Design for Biosecurity

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Lecture 23

MAB Production via Hybridomas

Welcome back to the 23rd lecture. So, today, we are initiating lecture 23, which will be monoclonal antibody production using phage display. So, in the last class, we talked about monoclonal antibody production via the hybridoma technique. Just to recap about the step before we move to the next technique. So, the first step in hybridoma is the fusion of B cells expressing unique antibodies with myeloma cells, creating a hybrid cell line called hybridoma. Clone screening identifies the clonally derived hybridoma cell lines, which produce high amounts of monoclonal antibodies.

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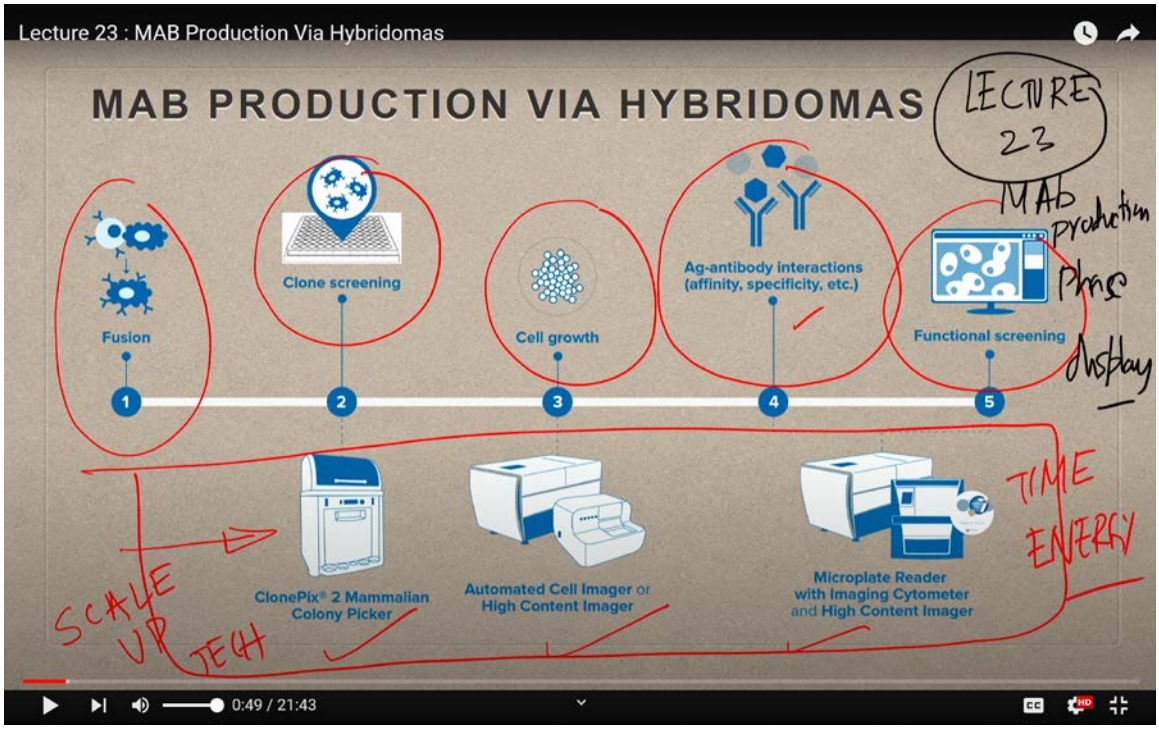

Remember one thing: why this clone selection is important: it is not necessarily all the spleen cells or B cells that are produced will have an equal propensity of producing monoclonal antibodies. Some of them will produce more, some of them will produce less. So, for higher productivity, we will have to pick up the ones that produce more monoclonal antibodies. That's why this step, clone screening and selection, is extremely important.

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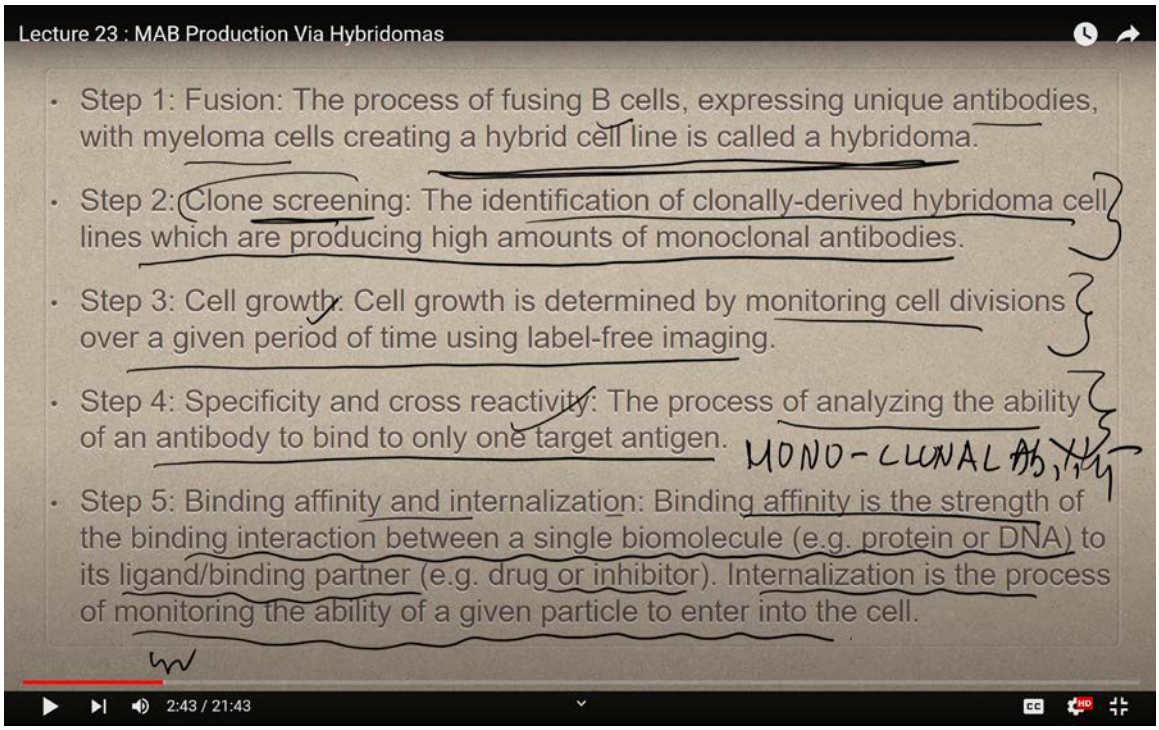

Part three is cell growth. Cell growth is determined by monitoring the cell division over a given period of time using level pre-imaging. Step four is specificity and cross-reactivity. This is the verification route, the process of analyzing the ability of an antibody to bind only one target antigen in order to prove its monoclonal ability. Binding affinity internalization.

Binding affinity is the strength of the binding interaction between a single biomolecule, that is, protein or DNA, to its ligand-binding partner, drug or inhibitor. Internalization is monitoring a given particle's ability to enter the cell. So, these screening processes are extremely important for monoclonal antibody production via the hybridoma technique. So

today's class, which is class 23, will be on monoclonal antibody production via phage display. So as I mentioned in the previous class, this technology is slightly more molecular biology intensive.

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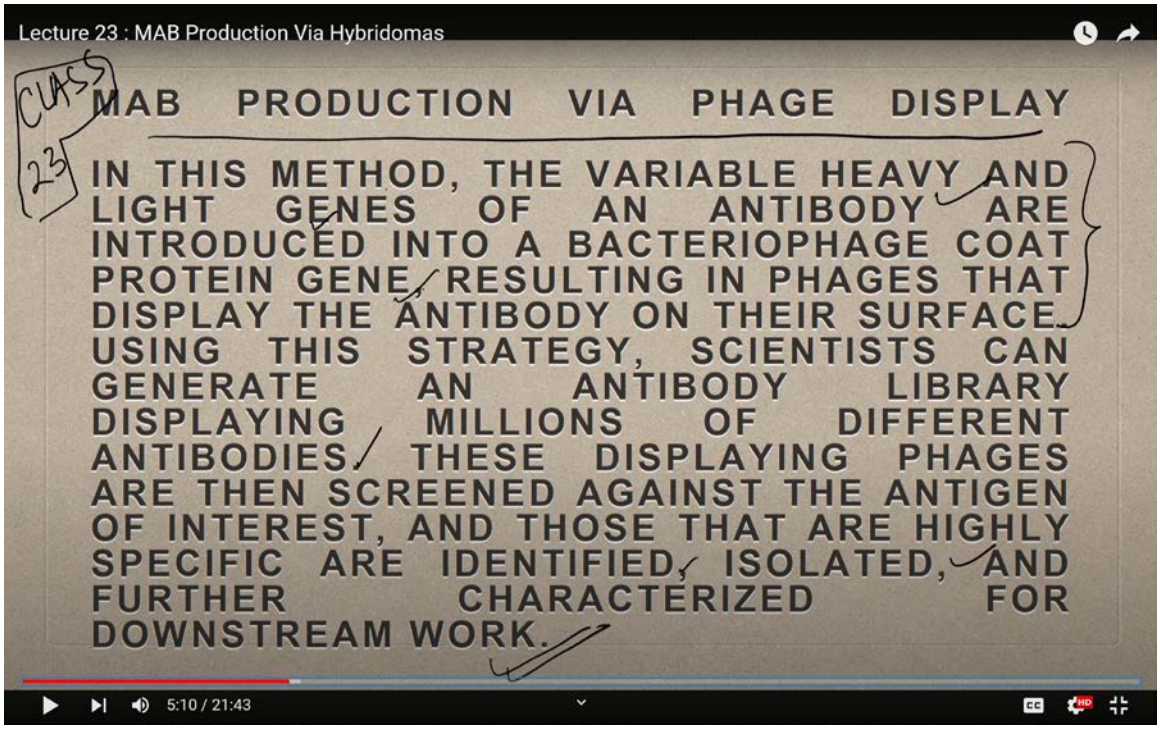

In this method, an antibody's variable heavy chain and light genes are introduced into a bacteriophage coat protein gene, resulting in phages that display the antibody on their surface. So, let me repeat it. In this method, the variable heavy and light genes of an antibody. So, if you look at the structure of an antibody, it has heavy chains and light chains, and there are genes that exclusively code or code for the heavy and light chains. So, what you are doing here is, now let me repeat, the variable heavy and light genes or the genes involved in the expression of variable heavy and light chains of an antibody are introduced into a bacteriophage coat protein gene.

So, you are introducing those genes; you are cutting that part of the DNA and introducing it into the bacteriophage protein gene, resulting in a phage that displays the antibody on the surface using this strategy. So, automatically, this bacteriophage is going to express itself.

So, you will see the antibody's signature on the bacteriophage's surface. So, it's a phage display technology. Scientists can use this strategy to generate an antibody library displaying millions of antibodies.

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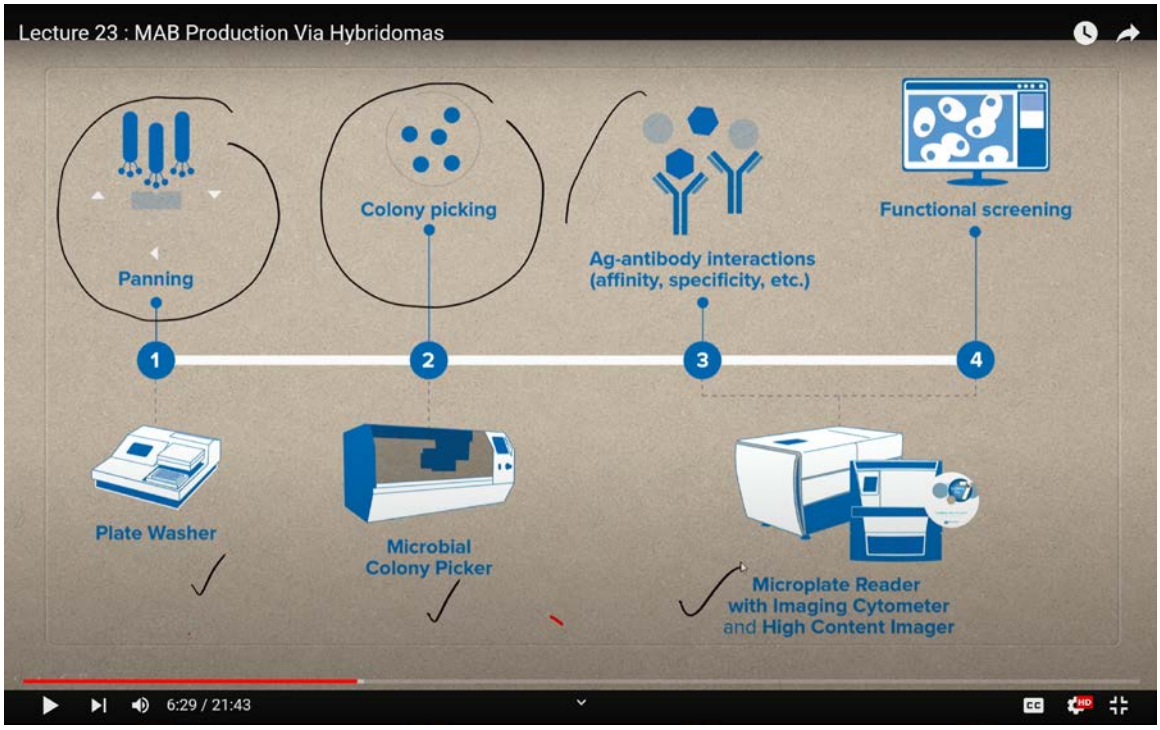

These displaying phages are then screened against the antigen of interest, and highly specific ones are identified, isolated, and further characterized for downstream work. So what you are essentially doing is that for every antibody, there are genes that are coding for those antibodies. It has a heavy chain, a light chain, a variable heavy chain, a variable light chain, and so on and so forth. So, you are trying to pick up that fragment of the DNA, integrate that particular DNA into an external code protein gene of the bacteriophage, and express it. So what you see on the top of the bacteriophage is the expression of those antibodies whose genes you have integrated into the bacteriophage system.

So you have a display on the surface of the bacterial cell, or bacteriophage, whereby you have a better window to screen for a wide range of antigens. So now, how it works is something like this. Using a plate washer, you have a panning process; then you have

colony picking, step two, antibody interaction or affinity interaction, and functional screening. And, of course, you have all the different technologies to do the process rapidly. So, panning is an iterative process for enriching phage within a population with high affinity binding to a target of interest compared to others.

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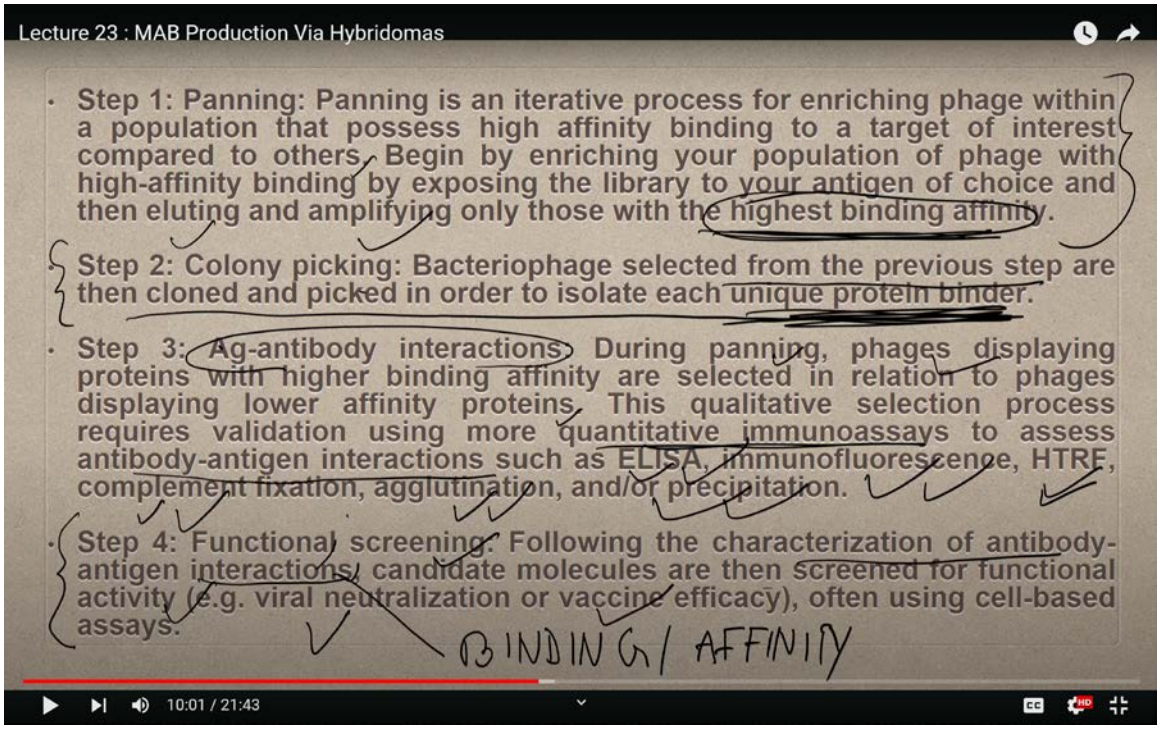

Begin by enriching your phage population with high affinity binding by exposing the library to your antigen of choice and then eluting and amplifying only those with the highest binding affinity. While discussing hybridoma technology, I told you that while screening, you should look for those particular hybridoma cells that produce the highest proportion or concentration of antibodies. So, you have to do something of that sort, which has the highest binding affinity. Then comes step two, which is colony picking. Bacteriophages selected from the previous steps are then cloned and picked to isolate each unique protein binder.

This is the colony-picking step. So, we talked about the planning step. Now, we are in the colony-picking step. Now comes the third step, which is antigen-antibody interaction, AG antibody. This is AG stands for antigen-antibody interaction.

During panning, phages displaying protein with higher binding affinity are selected over phages displaying lower affinity protein. So, this is that binding affinity assay. This qualitative selection process requires validation using more quantitative immunoassays to assist antigen-antibody interactions such as ELISA, immunofluorescence, HTRF, complement fixation, agglutination, and precipitation. So when you talk about binding affinity, we are talking about what force they are binding with each other. So, you need to have very specific assays or quantitative measures.

It's something like one finger. So, for example, versus like this, this is much stronger binding. So, unless you have a quantitative asset to figure out, is it just two fingers are binding with each other, or two fingers are binding with one, or two fingers are binding with two, or four fingers are binding with five, or the whole five fingers are binding with five, you have to have a quantification. Without that quantification, these selection processes cannot be validated in the long run. So those techniques, such as quantitative immune assays, assess our antibody-antigen interactions such as ELISA, immunofluorescence, HTRE, complement fixation, agglutination, and precipitation.

These technologies are of prime importance in terms of quantification. Next comes step 4, functional screening, following the characterization of antibody-antigen interaction, which is the binding interaction or binding affinity. Candidate molecules are then screened for functional activity, that is, viral neutralization and vaccine efficacy, often using cell-based assays. So, in the final game, can it neutralize your antigen? Can it neutralize the virus against which this antibody has been produced? So unless you do a cellular assay of viral neutralization, your technology will not be fully vetted. So, the vetting can only happen when you have a neutralization assay saying that it is okay.

This small amount of antibody neutralizes this small amount of virus. Why this is important from a biosensing perspective because when we are talking about a reaction on a chip. We should have a quantification; we should know how small amount of antibody or antigen

you are depositing on the top of the chip plate. If you know the number of molecules, you know how many opposite kinds of molecules will bind to them and how many signatures. If you remember, I told you that you can detect them using fluorescence, electrochemical, SPR, and AFM assays.

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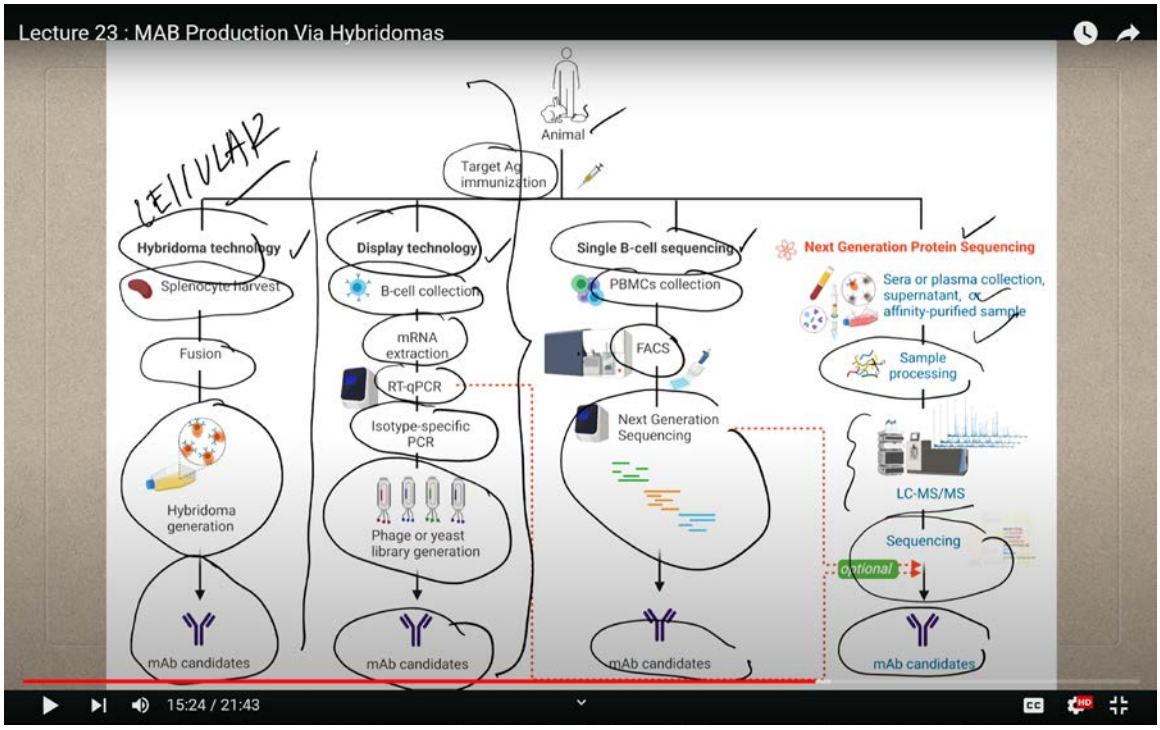

There are a series of assays you can use, but these are all signals. These interactions have to be quantified. So, if you have an assay to quantify them, then you will be able to say, okay, for X molecule, I need opposite kinds of Y molecules. And based on that, I will get this much signature or signal. That's why these individual assays, these individual steps, understanding the basic kinetics of the individual step, understanding how much fluorescent signals will come, how much SPR angle will shift, how much AFM will alter, how much quartz crystal microbalance changes will be able to evaluate, how much electrochemical impedance is going to show up are of immense significance.

So you understand, it's not only you need to understand the biology of the production of these antibodies, antigens, But you have to understand how you can detect them, how you

can quantify them. So that's why modern biosensing is very challenging, even as a course. You have to integrate everything to understand how critical each one of these steps is. And there is no one way you can quantify them. You have to use multiple ways to validate your result.

And that's why I'm repeatedly telling you we are heading into the era of multimodal detection. And this is the key. You have to understand that multimodal detection is changing the face of our understanding of several technologies. Now, if I summarize this whole thing, what I have talked to you about, we talked about hybridoma technology, we talked about display technology, and then, of course, included this single B-cell sequencing and the next generation of protein sequencing. So, here's the animal, target antigen immunization, and hybridoma; repeating it is melanocyte harvest, fusion with myeloma cells, hybridoma generation, and molecular antibody candidate.

This is the cellular technique of hybridoma. Then you have the display technology where you have B-cell collections, mRNA extraction, RT-qPCR, isotypes of specific PCRs, phage or yeast library regeneration, and molecular antibody or monoclonal antibody candidates. While we are doing monoclonal antibody candidates, you have Single B-cell sequencing, PBMC collection, FACS, fluorescent assorted cell screening, and next-generation sequencing assays; you have the MAB candidates. Similarly, you have the next generation of protein sequencing, where sera or plasma is collected, and supernatant and affinity purified samples. sample processing, then you go through LC-MS, MS, then you do a sequencing, and you have the MAB candidate.

So you look at it, there are multiple ways how you can generate monoclonal antibodies. This is just a snapshot of all the technologies that are available to you. You have to have a fair understanding of at least one of these two technologies because these two technologies, hybridoma and display technology, are pillars of understanding how monoclonal antibodies could be produced and utilized. Once you understand these two, the rest will follow. So you're observing that many of these techniques and technologies are extremely, extremely interdisciplinary, and you cannot really shy away from it because you have to understand the production.

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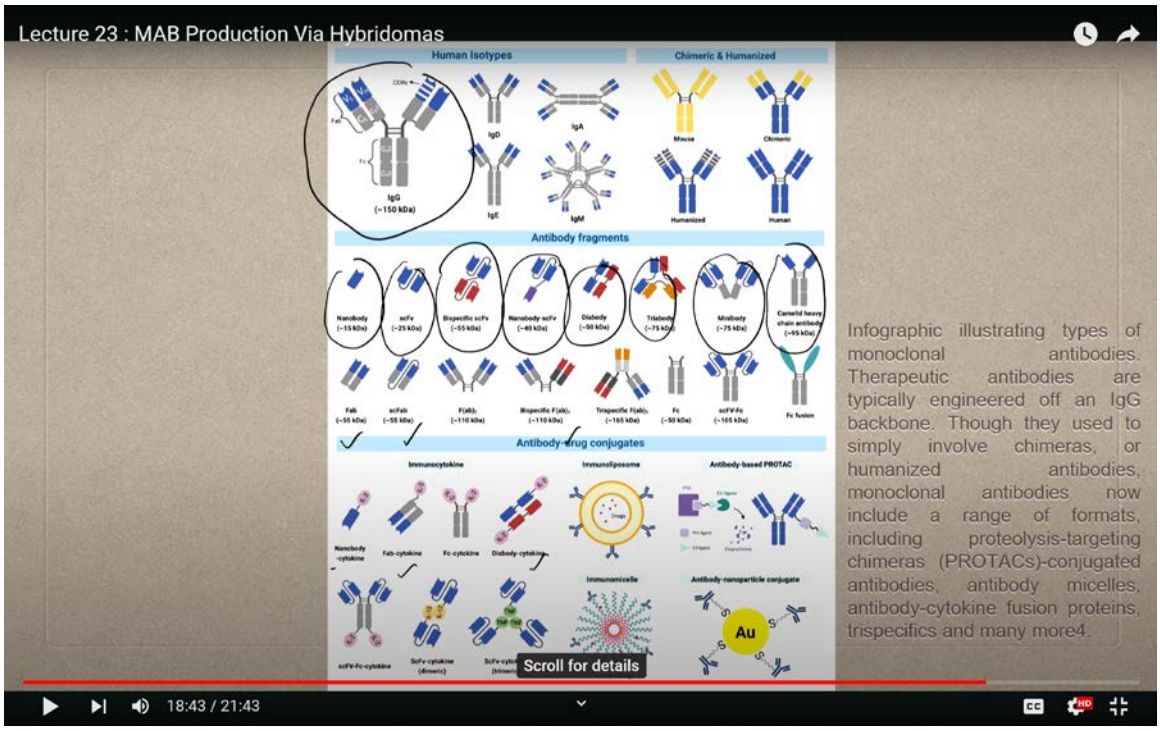

Once you understand the production, you'll have a better hang on the system and how these systems are working. In addition, I wanted to add this slide because there are a lot of terminologies that are coming up. So when we talk about antibodies, I was talking to you about antibodies, which is what the antibody looks like. It has a heavy chain, light chain, variable heavy chain, and variable light chain.

Now, there are terms like nanobody. If you follow this whole picture, you will realize you have SCFV. There are bispecific SCFV. Then, you have nanobody SCFV. These are newer and newer terminologies that are coming. Whenever you read a paper, you come across something and get scared.

Oh, I have no idea about this. But that's why I thought of putting this slide before you. This slide will kind of give you an idea of what are chimeric and humanized antibodies, humanized antibodies, human antibodies, and chimeric antibodies; if you are integrating mice and a human, you are making chimaeras. Then you have tri-bodies, dis-bodies, mini-bodies, camelid, heavy chain antibodies, FEB fragments, SC-FEB fragments, and

bispecific FEBs. Then you have immunocytokine, that is, nanobody, FEB cytokine, diabody cytokine, and you have the interactions. Some of these terminologies are so that you do not get kind of, you know, scared.

These are all newer and newer terminologies, which are basically from the fundamentals of immunology, that there are different fragments. You can isolate the DNA for a specific fragment. You can clone that. You can express it using a phage display or any other technique. And the molecules that are generated have a different kind of naming you are doing.

So, do not get bogged down by the nomenclature of all these things. They are simple nomenclature. They are all over the literature. But what I will highlight for you guys is to understand these two basic technologies, hybridoma and display technology, or fast display technology, because these are the two techniques that are of prime importance for you people. Of course, you should know some of these, or you should not get scared of these different names that are popping up, which will pop up from time to time.

That what is a nanobody, what is this, what, I mean, it is, literature is kind of, you know, completely flooded with this kind of terms, okay. So, do not get scared about these different terms that are being used. So, to summarize, before I move on to the next part of it, is to the basic slide. So, this is where the hybridoma technology started, and even before. So this is where we started this class: immunization process, fusion process of the preparation of myeloma cells, clone screening and picking, functional characterization, scale-up and winning, and expansion.

And somewhere down the line, we talked about this technology where we talk about the panning, and this is your fast display technology, colony picking, antibody interaction, affinity specificity, and functional screening. So these are the four basic steps involved. This is the summary sheet in front of you, where you have hybridoma, display technology, single B-cell sequencing, and next-generation protein sequencing, which are being used. So, as long as you know the framework of this whole process, you are good to go. And as I mentioned, there are multiple such words that will come.

They should not bother you. They are fine. You can deal with them. So you all can deal with these different words that are popping out as long as you understand. Different variants of antibodies and molecular techniques are used to express different parts of the antibody, such as varying low chain, variable low chain, variable heavy chain, or some other part of the antibody. They are being given newer and newer names, nanobodies, these bodies and likewise. So, do not get fumbled by these terminologies, but overall, you should understand this is the scheme of things where you have hybridoma technology and display technology, and these are the potent tools for antibody production and as long as you kind keep these in mind you are good to go.

Thank you, and we will move on to the next class after this.