Instructor Lab Guide

Athlete Gene PCR Experiment

Contents

Quick Overview

Explore human evolution by testing for the genetic marker associated with the "Athlete Gene": ACTN3. Students isolate cheek cell DNA, use allele-specific PCR to amplify parts of the ACTN3 gene, and use gel electrophoresis to identify genotypes linked to fast-twitch and slow-twitch muscle fibres that can influence athletic performance.

This experiment is an example of how PCR can be used to detect specific alleles that may contribute towards observable human traits, such as muscle performance; and how genetic variation can influence human physiology and potential athletic abilities.

Lab Skills and Scientific Concepts

Techniques	Concepts
• Pipetting • DNA extraction • Centrifugation • PCR • Allele-specific-PCR • Agarose gel electrophoresis	• Genotype vs Phenotype • Single nucleotide polymorphisms (SNP) • Gene expression and regulation • Human evolution and natural selection • Population genetics • Ethics and consent • Data privacy

Skills Required

To successfully complete this lab in the suggested timing, students should have experience pipetting using 20 µL and 200 µL pipettes.

Time Requirements

This experiment fits into two 60-minute periods with a minimum 2-hour break between sessions for the PCR run to complete. To keep Class 2 under 60 minutes, the teacher will need to cast the agarose gels before the session begins.

Extend the Lesson: If you have more time, you can expand this into two 120-minute sessions. This allows your students to cast their own gels, and provide more time to explore the underlying genetic concepts in detail.

Stopping Points (optional):

- **After DNA Extraction:** You can store DNA extracts in the freezer (-20 °C) for up to 8 days.
- **After PCR:** Amplified DNA is stable at room temperature for several days, or you can freeze it for long-term storage.

Materials and Equipment

What Is Included in the Kit?

Item(s)	Usage	Storage
5x HOT FIREPol® Master Mix Ready To Load 2 x 100 µL (50 reactions)	4 µL per PCR	**Unopened:** Stable at room temperature for up to 30 days *from shipping*, or at 2–8 °C for up to six months. **Opened/Long-Term:** Store at -20 °C.
PCR Grade Water 1 x 5 mL (50 reactions and excess for DNA extract dilution if required)	12 µL per PCR	**Unopened:** Stable at room temperature indefinitely. **Once Opened:** Store at 2–8 °C or -20 °C.
Primer Mix, Athlete's Gene, 1 x 400 µL (200 reactions)	2 µL per PCR	**Unopened:** Stable at room temperature for up to 30 days *from shipping*, or at 4 °C for up to nine months. **Once Opened/ Long-Term:** Store at -20 °C.

What Other Materials Will I Need?

Reagents and Chemicals	Suggested Amount
Household table salt	0.9 g per 100 mL (8 students), or 4.5 g per 500 mL (40 students).
Bottled or tap water	500 mL per 40 students.
Agarose Tablets or agarose power	1 x 0.5 g tablet per 25 mL of agarose gel, or according to your gel electrophoresis equipment instructions.
DNA stain (e.g. GelGreen®)	5 µL per 50 mL gel, or according to your DNA stain instructions.
100 bp DNA ladder	2 x 5 µL per gel row (first and last wells)
0.5x TBE buffer (or alternative running buffer)	100 µL per 50 mL gel, or according to your gel electrophoresis equipment instructions
Distilled or deionized water (1 L)	1 L per 10x 50 mL gels

What Plastic Consumables Will I Need?

Consumables needed	Per student
Transfer pipette (1 mL)	1
Pipette Tips 2-200 µL	One box per group of students. You will need a minimum of 7 tips per student, plus some extra for accidents; negative controls; DNA stain when making the agarose gels; and loading DNA ladder.
1.5 mL microcentrifuge tubes	1
200 µL PCR tubes	2
Disposable plastic cup	1
Nitrile gloves	1 set per student (of appropriate sizes) per session (minimum)

What Equipment Will I Need?

Equipment	Recommended Amount
Bento Lab Or A standard thermal cycler, centrifuge, and horizontal gel electrophoresis unit	Minimum 1 per a maximum of 6 students. Or Recommended 1 per group of 4 - 8, depending on equipment capacity.
Micropipette (adjustable, 2–20 µL range) Micropiette (adjustable, 20-200 µL range)	Minimum 1 of each per group
Centrifuge adaptors for PCR tubes: a 1.5 mL tube with a 500 µL tube inside, with lids cut off both tubes	Minimum 1 per centrifuge slot (ensure you have even numbers for balancing)
Plastic cup or shot glass	Minimum 1 per student
Plastic or glass beaker, 500 mL	Minimum 1 per gel casting
Microwave	1 per class
Bin for used pipette tips	Minimum 1 per group
Pocket weighing scales, eg. 0.01 g - 100 g	Optional: 1 per class, for weighing liquids during class preparation.

Scientific Background: The *ACTN3* Gene and Muscle Performance

Why are some people naturally better at sprinting and jumping, while others perform better in endurance sports? Part of the answer may lie in a small genetic difference in the **ACTN3 gene**.

One well-studied variation in this gene is known as R577X, also known as the ACTN3 rs1815739 polymorphism. This variation in a single DNA base allows or interrupts the production of a muscle protein called **α-actinin-3.**

Although absence of α-actinin-3 does not cause disease, it does influence how muscle fibres function, and by doing so it can contribute towards a person's athletic potential.

The Biology of Muscle Fibres

To understand how the *ACTN3* gene influences performance, it's necessary to examine the structure and function of human muscle fibres. Human skeletal muscle contains three main fibre types:

- **Type I (slow-twitch oxidative):** Fatigue-resistant and suited to endurance activities.
- **Type IIa (fast-twitch oxidative):** Intermediate in speed and endurance.
- **Type IIx (fast-twitch glycolytic):** Produce the fastest and most powerful contractions but fatigue quickly.
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Fast-twitch (Type II) fibres are especially important for sprinting and explosive movements. These fibres contain α-actinin-3, a structural protein that helps stabilise muscle during powerful contractions.

The protein α-Actinin-3 is mainly found in the fastest fibres (Type IIx); it is present in Type IIa fibres; and is absent from slow-twitch (Type I) fibres.

The Genetic Variation in ACTN3

This lab focuses on a single-base-pair change, or Single Nucleotide Polymorphism (SNP), located in the *ACTN3* gene, called R577X or the ACTN3 rs1815739 polymorphism

There are two possible **alleles** (variants) for the R577X polymorphism:

- **R allele (C):** The SNP is a C (cytosine), and this helps code for the amino acid arginine (**C**GA) which becomes incorporated into α-actinin-3 at that point in the protein synthesis. The protein α-actinin-3 is produced.

- **X allele (T):** The SNP is a T (thymine), which introduces a **nonsense mutation** (**T**GA) in the form of a premature **stop codon** (a sequence of three nucleotides that signals the

ribosome to stop translation), resulting in a truncated (shortened) and non-functional protein. As a result, no α-actinin-3 is produced.

Because we inherit two copies of the gene, three **genotypes** are possible:

- **RR / CC: Homozygous.** Full production of α-actinin-3.
- **RX / CT: Heterozygous**. Reduced production of α-actinin-3, but muscle structure and function remain largely normal.
- **XX / TT: Homozygous.** No α-actinin-3 production.

It's important to note that people with the XX genotype are healthy. Another protein, α-actinin-2, compensates for the missing α-actinin-3. However, their fast-twitch fibres tend to behave slightly more like slow-twitch fibres, with slightly lower power output and more endurance-like characteristics.

A Global Perspective

All R577X variants are generally common worldwide. Around 39% of people are RR, 43% are RX, and around 18% are XX.

However, the XX genotype is rare in some West African populations (as little as 1-5% of the population) and more common in European and Asian populations.

Studies show that RR and RX genotypes are modestly associated with sprint and power performance.

Evidence linking the XX genotype to endurance performance is less consistent, with some studies showing associations, while others show no associations.

Can R577X predict an individual's performance?

ACTN3 has only a small effect on predicting athletic ability in individuals. This is because performance depends on many factors, including training, nutrition, psychology, environment, and many other genes.

There are elite speed/power athletes with the XX genotype, and endurance athletes with RR or RX genotypes. No single gene can determine whether someone will become an elite athlete.

An Ongoing Field of Research

The R577X polymorphism has been studied for many years and remains an active area of research in genetics and sports science.

Many studies have shown that RR or RX genotypes are extremely common among elite sprint athletes, suggesting that even this small contribution to performance can be important at their level of competition.

However, studies investigating the XX genotype are less consistent in their findings: some studies have shown a modest association with endurance performance, while others have shown none, or even negative associations.

Scientists are still investigating how much the XX genotype influences endurance performance, how training and environment interact with ACTN3, and how other genes contribute to muscle performance.

These unanswered questions also show that athletic ability is complex and influenced by many factors, not just a single gene.

Key Terms

ACTN3 gene: A gene that provides instructions for making the protein α-actinin-3.

Allele: A different version of the same gene. In this case, the ACTN3 gene has R and X alleles.

Allele-specific PCR: A type of PCR that can distinguish between different alleles of a gene.

Codon: A sequence of three DNA or mRNA bases that codes for an amino acid or a stop signal.

DNA (Deoxyribonucleic acid): The molecule that carries genetic information in cells. It is made of a sequence of bases (A, T, C, G).

DNA polymerase: The enzyme that builds new DNA strands during PCR.

Fast-twitch muscle fibres (also known as Type II): Muscle fibres adapted for fast, powerful movements such as sprinting. They fatigue more quickly.

Gel electrophoresis: A method used to separate DNA fragments by size using an electric current.

Gene: A section of DNA that contains the instructions to make a protein.

Genotype: The combination of alleles an individual has (RR, RX, or XX).

Heterozygous: Having two different alleles (RX).

Homozygous: Having two identical alleles (RR or XX).

Nonsense mutation: A mutation that changes a codon into a stop codon, resulting in a shortened, non-functional protein.

PCR (Polymerase Chain Reaction): A laboratory technique used to make many copies of a specific DNA sequence.

Phenotype: The observable characteristics that result from the interaction between genotype and environment.

Primer: A short DNA sequence that's used to define the starting point of DNA replication in PCR.

Protein: A molecule made of amino acids that performs important functions in cells.

R allele (C allele): The version of the gene that allows normal production of α-actinin-3.

R577X: A specific SNP in the ACTN3 gene. The R version produces a functional protein, while the X version introduces a stop signal.

Slow-twitch muscle fibres (also known as Type I): Muscle fibres adapted for endurance activities. They contract more slowly but are more resistant to fatigue.

SNP (Single Nucleotide Polymorphism): A variation in a single DNA base at a specific position in the genome.

Stop codon: A codon that signals the end of protein synthesis.

X allele (T allele): The version of the gene that creates a stop codon, preventing production of α-actinin-3.

α-actinin-3: A protein found mainly in fast-twitch muscle fibres and involved in powerful muscle contractions.

Experimental Overview

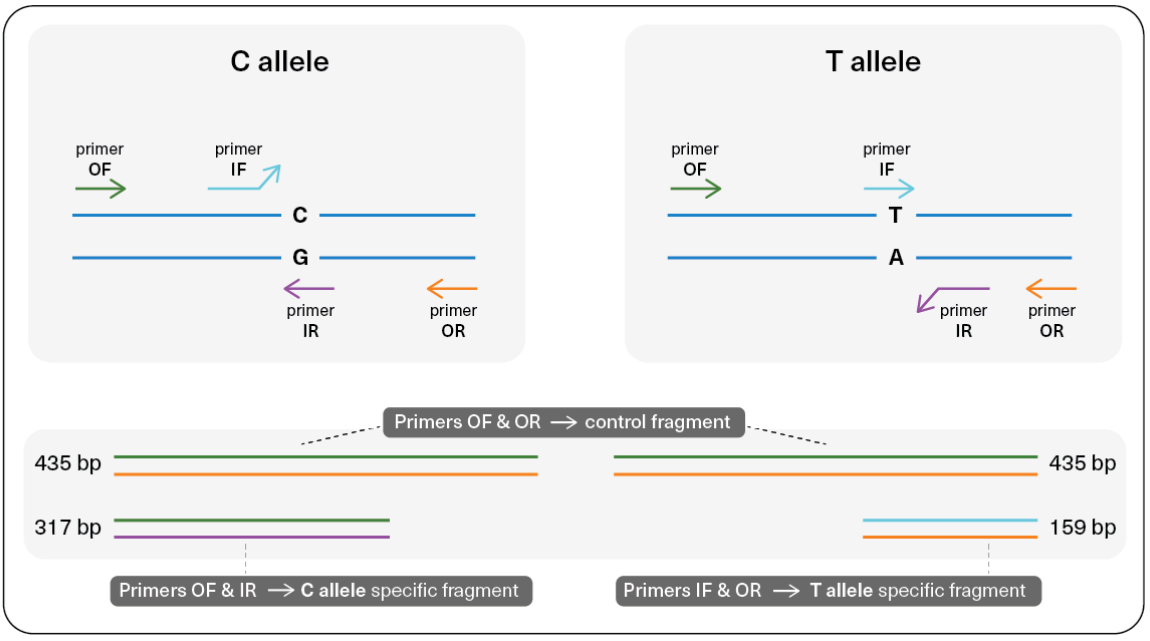
This lab uses a technique known as bidirectional **allele-specific PCR**. While standard PCR usually detects a large gene segment, allele-specific PCR is designed to identify a specific Single Nucleotide Polymorphism (SNP) – a change in just one "letter" of the DNA code – in a single reaction. The PCR is bidirectional because both SNP variants (if present) are detected by different primers that operate in different directions.

How the PCR "Reads" the DNA

The assay uses **four primers** working together. Two "outer" primers create the large control amplicon (amplified DNA fragment), while two "inner" primers are allele specific, meaning they will only extend and create an amplicon if they find a perfect match for either the C or T allele at the SNP site.

The figure below illustrates the way the primers interact to produce allele-specific amplicons.

- In all cases, primers OF and OR amplify the whole region, producing a control amplicon (435 base pair or bp size).
- For the C allele, primers OF and IR amplify a slightly smaller fragment (317 bp size) if the C allele is present, but primer IF (which is T-specific) cannot bind to the DNA.
- For the T allele, primers IF and OR produce an even smaller fragment (159 bp size), but primer IR cannot bind to the DNA.



What Does the PCR Produce?

A student's sample can produce either two or three distinct fragments on the gel:

- The **Control** Fragment **(435 bp):** A large, universal segment amplified by the outer primers to confirm the DNA extraction and PCR were successful.
- The **C Allele** Fragment **(317 bp):** A mid-sized fragment that only appears if the functional ACTN3 variant is present.
- The **T Allele** Fragment **(159 bp):** A small fragment that only appears if the nonsense mutation resulting in the non-functional ACTN3 variant is present.

Interpreting the Genotypes

By looking at the combination of bands on the gel, you can determine the specific genotype and associated **phenotype**:

Genotype	Definition	Bands on Gel	Typical Phenotype
CC	Homozygous dominant	435 bp + 317 bp	Produces α-actinin-3, may be slightly better adapted for speed/power athletics
CT	Heterozygous	435 bp + 317 bp + 159 bp	Produces some α-actinin-3, may be slightly better adapted for speed/power athletics
TT	Homozygous recessive	435 bp + 159 bp	Produces no α-actinin-3, may be slightly better adapted for endurance athletics

Suggested Schedule

We suggest running the lab over a **2 x 60-minute** schedule. To make this work, you will need to prepare materials before the sessions begin (detailed instructions are provided in the **Instructor Preparation** section).

If you have more flexibility, you can expand these into 120-minute sessions to allow students to prepare their own saline and cast their own gels. Longer sessions will also give you more time to discuss the science behind the techniques and topics.

Note on timing: Keep in mind that the PCR program requires a **2 hour** run time, which serves as a natural break between your two classes.

Session 1 (60 min)

Focus: DNA Extraction & PCR Setup

Before class: Instructor set-up of equipment and materials

Time	Activity	
0 - 10 min	Introduction	The instructor introduces the experiment and students consent.
10 - 30 min	DNA Extraction	Students produce a DNA template.
35 - 60 min	PCR Setup	Students set-up their PCR reaction tube, and the instructor set up the thermocycler.

After class: Instructor stores the PCR products after the PCR program is finished.

Session 2 (60 min)

Focus: Gel Electrophoresis & Analysis

Before class: Instructor casts a gel, and sets-up equipment and materials

Time	Activity	
0 - 15 min	Gel Loading	Assist students with loading their 3 possible fragments into the gel.
15 - 45 min	Running Gels	While gels run, review the theory and predict results.
45 - 60 min	Visualization	Take photos and help students identify the 435, 317, and 159 bp bands.

After class: Clean-up.

Instructor Preparation Guide

Preparation for DNA Extraction

1. Set Up and Check Equipment

Ensure the following equipment is available:

- **Thermocyclers:** One or more with enough capacity for one well per student.
- **Centrifuges:** Capable of speeds of at least ~2,700 xG. For quickest centrifugation use a centrifuge capable of >4,000 xG. and ideally use one per group of students.
- **Micropipettes (2-20 µL):** One for each small group, or ideally per student.

2. Program the Thermocycler

Program each thermocycler with the heatblock program below so it is ready to be run as soon as the students finish their pipetting.

2.1 Create and Save a Heatblock Program for Cell Lysis

- 10 mins at 95 °C
- 1 min at 15 °C

3. Prepare Reagents and Chemicals

3.1 Make 0.9% saline solution for DNA extraction

Add **4.5 g of non-iodized salt** to **500 mL of still bottled or tap water**. Allow the salt to dissolve completely, and shake to mix. This will make enough saline solution for 40 students.

3.2 Dispense 0.9% saline solution into cups and tubes

Each student will need:

- 10 mL of 0.9% saline solution in a plastic cup (for the mouthwash step)
- 1x 1.2 mL of 0.9% saline solution in a 1.5 mL centrifuge tube (for cell wash and resuspension steps)

ⓘ **Tip:** A quick and easy way to measure out the 10 mL volumes is by weight on small portable weighing scales.

4. Set Up the Student Benches

Materials for each **student group**

Equipment

- 20 µL pipette
- Fine permanent marker pen
- Bin for used pipette tips
- Centrifuge adaptors for PCR tubes: a 1.5 mL tube with a 500 µL tube inside, with lids cut off both tubes
- Centrifuge balance tubes (1x 1.5 mL tube filled with 1 mL water, and 1x PCR tube filled with 100 µL of water)
- A designated waste beaker for used saliva samples, clearly labelled as biological waste

Plastic consumables

- A box of 20-200 µL pipette tips (at least 10 tips per student to allow for errors)
- Lab gloves (of appropriate sizes)

Materials for each **student**

Equipment

- 1 mL transfer pipette

Reagents/chemicals

- 10 mL of 0.9% saline solution in a plastic cup
- 1.2 mL of 0.9% saline solution in a 1.5 mL centrifuge tube

Plastic consumables

- 1x 1.5 mL centrifuge tube for pelleting the extracted cheek cells

Preparation for PCR

ⓘ **Sense-check:** Make sure that you have enough PCR wells for your students' samples - you will need one well per student plus one well for a negative control. You may also want to run positive controls (using previously produced successful PCR products) - in which case allow well spaces for these.

1. Program the Thermocycler

Program each thermocycler with the PCR program below so it is ready to be run as soon as the students finish their pipetting.

Create and Save the PCR Program, "Athlete's Gene PCR"

- Initial Denaturation: 15 mins at 94 °C (to activate the HOT FIREPol Master Mix)
- 35 Cycles of:
  - Denaturation: 30 seconds at 94 °C
  - Annealing: 45 seconds at 67 °C
  - Extension: 45 seconds at 72 °C
- Final Extension: 120 seconds at 72 °C

2. Aliquot PCR Reagents

Each workstation should be supplied with enough PCR reagents for the number of students in the group.

Reagents should be aliquoted from the original tubes into labelled tubes for groups of students to pipette from. These tubes should include a small excess to allow for pipetting inaccuracies and mistakes.

Reagents to Aliquot for Each Student Group

PCR Reagent	Amount per PCR	Amount per Group of 4, plus excess for controls and mistakes (7 samples)
PCR Grade Water	12 µL	84 µL
5x HOT FIREPol® Master Mix	4 µL	28 µL
Athlete's Gene PCR assay primer mix	2 µL	14 µL

Aliquoted reagents should be kept in the fridge until the lab class.

ⓘ **Tip:** Volumes can be adjusted to allow for larger numbers of students, or for additional excess in case of mispipetting.

3. Set Up the Student Bench

Materials to set out for each student group

Equipment

- Micropipette 2-20 µL

- Micropipette 20-200 µL

Reagents

- 5x HOT FIREPol® Master Mix (aliquoted as above)
- PCR Grade Water (aliquoted as above)
- Athlete's Gene primer mix (aliquoted as above)

Plastic consumables

- 1x PCR tube per student, plus one for the negative control
- 20 µL pipette tips (2-200 µL tips unfiltered): one box per group of students. Allow around 10 tips per student, plus 4 tips per negative control PCR setup, 1 tip per gel, 2 tips per gel row (for the DNA ladder)

Preparation for Agarose Gel Electrophoresis

ⓘ **Sense-check:** Make sure that you have enough lanes for your students samples - you will need one lane per student, plus a lane for a negative control per PCR batch, and one or two lanes of DNA ladder per gel.

1. Preparing the Agarose Electrophoresis Running Buffer

Prepare agarose gel electrophoresis running buffer according to the manufacturer's instructions. Different electrophoresis equipment will require different concentrations and volumes of running buffer - please consult your manufacturer's recommendations.

If you are using Bento Lab, we recommend allowing up to 50 mL of 0.5x TBE buffer for casting a mini-gel, and up to 50 mL of 0.5x TBE for running the gel (100 mL total).

To make 0.5x TBE buffer from 10x TBE buffer, add 50 mL of 10x TBE buffer to 950 mL of distilled or deionised water, and mix thoroughly.

2. Casting the Agarose Electrophoresis Gel

Materials needed for each gel casting station

Equipment

1. Agarose gel electrophoresis gel casting apparatus
2. Agarose gel electrophoresis gel tank
3. Microwave oven
4. Plastic or glass beaker
5. Micropipette 2-20 µL

Chemicals and reagents

- 0.5x TBE buffer (or recommended buffer and concentration for your gel tank)
- Agarose tablets (0.5 g) or preweighed agarose powder of equivalent weight
- A safe DNA stain (e.g. GelGreen® DNA stain)

Plastic consumables

- 2-200 µL Pipette tips (1 tip needed per gel)

Cast your agarose gels according to the requirements of your agarose gel electrophoresis apparatus. Different equipment will use different volume agarose gels, and will allow different numbers of lanes per agarose gel.

If you are using Bento Lab, you can visualize up to nine PCR products plus one negative control and two DNA ladder lanes in a standard gel (using the 12 comb), or up to 19 PCR products plus one negative control and four DNA ladder lanes per gel if using an Add-A-Lane comb.

For best fragment separation and visualisation, we recommend casting 2% agarose gels using 0.5 g of agarose (tablets or powder) per 25 mL of agarose gel, stained with a safe in-gel DNA stain (e.g. GelGreen®) at the manufacturer's recommended concentration.

⚠ **Safety Warning:** Never microwave agarose in a tightly sealed container or bottle. The liquid can become superheated and boil over explosively when moved. Always use a loosely covered or open beaker.

⚠ **Safety Warning:** Hot agarose solution can cause severe burns. Handle beakers very carefully, and use heat-resistant gloves and eye protection. Allow the solution to cool to a safe handling temperature (~50 °C) before pouring.

Cast a 2% agarose gel for Bento Lab

1. Add 1x 0.5g agarose tablet to 25 mL of 0.5x TBE in a plastic or glass beaker. Allow it to completely break down into powder.
2. Microwave for around 30-45 seconds until the agarose just starts to bubble
3. Carefully remove the beaker and swirl it briefly
4. Microwave again for 10-20 seconds until the agarose starts to bubble again.
5. Carefully remove and swirl the beaker
6. Microwave again for 10-20 seconds until the agarose starts to bubble again.

ⓘ **Visual Check:** Once you have melted the agarose, hold the beaker up to the light. The molten agarose must be completely transparent, much like water. If you see any floating 'lenses', wavy refractive lines, or grainy specks, it needs more heating.

7. Allow the agarose and beaker to cool to around 50 °C.
8. Add 2.5 µL of GelGreen® DNA stain, and swirl carefully to mix thoroughly without introducing bubbles into the mix.
9. Set up the Gel Box with rubber dams and comb/s in place, and place it open on a level surface.
10. Carefully pour the agarose into the gel box, and allow it to set for 15-30 minutes.

ⓘ **Visual Check:** After pouring the liquid agarose into the tray, immediately check for small air bubbles, especially near the comb teeth. Use a clean pipette tip to push any bubbles to the edge or pop them before the gel sets.

11. Remove the dams and combs, and cover the gel with 0.5x TBE buffer with at least a few mm, ensuring the level of liquid is below the MAX line on the side of the tank.

ⓘ **Tip:** During the agarose melting procedure, some water can be lost through evaporation. To compensate for this, the beaker plus agarose plus buffer can be weighed before and after microwaving; the loss calculated from the difference in weights; and the water added back to the beaker as distilled water. Alternatively, adding back a few mL of distilled water should compensate for the majority of the evaporation loss.

Preparation for Loading and Running Agarose Gels

1. Setting up the gel loading station(s)

Materials needed **for each gel loading station**

Equipment

- Bento Lab and gel imaging hood, or:
  - Electrophoresis power supply
  - Agarose electrophoresis gel tank
  - Agarose electrophoresis gel casting apparatus
  - Blue light transilluminator and any gel imaging hood apparatus
- Micropipette (2-20 µL) - ideally a pipette dedicated to loading PCR products only (to minimise PCR product cross-contamination
- A smartphone camera or small compact camera for recording gel images
- Bin for used pipette tips

Plastic consumables

- 2-200 µL pipette tips (one tip needed per sample or DNA lane)

Reagents

- 100 bp DNA ladder
- Student PCR products, including negative control

Other materials

- Agarose gels cast in the previous step

Ensure that the gel tank/s are disconnected from any power supply, and that the power supply is off.

Place the students' PCR products and 100 bp DNA ladder in an appropriate place so that each student can either collect their own (if they are interpreting their own), or a random sample (if the class is randomising their samples).

For each gel loading station:

- Position the gel tank such that the gel and wells are sufficiently illuminated, and positioned in such a way that it is easy for students to look down at and pipette into the wells.
- For easier loading, a dark strip of card can be placed under the wells to increase contrast.
- Place the pipette tips on one side of the area, and the bin for waste pipette tips on the other side of the area.

Safety

Instructor Responsibilities

As the instructor, you are responsible for the safety of your students and classroom. We strongly recommend you perform a full risk assessment according to the specific requirements and policies of your institution, school, or district (e.g., a CLEAPSS-style risk assessment in the UK, or alignment with your district lab safety policy in the US).

Biological Safety: Working with Human DNA Samples

The DNA is extracted from the student's own buccal (cheek) cells using a saline mouthwash.

- Sample Control: Ensure students only extract and work with their own samples. All tubes must be clearly labeled immediately with a fine permanent marker pen.
- Contamination: Treat all saliva and cell extracts as potential biological waste.
- Waste Disposal: Follow local guidelines for the disposal of biological waste. At the end of Session 1 and Session 2, instructors must check all waste (saliva, DNA extracts, used tips, gloves, agarose gel, and running buffer) is disposed of properly.

Chemical & Reagent Safety

- Kit Reagents: The reagents in the Athlete Gene Teaching Lab Kit (PCR Master Mix, Primer Mix, PCR Grade Water) are generally considered non-hazardous. Safety Data Sheets (SDS) should be reviewed and made available as per your institution's policy.
- 0.9% Saline Solution: This is a simple salt-water solution.
    - Mouthwash: Instruct students not to swallow the 10 mL of 0.9% saline solution during the mouthwash step. The salt intake is low (0.09 g) if accidentally swallowed, but it is best practice to discourage ingestion. Ingestion of a large amount (e.g. 500 mL) may cause gastrointestinal discomfort or mild hypernatremia.
- DNA Stain: We recommend using a safe, non-mutagenic DNA stain such as GelGreen® to visualize the gel results. Use standard laboratory personal protective equipment (PPE), such as gloves, when handling the stain and stained gels.
- TBE Buffer: TBE (Tris/Borate/EDTA) buffer used for electrophoresis is generally low-hazard but should be handled with gloves.

Equipment & Process Safety

The following steps involve the use of standard laboratory equipment that requires instructor supervision:

Centrifuging (DNA Extraction):

- Balance: It is critical to check the centrifuge is correctly balanced before every run, using a balancing tube of equal weight when necessary. Unbalanced centrifuges can be a mechanical hazard.
- Speed/Time: Ensure the centrifuge is run at the correct speed and time as specified in the protocol (e.g., 4,000 xG for 120 seconds).
- **WARNING:** If the centrifuge begins to shake violently or makes a loud, unusual noise, **immediately press the stop button and alert the instructor.**

Thermocycler (PCR Setup & Run):

- Heat: The thermocycler block reaches high temperatures (up to 95 °C). Instruct students to be extremely careful when handling the PCR tubes after the heating steps, such as after the initial 10-minute 95°C heat incubation for DNA extraction. Use appropriate caution (e.g., gloves, gentle handling) as the tubes will be hot.

Casting an Agarose Gel:

- Microwave/Heat: Melting the agarose requires a microwave or other heat source. Hot agarose solution can cause severe burns. Use heat-resistant gloves and eye protection. Allow the solution to cool to a safe handling temperature before pouring.
- DNA stain: wear gloves, don't get in eyes, dispose of according to local regulations.

Running an Agarose Gel (Electrophoresis):

- Power Supply: The gel box must be closed with the lid and the leads correctly connected to the power supply before turning the power on. Do not touch the gel, running buffer, or leads while the power supply is on. Ensure the gel is run at the specified voltage for your electrophoresis equipment (e.g., 50V - 80V for Bento Lab's electrophoresis module).

Visualizing Results (Blue Light Transilluminator):

- Light/Eye Protection: While blue light transillumination is safer than UV, students should use the appropriate gel imaging hood or filter (if available) to minimize direct exposure to the blue light and to protect eyes. Do not stare directly into the light source.

Ethics & Consent

Working with Human Samples

Working with human DNA samples, even simple cheek cell extracts, raises ethical considerations that must be addressed clearly with students and guardians before the practical begins.

Informed Consent

- **Necessity:** Obtaining a DNA sample from a student requires their informed consent and, for minors, the consent of a parent or guardian.
- **Procedure:** You must explain the nature of the experiment—that it analyzes a single genetic variation (an ACTN3 gene SNP) linked to the production of α-actinin-3—and obtain permission before students collect their samples.
- **Right to Refuse:** Students must understand they have the right to refuse to participate without penalty. An alternative water-based "blank" sample should be provided for them to process through the protocol.

Anonymity of Results

The results of this experiment will determine the student's ACTN3 genotype, which is a private piece of personal information. While these data are not health-related, you may want to offer the class a choice regarding the visibility of these results:

- **Option 1:** Individual Interpretation (Identified Results): Students interpret their own results and keep them private. This is suitable for classes where the teacher is confident in managing a sensitive environment.
- **Option 2:** Class Pool (Anonymized Results): To check privacy, the instructor can mix up all the completed PCR reaction tubes after the setup stage, but before the gel run. Students would then load a random, unidentifiable product onto the gel. This allows the class to analyze the population genetics of the results without identifying any individual's genotype.

Data Handling and Storage

- **No Long-Term Storage**: DNA extracts and PCR products should be considered biological waste and disposed of immediately and properly at the end of the practical, as outlined in the Biological Safety section. Do not store or archive student samples.
- **Discussion:** Use the topic to discuss the wider ethical and societal implications of genetic testing and the importance of data privacy in science.

Troubleshooting Guide

DNA Extraction

Problem	Possible Cause	Solution to Try
Missing or small cell pellet	Not enough cells collected or not enough centrifugation.	Centrifuge for longer or at a higher speed. Repeat the mouthwash extraction, ensuring you rub your teeth against your cheeks.
The supernatant couldn't be removed from the pellet without it disintegrating	Insufficient centrifugation; ropey saliva strand in mouthwash extract	Centrifuge for longer; repeat extraction with another portion of the mouthwash extract
The cleaned supernatant is cloudy	Insufficient centrifugation at the sample cleanup stage	Repeat centrifugation with increased speed or time.

PCR

Problem	Possible Cause	Solution to Try
The labelling came off during PCR	Poor quality permanent marker; labelling the top of the tube only	Try a different permanent marker; label the side of the tubes
The PCR tube samples evaporated or decreased in volume	The tubes had a poor seal or were not firmly closed	Check the tubes are firmly closed or try a different brand of PCR tube if it happens again

Agarose Gel Electrophoresis

Problem	Possible Cause	Solution to Try
The DNA ladder is smeared and poorly resolved on gel	The agarose was not completely melted before casting the gel; excessive heat during run (voltage too high/long run); buffer issues	Ensure agarose is completely molten before casting; run at recommended voltage; check buffer composition and volume; recast and rerun gel
Smearing or poorly resolved PCR products on gel, but the ladder is well resolved	DNA overloaded; excessive heat during run (voltage too high/long run); buffer issues; non-specific amplification	Load less DNA; run at recommended voltage; shorten run time; check buffer composition/volume; recast gel if needed; check PCR protocol
The negative control contained amplified DNA	Mispipetting into the wrong well; DNA cross contamination from a previous PCR product	Run a new gel with the negative control to rule out mispipetting; decontaminate pipettes and use new reagents

The control fragment did not amplify, but smaller fragments did amplify	PCR inhibition; low DNA extract yield; DNA fragmentation during storage	Dilute the DNA extract by 10x with PCR Grade Water before using for PCR; repeat DNA extraction with a larger pellet
Primer dimers / ladder-like bands at bottom (non-specific)	Non-specific amplification early in PCR; primers annealing to each other	Reduce primer concentration. Use of hot start master mix should minimise primer dimer formation.
Unexpected additional bands (non-target amplicons)	Non-specific amplification due to suboptimal PCR conditions	Increase annealing temperature; adjust annealing/extension times; optimise primer design or get new primers.
Cloudy gel / bubbles / poor gel quality	Air trapped during casting; agarose not fully dissolved	Degas agarose before pouring; swirl to remove bubbles; check full dissolution pre-pour.
PCR products stuck in wells / smears near wells	Physical gel issues; DNA overloading; carryover contaminants from extraction; complex non-specific products	Check electrophoresis gel and ladder; dilute DNA extract; improve extraction cleanliness; recast gel.
No visible DNA ladder or bands from PCR product samples	No DNA stain added to the gel; degraded DNA stain; DNA stain exposed to light	Remake the gel and include DNA stain; use new DNA stain; do not expose the gel to too much bright light during the gel run
Uneven migration / crooked lanes	Uneven gel surface; unlevel electrophoresis tank	Ensure gel and tank are level; align electrodes properly before running.
Low template yield leading to weak target bands	DNA template concentration too low; inefficient PCR	Increase template DNA; repeat PCR with appropriate cycling conditions.
Excessive non-specific amplification without target band	Annealing temp too low; too many PCR cycles; incorrect extension time	Raise annealing temp; reduce cycle number; set extension time to ~60 s per kb target.