Course Name: Basics of Crop Breeding and Plant Biotechnology

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Week: 12

Lecture-53: Double Integration (Part I)

Hello everybody, welcome again to the SWAYAM NPTEL course on Basics of Crop Breeding and Plant Biotechnology. So, today we will discuss discussing the tandem and reverse tandem integration and Southern blot analysis. So, these are the concepts which will be covered under this particular topic. First of all, we will be discussing the tandem and reverse tandem integration. If our transgene is integrated in tandem orientation or reverse tandem orientation, how we should analyze the Southern blot or what should be the nature of the Southern blot, where should we expect the signals, those things will be discussed. Then we will discuss some problems on southern blot analysis.

I have mentioned earlier, that this course will be highly beneficial for those students who will be appearing in different competitive exams like CSR/UGC NET- JRF. So, this type of Southern blot related questions might come over there. Ok! So, let us try to understand these things. So, first of all we will be discussing about the double integration. Ok! And in double integration specially we will be discussing the tandem integration part.

So, before going to tandem integration let me draw a particular construct. Suppose in a construct we have a gene A and promoter of that gene. Ok! Suppose we are just analyzing this particular part. Ok! The size of this promoter is 1.5 kb and size of this gene is 1 kb. Suppose this thing has been integrated within the genome, over here the left border and right border regions are available. Ok!

This is just an example, in actual case, in a construct, in an over expression construct we

need a terminator part also. This is for your understanding I am just giving an example. Ok! Suppose 3 restriction sites have been used in this cloning, over here we have *Bam*HI site, over here we have *Eco*RI restriction sites, and over here we have *Hin*dIII restriction sites. Ok! So, the promoter has been cloned in *Bam*HI and *Eco*RI while, the gene has been cloned in *Eco*RI and *Hin*dIII site. Now, if tandem integration is taken place.

So, what type of banding pattern we should get? In tandem, what happens if you just think this whole thing in this way. Suppose, this is the whole chunk you are assuming. So, in tandem integration these things will be integrated in this way, within the genome at least 2 copies. Ok! In tandem means, in a, in this particular orientation our construct will be integrated within the genome, at least in 2 copies in this type of orientation. Ok! So, over here we will be having *Bam*HI in between we will be having *Eco*RI, over here we will be having *Hin*dIII.

Again the *Bam*HI will be here, then we will be having *Eco*RI and we will be having *Hin*dIII. Since same direction in same orientation this construct part will be repeated twice, within a particular locus of the chromosome. Let us assume, it is a chromosome, over here tandem integration has been occurred, means it has been integrated in this way. Now, how should analyze it? Ok! Now if we digest this particular construct with 3 different enzymes what type of banding pattern we should expect?

Suppose we are considering a particular plant P_1, in P_1 plant this tandem integration has been taken place, means the construct has been integrated in this way, anywhere within its genome. Ok! And, we are digesting it with *Bam*HI. So, if we digest it with *Bam*HI from this tandem integration, this part will come because one *Bam*HI and another *Bam*HI site is available. So, we know the size of this fragment 1.5 kb promoter and 1 kb gene is there. So, its size will be 2.5 kb, right? This 2.5 kb band would be there, I am talking about the digestion till now we have not used any probe. Ok! Till now we have not used any probe. Now if we carefully see this construct over here the next *Bam*HI site is available. So, somewhere wherever it has been integrated within the genome the chromosomal part will be in this side. So, one *Bam*HI and one *Bam*HI cut has been done

from this *Bam*HI site somewhere within the genome another *Bam*HI site might be there.

So, we will get that fragment also, we do not know the size of that fragment right, but its size will be anything equals to or greater than 2.5 kb right because the whole construct is there 2.5 kb construct is there thereafter some additional genomic part might be available. So, we may get a band at around 2.5 kb if *Bam*HI is just available next to the integration site or if *Bam*HI is available little bit away our size will be increased it will be more than 2.5. Ok! So, let us assume we are getting a band of 2.5 and 3.5. So, now in *Bam*HI digestion suppose we are getting these two bands from this particular construct from the endogenous genomic DNA lot of bands will be there we are analyzing this construct integration. So, we are just focusing on this particular part. Ok!

Now how 3.5 kb band could be there if *Bam*HI site is available in the 3′ end of the integration site this is the 5′ end this is the 3′ end of the integration site if in the 3′ end at 1 kb away the *Bam*HI site is available then this 3.5 kb band is expected. Now this is about the digestion things now, if we use a particular probe suppose we are using gene specific probe means this particular region is being used as a probe, gene specific probe. So, in this fragment our probe can bind while, over here in this fragment our probe can bind, right? So, over here our probe will bind and over here our probe will bind and, in the autoradiogram, we can see these two signals.

In digested one as we will see a smear like *Eco*RI things over here once the digestion will be done along with all endogenous DNA bands these two bands will be coming from the construct, and over here our specific probe can bind. Ok! So, what probe we have used here we have used gene A specific probe. If we use promoter specific probe, what we can see? If we use promoter specific probe, means if this part is used as a probe in hybridization, it can bind this fragment and it can bind this fragment. So, both of these bands will be visible. Ok! This is one thing you have to be think in a rational way, means once you will get some construct information, once you will get the information of different restriction sites, then first of all you have to think how it can be integrated as single copy, as double copy, if double copy tandem or reverse tandem, in most of the

cases we may expect at least two bands, in some cases we may not get two bands later on we will discuss. Ok! Now, if we digest it with the *Eco*RI restriction sites if you see this construct see carefully *Bam*HI is available in a particular site *Eco*RI is available in between.

So, if we use *Eco*RI what will be the scenario suppose over here the P_1 plant has been digested with *Eco*RI. Ok! So, one *Eco*RI fragment will be obtained from here if tandem integration is taken place, in tandem the construct is placed one after another. So, *Eco*RI site is here as well as, here in between these two constructs. So, a fragment will be coming from here what will be the size? Its size will be 1 this is 1 kb this is 1.5 kb means 2.5 kb. So, this 2.5 kb band could be there. So, here we have used promoter, specific probe right this whole thing for *Bam*HI digestion here we are discussing, *Eco*RI digestion. So, this fragment will be coming from the construct, next it has been integrated within the genome. So, we have to assume it that some *Eco*RI site might be available over here and some *Eco*RI site might be available over here in the genome right.

So, let us assume this size is 2 kb, let us assume. So, if it is 2 kb we should expect another *Eco*RI band from here, means its size is 1.5 and its size is 2 means total we will get a 3.5 kb band. Ok! In this side *Eco*RI is available here, and another *Eco*RI is available here. Ok!

Let us assume, the availability of the *Eco*RI site from the 3′ end of this construct is 3 kb away. Ok! So, this 3 kb and internal 1 kb, 1 kb and 3 kb. So, it will be a 4 kb band, at 4 kb another signal will be observed, means another band could be created then in addition to that, we will be having our different endogenous bands, because after digestion we will see a smear like things, right? These things will not be visible upon digestion. Now, we are using different probes. So, let us see how will the features be, if we use the gene specific probe, which fragments will be highlighted these things will be highly interesting and highly important. Ok!

So, if we use gene a specific probe, which of these fragments will be highlighted? If you

see about this particular fragment, which is just coming from the tandem integration over there our gene sequence is available. So, this 2.5 kb fragments over here our probe can bind.

So, in 2.5 kb we will get a signal. If we see about this fragment means this particular fragment which size is 3.5 kb. Ok! Because, the *Eco*RI site is 2 kb away from the 5′ end of the integration site. Ok! We are assuming it. So, in that case its fragment 3.5 kb will be available, but our probe will not bind there, because here only promoter region is there gene region is not available, right? Gene region is available here and it is available here.

So, in 3.5 kb our probe will not bind gene specific probe. So, let us see another fragment here from the integration site, at 3 kb away next *Bam*HI next *Eco*RI site was available. Ok! So, its fragment size is approximately 4 kb, can our probe bind, can the gene a specific probe binds there? Yes, because in this fragment our gene A, specific region is available. So, our probe will bind over here. Ok! So, we can get these 2 signals at 2.5 kb and at 4 kb. Ok! Over here it was 4, it was 3.5, it was 2.5. Ok! So, these 2 bands will be available in the autoradiogram, this one was also autoradiogram and this one was also autoradiogram. Now, if we use promoter specific probe to analyze this integration, what will be observed? Let us see, once we will use the promoter specific probe, then if you carefully see this part, the 2.5 kb, what we are getting from the tandem integration, both the construct parts are available their promoter region is also available. So, in this 2.5 kb over here this probe can bind while, in this fragment in the 3.5 kb fragment our probe can bind also, because over here the promoter region is available, if you draw these things by yourself then you can understand it in a better way. Ok! So, can we find this band using promoter specific probe? No, because in this band only the gene specific region is available and thereafter, the endogenous DNA is available right the chromosomal DNA is available.

So, our promoter region should not bind over there. So, in this way using different digestion, different restriction digestion, and using different probes, we can tell that, what type of integration might have occurred? If tandem integration is taken place, for your

remembrance ah, if tandem integration is taken place in most of the cases using 2 types of probes, like here 2 types of probes has been used, in using both types of probes at least one common band is available. Ok! So, just let me tell it once again if we use the *Hin*dIII restriction site what 3 will be the features over there? We had the construct like this, we had the *Bam*HI over here, we had the *Eco*RI over here, we had the *Hin*dIII over here, then tandem integration occurred again *Bam*HI, again *Eco*RI and again *Hin*dIII, *Bam*HI, *Eco*RI, *Hin*dIII, *Bam*HI, *Eco*RI, *Hin*dIII. Here the nature will be similar to the *Bam*HI site.

If we use *Hin*dIII restriction digestion, first of all this fragment will be coming from the construct its size will be 2.5 kb, right? And this 2.5 kb, will be highlighted by both the probe and over here somewhere within the genome the *Hin*dIII site will be available then, only we can get this fragment, right? Because, another *Hin*dIII is here. So, let us assume it is 3 kb away. So, what will be this fragment size here from, we are getting 2.5 and here from 3. So, it will be close to 5.5 kb. Ok! So, here also both the probes will bind the probe promoter specific probe will bind also, the gene specific probe will bind also ok. So, it will be just similar to the *Bam*HI digested scenario. Ok! But the band size or the signals will be different over here, we got a signal of 3.5 and 2.5, right, in *Bam*HI digestion, but in case of *Hin*dIII we may get different signals because, we do not know whether the *Hin*dIII is available, at the similar distance of like the *Bam*HI or not close to the integration site. Ok! So, in this way we have to analyze it.