

NPTEL VIDEO COURSE – PROTEOMICS

PROF. SANJEEVA SRIVASTAVA

LECTURE-12

Gel-based proteomics

TRANSCRIPT

So, welcome to the proteomics course. In today's lecture we talk about gel based proteomics. The gel-based proteomics includes different types of techniques which can be used in different aspects of life science research. Regardless of whatever field you work on every lab uses different type of gel-based approach to separate proteins in their day-to-day research. So, gel-based proteomics includes different types of techniques such as Sodium Dodecyl Sulphate Poly Acrylamide Gel Electrophoresis or SDS PAGE, 2 Dimensional Electrophoresis or 2DE. The advance form of 2 Dimensional electrophoresis also known as Difference in Gel Electrophoresis or DIGE. Then there are several advancement of it, variations of it such as Blue Native PAGE or BN PAGE. So, in the gel-based proteomics we will talk about different principles involved in operating each of these techniques and their applications. For example, the SDS PAGE can be used to separate the proteins based on their molecular weight, the 2 Dimensional Electrophoresis separates proteins based on both isoelectric point and molecular weight in the 2 dimensions. The advance form of 2 dimensional electrophoresis separates proteins in such a way that different types of artefacts and variations from 2DE can be overcome by adding different dyes and mix them together in the DIGE technology. So, all of these technologies will be discussed in the gel-based proteomics.

In today's lecture on gel-based proteomics, I will give you the concepts of different electrophoresis types for the 1 dimensional electrophoresis and 2 dimensional electrophoresis. During the description of 2 dimensional electrophoresis I will give you the workflow of several steps involved in 2 dimensional electrophoresis.

So, let me give you an outline for today's lecture. I will describe about gel-based proteomics, how electrophoresis is used, different types of electrophoresis such as one

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dimensional electrophoresis or SDS PAGE which is used to separate simple protein mixture but when you have very complex mixture and you want to separate proteins based on 2 properties then you can use 2 dimensional electrophoresis. I'll then describe you the workflow of two dimensional electrophoresis. There are different steps involved and we will walk you through all the steps. Also, during the process I will show you some animations to clarify your concept and some laboratory demonstrations to give you the real feel of the laboratory work being carried for performing the 2 dimensional electrophoresis.

So there are different steps which are involved in the analysis of the proteome. As I talked in the previous lecture that first-of-all it's very important to separate the proteins, which are present in the sample. Once you have a very good protein preparation made then the next thing is to separate the proteins by involving different techniques. You can use electrophoretic-based method such as 1 dimensional electrophoresis or 2 dimensional electrophoresis or one can also use different type of chromatography methods to separate the proteins. So, in proteomics you always have various options available to analyze your protein samples. It depends on your applications and the biological questions, which you want to ask, what information you want to obtain from that particular technique. So, if you want to have Just want to verify that your protein is very pure may SDS PAGE electrophoresis is good enough. If you want to determine the subunit compositions you can compare that on SDS PAGE and Native PAGE. If you want to resolve the proteins a very complex mixture of proteins obtained from whole cell lysate or tissue or the cell and which contains thousands of protein mixture at that time you can try different types of prefractionation methods by involving different chromatography as well as you can try different type of advanced electrophoretic methods such as two dimensional electrophoresis or DIGE. One can also use different mass spectrometry based or microarray based technologies to study these proteins. During the course you will come across all of these techniques, including mass spectrometry and microarrays but let's focus on the electrophoresis in this lecture.

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So, the gel-based proteomics involves different types of separation techniques, which includes sodium dodecyl sulfate polyacrylamide gel electrophoresis or SDS-PAGE, 2 dimensional gel electrophoresis or 2DE, the fluorescence 2-D difference in gel electrophoresis or DIGE, Blue native PAGE or BN-PAGE. But let me remind you, this is not a very descriptive list. These are just few very important techniques used for analysis of proteomes by applying gels.

There are different methods as well which have been developed to study the structure and functions of proteins and electrophoresis is very important in that direction, which is based on the principle of migration of charged proteins in a given electric field. The very gold standard of these SDS-PAGE or different type of electrophoretic method is because they have the ability to provide the information on protein structure and properties. This is one of the very power techniques to separate the proteins and visualize the separate protein by applying different types of staining methods.

Let me give the historical perspective of electrophoresis. This process was invented by Professor Tiselius in 1930. He developed moving boundary method to study electrophoresis of proteins. Since then lot of development happened in the field and during the 1950s and 60s this process was highly adopted in different laboratories. So here is Dr. Tiselius who is also known as father of electrophoresis. For his great contribution in chemistry he obtained the Nobel Prize in 1948.

Now I will describe one-dimensional electrophoresis, some of the concepts involved in that. The one-dimensional electrophoresis the separation is based on the charge-to-mass ratio and the molecular weight of the protein. Therefore on a given gel if you have smaller the proteins in the electric field they will move further down and the higher molecular weight proteins they will remain on the top. So, if you are applying some standard molecular weight markers which gives the known molecular weight of the protein then for a given unknown protein you can determine the molecular weight and it is expected higher molecular weight proteins will remain on the top and the smaller molecular weight proteins will reach towards the bottom of the gel.

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So, commonly employed one dimensional techniques include sodium dodecyl sulphate-PAGE (SDS PAGE) or other technique which is used for studying the protein in the native confirmation native form, which is known as Native polyacrylamide gel electrophoresis or PAGE.

The electrophoretic techniques can be used for protein characterization to study different properties. For example, the subunit composition of the proteins, what is the molecular weight of those subunits, what is the molecular weight of native proteins, different types of post-translational modifications? Again, only one of the techniques may not be able to answer all of these questions. So, often you have to involve more than one type of electrophoretic methods. For example, while comparing native and subunit composition forms you need both native electrophoresis and SDS-PAGE. If you want to look for post-translational modification then SDS-PAGE will be useful. If you want to compare the proteins based on the isoelectric point as well as the separation of the isoforms or PTM forms based on the molecular weight again two-dimensional electrophoresis will be useful. So, one needs to know the concept and laboratory way of using this technique so that by applying the combination of the technique one can study different types of properties.

However, the one-dimensional electrophoresis has different limitations. You cannot separate your complex proteome, complex protein samples. If you have thousands of proteins and have to study them, then one dimensional electrophoresis has limitations.

So, the one-dimensional electrophoresis is useful to separate few proteins or a simple protein mixture. But when you are studying a complex mixture then two-dimensional electrophoresis or DIGE or different mass spectrometry based methods will be more useful. So if you have complex samples like cell lysates or you have serum then separating those and comparing different types of samples on SDS-PAGE may not be a very good way. In that case you should apply different methods. So, one-dimensional electrophoresis is very useful and is being used in almost all the laboratories working in the life science area but it can give you few questions and it can address only those

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questions. But if you want to know that novel characterization then we have to apply different techniques. So to overcome these types of limitations and to obtain better resolution of protein separation the two-dimensional electrophoresis was applied.

So, now let's talk about two-dimensional electrophoresis or 2DE. This technique was earlier used in 1970s. So, it has a very long history of being used in laboratories from several years and interestingly two scientists Klose and O'Farrell, they applied two-dimensional electrophoresis to study different biological problems and simultaneously, they reported independently in 1975 that two-dimensional electrophoresis method can be used to separate the proteins based both molecular weight and isoelectric point. Now, since then two-dimensional electrophoresis field has seen many ups and downs. For example, several laboratories has started using two-dimensional electrophoresis. It was very easy method to be used and since one can visualize all protein spots on the gel it became very attractive way of separating the proteins. So, it was widely adopted. People started using it very often but when people started comparing the complex samples complex proteome and for example you have clinical sample where you have 20 patient samples and 20 healthy controls. In such a scenario you have to run large number of gels then two-dimensional electrophoresis shows lot of variations and reproducibility issues. Therefore, this technique started getting lot of criticism that there are some reproducibility issues; there are some manual artifacts. Then there was some technical issue inherent to this concept in the beginning like tube gel. So, all of these were a part of the things from 1970s to 1990s. And during that time people started advancing this field by applying new methods such as advancement if immobilized pH Gradient (IPG) strips. Furthermore, in the race of separating proteins in the complex proteome, people started applying different methods including mass spectrometry based methods. Then questions came that probably due to inherent issues of 2D electrophoresis. This technique may not be a powerful technique to study the proteome. But, then advanced form of 2D electrophoresis came such as difference in gel electrophoresis or DIGE.

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In this discussion I am trying to convey you the fact that from 1975 till date 2D electrophoresis has seen lot of criticism and appreciation of the technology. But still till date it remains one of the core technologies to separate the very complex protein mixtures and thousands of publications are providing a very good evidence for this technology being widely used.

So, in the two dimensional electrophoresis the first separation occurs based on the isoelectric point of the proteins and the second dimension occurs based on the molecular weight. In the first dimension separation you can separate the proteins in the isoelectric focusing unit. In the second dimension separation you can separate the proteins in SDS-PAGE.

I am giving you an overview of this process by showing this figure here, where; in the first dimension the isoelectric focusing process is occurring by applying immobilized pH gradient strip and now in the second dimension the SDS-polyacrylamide gel, you can place the first dimension separated proteins and with the decreasing molecular weight the proteins are separated. So, on the given gel, you can separate the proteins based on both molecular weight and isoelectric point.

I am showing you an image of two-dimensional electrophoresis gels or 2D gel where each spot is representing a protein which is separated in this case from a bacterial sample and each spot already provides you two information about its molecular weight and its isoelectric point.

Two-dimensional electrophoresis remains one of the core technologies to study the proteome and it is applied in various biological applications. It is relatively very easy technique to handle, it is not so costly, and it is still very much affordable. So, different laboratories use it widely. It is very high sensitive method to visualize the proteins.

As I mentioned earlier, two scientists Klose and O'Farrell independently investigated different problems by applying two-dimensional electrophoresis and in 1975 they reported about this method independently. Klose, he was investigating the

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heterogeneity of mouse lactate dehydrogenase isozymes. Independently O'Farrell was studying the complex proteins, which are present in the crude extract of the *E. coli*.

Here are these two scientists who developed the two-dimensional electrophoresis method; Prof. Patrick O'Farrell and Prof. Joachim Klose. They did a great contribution in the field of proteomics and a technology, which still remains one of the core techniques in the proteomics field.

2DE when it was started in 1970s it was not very easy process because protein separation in first dimensions involved casting the polyacrylamide gels containing ampholytes in the glass tubes. Lot of care and attention has to be taken at that time to prepare the tube gels. One of the major concerns was the reproducibility of the gels to gel. For example, if you want to compare a control and a treatment and you want analyze those two images lot of variations were observed by using the tube gel method.

So, the question is how two-dimensional electrophoresis became one of the very powerful methods? Various advancements took place in the course of two-dimensional electrophoresis. In that optimization and advancement of this technology, different milestone research happened which accelerated the pace of two-dimensional electrophoresis research. For example, the development of immobilized pH gradient strip or IPG strip. That was one of the major advancement in this field. Solubilization of hydrophobic proteins, which were difficult to separate on the gel. Then, reproducibility also advanced because of the advancement in the gel electrophoretic apparatus and gel-casting units. Again, lot of companies and manufacturers came into the play and they helped to improve the gel casting and electrophoretic units. Different type of staining methods were developed. We will talk about different types of stains and how they work during the course of two-dimensional electrophoresis workflow. But, very sensitive stains spearhead this process to visualize the proteins on the gel. Finally, how to analyze the images obtained from these gels. That was one of the major advancements; different types of software image analysis tools came into the play to analyze the gels.

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So, let's talk about immobilized pH gradient strip or IPG strip, which was one of major milestone development in the 2DE field. So, the development of IPG strip as compared to the tube gel removed lot of inconsistencies, which were involved earlier during the process of isoelectric focusing. The IPG strips, these are computerized, computer-controlled gradient formation and the pH gradient is covalently incorporated into acrylamide matrix and it is immobilized there. Reproducibility of one IPG strip to another IPG strip is very high. Now, they are supplied commercially from different manufacturers and made the 2DE process more efficient and it can be compared inter and intra-laboratory worldwide because these are computer-controlled gradients forms.

Let's talk about some of the advantages of IPG strips over tube gels. The IPG strips are more stable and durable as compared to the tube gels. They provide higher resolution and much better reproducibility for inter or intra-laboratory comparisons. The high loading capacity for micro-preparative two-dimensional electrophoresis is another advantage. Then, the separation of basic proteins under the equilibrium conditions. All of these are different advantages of using IPG strips over tube gels.

Now, I am introducing you to scientist Prof. Angelika Görg, who made a significant contribution for the development of IPG strip. It was her pioneer work in 1990s which eliminated lot of criticism which people were expressing due to the reproducibility of gel to gel. Along with the IPG strip several other advancements occurred in the field, which helped to make this technology more reproducible.

The concentrated protein samples can be applied on the IPG strips without causing gradient degradation, which is another advantage of using this over tube gel. You can take IPG strip and apply your protein sample so that it is absorbed on this chip and then protein can be separated in the electric field based on isoelectric point. The IPG strip eliminates several problems, which could be associated with top loading of a carrier ampholyte IEF tube gel, which is more sensitive to overloading than IPG strip.

People perform different types of experiments and depending on the type of problem they want to study, one can apply different type of IPG strip. For example, if you have

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no clue about what protein range you want to separate based on the pH gradient then one should start with a broad range of IPG strip, for example 3 to 10. But if you know that you are mainly interested in the physiological pH, may be 4 to 7 could be a better choice where you may lose few protein spots but your resolution and separation will be much better. Now, if you know your proteins are more rich in the acidic region or basic region, depending on those one can separate protein on the narrower range of the IPG strip. For example, one can use 3 to 6, 4 to 7, 5 to 8 and different types of gel patterns can be observed depending on your IPG strip and the preliminary result obtained then one can apply that to separate the proteins based on IEF, the first dimensional electrophoresis and SDS-PAGE, second dimension based on the molecular weight.

I'll now show you an animation for two-dimensional electrophoresis, which will give you an overview of different steps involved in 2DE experiment. In this animation I will describe you the two-dimensional electrophoresis process. Prior to isoelectric focusing in two-dimensional electrophoresis, the commercially available IPG strip must be rehydrated. This process can be done either by the passive rehydration or active rehydration. In passive rehydration, the IPG strip is placed with its gel side downwards in a well containing the protein sample reconstituted with a suitable buffer. As you can see in the animation you can apply the protein sample and then add the IPG strip so that it can absorb the protein solution. This process can be done for 10 to 20 hours depending upon your length of the IPG strip. This is then covered with mineral oil to prevent the gel from drying up and left over-night. Other process, known as active rehydration, where the protein sample is added to the strip via a sample cup followed by the cover fluid to prevent the gel from drying up. The protein sample being applied, a very low voltage condition is provided and then cover fluid can be added. This is then placed in the isoelectric focusing instrument and low voltage is applied. Process can be performed from 10 to 20 hours. These loaded strips are then focused on an isoelectric focusing unit by passing the current.

The various proteins of the sample mixture migrate in the electric field and come to rest when the pH is equal to their isoelectric point or pI. So, they become neutral and are no

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longer being affected by the electric field. The progress of electrophoresis is monitored by adding a tracking dye, which you can see in the animation moving ahead of the proteins.

The IPG strip is then equilibrated in a reducing agent such as DTT followed by an alkylating agent iodoacetamide, which prevents reformation of reduced bonds. This strip containing the separated proteins is then placed on SDS-PAGE gel slab for further protein separation in the second dimension-based molecular weight.

The proteins on the IPG strip are subjected to SDS-PAGE by applying direct current between 100 to 350 volts depending upon the size of the gel. Any protein that may have been present as a single band on the IPG strip; as you can see in the red circle; due to similar isoelectric point can then be separated based on the molecular weight, where the smaller proteins will migrate the farthest.

This is the view of a sample gel, which has been run by two-dimensional electrophoresis and stained with coomassie blue. Each spot provide the information about the molecular weight and the isoelectric point of the protein. I hope this animation was clear and you were able to understand the concepts involved in performing a 2DE experiment. So now let me give you few applications one can use by applying this technique.

People apply 2-DE for various objectives. For example, studying global protein expression. What is global protein expression? It means you want to identify or separate all the proteins present in a given protein sample. If you do not have information about all of the proteins, which could be present in your sample mixture. Then, first of all we need to create that fingerprint or blue-print of all the proteins and use that by the process known as global protein expression. Second thing, which is most commonly being used, is we want to compare the protein abundance from a given sample to a treatment sample. So, that is known as differential protein expression or also known as abundance based proteomics. You want to look at proteins which are either going up-regulated or down-regulated in their expression because of a given treatment or

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because of disease condition or because of your experimental condition. So, in both of these cases the two-dimensional electrophoresis to resolve the proteins from very complex mixtures. People also use to study different type of isoforms or the post-translational modifications, which occur in that process. One of the major advantages of this technique is the visual analysis of the proteins. When you stain a gel after two-dimensional electrophoresis process then you can see all the protein spots on the gel, which is not possible from the gel-free approaches such as mass spectrometry. Over there most of your analysis depends on your spectra and you have to rely on your analysis. In this case here, your all the proteins spots are already present and you can visualize that and then you can use each of these spots to further analyse and compare the images.

So, the protein profiling, if you want to compare your different sample types from condition A to condition B, you need to solubilize the proteins from the cell and separate all the proteins by applying the pH, which is shown here in the first dimension. After that you need to reduce and alkylate your samples and then separate that with the SDS-PAGE based on the second dimension and molecular weight and depending on your staining method being used whether coomassie brilliant blue or silver strain, different types of fluorescent strains or autoradiography you can visualize the protein spots. Now, one can compare these images from condition A to condition B and if there are some proteins, which are different in their expression those can be the proteins of your interest. One needs to analyse that from different types of gels, different replicates, both technical and biological and then obtain the statistical information for these spots. And if these are significant then this protein of your interest which you like to identify by using mass spectrometry.

So I am giving you a view of abundance based proteomics or differential expression profiling where you can separate the proteins and you can compare the abundance of each spot as shown in the 3D views and then the spot of interest could be trypsinized and it can be identified. The peptide spectra can be generated from mass spec.

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Now, I am giving you one application example. For example, if you want to study the prostate cancer and you have obtained the serum sample from these patients suffering from prostate cancer and you want to compare that with individuals who are healthy. So both the samples, I will talk about some of the details of the separation in following 2-DE workflow and you also have studied different type of protein extractions and depletion of serum in the previous lecture. Now, you can apply those together here that after obtaining the serum sample, as you have learnt from the last lecture, you can deplete the serum to remove the abundant proteins and then you can separate the proteins on the gel after obtaining different gel images you can compare and if there are some proteins which are showing difference in the abundance based on up or down regulation and statistical significance then these are the spots for further characterization.

Now let us go to the detailed part of the each of the processes involved in studying two-dimensional electrophoresis: the experimental workflow.

So, the first point in that workflow is isoelectric focusing, which is the first dimension separation of the proteins based on isoelectric point. Second step is equilibration of IPG strips. Whenever you are running an SDS-PAGE gel you make your protein denatured which you want to separate based on the molecular weight and heat the samples. You apply different types of denaturants. Now in this case when you are doing 2-DE your first dimension separation has occurred on the IEF in IPG strips. Now we want to take this strip and want to apply that on an SDS-PAGE gel, but you cannot do the heat treatment. So, we want to make these strips prepared to be separated based on the molecular weight in the second dimension and that's where the equilibration process is useful. Once you have done the equilibration of this strip then protein can be further separated based on SDS-PAGE. Now, fourth point in the workflow is staining the gel and visualizing the gel images and staining the gels with different kinds of gels. Image analysis, the fifth point, is very crucial because you need to analyze all the protein spots so that the abundance of each of these proteins can be calculated and more detailed statistical analysis can be performed. Once you are confident that these proteins are highly significant then you pick those spots by the process mentioned in the 6th point

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which is spot picking. After that, if you want to identify and know what this protein is then you need to do the in-gel digestion or enzymatic digestion of these proteins obtained from the gels. Then the last point will be the mass spectrometry analysis, which will be different series of lectures in the other module based on mass spectrometry.

Let's talk about the first point or IEF. In the IEF process, proteins are separated according to the isoelectric point. You can take the IPG strip and apply the protein solution on the IPG strip, so that the protein is absorbed on the protein strip and then after applying the electric field the proteins will migrate according to their charge. When a protein approaches its isoelectric point, where pH becomes equal to isoelectric point (pI), the proteins do not move in the electric field because of the lack of the charge.

Let me show you this process in this animation here. Here you have an IPG strip with pH 3 to 7 gradient. When it is moving in the electric field from the positive charge to the negative charge when pH becomes equal to its pI where there is no net charge proteins will remain over there. So now this will be the isoelectric point. In this view you can see that from low pH to high pH proteins are moving in an electric field and once they reach to a stage where pH equals pI then these discreet bands can be seen.

The rehydration is one of the important steps before applying the electric field in the IEF process and as we have seen in the previous animation that to rehydrate the IPG strip you can apply the protein solution on the IPG strip and different methods can be used for this. Whether passive rehydration where no voltage is applied or active rehydration where you apply the protein sample and very low voltage is applied to resolve the proteins. Now, we have already discussed that none of these methods can be compared and said as a superior method. One has to really use that in their own biological sample and see where the protein separation can be better based on passive rehydration or active rehydration. Once you have the rehydration process then one can apply the mineral oil to avoid any protein evaporation from these IPG strips.

Now, different type of strips can be used during the IEF process, whether it's pH gradient of 4-7, 3-10 or any other gradient. As I described in my previous slide,

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depending on your biological question, if you are really interested in separating the proteins in their biological pH range, then 4-7 could be a good choice where you can have better separation of the proteins but you may lose some proteins which could be in the extreme acidic and basic regions. Now, if you want know all the proteins which could be present in your sample, you can apply a very broad range strip like 3-10. Now, what type of length of IPG strip should be used? One can use the strips ranging from 7 cm till 24 cm. There different types of strips being manufactured from different commercial companies. When you optimize the protein extraction protocol then it is better if you use a small strip so as to know that the IEF process is going fine and your protocol is looking good and you can separate proteins. Once you know that your extract is good then you can apply that protocol for separating proteins using longer strips, for e.g. 17 cm or 24 cm. Now, these IEF units obtained from various manufacturers are capable of accommodating IPG strips of varying lengths. Few instruments accommodate a flexing length of 7-24 cm while the other instruments can accommodate different types of trays designed for each type of strip length. Depending on the amount of the protein in the sample mixture, it is always better to resolve that one a larger strip, for e.g. 18 or 24 cm. But if you do not have enough protein to separate then you have restrict yourself to smaller strips or one can still go with the large strip but apply more sensitive stain. So when I will talk about different types of staining methods, at that time we discuss even if you have low protein amount to be separated on the large gels but then apply more sensitive stains like silver or SYPRO Ruby. But overall the large gels are recommended for doing any type of differential proteomic analysis. When you want to compare your controls and treatment sample spots. But handling large gel is very tedious. So, I will show you the lab demonstration in a video, so that you can be well prepared to perform these experiments in your lab.

These are some recommendations for how much protein one should load on the IPG strip. For example, if you have a small strip like 7 cm, you can apply 100-300 μg of protein and maximum volume could be 150 to 160 μL . Usually coomassie is a good choice to stain this much protein. If you have very less amount of protein in your

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sample, for e.g. 10-100 μg , at that time you have to apply the sensitive stain such as silver or SYPRO Ruby. One can also apply the large strip like 17 cm or 24 cm strip but then you have to increase the amount of protein to be added and separation on the IPG strip. For example, 250-100 μg of protein can be applied on the large strip and one can visualize that by using coomassie stain. The total reaction volume should not be more than 350 μL . Even when you do not have much protein in your sample you still apply the sample to the large strip but then you have stain it with silver or SYPRO Ruby. In that case you can load between 100 to 1000 μg of protein.

So, I will give you the laboratory demonstration of isoelectric focusing so that you can be very clear about the intricate steps involved in performing IEF. Isoelectric focusing involves two major steps; rehydration of the IPG strips and IEF of rehydrated strips.

Rehydration of IPG strips

Step 1: Clean all the apparatus thoroughly to avoid any contamination.

Step 2: Add the reconstituted protein sample in a well of the rehydration tray.

Step 3: Then remove the IPG strip from its cover and place it carefully in the well.

IPG strips are used to separate proteins based on their isoelectric points. These are acrylamide coated plastic strips containing immobilins of various pH spread across it. They have successfully replaced conventionally used tube gels due to the reproducibility they can achieve.

Step 4: After around 30 min pour some mineral oil over the strip to prevent it from drying up.

Step 5: Cover the tray and leave it over night to allow rehydration to occur.

Isoelectric focusing

Step 1: Initialize the instrument and clean the instrument with a dust-free cloth.

Step 2: Place the focusing tray on the instrument and ensure that it is properly balanced.

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Step 3: Cut the paper wicks required for focusing to a suitable length and wet them with a small amount of water before use.

Step 4: Carefully remove the IPG strip from the rehydration tray and drain any excessive oil by blotting it with a tissue paper.

Step 5: Place the strip in the focusing tray and immerse it in mineral oil.

Step 6: Then place the 2 wicks at either ends of the IPG strip followed by an electrode at each end.

Step 7: Fill the adjacent wells with mineral oil to ensure uniform current flow.

Step 8: Then input the desired protocol on the instrument software along with details of strip length, pH range and number of strips and start focusing process. The voltage time curve will appear based on the protocol that has been set.

Protein separation occurs on the net charge of the protein. Protein will migrate along the IPG strip and come to rest at a point when the net charge becomes zero known as their isoelectric point.

So, in summary in today's lecture we have discussed about gel-based proteomics, different types of techniques being used in gel-based proteomics process. We have talked about different types of electrophoresis methods being used, including SDS-PAGE and two-dimensional electrophoresis. Then we started discussing about the details of 2-DE process and I have given you the workflow. In that workflow we talked about isoelectric focusing in more detail. You have seen animation for 2-DE electrophoretic process as well as the laboratory demonstration of IEF process. I hope, by now you are clear about the different types of techniques being used in gel-based proteomics. And what are the different workflow steps involved in performing this process. We will continue our next lecture on 2-DE workflow and study about the next steps, including the equilibration of the strips, SDS-PAGE, staining gels, obtaining images, image analysis, spot picking, etc. Thank you.