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 17 : FISH (Fluorescence in situ Hybridization)

Namaskar. Welcome back to the Comprehensive Molecular Diagnostic and Advanced Gene Expression Analysis. So, we were discussing the tools of molecular diagnostics and gene expression where in the previous class we have discussed DNA microarray. Today we are going to learn about FISH that is Fluorescence in-situ hybridization that is a technique. So, in this class we are going to discuss the basic concept of fluorescence in-situ hybridization, the basic principle, mechanism means the process how the FISH is done and finally, the application in the field of health care. So, in-situ hybridization is basically the group of molecular cytogenetic methods where we detect not only detect, localize a specific DNA sequence or gene inside a cell.

So, the detection is in-situ within the chromosome in a cell we are going to detect and localize the sequence as it is. Now, the with the advent of the in-situ hybridization experiments started in 1969 by Goll and Pardew, the technique has been modified in different way where the detection probe has been improvised in by different techniques. So, the current technique which is used mostly is in-situ hybridization using the probe which is tagged with fluorophore. So, this F stands for fluorescence in-situ hybridization.

What is the basic principle? It is just like the same which is used in southern blotting in DNA microarray that is hybridization based on the sequence complementarity. So, there is a probe that is a small piece of purified DNA which is tagged with a fluorescent dye that probe finds and binds the complementary sequence within the cell located over the chromosome. So, that is the basic principle of fluorescence in-situ hybridization. So, this is the figure by which we can learn the step by step technique of fluorescence in-situ hybridization. So, as we talked that there should be a probe, a label probe, a sequence a small sequence or fragment of DNA and that is labeled either directly with the fluorophore or indirectly with a half-time.

Now, when there is a direct labeling the fluorophores or the dye which is mostly used is fluorescein that is FITC fluorescein isothiocyanate different types of cyanines and AMCA that is amino methyl cumerin derivative of the dye. When we go for indirect

labeling there are different haptens, haptens like biotin, digoxygenin, dinitrophenol these are the non fluorescent haptens which are used in indirect labeling. Why we are saying this is indirect one? Because when these haptens further will be detected by definitely another fluorescent molecule and how these labelings are done by these methods, nick translation method, random primer labeling method, PCR using tagged nucleotides. So, these are all methods how we can tag a specific nucleotide with different molecules that can be a fluorophore that can be different types of hapten that can be one antibody like that. So, by this method we are tagging the probe.

So, there should be the another strand which is sample. So, one probe which we have designed to detect the target and the target is present as sample. In the sample there is DNA and that can be the sequence of DNA can be detected both in interface as well as metaphase chromosome. So, you are here you can see these are our tagged probes and from this is our sample where from we are getting single stranded DNA. So, we are denaturing the probe as well as the sample DNA we are getting the single stranded probe where this single strand you can see that is labeled and here is our sample which is also denatured using heat.

So, if this probe and this sequence or the sample sequence is complementary they will bind by definitely Sharap's rule. So, there is annealing of the complementary DNA sequence which causes the fluorescence and that is detected under fluorescence microscopy, but what happens when there is indirect labeling remember indirect levels are non fluorescent. So, to make them generate signal what there are two types of methods those are mostly followed one is enzymatic method where fluorochromes are tagged with enzyme which can detect and those fluorochromes when there is biotin the fluorochrome is tagged with streptavidin. Similarly, digoxygenin can be detected by the anti digoxygenin antibody. So, definitely there is another immunological method where antibody antigen antibody based on antigen antibody reaction we can detect the annealed complementary DNA sequences.

So, that is the basic principle of fluorescence in situ hybridization. Now, there are multiple types of probes for these fish and they mostly follow the same process that is the processing of the tissue for detection hybridization and post hybridization there are few post techniques or steps which we follow mainly. So, what are those? So, before hybridization or pre hybridization we need tissue preparation where we want to detect the target sample then there is hybridization and finally, post hybridization washing and detection method. Now, the samples can be of different type that is the what I can say that is the advantage of fish where we can use wide variety of samples the sample can be frozen section sample that can be paraffin embedded sections also that can be cells in suspension. And after hybridization what we do when there is after this probe and I mean binding of probe and the complementary target sequence unbound probe as well as

unbound other sequence those should be washed off.

So, after hybridization there is serial washing to remove the unbound probe as well as unbound sample. Remember during hybridization what we do we do a overnight incubation. So, the binding of the probe and the target needs time sometimes those are done within 6 to 12 hours and sometimes it needs overnight incubation. So, we were discussing about different types of probes as we said. So, there are different types of probes of fish based on what we want to do.

So, if we want to detect the whole chromosome the sequence present on the whole chromosome we use chromosome painting probes. So, what are chromosome painting probes? Those are DNA probes derived from a single type of chromosome. So, the in a single chromosome there are all the sequences of the DNA or the gene present over the chromosome. So, based on that multiple DNA probes are generated those are amplified by PCR and they are labeled. So, these are chromosome specific group or composite probe pool and what they do throughout the chromosome they detect the DNA sequences.

So, what happens the whole chromosome is painted. So, there is a homogeneous paint to highlight the entire chromosome and what we can detect by that we can visualize the entire chromosome in metaphase as well as interface and can detect the number of chromosome, the structure of chromosome by which we can detect the anomalies and simultaneously 24 chromosome all the human chromosome can be painted using this chromosome painting probe. The next probe is repetitive sequence probe. What happens there are different type different sequence in our chromosome which are present in repetitive pattern like telomeres. So, repetitive sequence probes are specific probes targeted towards those short sequences which are present in repetitive manner over the chromosome.

Pan telomeric probes which target these sequence TTAGGE sequence which is present tandemly at the end of every chromosome we can detect the telomere length which is important in aging and cancer. Then centromeric probes centromeric probes detect alpha and beta satellite sequence those are the sequences flanking sequences over centromere. Now what happens this centromeric alpha and beta satellite probes they are concentrated over the centromeric region in such a way that every chromosome are giving 2 signals one for the alpha and one for the beta. So, if there is I am talking about the normal cell. Now if there is monosomy there will be one only if there is trisomy there will be 3 aneuploid is leukemias in solid tumor all these can be detected by the centromeric probe.

Next is locus specific probe. So, the locus specific probe is basically detection for a specific sequence which is targeted for detection of different types of translocation

inversion deletion different mutations can be detected by this locus specific probes. So, what are the different application of FISH? So, what exactly FISH does? It detect the target DNA as it is present inside the chromosome in it is specific location. So, the target DNA is intact we are not in case of what in remember in case of DNA microarray we were isolating the DNA from the cell. So, basically the location is jeopardized, but here the cell is as it is it is spread over a thin and homogeneous flat preparation over which we detect the chromosomes sequence.

So, the target is intact and the position is accurate. Also the nuclear RNA and genes within the cell we can detect. Now what is the advantage? By this we can study the transcription as well as the RNA transport from inside the cell to outside or we can quantitatively detect it and that FISH which detect the RNA is known as RNA FISH. Also nuclear organization can be detected by high resolution information there is a type of FISH which is known as fiber FISH which are which we are going to discuss a bit later. So, by this we can get high resolution information of the nuclear organization and that is helpful to study the different cell cycle stages, how they are affected by different toxin different drugs different pathogens by this we can detect.

So, these are the study we can do based on the FISH application different chromosomal deletion, disomy, imprinting, translocation all these anomalies we can detect by FISH. And as we have already known about this term that is comparative genomic hybridization technique, we can detect the expression profile of the whole genome of a person via FISH. What we do? We use two types of dye consider there is a sample and there is a reference. So, we are taking the whole genome from a normal person the whole genome of a normal person which is tagged with green that is our reference gene pool, the sample which is tagged with red that is our sample gene pool. So, if and this of course, this reference gene pool is tagged with the fluorophore that is green and the sample gene pool is tagged with the red fluorophore that is tagged tagged and represent as red fluorescence.

Now if they are mixed what will happen if they are present the both the sample and the reference if they are present in equal amount we will get a hybrid color that is the yellow one. But suppose instead of yellow we are getting red fluorescence in higher quantity. So, it indicates that the expression of the gene pool in sample is higher. So, there is a comparison of the whole genome by hybridization technique through FISH. So, that is our comparative genomic hybridization.

And the remember this once again we are going to discuss in depth regarding this comparative genomic hybridization in the class of cancer. So, again if we go to the very specific application of FISH. So, there is cancer diagnostic and sub typing facilities by FISH because remember what happens the cancer cells if we want to detect the

chromosomal anomaly preparing one interface chromosome is very hectic from the cancer tissue. Instead of that in FISH just by the biopsy sample if we prepare a homogeneous and flat sample over the slide over that we can do interface FISH and that is very much helpful to detect the chromosomal anomalies related to different types of cancer. Also we can detect the minimal residual disease after the therapy if there is any residual cancer cells present via detecting those specific genetic abnormality.

Prenatal genetic testing can be done can be done there are different chromosomal anomalies which are detected by Down syndrome, trisomy 18 or trisomy 13 all this can be detected via FISH as I told you that those satellite probes or the probes which are used for the detection of the centromere those are helpful in detecting all these anomalies. Microbial detection and identification can be done and those are very rapid and specific detection of the microbial pathogen in sample. Also HPV virus human papilloma virus can be detected and this is a very commonly used technique which is helpful in detection early diagnosis of cervical cancer. Then pharmacogenomic research where the genetic variation by comparative genomic hybridization technique we can decide the drug response in individual and based on that we can design individualized therapy. Now, there are different types of FISH rather different modification on diversification of FISH where the probes are modified or the FISH is FISH is combined with different other molecular diagnostic techniques.

So, based on that we are going to discuss different types of FISH. So, multiplex FISH or M FISH. Now what happens here simultaneously we can identify multiple chromosomal regions or multiple genes over a chromosome by using distinct color for each target. So, remember there are when we are detecting the chromosomal probes chromosomal painting probes if the probes are or the specific DNA sequences are tagged with different colors those specific colors give I mean they paint the individual sequence and that can help in a comprehensive chromosomal analysis. Definitely there are 5 spectrally separable fluorochromes which by mix and match technique generates different colors.

Now this M FISH remember as I told you there are 24 human chromosomes simultaneous detection method, but this method is modified not only to identify chromosome only the chromosomal sub regions or the specific sequence centromere sub centromeric regions they can also be detected by using multiple colors. And the another modification for that is multi locus FISH or M L FISH where simultaneous use of multicolor multiple probes are there. Then instead of multicolor some this is the very common technique we use FISH that is D FISH or double color FISH where 2 color probes are used. Now as I told you if we have a red color and a green color if they merge there will be development of a secondary yellow color. So, based on the development of secondary color D FISH can detect different types of anomalies the commonest one is BCR ABL translocation which is very commonly present in CML or chronic

myelogenous leukemia.

Now you see what happens this is our CML sample where there is a translocation between chromosome 9 and chromosome 22. Remember this we are going to discuss in details in cancer class as well, but here you can see a very basic of how this D FISH can help in detect the CML by detecting this BCR ABL translocation. Now the flanking BCR flanking region so, this is our chromosome 9 and this is our chromosome 22. So, in the 9 region you can see it is the chromosome 9 the region is painted with the red fluorophore and the chromosome 22 is painted with the green fluorophore. Now what happens the if the that is a normal gene we will we can see they are a separate red fluorescence and a separate green fluorescence, but when there is translocation with the now when there is translocation the ABL flanking region from chromosome 9 is translocated near the this is our BCL over chromosome 22 and this ABL here you can see it is translocated over here.

So, we get a Philadelphia chromosome where the chromosome 22 is having BCL of its own as well as the ABL region from the chromosome 9. So, in the Philadelphia chromosome where the translocation has already happened we will get both the red and green fluorescence. Now what happens because of the very proximity of this red and green fluorescence they appear as yellow. So, you can see here there is a separate green fluorescence here we are getting yellow fluorescence. So, this is the translocated Philadelphia chromosome.

So, by this presence of secondary color we can detect the BCR ABL translocation. Also there is another modification of this D fish which is termed as break apart fish where absence of the secondary color is indicative of presence of pathology. Now what happens there is a locus specific probe you can see 2 of the loci are painted with different probes and if they are broken apart they will give the individual color. How is it you can see here is a green color, red color and on merging there is yellow color in between. So, I am just deleting this so, that you can see it properly.

So, you can see here is our green color, here is the red color and in between there is a yellow color. So, that is the locus specific probe where the adjacent loci where we are expecting that there can be a translocation the adjacent loci are painted with different probes. One is giving the yellow on superimposition the sides are red and green, but when there is translocation these 2 loci are separated creating individual color. So, if the chromosome is intact it will appear like this. Here you can see green, yellow and red all are present as it is, but if there is translocation you will see separate green and red color.

So, that is the concept of break apart fish. Now the fish can be used for quantitative estimation of the abundance of specific sequence and that we do for pantelomeric probe

where we detect the telomeres which are useful for assessing cellular aging cancer. Now in those probes sometimes we use PNA probes that is peptide nucleic acid probes. Now peptide nucleic acid probes are the modification of the DNA probes where synthetic DNA analog with a neutral peptide backbone. Remember the peptide backbone are negatively charged with the phosphates, but here the synthetic DNA analogs are having neutral peptide backbone which I mean it negates the charge problems related to charge which gives probes stability as well as improves binding affinity.

Then we have discussed the RNA fish which helps in detect in localized in quantify individual mRNA molecule. Their modification is telluris RNA fish or single molecule RNA fish where the single molecule of mRNA can be detected and quantified. Allele specific expression can be detected on a single cell basis. So, these are the modification of RNA for detection of RNA. Now fish can be combined with other techniques like indirect or direct immunofluorescence which is known as immunofish can be combined with flow cytometry for measurement of the telomeric signals in cell suspension that is known as flow fish.

And as I told you there is fiber fish for high resolution mapping of chromosome. Here what is done fiber means the chromosomal fiber and those chromosomal fibers are stretched over a slide for better resolution we I mean where we can quantify the chromosome specific location of DNA can map it due to over stretching. So, this is the summary for this class where we have discussed the basic principle of fish based on the complementarity of the DNA sequence and probe and it helps in in situ detection means where the chromosome is located inside the cell in that condition we are detecting the sequence of the gene. We have discussed different types of probe that is chromosomal painting probes where whole chromosome can be painted a specific sequence can be detected via the locus specific probe or some repeated sequence repetitive sequence which are present over the cell or the chromosome based on that we have designed the pantelomeric probes or the satellite probes directed towards centromeres and all these techniques or probes are mostly helpful in detection of cancer the chromosomal anomalies associated with cancer chromosomal anomalies which help in prenatal screening and that and also different modification of fish or and the merging of other molecular diagnostic techniques along with fish. These are my references see you in the next class. Thank you.