Introduction to Professional Scientific Communication
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Lecture – 12
Writing the Methods Section

Well welcome back to this course, professional scientific communication. So, in a week 3 we are looking at the structure of the manuscript how you go about you know, putting together your thoughts into you know a statements and words. And that finally, comes out as manuscript, right. So, today we will talk about how do you write the section called methods, right.

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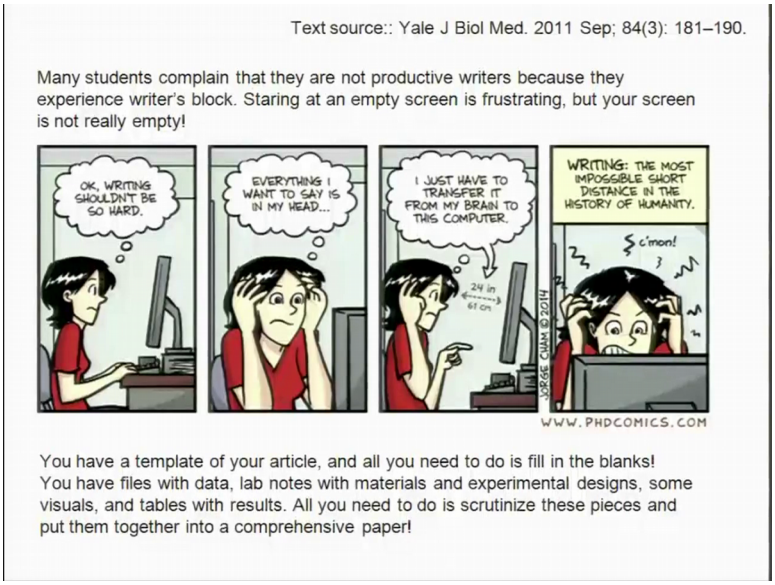

So, when you were asked to write a manuscript out of your users, one of the complaint that most of the students come up with is that, many students complained they are not productive writers, because they experience writers block, right. I told you that it is much easier doing than saying what you are done. Staring at an empty screen is frustrating, because you want to write you do not know what it is, because you know what where to start right that becomes a big challenging. So, it is not that the screen is really empty, that you need to understand that you are unable to put the words, together and convey what you are done, but it is not that you know the screen is empty.

So, you how material to start with but you need to know what start. That would give you confidence, because it is important that is that you have some confidence to start with. You have a template of your article, and you all you need is to fill in the blanks, because remember we discussed about the outline, I said that put the questions arrange them into different categories. So, you have these bullet us already you know, formed for each section.

So, what you need to do is that you have a expand them to you know sentences, sentences into paragraphs, paragraphs into sections, right. And what you also have is that you have files of data you have extra frames you have digital images, you have microscope images you have you know all the, you know a spreadsheet with data. And of course, your lab notebook is full of all the methods and material that have used.

So, you have all this data. So, you have to you know again pull out you know these information and put them into words, or make visual you know, for example, schematic or chart or bar diagram or line diagram or you know, take the gel images crop them make good figures. This is how you start. When you do that then it pretty much forms bulk of your results right.

So, all you need to do is to scrutinize your data that you have the pieces. And put together as a comprehensive paper. So, it is not that you do not have, it is not that the monitor is blank. You have everything is like a puzzle you have different pieces you know how to assemble them when you assemble possibly the board will now convey something. You know, a beautiful picture or beautiful story, that is exactly your scientific paper is. So, all you need to know is how do assemble the pieces.

Again, a going back to the same old paper, how to write the first research paper.

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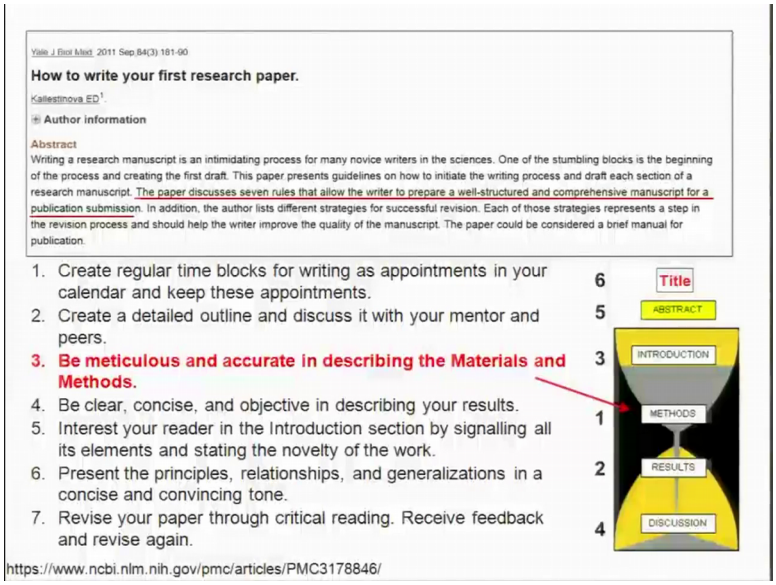

But that is what we are using as a basis to explain how to write, right because I am using one particular paper, therefore, you can go back and forth and consult that paper along with this you know lecture therefore, you can write better. I told that the third rule in this you know 7 rule theory of writing a paper is, be meticulous and accurate in describing the materials method section. Because this is something that you know you have done already. So, you know what are the materials you have used? what is the method that you have used.

So, the best way to start your research paper is to start writing the materials and methods section because really there is no intellectual challenge there. Because you have to put together a narrate everything, most often in their chronological order therefore, you can finish bulk part of your either a manuscript, or your thesis, because it is about 20 percent 25 percent of your paper materials method, right you can easily write. Another 20 percent paper or forty percent is results, which is again easier to write, because this is something the data you already have.

So, what do you want to discuss today in this lecture is how do you write the materials and methods section. And I told you often people start with that, because it is much easier among all the different sections of the paper. The materials in method section is easiest one. So, therefore, let us start with the materials method section.

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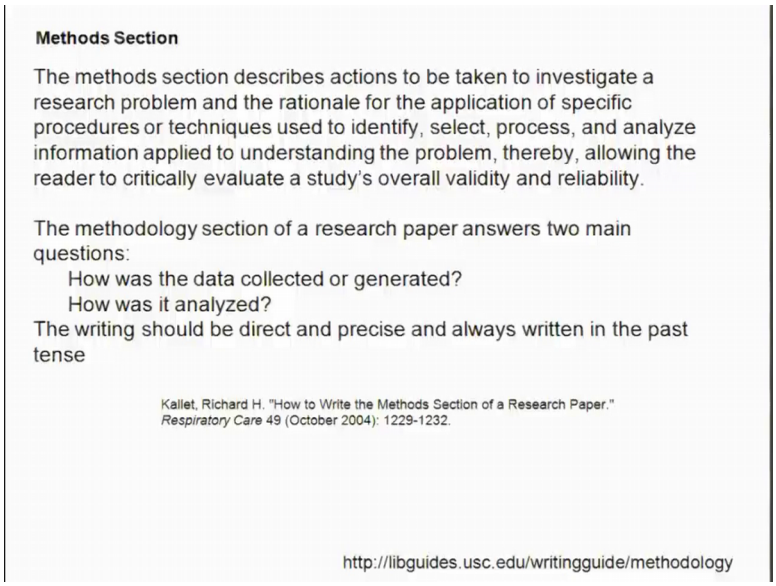

The method section describes actions to be taken to investigate a research problem, and the rationale for the application of specific procedural techniques used to identify select process and analyze information applied to understanding the problem. There way allowing the reader it is a pretty long sentence, right. What it says is that, it is not simply saying how you are done, is also important to sort of tell as to why you are done why you are used that approach and so on.

So, the methodology section of a depending on what kind of research you do. So, how was the data collected and generated. This is one important question. How was it analyzed? It is another important element of your method section. Because the writing should be direct precise, and always written in the past tense, right is very important, because you are narrating what you have done. Therefore, you have to write always in the past tense the methodology section invariably it will be in past tense and some of those elements that we will discuss now.

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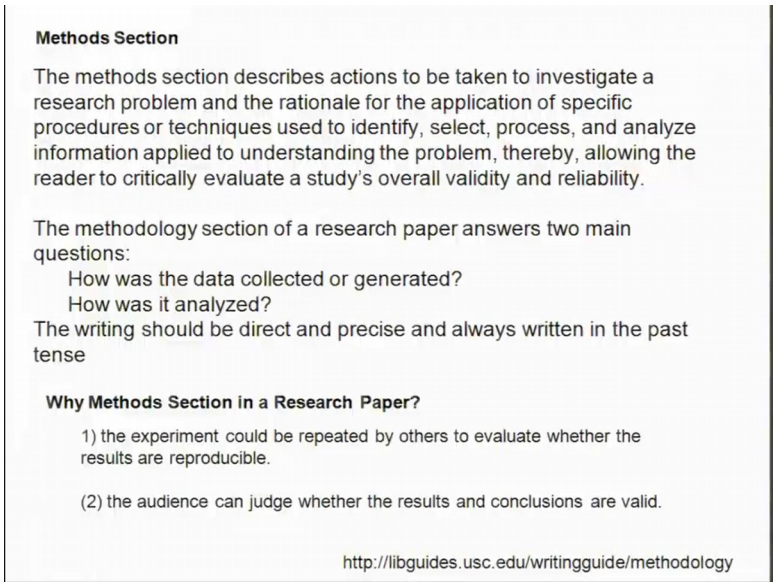

So, why you need to have methods section because you must have consulted many lab manuals because there are manuals available. For example, you have molecular cloning there are manual available for recombinant DNA technology. There are manuals available for protein expression purification; maybe you followed exactly what is given that right. So, why you need that?

So, it is important? Is that you know you have to narrate exactly what experience you have done to arrive at the results that you are present into the paper, therefore, other person can reproduce you can do the same thing and reproduce often when you carry out any experiment, even based on a well standardized you know kind of method. All the materials that have used for a given say for example, may had be identical because the method the volume the book, would never tell you that you have to buy this particular reagent from a given vender they will not do. Because they will only say use this reagent.

Now, this reagent can be a good quality or of bad quality, or the biden in the manufacturers made some difference to the reagent such that it becomes far more active, right. Therefore, you have to even mention as to what material you have used, what is the source of the material. Therefore, the other person can use the same therefore, you can also know get better results, because the material may work much better as compared to a material you have been you has been using before.

Therefore, the audience can judge whether the result and conclusions are valid another thing. Because you say what you are done, and what approach you have used to analyze the data even that is discussed in the methods section, therefore, they can go back to your raw data, and analyze and understand how you are done.

And then appreciate yes, and then they say that whatever your concluding based on this data is accurate because. They also agree that this is the best possible way to explain the results for that they should know how you analyze the data. That is why the methods and materials section is very important. And these are some of the you know again 5 major points that you should consider when you write the method section one.

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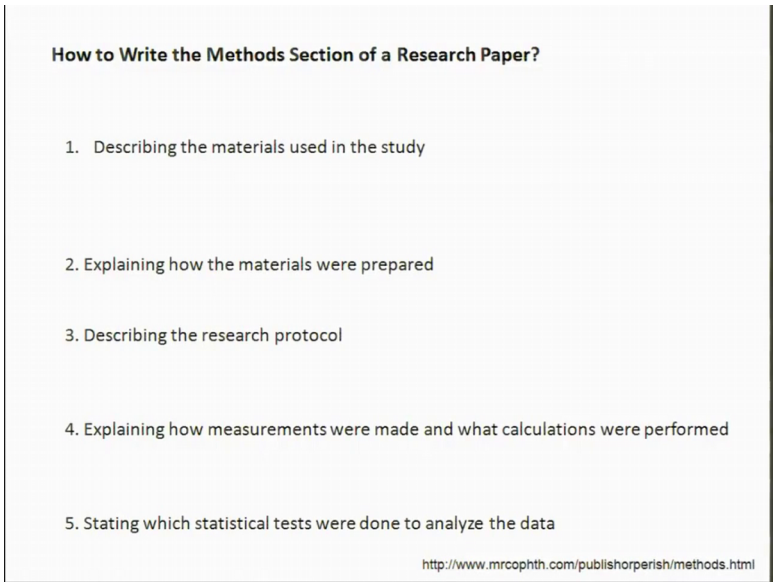

Describing the material used in the study. You know, the material, right? It could be chemical, right? It could be some bio molecules, it could be the samples, it could be the subjects, or it could be a you know a tool or a you know a online resource that you are used or it could be an algorithm. It could be anything right, but that is you know materials explaining how the materials were prepared if you have you know use certain novel material, that is prepared in your lab you want to explain that describe the research protocol.

You know, the step by step how you are done explaining how the measurement was done because often these are quantitative measurements. So, you have to say how it was done, and then how you are done the calculation. And finally, you know often your

experiments may require statistical test prove the difference significance and so on or you analyze the data, again what kind of analysis you have done, what kind of statistical tool that you have used, that makes the, you know, methods very precise and concise and as required.

Let us see some of those issues now.

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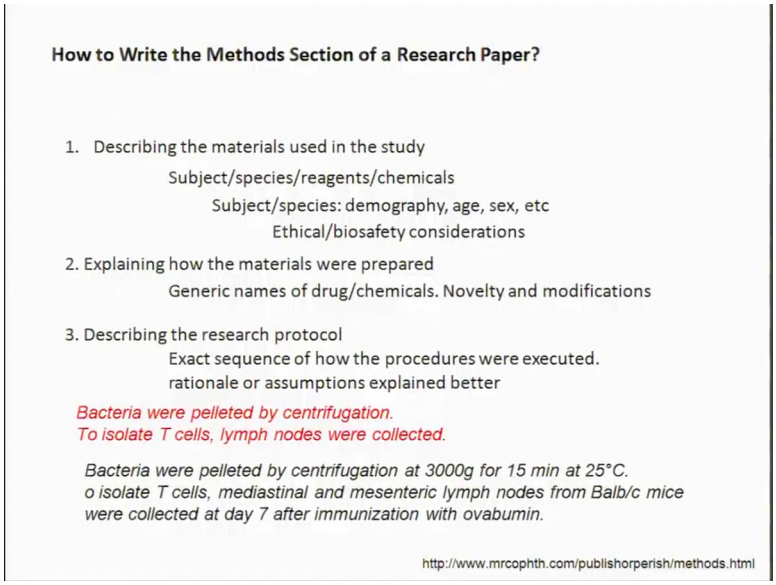

One describing the materials used in the study, right it could be subject for example, I told you like you know one of the examples that we are given earlier is that you know dementia in a patients, and we have used the particular you know therapy what do you call x therapy. And that was used to sort of forever tie title you know a better title. That was one topic that we described in the previous lecture.

Now, there we mentioned it is forty Japanese in a patients right. So, that is what subject is. So, when method section you have to say exactly you know who are they, for example, patients suffering from a given disease. And then you have to say what was the inclusion and exclusion criteria to call them that they are having a dementia, then you must have used certain parameters to say as long as a given patient 5 different symptoms, let us say all the patients show all the 5 simple then they are included as you know dementia. And then you are excluded you know for examples and criteria, the dementia could be secondary meaning you had some other ailment as a result you had some for example, their treatment. The treatment may have induced dementia.

Now, as a result dementia secondary is not the primary problem. So, then I should you know exclude. So, then you have what is called as inclusion criteria, exclusion criteria and that is what may subject what is the age group. What is this male only or female only, or both and what is the ethnicity? All these you know are involved in in what is called a subject.

Species for example, if you are using animal as a model, then you want you want to say what specie you cannot say that rat model. You have to say exactly what is the species that you have used? You cannot say is mouse you have to say which mouse pieces you have used. Because at times there are variation in terms of what kind of results come out, and it could be species specific, or strain specific then you have to mentioned that. And then you have to say what are the reagents that you are used what are the chemicals you have used often you say the source of the chemicals, source of the reagents, and if it is commercially available then you identify the company you have used often people ask you to put.

The cap number as well therefore, they can go and check as to what exactly was a purity of the compound, or how did they produce and so on these details are available in the company website, if I want to know I can go back and look at it.

Or if you have you know the chemical is given by somebody, other researcher then you mention his or her name, that it is you know a gift of somebody are you borrowed it from somebody and so on, if it is synthesized in their own lab if it is prepared in their own lab. But source must be mentioned therefore, people can go back and request them and so on.

So, that is very important. When I talk about subject I said you know these are you know demography. In a sense that what a population you are looking that what age group you are looking at is a male or female. In terms of for example, you are to looking at hygiene as one of the parameters. Are you looking at the low-income group or high-income group? Because it varies depending on whether you are looking at urban area or rural area or you are looking at slum area, you know and then what age you are looking at, what sex you are looking at you knows the male female.

So, there are so many other elements that you need to look into, because these are extremely important, because if you recall we discussed one data in the beginning where

we are discussing about the hypothesis and we give a hypothetical data talking about 2 different age groups.

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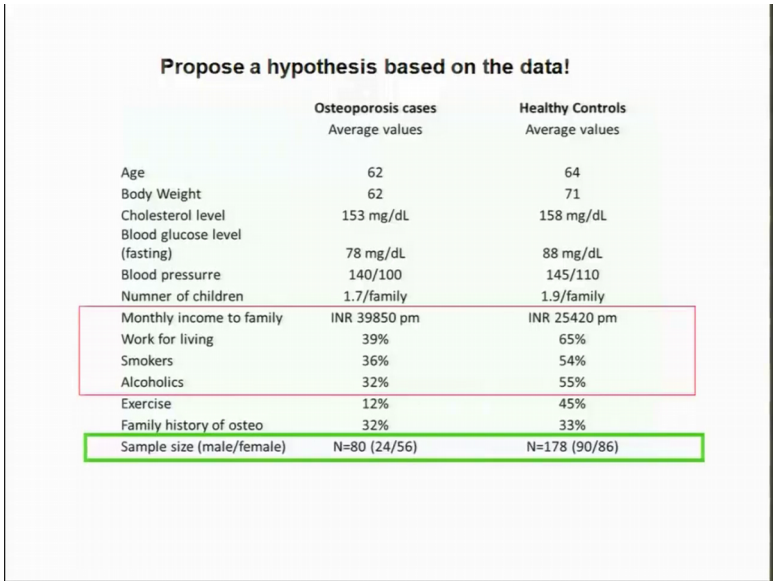

Population one is that are affected with osteoporosis, other only the healthy control and then you find that there is this you know your pseudo correlation, with the monthly income work for living whether you smoke alcohol and so on.

You know it is because there is a sample bias, you know you can see that in case of osteoporosis cases, you have had more female than male as a control it is pretty normal. So, this can pretty much you know you know lead to a false you know kind of a conclusion. Therefore, if the data was available presented in the paper, and even if I had made a wrong conclusion you as a reader someone else go into the data, and then say that this is wrong because your data basically is not accurate.

So, that is why it is very, very important, because once you have returned your paper published even if it is published, and people are not going to just blindly follow what you say. They going to row and look into your results, and say whether your results support what you are saying. If the results do not support what you are saying, they are going to question in their paper that that whatever you have made conclusion that is wrong. You paper may be cited, but for a wrong reason therefore, the data helps.

So, therefore, the methods material section should I have all these components, there for others can you know understand and appreciate that you have done a good job, are use that data to you know infer on their own the correct influence, right. And the third element in describing materials method used in your study is also what is called the ethical biosafety consideration.

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So, if you are using human samples, you know say you have drawn blood, and the serum level you are looking at several biomarkers for as a possible correlation with certain elements, then you know any time when you use a human as a subject for any research application then one need to get clearance from an appropriate body called as a review body, which looks into your objectives, looks into your methodology, looks into your statistical power and tell you whether you can go ahead and do the experiment or not. Unless that the review body allows you to do research in a given proposed area, you should not be doing any research involving you know samples or subjects you know human patients or even healthy controls.

For example, in India, there is a guidelines proposed by the Indian council of medical research it is called the ethical guidelines for biomedical research on human patients. And this guidelines pretty much tells you as to what are the in a circumstances on which you can do research using human subjects. And what is the regulatory body that can allow you to conduct such experiments.

So, before even you start your explosive you have to provide all the details to this regulatory body often it is called as institute human ethics committee, and then committee looks into your protocol. And then and looks into the number of subject that you are using. And then what kind of assays that you are going to do, and what kind of sample that you are going to draw from these subjects, and why this study is important, if this committee feel that this study is not going to add anything new, it is already in time it may say why are you arming a subject, because you are going to draw blood that is in in in be a secret invasive procedure you are going to put a needle and take the blood out.

And second most often the committee used to protect the subjects. Why do you need a committee? Because you know most often you know the human subject's commander you know it kind of what is called the clinical trial. So, if there is a new drug that is being developed by a pharmacy or company, before being marketed the drug has to be validated has to be it is safe and it is effective. And it can be given to patients suffering from a given disease right.

So, for this this drug has to go through what is called the clinical trial. So, in that what they do is that that identify some you know patient group, who are told about the drug, and these are the patients that otherwise you know you do not have an effective drug, drug to treat there for example, whatever disease that they have.

And in the company the doctors and research team convinces the patient that this drug has the potential to treat, and then they agree for that and the review board agrees that that can be you know under you know can be tested in this patient, if that is done, then these patients were given the drug. And they were monitored as to how do they react to this particular drug. Is it any adverse effect? Does it get any better? When they have such kind of clinical trial, you get to know that a given drug what is better than the existing one, and in that kind of observation replicated in multiple you know samples multiple groups in multiple countries.

Once you know that it is indeed good the clinical trials, convey that it has more beneficial effect than otherwise. Because no drug is free from any harmful effect you always have some adverse effect, but if the beneficial effects are much higher than the side effects, then that the drug is approved by the appropriate body; that it is, good for you know as a drug then only it comes in 2.

So, that is where the ethical guidelines are important, because the rights of the subject is you know is sort of governed by or taken care by the committee because whenever a drug trial comes in you know the pharma companies may have huge amount of money they really want to get the drug going, because they can get revenue out of the drug. Because that is how they are investing these are you know mostly these are profit making companies. And therefore, they want the drug to be tested, but what is that the patient is you know is going to be benefited what if the drug is having an adverse effect, right. What if they leave the patient and go?

So, what are the rights that patient has. So, these are the some of the, you know elements that are looked by this committee and gives you certain structure. And the whether the patient has a right to know what are happened, with the drug and what is biochemical parameter whether it has an adverse effect. And what are the what is the outcome of the study you know only he is one of the many, but he has every right to know how the drug has you know, what in the entire.

So, what are the rights that patient or the subject has. So, these are some of those elements that are discussed in the biomedical research ethics committee, and if you are involving any such research you need to know, what is that you know guidelines and obvious the committee functions, and why you should get the study protocol approved through the committee.

If you are using animals, then in India you have a different committee called institute animal ethics committee this is governed by the guidelines of for committee for the purpose of control and supervision of experimental experiments on animals, what is called as a CPCSCA. This committee parameters thus why should animal be used the question is for your own research why should you harm any animal, right.

So, there has to be compelling reason as that that this exponent should be done. So, the committee needs to be convinced, and the committee needs to convince that the number of animals that you are put you know, sort of you know proposing is required. Because yeah after all you are you are playing with the life of the animal, right. They may suffer because of the experiment you do, right although they may not be able to convey it to you, but they do suffer.

So, is it really justified? So, this what the committee looks into, and the committee also suggests various measures by which the animals can be, you know you know you know use animals for the experimentation without you know causing much pain to the animals. So, there are guidelines again this is governed by that.

The third one that is relevant to the bio research you seen, you know the biosafety committee, if you are using any recombinant protein. For example, this protein you may be expressing in for example, equal like which is a harmless bacterium. But the protein may be could be from for example, micro bacterium which causes the tuberculosis. Now one need to understand whether you know expressing your foreign protein in ecola would turn the ecola into something other than the normal ecoli. Or if you are doing any research that is by which you are putting a foreign gene into a plant, right. And the plant now is grown outside, now it is you know the pollen would carry all the transgenic you know gene, and how it is going to affect the environment the other plants that are otherwise non-transgenic.

So, these are the issues; that needs to be looked at. So, there is a committee which you know the guidelines set by the department of biotechnology. Now it tells you clearly what level of permission is required for what kind of project. So, it has to be cleared by them before you do then there are guidelines as to how to contain you know for example, such kind of you know microbes or plants that that you genetically modify and so on.

So, this you should know because there are guidelines. And it is important because when you submit a paper the journal would ask you whether this study has been you know vetted approved by any of the regulatory committees. And you have to say as it has been and if they demand. You have to give the clearance certificate; therefore, it must be done upfront even before you start the experiment right.

So, you should know that there are ethical considerations in terms of how do you do it as such. The second point is explaining how the materials were prepared. Say suppose you have generated a novel compound. Novel chemical by a synthetic process, right you need to tell how it was generated because this is not something that is available off their shelf from any of the companies. So, you have to give entire synthetic in a path you know pathway or schematic how you are generated it, and any novelty or modification you

brought in there for others can use it. Or even if you have purchase some chemicals from outside commercial source you need to give the antibodies.

For example, antibodies the source and cat number chemical source and cat number. The reason being you may have used an antibody that take a vendor or a company sold it as this antibody would recognize a given protein. So, you believe that indeed that is the case, and you have been using it in an experiment. And it possible that in addition to the protein that you are detecting it also detects in something else. And you are you know interpreting that as you know the other protein that you are interested in, and possibly it is a nonspecific band.

Now, by showing that what is the source what is the cat number, when someone else uses the same and then they find out that the other band what they are using or they are getting is not the one that is specific to this protein. You know, they are able to tell you that your results these bands are not because of you know the specific reaction is a nonspecific reaction, and since you have used a commercial source for, this you are not at fault you have went with what they have claimed certified to be an antibody that recognizes this protein. But it is because of the manufacturer could not validate it, it helps others to validate they give an antibody. That is why the cat numbers are given likewise, the chemicals the purity everything matters in redoing experiment confirming whether it is accurate.

Therefore, in your methods section you have to list all these things, which help you well others in arriving at you know replicating or shrunk gaining whatever conclusion that you are making. The final one is describing the research protocol, right. The exact sequence of how the procedure was executed you have to, write. Rational assumption of explain better. Because you need to see why I have done this you know it is very important. And then for example, these are the 2 statements bacteria were pelleted by centrifugation, to isolate t cells lymph nodes were collected.

Now, it may some that these are better way of writing, but indeed that is not the case. Bacteria were palliative a centrifugation, it does not say as to you know what speedy have used. To isolate t cells lymph nodes were collected. You know, how did you collect lymph node? These are not said. So, bacteria where piloted by centrifugation at 3000 g for 15 minutes at 25 degrees. This is very important, because what speed that you have

used for the centrifugation to pellet the bacteria, what is the same you know you know temperature. You used to isolate it cells you know, mediastinal and mesenteric lymph nodes from balsam mice you know it does not say the first sentence does not say from whom the human mouse rat.

So now you say balb c mice because this particular strain of mice collected at what age group at day 7 after immunization. You know, it gives you the detail these are very, very important when you document you know your method section.

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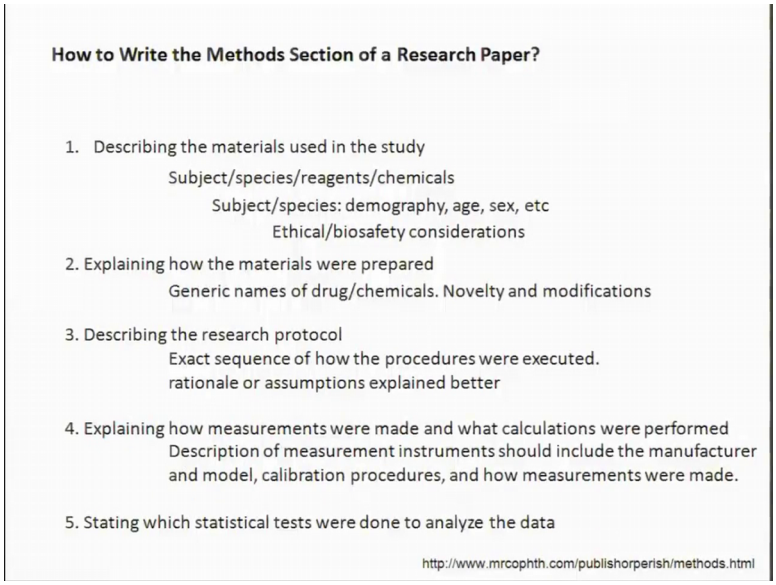

And starting with the statical tests you know, how what are the tests you are done to analyze your data. You have to narrate at the end of the method section as to how you are validated how you, you know looked at the significance of the variation differences that you found, right.

So now I am going to take few examples and explain as to how effectively you can write the method section.

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So, I am going to put something from again a publishers website, this from springer it says materials and methods. This section provides the reader with all the details of how you conducted your study; you should for example, use subheadings to separate different methodologies for example. There could be animal studies, there could be cell line studies that could be biochemical assays, each one should have different sub readings. Describe what you did in the past tense, because you are narrating what you are done. So, you cannot write what you will do, right.

So, it should be said like you know the cells were grown at 37 degrees for 24 hours, thereafter they were harvested, and the lysis were prepared and immuno blott was carried out. This is the past tense that have you should, right described new methods in enough detail that another researcher can reproduce your experiment. So, if you are improving an existing method, you may cite the original method, need not detail everything because already you are citing that is what one can go back and look how they have done it.

But you can say what changes you brought in. For example, you are using an antibody, for a immunoblotting to identify a specific protein or the level. So, they may have used the antibody at 37 degrees for one hour with the sample for the antibody antigen interaction. But maybe that was giving some nonspecific you know interaction. So, you went and then incubated the antibody at 4 degrees for longer time say 12 hours.

Now, in this case that you need to say that you know I have used the same antibody as detailed in the previous report. But instead for the interaction I have used 4 degrees for 12 hours. So, that is the you know novelty or chain that you have made that is important, because it may give you better results and others may appreciate that then they can go back and reproduce you know after reading your paper.

Describe established methods briefly, the point I just now explained. A well-known method already documented available in the literature you can briefly mentioned by giving the reference by citing and reference. Where readers can find more details, you do not need to rewrite everything, what has been done before state all statistical tests and parameters and these are where bottom taken from the springer website which houses which publishes a number of research paper.

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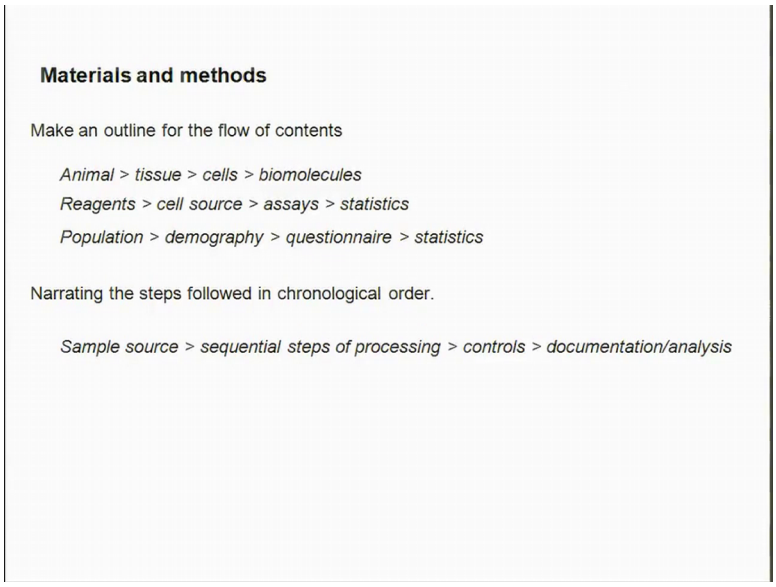

Let us see how do you write methods again you need outline, right. So, even for materials method you need to make an outline. This is always important, because you know when you start writing then you may forget something. So, even before you start writing you always make an outline as to what are the points that you need to cover in every section. Likewise, in methods and materials make an outline for the flow of content. This is very important.

So, you need to see for example, if my study involves animals, tissues, cells and biomolecules. Let us say I have done some animal study, I would have surgery, then I

have looked at certain tissue, we have done histopathology. Then I have cultured cells from the animals and done some study. Then I have extracted protein or mrna from the (Refer Time: 29:06) I have done some study. So, there are different levels.

So, when I write methods, I should always use what is again going back our original discussion like address. You start with which country, which state, which district with city and which street number, right. You start with animal and say what does animal that you are used. What is the line that you are used? How did you know, how is animal? What surgery you have done? And you are used tissue for dynasty pathology, your write about the tissue how you dissected how your dynasty pathology. And you have talked about cells, come next then you say how culture the cells.

And what kind of culture conditions were used, and then if you have extracted in a protein rna from the cells or tissues, comes at the end because, the approach that you use to analyze protein, whether it is from cell or tissue are from the animal. You know, all very similar because you take out tissue from the animal, you know you homogenized to make it like a single cell suspension. It is very similar to your cell culture condition, and then you lies the cells and then you have the protein.

So now the protein lysate is similar regardless whether you got it from the cell culture or from tissue, or directly the sected the animal from the tissue and so on. So, that can come at the end. So, you need to have certain you know some outline you have to think in a rational way. For example, you can you talk about certain bioassays, the second model that you offer. And put all the reagent that you are used. So, what are the reagents that you are used and what is the source what is the cat number. In list everything that you are procured right all fine chemicals antibodies are fine chemicals.

Then you talk about the cell lines that you have procured, from where you procure and then you discuss your essays, and then you discuss about your statistic. So, it all depends on what kind of work that you do. If it is like a population-based work, then you say the population, what is the study population, what is the number, what is the female male demography. And what kind of questionnaire you used to shortlist the patient group. And then the statistics are you to validate your findings.

So, each one you know it has got it all the includes for example, the ethical clearance if I use animal for experimentation, I have to mention, that whether the study was cleared by

the animal review committee; if I use human population, whether it was cleared by the review board that approved that protocol. So, this also should be part of your method. And then what once you have this outline then you have to narrate the steps followed in chronological order. Say, suppose I have done a given assay for example, let us talk about I have prepared a cell. I say then you have the start the chronological order, what you are done first I cultured the cells for 2 hours or 24 hours. After that I harvested the cells by using troops. In after that I lies the cells by mild homogenization which I did see.

After that I sent refused to remove the nuclei. Then I took the cytoplasmic extract, I boiled with sds you know, loading buffer to denature all the protein. Then I loaded in a gel separated them based on their molecular weight and then transferred it to a membrane. Incubated with the protein and then visualized the protein of my interest by using one or the other method.

So, this is a chronological order, that is exactly should be used. And finally, you have to say you know you have to say you know, how you are documented the result, right if it is in western then there is a colorimetric method, or aluminescent method chemiluminescent method. Then you have extra flame or a kami dog which gives you the signal or it sometimes is readout. You know, of some a cell assays then you have some values from your spectrophotometer, or from your by aluminator. You know kind of measurements then you have to have that. So, how do you document, and what kind of analysis your done that also becomes part of your method.

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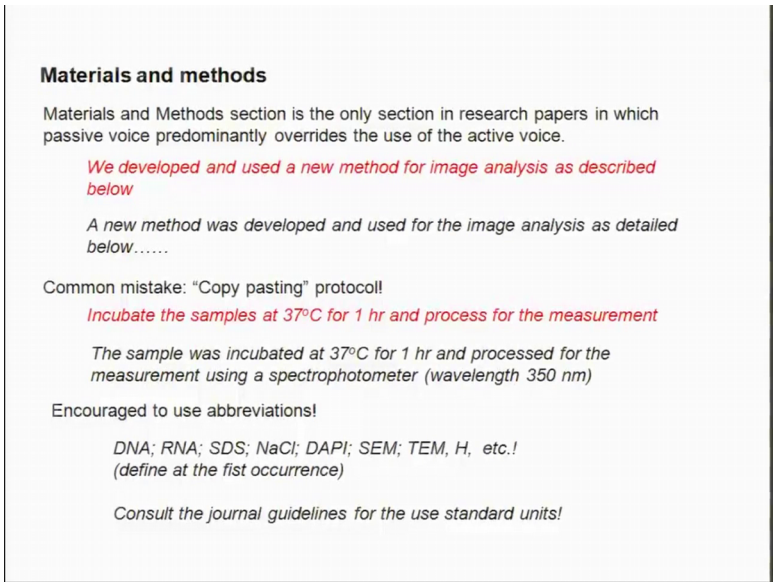

Go back to the same point that materials, and methods section is only section in research paper in which passive voice is predominantly, you know used. And the rest of the section you often use active voice. So, for example, we developed and used a method for image analysis as described below. So, you know that that is not the way you write. So, a new method was developed and uesd for the image analysis that I did below. There are different ways of writing you want to go, and look at common mistakes. Let us look into that.

There is a difference between methods under protocol. A protocol is a guide as to how you have to perform experiment. What is the next step and so on often one mistake people follow is that I use this method protocol to be carry out my experiment. Therefore, I can use the exactly the same as the method section. The answer is you know. For example, a protocol would say incubate the sample at 37 degrees for one hour and process for the measurement. But your method is something that tells you what you have done if the sample was incubated at 37 degrees. So, one hour because that is what you are done you are not done it for 2 hours. A protocol may say one hour at times you may change it.

So, you have to mention what you have done, rather than what you have been asked to do. And process for the measurement using a spectrophotometer, you say how it was measured you say what is the wavelength they used often we also give what make the

company the measurement that that you have used for this. Encouraged to use abbreviation the rest of the section of the manuscript you may not be encouraged, but method section which encourages you to use abbreviations, because there are you know chemical names that are pretty long. And you may have used this chemical often in that, you know in the different methods, by repeating the long names make it very difficult for people to read.

So, you can abbreviate for example, DNA RNA this is a well-known you know acronyms. These are, but abbreviations SDS for example, a religion that used for DNA testing. Protein mostly descendants SDS and then TAPI, again a stain used for nucleus same time for example, these are scanning electron microscope tunnel; each normally refers to the hour etcetera.

So, what is always advised is that you define the abbreviation the first occurrence or you have a separate. Section where all abbreviations are listed in your thesis it may be a table. And then after we you can use that abbreviation all over in your method section, is always encouraged and the and finally, consult the journal guidelines for the use of standard journal because every journal will have it is own way of writing for example, h 4 hour or hr for hour. So, they may say exactly what is the kind of (Refer Time: 35:45) that you have to used. There are many international standard units one I am showing here.

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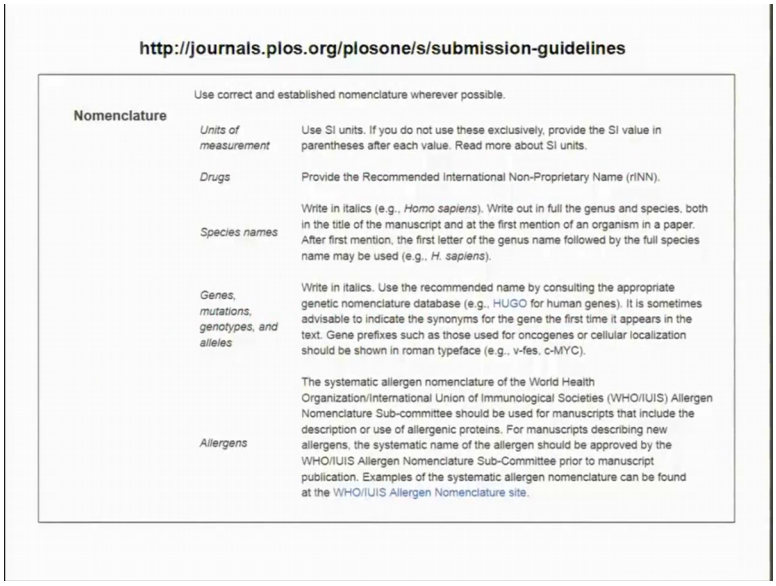

Again, from a journal web page units of measurement there are for example, often you know the centigrade is always used in scientific literature, when you are talking about temperature.

So, in the incubation for example, that 37 degree but if you go to us, you know they talk about the weather in Fahrenheit right. So, that is a, they use it for you know saying what is the temperature in India we use even the weather also, we always say today is 16 degree. You know centigrade in Kanpur. So, that is what it is. We do not say in Fahrenheit, but in you as they say that. But when it comes to scientific literature, there is a standard for temperature always a centigrade. And then you measure the weight always it is milligrams or grams or you know things like that. And then there are other forms for example, drug you know there are again non-proprietary names. For example, aspirin you take it. And that is a commercial name the chemical name is very different right.

So, when you use that chemical you have to use the chemical name not the commercial name. And species names again I said that, humans for example, homo sapiens likewise if I used mouse, lab mouse is mus musculus. And then there are strains that you have mentioned, there is a convention when you write species name. Genes, mutation genotypes, values you know when you talk about gene, there are convention as to how the gene there is a specific name for the gene. So, that should be used how to use it there are italics, you know if the slanting letters that is the way it should be written. And then there is again a convention for allergens for example, you are you are discussing about any human related work, and then there are allergens that are again there are stand standard nomenclature.

So, all these are there you have to follow that before you start writing because you need to know what should be used, right. That is very, very important.

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Neurobiol Dis. 2017 Apr;100:39-51. doi: 10.1016/j.nbd.2017.01.002.

Materials and methods

2.1. Reagents and antibodies

The following antibodies were used for the experiments: anti-Myc (11667149001; Roche India), anti-GFP (11814460001; Roche India), anti-γ-tubulin (T6557; Sigma-Aldrich), anti-β-actin (A2066; Sigma-Aldrich), anti-FLAG (F7425; Sigma-Aldrich) and anti-FLAG-HRP (A8592 Sigma-Aldrich) anti-DRP1 (85,700, CST), anti-Parkin (4211; CST) phospho-S616 Drp1 (3455; CST), anti-ubiquitin (BML-PW8805; Enzo Life Sciences), anti-malin (N85/18; NeuroMab), anti-TOM20 (42406, CST), and anti-Rhot1/Miro1 (ab188029, Abcam). The secondary antibodies were obtained from Jackson ImmunoResearch Inc, USA. CCCP (m-chlorophenylhydrazone), tunicamycin and BAPTA-AM [1,2-bis (2-aminophenoxy) ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid tetrakis (acetoxymethyl ester)] were purchased from Sigma-Aldrich India Pvt. Ltd. MitoTracker Red and Fluo3-AM were purchased from Life Technologies USA. MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide; thiazolyl blue) was from Sigma-Aldrich India Pvt. Ltd.

Now I am go to end this lecture by giving some examples, right. As to how people generally write methods. You can see here this is copy pasted, something from one of the publications. Now it talks about we are subheading under materials method that is reagents, and antibodies. All it narrates is what are the antibodies that have been used what is the cat member, what is the source you can see anthainik some number roche India. And the be beta acting some cat number sigma aldrich.

So, you tells you that what are the antibody they used what sold what cat number. Because the cat number tells you also relates to a particular clone from where they have generated antibodies. As long as you buy the same cat number and lot number, you know it is expected to behave the same way. So, then there are chemicals again, there are some important chemicals. Again, it shows from, where did you get it. And you can see one example is that, you know you talk about it the last line mtt, and this is an abbreviation for a long name of a chemical you can see that. So, often you abbreviate these things. So, again it says from which source you have obtained. And then you have generated something new.

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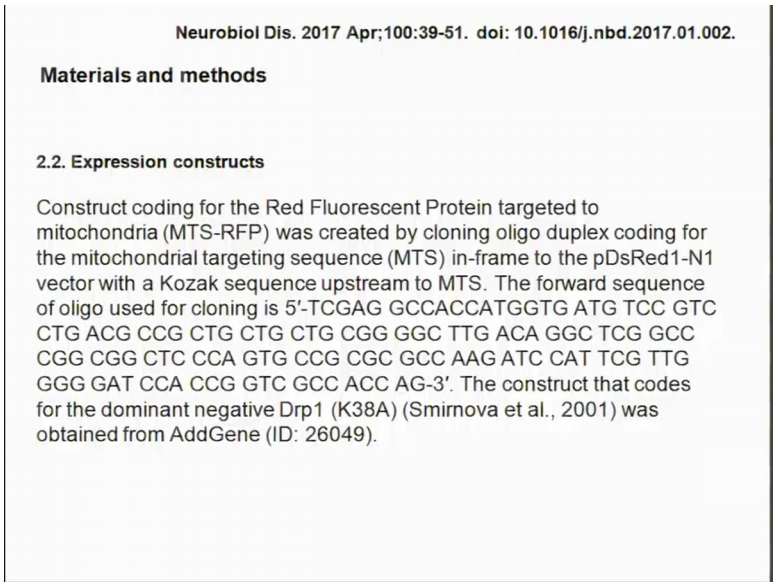

Right then you know then you have to say how you are generated the region.

So, this particular slide talks about the expression constructs. You know, some of them were obtained from somewhere, but some you have generated on your own. So, it gives you a nucleotide sequence, which was synthesized you know, and then they were put into a expression construct. Therefore, it produces a you know a protein variant which may have a given property. So, that is that is what it is like, how you generated constructs. Because it is something that you made it therefore, you need to tell how you have made it. So, that is very, very important.

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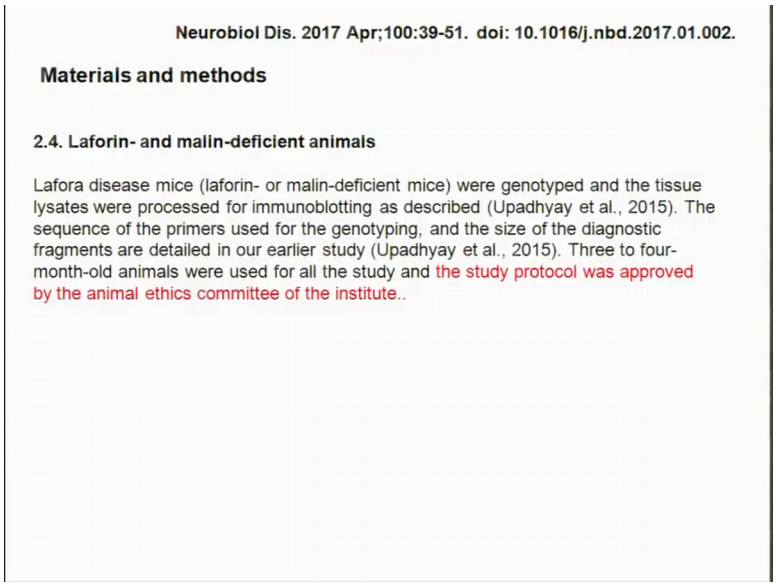

And then conditions of your experiments. Here we are talking about cell lines, for example, cos 7 neuro 2 a where obtained from the national center for cell science pune India.

So, you say which is the source for your, you know cell lines. And these are you know accredited national laboratories, which sort of you know routinely look at the cell line and say whether they are good for research. And they do all these biases to validate their cell line is good. So, when you mention that you know that you have obtained these lines from a lab which has the facility to certify that these are original cell lines and therefore, whatever the way they behave is expected of that particular cell lines. So, it is very, very important and then you mention how you have done all these experiments with regard to your whatever us is that you have done.

And another one we are talking about animals, you clearly say how do you know what is the source of animals, and whether the protocol was upload. So, you can see the one that is shown here in red color. It says the study protocols approved by the animal ethics committee of the institute clearly tells that this protocol was a front approved even before you started the work it has been approved. So, these are important elements that really look into.

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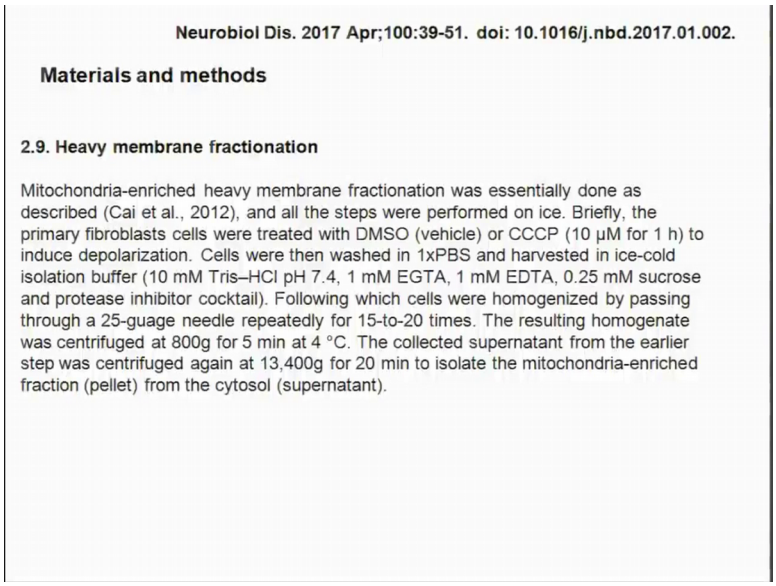

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Materials and methods

2.9. Heavy membrane fractionation

Mitochondria-enriched heavy membrane fractionation was essentially done as described (Cai et al., 2012), and all the steps were performed on ice. Briefly, the primary fibroblasts cells were treated with DMSO (vehicle) or CCCP (10 μM for 1 h) to induce depolarization. Cells were then washed in 1xPBS and harvested in ice-cold isolation buffer (10 mM Tris–HCl pH 7.4, 1 mM EGTA, 1 mM EDTA, 0.25 mM sucrose and protease inhibitor cocktail). Following which cells were homogenized by passing through a 25-guage needle repeatedly for 15-to-20 times. The resulting homogenate was centrifuged at 800g for 5 min at 4 °C. The collected supernatant from the earlier step was centrifuged again at 13,400g for 20 min to isolate the mitochondria-enriched fraction (pellet) from the cytosol (supernatant).

And when you talk about for example, you know some biochemical in purification, we often say centrifugation. So, in centrifugation often you say what is the certification basically, there is a rotor there is a, that rotates in a particular speed. So, in a centrifuge you set the speed.

But what you mentioned in methods is not the speed at which either what in the sample rotates. But it is the g force, that is calculated based on you know your formula, that is you must be knowing that is from the center of the radius of for the sample was, that determines the force applied on a tube, if it is closer to the radius less, and if it is far away it is much more for the same speed of rotation.

So, that is something that you need to know, because the rotor size may vary. But by having this g force you can arrive at the same g force regardless what centrifuge you are using in a lab. So, these are extremely important in the method section. So, what I am going to end is by giving a small schematic.

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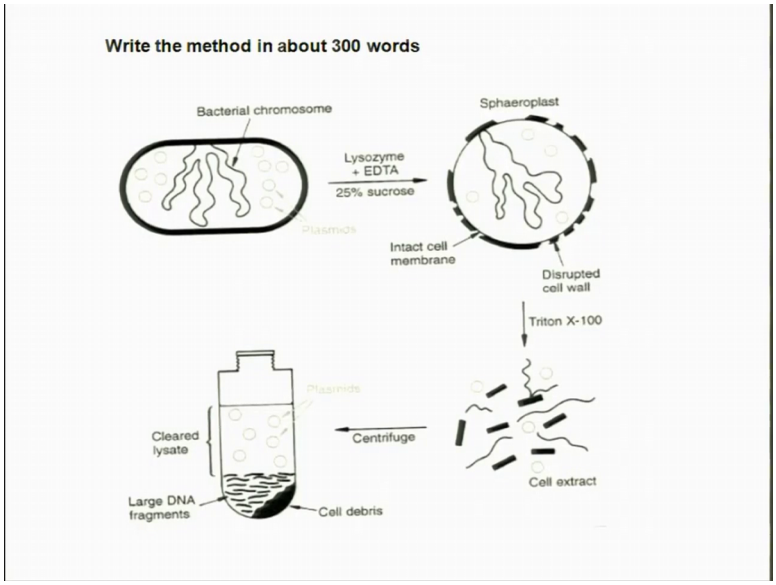

What is shown here is; obviously, those who have done some you know lab work you know that, this schematic is about plasmid d an isolation. This is a schematic, what I want you is to you know look at this and write a method as if you have done the experiment; that is, how would you know get the plasmid from the bacterium, if you have cultured it and carried out all these you know methods, steps. And in about 300 words you write a method the way that I have explained so that possibly would help you to learn how to write methods. So, with that we end this section. And will meet again next week with the introductory section, and discussion, and we will wrap up this course the next week.