0% autoclaved		
#8	LB-(amp)-ara	added after autoclaved (55C)		1.0% autoclaved	

	Plates - striped red/green/dark blue	Ampicillin	Glucose (add light blue stripe)	Sucrose (add 2nd green stripe)	Lactose (add orange stripe)
#9	LB-(amp)-ara	added after autoclaved (55C)			1.0% autoclaved
#10	LB-(amp)-ara	added after autoclaved (55C)	1.0% sterile filter added after autoclave		
#11	LB-(amp)-ara	added after autoclaved (55C)		1.0% sterile filter added after autoclave	
#12	LB-(amp)-ara	added after autoclaved (55C)			1.0% sterile filter added after autoclave

Appendix E

SOP – Produce sterile filtered arabinose for induction of overnight cultures

(Courtesy of Wendie Johnston, PhD)

1. Recipe for arabinose = 5 mg arabinose per mL of broth.
2. One overnight culture flask = 45 mL broth.
3. Determine how many mg of arabinose is necessary for one “induction dose”
4. One induction dose is contained in 1 mL of DI water.
5. Calculate how many overnight flasks need induction and make at least 1 more mL because the syringe filter will absorb some.
6. Weigh the total amount of arabinose
7. Put the total amount of water into a beaker large enough to allow the syringe to enter to aspirate the finished product.
8. Stir to get arabinose into solution
9. Label 1.5 mL sterile microfuge tubes as Ara and set them in a microfuge rack
10. Choose a syringe with appropriate capacity and aspirate the arabinose solution
11. Place the syringe filter on the syringe
12. Invert the syringe and expel trapped air.
13. Pick up an open microfuge tube and hold it at eye level
14. Push the syringe plunger to deliver 1.0 mL arabinose solution into the microfuge tube
15. Close the cap on the tube
16. Repeat until all tubes are filled.
17. Discard the syringe and filter into general lab trash.
18. Wash and replace the beaker and syringe.

Appendix F

Protocol for Plating Transformed E. coli using Cell Spreaders (Use with Grid 1 Appendix D)

1. Set the heat block to 42°C. Get a p-200 pipettor and tips.
2. Label one clean microfuge tubes “P–” and three “P+.”
3. Place the P– and 4 P+ tubes and tube with competent cells in floaters in slushy ice.
4. Add 50 µL of competent cells to the P– and P+ tubes. Before aspirating the cells, re-suspend them by gently pumping with the pipettor two times in the solution. Keep the tubes chilled.
5. Add 10.0 µL of Recombinant Plasmid (RP) to each tube labeled “P+.”
6. Keep the P– and P+ tubes on ice for 15 minutes.
7. While the cells are on ice, assemble the plates to be inoculated. For each experiment. Label plates 1 to 9 according to grid in addition to the 3 plates LB, LB/amp, LB/amp/ara.
8. Label each plate on the bottom with appropriate designation
9. Following the 15-minute incubation on ice, put the P– and P+ tubes in the 42°C heat block for exactly 45 seconds.
10. After the 45-second heat shock, immediately place the tubes back on ice for at least one minute.
11. Then add 150 µL LB broth to the P– and P+ tubes. Cap the tubes and flick gently 2-3 times to mix.
12. Allow the cells in the P– and P+ tubes to incubate at room temperature for 15 minutes.
13. Dispense 50 µL of P- tube contents into each P- plate and streak the plates suing a sterile spreader plate.). Place the used spreaders in the autoclave trash container.
14. Dispense 50 µL from the P+ tubes into each of the nine plates. Streak each of the plates with a sterile spreader Place the used spreaders in the autoclave trash container.
15. Allow the plates to sit closed and right side up for 5 minutes.
16. Incubate the plates upside down for 24 hours at 37°C.
17. Document results next day.

Protocol for Plating Transformed E. coli using Disposable Loops (Use with Grid 2 Appendix D) (Courtesy of Wendie Johnston, PhD)

1. Set the heat block to 42°C. Get a p-200 pipettor and tips.
2. Label two clean microfuge tubes "P–" and "P+."
3. Place the P– and P+ tubes and tube with competent cells in floaters in slushy ice.
4. Add 50 µL of competent cells to the P– and P+ tubes. Before aspirating the cells, re-suspend them by gently pumping with the pipettor two times in the solution. Keep the tubes chilled.
5. Add 10.0 µL of Recombinant Plasmid (RP) to the tube labeled "P+."
6. Keep the P– and P+ tubes on ice for 15 minutes.
7. While the cells are on ice, assemble the plates to be inoculated. For each experiment (1–12) use one control (LB-amp-ara) and 2 experimental plates. Label the plates to be inoculated – on the bottom around the edge with your name, date and number of experiment (#1–12; see clipboard).
8. With the plates closed, use a permanent marker to draw a line on the bottom of each plate to divide each plate in half. Label one half "P–" and the other half "P+."
9. Following the 15-minute incubation on ice, put the P– and P+ tubes in the 42°C heat block for exactly 45 seconds.
10. After the 45-second heat shock, immediately place the tubes back on ice for at least one minute.
11. Then add 150 µL LB broth to the P– and P+ tubes. Cap the tubes and flick gently 2-3 times to mix.
12. Allow the cells in the P– and P+ tubes to incubate at room temperature for 15 minutes.
13. With a disposable loop swirl content of P– tube to suspend the cells. Then with that loop, draw a line of cells on the P– side of the LB amp ara plate. Using the same loop, gather a loopful of P– cells and inoculate both P- sides of the experimental plates. (If one loopful will not do two inoculations, use a new loop for the second experimental plate.) Place the used spreaders in the autoclave trash container.
14. With a disposable loop swirl content of P+ tube to suspend the cells. Then with that loop, draw a line of cells on the P+ side of the control plate. Using the same loop, gather a loopful of P+ cells and inoculate both P+ sides of the two experimental plates. (If one loopful will not do two inoculations, use a new loop for the second experimental plate.) Place the used spreaders in the autoclave trash container.
15. Allow the plates to sit closed and right side up for 5 minutes.
16. Incubate the plates upside down for 24 hours at 37°C.
17. Document results next day.

Appendix G

An Investigative Study on the Effects of Various Sugars on the Expression of the RFP Gene of Para-R Plasmid

1) Describe the purpose of your study and why do you select certain sugars?

2) State a Hypothesis.

3) Refer to Appendices D, E, and F for Materials and Methods. Please describe any modifications.

4) Design a data table grid to record the results.

5) Record your results.

Discussion of Results

6) Which plates displayed red colonies? Which plates displayed white colonies?

7) Explain which sugars interacted with the pBAD promoter. How did these sugars interact with this promoter to induce or repress the expression of the red fluorescent protein?