Comprehensive Molecular Diagnostics and Advanced Gene Expression Analysis

Prof. Aritri Bir

Dr. B.C. Roy Multi-Speciality Medical Research Centre

Indian Institute of Technology Kharagpur

Lecture 19 : Methods to study DNA-protein interaction II

Namaskar. Welcome back. We are at the lecture series in the NPTEL platform, where we are discussing the Comprehensive Molecular Diagnostics and Advancing Expression Analysis different tools for this mechanism. In the last class, we have started methods to study the different DNA protein interaction. So, in the last class, we have learned different in vitro techniques. Today, we are going to learn different in vivo techniques by which we can study DNA protein interaction.

In the in vivo technique, the most important one is chromatin immunoprecipitation and along with that it has multiple modifications. So, let us learn something about chromatin immunoprecipitation. Now, chromatin immunoprecipitation in the classical approach, what is done the protein associated chromatin is basically sheared into smaller fragment. Basically, these are the 500 base pair approximate fragments of DNA, which is done by using either sonication or nucleus digestion.

After that immunoprecipitation of the DNA protein complex is done by specific protein targeted antibody and finally, the DNA protein complexes complex are basically isolated and then dissociated and then specifically enrich DNA segment. The segment which is actually interacting with the protein is analyzed by different amplification methods mostly PCR based amplification methods. This is the classical approach. So, once again what is done in chromatin immunoprecipitation? So, if that is our DNA bound with a protein, what happens? The protein associated chromatin fragment is basically sheared down in smaller fragments. Now, this smaller fragments are immunoprecipitated using protein specific antibody.

So, if we have multiple segments and in one region the protein is attached, when we immunoprecipitate it with a specific protein targeted antibody in the precipitated part we will get only this because this segment does not have the protein. So, the antibody will not bind with this region. So, this antibody bound protein DNA complex is precipitated using different sepharose beads after that these complexes are dissociated. So, we are having protein and DNA separately, then this DNA fragment is enriched after that

analyzed using PCR based amplification method. Now, this is the classical approach where we are using specific targeted antibody.

Instead there is halotax technology these days where we do not need a specific antibody. So, what is done in halotax technology basically transfection of halotax vectors containing halotax are fused with the protein of interest. After that it is expressed in the mammalian cell line. Now, in the cell these are cross linked with the DNA finally, this DNA protein in complexes are captured over the halolink resin after that the process remains same. So, over the halolink resin the DNA protein complexes are separated, then they are d cross linked means the dissociation is done after the DNA is purified and amplified by PCR and then identified.

So, this is the technique where we are basically immunoprecipitating the chromatin to identify the specific DNA protein interaction. Now, this chromatin immunoprecipitation has different type of modification like proteins which are weakly bound to the DNA or when the if you remember there is a term known as nucleosome where the DNA is bound over the histone protein basically the histone proteins are wrapped by the DNA. So, when there is the rearrangement of nucleosome the binding of histone and the DNA can be changed also the weak proteins which are binding over the DNA they can be released. So, to avoid that freezing of this DNA protein interaction is done by cross linking agents the commonest one is formaldehyde. Now, formaldehyde basically forms covalent cross link between the specific protein to the DNA it actually reacts with the primary amines located on the amino acid and the basis of the DNA or RNA molecule.

Now, what happens? Protein DNA complex is now immunoprecipitated then this cross link is reversed by river heating then the protein is removed by proteinase K digestion after that the DNA portion is purified and identified by the PCR. So, here we can avoid nucleosomal rearrangement because the DNA protein interaction is fixed or we can analyze those protein which has the tendency to dissociate from the DNA very easily, but because of this cross linking the nature of the DNA can be changed or the interaction can be changed or the proteins characters can be changed. So, there are native chip as well or n chip where those proteins which are actually strongly bound to DNA they do not need this cross linkers because they have actually high affinity for DNA. So, the core histone proteins they are having high affinity for the DNA. So, these in these chromatin immunoprecipitation we do not need the cross linking with directly go to immunoprecipitating the complex.

Next is fast chip. So, it is evident by the name that the experiment is done taking less time for what basically when there are large number of cells where we need to study the DNA protein interaction the process needs to be conducted in fast faster speed. So, the steps which are taking much time in conventional chip are basically avoided. Now what

are those this cross linked DNA protein they are dissociation is basically having very low recovery rate. So, from the total DNA the cross linked part are basically very low in amount.

So, what happens the quantity is very low. So, for those we can do fast chip not only that there are steps for multiple washing to procure the DNA segment and that also causes loss of specific interaction. Here a sonicating water bath is basically used in fast chip for the antigen antibody binding. So, the rate is basically improved. So, the lesser though.

So, lesser time is required also the recovery efficiency is increased by using a resin that is telex resin which basically combine the cross reversal of cross linking as well as DNA purification. So, these two steps basically helps help in faster assay of the chromatin immunoprecipitation. After that the tubes are spun and DNA containing the supernatant can directly be acid in PCR. So, basically in conventional chip where we need around 2 to 3 days to complete the assay here in fast chip we need only 4 hour. Then carrier chip now carrier chip is for required for the study where we are having very few amount of cell like 100 cells count.

And studies like histone modification for different gene regulation or developmental gene regulation they in those cases this carrier chip is required. Now, this small amount of chromatin which we need to identify is basically immunoprecipitated by using another carrier chromatin. Now, what is that carrier chromatin? Basically carrier chromatin is one heterogeneous chromatin which is originated from a species which is evolutionally distant from the species we are investigating the commonest one is Rosophila. So, by using the carrier protein we can immunoprecipitate some small this small amount of chromatin of our interest. After that the detection is same like radioactive PCR or phosphor imaging, but the problem is we need to design the primer with high specificity to prevent any spurious amplification of the carrier DNA.

So, that is the reason we need to identify one carrier carrier chromatin which is not at all matching with our target of interest that should be evolutionally distant from the species which we want to identify. So, that is carrier chip. After that we are at chip-chip. So, the first chip is basically chromatin immunoprecipitation. The second chip if you remember is the chip we use in microarray technology.

So, one chip is for chromatin immunoprecipitation which is combined with another chip used in microarray technology. So, basically what is done? So, the immunoprecipitated DNA fragment is labeled by a fluorescent dye that can be Cy 5 or Alexa 647 like that and it is combined with genomic DNA which is labeled with a different dye that can be Cy 3 this is the reference DNA. So, if the probe mixture is applied over the microarray chip

there will be hybridization the microarray chip contain the whole genome labeled with suppose Cy 3. So, what we will get? We will get the expression of Cy 3, Cy 5 based on the significant attachment. So, those regions which are enriched by this isolation over the chip can be immunoprecipitated.

The advantage is we can use multiple types of probes multiple types of sequence to identify multiple types of protein binding also parallel analysis of different genes can be done and a large number of genomic region can be probed in a single experiment. So, chromatin immunoprecipitation is basically here combined with microarray technology it can again be combined with sequencing. So, we need to generate a library. So, what happens? The enriched DNA fragments which is basically isolated by immunoprecipitation are blunted and phosphorylated using K4 kinase after that adenine is added using cat polymerase and adaptor is ligated to both ends of the fragment. So, the isolated DNA basically is forming the chip sequence library after that the whole library is amplified using the PCR.

Then the DNA fragment of around 100 to 300 base pair tag are selected and sequenced. Now, these tags are analyzed computationally with different alignment tool and is matched with our reference genome to identify what are those enriched sites. So, those are the enriched sites which are actually interacting with the protein. Now, this chip sequencing method is basically very helpful in detecting histone modification, nucleosomal positioning, mapping of the binding site of different DNA binding protein also it is helpful in distinguishing alleles on the basis of different single nucleotide polymorphism. So, basically after the immunoprecipitation of protein and DNA the DNA part is d cross linked and separated then it is sequenced by matching it with the different probes in our chip sequence library.

It is required for very minute changes like single nucleotide polymorphism here very minute changes is causing the mutation which can alter the protein interaction with the target DNA. So, these are all of the in vivo techniques to study DNA protein interaction where we have learned the principle of chromatin immunoprecipitation and its various modification like x chip where we cross link the DNA protein interaction by different cross linker like formaldehyde native chip where we do not need the cross linker because the interaction of the protein and DNA are basically very strong. Then carrier chip is required for small amount of DNA or cell to be studied where we use one additional carrier oligonucleotide or chromatin which is completely unrelated to the target. Then chip-chip where we can combine the chromatin immunoprecipitation with microarray and also we can combine the chromatin immunoprecipitation with sequencing. So, by chip-chip we can identify different in a genome different segments in a small in a single experiment we can identify different segments which are interacting with different types of protein.

So, the genes can be of different interest or the proteins can be of different interest or different types of genome can also be studied whereas, the sequence which is actually interacting with the protein can be sequenced by chip sequencing. So, these are all of in vivo techniques to study DNA protein interaction these are my references and see you in the next class. Thank you. .