Design for Biosecurity

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Lecture 22

Monoclonal Antibody Production

So, welcome you all once again for the 20th lecture, and it is the second class in the fifth week. So, the title of this class is Monoclonal Antibody Production. So, in the previous class, we decided that we'd be exploring all the different recognition elements like monoclonal antibodies, single-chain antibodies, aptamers, peptides, and phage displays. So, regarding that, we talked about the monoclonal antibodies.

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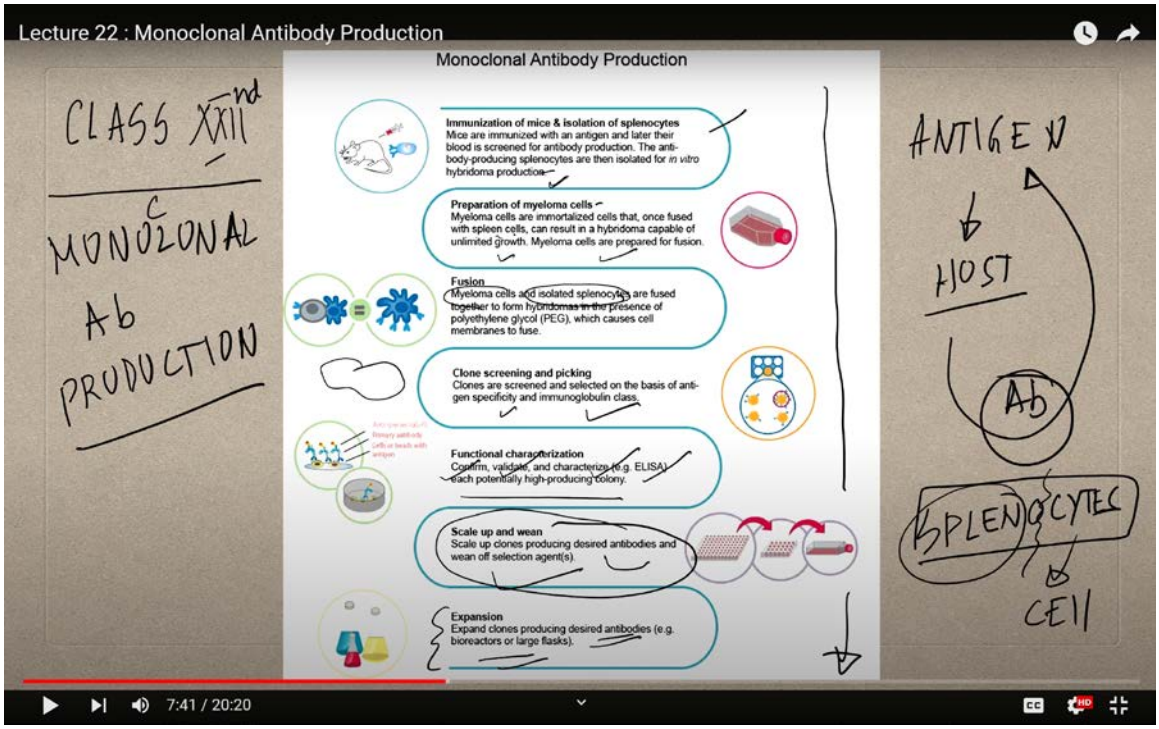

So, today, we will talk about how these monoclonal antibodies are being produced. A brief understanding of monoclonal antibody production is essential for the next generation of

biosensing experts because I believe that it will bolster their capabilities and help them to understand and use this technology with far more perfection.

Now, regarding monoclonal antibody production, the very first step is out here. Immunization of mice and isolation of splenocytes is you are immunizing the mice with an antigen. So you take a foreign material, and you inject it into the mice, as the drawing says. Later, their blood is screened for antibody production.

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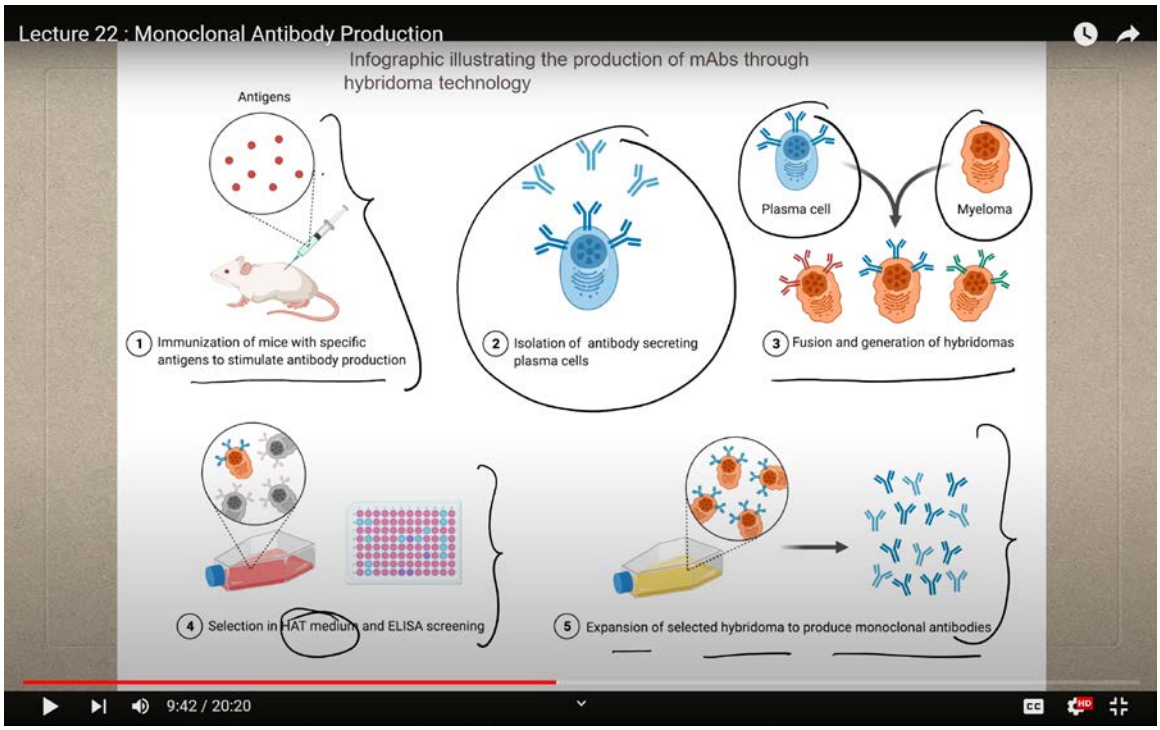

The antibody-producing splenocytes are then isolated for in vitro hybridoma production. So whenever you inject an antibody or an antigen or a foreign element, you inject a foreign element called an antigen into a host. This host could be anything: a mouse or even a human. The body will be producing antibodies against this antigen. So, this is exactly what we are talking about, and splenocytes are the ones that are generating the antibodies.

It's a preparation of myeloma cells. This is stage two. Myeloma cells are immortalized cells. Myeloma is something like a cancerous cell. Once fused with spleen cells, spleen cells are the splenocytes.

If you come across the word splenocytes, it means spleen cells. Cytes means cells. Spleno means spleen. Okay? Esplenocytes. So now, these primary splenocytes or regular splenocytes of your body have a limited life cycle in order to produce unlimited antibodies.

So, say, for example, I inject an antigen into a mouse or a human, and then I isolate the antibody-producing splenocytes. So, I know this particular splenocyte or spleen cell produces the antibody. Now, what I do after screening that particular cell and isolating that cell, I will make a clone of it. I will immortalize that cell. How will I immortalize that cell? Using cell technologies, I will cross it with a cancerous myeloma cell.

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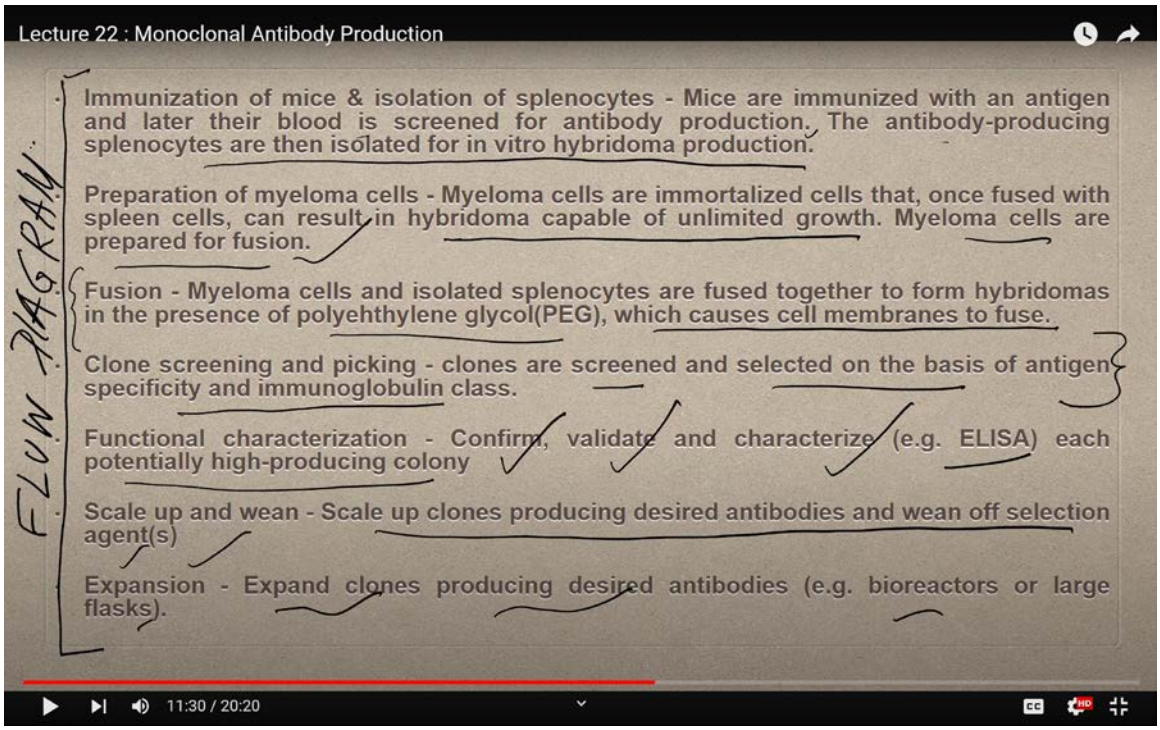

By fusing it with a myelomucil, what happens is that this particular splenocyte producing that specific antibody is now immortalized. I can freeze it, I can thaw it, I can grow it, I can keep it for years together. After 30 years, 100 years even, I can pull it out and make it work. And I'll know with absolute certainty that if the DNA is intact and there is no freezing damage of DNA or something, then that particular splenocyte will produce that specific

monoclonal antibody. So now, coming to the second step again, the preparation of myeloma cells.

Myeloma cells are immortalized cells that, once fused with the spleen cell, can result in a hybridoma capable of unlimited growth. Myeloma cells are prepared for fusion. So when we talk about hybridoma technology, it is a hybrid between myeloma and the spleen cells or antibody-producing cells. And as I mentioned, always remember it like this. CITES stands for cells, and it could be anything.

It could be some other tissue. It will be CITE. Now comes the fusion. As I mentioned, the myeloma cells and isolated splenocytes are fused to form hybridomas in the presence of polyethene glycol, which causes the cell membrane to fuse. So you are fusing both of them.

So you get something like this. Now, clone screening and picking. Clones are screened and selected based on antigen specificity and immunoglobulin class. Antigen specificity means which particular antigen they are neutralizing. So you know that this particular clone is working in that direction.

Now comes the functional characterization, where you confirm, validate, and characterize each potentially producing colony, that is, ELISA. There are multiple colonies that are produced, so you have to check for fidelity in terms of confirmation, validation, characterization, scale-up and wean. Scale up clone producing desired antibodies and wean off selection agent. You're slowly narrowing down, scaling it up, and weaning out the unnecessary ones. Finally, the expansion.

Expand clones producing desired antibodies in the bioreactors or large flask. This whole monoclonal antibody production process is a cellular engineering marvel, where using different cellular technologies, you're fusing cells, you're producing them in large numbers, you're isolating them, you're propagating them. It's an industry of its own. These industries are now merging with the biosensing industry, and a new change or a wave is coming in the whole area of bioengineering and Biodesign, where a lot of these kinds of sensors are being made because you have easy access to a lot of antibodies that are extremely specific for specific pathogens. In terms of the infographic illustrating the process, here you have

the antigen to give you a better idea of those people who have come from different background or who never got a chance really to visualize the whole thing.

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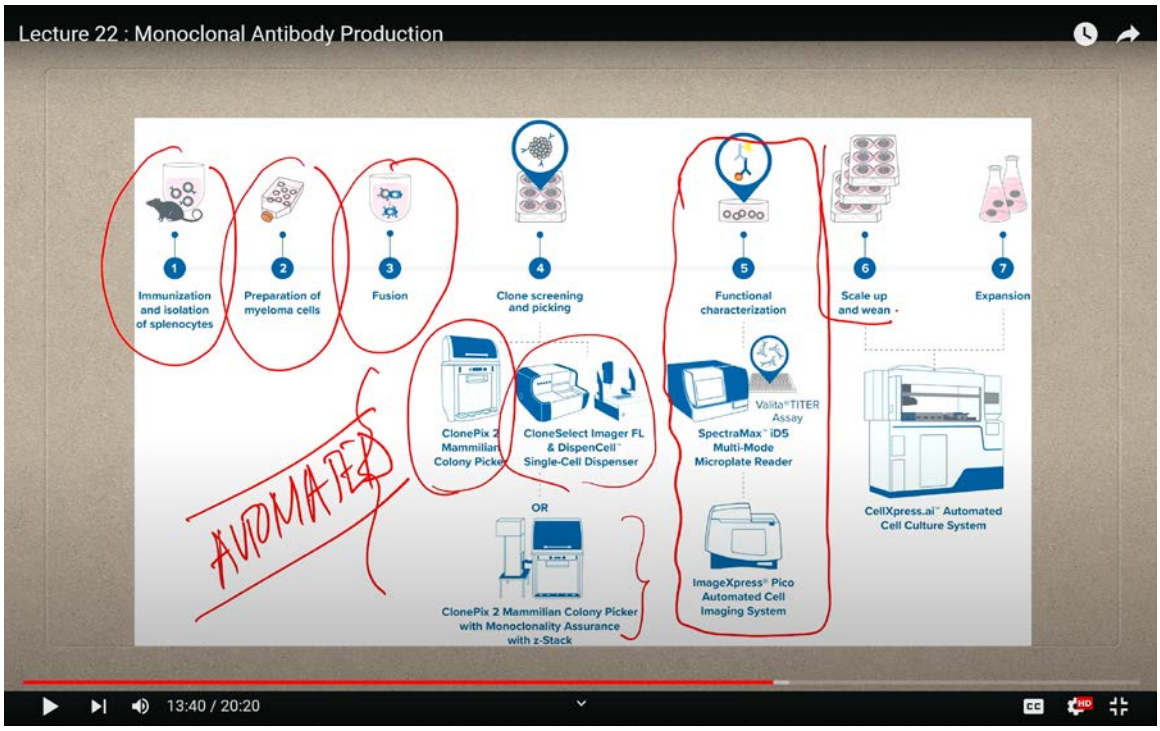

Immunization of mice with a specific antigen to stimulate antibody production. Isolation of antibody screening plasma cells. These plasma cells are then fused with the myeloma cells and fusion and generation of hybridoma. So now, at this stage, you have to screen which one has all the potential of what you are looking forward to. Then comes selection in HAT medium.

So do not worry about HAT medium or ELISA screening because these are cellular technologies where you use a specific medium to grow or select a specific clone or population. There are other enzyme-linked immunoabsorbent assays for screening and, thereafter, expansion of the selected hybridoma to produce monoclonal antibodies. So this is one simple way for him. Other ways will be coming, but this is an overall understanding you will need about the monoclonal antibody production system. Now, we are talking about the steps of immunization of mice and isolation of splenocytes.

Mice are immunized with an antigen, and later, their blood is screened for antibody production. The antibody-producing splenocytes are then isolated for in vitro hybridoma production. In vitro means you are producing it outside the body. So the first part was inside the body. Part 2 is outside the body.

That is why it is called in vitro. Preparation of myeloma cells. Myeloma cells are immortalized cells that, once fused with the splint cells, can result in hybridoma capable of unlimited growth. Myeloma cells are prepared for fusion. Next comes the step of fusion.

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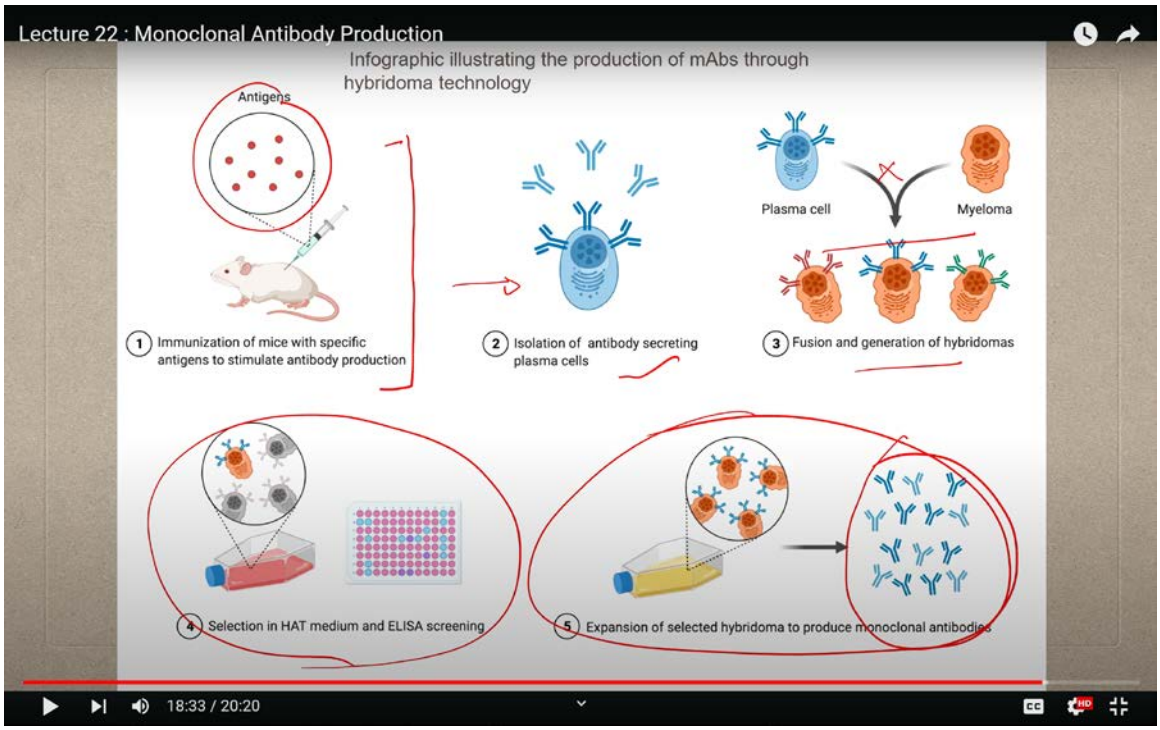

Myeloma cells are isolated splenocytes and fused to form hybridomas in the presence of polyethene glycol, which causes the cell membrane to fuse. Then comes clone screening and picking. Clones are screened and selected based on antigen specificity and immunoglobulin class. Then comes the functional characterization, where we confirm, validate, and characterise them using ELISA or potentially high-producing colonies. Scale up and wean, as I mentioned.

Scale up the clones producing desired antibodies and wean off the selection agents and expansion. Expand the clones, producing desired antibodies, bioreactor, or large flask. So, this is the overall scheme of things or flow diagram that one must remember while producing monoclonal antibodies. So, if you summarize the whole thing, the process of immunization, preparation of myeloma cells, fusion of the myeloma, clone screening. There are a lot of technologies currently being used for clone screening.

There are different vendors like mammalian colony pickers; then you have clone select imagers, single-cell dispensers, mammalian whole colony pickers and monoclonal assurance with the Z stack. As I mentioned, a series of multi-modalities are now used for clone selection. Otherwise, it used to be a very tedious process. Well, you have to go under the microscope and observe each and every colony, and you have to pick it up. But now, this whole process has been automated.

But the basics are the basics. The basics haven't changed. It is just for ease and time-saving. The technology has been automated over a period of time. From here, you have the functional characterization. Again, a huge amount of automation has happened.

Like, these were not there, at least I remember during my postgraduate days when these technologies were picking up back in the 90s. All these automation and use of robots were brewing in the R&D wings. But today, almost now 30 years down the line, things have changed. Things are much more automated and much more simple, yet the basics are the same.

So always remember the basics. Try to understand the basics. How can you operate in this situation if you do not have these automated systems? And then comes the scaling up. Again, there are a lot of very good quality bioreactors that have developed and are expanding using different kinds of multimodal equipment. So, overall, if you look at it, this is a very basic fundamental process. You inject an antigen, the body generates its antibodies.

So, you isolate the antibody-producing splint cells. You take these splint cells and isolate the specific antibody-producing ones, which you are looking for because different splint

cells will be producing different kinds of antibodies. Now, that particular splint cell, you fuse it with a myeloma cell using polyethylene glycol where the membrane merges. And this we call as a hybridoma. This hybridoma is further being screened for the production of monoclonal antibodies.

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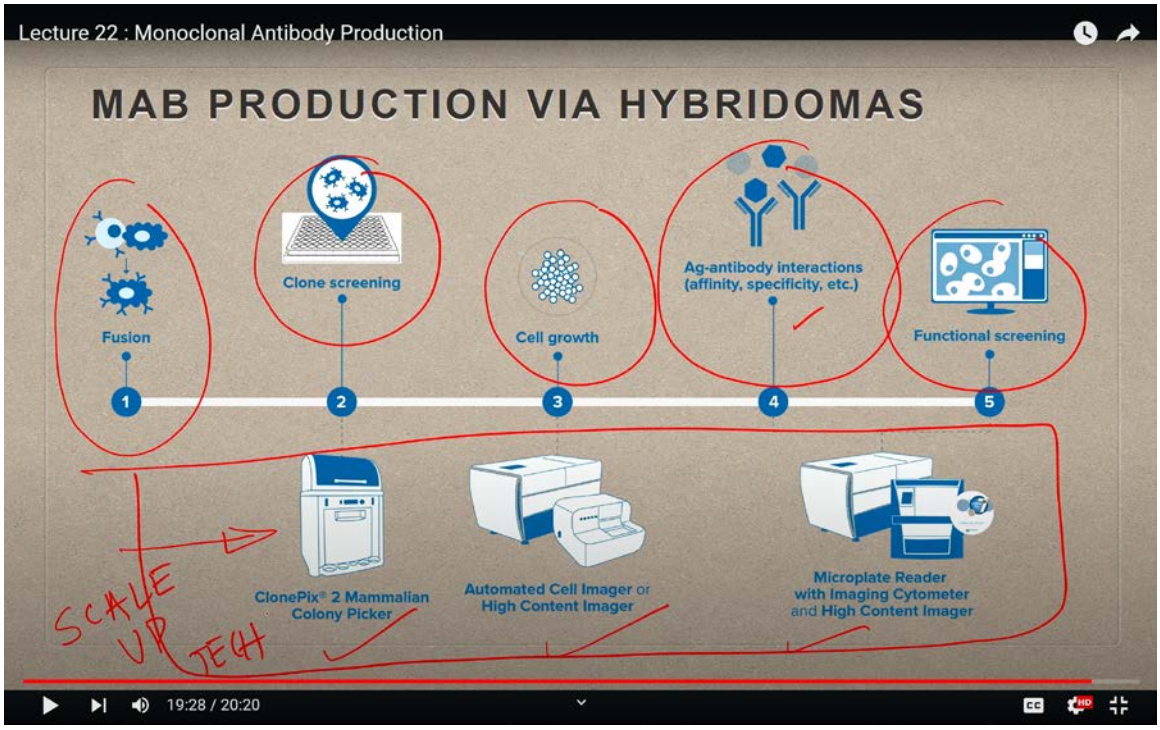

This is the whole spectrum of things. Now, during this process, there are zones where there is a lot of room for automation in terms of how you are selecting the clones, how you are screening the clones, how you are propagating the clones, how you are weaning off the unnecessary, or at that particular instance, the ones which are not important, and how you scale up or jack up the production of these clones. These technologies have made monoclonal antibody production a much easier technique. So, this particular technology is hybridoma-based. There are other technologies for monoclonal antibody production. What we'll be discussing is called phage display.

Further selection methods, which are a little bit more molecular biology intensive techniques, unlike hybridoma, a cell-based technique, are being used. And further, this

monoclonal antibody production has revolutionized the whole field of immunocytochemistry, especially in development biology. And all other fields of disease progression because whenever we see a signature, these are all recognition elements; whenever we see a specific signature, we produce that monoclonal antibody. So, in a way, immunology has equipped us with very specific tools for identifying pathogens, identifying how the pathogen reacts and what kind of reactions. It shows how the anti-molecule towards the pathogen or an antibody fights against it and where the antigen changes its properties and evades or tricks the antibody system.

And we have learned a lot over the last three or four decades in terms of the AIDS virus, in terms of prion disease, in terms of COVID, in terms of some tricky cancers, in terms of neurodegenerative disorders. All these situations are situations where the body fights. And whenever we say body fights, it means the body is producing antibodies. So, the antibody-antigen reactions always bring about the orchestra of the body fighting for its survival. And this technology is an asset for biosensor production.

So overall, the basics remain the same. If we go back and check this, this is step one, where the body is doing its own work of fighting against the particular antigen that is getting injected. Isolation of the plasma cells, the crossing of the plasma cells with myeloma cells, fusion, and then selection. Then, expansion and further production of these monoclines, right? So, this is the overall spectrum of hybridoma technology for monoclonal antibody production. And if you look at it graphically, there's a fusion process, clone screening process, cell growth, antibody interaction using affinity specificity, and functional screening. These are the different technological breakthroughs in terms of mammalian colony picker, automated cell imager, high content imager and microplate reader, imaging cytometer and a high content imager.

So, if you look at it, this bottom line out here is scaling up technologies that are revolutionizing hybridoma production and saving time and energy. So, in the next class, we will move on to the next technology for monoclonal antibody production, which is a fog display technology. Thank you.