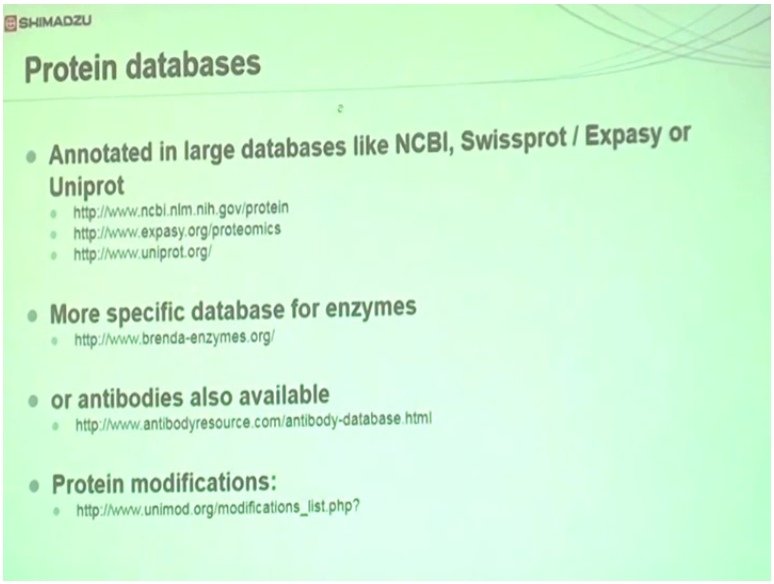
Introduction to Proteomics
Dr. Sanjeeva Srivastava
Department of Biosciences and Bioengineering
Indian Institute of Technology - Bombay

Lecture - 40
Lab session Targeted Proteomics: Multiple Reaction Monitoring

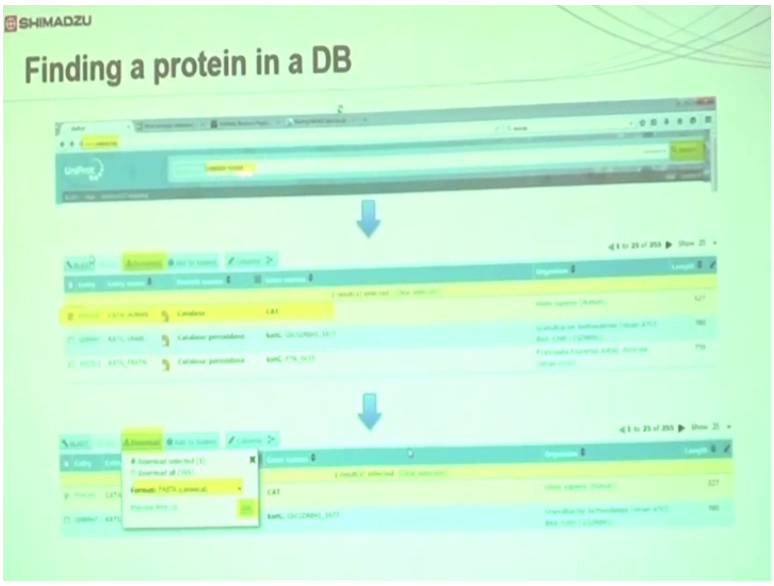
Welcome to the proteomics course.

(Refer Slide Time: 00:32)



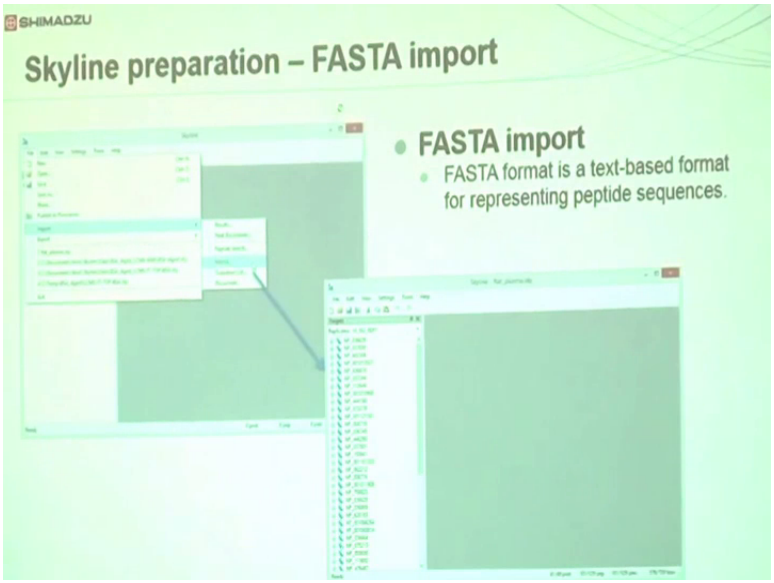
All you need to find out the fastest sequence for given protein, so that you can search many of the databases for this purpose. Most commonly used databases can be Uniprot database.

(Refer Slide Time: 00:44)



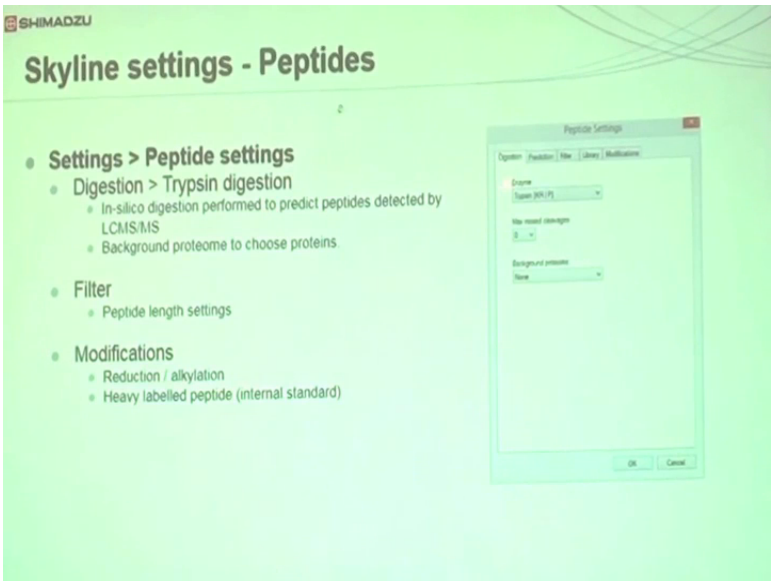
So here you just have to give a name of the protein, and then you can download the faster file, and then you can import that faster file in a Skyline software

(Refer Slide Time: 00:55)



So this is the screenshot of Skyline software, so once that faster file is imported, then you can see the list of peptides it has generated, you can actually give the filtration criteria for this generated list.

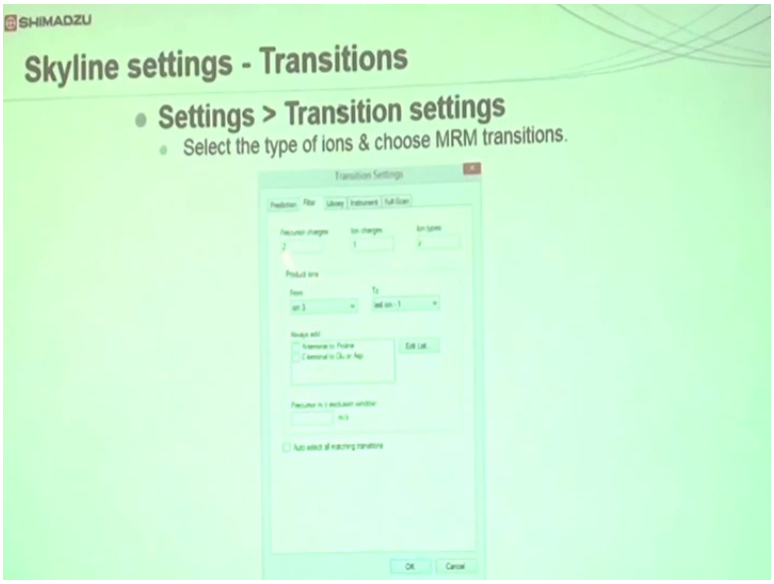
(Refer Slide Time: 01:18)



This is peptide selection criteria, here you can specify which enzyme you are using for digestion, you will be selecting with cleavage as zero to get better accurate results. Then you can select peptide length, what should be the ideal length of protein for triple quadrupole system, most commonly it will be 825 amino acids and if modification is tend to happen in your peptide. Then that modification you can specify like oxidation, phosphorylation.

If you are using heavy labelled peptide then you can specify that as well in peptide selection criteria.

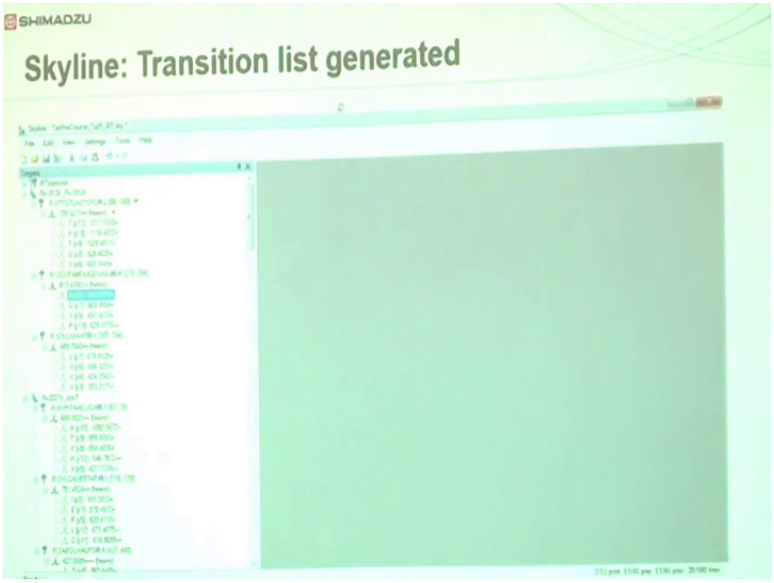
(Refer Slide Time: 01:55)



Once the peptide selection criteria are set, then go for transition selection criteria. So in that case you will select what should be the charge on precursor m z, what should be the charge on your product m z? You will select which type of ions you want to monitor because when peptides get fragmented there are A, B, C and X, Y, Z all these six types of ions will form, but out of these Y and B ions are most predominant in that way and B also, Y are most predominant.

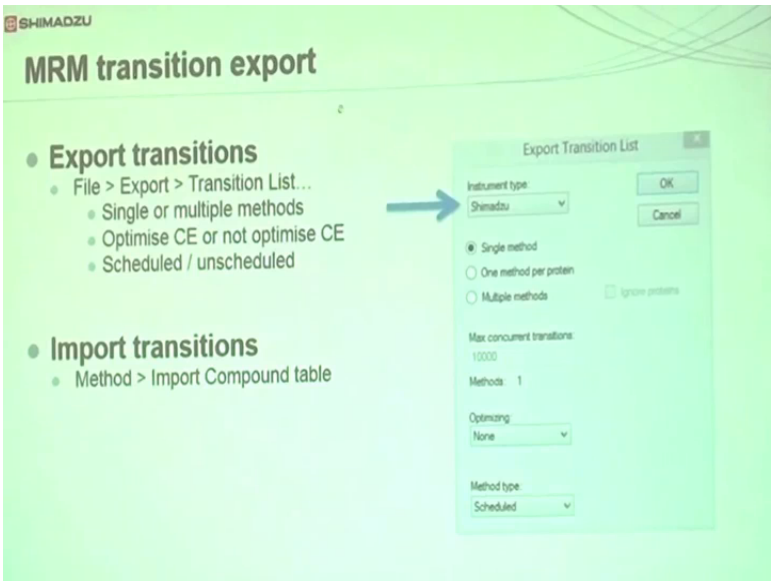
So in majority of cases you will prefer to go with only Y type of ions, then you will select what should be the length of product ion and that should be considered during the fragmentation. So you can specify here ion 3 to last, ion-1 or ion 2 last ion-2 depending upon your requirement.

(Refer Slide Time: 02:59)



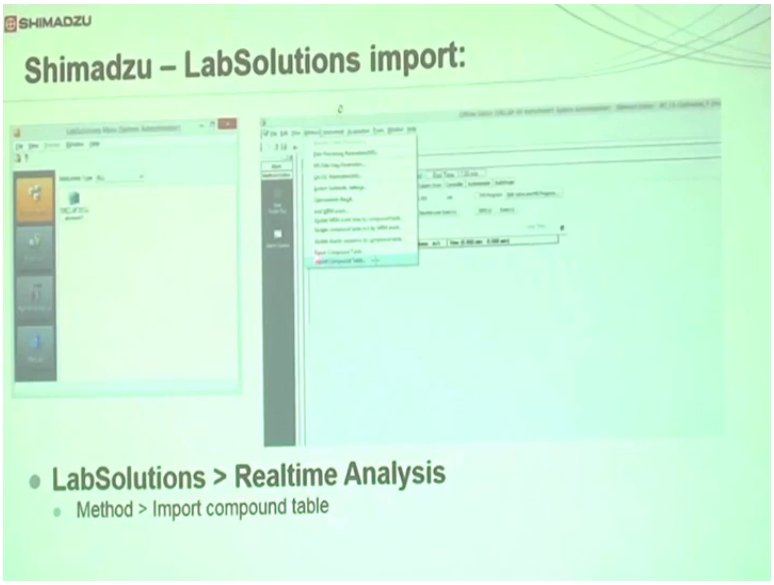
Once that is set you can see the list of transitions generated like this, so you can see here almost 160 transitions generated for selected protein. After this you will just export this transition list.

(Refer Slide Time: 03:13)



Here, you will just select the Shimadzu instrument, because if you are performing your analysis using CMAT suitable quadrupole instrument you have to specify the Shimadzu triple quadrupole instrument you have to specify the Shimadzu instrument in one method you will be exporting all these transitions.

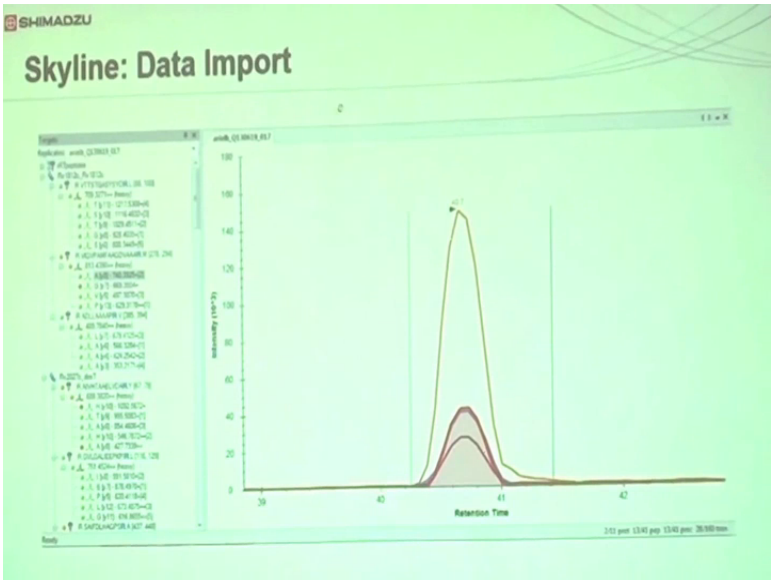
(Refer Slide Time: 03:35)



Once those transitions are exported you will open your lab solution software which is nothing but acquisition software of a Shimadzu triple quadrupole instrument, in that solution software you will import all these transitions, after that you will specify what should be the time program HPLC time program, whether you want to do staggering that staggering you can specify what column you want to use that you can specify that.

After all these parameters optimization, you can give a run and perform the analysis, after the analyses is over, you can import that result or data file in Skyline software again, and you will be seeing here different colored symbols.

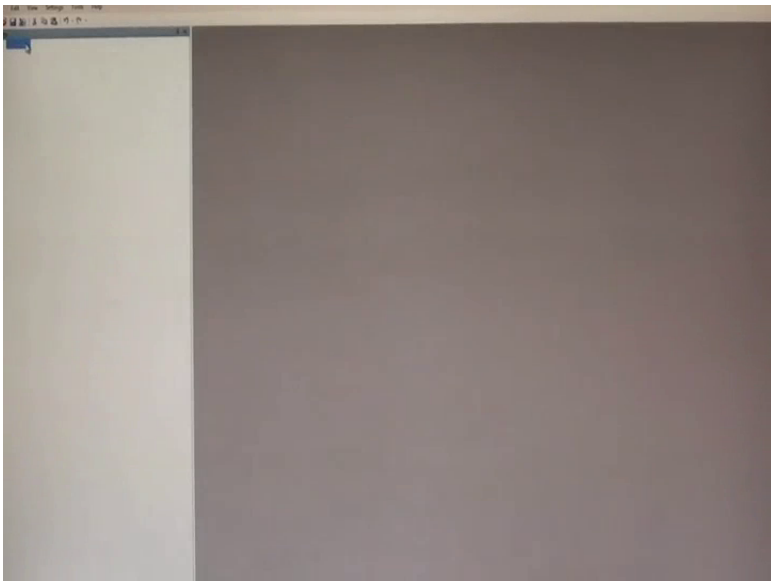
(Refer Slide Time: 04:11)



For a given peptide if all the transitions which are generated by Skyline show positive results, then that peptide will appear in green color. Suppose, if any of the transition is not generated any response but remaining others are generating response, then that peptide will appear yellow in color. If none of the peptides has generated the response then that peptide will give red signal, so depending upon your selection criteria you will select which peptides have to be kept for further analysis and which have to be removed.

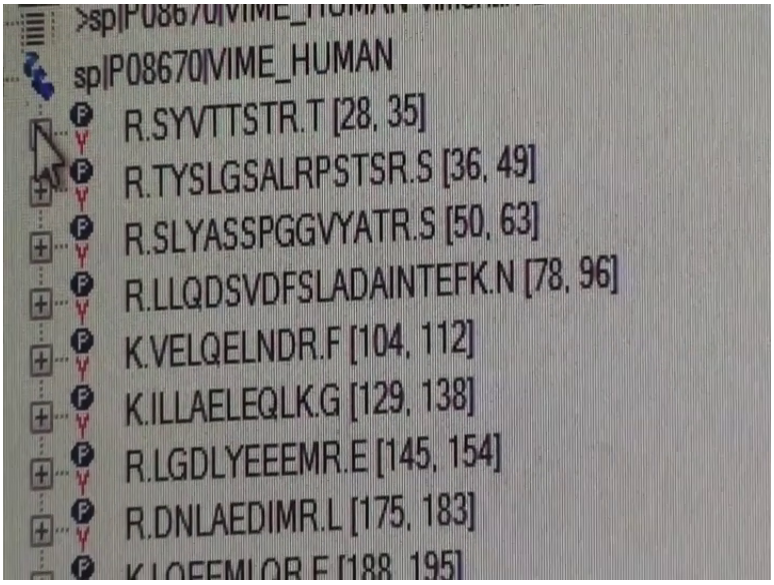
So most of the peptides which are giving you red color symbol you will refine them in the filtration, and you will keep only those peptide which are giving green color symbol, and then those transitions you can use for further analysis on that method you will be analyzing all of your samples. And then you can compare the peak areas for all the samples and perform the relative quantification analysis.

(Refer Slide Time: 05:18)



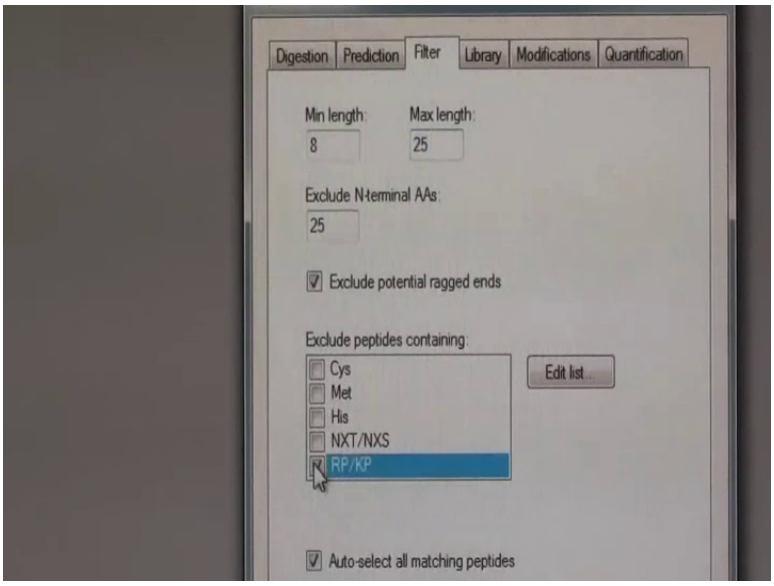
Now let us see how to do this thing practically, so this is blank Skyline document, now we want to find the faster sequence for protein called as Vimentin that we can get from Uniprot site. This faster sequence you will copy and you will paste it into Skyline file.

(Refer Slide Time: 05:55)



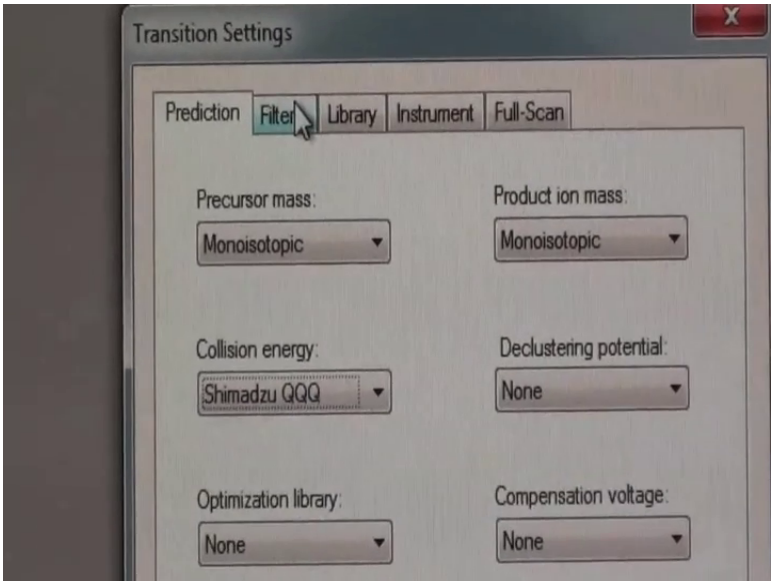
Once the faster sequence is pasted in your skyline file, you will see a huge number of peptide sequence transition list generated by Skyline.

(Refer Slide Time: 06:32)



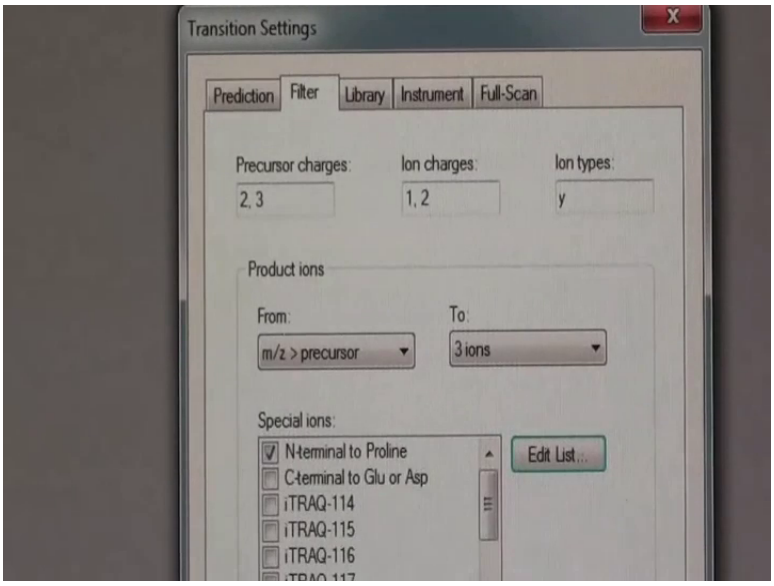
Now you can use some refinement of filtration criteria, you will go to setting change peptide setting, you will specify which enzyme you have used for digestion maximum missed cleavages will be zero. In filter you will specify what should be the length of the peptide it will be generally at 25, you want to exclude potentially ragadens, and if you want to exclude peptide containing some special amino acid you can just click on that amino acid here.

(Refer Slide Time: 08:16)



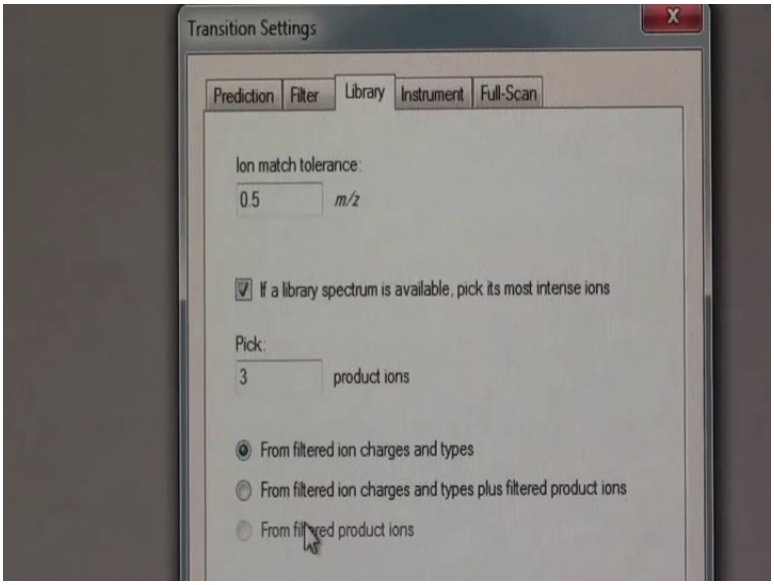
In that case you will select precursor and product ion mono isotopic, system will be Shimadzu triple quad system, collision energy is already optimized for Skyline and Shimadzu triple quad system by Shimadzu research and development center depending upon the transition it will tell you what will be the best optimized energy.

(Refer Slide Time: 08:40)



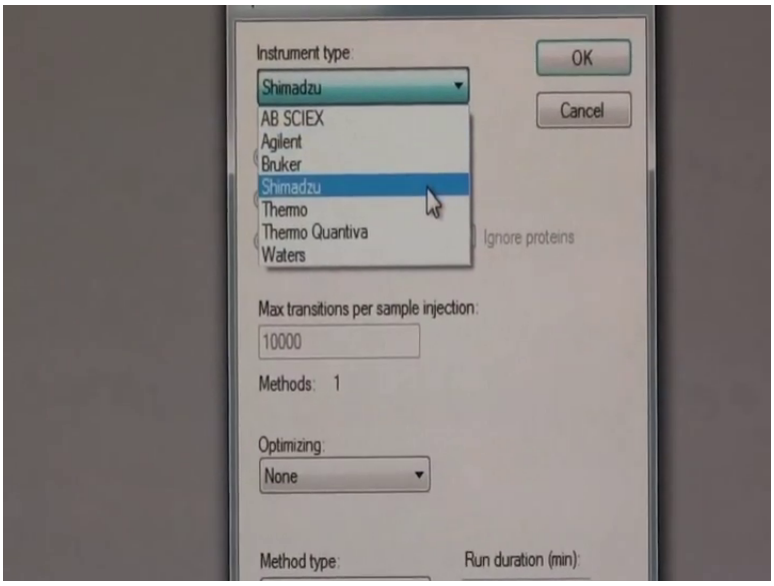
In filter criteria you can specify charges on precursor 1, 2, 3, you can specify charges on product ions, and one can select which type of ion you will be using for quantification. Most commonly used is Y type of ion, then you can specify the length of the product ion which needs to be considered during the fragmentation which is ion 2 last ion-2.

(Refer Slide Time: 09:02)

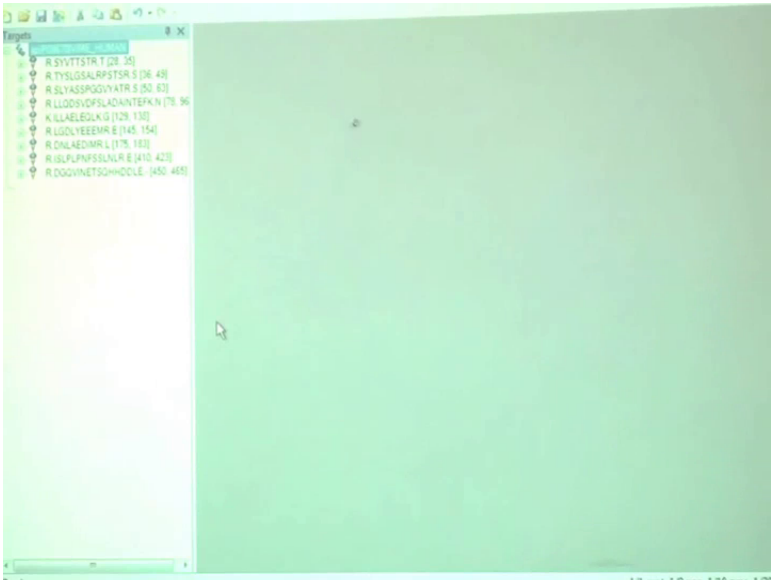


In case if you want to mention some specific modification, you can just check on that modification. Now you will see this file and you can export the transition list, now you will go to lab solution software which is Shimadzu acquisition software for triple quadrupole instrument.

(Refer Slide Time: 09:49)

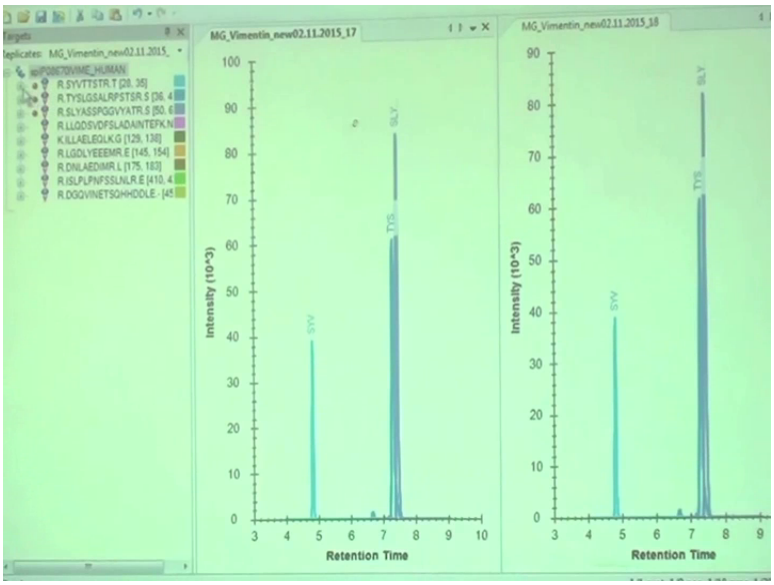


(Refer Slide Time: 10:20)



Once analysis is done, now you will open those data files which you have analyzed in your vimentin Skyline file that you will go to file import results.

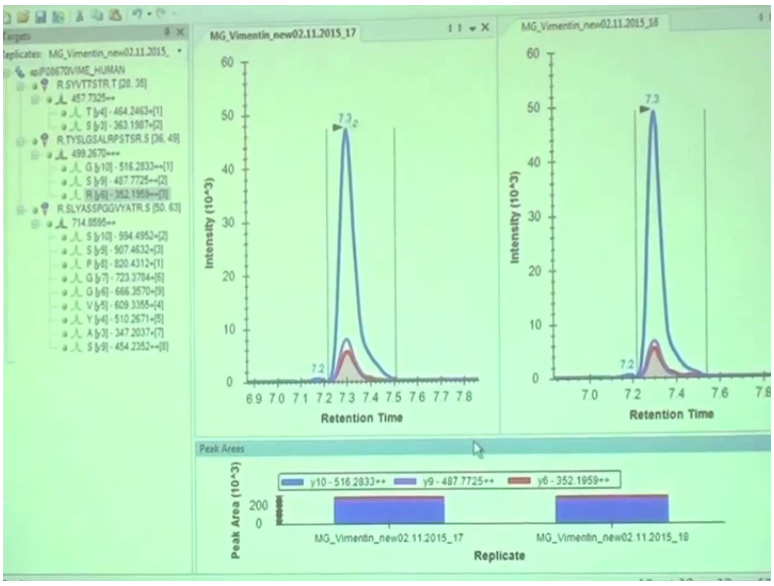
(Refer Slide Time: 10:51)



You can see results have been reported in the Skyline file for both these data files, so you can just adjust the data files and here you can see in the remaining peptides there is no response generated. And for these three peptides there is response generated, but still they are looking red because for some of the transitions there will not be any response, so here only for these two transitions response is there.

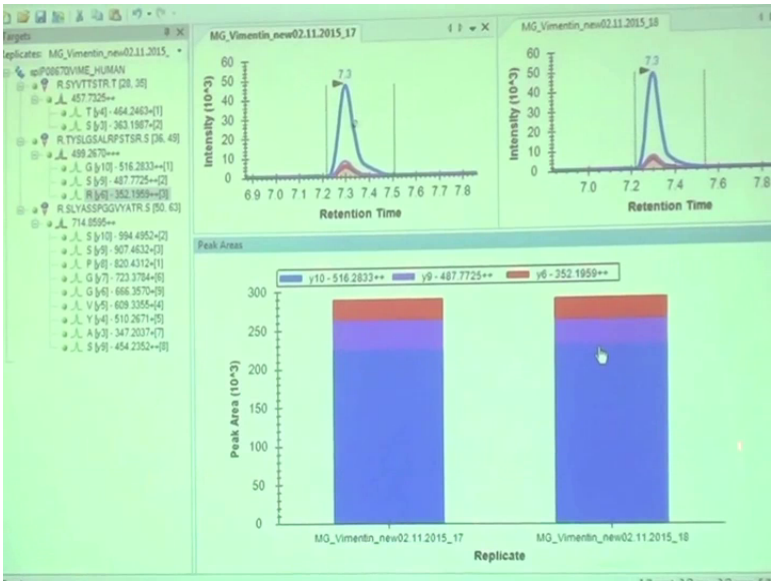
Here, also for 3 transition response is there, and in this case so there are many transitions for which responses is generated. Now you want to refine these results, so you will go to edit refine remove missing results, all the amazing results are disappeared and only those transitions for which response is generated in triple quad.

(Refer Slide Time: 12:07)



So you can see the peak shapes for both these data files and you can scroll down to check how peaks are looking. If you see for a given peptide all the transitions are properly correlating at the same time retention time, its peak shape is also good and if you want to compare the peak areas you can just have this graph.

(Refer Slide Time: 12:39)



Both of these samples belong to same grade of cancer, so there accounts are almost similar, you can actually compare peak areas for different grades of cancer and see if area accounts are increasing or decreasing, and you can check what is the relative expression of given protein in case of cancer samples.