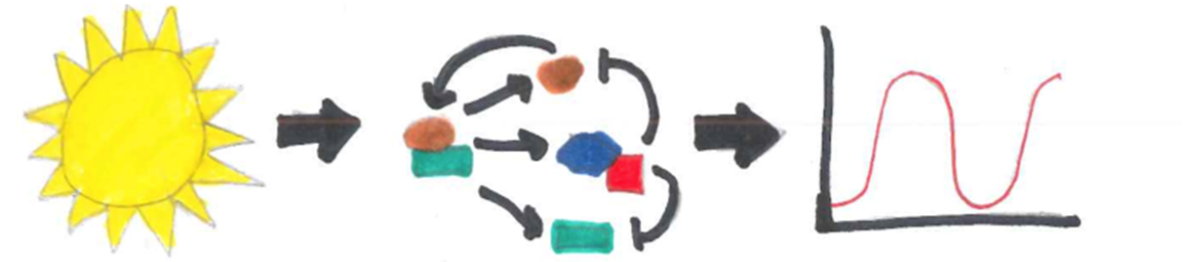
## 4. Background and significance

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 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*.



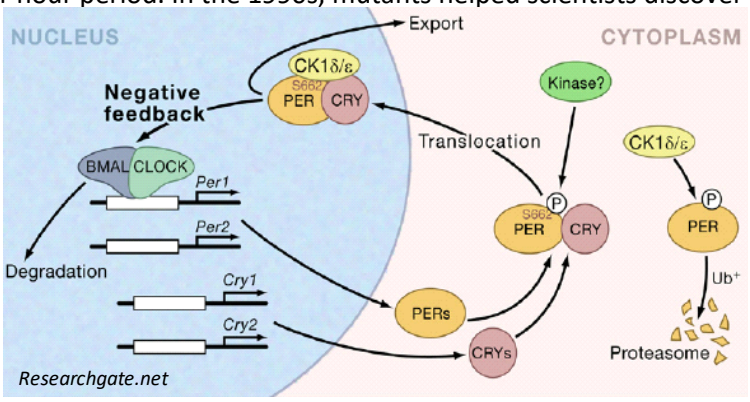

A fascinating feature about the circadian clock is that it operates at the cellular level; yes, individual cells in your body can keep and tell time! This is possible because the circadian clock is controlled by a genetic feedback loop, where proteins regulate gene expression with 24-hour periodicity. 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**: Transcription/translation feedback loops

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! To learn more about these and other circadian clock genes, read this review article by circadian clocks geneticist Joe Takahashi.

### 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.

## About 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!

**Let's get started with inquiry-based science**

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 ~350 base pair fragment, while 5-repeat segments will amplify as a ~400 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.)**

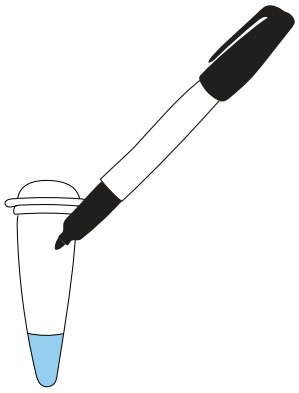
We hope that **matching chronotypes to genotypes across many students**, and later aggregating the data, will help us shed some light on the question of this reported genetic association between the *Per3* VNTR and circadian preferences. Through this lab everyone can become a circadian biology researcher!

**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 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.

# Laboratory guide

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

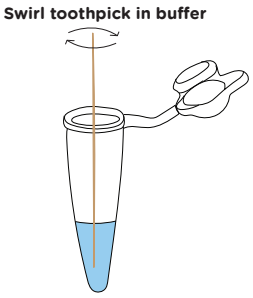
## DNA extraction



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

1. **You should receive a tube with 50 µl extraction buffer in it. Label the tube with your initials**
   - Label tubes on the upper sidewall, as writing on the cap or lower sidewall may rub off.

2. **Collect cheek cells**
   - Gently scrape the inside of your cheek 3-4 times with a flat-end toothpick.
   - It shouldn't hurt.




3. **Transfer cheek cells to tube with buffer**
   - Dip the end of the toothpick with your cheek cells in the X-Tract™ buffer.
   - Swirl the toothpick to dislodge the cells.
   - Dispose of the toothpick.

4. **Cap your tube**

5. **Incubate your tube for 10 minutes at 95°C**
   - You can use a miniPCR® thermocycler in Heat Block mode, a water bath, or other heat block.
   - Note: If using a heat block or water bath, weigh down the lids of your tubes so they don't pop open.

6. **Remove your tube from heat and immediately use DNA extract for PCR**
   - Note: The DNA extract is stable for up to two hours, but the sooner you use it for PCR the better.

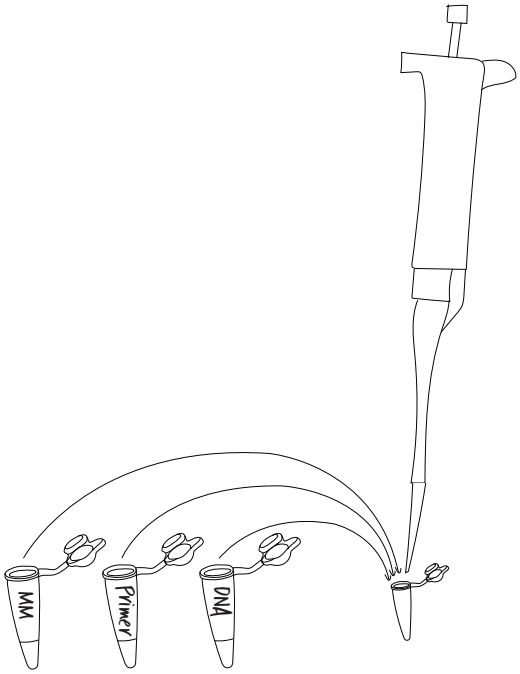
# PCR setup

1. **Label a PCR tube (200 µl tube) with your initials followed by "P" for PCR**
   - Note: label tubes on the upper sidewall, as writing on the cap or lower sidewall may rub off during PCR.

2. **Add PCR reagents to your labeled PCR tube**
   - Change pipette tips between samples to prevent contamination.

| Reagent | Volume |
|---|---|
| Sleep Lab Primers | 20 µl |
| 5X EZ PCR Master Mix™ | 5 µl |
| Student DNA sample | 3 µl |
| TOTAL VOLUME | 28 µl |

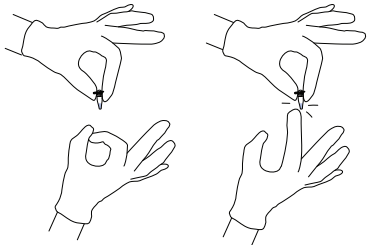
Note: EZ PCR Master Mix™ contains:
• Taq DNA polymerase • dNTPs
• PCR buffer with $Mg^{2+}$ • Gel loading dye



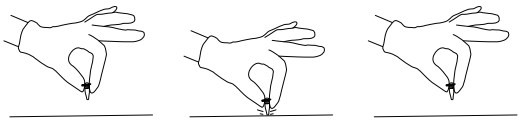

Use a micropipette to add each of the reagents. Remember to change tips at each step!

3. . **Cap the tubes and ensure the reagents mix well**
   - You may flick each tube with your fingers to ensure proper mixing.
   - Gently tap tubes on your bench to collect liquid at the bottom.

**Flick to mix**



**Tap to collect liquid at bottom**



4. **Place the tubes inside the miniPCR® machine**
   - Press firmly on the tube caps to ensure a tight fit.
   - Close the PCR machine lid and tighten it gently.

# PCR programming

*These instructions are illustrated using miniPCR® software on a Windows PC. Software interfaces vary slightly by operating system. See the miniPCR® User's Guide for more details.*

*If using a different thermal cycler, PCR protocol parameters should remain the same (step 7).*

**IMPORTANT NOTE:** CHECK YOUR KIT'S LOT NUMBER: Before proceeding further, please check that your Sleep Lab lot number (as labeled on the outside bag) is 211001 or starts with 22_ _ _ _ . In October 2021, Sleep Lab Primers were updated for improved *per3* gene detection. It is essential that you check the lot number on your kit! The protocol below is for kits with lot number 211001 or a lot number that starts with a number ≥ 22 (e.g. 22 _ _ _ _). If your kit has a prior lot number, the annealing temperature should be 65°C instead of 57°C. All other program parameters can remain the same.

1. **Open the miniPCR® app and remain on the "Library" window**

2. **Connect your miniPCR® thermal cycler to your device using the supplied USB cable or via Bluetooth®**
   - Note: Bluetooth® is only available on certain models. To connect via Bluetooth®, select the (m) icon, located by "Devices" at the left of the desktop app or at the top of the mobile app.

3. **Make sure your miniPCR® thermal cycler is plugged in and that the power switch is turned on**
   - Note: If your machine begins running a previously loaded protocol, you may stop it by clicking or tapping the "X" symbol in the top left box of the "Monitor" window.

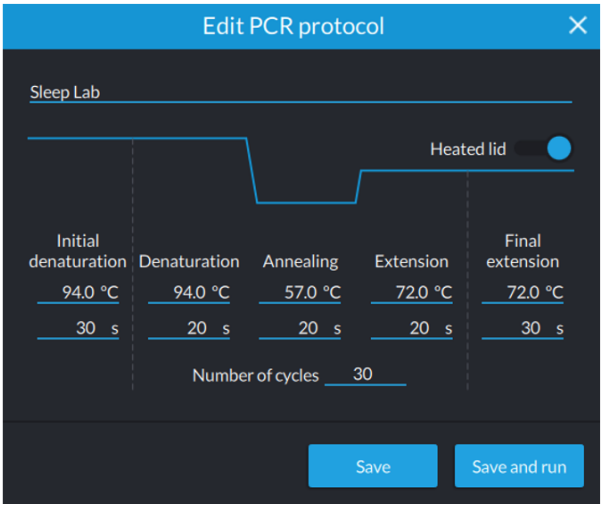
4. **While in the "Library" window, click the ⊕ button to create a new protocol**
   - Button is located in the upper right hand corner of the window.

5. **Select "PCR" from the drop-down menu**

6. **Enter a name for the protocol; for example: "Sleep Lab"**

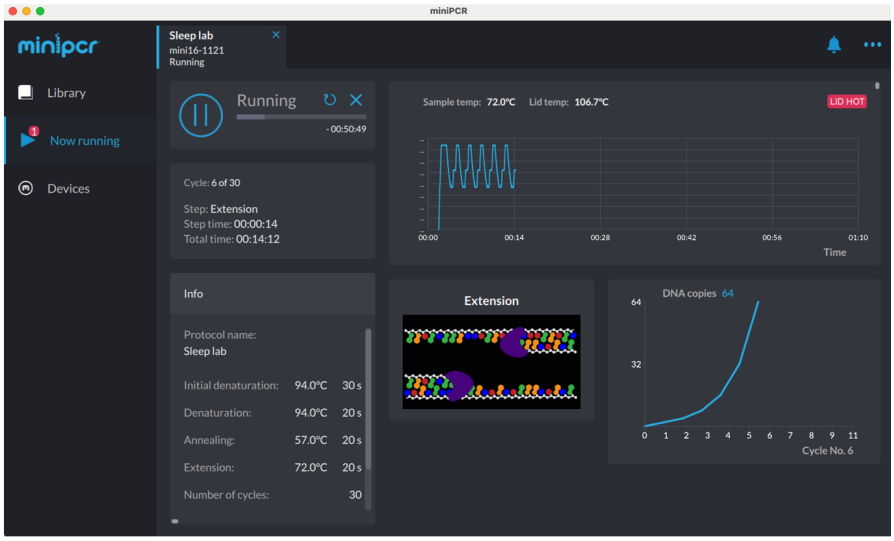
7. **Enter the PCR protocol parameters:**
   - Initial denaturation 94°C, 30 sec
   - Denaturation 94°C, 20 sec
   - Annealing 57°C, 20 sec
   - Extension 72°C, 20 sec
   - Number of cycles 30
   - Final extension 72°C, 30 sec

   Note: The "Heated lid" slider should be in the on position.



8. **Click "Save and run" to start the protocol**
    - If connected to more than one machine, choose the serial number of the miniPCR® thermal cycler you are using. If asked "Do you want to stop the current protocol...?", click "Yes".
    - The lights on the front of the miniPCR® thermal cycler will blink 3 times to indicate that the protocol has been loaded.
    - Note: If needed, you may unplug the USB cable or disconnect Bluetooth® once the protocol has been loaded. Even if disconnected from your device, the protocol will continue to completion as normal.

9. **Choose "Monitor" window**
    - The "Monitor" window can be selected on the left column in the desktop app and at the top in mobile app.
    - If more than one miniPCR® thermal cycler is connected to the same device, choose which machine you would like to monitor using the tabs at the top of the window (desktop app) or bottom of the Library (mobile app).




*The miniPCR® software allows each lab group to monitor the reaction parameters in real time.*

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10. **When the PCR run has completed (approximately 60 min), app status will show "Finished" and the red, yellow, and green LEDs on your miniPCR® thermal cycler will light up and stay on**

Be careful not to touch the metal lid which may still be hot.

11. **PCR product is stable at room temperature for several days. For longer term storage, move tubes to a fridge or freezer**
    - Tubes may remain inside the miniPCR® thermal cycler for several days following protocol completion.

# Laboratory guide

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

## Gel electrophoresis - Pouring gels (before or during class period)

*Important Gel Pouring Notes*

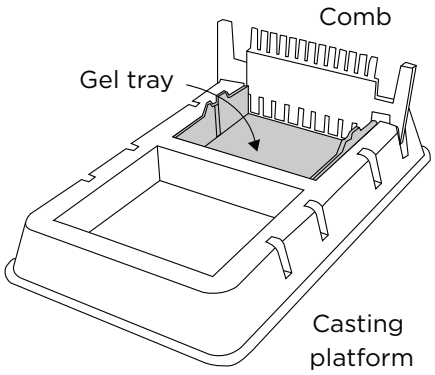
Gels can be prepared up to three days ahead of time and stored at ambient temperature, covered in air-tight plastic wrap and protected from light.

You will need one lane per student plus one lane for ladder per gel.

These instructions are designed for use with the blueGel™ electrophoresis system by miniPCR bio™. If using another electrophoresis system, these instructions may need to be adjusted according to the manufacturer's instructions.

1. **Prepare 1X TBE buffer (to be completed by teacher in advance)**
   - TBE buffer is often provided as liquid concentrate or powder.
   - Follow manufacturer's instructions to prepare 1X TBE buffer solution.

2. **Prepare a clean and dry casting platform with a gel tray and comb**
   - Place the clear gel tray in the white casting platform.
   - Place a well-forming comb at the top of the gel tray.




3. **Prepare a 2% agarose solution with a fluorescent DNA stain (e.g., SeeGreen™ or GelGreen®) using the method indicated by your instructor**

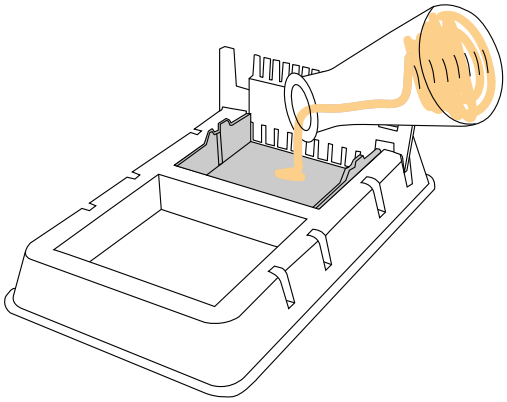
**IMPORTANT NOTE: There are several ways to prepare agarose gels**

- Scan the QR code for detailed instructions on how to prepare agarose gels.
- Both written and video instructions are available.

www.minipcr.com/agarose-gel/

4. **Pour the agarose solution into the prepared casting platform with a gel tray and comb**
    - The agarose solution should cover the bottom of the gel tray and the bottom 3 mm of the comb (roughly the bottom 1/3 of the comb).

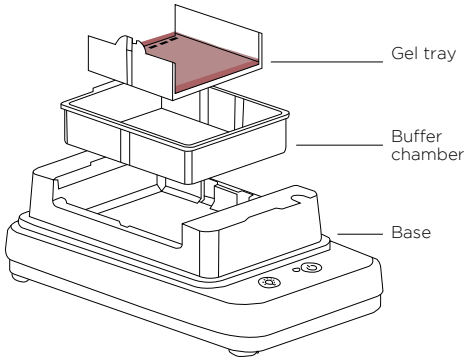
5. **Allow gel to solidify completely and remove the comb by pulling firmly upwards**
    - Gels will typically be ready in about 10 minutes.
    - Gel is ready when cool and firm to the touch.



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

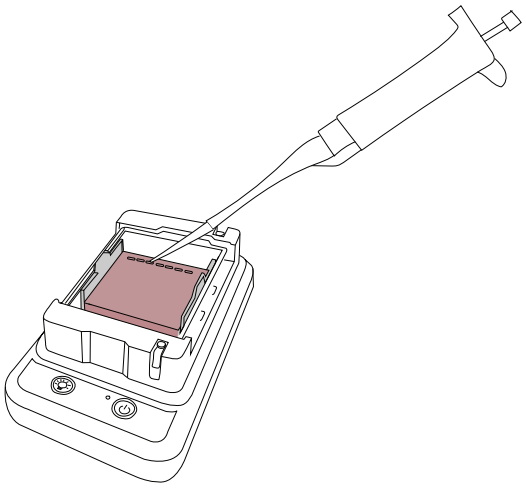
# Gel electrophoresis - Running the gel

These instructions are designed for use with blueGel™ electrophoresis system by miniPCR bio™. If using another electrophoresis system, these instructions may need to be adjusted according to the manufacturer's instructions.




1. **Place the gel tray containing your gel in the buffer chamber**
   - Ensure that the clear buffer chamber is inside the blueGel™ electrophoresis system.
   - The wells of the gel should be on the same side as the negative electrode, away from the power button.

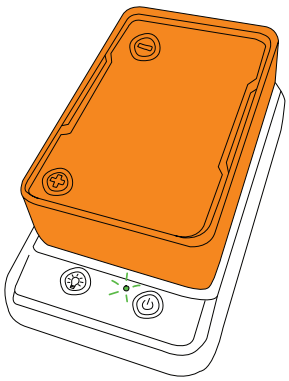
2. **Add 30 ml of 1X TBE electrophoresis buffer**
   - The buffer should just cover the gel and fill the wells.
   - Ensure that there are no air bubbles in the wells (shake the gel gently if bubbles need to be dislodged).



3. **Load samples onto the gel in the following sequence**
   - **Lane 1:** 10 µl DNA Ladder
   - **Lane 2:** 15 µl Student 1 PCR product
   - **Lane 3:** 15 µl Student 2 PCR product
   - **Lane 4:** 15 µl Student 3 PCR product
   - **Lane 5:** 15 µl Student 4 PCR product

   Note: Change pipette tips between samples to prevent contamination.

4. **Place the orange cover on the blueGel™ electrophoresis system**
    - To prevent fogging, make sure that ClearView™ spray has been evenly applied to the inside of the orange cover.
    - Match the positive and negative electrode signs on the orange lid with the corresponding positive and negative signs on the blue base.
    - The electrodes of the lid should be aligned with the metal leads on the base.
    - The orange lid should sit flush with the blue base using littl force.



5. **Press the "Run" button**
    - Check that the green light beside the power button remains illuminated.

6. **Conduct electrophoresis for 35-45 minutes**
    - Note: Check the gel every 10 minutes to monitor sample migration.
    - Longer electrophoresis times will result in better size resolution. However, if run too long, small DNA fragments can run off the end of the gel or lose fluorescence.

# Chronotype questionnaire

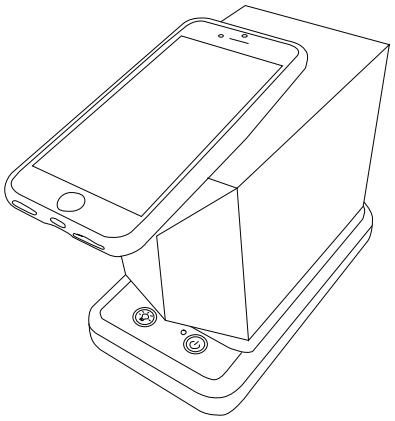
1. **While your gel is running complete the chronotype questionnaire on page 27.**

# Gel electrophoresis – Visualizing results

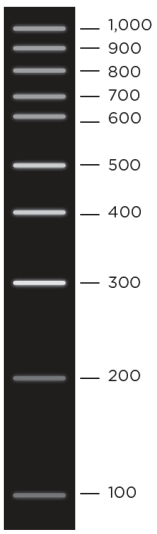
1. **Press the "light bulb" button to turn on the blueGel™ transilluminator**
   - For best viewing, dim lights or use Fold-a-View™ photo documentation hood with a smartphone camera.
   - Gels may be viewed at the end of the run or periodically throughout the run.
   - If the image appears hazy, wipe off the inside of the orange cover and reapply ClearView™ spray.

2. **Ensure that there is sufficient band separation in the 300-500 bp range**
   - Run the gel longer if needed to increase resolution.

3. **Document your results**
   - Place Fold-a-View™ photo documentation hood on the blueGel™ electrophoresis system to take a picture with a smartphone or other digital camera.
   - Compare the bands from the DNA samples to the ladder to obtain size estimates.



**100 bp Ladder**



## 6. Study questions

*Teachers may email team@miniPCR.com to request answer key.

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**

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

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

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

4. 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?

Let's now look at class data slightly differently.

5. 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.

**6.** What percentage of the total alleles for each phenotype 4-repeat alleles?

**7.** 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.

**8.** What percentage of the total alleles for each phenotype 4-repeat alleles?

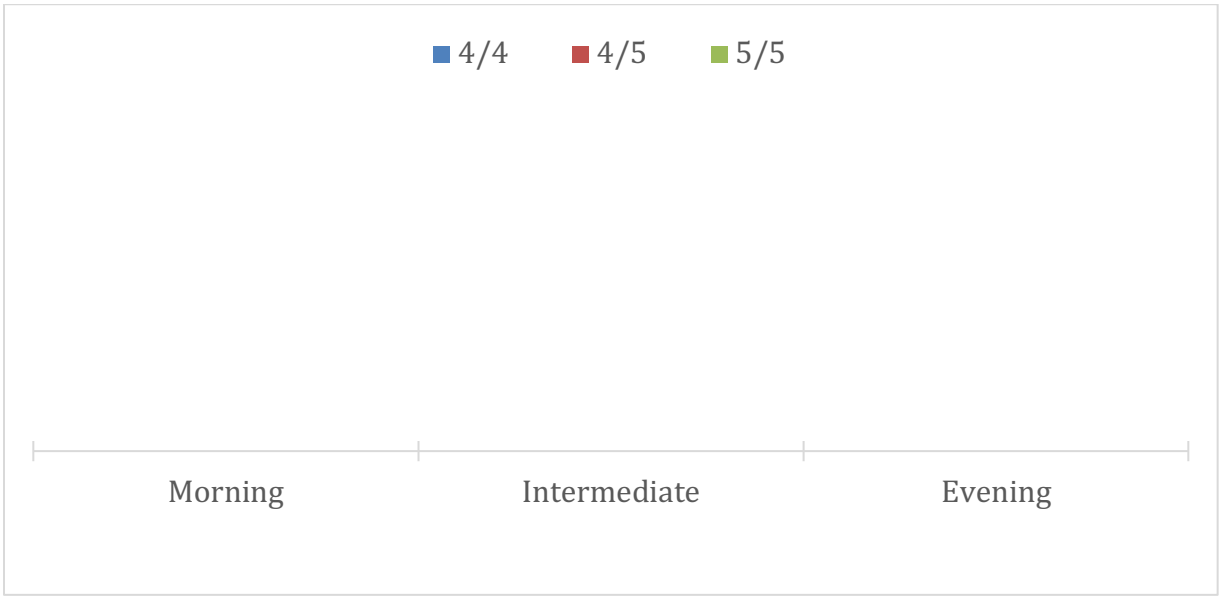
**9.** Compare the two sets of percentages. Do you see any trend from the data?

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

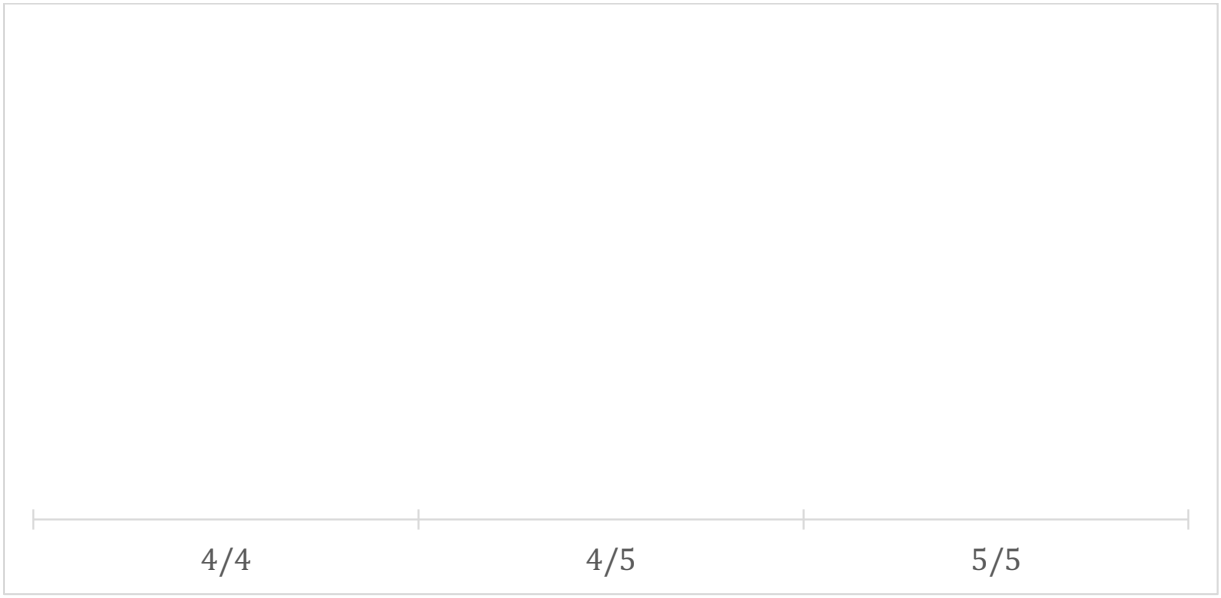
**11.** Why would you need to use large sample sizes and a statistical test to establish whether an association is real or not?

## *Data Analysis*

*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.)*




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



## 7. Morning-Eveningess Questionnaire6

This self-assessment questionnaire to determine your circadian rhythm *chronotype* can be carried out during the PCR or gel electrophoresis runs.

Name (or sample number): _____ Date: _____

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.

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 AND USING YOUR MORNINGNESS-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 |

## *Share your data:*

## *Help towards a better understanding of this postulated genetic association.*

Most phenotypes are complex traits, with multiple genetic and environmental components. Genetic associations are difficult to establish unequivocally, and association studies are notoriously hard to reproduce, especially due to limitations in sample size. By compiling anonymized data across our classrooms, we can contribute to a deeper understanding of this postulated association between *Per3* alleles and sleep preferences.

If you're interested in contributing your classroom's anonymized data to this ongoing investigation, please contact: team@minipcr.com.

We are happy to provide guidance on data gathering, anonymization, and sharing (in the format provided here: https://www.minipcr.com/wp-content/uploads/sleep-lab-data.xlsx).

## 8. Extension activity: Hypothesized mechanism[5]:

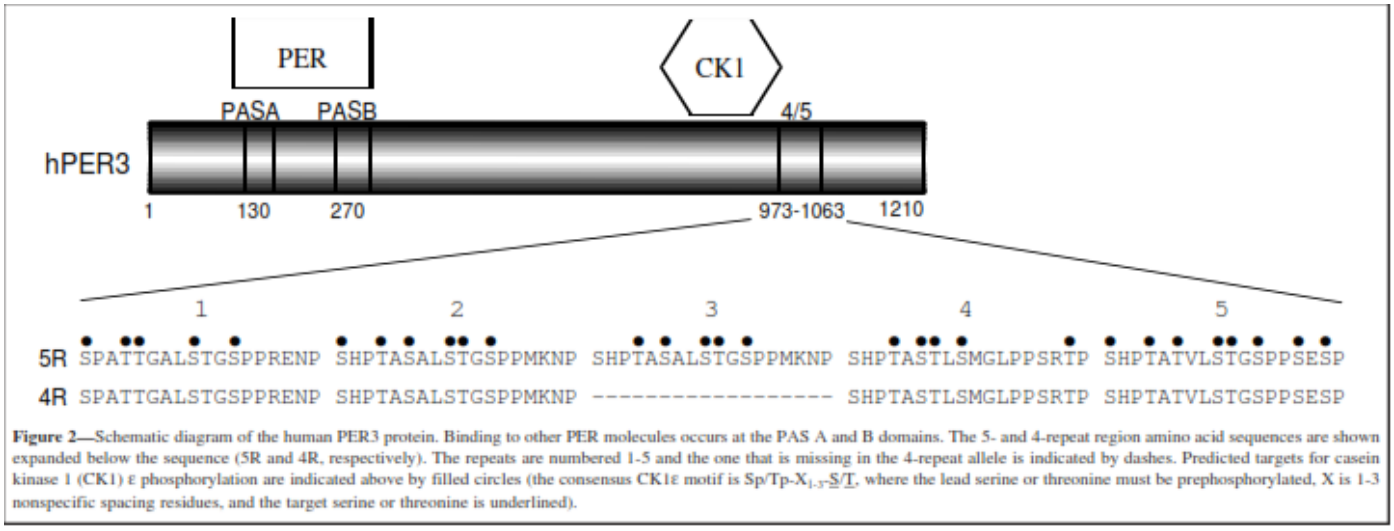



**Figure 2**—Schematic diagram of the human PER3 protein. Binding to other PER molecules occurs at the PAS A and B domains. The 5- and 4-repeat region amino acid sequences are shown expanded below the sequence (5R and 4R, respectively). The repeats are numbered 1-5 and the one that is missing in the 4-repeat allele is indicated by dashes. Predicted targets for casein kinase 1 (CK1) ε phosphorylation are indicated above by filled circles (the consensus CK1ε motif is Sp/Tp-$X_{1-3}$-S/T, where the lead serine or threonine must be prephosphorylated, X is 1-3 nonspecific spacing residues, and the target serine or threonine is underlined).

As a reminder, CLOCK and BMAL1 are transcription factors activating the expression of circadian clock genes, including *Period* (PER) and *Cryptochrome* (CRY). PER and CRY proteins are in turn repressors of CLOCK/BMAL1 activity, shutting down their own activation.

PER and CRY proteins accumulate throughout the circadian day, peaking in the early night, when they move into the nucleus and turn off their own transcription by blocking Clock and Bmal1 (see p. 10 inset for a schematic model).

PER proteins undergo phosphorylation by two *kinases*, enzymes that phosphorylate (covalently link phosphate groups) other proteins as part of signaling processes within the cell. Kinases in the circadian feedback loop are Casein kinase 1 δ and ε (CK1δ and CK1ε), and it is known that by phosphorylating PER and CRY proteins they target them for degradation, which results in decreased PER and CRY protein levels in the early circadian day. With low PER and CRY levels, repression of Clock/BMAL1 is lifted, and transcription can begin again. Thus, a new circadian cycle starts early in the prospective day.

> Hypothesizing a model where PERIOD3 undergoes phosphorylation in the same manner described above, and that the 5-repeat VNTR *adds* new phosphorylation sites to the PER3 protein, **ask students to draw a model for how the *Per3$^{5/5}$* polymorphism may result in a morning lark phenotype**.

[5] Figure from Archer SN, et al. "A length polymorphism in the circadian clock gene Per3 is linked to delayed sleep phase syndrome and extreme diurnal preference." Sleep. 2003 Jun 15;26(4):413-5.