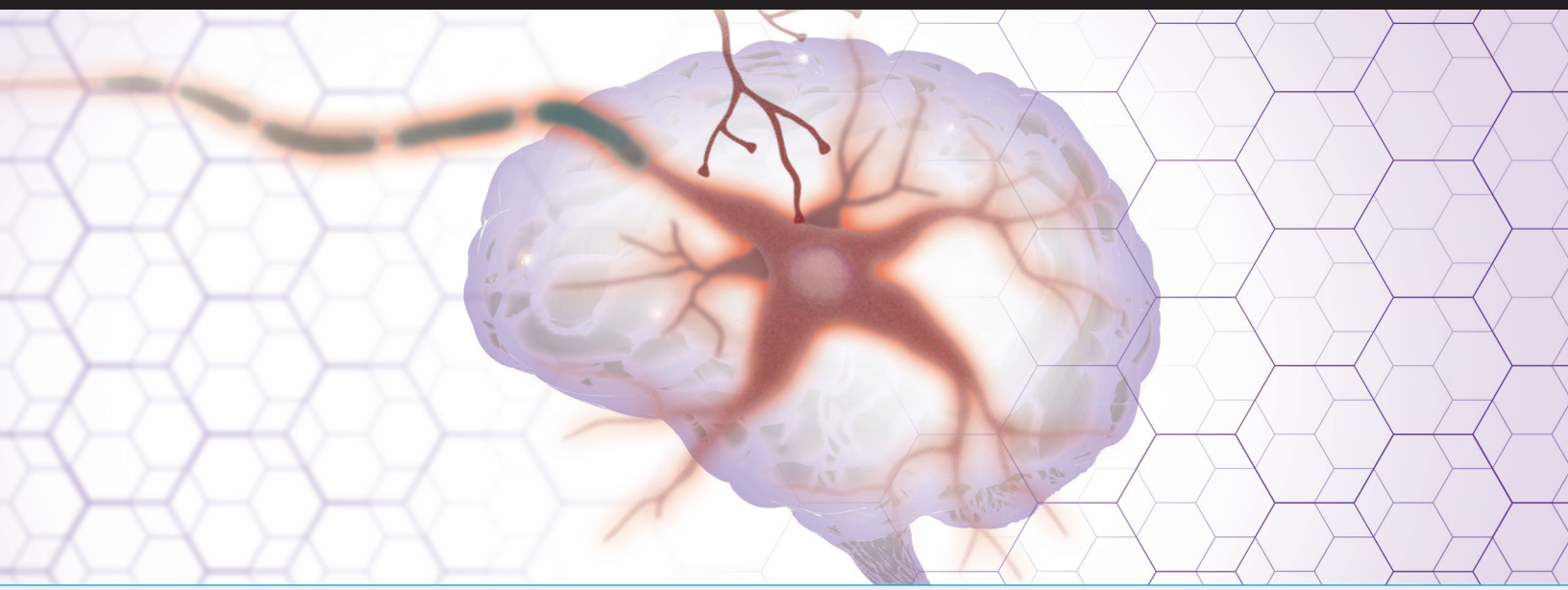
BIO-RAD Explorer



The Science of Opioid Dependence kit

Student Guide

BIO-RAD

Activity 1

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

Opioids, Pain, and Reward Pathways

What are opioids? Natural opioids, like endorphins, are neuropeptides that activate reward pathways in the brain by reducing pain and increasing feelings of well-being. Synthetic or plant-based opioids, like oxycodone or heroin, mimic endorphins to activate the same reward pathways.

All opioids act by binding opioid receptors in the midbrain, where they cause the release of dopamine, a neurotransmitter. Dopamine then floods into many regions of the brain, activating neurons and producing feelings of euphoria (see Figures 1 and 2).

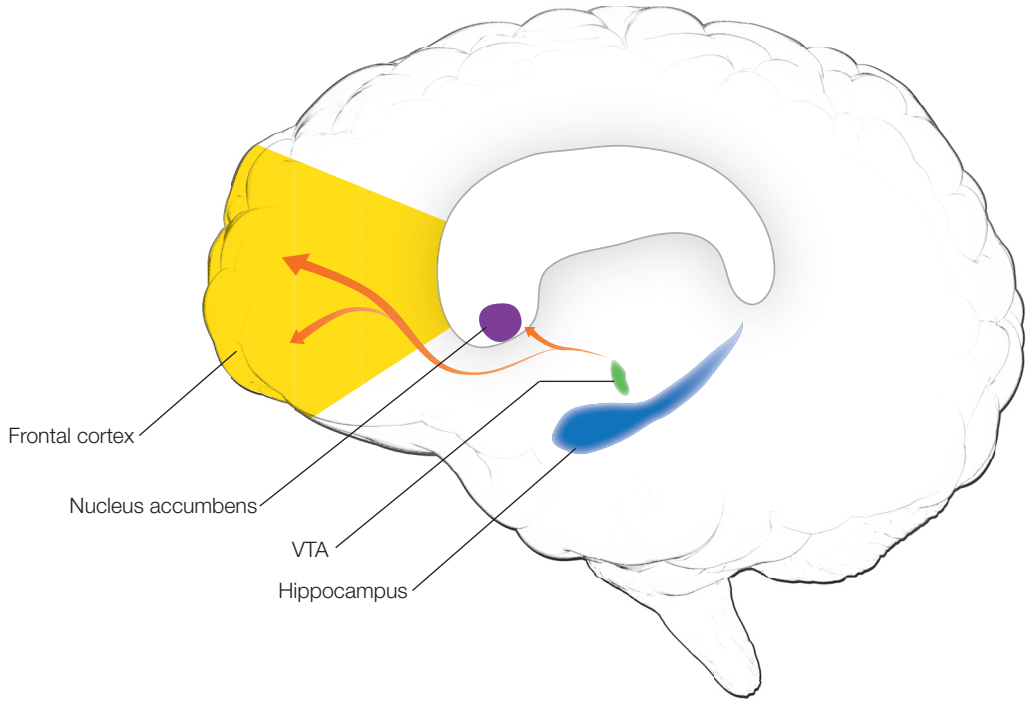


Figure 1. Opioids activate the reward pathways by stimulating the ventral tegmental area (VTA) to release dopamine. This flood of dopamine sends signals to many areas of the brain including, the nucleus accumbens and the prefrontal cortex where it produces feelings of euphoria.

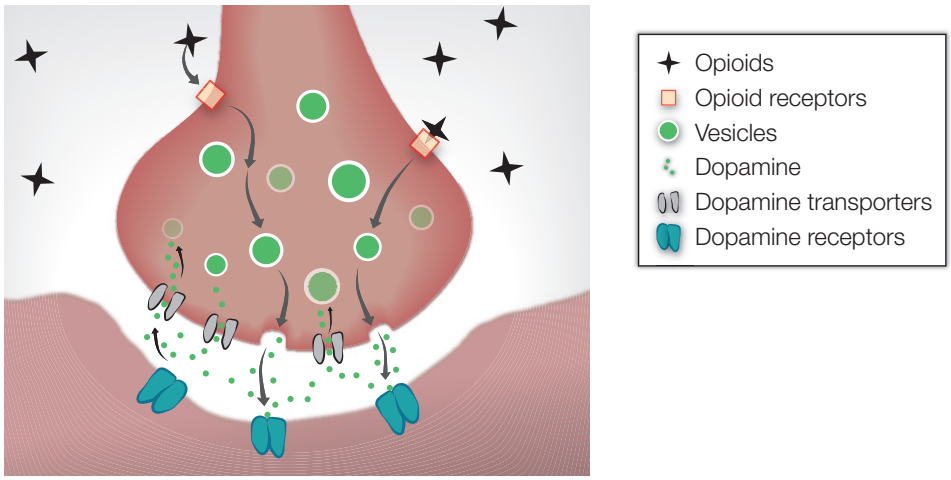


Figure 2. Opioids stimulate opioid receptors. This triggers dopamine-containing vesicles in the sending (presynaptic) neurons to increase the rate of dopamine released into synapses. Dopamine in the synapse binds to and activates dopamine receptors. This can lead to an overactive reward pathway response that may overwhelm dopamine receptors. Dopamine is released at lower rates in a drug-free brain.

Some synthetic opioids may be prescribed to treat severe or chronic pain, such as pain from surgery or cancer. While managing pain is vital for good patient recovery, opioids can also cause serious side effects, including drug dependence and respiratory arrest.

Many people who are dependent on or abuse opioids began with a prescription for pain medication (see Table 1). Doctors must take this risk of dependence into account and prescribe opioids only when they are necessary.

Table 1. Common pain medications.

Commonly Prescribed Pain Drugs	Pain Type
Nonsteroidal anti-inflammatory drugs (NSAIDs) (ibuprofen, aspirin, naproxen, etc.)	• Mild to moderate pain accompanied by swelling and inflammation • Arthritis pain • Pain resulting from muscle sprains and strains, back and neck injuries, overuse injuries, and menstrual cramps
Antidepressants	• Neuropathic pain • Chronic daily headaches • Fibromyalgia • May be considered for chronic low back pain
Opioids	• Acute pain, such as pain that follows surgery or a bone fracture • Long-term or chronic pain related to cancer • Pain that has been unresponsive to other treatments

Modified from mayoclinic.org/chronic-pain-medication-decisions/art-20360371.

The Opioid Risk Tool

The opioid risk tool (ORT) is a screening tool designed for healthcare providers to assess the risk of opioid abuse by adult patients who are prescribed opioids to treat pain. The tool uses patient information about environmental and personal factors to calculate a risk score of whether that person might abuse opioids. The factors do not necessarily cause a person to abuse opioids, but they are correlated to the level of risk. A total score of 3 or lower indicates low risk of future opioid abuse, a score of 4–7 indicates moderate risk, and a score of 8 or higher indicates high risk.

Table 2. Opioid risk assessment grid.

Mark each box that applies			Item values Female	Item values Male
Family history of substance abuse	Alcohol	❑	1	3
	Illegal drugs	❑	2	3
	Rx drugs	❑	4	4
Personal history of substance abuse	Alcohol	❑	3	3
	Illegal drugs	❑	4	4
	Rx drugs	❑	5	5
Age 16–45 years		❑	1	1
History of preadolescent sexual abuse		❑	3	0
Psychological disorder	Attention-deficit disorder (ADD), obsessive-compulsive disorder (OCD), bipolar disorder, schizophrenia	❑	2	2
	Depression	❑	1	1
Scoring totals				

Adapted from Webster et al. (2005).

Use Table 2 to calculate the ORT scores for two of the patients on the next page and determine their risk for opioid abuse. Then recommend a prescription for their pain symptoms and explain your reasoning. Record your answers in Table 3.

Table 3. Prescription recommendations for pain patients.

Patient	ORT Score	Risk of Opioid Abuse, High/Moderate/Low	Recommended Prescription	Reasoning for Recommendation

Patients Experiencing Chronic Pain

Patient Background

Name: Javier

Pain report: Presented with acute pain from recent dental work

ORT response:
- 36-year-old male

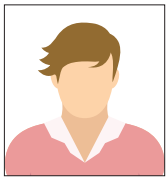
Patient Background

Name: Lin

Pain report: Recently had a baby by C-section; experiencing pain during recovery from the surgery that is not responding to NSAIDs

ORT response:
- 34-year-old female
- Preadolescent sexual abuse
- Diagnosed with ADD

Patient Background

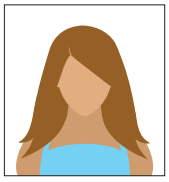


Name: Max

Pain report: College football player whose shoulder was fractured in a tackle, and who is experiencing pain during recovery from multiple surgeries over several months

ORT response:
- 21-year-old male
- Personal history of alcohol abuse
- Depression
- Family history of prescription drug abuse

Patient Background

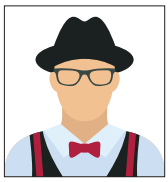


Name: Lianne

Pain report: Experiencing chronic pain since falling off a ladder at work 7 years ago

ORT response:
- 46-year-old female
- Personal history of prescription drug abuse

Patient Background

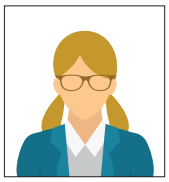


Name: Elmer

Pain report: Experiencing pain associated with lung cancer and several surgeries over the last year related to the cancer

ORT response:
- 50-year-old male
- History of ADD
- Personal history of alcohol abuse
- Family history of alcohol abuse

Patient Background



Name: Sasha

Pain report: Experiencing menstrual cramp pain consistently for six months

ORT response:
- 17-year-old female
- Family history of alcohol abuse

Patient Background

Name: Taj

Pain report: Experiencing chronic headaches

ORT response:
- 18-year-old male
- Family history of alcohol abuse

Patient Background

Name: Zem

Pain report: Reports elbow pain, from playing tennis, that keeps her up at least two nights a week

ORT response:
- 40-year-old female
- Depression
- Family history of alcohol abuse

Activity 2

Designing a Human Genetic Research Study

Genes Involved in Opioid Signaling

If there are genetic elements in susceptibility to opioid dependence or abuse, then the genes involved in opioid reward pathways may be involved.

There is one area, or locus, on the long arm of chromosome 11 that contains the D2 dopamine receptor (DRD2) gene and another gene involved in brain signaling called ANKK1 (for ankyrin repeat and kinase domain containing 1) (see Figure 3).

The **genome** is the complete set of genes or genetic material present in an organism.

A **SNP** is a variation in a single nucleotide that occurs at a specific position in the genome. Each variation is present to some appreciable frequency within a population.

To find genetic differences, scientists compare the **genomes** of different people. And while most DNA sequences are identical between people, there are thousands of differences in single base pairs. These differences are called **single nucleotide polymorphisms, or SNPs** ("snips"). Figure 3 shows six known SNPs in the DRD2/ANKK1 locus. In this study you will investigate the role of one of them, rs1800497, in opioid dependence. SNP rs1800497 has a C allele or a T allele, depending on an individual's genotype.

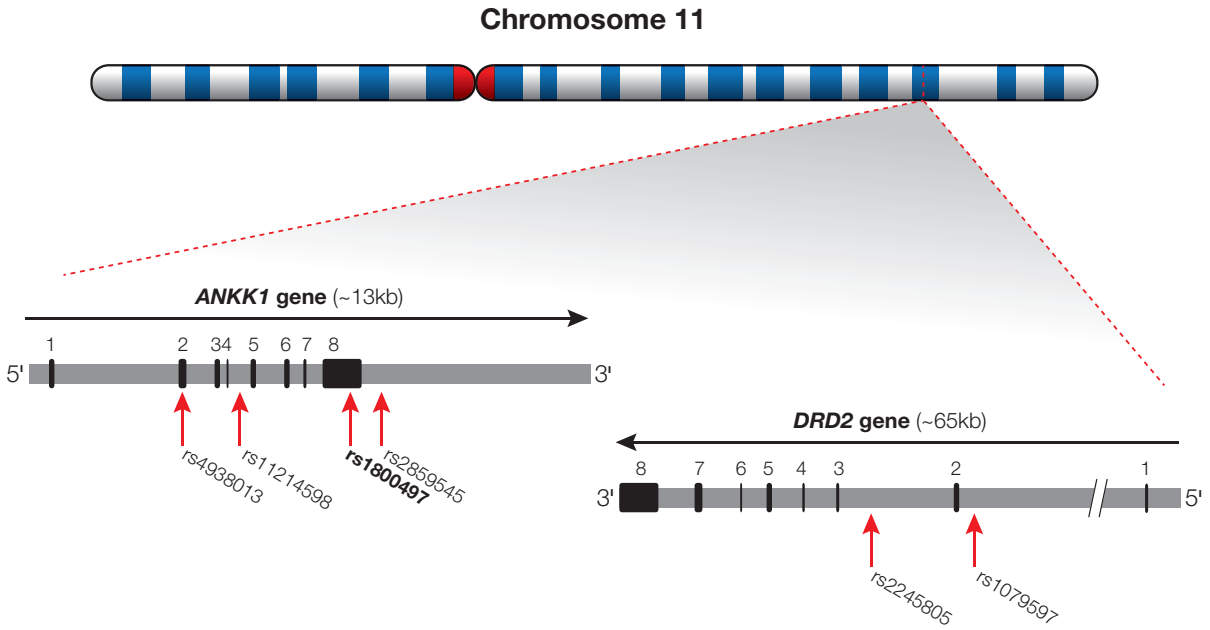


Figure 3. Location of six SNPs in DRD2 and ANKK1. Black bars are exons for DRD2 and ANKK1.

Testing for the rs1800497 genotype

To determine someone's genotype for rs1800497, scientists take advantage of a **restriction site** found in the C allele but not in the T allele. In the assay, the rs1800497 region of an individual's genome is amplified, or copied many times, using the **polymerase chain reaction (PCR)**. Then, the DNA PCR product is digested with the **restriction enzyme** TaqI (see Figure 4). TaqI will cut the PCR products from the C allele directly after the "T" in the sequence "TCGA" (see Figure 5). TaqI will not cut PCR products from the T allele.

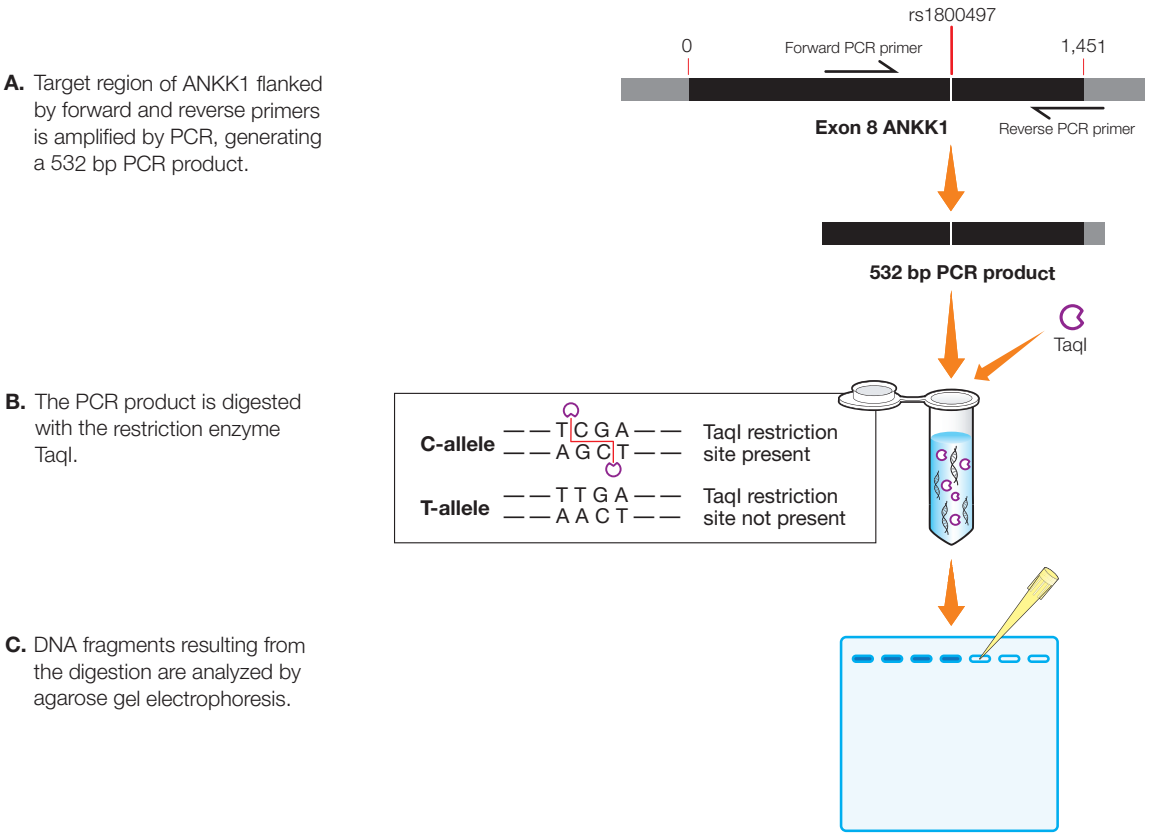


Figure 4. Assay to identify which alleles of SNP rs1800497 an individual possesses. **A,** the region of the ANKK1 gene containing the SNP is amplified using PCR. **B**, the PCR product is digested with the restriction enzyme TaqI. **C,** the results are analyzed using agarose gel electrophoresis.

DNA sequence of the PCR product of the rs1800497 region

5' *AGACAGGGTTTTG*CCATGTTGGCCAGGCTGGCCTCAAACTCTTGATATCAGGTGATCTGC-60
CTGCCTCAGCCTCCCAAAGTGCTGGGATTACAGACGTGAGCCACCACGGCTGGCCAAGTT-120
GTCTAAATTTCCATCTCGGCTCCTGGCTTAGAACCACCCAGAGTGGCCACTGACGGCTCC-180
TTGCCCTCTAGGAAGGACATGATGCCCTGCTTTCGGCTGCGGAGGGCCAGTTGCAGGGGT-240
GTGCAGCTCACTCCATCCTGGACGTCCAGCTGGGCGCCTGCC**TCGA**CCAGCACTTTGAGG-300
ATGGCTGTGTTGCCCTTGAGGGCGGCCAGGTGGGCGGGTGTCCAGCCCACCTTGTTGCGG-360
GCGTGGACATTTGCGTGATGTTCTAGGAGGTTGATGACACTCAGGAAGGTGCTCCTCTGG-420
ACCGCCAGGTGGAGGGGTGTCCAGCCTGACTGCTCTGCAGCATTGGGGTCAGCCCCACAC-480
TGCAGCAGTGCTGACACCACCGCCTCCTCCCCGTG*GCGTGCAGCTAGGTGCA* 3'

Figure 5. PCR product for the C allele of the SNP at rs1800497. Underlined base indicates the SNP location. Bold bases indicate the TaqI restriction site. Italic and underlined bases indicate PCR primer sequences.
Note: Sequence is shown as reverse complement.

Design and plan your study

You are part of the team of researchers conducting a study to investigate whether there is a genetic association with opioid dependence.

Write an investigation question (1–2 sentences):

Summarize relevant background information (3–6 sentences):

Define your dependent and independent variables:

Selecting study participants

In your study, you will compare case and control groups to determine whether there is a genetic association with susceptibility to opioid dependence. You will need to decide how many individuals you want to include and how to assign them to your case and control groups. It is important to select the right individuals for each group to control for other factors that may influence the results.

Case and Control Groups

Cases: people with the condition you are investigating.

Controls: people who represent the general population.

Think back to the ORT for ideas about variables for which you may want to control.

What considerations and attributes are important when defining your case group? Describe your case group.

What considerations and attributes are necessary when defining your control group? Describe your control group.

Planning the protocol

For this study you will have access to DNA samples from the study participants. Design a protocol that will provide evidence to help answer your investigation question.

Available materials

- Study participant DNA samples. The samples are PCR products amplified from the rs1800497 locus and treated with TaqI; the samples are ready to load onto an agarose gel
- Molecular weight ruler with DNA sizes 1,000, 700, 500, 200, and 100 bp
- Control DNA from an individual with CT genotype
- Agarose gels, running buffer, and DNA stain
- DNA electrophoresis cells, power supplies, and micropipets

Write out the protocol steps you will use in the investigation.

Describe the data that will be collected.

How will you analyze your data?

Predict the answer to your research question and write it down.

Sketch an agarose gel showing an experimental result that would support your prediction. Write a short explanation of how the data support your prediction. Identify the samples on each lane of the gels.

Sketch a result that would show your prediction was incorrect. Write an explanation of how the data refute your prediction. Identify the samples on each lane of the gels.

Go back and refine your protocol design if necessary.

Activity 3

Conducting the Research Study

A local university is conducting your human genetic study and has recruited 127 individuals to participate. Your class will help the university research team determine the participant genotypes. Each student group will determine the genotypes of a different set of samples. The genotype results that your class collects will be combined with the results from the university research team. You will then analyze and interpret the full data set.

Study participants

The case group includes 69 unrelated, heroin-dependent patients from three drug treatment centers in the U.S. Case patients and their samples are numbered 200 through 269. Diagnosis as heroin-dependent was made based on Diagnostic and Statistical Manual of Mental Disorders, 5th Edition (DSM-5) criteria (American Psychiatric Association 2013).

The control group includes 58 healthy individuals age- and gender-matched with the case group. Control members and their samples are numbered 100 through 158. These participants had no psychiatric disorders.

The research protocol was been approved by the University Internal Review Board (IRB). All participants were over the age of 18 at the time of inclusion in the study. All participants provided written informed consent.

Conduct your investigation

Follow your protocol design to determine the genotypes of your assigned samples. Refer to the steps below to load, run, and stain an agarose gel. Important: record which sample is loaded into each well.

Student Workstation

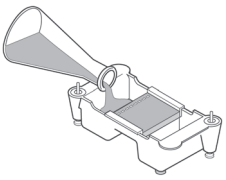
Materials	Quantity
Molecular weight ruler (MWR), 20 µl	1
Study participant DNA samples, 10 µl	6
PCR control DNA (CT), 10 µl	1
TAE electrophoresis buffer	300 ml
100x Fast Blast DNA Stain (if using)	50 ml
1% TAE agarose gel with 8 wells	1
Horizontal gel electrophoresis chamber	1
Power supply (may be shared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

Running DNA on an Agarose Electrophoresis Gel

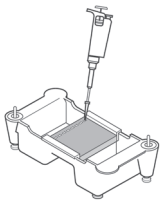
1. *Sketch your agarose gel setup to the right. Label each of the eight wells with the sample that will be loaded.*

2. *Place a 1% TAE agarose gel into the electrophoresis chamber. Be sure that the gel is oriented so that the wells are closest to the black (–) electrode, or cathode.*

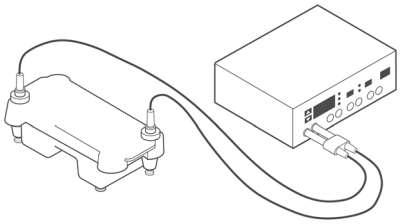
3. *Fill the electrophoresis chamber with enough TAE buffer to cover the gel by about 2 mm.* →



4. *Using a fresh tip for each sample, load 10 µl of each DNA sample and 20 µl of MWR into your gel according to your sketch.* →



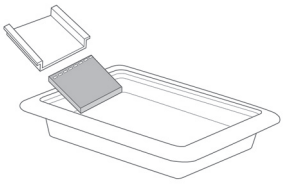
5. *Place the lid on the electrophoresis chamber and connect the electrical leads to the power supply, red to red and black to black.* →



6. *Turn on the power and run the gel. Ask your instructor for the run conditions.*

7. *When the electrophoresis run is completed, turn off the power and remove the lid of the chamber.*

8. *Carefully remove the gel from the electrophoresis chamber and transfer it to a gel staining tray. Be careful — the gel is very slippery.* →



9. *Stain and/or visualize your gel as directed by your instructor.*

Activity 4

Analyzing Data and Making a Claim

1. Draw your gel results

2. Record your data below:

Table 4. Study participant data collection table.

Participant or Tube Number	Opioid Dependence Status	Genotype

3. Summarize the class data in Table 6 below.

Table 5. Summarized class data on participant genotypes.

	Total	CC	CT	TT
Control participants				
Case participants				

Table 6. Summarized university research team data on participant genotypes.

	Total	CC	CT	TT
Control participants (#100–136)	36	17	15	4
Case participants (#200–243)	43	14	22	7

4. Combine the class data and the university study data in Table 7 below.
5. Calculate and record the genotype and allele frequencies for the combined data in Tables 7 and 8 below.

Table 7. 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, %
CC genotype				
CT genotype				
TT genotype				
Total				

Table 8. 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, %
C allele				
T allele				
Total				

Rewrite your investigation question:

Write a **claim** that answers your investigation question:

Provide **evidence** to support your claim:

Explain your **reasoning** for why the evidence supports your claim:

Summarize your results in a **scientific explanation**:

Activity 5

Establishing Confidence in Data and Addressing the Opioid Crisis

Data Confidence and Published SNP Frequency Data

How confident are you that the data support or refute your claim? Could any differences you identify occur just by chance? How might you find out?

Probability values

Looking at how many data points you have and using statistical methods to determine how much difference there is between their values can answer these questions. The chi square test can be used to determine whether the differences are real (significant) or by chance. One output of chi square analysis is the probability value, or p-value. These p-values are a measure of how likely it is the difference happened by chance.

A p-value of less than 0.05 is usually considered significant and means that 19 times out of 20 the difference or relationship between the identified data points is real and only one time out of 20 would the difference occur by chance. Also, while a p-value of 0.05 or less is considered significant, p-values can go much lower, indicating higher significance the lower they go. So a p-value 0.0001 is much more significant than 0.05 and offers much stronger evidence that the difference is real.

Table 9. Published data on SNPs tested for association with opioid dependence.

Gene	SNP	Control subjects, n	Case subjects, n	Frequency of Allele in Control Group	Frequency of Allele in Case Group	p-Value	Reference
ANKK1	rs1800497 C/T	279	313	38% T	44% T	0.04	Zhang J, et al. (2018)
DRD2	rs2245805 T/G	279	314	54% T	49% T	0.06	
Dopamine receptor D3 (DRD3)	rs6280 G/A	70	54	29% G	35% G	0.16	Duaux E, et al. (1998)
Mu opioid receptor (OPRM1)	rs1799971 A/G	156	126	12% G	31% G	0.0001	Kapur S, et al. (2007)
	rs1799972 C/T	57	123	89% C	83% C	0.121	
Delta opioid receptor (OPRD1)	rs1042114 G/T	426	98	13% G	21% G	0.005	Zhang H, et al. (2008)
	rs12749204 A/G	376	105	79% A	84% A	0.07	
Brain-derived neurotrophic factor (BDNF)	rs56164415 C/T	492	487	4.8% T	3.6% T	0.191	Jia W, et al. (2011)
	rs6265 G/A	492	487	54% G	61% G	0.001	
	rs13306221 G/A	492	487	6.9% A	4.0% A	0.005	

1. ***Write a brief summary of your analysis of the published data set.***

2. ***Briefly state whether these additional data support or refute your previous claim and why.***

How to address the opioid crisis

1. ***Go back to the patients in Activity 1. Review your recommendation for whether the doctor should prescribe the patient opioid pain medication. Discuss with your group how what you have learned might affect your recommendation. Explain your reasoning.***

2. ***Why is it inaccurate to say that if someone has a T allele for the rs1800497 SNP, they will become dependent on opioids?***

3. ***What do you think can and should be done to address the opioid epidemic?***

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Bio-Rad Laboratories, Inc.

Life Science Group

Web site *bio-rad.com* ***USA*** *1 800 424 6723* ***Australia*** *61 2 9914 2800* ***Austria*** *43 01 877 89019* ***Belgium*** *32 03 710 53 00* ***Brazil*** *55 11 3065 7550* ***Canada*** *1 905 364 3435* ***China*** *86 21 6169 8500* ***Czech Republic*** *36 01 459 6192* ***Denmark*** *45 04 452 10 00* ***Finland*** *35 08 980 422 00* ***France*** *33 01 479 593 00* ***Germany*** *49 089 3188 4393* ***Hong Kong*** *852 2789 3300* ***Hungary*** *36 01 459 6190* ***India*** *91 124 4029300* ***Israel*** *972 03 963 6050* ***Italy*** *39 02 49486600* ***Japan*** *81 3 6361 7000* ***Korea*** *82 2 3473 4460* ***Mexico*** *52 555 488 7670* ***The Netherlands*** *31 0 318 540 666* ***New Zealand*** *64 9 415 2280* ***Norway*** *47 0 233 841 30* ***Poland*** *36 01 459 6191* ***Portugal*** *351 21 4727717* ***Russia*** *7 495 721 14 04* ***Singapore*** *65 6415 3188* ***South Africa*** *36 01 459 6193* ***Spain*** *34 091 49 06 580* ***Sweden*** *46 08 555 127 00* ***Switzerland*** *41 0617 17 9555* ***Taiwan*** *886 2 2578 7189* ***Thailand*** *66 2 651 8311* ***United Arab Emirates*** *971 4 8187300* ***United Kingdom*** *44 01923 47 1301*

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