A **bristlecone pine** is one of three [species](https://en.wikipedia.org/wiki/Species) of [pine](https://en.wikipedia.org/wiki/Pine) trees (family [Pinaceae](https://en.wikipedia.org/wiki/Pinaceae), genus [*Pinus*](https://en.wikipedia.org/wiki/Pinus), subsection *Balfourianae*). All three species are long-lived and highly resilient to harsh weather and bad soils. One of the three species, *Pinus longaeva*, is among the longest-lived life forms on Earth. The oldest *Pinus longaeva* is more than 5,000 years old,[[1]](https://en.wikipedia.org/wiki/Bristlecone_pine#cite_note-oldest-1) making it the oldest known individual of any species.

Recently, a Bristlecone pine growing in an undisclosed location in a National Park in Nevada was illegally cut down. Annual ring data suggests the tree is about 4600 years old.



National Parks and wildlife Service officials recently made a sweep of seven different suspects homes where wood and debris from ancient Brisltlecone pines were found. DNA samples were obtained from timber at each of these seven sites. Your task is to determine if any of the seven DNA samples are in fact DNA from the same tree that was illegally cut down and removed. A sample of DNA was obtained from the trunk of the illegally cut Bristlecone Pine, and will be provided.

 PreLab:

1. Research the geographical range of the Bristlecone pines. Describe the habitat and identify the species niche.
2. Provide a brief summary of the conservation status of the plant. List 10 interesting facts you encountered regarding the species.
3. How bad is their plight?
4. What is your recommendation for their future?

**Activity: Using Gel Electrophoresis to Solve a Crime**

In this section you will take the DNA samples of the seven trees as well as the DNA obtained from the trunk at the crime scene and use gel electrophoresis to determine which homeowner has the illegally cut tree.

* Each table will have one electrophoresis chamber.
* The four students per table will break up into two groups with each group having there own gel wells.
* The first group students will run solutions A, B, C of the suspects and CS (DNA left at the crime scene).
* The second group will run D, E, F, and G of the remaining suspects.
* When the activity is completed, the two groups will compare their results to determine who left the blood sample on the glass shard.

**The Crime Scene (CS) DNA sample and the DNA of the Seven Suspects**

 CS Crime scene DNA (known DNA from ***illegally cul bristlecone pine)***

 A

 B

 C

 D

 E

 F

 G

**Group 1**

1. Label four microfuge tubes- CS, A, B, and C.
2. Set the P-20 micropipette to 2 uL and dispense 2 uL dH2O to each microfuge tube.

CS, A, B, C.

1. Add 8 uL CS (crime scene) DNA to the labeled CS microfuge tube.
2. Eject the tip into the waste container and replace it with a fresh tip.
3. Follow steps 2 and 3 for the microfuges labeled A, B, and C.
4. Place all four microfuges tubes in the microcentrifuge for 10 seconds

**Group 2**

1. Label four microfuge tubes*-* D, E, F, and **G.**
2. Set the P-20 micropipette tip to 2 uL and dispense 2 uL of dH2O into each microfuge tube:

D, E, F, G.

1. Add 8 uL of suspect D DNA to the microfuge tube labeled D.

1. Eject the tip into the waste container and replace it with a fresh tip.
2. Follow steps 2 and 3 for the microfuges labeled E, F, and G.

1. Place all four microfuges in the microcentrifuge for 10 seconds.

 **Loading the DNA into the Gels**

1. Pour the melted agarose into a gel casting tray. Make sure you place **two-eight teeth combs** into the gel casting tray. Group 1 will use the first set of wells, and group two the second set of wells. Allow the gel to solidify before removing the two combs.
2. Place the agarose gel into electrophoresis chamber. Make sure the gates are down on both sides before placing the gel into the cast tray. The wells should be located next to the negative electrode (black).
3. Slowly add the 1X SB buffer into the electrophoresis chamber until the buffer covers the gel by 1-2 millimeters. Make sure the gel wells are filled with buffer. Do not connect the electrodes at this point.

**Group 1**

1. Set the micropipette to 10uL and slowly load each sample into a separate well as indicated below. Use a fresh tip for each sample.

 Well 1 add 10 uL sample CS

 Well 3 add 10 uL sample A

 Well 5 add 10 uL sample B

 Well 7 add 10 uL sample C

1. When loading each sample, center the pipette over the well and gently depress the micropipette plunger to slowly expel the sample. Use your other hand to help support your pipette hand to avoid shaking.

**Group 2**

1. Your group will be using the second set of wells. Set the micropipette to 10uL and slowly load each sample into a separate well as indicated below. Use a fresh tip for each sample.

 Well 1 add 10 uL sample D

 Well 3 add 10 uL sample E

 Well 5 add 10 uL sample F

 Well 7 add 10 uL sample G

1. When loading each sample, center the pipette over the well and gently depress the micropipette plunger to slowly expel the sample. Use your other hand to help support your pipette hand to avoid shaking.

**Turning on Power Supply**

1. Close the cover tightly over the electrophoresis chamber. Connect the leads to the power supply, black to black and red to red.
2. Turn on the power supply and set the voltage to 130 v. Press the “run” switch to begin the process. Look for tiny bubble rising in the chamber.
3. Stop the process in approximately 15 minutes and unplug the electrodes from the power supply. Carefully remove the gel from the gel tray from the chamber and place it on a piece of paper toweling. Compare the pattern of both gels.

**Post-Lab Questions**

1. Compare the dyes in the lanes of both wells to determine whose DNA matches the sample left at the crime scene. Who committed the crime?
2. The crime scene suspect left behind a sample of blood at the crime scene that was used to construct a DNA profile. Are there other ways that the perpetrator could have left a DNA sample for forensic Identification?
3. Besides DNA evidence, list other types of non DNA evidence that a crime suspect could leave behind that might be used as forensic evidence?

**Teacher Key**

**The solutions contain various combinations of Bromophenol blue (purple) , Xylene cyanole (blue), and Orange G (orange).**

 **Suspect Colors on Gel**

**A Porterford; the butler Orange, purple**

**B Colonel Hornblower; Army officer, retired Blue**

**C Franz; the cook Orange, purple**

**D Vance; the chauffeur Blue, purple**

**E Martini; the carpenter Orange, purple, blue**

**F Senorita Esmerelda; the maid Purple**

**G Juan; the gardener Orange**

**E Crime Scene Orange, purple, blue**

**Some Examples of DNA Uses for Forensic Identification**

* **Identify potential suspects whose DNA may match evidence left at crime scenes**
* **Exonerate persons wrongly accused of crimes**
* **Identify crime and catastrophe victims**
* **Establish paternity and other family relationships**
* **Identify endangered and protected species as an aid to wildlife officials (could be used for prosecuting poachers)**
* **Detect bacteria and other organisms that may pollute air, water, soil, and food**
* **Match organ donors with recipients in transplant programs**
* **Determine pedigree for seed or livestock breeds**
* **Authenticate consumables such as caviar and wine**

**Is DNA effective in identifying persons?**
 [answer provided by Daniel Drell of the U.S. DOE Human Genome Program]

* **DNA identification can be quite effective if used intelligently. Portions of the DNA sequence that vary the most among humans must be used; also, portions must be large enough to overcome the fact that human mating is not absolutely random.**
* **Consider the scenario of a crime scene investigation . . .**
* **Assume that type O blood is found at the crime scene. Type O occurs in about 45% of Americans. If investigators type only for ABO, finding that the "suspect" in a crime is type O really doesn't reveal very much.**
* **If, in addition to being type O, the suspect is a blond, and blond hair is found at the crime scene, you now have two bits of evidence to suggest who really did it. However, there are a lot of Type O blonds out there.**
* **If you find that the crime scene has footprints from a pair of Nike Air Jordans (with a distinctive tread design) and the suspect, in addition to being type O and blond, is also wearing Air Jordans with the same tread design, you are much closer to linking the suspect with the crime scene.**
* **In this way, by accumulating bits of linking evidence in a chain, where each bit by itself isn't very strong but the set of all of them together is very strong, you can argue that your suspect really is the right person.**
* **With DNA, the same kind of thinking is used; you can look for matches (based on sequence or on numbers of small repeating units of DNA sequence) at many different locations on the person's genome; one or two (even three) aren't enough to be confident that the suspect is the right one, but thirteen sites are used. A match at all thirteen is rare enough that you (or a prosecutor or a jury) can be very confident ("beyond a reasonable doubt") that the right person is accused.**