Course Name: Basics of Crop Breeding and Plant Biotechnology

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Lecture-54: Double Integration (Part II)

Welcome back. So, we will continue again. Let us discuss, the reverse tandem integration part. Here also we have to initially make a construct. Suppose this is our, here onwards we are talking about 2 genes, suppose this is the gene A part and this is the gene B part. So, its size is 1.5 kb, its size is 1 kb over here we had *Bam*HI, over here we had *Eco*RI restriction site and over here we had *Hin*dIII restriction site. So, now suppose reverse tandem integration has been taken place. Now, once we have to assume these things we have to think about the construct in this way. Suppose this is our construct orientation.

It is starting from *Bam*HI and it is ending in *Hin*dIII. In reverse tandem 2 types of integration possibilities might be there. One could be in this way; another one could be this way. In the first one we had *Bam*HI over here, and we had *Hin*dIII over here. Again, *Hin*dIII is available over here and *Bam*HI is available over here.

While this is also reverse tandem, in this case, we are having *Bam*HI over here, *Bam*HI and over here we have *Hin*dIII, over here we have *Hin*dIII. So, we will analyze this particular reverse tandem integration using the internal restriction sites. Ok! So, let us assume transformation has been done. This type of reverse tandem integration has been taken place in the plant, and we are analyzing the plant using *Eco*RI enzyme. So, let us see what we have over here, a *Eco*RI site and over here another *Eco*RI site is available. right?

So, this distance is 1.5 kb, this is 1 kb, this distance is 1.5 kb, this is 1 kb. Over here also one *Eco*RI site will be here, and one *Eco*RI site will be here. The size of the promoter was 1.5. So, 1.5, 1.0, this one was 1.5, 1.0. So, in these two cases can we expect similar banding pattern or not? Let us see. So, suppose we are considering two different plants. Ok! In plant 1, this type of integration has been taken place and in plant 2 this type of integration has been taken place. So, their genomic DNA has been isolated, it has been digested with *Eco*RI restriction enzyme. Ok! So, in P_1 what will be the scenario? In P_1, here from let me take a different color, one fragment you will get from here, right?

Its size will be 2 kb. Then in P_1 plant another *Eco*RI fragment will be coming from here, we do not know where is the next *Eco*RI site is available. Ok! Based on that it will be at least 1.5 kb or more than that, it will be at least 1.5 kb or more than that. Ok!

So, suppose over here 3 kb away from the integration site next *Eco*RI site is available. Ok! So, this fragment will be 4.5, 4.5 kb we can expect this band. And over here suppose another *Eco*RI site is available another *Eco*RI site is available 2 kb away, this distance is supposing 2 kb.

So, 2 and 1.5 these things will be the size of this band 3.5 kb. So, this 3.5 kb band will be generated from this construct related part. In addition to that, different endogenous bands will be also there.

So, this is the thing which we can see in case of plant 1. In plant 2 what will be the scenario? In plant 2 for your better understanding let me assume that, over here also the, over here also the *Eco*RI site in this side is available at similar distance, at 3 kb away. And over here next *Eco*RI is available, at 2 kb away. So, what will be the scenario? One *Eco*RI fragment will be coming from here, its size will be 1.5 + 1.5 = 3 kb. Ok! So, at 2 kb we should not expect any band at 3 kb, 1 band could be generated from here, because their integration pattern is different right. Then another fragment what will be coming from here, its size will be 3 kb + 1 kb means at 4 kb we should expect another band, 4 kb

this one was 3 kb. And over here this band will be also 3 kb. In addition to that, we will be having other different endogenous digested DNA fragments.

Till now we have done the digestion only we have not used any probe. Now let me use different probes. Suppose over here this *Eco*RI digested Southern blot has been prepared means, gel casting has been done electrophoresis has been done and we are using gene A specific probe. We are using gene A specific probe. Ok! So, what thing could be visible over there. Ok!.

So, if we use gene A specific probe, gene A, size is 1.5 kb gene A is available over here and gene A is available over here. Right? So, only 3.5 kb and this 3.5 kb bands will be detected over here, and this 4.5 kb bands will be detected over here because, gene A can bind here, and gene A probe can bind here in P_1 plant, right? If we use gene A probe, in P_2 in plant 2 where it will bind? In plant 2 gene A is available will bind here and here in these 2 parts. So, the 3 kb band will be highlighted by gene A while, here we are getting 3.5 and 4.5 kb fragments. Ok! In this fragment the gene A probe cannot bind in this fragment, the gene A probe cannot bind, right? Because, 1.5 kb region is available in this side. Now if we use a different probe suppose we are using gene B as a probe, P_1, P_2 we are using gene B probe. Ok! So, suppose gene B is, we are showing with purple color. Ok! Now we are using the gene B probe in this particular *Eco*RI digested Southern blot where, it will bind.

If you see gene B its size was 1 kb right. Over here in this fragment, that region is available, right? Means, at this 2 kb fragment we can see this band. Can you see any other signal in the autoradiogram using gene B probe? No because over here our gene B is available neither in this side, nor in this side our gene B probe can bind right. While let us see what will be happening in plant 2. In plant 2 over here the gene B probe can bind. Ok!.

What is the size of that? Its size is approximately 4, its size is 4 kb right. So, this 4 kb band it will be available in plant 2, we are talking about plant 2 now. While, in this

fragment over here also gene B or probe B can bind. So, its size is 3 kb. So, we can see these 2 signals, 4 kb and 3 kb in plant 2.

So, in this way if we use a restriction enzyme that is available in between of the 2 gene or if we use 2 different probes which are available in 2 sides of our restriction enzyme what we are using for Southern analysis then we can easily analyze this. Ok! In reverse tandem integration in most of the cases. Ok! once we will change the probe then our banding pattern will be changed. Like in probe A, using probe A in plant 1 we are getting 2 signals while in using probe B we are getting only 1 signal and vice versa, in plant 2 using probe A we are getting 1 signal while using probe B in plant 2 we are getting 2 signals. Ok! So, in this way the reverse tandem integration could be analyzed. Because if you get any construct information you have to visualize it that it is located within the chromosome then we must need other restriction sites within the genome to get the fragments. Ok!

Now one thing I would like to mention suppose, a reverse tandem integration has been taken place. Ok! over here its size is 1.5 kb, its size is 1 kb, and it has been integrated in this way, its size is 1 kb, its size is 1.5 kb suppose, over here *Eco*RI restriction site is available here suppose *Bam*HI was available and suppose, over here *Hin*dIII site was available. Ok! So, 2 *Bam*HI sites will be there, if this type of reverse tandem integration is taken place. So, suppose here we are analyzing a Southern blot. Ok! in a particular plant we are digesting, we have digested the genomic DNA with *Eco*RI restriction enzyme.

So, this part we should expect this fragment, we should expect from here, its size will be 2 kb, we should expect another fragment from here and another fragment from here. Because, we do not know where on the genome our next *Eco*RI site is available, because *Eco*RI site is being used in this Southern hybridization, right? So, suppose *Eco*RI site may be here that is let us assume 3 kb away. So, its overall size could be 4.5 in another case, in another site some *Eco*RI has to be there. Ok!

Now we have found a particular banding pattern in autoradiogram where this particular part has been used as a probe. Ok! Means initially let me tell you about, the construct once again we had this construct. Ok! Its size was 1.5 kb and its size was 1 kb. Ok! This construct was used right. Over here we had *Hin*dIII site over here, we had *Eco*RI site and we had *Bam*HI site. Ok! This construct was used for plant transformation and we are using this particular probe.

Suppose this is probe A and this one is B part. Ok! So, if we use probe A what we should expect? This part will not be detected, because this part will be detected using probe B only. Autoradiogram using probe A, we are making another autoradiogram of probe B. In probe B we will get only a signal at around 2 kb, right? P_1 plant only a signal at around 2 kb will be detected. While in case of probe A we should expect at least these two bands one band is supposed to come from here, and one band is supposed to come from here, because probe A will be available over here and it will be available over here.

Now we should expect the size of both of these bands will be more than 1.5 kb, right? So, suppose one band we are getting in the autoradiogram at around 4.5 kb, that is coming from here, that is highly possible and, another band we are getting at around 1 kb that is less than the size of the gene A, part gene A part was 1.5 kb, but here we are getting 1 kb, is it possible?

It is also possible in *Agrobacterium* mediated transformation, because during the transformation process sometimes the whole construct is not delivered or is not integrated properly. So, it may be shared. So, if we see these types of things our analysis should be in that way, we have to tell that, the based on this banding pattern we have to tell that, as two bands are detected using a particular probe, and one band is detected using another probe. So, we may expect the reverse tandem integration has been occurred, first of all. Next of all, we have to visualize these things we have to make the diagram and we have to tell that from the, 5′ end of this construct, from the 5′ end of this construct, the partial integration has been taken place or the full-length construct has not been delivered. Ok!

Some truncation has been occurred in the 5′ end. The full-length construct has not been delivered and truncation occurred at the 5′ end of the construct. Ok! Because we do not know, whether from here we will be getting 4.5 or from here we will be getting 4.5, but in the signal, we are getting one band is 4.5, one band is less than 1.5. It means some truncation occurs over here. So, in this way basically we need to analyze different Southern related problems. Now let us start about a particular problem. In this particular problem a construct has been shown, the following construct has been used for plant transformation. Then genomic DNA isolated, from two putative transform plants were digested, with *Hin*dIII restriction enzyme genomic DNA were digested *Hin*dIII is available, in this side followed by Southern hybridization using two different probes.

So, two probes have been used probe A and probe B. Ok! Depicted the following autoradiogram as mentioned below. So, which of the following possibilities could be there? How can we specifically tell that which of the following possibilities are actual? So, first of all it is being asked that which of the following possibilities could be there? Means maybe a number of possibilities could be true or a single possibility could be true. Ok! Next one how can I specifically tell which of the following possibilities are actual? So, let us see so, this is the autoradiogram. Ok! This is the autoradiogram. So, in P_1 plant we got a band at 4 kb, using probe A and, in probe B also we have got a band of 4 kb. In P_2 plants also a band at 6 kb, using probe A and in P_2 plants we have got a not band those are not bands in autoradiogram we get basically signals in P_2 plants also we have got a signal at 6 kb.

So, this is the scenario if we see about the construct, in the construct one part was 1 kb, one part was 2 kb. Ok! So, this part is probe A belong to 1 kb part and probe B for 2 kb part. Ok! So, now as from the signals available on the autoradiogram we are getting that only single signal is available, using two different probes. So, one possibility might be that single integration has done in both the plants, that might be a possibility the single integration has done in both the plants. Ok! Another possibility is written here double integration has done in both the plants double integration in both the plants may be there

may not be there.

Third one P_1 is having single integration and P_2 is having double integration. Ok! P_1 and P_2 are two different plants. So, let us see if any reverse tandem integration is taken place what could be the possibilities this is the *Eco*RI site this is the *Hin*dIII site. Ok! Its total size will be 3 kb right. Suppose a reverse tandem integration has occurred in this orientation another *Eco*RI has come here and *Hin*dIII site is available over here. So, this whole 6 kb chunk will come out after *Hin*dIII digestion because in between no *Hin*dIII site is available right. So, this whole chunk from this construct could come out and if this thing occurs, we may get a 6 kb band.

If you see carefully in plant 2, we are getting 6 kb band. So, in this 6 kb band, what could be available in this 6 kb band? Our probe A can bind as well as our probe B can bind, right? So, using both of this probe we can get this particular signal. So, this possibility might be true that P_1 is having single integration and P_2 is having double integration, right? So, if P_1 is having single integration, I think you guys can understand the process because here digestion has been done using *Hin*dIII restriction enzyme. Ok!

So, what was our construct? Our construct was like this, this one was our construct at its one end we had *Hin*dIII site, its size was 3 kb, in total size. So, it has been integrated on the chromosome, somewhere on this chromosome another *Hin*dIII will be available maybe 1 kb away. So, this band will come its size will be 4 kb and it will be highlighted by both the probes, the probe A as well as probe B. So, single integration could be explained easily. The single integration may be taken place in plant 2 also right.

How in plant 2 if this thing, suppose this one was our construct over here the *Hin*dIII site was there its size was 1 kb its size was 2 kb. So, suppose it has been integrated on the chromosome and somewhere this *Hin*dIII is available that is 3 kb away. So, here also we may get a 6 kb band. So, in this way in plant 2 we may get such type of integration pattern in case of single integration also. So, such type of signal pattern in autoradiogram in case of single integration also right.

So, because both the probes will bind to these fragments, 6 kb fragments. So, in single integration it is possible. So, these two possibilities might work. Now if we recall, the question how can we specifically tell that, which of the possibilities are actual? If you have to specifically tell it, we need to digest it with this restriction enzyme which is available in between these two probes.

So, I think you guys can make such type of construct and try to digest it, with *Sal*I restriction enzyme and then only your understanding will be clearer. Ok! So, these two possibilities will be true and the answer of this one, how can you specifically tell that? So, you can specifically tell by using the restriction enzyme which is available in between these two probes. Because if we digest it with *Sal*I then, this will be one side and this will be another side, definitely the banding pattern will differ using both the probes. Ok! And if reverse tandem integration is there, then we must see a common signal using both the probes at least, at least in case of some particular probe. Ok!

No basically, in tandem integration the same banding pattern is available in reverse tandem, that thing will not be available. Ok! Now it is another question, this following construct has been used for generating transgenic plants P_1 and P_2 its size is like this 1.5 kb probe A and 1 kb probe B to confirm the transgenic plants Southern blotting was conducted, and following autoradiograms were found using probe A and probe B, which of the following situations will explain the result? So, these are the things here in plant 1 we got a signal at 2 kb in plant 2 we got a signal at around 2.5 kb, both the, in both the cases *Bam*HI digestion was done while in probe B in plant 1 we are getting a signal at 2 kb in plant 2 we are getting 2 signals.

It means at least double integration may have occurred in plant 2. Ok! So, let us see the possibilities single integration has been done in both the plants it could be rejected, if single integration we should not see 2 signals in the autoradiogram, until and unless within the probe the restriction site is not available. Ok! So, here what we are using as a probe different probe. So, in between the probe the restriction site is not there because,

*Bam*HI is being used as a restriction enzyme. Next one the double integration has been done in P_1 and single in P_2 this could be rejected also in P_1 we are getting almost single bands, in P_2 we are getting double signals. Ok!.

Third one double integration has been done in P_2 and single integration in P_1. So, double integration in P_2 and single in P_1 that could be true and, fourth one at least one tandem integration has been taken place in any of these transgenic plants. So, if tandem integration is taken place we need to assume it in this way. Suppose this is our construct, this is probe, this is A part, this is B part, in tandem integration it will be integrated in this way AB, AB in this way. So, over here over here we had *Bam*HI site over here we have *Bam*HI site, right?

So, this fragment will be coming, its size will be 2.5 kb this will be highlighted by both the probes it will be highlighted by this probe, as well as it will be highlighted by this probe. So, in case of tandem integration only both the probes can show similar signal at a particular position. Ok! So, these two bands these two signals will be common. Now using probe, A, using probe A we should get another signal from here because somewhere within the genome, somewhere within the genome the *Bam*HI site will be available. So, let us assume that this size, sorry, let us assume that, this size is 1 kb its size, is 1.5 that is known to us. So, this 2.5 kb band will be generated from here. Ok! So, using probe A this part will be highlighted as well as this part will be highlighted at 2.5 kb we can get a strong band. While using probe B what could be the scenario in probe B it will bind the probe B is this part.

So, probe B will bind over here means the 2.5 kb will be available, in probe B and this side somewhere within the genome next *Bam*HI site is available. So, there from we should expect another band. So, let us assume that it is 4 kb away this difference is 4 kb.

So, this is 1 kb is known to us. So, this 5 kb will be detected over here. So, in this way we have to analyze different Southern related problems. I hope that you have understood

this if you have any doubt, you may ask us through email or some other means or during the live sessions. Thank you.