Human Molecular Genetics
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Module - 02
Lecture - 10
Practice session – 2
Problems related to Restricted Fragment Length polymorphism
and its applications

Hello everybody, hope you enjoyed the pedigree problem solving session. So, now we will solve some RFLP, restriction fragment length polymorphism problems. Hope you enjoy that too.

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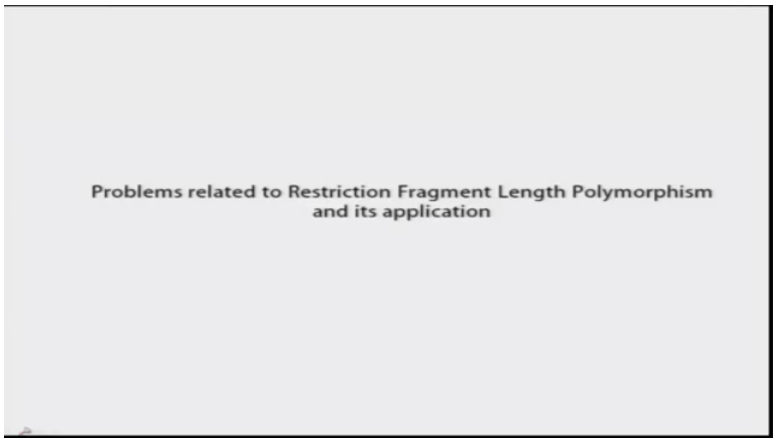

So, RFLP, like you have already studied in one of the previous lectures is important to study, because it tells you the relative positioning of the restriction enzymes on a DNA sequence and that is important when we want to clone a gene or sub clone a gene into a vector. The exact location of the enzymes, the restriction enzymes is important to know.

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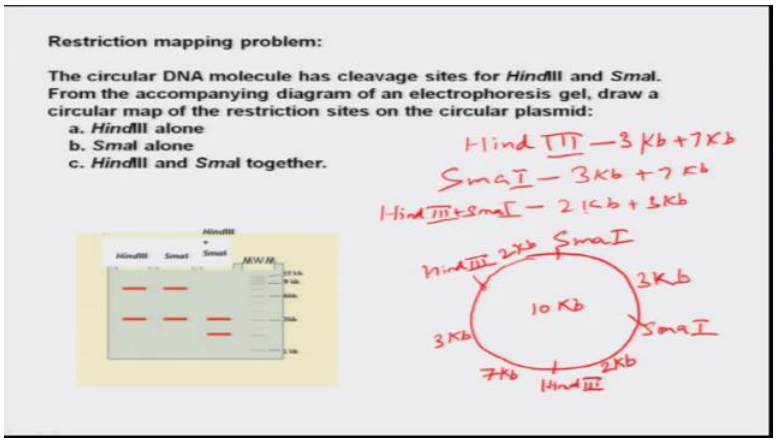

So, one of the problems I will read here. The circular DNA molecule has cleavage sites for HindIII and SmaI. From the accompanying diagram of an electrophoresis gel, draw a circular map of the restriction sites on the circular plasmid. So, as we see in the electrophoretic gel, it has been digested, singly digested with HindIII, SmaI and double digested within HindIII and SmaI. So, a single digestion with HindIII gives a band of 3 Kb and 7 Kb. Also, as you see in the second lane, a single digestion with SmaI has been done. Also it gives a 3 Kb plus a 7 Kb band and a double digestion with HindIII and SmaI gives two bands; one of 3 Kb and one of 2 Kb. So, the total size of the vector here is 10 Kb.

So, first let's draw a circle and the total size which is 10 Kb, ok. So, first let's randomly draw a site for one of the restriction enzyme, which is SmaI. So, here we draw. So SmaI gives two bands, one of 3 Kb and one of 7 Kb. So, that's what we draw here, a 3 Kb, say suppose, one site is over here and the other site is over here in the circular map and so, this is a 3 Kb band and this would 3 Kb and this would be the 7 Kb band. Now, if you look at the double digested products of HindIII and SmaI, they yield a 2 Kb and 3 Kb band which is, the total of which is not 10 Kb. So, that means that there should be two 2 Kb and two 3 KB bands to make, which adds up to the sum of the vector, which is 10 Kb.

So, drawing a HindIII site over here, which would give a 2 Kb band according to the double digestion for HindIII and SmaI and another site if we draw it over here, so that will give us a band of 3 Kb, a fragment of 3 Kb and a HindIII SmaI fragment of 2 Kb as mentioned here. So,

now if we add up add up all the fragment, if we look at the individual sizes of the fragments and see if the total is adding up to the vector, then we will know that our map is, the map we have drawn is correct. So, SmaI yields a 3 Kb and a 7 Kb fragment. So, we will see here. this is a 3 Kb fragment and this is to 2 + 3 +2, so this is a 7 Kb fragment. Again HindIII gives a 3 Kb and 7 Kb fragment, so this is a 3 Kb fragment for HindIII and this is a 7 Kb fragment, 2+3+ 2 fragment for HindIII and again double digest, we are getting HindIII SmaI 2 Kb and HIndIII SmaI 3 Kb fragments we are getting. So, this is a problem for a circular restriction map.

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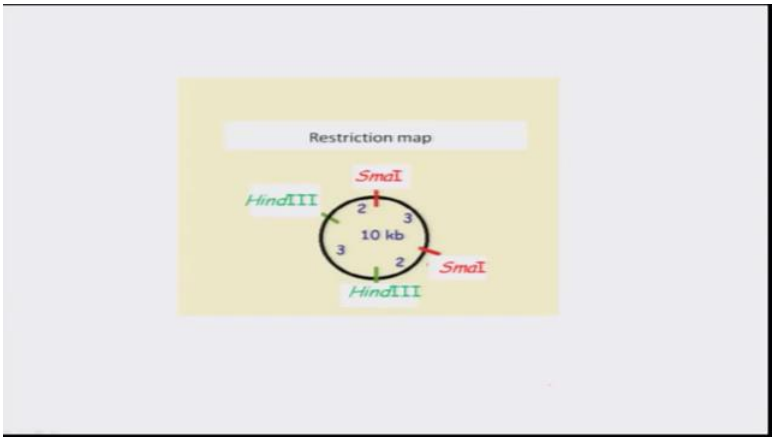

This is the map that we just drew and it looks exactly the same. So, next I will tell you about, how to draw a linear map. So, the strategy is that one should look at the double digest, the new bands in the double digestion first to look at the intermediate sites present between the bigger fragments.

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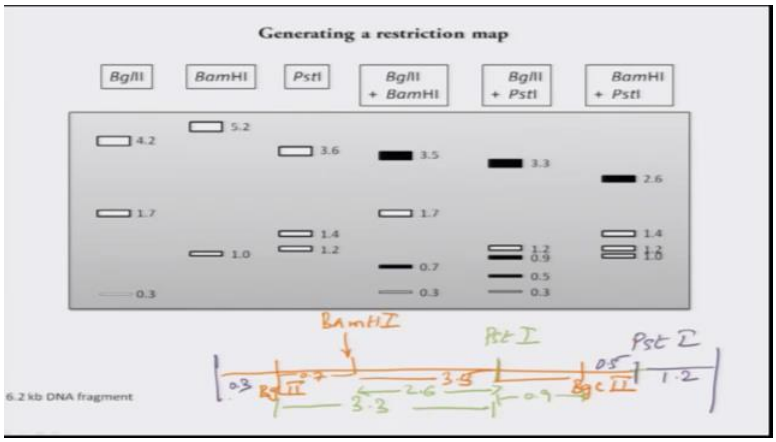

So, let's draw linear line, because it i been a linear fragment and that if you look at the double digestion for BglII and BamHI first, we see that the new bands which are black in colour is a 3.5 Kb band and a 0.7 Kb band. So, now if you look at the BglII single digestion, there is a 4.2 Kb band. So, it seems that that has been cleaved by BamHI site into two, 3.5 and 0.7. So, that's what we draw here. It's a BglII site and one BglII site over here, okay and this has been cleaved by a BamHI site over here. Say, suppose here is the BamHI site which is giving the BglII, BamHI 0.7 Kb fragment and this is the one which is giving a 3.5 Kb fragment, okay. So, next if we look at the double digestion for the BamHI and PstI, here there is one single band that we see which is of 2.6 Kb. So, if we draw PstI site over here, let me change the colour of the pen for your ease, so here if we, if you draw PstI site over here, so that would give us a 2.6 Kb band for BamHI and PstI over here, okay.

Now, let's look at the third double digest for BglII and PstI. Here we get three new bands 3.3, 0.9 and .5 Kb rest are all there in the single digest. So, if you look at the Pst, BglII single digest site, there is a 4.2 Kb band in the BglII single digest and it seems that that has been cleaved into a 3.3 and 0.9 Kb band by a PstI site. So, if we subtract 2.6 from 3.5 that leads to 0.9, so this seems to be the, this seems to be the 0.9 new fragment, and 2.6+0.7, which is equal to 3.3 is the, of the other BglII Pst fragment. So, what is left now, there is another band of 0.5 Kb in the BglII PstI digestion and so that means there is another PstI site and it should be on the other side of BglI, hopefully.

So, this is the change, let me change the colour again. If this is the PstI site, this could be the other 0.5 the Kb fragment and if we see there is a, there are, in the singled digest Pst 1, there is 1.2 Kb band, which doesn't come up in any of this calculation. So, this would be the 1.2 single, since it is a linear cut DNA, this would be the 1.2 Kb fragment for PstI and the single digest of BglII, shows a .3 Kb band, which should come here. Since it's not coming anywhere in between, this would be the 0.3 Kb fragment.

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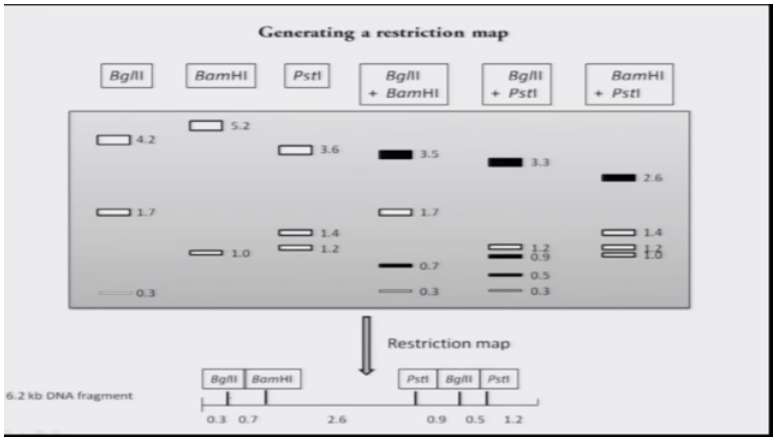

So, now if we add up all the, add or subtract all the combinations that we have drawn, hopefully it draws, it comes up with the electrophoresis band pattern that we see here and this is precisely what has been shown as the answer and you can always practice it again and see if it comes up well.

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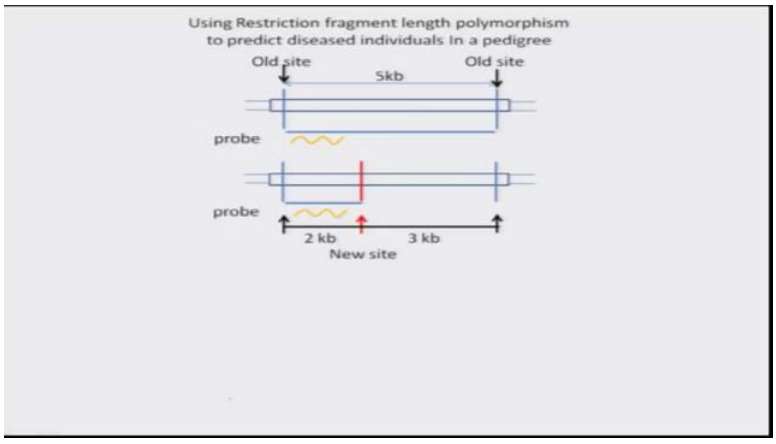

So, restriction fragment length polymorphism can be used to, can, has multiple applications. One of those is to predict, are there any diseased individuals in a pedigree, if the parents are suffering? So, when does that happen? So, there are like, we have heard in the lectures about polymorphisms. These are mutations happening in the genome which introduces or deletes restriction sites in a gene and which are also the basis of evolution, so it can, the basis of evolution as well as it can cause diseases. So, like I show you over here, this is the diagram, cartoon of a gene which I have drawn, a 5 Kb fragment and it has two restriction sites on either sides which, if the gene is digested with those, it leads to the 5 Kb fragment and here, I have shown that a probe against one of specific region of the gene, as you have learnt in the Southern blot technique in the previous lectures, will give you a band on the auto radiogram of 5 Kb.

But, if there is a polymorphism occurring in this gene in some population or in case of a disease, then, it might introduce a new site into that gene which leads to the formation which might not, now when this gene will be digested with these two restriction enzymes it will, apart from the 5 Kb band, apart from generating a 5 Kb band upon a partial digest, it will also lead to generation of two other bands on full digestion, one of 2 Kb and other of 3 Kb.

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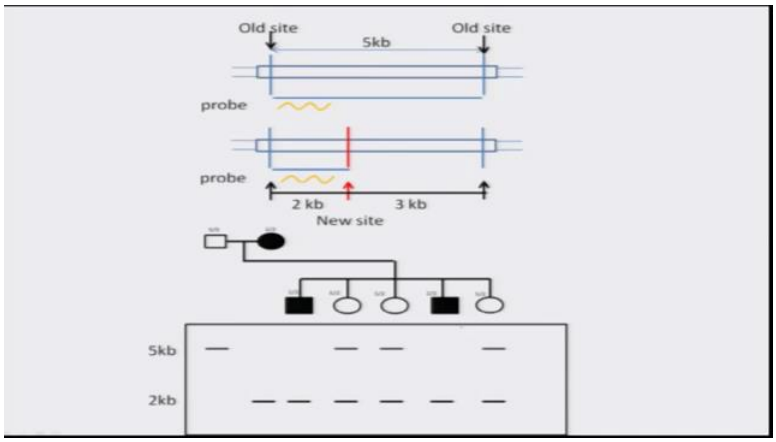

So, let's see. This is a pedigree of a diseased family which has a polymorphism, female of the family is suffering and if you see the, so what we know was, what we want is that in the next generation we want to predict that whether there is a diseased individual, will there be a diseased individual in the next generation? So, if you look at the banding pattern that we got from the auto

radiogram that we got from the probe detections in Southern blotting, the father gives a 5 Kb band which means, both the alleles, none of the alleles have the that particular mutation which generates that new site and hence there is only one single band. Both the alleles give the same, you know, amplify at the same range, same size. The diseased female however gives only one single band at 2 Kb which means that, because of the restriction site generation, the probe can now detect only that particular fragment and not the 3 Kb fragment, all right?

Now, let us look at the next generation. So, the first individual is a male and it, it generates a single 2 Kb band, which is similar to the diseased phenotype of the mother. So, that hints us that this might be a diseased individual. The other females in the family all have both 2 Kb and a 5 Kb band, which means that one of the alleles is generating a 2 Kb band while the other allele is generating a 5 Kb band, which means these are carriers for the, that particular polymorphism and however they are not manifesting the disease. But the males in the generation have only a 2 Kb band which, which hints us that these two individuals might show the disease, might manifest the disease. So this, this is how restriction fragment length polymorphism helps us in diagnosing problems.

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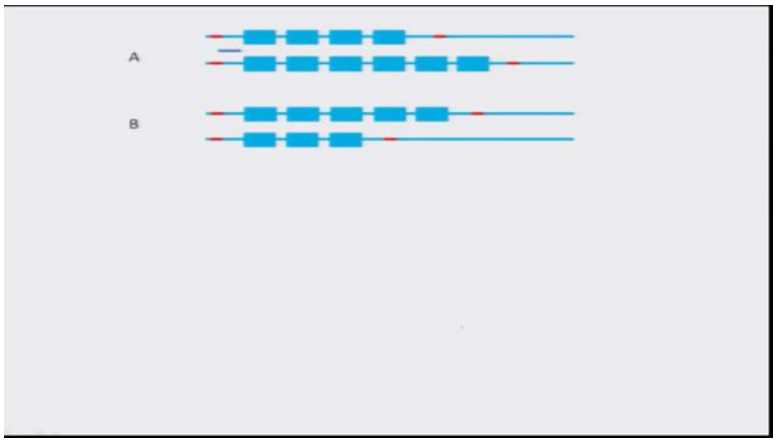

Apart from this application, we all have heard about DNA fingerprinting or also called as DNA profiling. Now, what is the basis of DNA profiling? We have, like you can see in this diagram that I have drawn, these are gene segments which look pretty similar to each other. So, these are

nothing but, tandem repeats, repeats which are tandemly arranged in a region of the genome and these repeats are highly variable.

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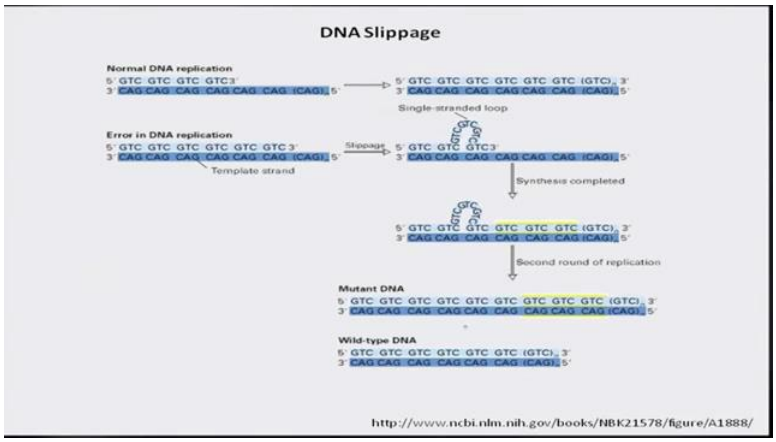

The reason for them being variable is that, is one of the reasons is that during DNA replication, one of the strand, it straggles behind and hence there is a problem in the pairing, in the base pairing. So, what happens is that there is a slippage that happens and there is a miss pairing, between the, pairing happens between the non-contiguous repeats. So, that leads to either the deletion of repeats on a strand or introduction of duplication of repeats and that is what results in the variable number of the repeats and that is the, that is the phenomena called as variable number tandem repeats VNTR that, that forms the basis of DNA fingerprinting.

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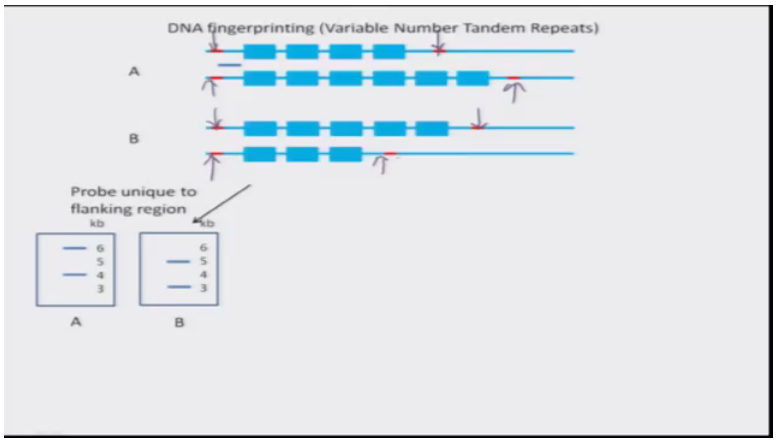

So, here I want to show that what happens, what happens as a result of this variable number? So, you see in the, in the upper panel there are restriction sites present flanking to both, present flanking to these regions in the orange colour and if we, if we use a probe which is specific to the flanking regions, we get a unique, probe unique to the flanking regions, we will get a unique banding pattern in both the, unique banding patterns. So, as you see, in figure 'a' it is giving, since there are 6 repeats in one of the alleles and there are 4 repeats in the other allele, so the restriction enzyme cuts at these restriction sites and hence generates different length of fragments. So, this is what we see 6 and 4 for A and 5 and 3 for the, for B.

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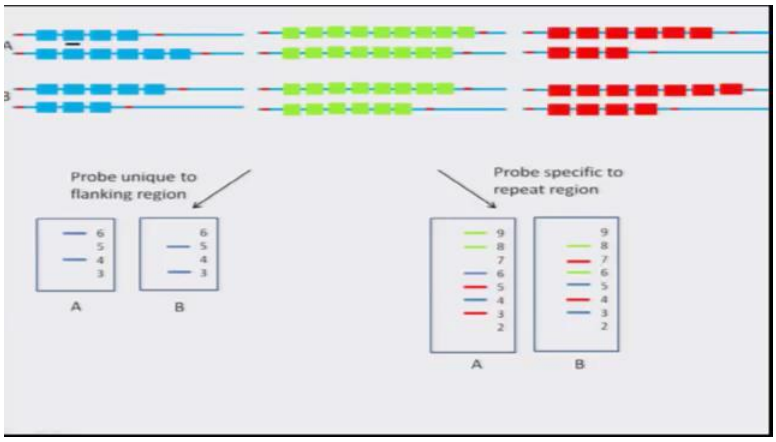

But, what if we use a probe which is specific to one of the repeats and not the flanking regions, specific, rather specific to the repeat region and not the flanking region? We will, this is just three VNTR loci which I have shown, but we will get multiple banding pattern, because the repeat might be present at variable regions in the genome and this is what I have shown over here that if we use a probe, which is specific to the repeat region, it will show, for example I will show, I will give, I will explain you the green one; so, the green one has 9 repeats in one of the allele and 8 in the other. So, that is what we see over here and in the visual B, the green one show 8 repeats in one of the alleles and 6 in the other. So, this is the binding pattern that it shows upon amplification, upon detection. So, this is the kind of DNA profile what we get of an individual, obviously, in much larger number of bands that you see in a real DNA profile, This is just a cartoon to show you, explain you the concept and to show you, to tell you about the application, let's get into a crime scene.

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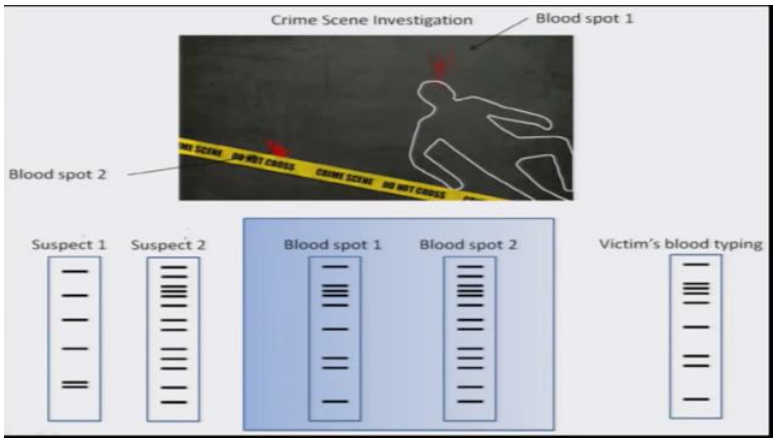

So, here you see an individual who has been murdered and there are two blood spots as indicated in the surrounding, one near his head and one in the close proximity. So, we call them, so we type these two as a blood spot 1 and blood spot 2 and what, what, the aim is to, you know, see whose blood is this and does the blood help us in telling anything about the suspect who killed this man? So the first step is, type DNA profile or like what I just told you in the previous slide, type by the blood type or the fingerprint of these two blood spots. So, this is a particular banding pattern that you see in, for both the blood spots. It is it is quite different, okay. So, that tells us that it comes from two different individuals, it is not just, and it does not just belong to the person who is killed. So now, which one of these belongs to the person who has been killed? For that simply type the pattern for the victim's blood and we see that the victim's blood is very similar to that of blood spot 1, which is near the head of the victim, which tells us that this is the blood, this particular blood spot comes from the victim who has been murdered.

But, what about the other blood spot? So, in this case, there were two suspects, which in the vicinity. The police suspected two people, but it was not sure that which one of them killed this individual. So, blood was taken from both these individuals and their DNA profile was done profiling was done. So, this is the DNA profile of the two suspects and now we have to see that which banding pattern looks, blood spot 1 is already of the victim, so we have to match the blood spot 2 profile with these two suspects and we see that the suspect 2 DNA profile matches almost exactly to the blood spot 2 pattern, which tells us that suspect 1 is innocent and 2 is the one, who committed the crime.

So, this is how DNA profiling using or DNA finger printing is done for paternity cases or for murder mysteries. One of the very popular, the infamous case of Rajiv Gandhi assassination, where his murderer, Dhanu was DNA profiled; so, what was done is that the blood from all over the, after the explosion the blood, her blood was all over the crime scene and that was taken and that was matched with the tissue on the belt, the bombing belt what she was, which she was wearing and that was matched and that was how it was confirmed that she was the one who was, who detonated the bomb and she was the one who was responsible for the explosion. So, that is how it helps in real life problems. So, this is all for today and I really hope you enjoyed these problems and see you in week III. Thank you.