Blackett Family DNA Activity

Introduction

Welcome to the Blackett Family DNA Activity. Bob Blackett is a DNA analyst. As part of his training, he made a DNA profile of his own family using a technique called RFLP analysis. Family studies are a good way to learn about DNA profiling and RFLP analysis because you can follow the inheritance of DNA markers (alleles) from one generation to the next.

In this activity, you will:

1. Learn the concepts and techniques behind DNA profiling
2. Interpret DNA auto radiograms
3. Evaluate DNA profiles to determine familial relationships

Restriction Fragment Length Polymorphism analysis

In **RFLP** analysis,

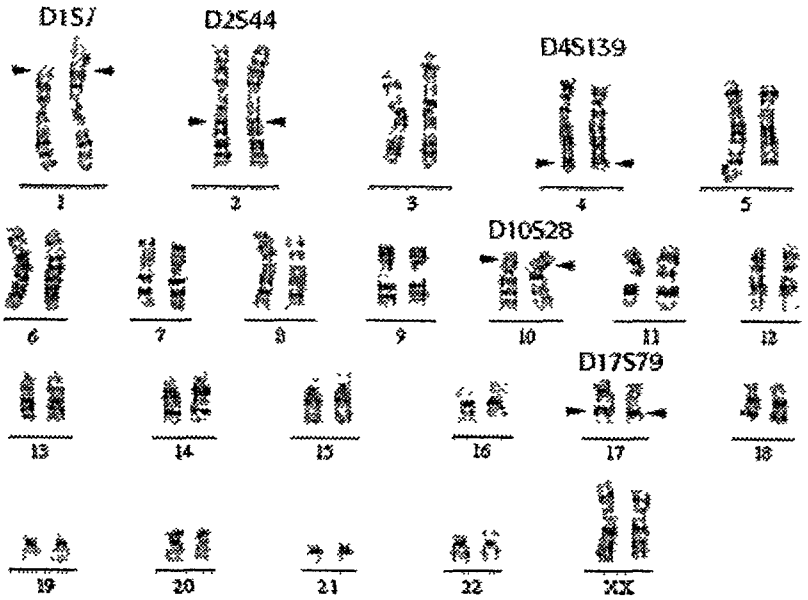
> **RF** stands for Restriction Fragments. Those are the fragments of DNA that were cut by restriction enzymes.
>
> **L** stands for Length, and refers to the length of the restriction fragments.
>
> **P** stands for Polymorphism, a Greek term that literally means "many shapes". The lengths of some of the restriction fragments differ greatly between individuals, thus there are many shapes, or lengths, of DNA possible.

Molecular biologists have identified regions of the human genome where restriction fragment lengths are highly variable between individuals. These regions are called RFLP markers.

Inheritance of RFLP markers

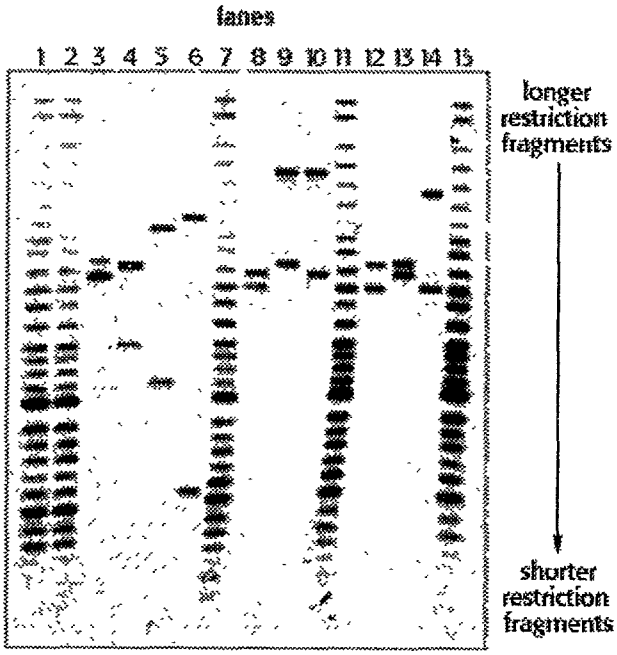
Humans have a total of 23 pairs of chromosomes. Each pair contains one chromosome from mom and one from dad. The RFLP markers most commonly used for DNA profile analysis are found on chromosomes 1, 2, 4, 5, 10 and 17. These RFLP markers are named after their locations on these chromosomes. For example, the marker on chromosome 2 is called D2S44 (section 44 of chromosome 2). These chromosomal locations are also referred to as DNA loci (from Latin: locus is singular, loci is plural). The DNA loci used in profile analysis are shown on the karyotype below.

Chromosomal locations of RFLP markers used in DNA profiling



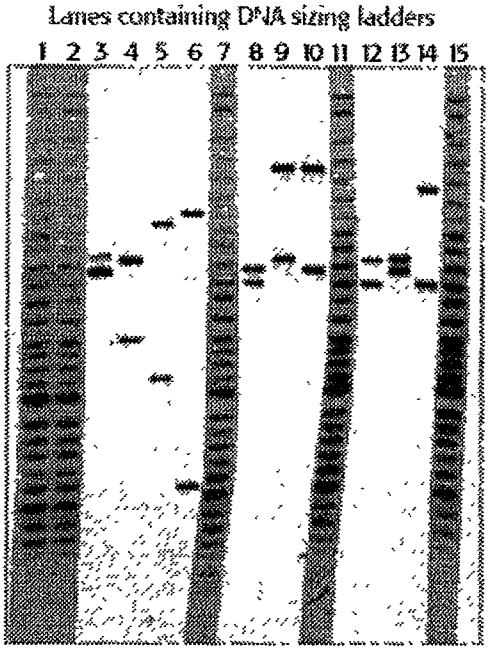
Human Female Karyotype

Anatomy of the autorad



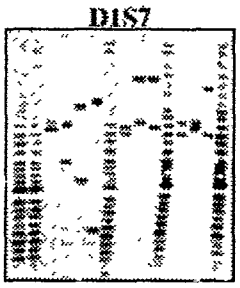
Autorads are x-ray films with dark bands representing RFLP markers. The bands are found in lanes, and each lane in this autorad contains DNA fragments from a different source. In the autorad to the left, the tops of the 15 lanes are numbered. Bands containing longer fragments of DNA are toward the top of the autorad and bands containing shorter fragments are toward the bottom.

This is where the "Length Polymorphism" of RFLP is important; Because different individuals will have many different lengths of DNA possible at the location of an RFLP marker, different people will have bands at different places.



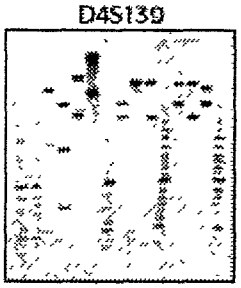
Every autorad also has several lanes containing DNA ladders. Each band in these lanes contains a known length of DNA. The ladders are used to determine the length of the DNA in bands in other lanes.

Evaluating the DNA profiles

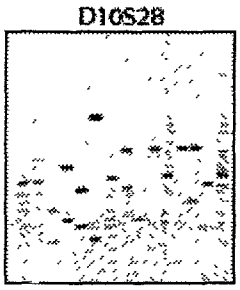


D1S7

D2S44



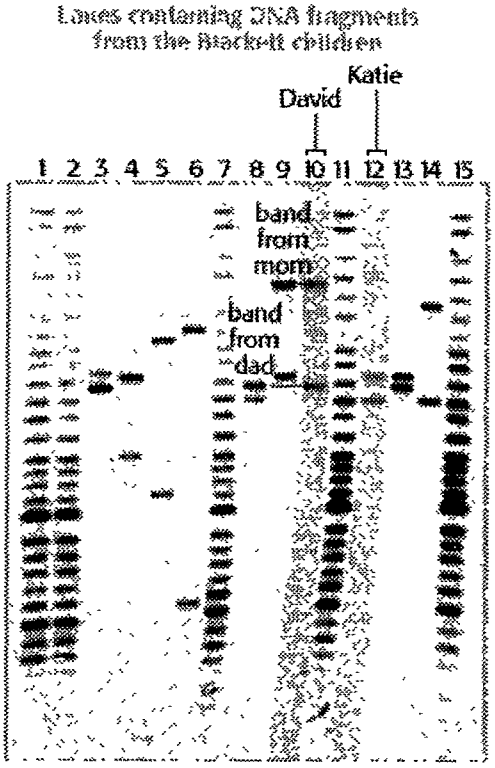
D4S139



D10S28

Here are some questions to further your understanding of the Blackett Family Activity.

1. Are the grandparents maternal or paternal? See hint 1
2. The autorad contains 8 alleles for the siblings tested. Which of the alleles and how many are shared between each of the siblings? See hint 2
3. Are any of the unknowns related to the family? If so, which ones? See hint 3
4. Are any of the other unknowns tested related to each other? If so, which ones? (hint 4)
5. Are there any 1-locus matches between non Blackett family members? See hint 5

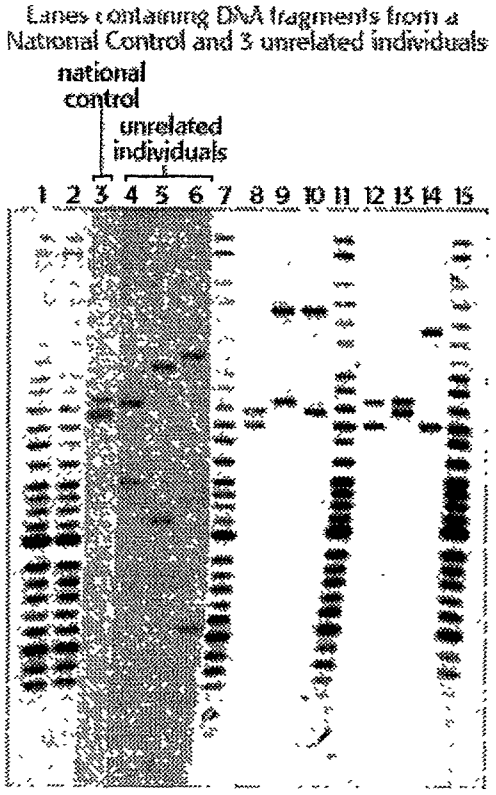


As mentioned earlier, children inherit 23 chromosomes from their mother and 23 from their father. In this autorad, we are looking at the RFLP marker D1S7 located on chromosome 1.

In lanes 10 and 12, the DNA analyst loaded DNA from his children David and Katie. Each child inherited one copy of the D1S7 marker from their mother and one copy from their father. These markers show up as bands on the autorad. Most people have two bands because they inherit one band from each of their parents.

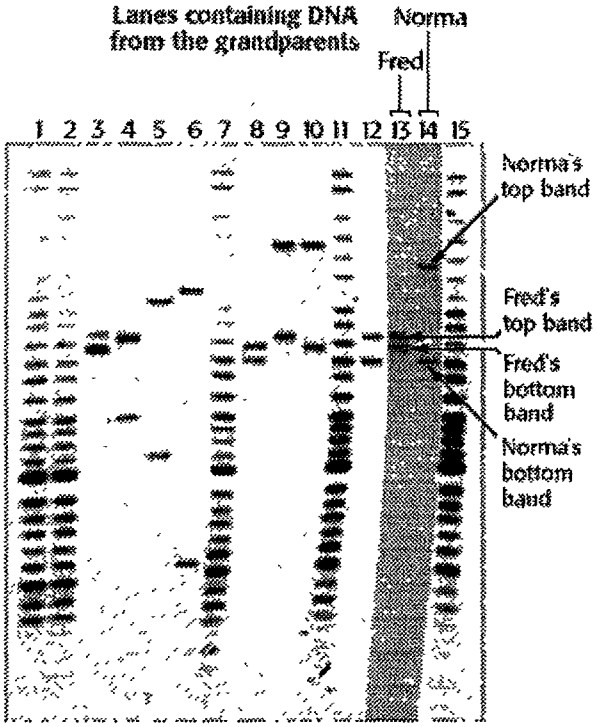
Note that David inherited his mother's top band and his father's top band. David's sister Katie, however, inherited mom's bottom band and dad's bottom band.

Sometimes siblings will inherit the same bands from their parents, although this is not the case for David and Katie at this DNA locus (*i.e.*, they share no bands at D1S7).



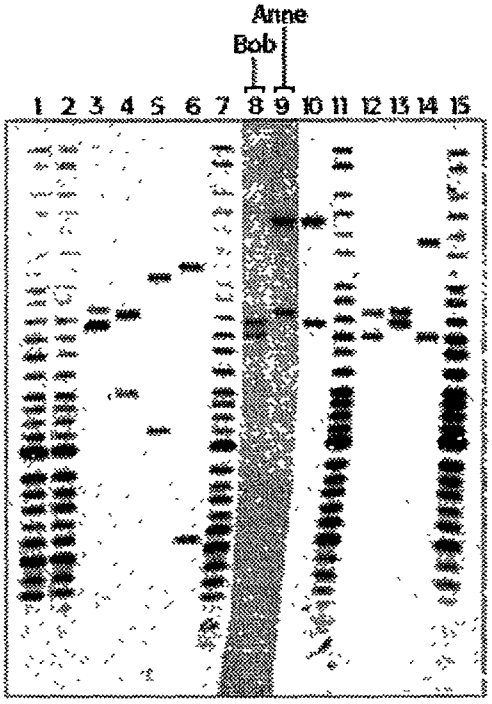
In lanes 4, 5, and 6 the DNA analyst loaded DNA from 3 unrelated individuals. Notice how none of their bands match with one another. Unrelated individuals will, however, occasionally share bands. For example, in this case, it appears that the top band in lane 4 could match the bottom band in lane 9 (Bob's DNA).

DNA analysts are careful to always use a control when performing DNA analysis. In lane 3, Bob loaded a DNA sample that should always have bands in the same place on an autorad. If the control bands do not appear where the analyst expects them to be, the integrity of the rest of the information in the autorad is often questioned. If the control bands do appear where they should be, then the analyst has confirmation that the autorad contains usable information. In this case, the control bands were good



In lanes 13 and 14, the DNA analyst loaded in DNA from the grandparents. Notice that Norma and Fred do not share any bands. Although they are married, they are not "blood relatives" and you would generally not expect unrelated individuals to have the same bands.

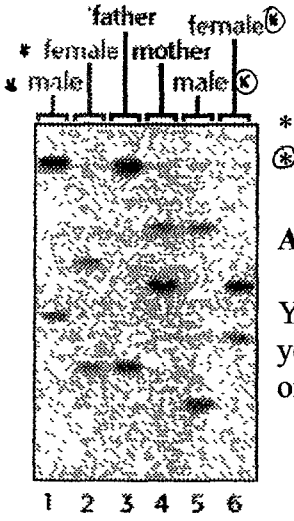
Lanes containing DNA fragments from the parents Bob and Anne Blackett



In lanes 8 and 9, the DNA analyst loaded DNA fragments from himself (Bob) and his wife (Anne). Notice that Bob and Anne each have 2 bands in their respective lanes. At any given DNA locus, most people have two bands. Sometimes they only have one band and sometimes they actually have three.

Also notice that like Fred and Norma, Bob and Anne do not share any bands at this DNA locus. Although Bob's top band is close to Anne's bottom band, they are not close enough to consider them a match.

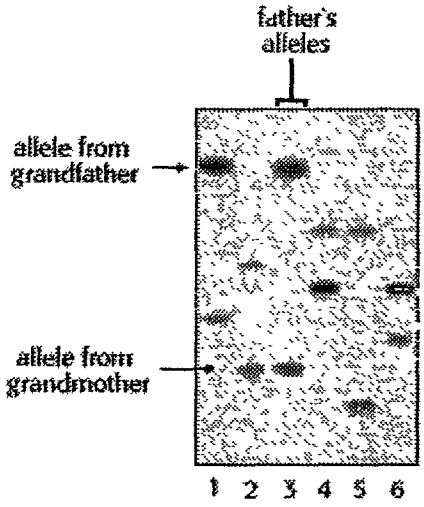
Problem 1: Hint



* Paternal grandparents
Ⓚ Maternal grandparents

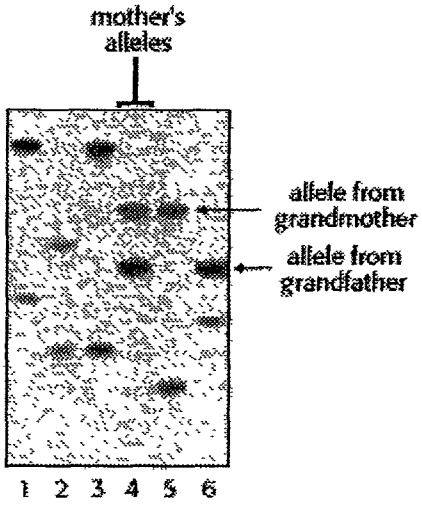
Are the grandparents maternal or paternal?

You already know that maternal parents are your grandparents on your mother's side. Therefore, paternal parents are your grandparents on your father's side.



Father's alleles

Remember, that your mom and your dad each contributed one set of alleles to your genome. Therefore, your grandmother and your grandfather on your father's side each contributed a set of alleles to your father. Notice, highlighted in red and blue, that indeed one allele was contributed from each grandparent to the father. It is important, however, to observe each loci given to make sure that the match is correct.

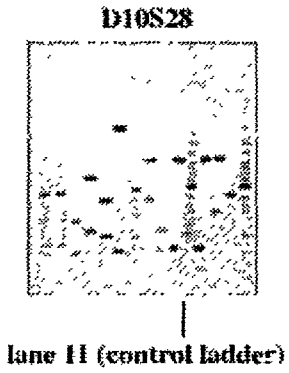


Mother's alleles

Here again, you will observe that each grandparent contributed one allele to the mother's lane. For accuracy, click on all four loci pictures listed above the questions page to confirm your answer. With this information, you should be able to determine if the grandparents on the autoradiograms are paternal or maternal.

Problem 2: Hint

The autorad contains 8 alleles for the siblings tested. Which of the alleles and how many are shared between each of the siblings?



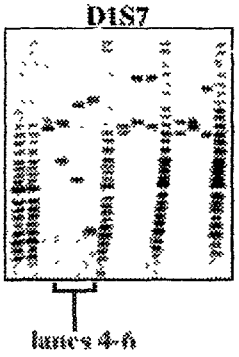
This question may sound confusing. The Blackett activity contains four loci. Each loci contains two bands per lane for each autorad thus giving the total number of eight alleles per child. If that is understood, simply compare the alleles of the two children for all four loci. For an allele to be shared, the band must have migrated the same distance down the gel.

Notice loci D10S28 on your left Both children share the same bands inherited from their parents. At the D10S28 loci, both pair of alleles are shared. Be careful when examining the gels Notice that in D10S28, the lanes curve to the left at the bottom of the gel. There are many reasons why a gel might appear to be curved One explanation could be when the gel was being cast, the gel electrophoresis box may not have been placed on a level surface.

Compare the bands by using the control ladder in lane 11 as a reference for distance traveled. This control ladder should aid you in your search for shared alleles.

Problem 3: Hint

Are any of the unknowns related to the family? If so, which ones?

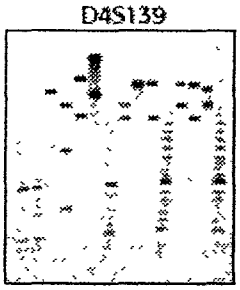


Lanes 4-6 contain the lanes for the unknowns. Determine if any of the unknowns contain matches for any of the Blackett family members.

If you find a match, then examine the other 3 loci to determine if the match is still present. If a match can be found between the same lanes on all four loci, a match exists.

Problem 4: Hint

Are any of the other unknowns tested related to each other? If so, which ones?

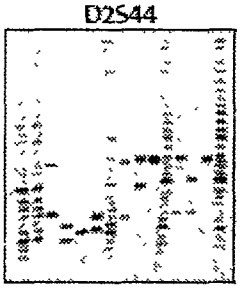


At times, there could be a chance that an unrelated individual could contain a match for the individual being tested. However, the chance that an unrelated individual will share the same band match on a different probe is not as common. For example, an unrelated individual might share a same band with Bob Blackett. If one examines other loci, the chance of that shared allele decreases if they are not related.

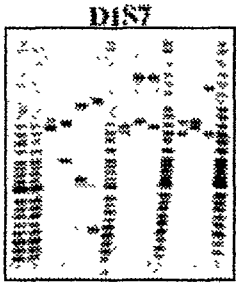
For this problem, determine if any of the unrelated individuals share identical bands. Score the autorads per loci. Then compare all related loci together. Chances are that you will observe matches for the same loci. However, matches between different loci are not as common.

Problem 5: Hint

Are there any 1-locus matches between non-Blackett family members?



Lanes 4-6 are unrelated individuals. At times, bands in these lanes will match other individuals tested. There is always a chance of having matched alleles for unrelated individuals.

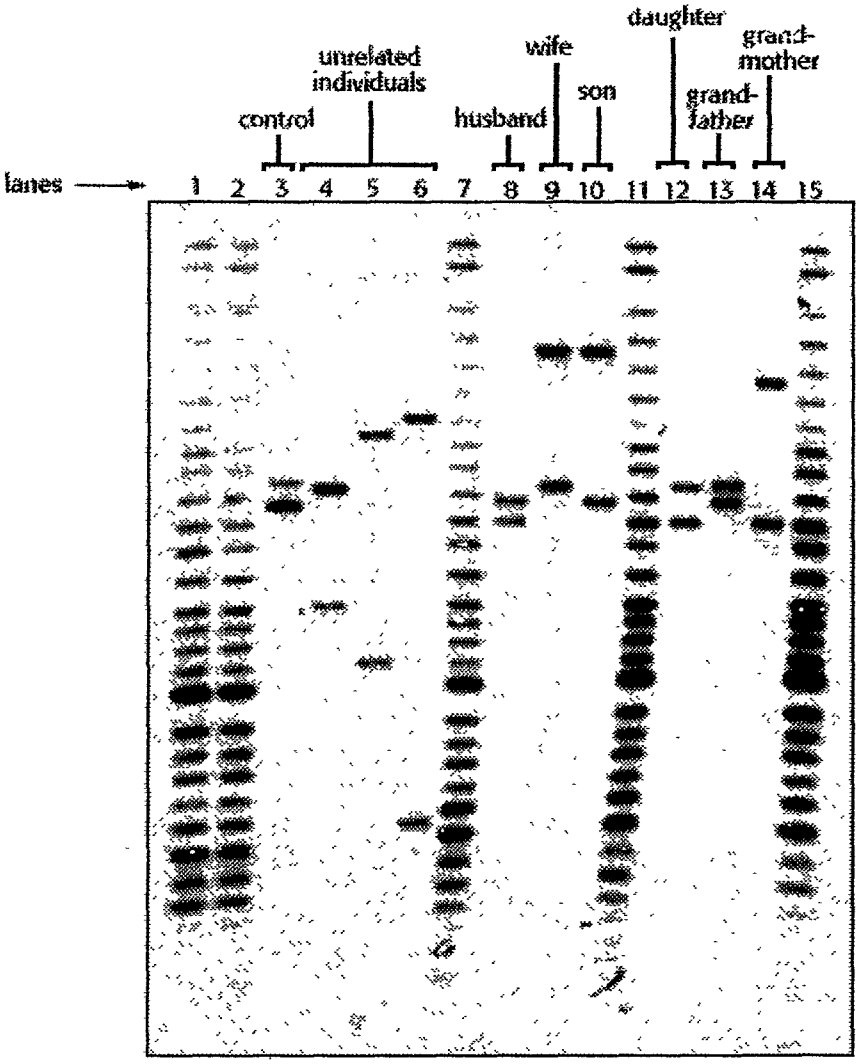


For example, you determine that an unrelated individual has a match for a band with a Blackett family member. If you were the DNA analysist for this probe on the left, what is one way to determine whether or not lane 4 is not related to lane 9? Remember, the question above is asking for any "1-locus matches".

The best way to answer paternity for this question is to determine if that same individual (lane 4) has the same band match with another 1-locus probe. If the lane 4 continues to share bands with lane 9 on different one-locus probes, then these two individuals might be related

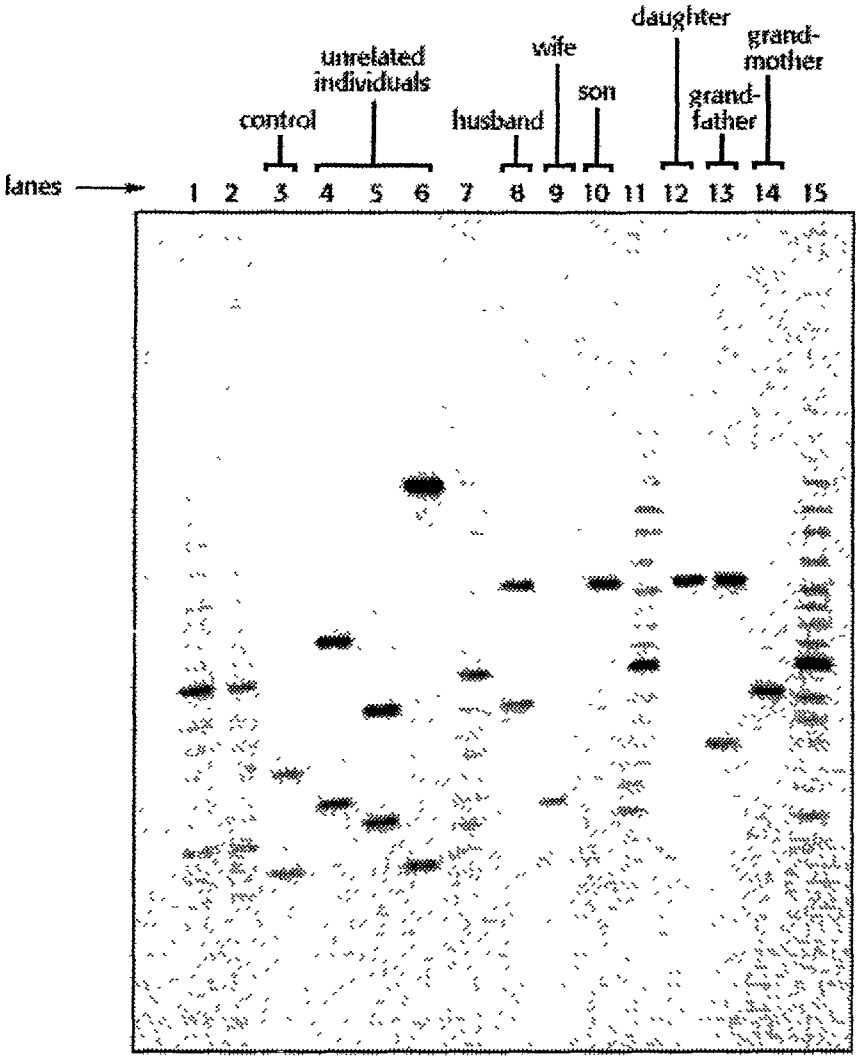
The Blackett Family Profile

DNA Locus: D1S7



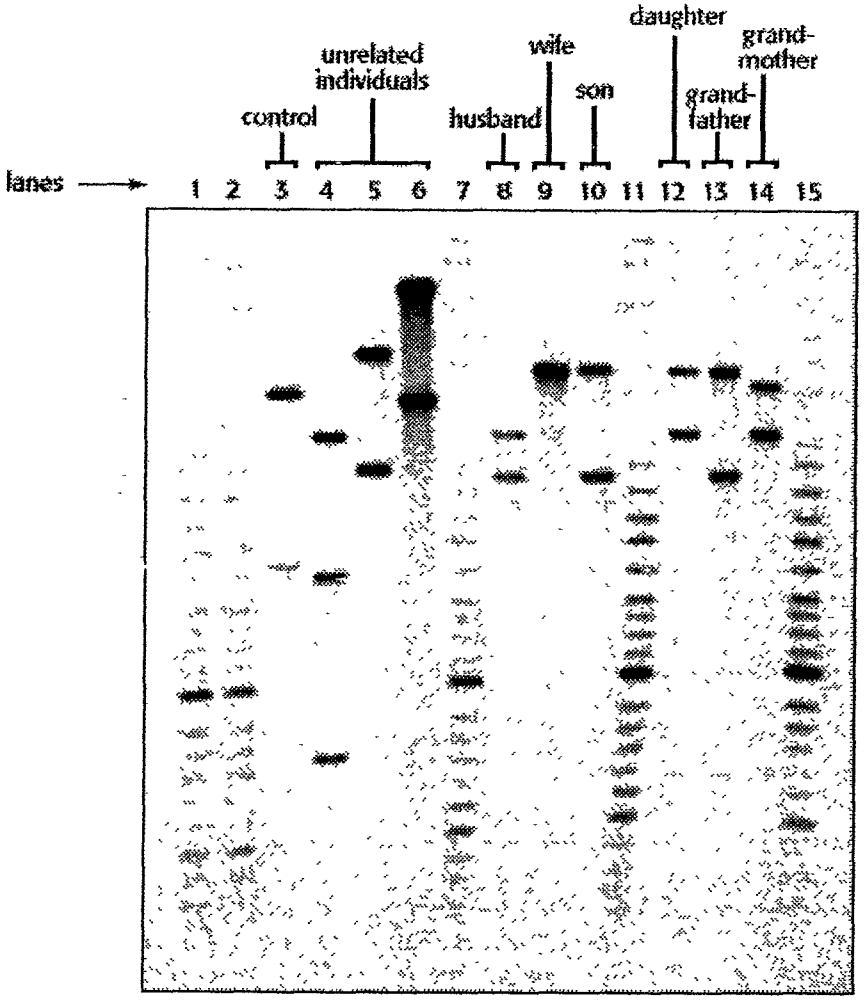
The Blackett Family Profile

DNA Locus: D10S28



The Blackett Family Profile

DNA Locus: D4S139



The Blackett Family Profile

DNA Locus: D2S44