miniPCR Sleep Lab™

Morning Lark or Night Owl?

Contents

At a glance

Lab overview

Participate in an authentic open inquiry investigation on the genetic underpinnings of sleep. Students will determine their genotype for a gene that has been associated with sleep behavior in humans. Students will also assess their sleep phenotypes through a simple circadian questionnaire, with the ultimate goal of learning about circadian clocks and the study of genetic associations.

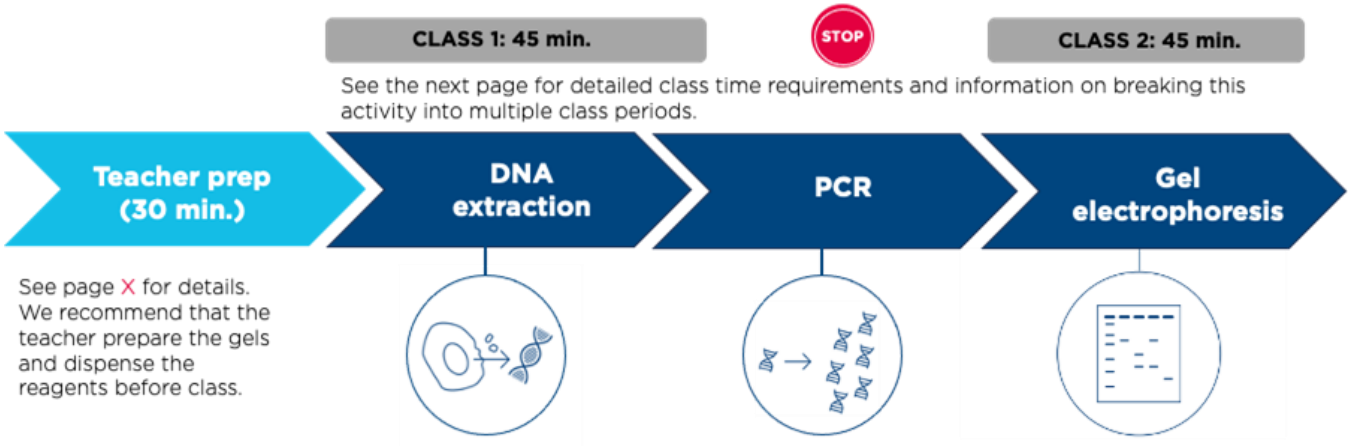
TECHNIQUES	TOPICS	LEVEL
Micropipetting	Inheritance	Advanced high school
DNA extraction	Genetic associations	College
PCR	Circadian rhythms	
Gel electrophoresis		

Required lab skills

- Students must be proficient in accurately pipetting liquids in the 2-20 µl range.
- Instructional videos, worksheets, and free activities to help students build micropipetting skills can be found at https://www.minipcr.com/micropipetting/

Planning your time

- This activity has three parts that will likely take place over multiple days.
- The entire experiment can be completed in a single 150-minute block.
- Refer to the next page for detailed information on splitting this activity into several classes.
- The most common classroom implementation timeline is shown below.



Technical support

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

Class time requirements

This protocol offers some flexibility to help you manage the class time needed.

Steps		Time required
1	DNA extraction	15 minutes
STOP	Optional stopping point: The DNA extract can be stored in the refrigerator for up to eight days before proceeding to PCR.	
2	PCR	
	A. Set up samples and start PCR program	20 minutes
	B. Run PCR	60 minutes The PCR program can be started during class and left to run without being monitored. Once the program is complete, samples are stable at room temperature for several days and can be left in the machine.
STOP	Optional stopping point: The PCR product is stable at room temperature for several days. For longer-term storage, place it in the freezer.	
3	Gel electrophoresis	
	A. Load gel	10 minutes
	B. Run gel	20-30 minutes
	C. Interpret results	5 minutes

Materials needed

Supplied in kit (KT-1005-01)

- Kit contains PCR reagents for 32 students.
- If kept in the freezer, reagents can be stored for 12 months after receipt. If kept in the refrigerator, reagents can be stored for 1 month after receipt.
- Reagents for preparing gels, plastic tubes for distributing reagents to individual groups, plastic tubes for PCR, and pipette tips are sold separately. See below for details.

Contents	Provided	Required per student	Storage
X-Tract™ Buffer	1,200 µl x two tubes	50 µl	Freezer
5X EZ PCR Master Mix, Load-Ready™	240 µl	5 µl	Freezer
Sleep Lab Primers	960 µl	20 µl	Freezer
100 bp DNA Ladder, Load-Ready™	100 µl	12 µl per gel	Freezer

Electrophoresis reagents and plastics sold separately

- This lab requires:
  - 2% agarose gels with a fluorescent DNA stain (*e.g.*, SeeGreen™ or GelGreen®).
  - Plastic tubes for distributing reagents to individual groups and 0.2 ml PCR tubes for running PCR.
- The Learning Lab Companion Kit (KT-1510-01) provides sufficient reagents to make and run eight gels when using the blueGel or Bandit electrophoresis system, as well as plastic tubes for distributing reagents to individual groups and plastic tubes for PCR.
- Alternatively, bulk electrophoresis reagents and plastics (tubes, pipette tips) are available for purchase from miniPCR bio.
- Gel electrophoresis reagents and plastics can also be purchased from other suppliers.

Required equipment

- This lab is compatible with any thermal cycler.
- This lab is compatible with any horizontal gel electrophoresis system in combination with:
  - A fluorescent DNA stain (*e.g.*, SeeGreen™ or GelGreen®)
  - A transilluminator that is compatible with the DNA stain used. Fluorescent DNA stains typically require blue light (~470 nm) or UV (~260 nm) illumination.
- The table below outlines equipment from miniPCR bio that meets these requirements:

Other materials supplied by user

- Distilled water
- Microwave or hot plate
- Heat-resistant flask or beaker
- Plastic tubes for dispensing reagents (1.7 or 0.2 ml tubes can be used)
- 0.2 ml PCR tubes
- Disposable laboratory gloves
- Protective eyewear
- Fine-tipped permanent marker

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

Teacher prep

Overview

- This activity has three parts that will likely take place over the course of multiple days.
- The table below provides an overview of the teacher prep, and the subsequent pages provide detailed instructions.

Prep	Time required	Timeline
Dispense reagents	10 minutes	Can be completed up to one week before use.
Prepare electrophoresis buffer and agarose gels	20 minutes	Varies - If using gel reagents from miniPCR, gels can be prepared and stored for up to five days before use.

Dispense reagents

- Reagents can be dispensed up to one week in advance and stored in the refrigerator until use.
- This kit provides sufficient reagents for 32 students.

Materials needed

From the lab kit (stored in the freezer):

- X-Tract DNA Extraction Buffer
- 5X EZ PCR Master Mix
- Sleep Lab Primers
- 100 bp DNA Ladder

Supplied by user:

- Plastic tubes for dispensing reagents (1.5 ml or 0.2 ml tubes can be used)
- PCR tubes (0.2 ml)
- 2-20 µl micropipette and tips
- Fine-tipped permanent marker

1. Thaw reagents by placing tubes at room temperature.
   Note: The kit components are stable at room temperature for several hours and do not need to be stored on ice while you are dispensing reagents.
2. Collect the liquid at the bottom of each tube. Either spin briefly in a microcentrifuge or shake the liquid down with a flick of the wrist.
3. When you open each tube, check for liquid stuck inside the cap. If necessary, put the cap back on and repeat step 2.
4. For each **student**, dispense 50 µl of X-Tract DNA Extraction Buffer into a labeled plastic tube that fits your 95 °C heat source. If using a miniPCR thermal cycler for the DNA extraction, use 0.2 ml PCR tubes.
5. For each **group of four students**, dispense the following reagents into labeled plastic tubes. 1.5 ml or 0.2 ml plastic tubes can be used.
   - 5X EZ PCR Master Mix (label tube as “M”) 25 µl
   - Sleep Lab Primers (tube P) 100 µl
   - 100 bp DNA Ladder (tube L) 12 µl
6. If you are dispensing the reagents more than 24 hours before class, store the tubes in the refrigerator until use. Reagents can be stored in the refrigerator for one week.

Prepare electrophoresis buffer and agarose gels

1. Prepare electrophoresis buffer.
   - Follow the manufacturer’s instructions to prepare buffer solution.
   - The volume of buffer needed varies depending on the gel electrophoresis system.
     - For the blueGel and Bandit electrophoresis systems, 600 ml of TBE buffer is sufficient for at least eight gel runs.
     - For other systems, refer to the manufacturer’s instructions for:

(1) The buffer volume needed to prepare agarose gels.
(2) The buffer volume needed for use as running buffer.

2. Prepare 2% agarose gels with fluorescent DNA stain.
   - You will need one lane per student plus one lane for DNA ladder per group. If groups are sharing gels, a single lane for ladder per gel is sufficient.
   - This lab kit is compatible with any molecular grade agarose and fluorescent DNA stain (*e.g.*, SeeGreen™ or GelGreen®).
   - The volume of gel needed varies based on the gel electrophoresis system you are using. Refer to the manufacturer's instructions.
   - If using gel electrophoresis reagents from miniPCR bio, gels can be prepared up to five days in advance. Store prepared gels at room temperature in an airtight container protected from light. Do NOT soak the gels in buffer or wrap them in paper towels.

Student workstation setup

Part 1: DNA extraction

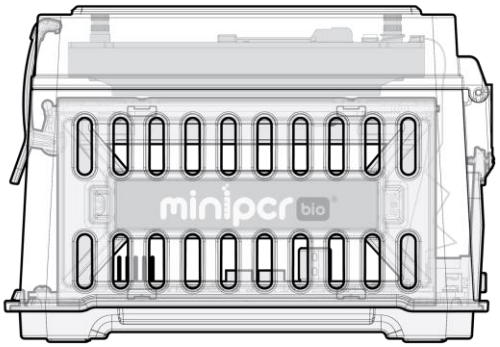
	Per student
X-Tract DNA Extraction Buffer (Tube E)	50 µl
Flat-end toothpicks	1
2-20 µl micropipette and tips	
Fine-tipped permanent marker	
Access to a 95 °C heat source (you can use a miniPCR in heat block mode)	

Part 2: PCR

	Per student	Per group of 4
DNA extract from previous step	Each student will have their personal sample	
5X EZ PCR Master Mix, Load-Ready™	5 µl	25 µl
Sleep Lab Primers	20 µl	100 µl
PCR tubes (0.2 ml)	1	4
2-20 µl micropipette and tips		
Access to a thermal cycler 2-20 µl micropipette and tips		

If using miniPCR thermal cyclers:

- Groups will need access to a miniPCR thermal cycler and power supply.
- Download the miniPCR app from the app store or at www.minipcr.com/downloads.
- Machines can be programmed ahead of time by the teacher or during class by the students.
- Once the program has started, the miniPCR will complete the program even if disconnected from the device running the app.
- If you want to monitor the reaction in real-time during the run, the miniPCR thermal cycler must remain connected to the device running the app.



Part 3: Gel electrophoresis

	Per group
PCR samples from previous class	Each student will have their personal sample
100 bp Ladder (tube L)	12 µl
Electrophoresis buffer *Volume depends on your electrophoresis system	30 ml TBE if using a blueGel or Bandit
2-20 µl micropipette and tips	
2% agarose gel with fluorescent DNA stain	1 well per student plus an additional lane for ladder

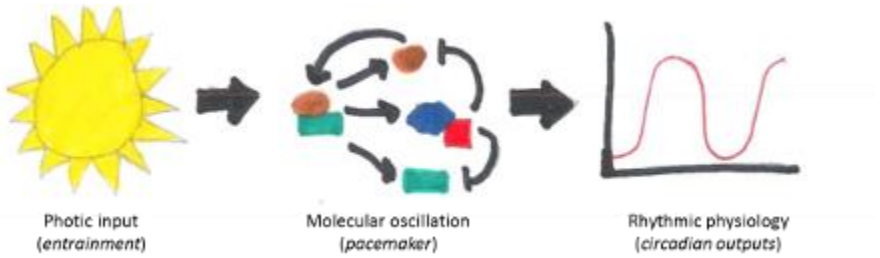
Student guide

Background information

Circadian clocks

Have you ever felt jet-lagged after traveling far from home, and found it hard to sleep? Do you usually find yourself feeling tired at the same time of the day every day? Both of these phenomena are controlled by your circadian clock. Circadian clocks are internal timekeepers that regulate our body's physiology and behavior on a cycle that repeats each day. Our internal clock dictates what times of day you feel sleepy, energized, or hungry, and controls important bodily functions such as our blood pressure, body temperature, hormone release, and metabolism. In humans, the circadian clock is set to be a little longer than 24 hours. Other animals, plants, fungi, and even unicellular organisms have circadian clocks too, which helps them anticipate the daily transitions between light and darkness.

Any good clock has two important features. First, it needs to keep regular time, and second, it needs to be able to be set and reset. Our circadian clock has both of these features. Our clocks are set to roughly 24 hours, but they can also be reset by external factors, especially sunlight; this ensures that your internal timekeeper (naturally a little longer than 24 hours) stays synchronized with the natural day (24 hours). It is also why when you travel to a new time zone, your internal clock is initially off, causing jet lag, but within a few days resets to the new time zone you are in. We call this ability of the circadian clock to synchronize to the environment entrainment.

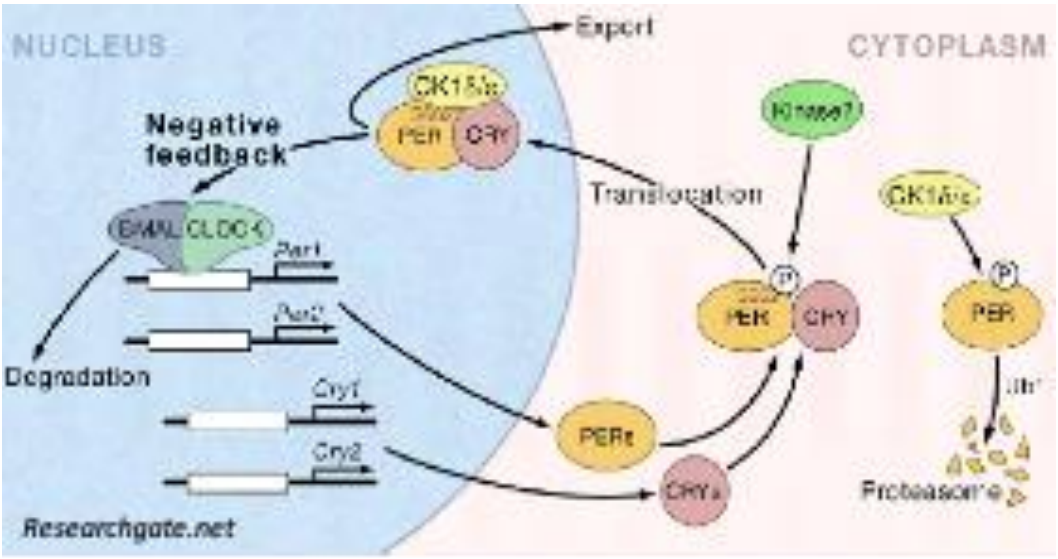


A fascinating feature about the circadian clock is that it operates at the cellular level; yes, individual cells in your body can keep and tell time! This is possible because the circadian clock is controlled by a genetic feedback loop, where proteins regulate gene expression with 24-hour periodicity. Circadian clock proteins exhibit negative feedback, meaning the proteins produced by the clock genes, in turn, turn off the genes that produced them. Production of circadian clock proteins will therefore continue until the proteins reach a certain concentration in the cell, at which time their production will cease. Over time as these circadian clock proteins are degraded at a regular rate, the concentration of clock proteins will go down until it is low enough that gene expression can resume. Here the cycle will continue – producing proteins until expression is halted, degrading proteins until expression begins again. Amazingly, the daily cycle of expression and degradation of these proteins has been so fine-tuned by evolution that it closely matches a 24-hour Earth day.

Molecular mechanism of the clock

We live in an environment that is cyclic due to the Earth's rotation around its axis. Many different living systems have evolved *biological clocks* that can predict the Earth's rotation and the 24-hour light-dark cycle. This biological clock controls metabolism, biochemistry, and many functions inside the body, including our activity-rest cycles.

Individual cells in our bodies have internal clocks. In humans and other mammals, the master clock is a tiny structure in the hypothalamus called the *suprachiasmatic nucleus* (SCN). Cells in the SCN (and many tissues in the body) can each oscillate with a ~24-hour period. In the 1990s, mutants helped scientists discover *clock genes* (such as *Clock*, *BMAL*,*Period*, etc.) which are fundamental in generating circadian rhythms. We now understand the genetics of circadian behavior in remarkable detail. The molecular mechanism of the clock involves transcription-translation negative feedback loops of multiple genes. The transcription factors BMAL1 and CLOCK form heterodimers, which activate transcription of Cryptochrome (*Cry*) and Period (*Per*) genes by binding to their promoters. CRY and PER proteins gradually accumulate in the cytoplasm. CRY, PER, and other proteins form complexes that translocate to the nucleus and shut down BMAL1–CLOCK mediated expression of the *Cry* and *Per* genes. This transcription/translation negative feedback loop repeats itself every 24 hours inside your cells!



Genetic variation and the clock

One important gene in these feedback loops is the Period 3 circadian clock gene (*Per3*). Research has found that there is variation in this gene among humans, *i.e.*, the gene is polymorphic. Further studies have found that a specific form of variation in this gene, a variable number tandem repeat (VNTR) in *Per3* can affect how people's circadian clocks are set. A VNTR is a short sequence that repeats itself several times in succession within a gene. In the case of *Per3*, there is a 54-base pair sequence that is repeated 4 times in one allele, and 5 times in another variant. Research has found that carrying 4 copies of this repeat may be associated with a preference for evening activity, while having 5 repeats may be associated with a preference for activities in the morning. It would seem from these studies that your genes can influence whether you prefer to be a morning lark or a night owl!

Since polymorphisms (variation) in this repeat change the length of this gene, the difference between 4-repeat and 5-repeat *Per3* genes can be seen in gel electrophoresis. Of course, we first need to amplify (make a lot of copies of) this gene to make it visible on a gel.

Genetic associations

Most phenotypes are complex traits with multiple genetic and environmental components (i.e., not determined by a single gene). Genetic association studies are used to find candidate genes or genome regions that contribute to a given trait by testing for a correlation between that trait and genetic variation. A higher frequency of a given allele (or genotype) in a sample of individuals who express the trait can be interpreted as meaning that the allele increases the probability of having that specific trait. Associations are difficult to establish unequivocally, and require obtaining large datasets to increase the statistical confidence in the possible association.

Traditional genetics techniques tend to look for mutant or nonfunctional versions of genes to try to determine the function of the gene. While this has been a fruitful approach, until only very recently, it was limited to use on model organisms grown in labs. This limits the ability of scientists to study human genetics in this way. Also, traditional genetics techniques typically do not capture real-world variation affecting real-world phenotypes. The power of association studies lies in taking human phenotypic variation and being able to associate it with actual genetic variation present in populations.

Associations can be difficult to establish, however, because most traits are controlled by many factors. Let's take a trait like height as an example. While you have no doubt learned about phenotypes being controlled by dominant and recessive alleles, complex traits like height are controlled by hundreds of genes. Each one of those genes has different alleles that may influence you being a little taller or a little shorter. For the vast majority of these we have no idea if an allele is dominant, recessive, or displays some other dominance relationship. You may know that for one particular gene you have an allele that has been associated with increased height. But it's only when you add together the effect of all the alleles for all those genes and include outside influences such as diet that your actual height is established.

This is what we are trying to test today. There are reported associations between the *Per3* alleles and morning or evening preference, while other studies found none. Whether or not this is a real association and how strong an association it may be is still an open question. As is true with all association studies, sufficient sample size and statistical tests are needed to establish a true correlation. In this lab, your class can contribute the data you collect to help establish how important the *Per3* gene is to determining morning or evening preference!

Today's lab

In this lab, you will use PCR to amplify a segment of the *Per3* gene (from your own DNA) and gel electrophoresis to directly observe whether you carry the 4-repeat or 5-repeat genotype in this VNTR within a circadian clock gene. 4-repeat alleles will be amplified as a ~250 base pair fragment, while 5-repeat segments will amplify as a ~300 base pair fragment. We will also take a simple self-assessment questionnaire that will help us determine our chronotype, or phenotypic circadian preference (whether you're a morning or evening type.)

We hope that matching chronotypes to genotypes across many students, and later aggregating the data, will help us shed some light on the question of this reported genetic association between the *Per3* VNTR and circadian preferences. Through this lab, everyone can become a circadian biology researcher!

Please remember that just as is true for many other traits, your circadian clock and sleep patterns are not solely determined by genetics. Modern society is filled with artificial light, caffeine, changing feeding schedules, work or school obligations, and other external signals which interact with people's intrinsic sleep behaviors (chronotypes). While your genes may predispose you to certain sleep behaviors, your environment still changes those patterns in ways you may not expect.

Student lab protocol

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

DNA extraction

For best results, don't eat or chew gum for ~20 minutes prior to cheek cell collection.

1. Each group member should receive a tube containing extraction buffer (tube E). Label the tube with your initials. Write on the upper sidewall of the tube.
2. Collect cheek cells by gently scraping the inside of your cheek 3-4 times with a flat-end toothpick. It should not hurt.
3. Dip the end of the toothpick with your cheek cells into the extraction buffer in your individual tube. Swirl the toothpick to dislodge the cells, then dispose of the toothpick.
4. Close the cap on the tube. When it is closed correctly, you should feel the cap "click" into place.
5. Incubate your tube for 10 min at 95 °C. You can use a miniPCR thermocycler in Heat Block mode, a water bath, or another heat block.
6. Remove your tube from heat and proceed to setting up the PCR.

Optional stopping point: The DNA extract can be stored in the refrigerator for up to eight days before proceeding to PCR.

Set up PCR samples

1. Label a new 0.2 ml PCR tube with your initials followed by “P” for PCR.Write on the upper sidewall of the tube.
2. Add PCR reagents to the labeled tube according to the table below. To prevent contamination, use a new tip for each addition.

Primer Mix (tube P)	20 µl
Master Mix (tube M)	5 µl
Student DNA sample from the previous step	3 µl
Total volume	28 µl

3. Close the cap on the tube. When it is closed correctly, you should feel the cap “click” into place.
4. Flick the tube to mix the contents. If available, a vortex mixer can be used.
5. Make sure all the liquid is at the bottom of the tube. If there is liquid stuck on the sides of the tube, shake it down with a flick of the wrist or a brief spin in a microcentrifuge.
6. Proceed immediately to the next section of the protocol.

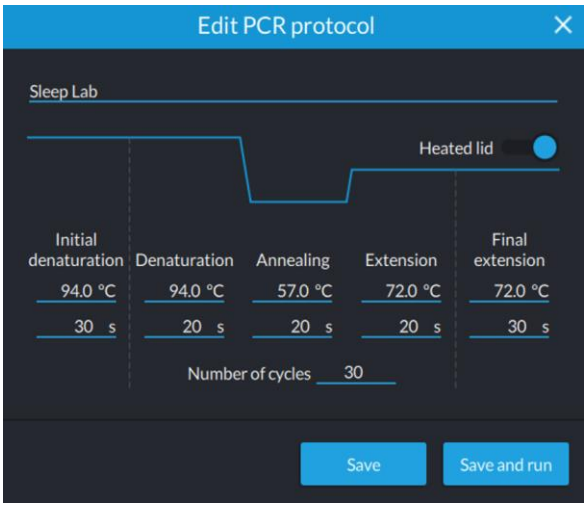
Run PCR

- Program your thermal cycler with the following parameters:

Initial denaturation	94°C, 30 sec
Denaturation	94°C, 20 sec
Annealing	57°C, 20 sec
Extension	72°C, 20 sec
Number of cycles	30
Final extension	72°C, sec

- The PCR takes approximately 60 minutes when using a miniPCR® thermal cycler.

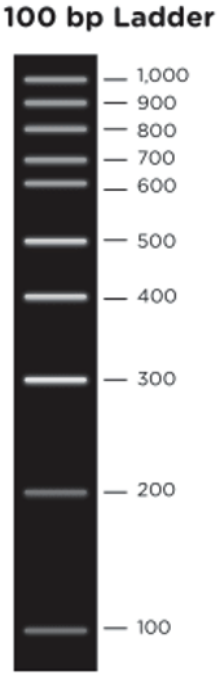
- Optional stopping point: PCR product is stable at room temperature for several days. For longer-term storage, move tubes to a freezer.



Gel electrophoresis

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

1. Place the prepared gel into the electrophoresis chamber.
2. Add enough electrophoresis buffer to fill the chamber and just cover the gel.
   - You will need 30 ml of TBE buffer for a blueGel or Bandit electrophoresis system. Do not overfill the chamber.
   - If using another electrophoresis system, refer to the manufacturer's instructions for the recommended buffer type and volume.
3. Use a micropipette to load samples in the following order. To prevent contamination, use a new tip for each sample. Note the order in which you loaded your group's samples below:
   - Well 1: 10 µl DNA Ladder (tube L)
   - Well 2: 14 µl Student name _____
   - Well 3: 14 µl Student name _____
   - Well 4: 14 µl Student name _____
   - Well 5: 14 µl Student name _____
4. Run the gel for 30 minutes.
   - The blueGel and Bandit electrophoresis systems run at a fixed voltage.
   - If using another gel electrophoresis system, set the voltage in the 70-90 V range.
5. To visualize the DNA samples, turn on the blue light in your electrophoresis system, or move the gel to a transilluminator.
6. If needed, continue to run the gel until there is sufficient separation between the 200-400 bp bands in the ladder to interpret the results.
7. If desired, take a photo to document the results.
8. Compare the bands from the DNA samples to the DNA ladder to obtain size estimates.



Morning-eveningness questionnaire

This self-assessment questionnaire to determine your circadian rhythm *chronotype*. For each question, please select the answer that best describes you by circling the point value that best indicates how you have felt in recent weeks. (Adapted from Horne & Ostberg, 1976).

You can access an electronic version of this questionnaire here.

1. Approximately what time would you get up if you were entirely free to plan your day?
   [5] 5:00 AM–6:30 AM (05:00–06:30 h
   [4] 6:30 AM–7:45 AM (06:30–07:45 h)
   [3] 7:45 AM–9:45 AM (07:45–09:45 h)
   [2] 9:45 AM–11:00 AM (09:45–11:00 h)
   [1] 11:00 AM–12 noon (11:00–12:00 h)

2. Approximately what time would you go to bed if you were entirely free to plan your evening?
   [5] 8:00 PM–9:00 PM (20:00–21:00 h)
   [4] 9:00 PM–10:15 PM (21:00–22:15 h)
   [3] 10:15 PM–12:30 AM (22:15–00:30 h)
   [2] 12:30 AM–1:45 AM (00:30–01:45 h)
   [1] 1:45 AM–3:00 AM (01:45–03:00 h)

3. If you usually have to get up at a specific time in the morning, how much do you depend on an alarm clock?
   [4] Not at all
   [3] Slightly
   [2] Somewhat
   [1] Very much

4. How easy do you find it to get up in the morning (when you are not awakened unexpectedly)?
   [1] Very difficult
   [2] Somewhat difficult
   [3] Fairly easy
   [4] Very easy

5. How alert do you feel during the first half hour after you wake up in the morning?
   [1] Not at all alert
   [2] Slightly alert
   [3] Fairly alert
   [4] Very alert

6. How hungry do you feel during the first half hour after you wake up?

[1] Not at all hungry
[2] Slightly hungry
[3] Fairly hungry
[4] Very hungry

7. During the first half hour after you wake up in the morning, how do you feel?
   [1] Very tired
   [2] Fairly tired
   [3] Fairly refreshed
   [4] Very refreshed

8. If you had no commitments the next day, what time would you go to bed compared to your usual bedtime?
   [4] Seldom or never later
   [3] Less than 1 hour later
   [2] 1-2 hours later
   [1] More than 2 hours later

9. You have decided to do physical exercise. A friend suggests that you do this for one hour twice a week, and the best time for him is between 7-8 AM (07-08 h). Bearing in mind nothing but your own internal "clock," how do you think you would perform?
   [4] Would be in good form
   [3] Would be in reasonable form
   [2] Would find it difficult
   [1] Would find it very difficult

10. At approximately what time in the evening do you feel tired, and, as a result, in need of sleep?
   [5] 8:00 PM–9:00 PM (20:00–21:00 h)
   [4] 9:00 PM–10:15 PM (21:00–22:15 h)
   [3] 10:15 PM–12:45 AM (22:15–00:45 h)
   [2] 12:45 AM–2:00 AM (00:45–02:00 h)
   [1] 2:00 AM–3:00 AM (02:00–03:00 h)

11. You want to be at your peak performance for a test that you know is going to be mentally exhausting and will last two hours. You are entirely free to plan your day. Considering only your "internal clock," which one of the four testing times would you choose?
   [6] 8 AM–10 AM (08–10 h)
   [4] 11 AM–1 PM (11–13 h)
   [2] 3 PM–5 PM (15–17 h)
   [0] 7 PM–9 PM (19–21 h)

12. If you got into bed at 11 PM (23 h), how tired would you be?
   [0] Not at all tired

[2] A little tired
[3] Fairly tired
[5] Very tired

13. For some reason you have gone to bed several hours later than usual, but there is no need to get up at any particular time the next morning. Which one of the following are you most likely to do?
    [4] Will wake up at usual time, but will not fall back asleep
    [3] Will wake up at usual time and will doze thereafter
    [2] Will wake up at usual time, but will fall asleep again
    [1] Will not wake up until later than usual

14. One night you have to remain awake between 4-6 AM (04-06 h) in order to carry out a night watch. You have no time commitments the next day. Which one of the alternatives would suit you best?
    [1] Would not go to bed until the watch is over
    [2] Would take a nap before and sleep after
    [3] Would take a good sleep before and nap after
    [4] Would sleep only before the watch

15. You have two hours of hard physical work. You are entirely free to plan your day. Considering only your internal "clock," which of the following times would you choose?
    [4] 8 AM–10 AM (08–10 h)
    [3] 11 AM–1 PM (11–13 h)
    [2] 3 PM–5 PM (15–17 h)
    [1] 7 PM–9 PM (19–21 h)

16. You have decided to do physical exercise. A friend suggests that you do this for one hour twice a week. The best time for her is between 10-11 PM (22-23 h). Bearing in mind only your internal "clock," how well do you think you would perform?
    [1] Would be in good form
    [2] Would be in reasonable form
    [3] Would find it difficult
    [4] Would find it very difficult

17. Supose you can choose your own work hours. Assume that you work a five-hour day (including breaks), your job is interesting, and you are paid based on your performance. At approximately what time would you choose to begin?
    [5] 5 hours starting between 4–8 AM (05–08 h)
    [4] 5 hours starting between 8–9 AM (08–09 h)
    [3] 5 hours starting between 9 AM–2 PM (09–14 h)
    [2] 5 hours starting between 2–5 PM (14–17 h)
    [1] 5 hours starting between 5 PM–4 AM (17–04 h)

18. At approximately what time of day do you usually feel your best?
    [5] 5–8 AM (05–08 h)
    [4] 8–10 AM (08–10 h)

[3] 10 AM–5 PM (10–17 h)
[2] 5–10 PM (17–22 h)
[1] 10 PM–5 AM (22–05 h)

19. One hears about “morning types” and “evening types.” Which one of these types do you consider yourself to be?
[6] Definitely a morning type
[4] Rather more a morning type than an evening type
[2] Rather more an evening type than a morning type
[1] Definitely an evening type

Interpreting your morning-eveningness score

This questionnaire has 19 questions, each with a number of points. First, add up the points you circled and enter your total morningness-eveningness score here: _____
Scores can range from 16-86.

- Scores of *41 and below* indicate *"evening types."*
- Scores between *42-58* indicate *"intermediate types."*
- Scores of *59 and above* indicate *"morning types."*

16-30	31-41	42-58	59-69	70-86
Definite evening	Moderate evening	Intermediate	Moderate morning	Definite morning

Occasionally a person has trouble with the questionnaire. For example, some of the questions are difficult to answer if you have been on a shift work schedule, if you don’t work, or if your bedtime is unusually late. Your answers may be influenced by an illness or medications you may be taking.

One way to check this is to ask whether your morning-eveningness score approximately matches the sleep onset and wake-up times listed below:

Score	16-30	31-41	42-58	59-69	70-86
Sleep onset	2.00 am-3.00 am	12.45 am-2.00 am	10.45 pm-12.45 am	9.30 pm - 10.45 pm	9.00 pm – 9.30 pm
Wake up	10.00 am - 11.30 am	8.30 am-10.00 am	6.30 am - 8.30 am	5.00 am – 6.30 am	4.00 am – 5.00 am

Pre-lab questions

1. What are some physiological processes that are controlled by your circadian clock?

2. People often have to get up earlier in the morning on weekdays than they do on weekends. Because of this, people often go to bed early on weeknights, but stay up late on the weekends. Based on what you have learned about circadian clocks, what do you think about having a different schedule for different times in the week?

3. People who work night shifts are often diagnosed with what is known as “shift work disorder”. The disorder is characterized by being excessively sleepy when trying to be awake (while working late at night) and not being able to sleep when trying to (during the day). This is despite the fact that such workers will often try to keep the same schedule for long periods of time. Why might it be difficult to switch your circadian rhythm to be awake at night and asleep during the day even when you are attempting to get the same total amount of sleep as normal?

4. The circadian clock is described as a “transcription-translation negative feedback loops”. What is a negative feedback loop? Can you describe a transcription-translation negative feedback loop in simple terms?

5. What are the two alleles being discussed in this lab? How do they differ genetically?

6. What are some reasons that having an allele that shows an association with a certain trait may not mean that a person actually displays that trait?

7. Is being a “morning person” or an “evening person” solely dependent on your circadian clock?

8. Reverse genetics uses techniques such as knocking out a gene (turning it off) to learn what effects that has on the organism. What might be some advantages of doing association studies over reverse genetic techniques? What might the limitations be?

Post-lab questions

Interpreting results

1. Use the schematic gel on the right to draw what your results look like. For each sample, draw the bands that you see on your actual gel.

2. Next to each band, write approximately how long (in base pairs) the DNA in that band is. Use the image of the ladder from page x to help you.

3. Use your gel electrophoresis results to complete the table below:
   A. Use checkmarks to record the gel electrophoresis results in the first two rows of the table.
   B. Record each person's genotype and chronotype (from the survey).

	Student 1	Student 2	Student 3	Student 4
Per3 5 allele (308 bp)				
Per3 4 allele (254 bp)				
Per3 genotype (5,5 / 5,4 / 4,4)				
Chronotype (Morning/intermediate/evening)				

Critical thinking

4. According to the questionnaire, are you an “evening type”, “morning type”, or “intermediate type”? Does your result match how you normally think of yourself?

5. What is your genotype and, assuming that the genetic association holds true, what does this suggest about your expected phenotype?

6. What are some reasons your genotype and perceived phenotype may not have matched?

Advanced questions

7. Now look at your class data. What is the average morning-eveningness score for students who are 4/4 homozygotes, 4/5 heterozygotes, and 5/5 homozygotes? Why is it more important to look at group averages than individual scores?

8. How many total **4-repeat** alleles were there in your class data? Remember that 4/4 homozygotes each have 2 alleles. 4/5 heterozygotes will have only one.

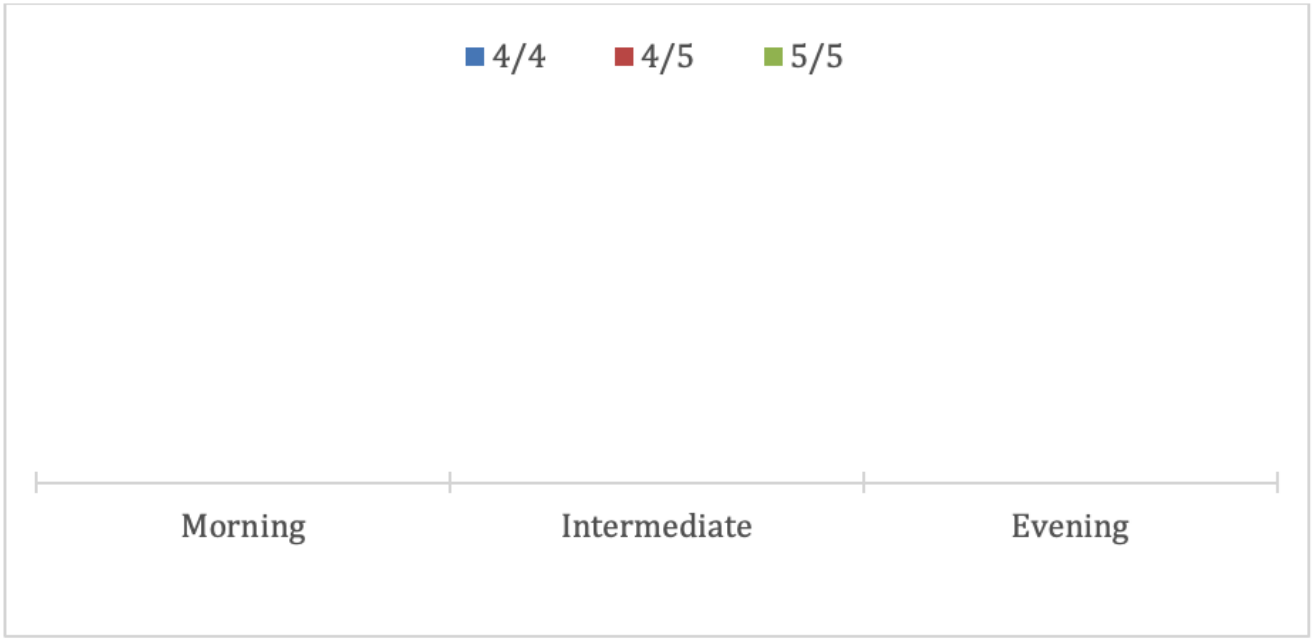
9. How many total **5-repeat** alleles were there in your class data? Remember that 5/5 homozygotes each have 2 alleles. 4/5 heterozygotes will have only one.

10. Calculate the percentages:
    A. What percentage of the total alleles for each phenotype 4-repeat alleles?
    B. What percentage of the total alleles for each phenotype 5-repeat alleles?
    C. Compare the two sets of percentages. Do you see any trend from the data?

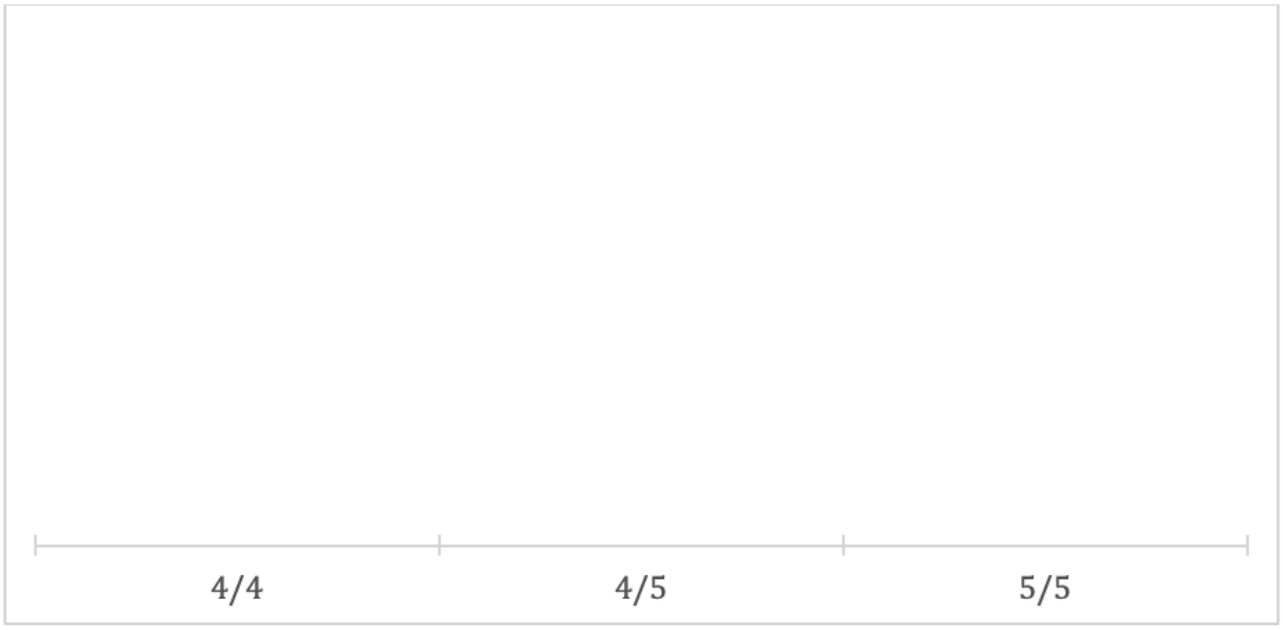
11. Does your class data show a possible association between either *Per3* allele and morning or evening preference?

12. Why would you need to use large sample sizes and a statistical test to establish whether an association is real or not?

13. Create a bar graph of your class data. For each chronotype, plot how many of each genotype were present in your class. (Each chronotype should have three bars.)



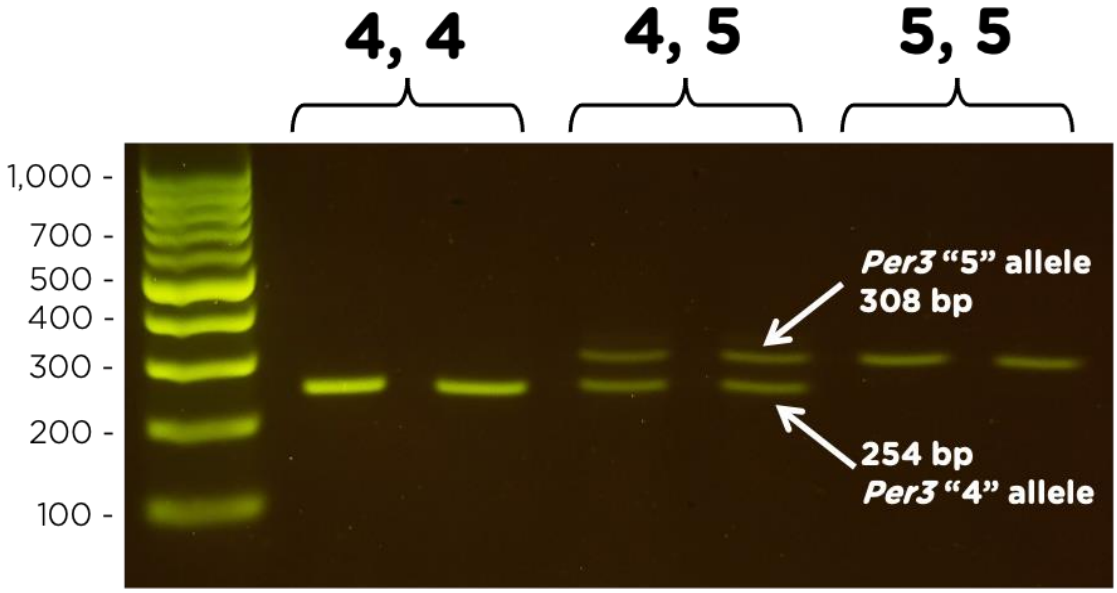
14. Create a graph showing the average Morning-Eveningness Score for each genotype.



Instructor guide

Expected results

Gel electrophoresis results are expected to resemble the photo below:



This photo represents results obtained from a 30-minute gel run using a blueGel.

Note that it is likely that the top of the 100 bp ladder will not fully separate with a short gel run, but this is ok! You only need the ladder to separate in the 200 to 400 bp range to be able to interpret the experimental results.

Remember that this activity investigates a **possible genetic association** between *Per3* genotype and morning-evening preference. Association does not imply causation—further studies are needed to confirm whether *Per3* genotype directly influences sleep phenotypes. Therefore, it is NOT expected that there will be a perfect correlation between *Per3* genotype and the morningness-eveningness score!

Unexpected results and troubleshooting

If fluorescent DNA bands are faint or entirely absent from one or more experimental samples, the following may have occurred:

- Too much tissue: The most common source of error in this protocol is the DNA extraction. Repeat the DNA extraction, and ensure students only lightly scrape their cheeks, as too much DNA can inhibit PCR.

- Suboptimal PCR amplification: Pipetting errors during PCR setup can lead to suboptimal amplification for individual student samples.

- Failure to load the DNA samples on the gel: Loading DNA samples for gel electrophoresis takes a little practice. The bands will appear faint if students do not successfully deposit the full sample volume into the well. Refer to https://www.minipcr.com/how-to-load-a-gel-electrophoresis/ for gel loading tips.

If fluorescent DNA bands are <u>not</u> visible on the gel, even for the DNA ladder, the following may have occurred:

- Failure to use a fluorescent DNA stain: This lab requires agarose gels made with a fluorescent DNA stain (*e.g.*, SeeGreen™ or GelGreen®). DNA stains that reveal DNA with a visible blue compound are less sensitive and are <u>not</u> compatible with this lab kit.
- Incorrect visualization conditions: Fluorescent DNA stains (*e.g.*, SeeGreen™ or GelGreen®) must be viewed using a blue light or UV transilluminator. The blueGel system has an integrated blue light transilluminator. For DNA visualization, ensure that you have turned on the blueGel's blue light by pressing the light bulb button.
- Samples were run off the gel: If you run the gel too long, DNA samples may migrate off the gel. Monitor progress by occasionally checking the DNA samples under a transilluminator or tracking the loading dye, which is visible to the eye. Stop the run before the colored loading dye reaches the end of the gel.
- Reagents were stored improperly and/or are expired: The lab kit can be stored in the freezer for up to twelve months after receipt. Storage under different conditions or in excess of this guidance may impair performance.

For tips on picture-perfect gels, see https://www.minipcr.com/gel-electrophoresis-troubleshooting/

For additional technical support, contact support@minipcr.com

Additional student supports

miniPCR tutorials: Access an extensive set of free resources to help your students succeed in molecular biology techniques. Visit https://www.minipcr.com/tutorials/. The resources most relevant to this lab are listed below.

- **Micropipetting:** Video, worksheet, and hands-on activity resources to train students in the basic use of a micropipette.
- **PCR:** Video and worksheet activity instructing students on the fundamentals and practice of PCR.
- **Gel electrophoresis:** Video and worksheet activity instructing students on the fundamentals and practice of agarose gel electrophoresis.

miniPCR Digital: Interactive tools for experiment-based learning with or without hands-on lab kits. Visit https://digital.minipcr.com/

Learning goals and skills developed

Student learning goals

- Explore the genetic underpinnings of circadian rhythms
- Understand the concept of genetic association studies

Scientific inquiry skills

- Identify or pose a testable question
- Formulate hypotheses
- Follow detailed experimental protocols
- Create tables or graphs to present their results
- Interpret data presented in a chart or table
- Use data to evaluate a hypothesis
- Make a claim based in scientific evidence
- Use reasoning to justify a scientific claim

Molecular biology skills

- Micropipetting
- DNA extraction
- Principles and practice of PCR
- Agarose gel electrophoresis

Citations

Archer, S.N., Robilliard, D.L., Skene, D.J., Smits, M., Williams, A., Arendt, J., and Von Schantz, M. (2003). A Length Polymorphism in the Circadian Clock Gene Per3 is Linked to Delayed Sleep Phase Syndrome and Extreme Diurnal Preference. Sleep 26, 413–415. https://doi.org/10.1093/sleep/26.4.413
Ebisawa, T., Uchiyama, M., Kajimura, N., Mishima, K., Kamei, Y., Katoh, M., Watanabe, T., Sekimoto, M., Shibui, K., Kim, K., et al. (2001). Association of structural polymorphisms in the human period3 gene with delayed sleep phase syndrome. EMBO Reports 2, 342–346. https://doi.org/10.1093/embo-reports/kve070.
Horne, J.A., and Ostberg, O. (1976). A self-assessment questionnaire to determine morningness-eveningness in human circadian rhythms. Int J Chronobiol 4, 97–110.
Osland, T.M., Bjorvatn, B., Steen, V.M., and Pallesen, S. (2011). Association Study of a Variable-Number Tandem Repeat Polymorphism in the Clock Gene PERIOD3 and Chronotype in Norwegian University Students. Chronobiology International 28, 764–770. https://doi.org/10.3109/07420528.2011.607375.

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