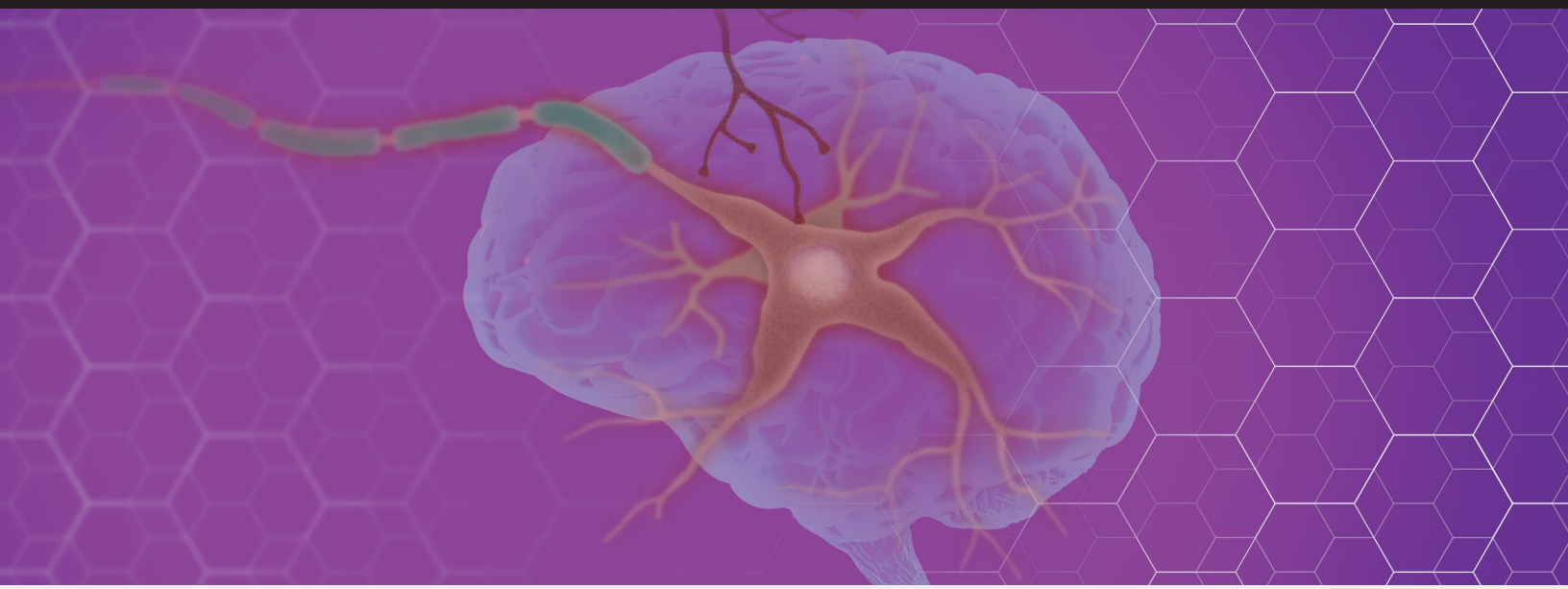
BIO-RAD Explorer



The Science of Opioid Dependence kit

Catalog #17005316EDU

Instructor's Guide

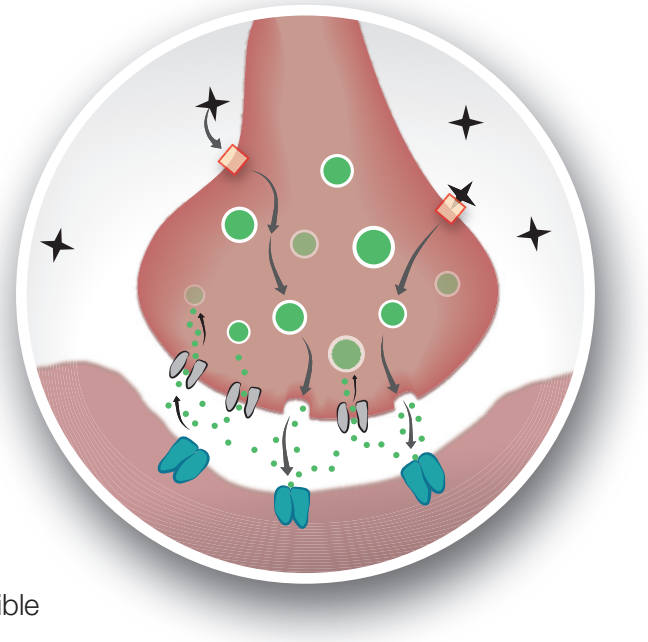
For technical services, call your local Bio-Rad office, or in the U.S., call 1-800-424-6723.

BIO-RAD

Dear Instructor

Thank you for fostering curiosity in our future scientists and citizens as they prepare for an exciting future in which they must think critically, solve problems, collaborate, and communicate effectively.

Susceptibility to opioid dependence is a very complex trait and is an example of a latent phenotype that relies upon environmental factors for its expression. If an individual is never exposed to nonendogenous opioids, they will never display dependence. Conversely, an individual with some genetic predisposition to dependence may be more likely to become dependent if exposed to alcohol, drug, or sexual abuse in their home environment.



The Science of Opioid Dependence Kit is designed to emphasize the complexity of opioid dependence and the many environmental, behavioral, and genetic factors that impact it. Students are first led through environmental factors influencing opioid dependence in the context of prescriptions for pain. Then, they design a human genetic research study to investigate a possible genetic effect. As part of the study, they will use agarose gel electrophoresis on simulated patient DNA samples to determine the genotype for a SNP implicated in opioid dependence. Students use statistical reasoning to analyze the results of their investigation, as well as published data on other genetic links, and construct evidence-based explanations. Finally, your students will examine and discuss the broader ethical questions around the issue of opioid dependence.

We strive to continually improve our curriculum and products, and your input is extremely important to us. We welcome your stories, comments, and suggestions.

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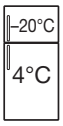
Contents

Kit Storage

When you receive the Science of Opioid Dependence Kit:

1. Put the **DNA Electrophoresis Samples bag** in the freezer (–20°C). For short-term storage, the reagent bag can be stored in the refrigerator (4°C) for up to 1 month.



2. Store the agarose, **50x TAE buffer, and Orange G Loading Dye** at room temperature (20°C).

3. If using, **UView 6x Loading Dye and Stain** should be stored at 4°C.

4. Visit bio-rad.com/opioidmanuals to download the Instructor's Guide and Student Guide and to find additional resources.

Technical Support is available at support@bio-rad.com or 1-800-4BIORAD, option 2.

Safety Guidelines

Proper safety precautions such as no eating or drinking should be practiced. Protective eyewear and gloves are recommended. Students should wash their hands with soap before working in the laboratory. If any of the solutions get into students' eyes, flush with water for 15 minutes. Fast Blast DNA Stain is not toxic but will stain hands and clothes. Gloves and lab coats are recommended to prevent stains. Use appropriate personal protective equipment when using UV transilluminator, UV light can irradiate skin and eyes.

Considerations for Sensitive and Complex Subject Matters

Some of your students may have personal connections to the issues around opioid dependence. They may know someone who has been affected or they may harbor misconceptions about the causes of dependence. Be sure that all instruction is focused on the simulated patients and study participants in the activities and not your students. Before addressing this sensitive topic in the classroom, you may want to prepare yourself to provide additional support for your students. Appendix D has some resources to assist.

Recently, researchers have demonstrated genetic associations with opioid use disorder (OUD). However, the genetic contribution is complex and the clinical use of this information is still controversial. It is important to help students understand that addiction is a recognized neurological condition. It is not due to a lack of willpower nor is it a moral failing, and it is not done on purpose. Also, genetic factors only *contribute* to the risk of dependence, they do not ensure it.

These discussions with your students can provide great opportunities to discuss genetic privacy, precision medicine, and genetic counseling in a new context. Be sure students understand that these topics are not straightforward and that research and policies are still in development. See Appendix D and References for additional resources.

Kit Components

Each kit contains materials for 8 student workstations.

Science of Opioid Dependence Kit	**Quantity**
1.5 ml EZ Micro Test Tubes	90
Molecular weight ruler	200 µl
DNA Sample 1	215 µl
DNA Sample 2	250 µl
Orange G Loading Dye, 5x	1 ml
Fast Blast DNA Stain Electrophoresis Reagents	
Fast Blast DNA Stain	100 ml
Certified Molecular Biology Agarose	5 g
TAE Electrophoresis Buffer, 50x	100 ml
UView 6x Loading Dye and Stain Electrophoresis Reagents	
UView 6x Loading Dye and Stain	200 µl
Certified Molecular Biology Agarose	5 g
TAE Electrophoresis Buffer, 50x	100 ml

Required Materials (not included in this kit)	**Quantity**
20–200 µl adjustable-volume micropipet and tips	1
2–20 µl adjustable-volume micropipet and tips	8
or 10 µl fixed-volume micropipet and tips	8
Microcentrifuge tube rack	8
Horizontal gel electrophoresis chamber	4–8
Power supply	1–8
UV transilluminator (if using UView 6x Loading Dye and Stain)	1
Marking pen	8
Waste container	8
Gel staining tray	4–8
Calculators	8–32

Microcentrifuge tubes, molecular weight ruler, DNA Sample 1, DNA Sample 2, and Orange G Loading Dye, 5x

Fast Blast DNA Stain, Certified Molecular Biology Agarose, and TAE Electrophoresis Buffer, 50x

UView 6x Loading Dye and Stain, Certified Molecular Biology Agarose, and TAE Electrophoresis Buffer, 50x

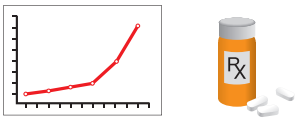
Ordering Information

Catalog #	Description
17005316EDU	**Science of Opioid Dependence Kit**
17005313EDU	**Science of Opioid Dependence Kit plus UView Electrophoresis Reagents**
17005297EDU	**Science of Opioid Dependence Kit plus Fast Blast Electrophoresis Reagents**
1660450EDU	**Small Fast Blast Electrophoresis Reagent Pack**, includes 25 g agarose powder, 100 ml 500x Fast Blast DNA Stain, 100 ml 50x TAE electrophoresis buffer
1660462EDU	**Small UView Electrophoresis Reagent Pack**, includes 25 g agarose powder, 1 ml UView 6x Loading Dye and Stain, 100 ml 50x TAE electrophoresis buffer
1613116EDU	**Certified Molecular Biology Agarose**, 5 g
1613100EDU	**Certified Molecular Biology Agarose**, 25 g
1660742EDU	**TAE Electrophoresis Buffer, 50x**, 100 ml
1665111EDU	**UView 6x Loading Dye and Stain**, 200 µl
1665112EDU	**UView 6x Loading Dye and Stain**, 1 ml
1613015EDU	**1% TAE Mini ReadyAgarose Precast Gel**, 7.1 x 10 cm, 8-well
1660551EDU	**Classroom Adjustable-Volume Micropipet**, 2–20 µl
1660512EDU	**Fixed-Volume Micropipet**, 10 µl
1660506EDU	**Professional Adjustable-Volume Micropipe**t, 2–20 µl
1660507EDU	**Professional Adjustable-Volume Micropipet**, 20–200 µl
1660531EDU	**UView Mini Transilluminator**
1645050EDU	**PowerPac Basic Power Supply**
1664000EDU	**Mini-Sub Cell GT Cell**
1660481EDU	**Green racks**, set of 5
2239480EDU	**1.5 ml EZ Micro Test Tubes**, clear, 500

Kit Activity Overview

Activity 1

Learning about Opioids, Reward Pathways, and Environmental Factors in Dependence



Engage in the topic of the opioid crisis and reward pathways

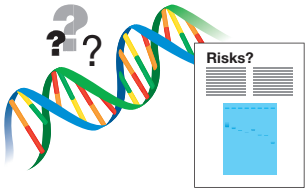
Students review information on the opioid crisis, reward pathways, and pain medication and raise ideas and questions about causes and solutions.

Use an opioid risk tool (ORT) to make prescription recommendations

Students review patient responses on an opioid risk tool to assess risk of future opioid dependence and make a recommendation on prescribing opioids.

Activity 2

Designing a Human Genetic Research Study



Explore other factors for assessing risk

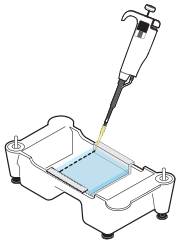
Students identify genetics as a potential factor in assessing risk of dependence.

Design and plan the investigation

Students design a human research study to determine whether SNP rs1800497 is associated with opioid dependence.

Activity 3

Conducting the Research Study



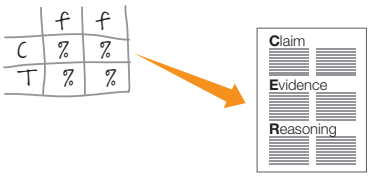
Genotype participant DNA samples

Students complete their experimental design and perform DNA electrophoresis to genotype study samples.

Kit Activity Overview

Activity 4

Analyzing Data and Making a Claim



Collate data, calculate frequencies, and write a scientific explanation

Students collect and collate experimental data and calculate allele and genotype frequencies. They then write an evidence-based scientific explanation.

Activity 5

Establishing Confidence in Data and Addressing the Opioid Crisis

Calculate probability values and review published SNP data

Students learn the concept of probability values (p-values), analyze published data about multiple genes, and reassess their claim.

Address the opioid crisis

Students discuss the broader question of what should be done to address the opioid crisis.

Curriculum Fit and Inquiry Support

Required prior knowledge

- DNA's structure in chromosomes and its role in encoding heritable information
- The role of genes as instructions for an organism to function and develop
- How genes encode proteins, which do much of the work of the cell
- The effect on protein function of mutations in gene DNA sequence
- The basic principles of DNA electrophoresis, restriction digestion, and PCR

Curriculum fit and topic considerations

- **Genetics** — opioid dependence is associated with both environmental and genetic factors. Differences in genotypes can affect how individuals in the population respond to environmental factors. Susceptibility to opioid dependence is a complex genetic trait and research is ongoing to elucidate the genes and SNPs involved. DNA technology allows scientists to investigate these phenomena
- **Neurobiology** — opioids activate reward pathways in the midbrain. Opioids bind to and activate neuron receptors in the reward pathway that communicate feelings of pleasure via cell signaling and dopamine release. Repeated activation of this system produces and perpetuates behaviors that are associated with drug dependence
- **Science practices** — in these activities, students will ask questions to define a problem, then design and plan an investigation to answer an investigation question. As students analyze their data, they will identify patterns and relationships and apply statistical methods to evaluate datasets
- **Ethical, legal, and social issues** — there are many broader issues associated with the opioid crisis, including genetic privacy, government policy, scientific consensus, and medical diagnosis as well as various treatments

Activity timelines

The activities are designed to take 5 days but the timeline can be adjusted as needed. Table 1 offers suggested ways to shorten the activity.

Table 1. Alternative timelines.

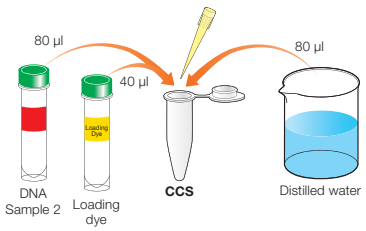
# of Days	In-class Activities	Out-of-Class Assignments
Two	Activity 1 Activity 3 using fast gel results; begin Activity 4	Activity 2, Part 1 Remainder of Activity 4
Three	Activity 1 Activity 2, Part 2 Activity 3 with fast gel results; begin Activity 4	Activity 2, Part 1 Remainder of Activity 4
Four	Activities 1–4 Or Activities 1, 3, 4, and 5	Activity 5 Activity 2, if student study design is desired
Five	As prescribed in the full manual	As prescribed in the full manual

Preparation Instructions

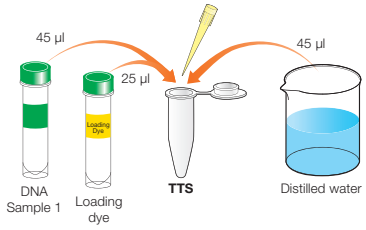
Reagent preparation will take 1–2 hours and may be done up to one week ahead of Activity 3. Store the DNA samples in the refrigerator for one month or at –20°C for up to one year.

Prepare DNA sample stocks and molecular weight ruler

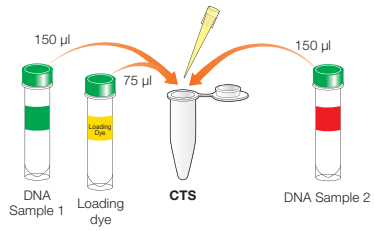
1. **CC DNA stock.** Label a microtube **CCS**. Using a fresh tip each time, add 80 µl DNA Sample 2, 80 µl distilled water, and 40 µl Orange G Loading Dye or UView 6x Loading Dye and Stain; pipet to mix.



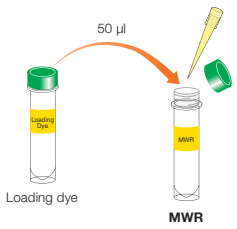
2. **TT DNA stock.** Label a microtube **TTS**. Using a fresh tip each time, add 45 µl DNA Sample 1, 45 µl distilled water, and 25 µl Orange G Loading Dye or UView 6x Loading Dye and Stain; pipet to mix.



3. **CT DNA stock.** Label a microtube **CTS**. Using a fresh tip each time, add 150 µl DNA Sample 1, 150 µl DNA Sample 2, and 75 µl Orange G Loading Dye or UView 6x Loading Dye and Stain; pipet to mix.

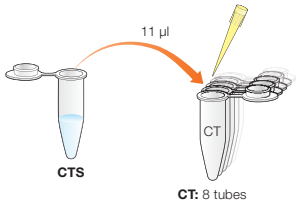


4. **Molecular weight ruler.** Using a fresh tip add 50 µl Orange G Loading Dye or UView 6x Loading Dye and Stain to the molecular weight ruler; pipet to mix.

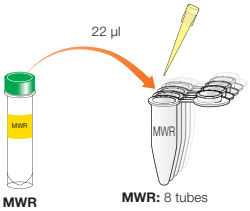


Dispense the DNA samples and molecular weight ruler

5. **CT PCR control DNA samples.** Label 8 microtubes **CT** and add 11 µl of CT DNA stock (**CTS**) to each tube.



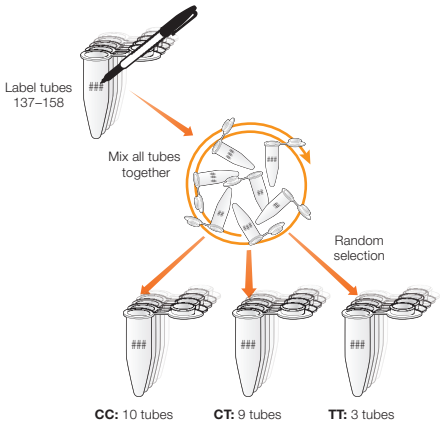
6. **Molecular weight ruler.** Label 8 tubes **MWR**. Use a fresh pipet to add 22 µl of molecular weight ruler to each tube.



7. **Participant DNA samples**.

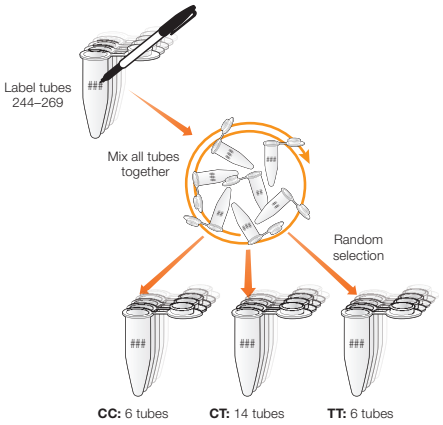
Control samples

a) Label 22 tubes 137 through 158. These will be control samples.
b) Randomly select tubes from this set for each genotype according to the numbers in Table 2 below.
c) Record the genotype for each tube in Table 3.



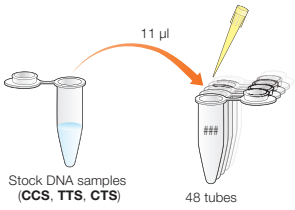
Case samples

d) Label 26 tubes 244 through 269. These will be case samples.
e) Randomly select tubes from this set for each genotype according to the numbers in Table 2 below.
f) Record the genotype for each tube in Table 3.



All samples

g) Add 11 µl of the appropriate DNA sample stock (**CCS**, **TTS**, **CTS**) into each tube.



Note: The DNA used in this kit simulates the PCR products of a genetic test by producing representative bands on an agarose gel following electrophoresis. The actual DNA sequence does not align with the region of the rs1800497 SNP.

Table 2. Number of tubes per genotypes.

	Genotype		
	CC	**CT**	**TT**
Control samples 137–158	10 tubes	9 tubes	3 tubes
Case samples 244–269	6 tubes	14 tubes	6 tubes

Note: Maintaining the proportion of genotypes within the categories Control and Case is required for the genotype and allelic frequencies to align with published data. Record the tube number for each genotype in Table 3.

Table 3. Instructor's record of tube numbers and genotypes.

Control sample tube numbers				Case sample tube numbers			
Control #	**Genotype**	**Control #**	**Genotype**	**Case #**	**Genotype**	**Case #**	**Genotype**
137		150		244		257	
138		151		245		258	
139		152		246		259	
140		153		247		260	
141		154		248		261	
142		155		249		262	
143		156		250		263	
144		157		251		264	
145		158		252		265	
146				253		266	
147				254		267	
148				255		268	
149				256		269	

Prepare agarose gels, TAE buffer, and stain (Fast Gel Protocol only)*

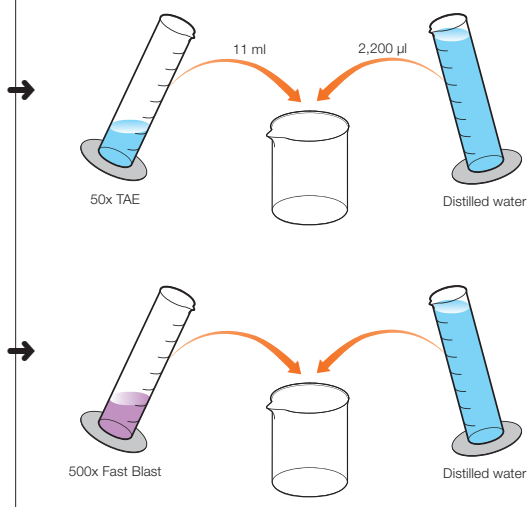
8. Prepare eight 1% TAE agarose gels with 8 wells each. It is essential to use 1x TAE (not 0.25x TAE) to make these gels. See Appendix B for gel casting instructions.

9. Prepare 2.2 L of 0.25x TAE electrophoresis buffer by adding 11 ml of 50x TAE to 2,200 ml of distilled water to use with the Fast DNA Gel Protocol.*

   Note: the correct volume of distilled water would be 2,189 ml, but the difference does not affect results.

10. If using, prepare 100x Fast Blast DNA stain for overnight staining of agarose gels. See Appendix B for instructions.

    Note: Fast Blast DNA stain is not required if using UView 6x Loading Dye and Stain for visualization.



* This activity is designed to take advantage of Bio-Rad's Fast Gel Protocols using 0.25x TAE running buffer and higher voltages. See Appendix B for details and alternate electrophoresis options with preparation instructions.

Activity 1

Learning about Opioids, Reward Pathways, and Environmental Factors in Dependence

Classroom Preparation

- Prepare video or fact sheets
- Print the pages for Activity 1 from the Student Guide for students

Part 1 (20 min): Engage students in the topic of the opioid crisis and reward pathways

Goal: Students engage in the topic, generate questions, and learn some background information.

1. Show students a video, fact sheet, and/or a prompt such as the data shown in Figure 1 to elicit their thoughts, questions, and prior knowledge about opioid dependence. See Appendix D for resources.

2. As a class, discuss student observations and record their ideas. "Do your observations signify a crisis or epidemic? Why? Why do you think the opioid crisis has grown so quickly in the U.S.?"

3. Have students review the reading in the Student Guide, *Opioids, Pain, and Reward Pathways.* Revisit their questions from the prior discussion.

Part 2 (25 min): Use an opioid risk tool (ORT) to make prescription recommendations

Goal: Students understand some of the environmental and behavioral factors involved in the risk of opioid dependence.

1. Explain the opioid risk tool to students.

2. Have each student review two patient profiles, calculate their ORT scores, and make recommendations for whether a doctor should prescribe opioids for the patients.

3. Ask students to share their findings and record information for all/most patients as a class.

Number of Deaths by Cause in the United States

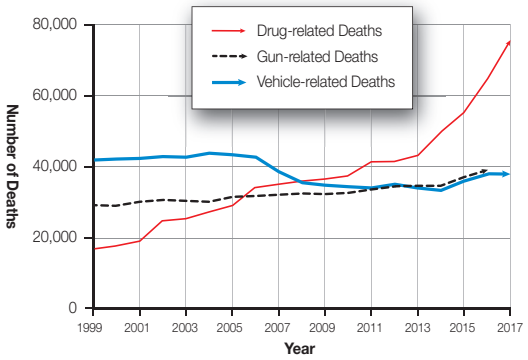


Figure 1. Historical data on deaths from the Centers for Disease Control and Prevention.

Background Information

The Opioid Risk Tool and Prescribing for Pain

Pain is a significant factor in patient recovery. Doctors must balance multiple factors when deciding how to treat a patient's pain, including level, duration, and type of pain, as well as the potential for opioid abuse.

The ORT is one clinical tool available to doctors to quickly assess a patient's risk of developing an opioid use disorder. The ORT calculation is based on multiple factors associated with opioid abuse, including age, a history of sexual abuse, personal and family history of substance abuse, and psychological disorders. Doctors use patient ORT responses to categorize a patient's risk for opioid use disorder as low, medium, or high.

To lower the chance of opioid dependence, doctors can restrict their prescriptions by prescribing a limited dose, requiring light monitoring such as pill counts or urine screening, or giving referral to a pain treatment center that specializes in treating high-risk patients. However, these limitations make treatment plans more cumbersome for patients and can significantly increase treatment costs.

Activity 2

Designing a Human Genetic Research Study

Classroom Preparation

- Print the pages for Activity 2 from the Student Guide for students; *do not distribute until Part 2.*
- (Optional) Have experimental materials available for students to review

Part 1 (10 min): Explore other factors in assessing risk

Goal: Students discover connections between genetics, reward pathway signaling, and susceptibility to opioid dependence.

1. Hold a class discussion about the opioid risk tool (ORT). *Do not distribute handouts until after this discussion.*

- How useful is the ORT for doctors?
- What are the strengths and weaknesses of the ORT? What if patients do not reveal important information?
- What other factors may help doctors assess the risk of drug dependence? If necessary, lead them to genetics
- What sorts of genes might be associated with pain, medication, and drug dependence? How might differences in those genes cause individuals to respond differently to drugs and pain?

2. Have students complete the background readings *Genes Involved in Opioid Signaling* and *Testing for the rs1800497 Genotype* in the Student Guide.

Part 2 (35 min): Design and plan the investigation

Goal: Students apply genetic concepts and research techniques to design a human genetics research study.

1. Introduce the scenario: students will be working with a local university research team to investigate whether there is a genetic association with susceptibility to opioid dependence.

2. Guide student groups as they design investigations to answer the overarching research question "Is there a genetic association with opioid dependence?" using the information and materials provided. Review their designs to ensure they are ready for Activity 3.

Tips & Tricks

Identify student misconceptions

Look out for student language that indicates they harbor the misconception that a person with a high ORT score is sure to have a genotype linked to opioid dependence or vice versa.

Students who have this misconception may not understand that the environmental and genetic factors contribute to risk independently of each other.

Background Information

Environmental Effects on Phenotype Expression

Opioid dependence is an example of a latent phenotype that is influenced by both genotype and environmental factors but cannot be expressed without another specific environmental factor, in this case, access to external opioids. The lab activity and genetic studies reviewed in this exercise demonstrate that when exposed to external opioids, populations with distinct genotypes at a particular SNP respond differently and are more or less likely to become opioid dependent.

Investigation notes when designing the study

Example student investigation questions:

- Is there a genetic association with susceptibility to opioid dependence?
- Is one SNP rs1800947 genotype found more often in people who are dependent on heroin than in healthy people?

- Ask students to make observations about the control and case groups, which represent different populations, and to discuss how the characteristics of the groups can help them to address their investigation questions
- Students should conclude that they need to use agarose gel electrophoresis to determine the genotype of the samples; they should compare the sample genotypes between the control and case groups
- The provided background information is necessary for students to predict the sizes of the DNA fragments and the banding pattern on their gels. Ensure they make predictions for each of the three genotypes, CC, CT, and TT, so they are prepared to interpret their gel results
- Student predictions will vary. Allow them to discover for themselves the associations between genotypes and phenotype as well as the degree of association

Background Information

Taq1 Assay Electrophoresis Results

PCR products from the Taq1 assay will be 532 bp. After the restriction digestion the product from the C allele can be cut by Taq1 and will yield 284 and 248 bp fragments, while the T allele cannot be cut and will remain 532 bp. If a study participant is CT heterozygote, their DNA sample would have 532, 284, and 248 bp fragments. The PCR Control CT represents a heterozygote. See Figure 2.

The two smaller bands, 284 and 248 bp, cannot be distinguished on a 1% TAE agarose gel and would appear as a single band. Students may not realize this at this stage.

This kit includes a single DNA fragment that simulates the merged gel band for the 284 and 248 bp DNA fragments. Separate bands cannot be resolved.

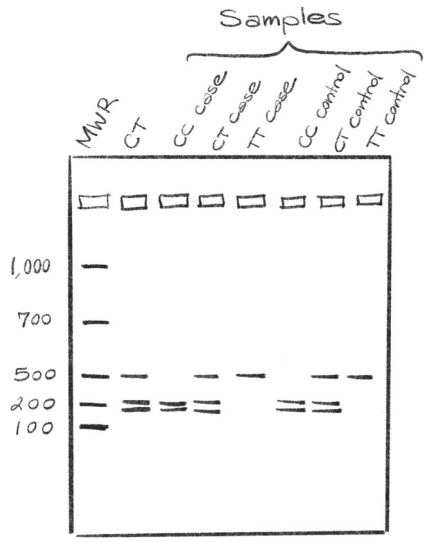


Figure 2. Example of a student's predicted results showing expected DNA band sizes.

Activity 3

Conducting the Research Study

Classroom Preparation

- Prepare student and common workstations (below)
- Print the pages for Activities 3 and 4 from the Student Guide for students

Student Workstation

Materials	Quantity
Molecular weight ruler (MWR), 20 µl	1
Study participant DNA samples, 10 µl	6
PCR control DNA (CT), 10 µl	1
TAE electrophoresis buffer*	300 ml
100x Fast Blast DNA Stain (if using)	50 ml
1% TAE agarose gel with 8 wells	1
Horizontal gel electrophoresis chamber	1
Power supply (may be shared by multiple workstations)	1
Microcentrifuge tube rack	1
Micropipet and tips	1
Gel staining tray (optional)	1
Waste container	1

* Required for Fast Agarose Gel protocol. Using 0.25x TAE buffer with a 1% agarose gel allows for higher voltage and faster gel runs. See Appendix B for details.

Goal: Students carry out their plans and practice experimental techniques.

1. Review the available materials and procedure with students. Introduce the expanded scenario: students will be collaborating with a local university research team to conduct the human genetic study. By doing so, they will have a larger dataset to analyze.

2. Remind students to load 20 µl of MWR and 10 µl of PCR Control DNA along with 10 µl of each of their six participant DNA samples on their gel.

3. Distribute six DNA samples to each student group. *Ensure students record their sample numbers.*

4. Have students conduct their investigations and visualize their DNA gels using Fast Blast DNA Stain or UView 6x Loading Dye and Stain (see Figure 3). See Appendix B for visualization options and protocols.

Tips & Tricks

Taking accurate records

As students complete their investigations and record data, ensure they record any changes to the methods or materials used in their investigation design. A common error is to incorrectly record what was loaded into each well of their gel.

Tips & Tricks

Assigning participant DNA samples

Collectively, your class should run all 48 participant samples. After all the experiments are complete, students will pool their data with the entire class. The data in this kit are designed to be analyzed as a full class set, not per student group. Therefore, how samples are distributed among students for gel analysis is up to you and your students.

Background Information

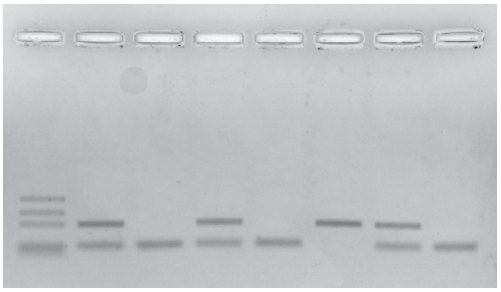


Figure 3: Example of a student gel. Lane 1, MWR with DNA sizes 1,000, 700, 500, 200, and 100 bp. Lane 2, PCR Control DNA (CT) with the full-length PCR product of 532 bp and the merged band for the 248 and 284 bp fragments. Lanes 3–8, six participant DNA sample genotypes. From L to R: CC, CT, CC, TT, CT, and CC.

Activity 4

Analyzing Data and Making a Claim

Classroom Preparation

- Print the pages for Activity 4 from Student Guide for students
- Make students' stained gels available
- Ensure calculators are available

Part 1 (45 min)

Goal: Students interpret agarose gel results, analyze the dataset, and write scientific explanations.

1. Have students sketch and interpret their gel results as directed in the Student Guide. At this stage there should not be any significant patterns.

2. Collect student data in a class data table,* or other shared space; have students summarize the class data in the tables in the Student Guide.

3. Have students combine the class and university data in Tables 6, 7, and 8 in the Student Guide, and then calculate and record the allele and genotype frequencies for the class data.*

- Invite students to highlight any patterns or aspects of the data that support or conflict with their predictions

4. Direct students to answer the claim/evidence/reasoning prompts in the Student Guide and write a scientific explanation based on the collected evidence.

* See Appendix A for a blank class data table and expected results.

Background Information

Example calculations

Genotype frequency

$$\textbf{CC genotype frequency} = \frac{\text{\# participants with CC genotype}}{\text{total \# participants}}$$

Allele frequency

$$\textbf{C allele frequency in controls} = \frac{\text{\# participants with CT genotype} + 2 \times \text{\# participants with CC genotype}}{2 \times \text{total \# participants}}$$

The gene and allele frequencies of SNP rs1800497 in the student samples align with those published in Zhang et al. (2018).

Tips & Tricks

Interpreting complex data

The Identify and Interpret (I2) Strategy from BSCS can help students interpret complex data.

media.bscs.org/icans/Icans_I2_SE.pdf

Activity 5

Establishing Confidence in Data and Addressing the Opioid Crisis

Classroom Preparation

- Print the pages for Activity 5 from the Student Guide for students
- Ensure students' work from Activity 1 is available

Part 1 (20 min): Calculate data confidence and review published data

Goal: Students use statistical tools to further interpret their data and consider the complexity of opioid dependence.

1. Discuss and review student insights and questions from their scientific explanations. Ask how confident they are about their conclusions.

2. Have students read the text on p-values in the Student Guide.

3. Have students use chi square analysis to calculate p-values for the allele and genotype frequencies in the class data or provide the p-values from Appendix A. The data have been engineered so that p = 0.048 and the frequencies are aligned to the results in Zhang (2018).

4. Review the published frequency and p-value data in Table 9 in the Student Guide with students, and ask for their observations. "How do the data for rs1800497 compare with ours? Which studies are significant? Do these data strengthen or weaken your claim?"

- Students may notice the data for rs1800497 have a lower p-value but similar frequencies — there is a larger number of participants in the published study
- 6 out of the 10 alleles show significant ($p < 0.05$) differences between healthy and opioid-dependent individuals, 4 do not

5. Have students use the data to reexamine their research questions and support or refute their claims with evidence and reasoning.

Tips & Tricks

Scaffold student learning

As an intermediate step toward student proficiency while using chi square analysis, provide students with a link to a chi square calculator, such as socscistatistics.com/tests/chisquare/.

Part 2 (20 min): Addressing the opioid crisis

Goal: Students consider the real world implications of their findings and identify opportunities to influence change.

1. Ask students to use all the information they have learned and review the recommendations they gave for the patients in Activity 1.

2. Discuss their recommendations and revisit factors, including personal, family, and behavioral history and the multiple genes and SNPs involved in the opioid reward pathway.

   - Emphasize that simply having the T allele for the rs1800497 SNP does not mean a person will become opioid dependent

3. Invite and discuss suggestions to address the opioid epidemic.

   - What role does the government have in addressing an issue such as the opioid crisis?
   - What responsibilities do consumers have in a healthcare system?
   - What role do pharmaceutical companies play in a healthcare system?
   - What role does the prescribing physician have in the opioid crisis?

Tips & Tricks

Reading published perspectives

Students may suggest that patients from Activity 1 receive genetic testing before prescribing. Have students read and analyze published articles as they decide whether to recommend genetic testing for patients. Articles can provide other viewpoints for students to consider such as those around genetic privacy and confidence levels in published data. See Appendix D and References for example articles.

Appendix A

Class Data Template and Published SNP Frequency Data

Table 4. Class data table template. Capture genotypes for each study participant from the student groups.

Control sample tube numbers				Case sample tube numbers			
Control	**Genotype**	**Control**	**Genotype**	**Case**	**Genotype**	**Case**	**Genotype**
137		150		244		257	
138		151		245		258	
139		152		246		259	
140		153		247		260	
141		154		248		261	
142		155		249		262	
143		156		250		263	
144		157		251		264	
145		158		252		265	
146				253		266	
147				254		267	
148				255		268	
149				256		269	

Table 5. Expected class data (using 48 samples).

Participant Group	Total	CC	CT	TT
Control	22	10	9	3
Case	26	6	14	6

Table 6. Combined class and university data on genotype frequency.

Genotypes	Number of Participants in Control Group, n	Frequency of Genotype in Control Population	Number of Participants in Case Group, n	Frequency of Genotype in Case Population	p-Value
CC	27	46.5%	20	29.0%	0.115
CT	24	41.4%	36	52.2%	
TT	7	12.1%	13	18.8%	
Total	58		69		

Note: Chi square analysis was performed to determine p-values.

Table 7. Combined class and university data on allele frequency.

Alleles	Number of Alleles in Control Group, n	Frequency of Allele in Control Population	Number of Alleles in Case Group, n	Frequency of Allele in Case Population	p-Value
C	78	67.2%	76	55.1%	0.048
T	38	32.8%	62	44.9%	
Total	116		138		

Note: Chi square analysis was performed to determine p-values.

Appendix B

Fast Gel Protocol and Electrophoresis Preparation Instructions

Fast Gel Protocol

There are multiple ways to hasten visualization of DNA bands on an agarose gel using modified conditions and alternate reagents. Options, as well as the required materials and protocols, are provided below.

1. Cast 1% agarose gels with 1x TAE buffer.
2. Prepare 0.25x TAE electrophoresis buffer.
3. Load samples, run gel using conditions in Table 8, and visualize DNA using one of the stain options below.

Table 8. Electrophoresis options.

Electrophoresis Buffer and Voltage	Electrophoresis Time
0.25x TAE at 300 V*	10 min
0.25x TAE at 200 V	20 min
1x TAE at 100 V	30 min

* Requires power supply capable of voltages over 200 V, such as the PowerPac Basic Power Supply (1645050EDU).

UView 6x Loading Dye and Stain: Prepare DNA samples for electrophoresis using 6x UView Loading Dye and Stain. After gel electrophoresis visualize instantly with a UV transilluminator or a handheld UV lamp in the dark.

Fast Blast DNA Stain: Prepare DNA samples before electrophoresis with 5x Orange G Loading Dye. After electrophoresis, stain DNA with Fast Blast DNA Stain, and visualize the next day.

Preparing Agarose Gels

Cast either eight 7 x 7 cm gels with one 8-well comb for eight workstations or four 7 x 10 cm gels with two 8-well combs each to be shared between two workstations. Table 9 provides measurements for a variety of options.

Table 9. Volumes and quantities of reagents for agarose gels.

Number of Gels	1	4	8	16
1% TAE Agarose Gel (7 x 7 cm) — serves one workstation				
Purified water, ml	39	156	312	624
50x TAE, ml	0.8	3.2	6.4	12.8
Agarose, g	0.4	1.6	3.2	6.4
Total, volume of molten agarose, ml	40	160	320	640
1% TAE Agarose Gel (7 x 10 cm) — serves two workstations				
Purified water, ml	49	196	392	784
50x TAE, ml	1.0	4.0	8.0	16.0
Agarose, g	0.5	2.0	4.0	8.0
Total, volume of molten agarose, ml	50	200	400	800

Prepare molten agarose

1. Add the appropriate amount of agarose powder and then the liquids to a suitable container; fill to less than 75% of the container volume. Swirl to mix.

   Note: If using an Erlenmyer flask, invert a small 25 ml flask over the opening to minimize evaporation. If using a bottle, loosen the cap so that air and steam can escape.

2. Place the agarose solution into the microwave. Microwave for 3 min. Continue to boil in 30 sec increments until the solution boils and all agarose has dissolved.

   Caution: Always wear heat-protective gloves, goggles, and a lab coat while preparing agarose gels. Hot molten agarose can cause severe burns.

3. Let the agarose cool to 60°C before pouring the gels.

Cast agarose gels

There are a variety of ways to cast agarose gels. This section outlines the tape method. Consult the instruction manual for your horizontal electrophoresis system for alternate methods.

1. Firmly seal the ends of a gel tray with standard laboratory tape (not regular sticky tape).

2. Place the comb into the appropriate slot in the gel tray. If pouring a double-well gel, place a comb at one end of the tray and another in the middle.

3. Once the molten agarose has cooled at least to 60°C, pour enough agarose to cover the gel comb teeth or to a depth of 0.5–0.75 cm.

4. Allow the gel to solidify at room temperature for 10–20 min — it will be opaque when ready to use.

5. Carefully remove the comb(s) and the tape.

6. Store gels in a sealable plastic bag at room temperature for up to 1 day or in the refrigerator for up to 1 week.

Preparing TAE Buffer

Conventionally, 1x TAE buffer is used both for gel casting and as running buffer. The electrophoresis time can be greatly reduced by instead running the gels with 0.25x TAE buffer at 200 or 300 V. When using this faster protocol, gels should still be cast using 1x TAE buffer. See Table 8.

1. Combine distilled water with the volume of 50x TAE buffer indicated in Table 10 and mix well.

   Note: If you are using 0.25x TAE buffer sequentially between classes, ensure the buffer is at or below room temperature before using. If the buffer starts out warm, it may become hot enough to melt the agarose gel during a high voltage run.

Table 10. Volumes and quantities of reagents for electrophoresis buffer.

Number of Electrophoresis Chambers	1	4	8	16
0.25% TAE Buffer				
Purified water, ml	274	1,094	2,189	4,378
50x TAE, ml	1.4	5.6	11	22
Total, volume of 0.25% TAE buffer, ml	275	1,100	2,200	4,400

Visualizing DNA

UView 6x Loading Dye and Stain

No additional preparation is needed when using UView 6x Loading Dye and Stain.

1. Replace Orange G Loading Dye with UView 6x Loading Dye and Stain when preparing DNA samples.

2. Directly after electrophoresis, carefully place gels on a UV transilluminator, lower UV shield, and turn on UV light to visualize.

Fast Blast DNA Stain

1. Prepare 100x Fast Blast DNA Stain according to volumes in Table 11.

2. Add ~50 ml of 100x stain per gel in a gel staining tray and gently rock for 2–3 min.

3. After 2 min pour off stain and retain for future use.* Staining longer will greatly increase background.

4. Rinse gel with tap water for 30 sec to 1 min or until all surface stain is removed. Completely cover the gel with a large volume of tap water and gently rock overnight to destain. DNA will be visible as dark blue bands against a lighter blue background after a few hours with contrast gradually increasing overnight.

   Note: Using 100x Fast Blast DNA stain prevents the small DNA fragments from diffusing in the gel during an overnight stain. Because it is lower concentration, 1x Fast Blast DNA stain will not prevent DNA fragment diffusion and so should not be used for overnight staining.

* 100x Fast Blast DNA Stain can be reused at least six times.

Table 11. 100x Fast Blast DNA Stain Preparation.

Number of 7 x 10 cm gels to stain	1	4	8	16
500x Fast Blast DNA Stain, ml	10	40	100	200
Distilled water, ml	40	160	400	800
Total volume of 100x Fast Blast DNA Stain, ml	50	200	500	1,000

Appendix C

Neurobiology Extension Activity

Use the following extension to include a more detailed study of cell signaling and neurobiology in the activity sequence.

Prior to Activity 1

1. Have students draw a model of a brain from a person who is not opioid dependent and a brain from a person who is opioid dependent. Ask them to use their prior knowledge and think about:

   - Regions of brain involved
   - Pathways and molecules involved

After Activity 1

2. Have students revise their models of the brain with any information they learned in Activity 1.

3. Ask students how reward pathways in the brain are beneficial for survival.

4. Reward pathways in some individuals activate at weaker levels than in other individuals.

5. Ask students, “In early humans, how might you expect the varying levels of activation to affect behavior?” How might the differences affect their likelihood to survive?”

   Further extend students’ understanding in these topic areas:

   - Feedback mechanisms in the human brain — feedback mechanisms inhibit risky behaviors, such as drug taking — may be less effective in people who become addicted to drugs
   - Action potential
   - Chemical synapses involving dopamine as well as details about structure and function of the opiate and dopamine receptors
     - Opioids excite dopamine-containing neurons in the VTA, which causes the neurons to produce more action potentials. The increase in action potentials triggers increased amounts of the neurotransmitter dopamine being released into the synapse

   Students can continue to add to and revise their models as they learn.

6. Ask students to use their models to think about the question, “If a dopamine receptor was changed such that the receptor protein bound dopamine less tightly, how might this affect reward pathway activation? Explain your reasoning.”

   Learn Genetics and the Society of Neuroscience have excellent educational resources on basic neuroscience and the genetics of addiction; see Appendix D.

Appendix D

Resources

Organization	Link/Reference	Type of Resource	Description
Bio-Rad	bio-rad.com/opioidkit for additional supporting information	Supplementary documentation	Instruction manual, student guide, bulletins
Newsweek	newsweek.com/new-painkiller-powerful-morphine-sideeffect-1097960 Accessed December 2018.	Video (48 sec)	The Opioid Epidemic By The Numbers. Student engagement video option for Activity 1
MedPage Today	medpagetoday.com/genetics/genetictesting/58207 Accessed December 2018	News article	Potential student reading on genetic testing for drug addiction/treatment predisposition
Public Broadcasting Service (PBS)	pbs.org/wned/opioid-epidemic/for-educators Accessed December 2018.	Videos and educator resources	Lesson plans and short videos from the PBS series, Understanding the Opioid Epidemic
Centers for Disease Control and Prevention (CDC)	cdc.gov/rxawareness/stories/index.html Accessed December 2018.	Videos (30 sec); government website	10 short stories on the personal impact of prescription pain medication
National Institute on Drug Abuse (NIDA)	drugabuse.gov/drugs-abuse/opioids Accessed December 2018.	Government website	Up-to-date and background information. U.S. government resource on opioid abuse. Has clinical advice and data summaries
NIH HEAL Initiative	nih.gov/research-training/medical-research-initiatives/heal-initiative Accessed December 2018.	Government website	Cross-agency U.S. government initiative to hasten scientific solutions to address the opioid crisis; launched April 2018
Opioid Risk Tool	drugabuse.gov/sites/default/files/files/OpioidRiskTool.pdf Accessed December 2018.	PDF document	Opioid risk tool table and instructions
Genetics Science Learning Center	learn.genetics.utah.edu Accessed December 2018.	Educator website with interactive lessons for students, videos, and handouts	Learn Genetics: The Science of Addiction: Genetics and the Brain, Basic Neuroscience, Precision Medicine Extend topics discussed in this activity using resources from GSLC
Society of Neuroscience	brainfacts.org/diseases-and-disorders/addiction Accessed December 2018.	Educator website with videos, articles, and educator resources	Educational website of Society for Neuroscience. Extend topics in brain and neuronal signaling and addiction science
Biological Sciences Curriculum Studies (BSCS)	media.bscs.org/icans/Icans_I2_SE.pdf Accessed December 2018.	Instructional support document	Identify and Interpret (I2) Strategy
Social Science Statistics	socscistatistics.com/tests/chisquare/ Accessed December 2018.	Online calculator	Chi square calculator for simple 2 x 2 contingency calculator. Can use to calculate p-values in Activity 3

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