



Dye Electrophoresis Lab

Princes in the Tower



Student guide



Background information	P. 11
Today's lab	P. 17
Glossary	P. 22
Student lab protocol	P. 23
Post-lab questions	P. 24
CER table	P. 26



Background information

The Princes in the Tower

1

- You are probably familiar with scientists using DNA analysis to solve crimes.
- We can use the same techniques to investigate centuries-old mysteries, like the Princes in the Tower.

2

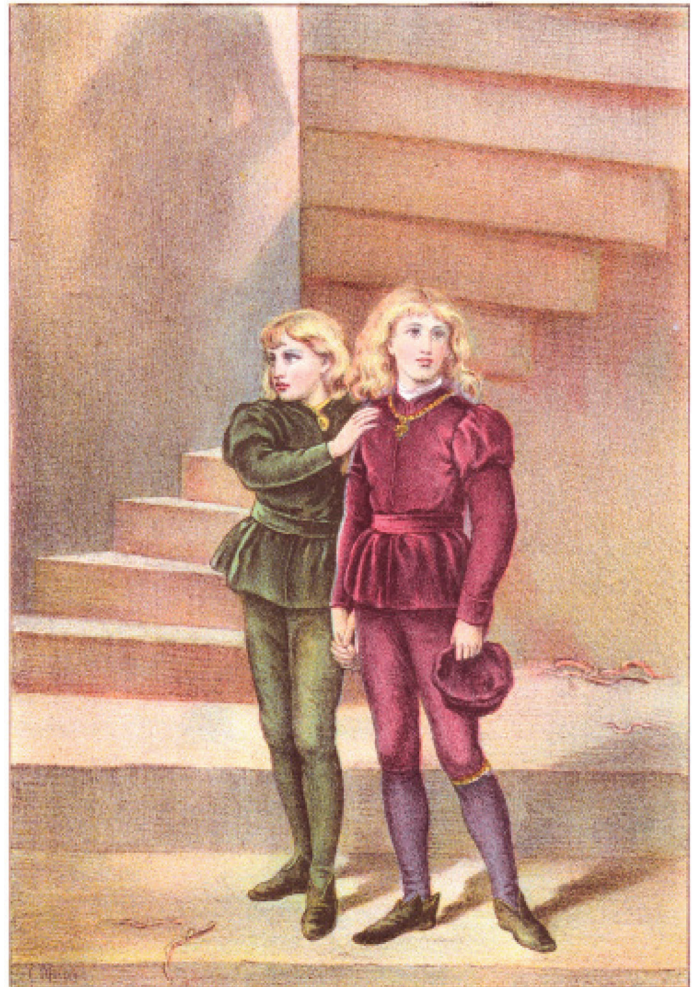
- King Edward IV was the English monarch from 1461 until his sudden death in 1483.
- At the time of King Edward IV's death, his heirs, Prince Edward and Prince Richard, were only 12 and 9 years old, respectively.

3

- On his deathbed, King Edward IV appointed his brother, Richard of Gloucester, to act as head of state until Prince Edward came of age.
- Unfortunately, the princes were declared illegitimate, and their uncle was crowned King Richard III.

4

- Prince Edward and Prince Richard were being held in the Tower of London but mysteriously disappeared in the summer of 1483.
- No one knows what happened to the princes, but many suspect they were murdered by order of their uncle, King Richard III.



5

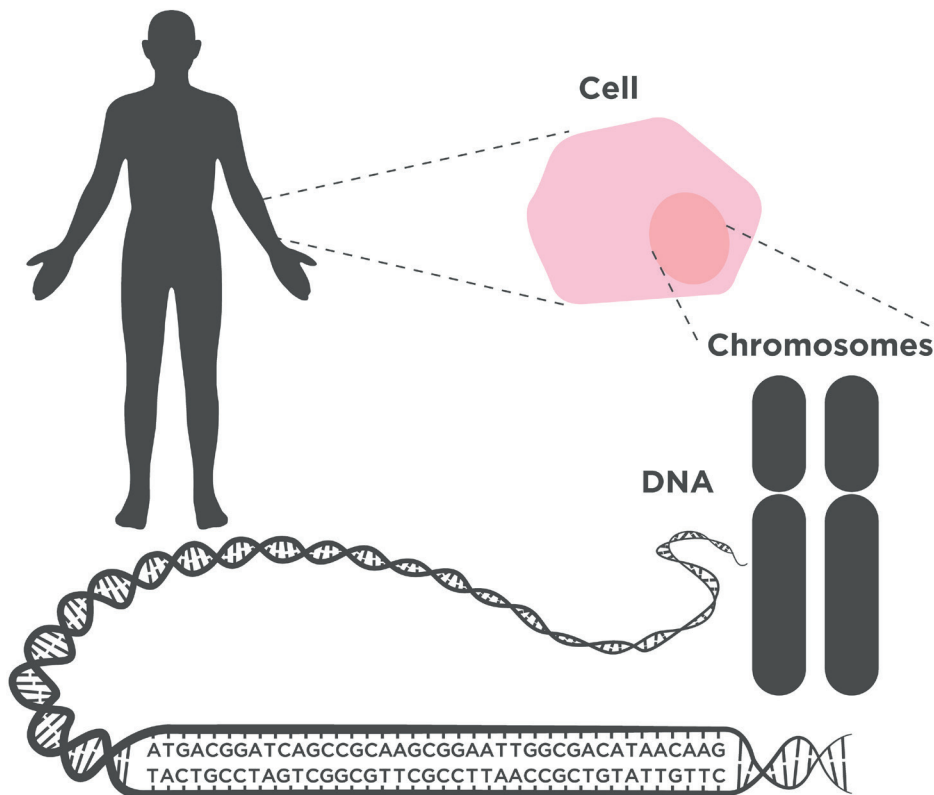
- Two pairs of remains have been discovered, each of which could potentially be the missing princes.
- Your goal in today's lab is to use DNA analysis to investigate this historical mystery!



Using DNA for identification

1

- Scientists use DNA analysis to solve crimes and identify remains.
- To understand why DNA analysis is the most powerful tool for identification, we need to review some background on DNA.



2

- **DNA** contains the instructions for the cell and is found in structures called **chromosomes**.
- DNA is made of four different building blocks, usually referred to as **bases** and abbreviated A, T, C, and G. The order of the As, Ts, Cs, and Gs is referred to as the **DNA sequence**.
- Every person has a unique DNA sequence, which means that a person's DNA sequence can be used like a fingerprint to uniquely identify them.



Short tandem repeats

1

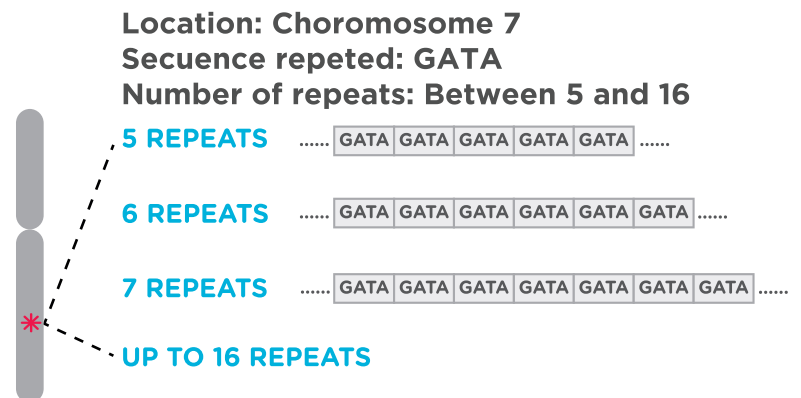
- While every person has a unique DNA sequence, over 99.9% is the same in all humans.
- To identify someone using DNA, scientists examine areas known to differ between people, typically areas of DNA referred to as short tandem repeats.

2

- **Short tandem repeats (STRs)** consist of short sequences, usually two to five DNA bases, that repeat several times in a row.
- While the chromosomal location and repeating bases are consistent among individuals, the number of repeats can vary.

3

- There are many places in our DNA that contain STRs. We will refer to a specific location that contains an STR as an **STR region**.
- Let's examine a specific STR region scientists use for identification! For simplicity, we will only examine one strand of the DNA sequence.
- This STR region is located on chromosome 7, and the sequence "GATA" is repeated.
- The number of "GATA" repeats can vary from as few as five to as many as sixteen!



4

- Because the number of times the bases repeat varies between people, the length of the DNA at a given STR region can also vary.
- Scientists take advantage of this and compare these length differences when using DNA for identification.



Background: Stop and think

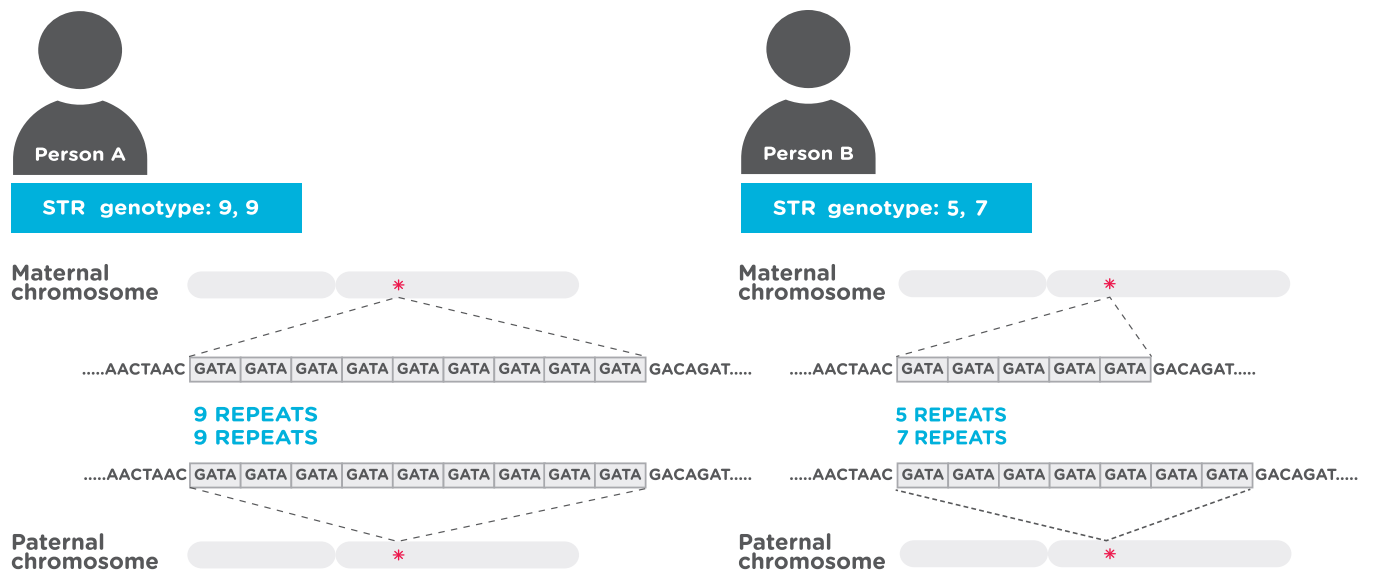
- Q1. Why do scientists analyze STR regions for genetic identification?
- The location of each STR region can vary across people.
 - The length of each STR region can vary across people.
 - The sequence that is repeated for each STR region can vary across people.
 - All of the above



STR genotypes

1

- Scientists use the term **allele** to refer to different versions of the DNA sequence at a given chromosomal location.
- Because the number of tandem repeats can vary at an STR region, each STR region has multiple alleles.
- Scientists refer to **STR alleles** by the number of repeats they contain.
- In the example below, Person A has 9 GATA repeats at this STR region, so we would say this person has the “9” allele.



2

- Humans have two copies of each chromosome, one inherited from each biological parent. Therefore, people have two alleles for each STR region. Scientists use the term **genotype** to refer to different combinations of alleles that a person could have.
- It is possible for someone to have two copies of the same allele for a given STR region. That is the case for Person A in the example above. They have two copies of the “9” allele, so their genotype for this STR region, or their **STR genotype**, would be “9, 9.”
- It is also possible for someone to have two different alleles for a given STR region. That is the case for Person B in the example above. They have one copy of the “5” allele and one copy of the “7” allele, making their STR genotype “5, 7.”

3

- By comparing STR genotypes at 20 STR regions, forensic scientists create DNA profiles that can be used for identification.
- For simplicity, in this activity we will focus on a single STR region.



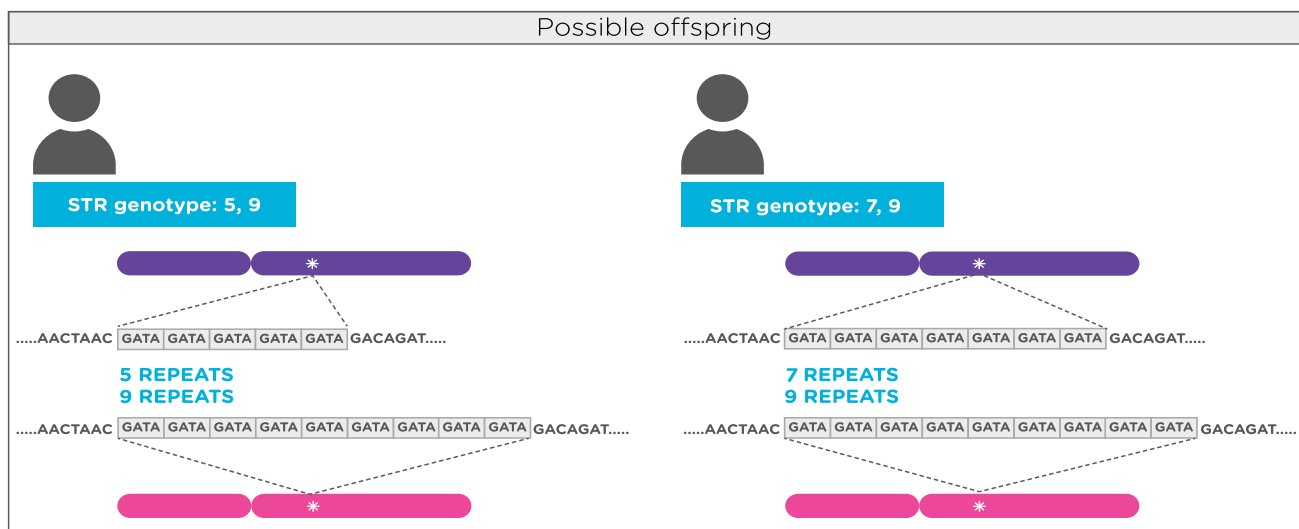
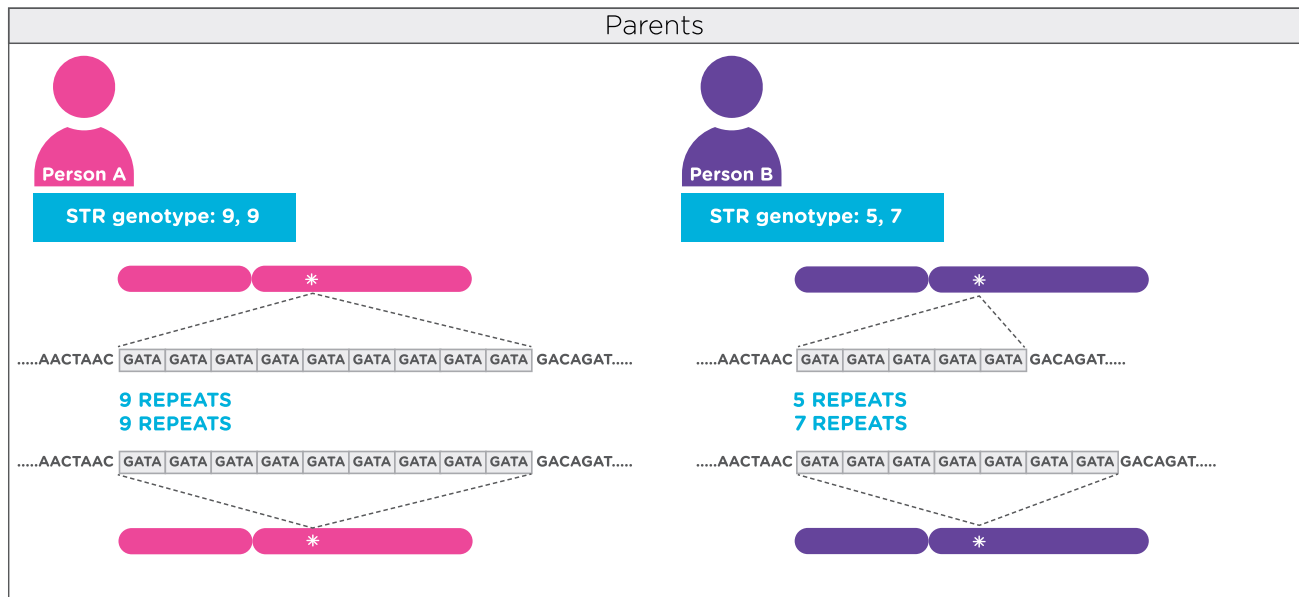
Using STR genotypes for familial matching

1

- Because individuals inherit one copy of each chromosome from each biological parent, they also inherit one STR allele for each STR region from each parent. This means that STR genotypes can be used to test biological relationships like paternity.
- Further, when the identity of unknown remains is suspected, forensic scientists can attempt to identify the remains through familial matching.

2

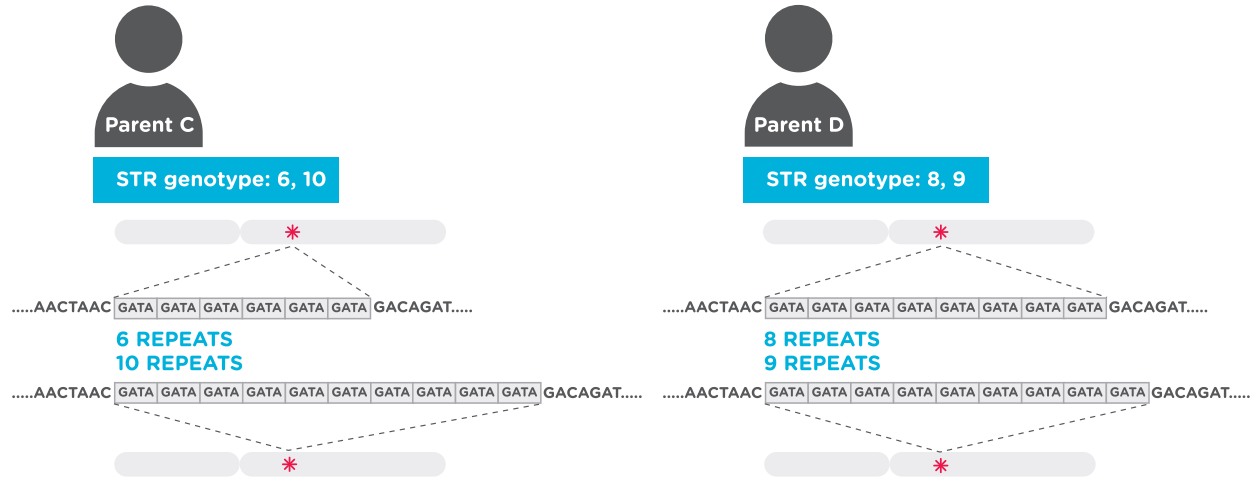
- Imagine the two people from the previous page had children.
- To visually track the inheritance of the STR alleles, Person A's chromosomes have been color-coded pink, and Person B's chromosomes color-coded purple.
- Their offspring could only have two genotypes for this STR region: 5, 9 or 7, 9.





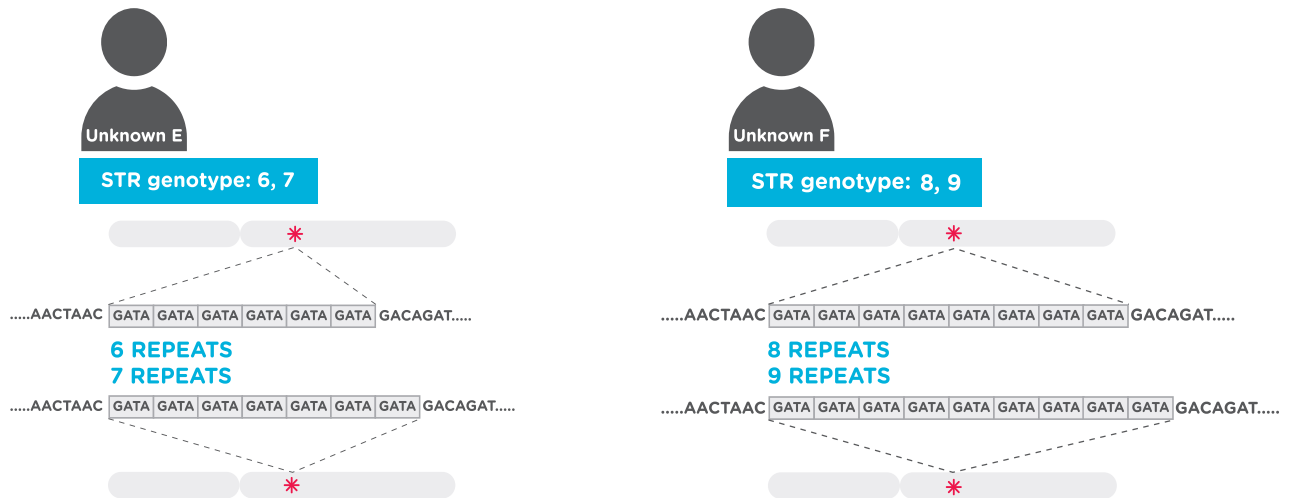
Background: Stop and think

Q2. Imagine that an offspring of these two individuals was missing, and there were two sets of unidentified remains that could potentially belong to them.



Could either of these unidentified remains belong to the missing offspring of Parent C and Parent D?

- A. Unknown remains E
- B. Unknown remains F
- C. Both
- D. Neither





Today's lab

1

- Recall that two pairs of remains have been discovered that could potentially belong to the lost princes.
- In 1674, a pair of remains was found under a stairwell in the Tower of London, the last known location of the two princes.
- As it was believed at the time that these were the remains of Prince Edward and Prince Richard, the remains were buried in Westminster Abbey.
- Preliminary forensic analysis performed in 1933 determined that the remains belonged to two children of the correct ages to have been the young princes.



2

- In 1789, two unidentified child-sized coffins were found in a secret, walled-off vault connected to King Edward IV's burial chamber in St. George's Chapel at Windsor Castle.
- While these remains were found in a chamber adjacent to the princes' parents, no forensic analysis has yet been performed.

3

- DNA analysis could determine if any of the unknown remains might belong to the lost princes.
- However, in order for royal remains to be analyzed, the sitting monarch must give consent, and this has not happened.



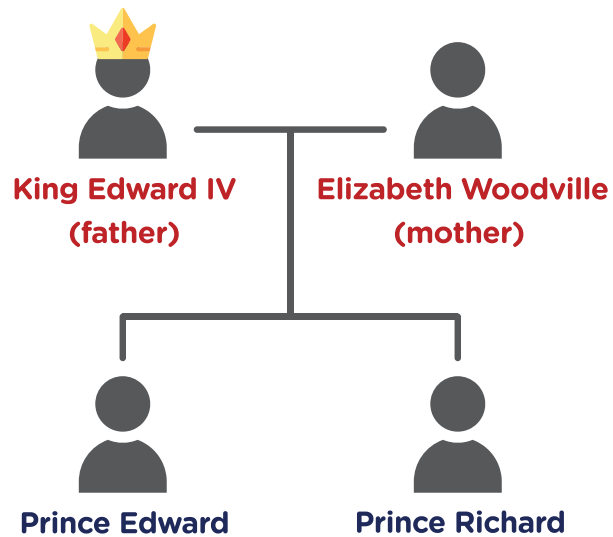
Scenario

1

- Imagine that the English monarch has decided that DNA analysis should be performed on the two pairs of unknown remains to determine if they may belong to Prince Edward or Prince Richard.
- You work in a lab specializing in DNA analysis of historical samples, and your team has been tasked with the testing!

The lost princes' parents, buried at St. George's Chapel

The lost princes, location unknown



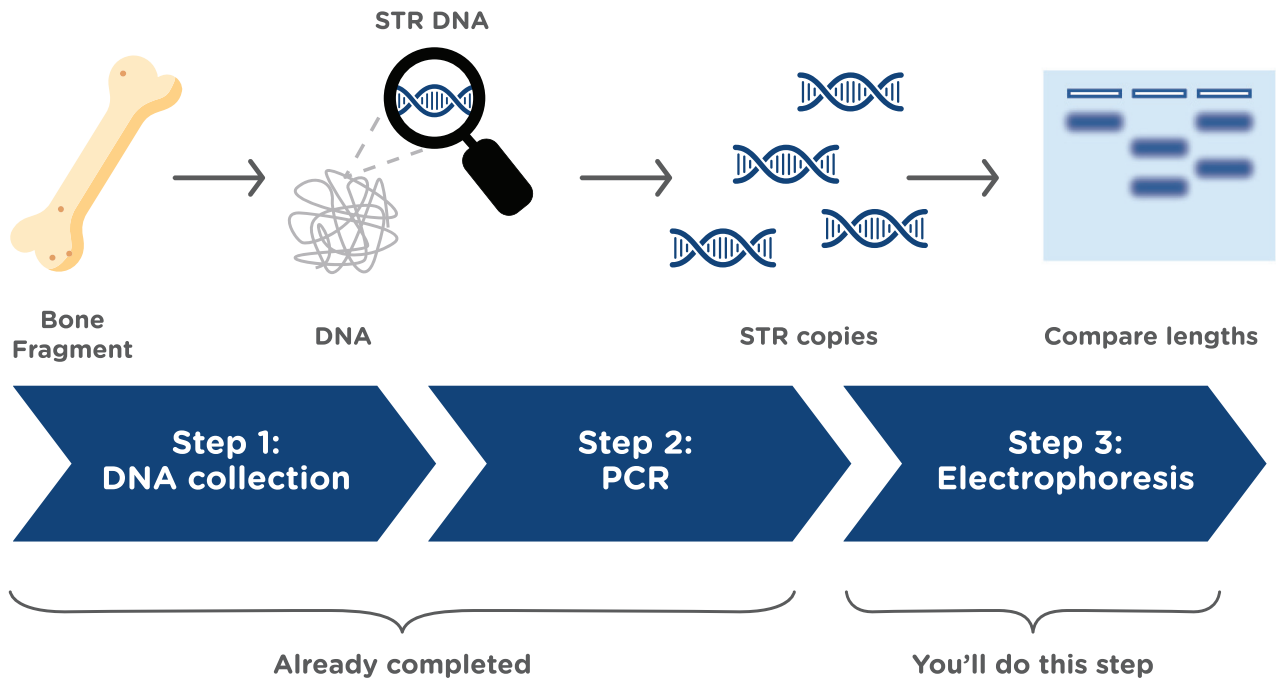
2

- The princes' parents are buried at St. George's Chapel. By comparing the parental STR genotypes to those of the unknown remains, you can determine if any could belong to the Princes in the Tower.
- You will perform DNA analysis on six samples:
 - Remains of King Edward IV (father of the two princes)
 - Remains of Elizabeth Woodville (mother of the two princes)
 - Unknown remains 1, from the Tower of London
 - Unknown remains 2, from the Tower of London
 - Unknown remains 3, from St. George's Chapel
 - Unknown remains 4, from St. George's Chapel



DNA analysis

DNA testing involves several steps. The following procedure will be used to analyze samples from the remains.



1

First, DNA is collected from a tiny bone fragment from each set of remains. As little as 2.5 grams of bone are needed.

2

Next, a process called **Polymerase Chain Reaction (PCR)** is used to make billions of copies of the STR DNA for analysis.

3

You will perform the last step of the DNA testing to determine if any of the unknown remains may be ruled out as being the Princes in the Tower.



Interpreting gel electrophoresis results

1

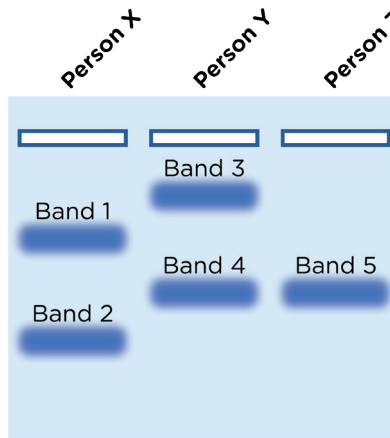
- You will use gel electrophoresis to study the STR DNA. **Gel electrophoresis** separates pieces of DNA by size. At the end of a gel electrophoresis experiment, smaller pieces of DNA will have traveled farther through an **agarose gel** than larger pieces of DNA.
- Because STR alleles are different lengths, you can use gel electrophoresis to separate STR DNA. STRs of the same length will travel the same distance and appear as **bands** on the gel.

2

- Let's walk through an example gel that shows the alleles for three people at a single STR region.
- The bands on the gel have been numbered to make them easier to refer to.

3

- We will read the gel from left to right, starting with Person X.
- There are two bands for Person X, indicating two different lengths of DNA at this STR region.
- Band 2 represents an STR allele with fewer repeats than Band 1, as smaller DNA fragments travel farther during gel electrophoresis.



5

- There is a single band for Person Z, indicating that all DNA at this STR region is the same length.
- Additionally, Band 4 from Person Y and Band 5 from Person Z traveled the same distance on the gel, which tells us that Person Y shares one allele with Person Z.

4

- There are also two bands for Person Y, indicating two different lengths of DNA at this STR region.
- These two alleles differ from those in Person X, as they traveled different distances on the gel.



Background: Stop and think...

- Q3. Why is electrophoresis a good tool for studying the different alleles of an STR region?
- Because it allows you to make copies of the STR alleles to study.
 - Because humans have two copies of each chromosome.
 - Because it allows you to extract DNA from cells.
 - Because the STR alleles are different lengths.



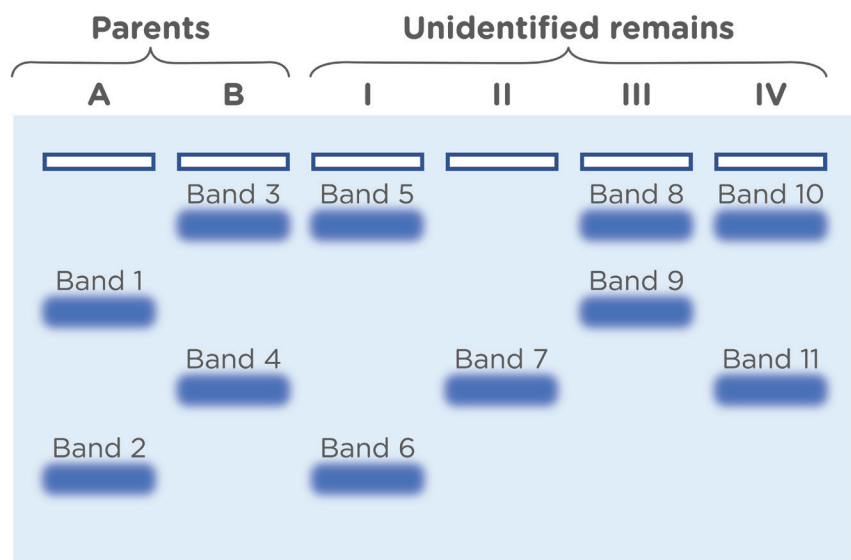
Your goal

- You will compare the band patterns from the parents of Prince Edward and Prince Richard to the band patterns from the four sets of unknown remains.
- The results will allow you to determine if any of the unknown remains can be ruled out as possibly belonging to the missing princes.



Background: Stop and think

Q4. To the right is an example of the gel electrophoresis results you might obtain when comparing STR alleles from unknown remains to a known set of parents. Fill in the table below to indicate whether any of these unknown remains could be the offspring of this set of parents. The bands have been numbered to make them easier to refer to.



	Unidentified remains I	Unidentified remains II	Unidentified remains III	Unidentified remains IV
Possible biological offspring (yes/no)				
Justify your answer				



Glossary

DNA: A molecule passed down from parent to offspring that contains the instructions for the cell. DNA includes four different building blocks, usually referred to as bases and abbreviated A, T, G, and C.

Chromosome: The structures that store DNA in the cell. Humans have 23 pairs of chromosomes. For each chromosome pair, one copy is inherited from each biological parent.

Bases: The building blocks of DNA. There are four different DNA bases: adenine (A), thymine (T), cytosine (C), and guanine (G).

DNA sequence: The order of bases in a DNA molecule. Every person has a unique DNA sequence, making DNA a powerful way to identify people.

Short tandem repeats (STRs): DNA sequences of two to six bases that repeat in a head-to-tail manner. While the chromosomal location and the sequence that repeats are the same in all people, the number of times the sequence repeats can vary. Scientists can identify people by cataloging the number of repeats a person carries at multiple STR regions. Forensic scientists can use these DNA profiles to analyze DNA evidence and to identify unknown remains.

STR region: A specific chromosomal location that contains a short tandem repeat. The human genome contains many STR regions, some of which have been selected to be used for genetic identification.

Allele: One of two or more alternative versions of the same DNA region. This activity focuses on STR alleles, which differ in the number of tandem repeats they contain.

STR allele: A given form of an STR region characterized by a specific number of repeated units. In forensics, identifying STR alleles is crucial, as the unique combination of alleles in an individual can be used for genetic identification.

Genotype: An organism's genetic makeup.

STR genotype: The STR alleles a person carries at a given STR region.

Polymerase chain reaction (PCR): A method used to make many copies of a DNA segment for study. In this lab, PCR was used to copy an STR region. For more detailed information on PCR, refer to <https://www.minipcr.com/polymerase-chain-reaction>.

Gel electrophoresis: A method that separates pieces of DNA by length. In this lab, gel electrophoresis is used to examine STR DNA. For more detailed information on gel electrophoresis, refer to <https://www.minipcr.com/gel-electrophoresis/>.

Agarose gel: A type of gel commonly used for electrophoresis. At the microscopic level, the inside of an agarose gel looks like a porous web. Small molecules can move through the holes with relative ease, but larger molecules get slowed down. This allows scientists to separate molecules of different sizes.

Band: A visible group of molecules of the same size that traveled together through an electrophoresis gel.



Student lab protocol

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

1. Add enough electrophoresis buffer to fill the chamber and just cover the gel.
 - You will need 30 ml of TBE buffer for a Bandit™ or blueGel™ 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.
2. Use a micropipette to load samples in the following order:
 - Lane 1: 10 µl King Edward IV (father of the two princes)
 - Lane 2: 10 µl Elizabeth Woodville (mother of the two princes)
 - Lane 3: 10 µl Unknown remains 1, from the Tower of London
 - Lane 4: 10 µl Unknown remains 2, from the Tower of London
 - Lane 5: 10 µl Unknown remains 3, from St. George's Chapel
 - Lane 6: 10 µl Unknown remains 4, from St. George's Chapel
3. Connect the electrodes and turn on your gel electrophoresis system.
4. Run the gel for 20-30 minutes, or until the bands have separated enough to interpret the results.
 - Times are based on Bandit™ and blueGel™ electrophoresis systems. If using other gel electrophoresis systems, separation time may vary.
 - 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. If desired, take a photo of the results. Placing the gel over a white background will make it easier to see your results.

Detailed operating instructions for miniPCR electrophoresis systems



Bandit

<https://www.minipcr.com/bandit-assembly/>



blueGel

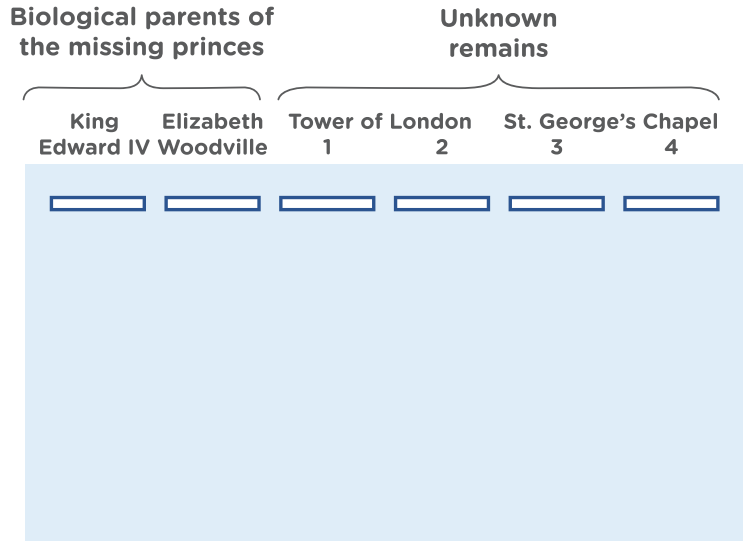
<https://links.minipcr.com/blueGelRun>



Post-lab questions

Interpreting results

- Use the image to the right to draw your results. For each sample, draw the bands you see on the gel.



- Using the information from the gel, determine if any of the unknown remains carry alleles that could be inherited from the parents of the princes.

Sample	Could these alleles have been inherited from King Edward IV and Elizabeth Woodville? (yes/no)
Unknown remains 1, from the Tower of London	
Unknown remains 2, from the Tower of London	
Unknown remains 3, from St. George's Chapel	
Unknown remains 4, from St. George's Chapel	

- Can you remove any unknown remains from consideration as being the Princes in the Tower? Explain your reasoning.

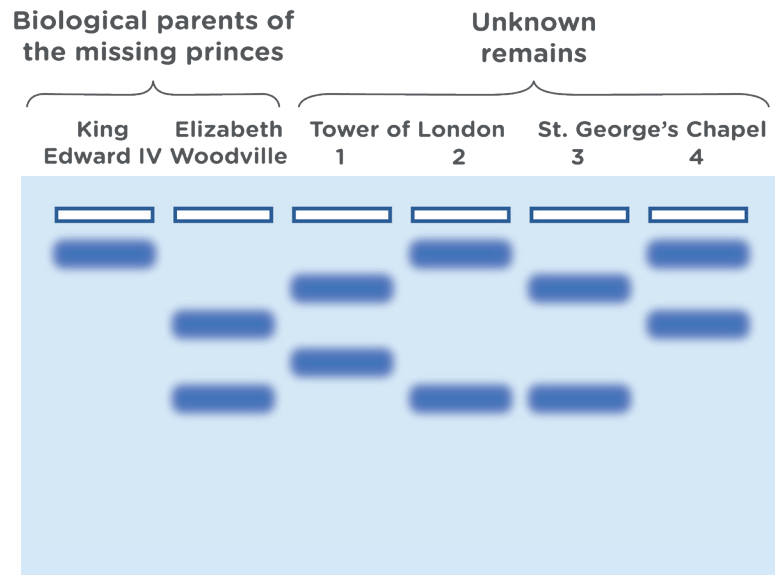


Critical thinking

4. What would your next steps be if you were working on this mystery? Explain your reasoning.

Advanced questions

5. When identifying individuals using STRs, scientists analyze multiple STR regions. Imagine that all six sets of remains were analyzed for a second STR. The gel to the right shows the results of this analysis. Based on these results, could you further remove any unknown remains from consideration as being the Princes in the Tower? Explain your reasoning.



6. What does this tell you about the importance of analyzing multiple STR regions when using DNA analysis for identification?



CER table

Fill in the table based on your results from the lab. Refer to the rubric on the next page for guidance.

Question:

Based on your results, can any of the unknown remains be removed from consideration as possibly belonging to the Princes in the Tower?

Claim

Make a clear statement that answers the above question.

Evidence

Provide data from the lab that supports your claim.

Reasoning

Explain clearly why the data you presented supports your claim. Include the underlying scientific principles that link your evidence to your claim.



Score	4	3	2	1
CLAIM A statement that answers the original question/problem.	Makes a clear, accurate, and complete claim.	Makes an accurate and complete claim.	Makes an accurate but incomplete or vague claim.	Makes a claim that is inaccurate.
EVIDENCE Data from the experiment that supports the claim. Data must be relevant and sufficient to support the claim.	All of the evidence presented is highly relevant and clearly sufficient to support the claim.	Provides evidence that is relevant and sufficient to support the claim.	Provides relevant but insufficient evidence to support the claim. May include some non-relevant evidence.	Only provides evidence that does not support claim.
REASONING Explain why your evidence supports your claim. This must include scientific principles/knowledge that you have about the topic to show why the data counts as evidence.	Provides reasoning that clearly links the evidence to the claim. Relevant scientific principles are well integrated in the reasoning.	Provides reasoning that links the evidence to the claim. Relevant scientific principles are discussed.	Provides reasoning that links the evidence to the claim, but does not include relevant scientific principles or uses them incorrectly.	Provides reasoning that does not link the evidence to the claim. Does not include relevant scientific principles or uses them incorrectly.

We recommend that teachers use the following scale when assessing this assignment using the rubric. Teachers should feel free to adjust this scale to their expectations.

Rubric score	3	4	5	6	7	8	9	10	11	12
Equivalent	55	60	65	70	75	80	85	90	95	100