LECTURE-14

Gel-based electrophoresis

Two-dimensional electrophoresis workflow: Staining and image analysis

TRANSCRIPT

So welcome to the proteomics course. In the last lecture we discussed about the gel based proteomics. How different techniques are used to separate proteins from complex protein mixtues and I mentioned that SDS-PAGE BN-PAGE, 2-DE and various advance forms of gel-based proteomics. They all together help to study different types of problems by applying gel based approaches in proteomics. In today's lecture we will talk about the workflow of how to perform 2-DE. I give you a stepwise method in which the first step was to do the isoelectrofocusing second step equilibrating the IPG strip to make it compatible for the second dimension separation in SDS-PAGE. Third step was SDS-PAGE to separate proteins in molecular weight. Fourth is to visualize to protein spots on the gel, staining methods. In the last lecture we discussed about two staining methods. One was coomassie brlliant blue and other was silver staining.

So now, in the same workflow today I will continue on different types of staining methods and describe you some more sensitive stains and the stains used for different applications such as SYPRO Ruby, Pro-Q Diamond, cyanine dyes.

Each one of these are used for various applications. For example, the cyanine dyes are used in one of the advance form of gel electrophoresis which is known as difference in gel electrophoresis. In this method you mix the cy-dyes together and spearate the proteins on one gel itself. SYPRO ruby is one of the sensitive stains and this stain is also used for looking at different types of protein patterns such as it can used for dual staining. Now, if you want look at post-translational modifications few stains such as

Pro-Q diamond can be used but if you want to look at the overall protein pattern then you can do dual staining with SYPRO Ruby or some other stain.

So let's continue our today's lecture on different types of staining methods being used. So lets start with SYPRO Ruby staining. This is one of the flourescent stains which is usually visualized on the gel in the UV or Blue light transluminescence. This stain is very sensitive and it is endpoint stain. So very little background is used unlike the silver stain where there is lot of issues with the very dark background. The SYPRO Ruby has overcome those issues. This is also one of the very sensitive stains and it can also be used for studying different types of post-translation modifications such as glycoproteins, often low low molecular weight proteins which are low abundance proteins; those can also be visualized by using this type of stain. So SYPRO Ruby is one of the very useful stain, which is very compatible for doing further processing with the mass spectrometry. So, if you want to separate the proteins you have to visualize them. After that you end aim is to characteraize those proteins. What these proteins are and further you want to do experiments on those. In that light, SYPRO Ruby is very compatible with mass spectrometry and you can further characterize your proteins.

Now I will show you the steps involved in performing SYPRO Ruby staining. SO, I will show you one animation.

SYPRO Ruby red: The completed gel is first placed in a fixing solution of methanol and acetic acid that fixes the protein bands in the gel and minimizes any diffusion. This must be subjected to gentle shaking for around 30 min.

Fixing the protein bands in the gel

After the fixation step the gel is soaked in the flourescent SYPRO Ruby Red stain solution. Gel should be kept on the mechanical rocker for uniform shaking. During the process the gel gets uniform staining with deutherium based chelate dye.

Staining the gel

After this overnight step of staining the gel can be washed with methanol and acetic acid solution. Again, you need to keep it on rocker so that uniform destaining can be performed. The MS compatible gel is then viewed using UV or laser scanning instrument with an excitation wave length near 280 or 450 nm. This stain has two excitation maxima and an emission maxima of 610 nm. In this animation you can see a gel pattern which is showing you the staining obtained after SYPRO Ruby Red and it is showing separation in two dimensions based on the molecular weight and isoelectric point.

So now, after looking at this animation let's continue on second staining method which is use of cyanine dyes. As I mentioned these are very sensitive dyes and these are water soluble, derivative of N-hydroxysuccinimide which is covalently bound to the alpha-amino groups of a protein's lysine residue. Now, since this labeling is minimal labeling your overall mass is not going to change by adding different types of cy-dyes such as cy2, cy3 and cy5. Protein samples can be labeled with these dyes and then mixed together to separate the proteins on one gel. Now, unlike your 2-DE where you have 2 gel separately your comparing a control and a treatment gel and those two are separately in this case whole sample is run on one single gel. Now, when you are separating everything on one gel and just you are changing the flourescence scanning parameter. Then there is no variation overall and at the end you can obtain from the same gel 3 different images based on the emission flourescence spectra. So this method is employed in DIGE which is one of the very advanced forms of 2-DE and it eliminates many gel-to-gel variation problems.

I'll describe this technique in much more detail in the latter part of the course. But briefly I will show you this animation for cyanine dye staining.

Cyanine Dyes:
In this detection technique the dyes are mixed with the samples prior to electrophoresis. The first sample was labeled with cy3 dye and second sample is now mixed with cy5 dye. Now, an internal pool made from both samples 1 & 2 is labeled with 3rd dye which is cy2 dye. Each protein sample as well as the standard internal pool is labeled with a

differently fluorescent cyanine dye, which allows all the protein samples to be simultaneously run on a single 2-DE gel. After dye labeling and mixing all the samples together the isoelectric focusing can be performed. Followed by the second dimension separation in SDS-PAGE.

The gel can be further scanned and viewed by illuminating it alternately with excitation wave length corresponding to the various cy-dyes. This technique minimizes the gel-to-gel variations and allows a large number of samples to be run by using lesser number of gels. Therefore, it provides very useful solution to various proteomics applications.

Now, after looking at this animation you are familiar that different types of staining methods can be used. Let me introduce you to another stain, Pro-Q diamond which is used for studying phosphorylation of the post-translationally modified proteins. Now, Pro-Q diamond is a flourescent dye which is capable of detecting phosphorylation. So, if your proteins having some phosphorylation addition of certain phosphate residues. Now this stain is quite sensitive to pick up those patterns of phosphorylation of post-translational modifications in your gel. This is very suitable for use in electrpphoresis and it offers sensitivity in nanogram level. Now, let's say you want to look at the post-translationally modified forms of the protein. But after that you also would like to know which proteins belongs to you would like to do mass spectrometry on these. So, often it becomes very difficult if you have just done Pro-Q diamond one gel. So, you would like to do the dual staining. So, first you like to detect only your post-transnationally modified form of the protein or the phosphorylated forms. Then followed by the same gel can be used for further staining with some endpoint stain such as SYPRO Ruby. Now, you take the same gel and further stain with the SYPRO Ruby. So you will obtain two images for the same gel where few spots can show the PTM form and the overall the global pattern of proteins can be visualized on different gels. So, in this regard by comparing the PTM form or the phosphorylated form along with the complete profile of the gel one can obtain the very comphrehensive picture, these many proteins are separated on the gel, among those certain numbers are phosphorylated. One can then further excise those spots and do the mass spectrometry for its further identification and characterization.

Now let me show you this animation for doing Pro-Q Diamond staining followed by dual staining.

Pro-Q Diamond:
This is very useful staining technique for detection of phospho-proteins and when used in combination with SYPRO Ruby red it also provides a very comparative profile of total protein contents and phospho-protein contents. The first step is to perform the fixation followed by addition of Pro-Q diamond stain. The staining procedure to the SYPRO Ruby red which was earlier described. This flourescent dye offers more sensitivity down to nanogram level and detects specifically the phosphorylation taking place at serine, threonine or tyrosine residues. For uniform staining you need to add the staining solution for atleast 8 hours or overnight. Now the staining solution can be removed and further washed with methanol and acetic acid. For uniform destaining it is a good idea to keep on gentle shaking for atleast 6 hours. Gel can be further scanned and image can be procured especially at emission maxima of 580 nm and it provides again on the 2 dimensions decreasing molecular weight and increasing pH information for all of these protein spots. Often people combine this phospho stain along with SYPRO Ruby stain to obtain dual staining information which was described in the lecture.

So, in the animation you have seen that how Pro-Q diamond stain can be used to detect PTM form or phosphorylated forms of the proteins and then other staining procedures such as SYRO Ruby can be used for dual staining. Now, dual staining allows more than one detection protocol on the same gel. So it has lot of merits and I hope after watching this animation you must be able to appreciate that. So after discussing about different types of staining procedures which are available now you have separated different protein spots on the gel, now you have the visualized that by using different types of stains. Again the selection of the stain depends upon the type of application you are looking for. Often you have limitations of your protein, if you did no have enough protein you probably used silver or SYPRO Ruby. If you had enough protein you went with coomassie brillaint blue staining. You were interested in looking for certain form of PTM you used Pro-Q diamond or other specific stains. Now if you want to do some more

advance form to eliminate lot of gel artefacts probably you used cyanine dyes. After all of these types of staining detection now you are ready to scan the image. Because now you can see all the protein spots available on the gel in different colours whether its blue in coomassie or brown in silver, pinkish in SYPRO Ruby or different types of flourescence patterns in cyanine dyes and Pro-Q diamond.

Now let me describe you some staining comparisons of available for various stains which we discussed before we move onto the next part on the image scanning. In a nutshell here you can obtain information for various stains, which are available, their properties and their sensitivities. Please note the sensitivity is only approximate. So coomassie blue is one of the most commonly used stain, which is employed in most of the laboratories worldwide. It is compatible for mass spectrometry. Sensitivity is in the range of 40-100 ng. Biosafe coomassie is also MS compatible. Another advance form of coomassie staining which is environmental friendly, non hazardous and offers sensitivity in the range of 10 ng. We have talked about silver stain when you have low protein in your extract probably silver stain is a method of choice or SYPRO Ruby. In that case silver stain is very sensitive. It offers sensitivity in the range of 1 ng. It is having some issues with MS compatibility but people have overcome those limitations by changing certain reagents and now there are advance forms of silver stain available such as silver stain plus which sensitive as well as compatible with the mass spectrometry. Next we will talk about SYPRO Ruby. This is again a very sensitive stain compatible for mass spectrometry and it gives very low background on the gel. So it offers linear order of 3 orders of magnitude. So you can see your signal in much clear white background. By looking at this table overall you can compare different type of stains available and which one you would like to use for your application.

Next I'll talk about laboratory demonstration of staining two-dimensional gels. So in this video I'll show you how you can use coomassie or silver stain to stain your 2-DE gel and then what steps you need to perform to obtain this pattern and finally what type of image you can acquire after the staining.

Video demonstration. Gel staining and de-staining:
This process involved removal of the gel from the electrophoresis assembly followed by the treatment with fixing solution, staining solution and finally the de-staining solution.

Gel removal and protein fixation:
Soak the gel present the between the glass plates in fresh distilled water to remove any bound SDS. Pour the fixing solution containing methanol and acetic acid into another tray. Open the glass plates and carefully remove the IPG strip from the gel. Then transfer the gel into the fixing solution by gently sliding off from the glass plate. Place the tray on a mechanical shaker for an hour to ensure that protein spots get fixed on the gel, thereby minimizing any diffusion.

Gel staining:
Transfer the gel carefully into another tray containing the coomassie blue staining solution and place it on the shaker to ensure uniform contact of the gel with the solution. The negatively coomassie dye interacts with proteins through ionic and other non-covalent interactions thereby staining them with a blue color. Add some more staining solution such that the gel is completely immersed and leave it overnight for around 8-10 hours.

De-staining:
Drain out the staining solution from the tray and pour the de-staining solution consisting of methanol and acetic acid into the tray. Place the tray on the shaker for around 6-8 hours until the background stain of the gel is completely removed and spots are clearly visible.

Another commonly employed technique for visualization of protein bands is the use of the silver staining solution. In this procedure the gels are first treated with the fixing solution followed by the silver stain and finally with the developing solution where the silver ions get reduced to metallic silver by an alkaline solution of formaldehyde and sodium carbonate. The dark protein bands can be viewed well against a light background. Here we are showing gels containing separated proteins from serum,

bacteria and plant samples that have stained with coomassie blue dye as well as plant protein sample that has been stained with silver dye.

So after looking at the laboratory demonstration now you are clear that what are the steps involved in doing the staining procedure. Now, in this image which is a 2-DE gel image obtained from human serum, you can see the molecular weight and isoelectric point of proteins separated in two dimensions. The first dimension is isoelectric point and the second dimension is the molecular weight. For each of the protein spot you can obtain both informations for isoelectric point and molecular weight. This is a gel stained with coomassie stain.

So now, let's see how we acquire these images and what type of scanning methods available. So image analysis is another one of the very important aspects of the 2-DE workflow.

There are different types of image scanners available from different vendors such as molecular imager densitometer and Typhoon variable mode imager. There are many staining image scanners available.

How to analyse these image? You want to do the things manually, so you can take your gel patterns and sit together and say this is my protein control and this is your protein in treatment. Now I am going to look at each spot manually and going to size the spot based on this comparison. That is going to be very tedious work and you will not have any information about the whether your spots having any statistical significance or not. How reprodicible those are? So you need to scan it by using good scanners and then finally you need to analyse your image from different software which are available.

There are many commercially available softwares for doing 2-DE gel analysis. I will give a comparative table at the end. But almost all of these software take the scanned images and analyse your gels by using various steps. So all softwares enable the spot identification, comparison of the gels, overlaying of the images from your control and

treatment, cropping the gel the part of which you want to compare and further doing the statistical analysis.

The crop tool that is the first part which you like to use. If in your gel you have some extra regions where you do not have any spots of interest probably you would like to crop those regions and crop both your control and treatment gels uniformly. This crop tool allows for a very specific defined region of the gel to be cut from the entire gel. It helps to select the region with the high spot density which can be used for doing further gel analysis. Next, you would like to see your spots in more detail, so you like to use zoom tool which can expand a specific area of the gel for doing further analysis.

Image overlaying is very important aspect because if you are comparing two gels; one control and other treatment you like to overlay those images together to compare the spot pattern present on two different gels. Because you have acquired two separate images. Now you need to overlay those so that it can appear as a single merged image. Now, spots which are going to coincide on top of each other; whereas you can also locate their original position from the each of the individual images. Image overlaying is important aspect where you can merge your control and your treatment gels. Then you would like to do the spot analysis, where it is possible that you can obtain physical and statistical parameters for each spot which is present on the gel. You can look at the three dimensional views of each of the spots, how they differ from the control to the treatments. And then you allow the comparison of the gels spot-by-spot basis. Often running a gel or acquiring images and generating lot of data is much straight forward as compared to doing the analysis which is more tedious step. One has to really sit and go through the gels usually spot-by-spot to analyse the gels.

Now, I am showing you a gel pattern of control and its comparison with the treatment gel. These are taken from one of the healthy control and a disease sample and each of the spots is compared from the control to the treatment and one can look at from different healthy controls and different disease sample, what is happening to each of the spots and if there is any statistical significance for their overall change. If it is going up

or going down on the gels, is that uniform in all the gels? And how significant that is? All of this analysis can be performed by using different software.

As you can see in this image I am showing you one spot which you like to compare across 16 gels. Now you have zoomed into that particular region and further you are looking at the 3D pattern of each of the spot, how they differ from the control shown in the black and the blue spots and the treatment which is shown on the black background in the red spots. So, after looking at 3D views of this particular protein you can confidently say that this protein expression is changing and it's going higher amount in the treatment. Now, one can look at each of these spot's intensity in much detail and then followed by plot different type of parameter for percentage volume or spot intensity to compare their values and do the statistical comparison.

There are various commercial softwares that are available for comparing the 2-DE gels such as image master 2D platinum from GE lifesciences, PDQuest from Biorad, Delta 2d from Decodon, Dymension from Syngene, Ludesi 2-D gel image analysis and Progenesis from Nonlinear. These are just a very few softwares that I have mentioned that are commonly used but they are many other available which one can use to analyse these gels.

Now, let me show this animation for performing 2-D gel analysis, how to go step by step to analyse your 2-D gels.

2-D gel analysis software:
In this animation I will describe you how to analyse the 2-D gel images by using a generic software layout. So, first you need to load a gel image. It is possible to load either a single or multiple gel images simultaneously. This can be done by means of the laod option in the file menu. You can save the gel images and then you can crop the area depending upon what area you want to analyse. There are several tools which are available for the analysis of the gel.

It is possible to crop the gels by selecting a specific region that is to be studied and then selecting the “crop gel” function. Cropping gel helps in the selection of region with high spot density or to reduce the regions which contain high background stains with no spot.

Zooming into a selected region; if you want to expand a specific region, you can use zoom tool. Specific selected region of the gel can be zoomed into viewing the spot more closely and for comparison of spots between the two gels. This is particularly useful for gels with large of spots. Once we have seen the area to like to overlay the images. Overlaying of images is a particularly useful tool for the comparison of two gels. The gels are overlayed such that they appear merged and the spots that coincide will overlap with each other. This is extremely helpful while comparing the large clinical samples of controls and treatments so that you can obtain the clear indication of the proteins which are differentially expressed. Now, after the analysis one can look at the graphical representations of these 3D view of the spots. The spots on the gels can be displayed as 3D graphs. Either the entire gel can be chosen or particular region can be selected for this representation. The peaks obtained in the graphical representation are directly related to the spot intensity.

Next we talk about the spot analysis. Every spot on the gel can be detected by selecting the “detect spot” option. Various parameters such as smoothness, saliency and minimum area must be suitably adjusted for maximum clarity. Once this done, each spot will either be encircled or marked with a cross depends upon the setting along with the spot numbers. Now, I’ll describe you the gel matching. This software facilitates interpretation of gel images by matching two different gel images which were obtained in your experiment. The matching spots are marked and after the matching is done any variation in the spot intensity, spot position can be indicated by the blue line as shown in the animation. This provides an understanding about the reproducibilty across the gels.

Once you have analysed the gel one can obtain the detailed information for these spots from spot table. Information regarding various physical parameters of each spot can be obtained with spot table which provides spot number, intensity, area and volume of the

spots as well as the saliency of the spots. These parameters help to judge the quality of a gel.

In addition to the physical parameters various statistical parameters can also be computed for each gel and each spot on the gel such as central tendency, mean, median, dispersion, coefficient of variation, standard deviation or other statistical parameters. The scatterplots and histograms can also be plotted for clear data analysis. These can provide information regarding inter and intra gel variations.

The Spot comparison: It is possible to specifically compare a particularly selected spot across the gels. When the gels are run with molecular weight markers with molecular weight of unknown protein can be estimated from this information. For example, as you see in the animation on the left hand side you first load the molecular weight marker and now from that information you can compute the information for the unknown protein to calculate its molecular weight and isoelectric point. These parameters in addition to the other physical and statistical parameters can be obtained for each spot. I'll describe you a different analysis which is DIGE. Although we have briefly touched upon the use of cyanine dyes and I'll describe you this technology in more detail in the next lecture. But briefly, I'll introduce you here that DIGE analysis can be performed with specialized software which can overlay the images obtained from the scanners for Cy3, Cy5 and Cy2 labeled samples. It can compare 3 gels simultaneously for which one is typically the pooled internal standard containing all spots labeled with Cy2 and your control and treatments are labeled with either Cy3 or Cy5. Any changes implemented one gel such as cropping, spot selection, etc. will be implemented across all the three gels in DIGE. Other features and tools for DIGE analysis are similar to those used for 2-DE analsis. The physical and statistical parameters of all the spots on the gels can be determined through their corresponding reports.

After looking at this animation now you are clear that you can do the comparison of your control and treatment gels and you can identify few statistically significant proteins which can be further used to do the spot picking and further mass spectrometry

analysis. So the sixth step in this workflow is the spot picking. How to excise your spot of interest from the gels. Now, most of the time people use the comparison of their control and treatment gels which is abundance based proteomics, where you are looking at only change in few proteins because of a given treatment or a given disease or a given condition. Now, if you want to compare this then the number of protein changes which are going to happen overall is not going to be very much. May be you will have 50-100 proteins maximum which will alter their overall pattern because of a given treatment. So it's very easy if you have less number of protein spots to excise the spot from the given gel even manually. If you print the spot map or the 2D gel image and keep it side by side along with the gel, it is easy and possible to locate those spots on the given gel and then you can excise those manually. You have to obviously ensure various quality control checks over there to avoid any contamination but that can be done. Now, other way of doing is to use the robot picker and excise the spot of interest. That's more precise but more costly. Many times when people want to know the global expression of all the proteins, for eg., for a given sample or for a given organism if the proteome is unknown and you were the first one who want to characterize, identify all the proteins from that sample probably you would like to know what all proteins present in that given sample. In that case you would like to identify all the proteins which present on your gel. So you need to do the mass spectrometry for each of those protein spots. That is not possible to do manually. In that case you have to rely on spot pickers.

I'll show you two methods here for the spot picking. One is the manual excision of the spot where user can take a scapel and make a cut on a particular gel for the given spot of interest and then transfer that into an eppendorf tube, label that and transfer that to a 96 well plate along with water. You have to ensure that you are using milliQ water or autoclaved water or very purified form of the water. Your using sterile scalpel. Your glass plate on which you keeping the gels are also very clean. You do not want to introduce any contamination from here or from gloves or any other dust particles available in the environment. Preferrably do these steps in the sterile conditions in the lab under laminar hood. By doing that you should be able to pick up the spots of your

interest and transfer in the 96 well plates. Further, which can be used for doing other steps such as in gel digestion and mass spectrometry.

Now, second part is robotic spot picking, where as I mentioned that is more accurate, more precise but its more costly. If you are doing global profiling you have to rely on the robotic spot picking. Often if you are looking at Cy-dye or some sort of flourescent dyes where you can even visualize the spots then you also you have to rely on the robotic spot picking. For each selected spot, the robotic arm moves and picks up the individual spot. Now, you can set-up the XY coordinates in the software and then based on the location on the map of the spots present on the gel it can go and excise the spots from the gel. Then it can be transferred to the 96 well plate for further processing.

Now I have described you the workflow of performing 2-DE and in this workflow today we have talked about staining methods as well as how to scan those images and analyse those images by using various softwares. After that you have completed the workflow of 2-DE but I'll still try to teach you other gel based methods which can be employed to study the proteome. So based on these gel based methods there are new methods which have emerged for different proteomic applications.

The conventional methods or traditional methods of 2-DE and SDS-PAGE we have talked in detail. 2D-BN gel electrophoresis also we have described and discussed in the last lecture. OFFGEL electrophoresis that is another new technology introduced from Agilent that we will talk briefly in this lecture. And 2-D flourescence difference in gel electrophoresis or DIGE; this will be covered in the following lecture.

Let us briefly talk about OFFGEL electrophoresis. As the name suggests in this method you are avoiding the separating proteins based on the gel. OFFGEL electrophoresis can be performed on the IPG strip but you do not need to use the gel approaches for separating the proteins and this method is mostly used for liquid chromatography based mass spectrometry applications, where people want to separate the proteins to reduce their complexity based on the isoelectric point but you do not want to separate the proteins based on the gel due to various artifacts which gel based methods can offer.

So, OFFGEL electrophoresis spearates proteins based on the isoelectric points of the proteins and the peptides. We have talked about how to do isoelectric focusing in the previous lectures. These separated components which are separated based on the pI values can be recovered in the liquid phase itself. While the IEF has finished your protein samples are already present in the liquid phase and you can remove aliquotes in different pH range so that you have the proteins separated based on the pI values in the liquid phase itself. Now, this method is very compatible for further down stream processing in LC/MS MS or doing immunoprecipitation.

So I briefly mention here the fractionation principle involved in OFFGEL electrophoresis method where IPG strip can be re-hydrated and tightly sealed against the frame of the well in OFFGEL instrument. I am giving you here example of 3 proteins. Let's say you want to separate protein A, protein B and protein C. Each of these proteins have different isoelectric points, pI 4, 8 and 9. Now in the IEF instrument when you add your IPG strip you apply your protein sample, you have tighten the wells and now protein samples will be equally distributed in the wells throughout on the IPG strip. There you need to apply a cover slip so that you can avoid any evoporation issues.

Now the proteins and peptides in the electric field will migrate because of the high voltage applied and proteins will start moving based on their isoelectric points. When they will reach to a place where pH is equal to the isoelectric point then there will be no protein migration. This is the protein separation which you wanted to happen. For example, A, B and C you had 3 proteins with different pI and you want to separate those in this method. Now you know in the pH range 4, 8 and 9, you have those fractions which you can collect after doing the isoelectric focusing. Proteins can be separated based on the pI and it can be removed in the liquid phase. Now, you have seen very easy example of 3 proteins, ideally people use this for very complex protein mixtures to reduce the complexity of overall proteome and to separate or fractionate the overall proteome based on the isoelectric point and collect each of the fractions which can be separately further analysed using LC/MS approaches.

Now, OFFGEL itself is sufficient and used widely for various LC/MS applications but same manufacturer has introduced another technology so that one can separate proteins in two dimensions both in isoelectric point and the molecular weight. So OFFGEL and on-chip electrophoresis methods can act as substitute for gel based protein separation in the isoelectric point and the moelcular weight. Now, the OFFGEL fractionation can be combined with high sensitive on-chip electrophoresis bio-analyzer which enables 2-DE type of analysis with very high resolution and high sensitivity. This method is very suitable for studying differential protein expression for different applications. Similar applications which one can do on 2-DE can also be performed by applying these two methods together; the OFFGEL and on-chip electrophoresis.

So in summary, in today's lecture we have talked about different types of staining techniques available which depending upon your application you can use. How to compare and analyse your 2-D image obtained from different samples; control and treatment, how to excise the spots and then we had briefly looked at some variations, advanced forms of gel based proteomic methods such as OFFGEL fractionation. We will continue this in the next lecture with more advanced form of electrophoresis such as two-dimensional difference in gel electrophoresis. I hope you are able to appreciate the various types of methods to analyse the protein samples, the complex proteome and how one can depending upon their challenging samples apply different types of techniques. So we will continue in the next lecture. I hope you are able to appreciate the gel-based proteomics and it's potential. So we will talk about 2-D DIGE in the next lecture. Thank you!