Steve Lewis 00:10
Welcome to Speaking of Mol Bio, a podcast series from Thermo Fisher Scientific about molecular biology and its training applications in life sciences. I'm Steve Lewis, and today I'm delighted to welcome Dr. Leif Larsen to the show. Leif is the Director of Biology at Vipergen, a small molecule drug discovery services provider using DNA encoded library technologies to find hits and leads. This conversation is a fascinating look at how molecular biology and innovation in screening technologies are reshaping drug discovery. We begin by asking Leif about his professional background and the areas that he's explored during his career. I hope you enjoy.

Leif Kongskov Larsen, PhD 00:57
So I'm trained as a molecular biologist back to the university days in in the 80s or and 90s. So, where I've been working with transcription regulation and so on. And then I have been working, actually at Vipergen for, since 2008. But before that, I was in other CROs, been working a lot on assay development, assays for different compounds, interaction with proteins and so on. So, it's like my whole career has been focused on drug target interaction I'd say.

Steve Lewis 01:36
That's a really cool area, and something I definitely would love to learn more about and I think drug discovery is very much a area of interest for our listeners. Very, very difficult area of science. What got you into it in the first place?

Leif Kongskov Larsen, PhD 01:54
I think in so the drug discovery part, what got me into that was that it was that I got a really exciting possibility to work with these DNA encoded libraries and the screening of those and, I mean, it's actually quite fascinating for me. A lot of molecular biology, I mean, it's really, it's so complicated. But in a way, you can also, if you can visualize it inside your head, it's actually very much like building Lego. That's also, that's also a Danish company actually. Making small blocks that you can put onto each other and then just put them together and build stuff. And actually, that's, that's, that's really satisfying way of thinking of something that is very complex but actually trying to put it down to how to put these blocks together to get it to work. So, and that's really fascinating for me. At Vipergen, what we're doing is that we are screening the targets that that our customers provide us with against DNA encoded libraries. So that's very large chemical libraries. That's a service that we provide. What is really special about what we are doing is that we are doing this screening in living cells, which is quite difficult for some reasons that we will also come into, I think. And we are using some very large cells for that, because then we can micro inject. DNA encoded libraries have the problem that they are difficult to get into a cell, because large pieces of DNA are not just readily taken up by cells. But we can micro inject into oocytes, and in that way, we can get the DNA in and do the screening in the cells, which is really a significant different thing from what everybody else is doing.

Steve Lewis 03:54
And it's a it's a platform technology. So in your group, it sounds like you focus on everything from assays to sequencing, and then also, of course, screening, as you mentioned?

Leif Kongskov Larsen, PhD 04:06
Yeah. The screening is really what we focus on and what we're trying to improve all the time, the methods we're using and so on. And maybe, just to start from the beginning, maybe we should talk a bit about what is a DNA encoded library?

Steve Lewis 04:22
Please.

Leif Kongskov Larsen, PhD 04:23
Because I think that it's important to have that concept in place to understand the rest of it. So, imagine when you have a target and you want to find a compound that interacts with that target. That could be the target, could be a protein, for example. And you want to find compound. And what people have been doing is sort of some sort of setting up some sort of assay and doing some high throughput screening and see how that can be done. And that mean you have to make a lot of compounds; you have to administer a lot of wells and a lot of microplates and so on. But at DNA encoded library, the philosophy is sort of put upside down. So what you do there is you synthesize a lot of different compounds, but each compound is actually put onto a piece of DNA, and the piece of DNA works as a barcode for exactly that compound. And that means that you can, you can synthesize the compounds, and you can do that by mix and split, and you can do it in some other means, but, but you can synthesize them and have them all in one in the same tube, so to speak. So actually, what we use our whole library, or the libraries we're using, they are just stored in one small PCR tube. That PCR tube can actually contain, like we normally work at, compound libraries that have the size of 500 million different compounds. Imagine 500 million different compounds with one single PCR tube, but each with a unique DNA barcode that if you sequence that barcode, then you can find out what was that exact compound.

Steve Lewis 06:03
So it's a very efficient way to screen large compound libraries?

Leif Kongskov Larsen, PhD 06:08
Yeah, and what, what, what, what DEL screening actually is, is all about, is that you try to identify the compounds, and the compound and compounds often is more than one, in that set of compounds that are able to bind the target. What we do is so we have set up. We can do it either for targets that are just a purified protein. That's actually the more difficult thing to do, in a way, because what we do there is that we can, we can label the target with another piece of DNA, and then we can mix the target, the DNA label target, together with the DNA encoded library. Allow them to bind. We dilute it very much, and then we make an emulsion. And in those droplets, the library molecule can end up in a droplet together with the target. And in principle, if we make enough droplets, then that will only happen if those two are bound together during the binding of it. And then what we do next step is to activate the DNA ligase so we can ligate those two pieces of DNA. And then it's just a matter of actually a lot of DNA purification, breaking the emulsion first, then purify the DNA, do a PCR in order to isolate those DNA fragments that have sort of both ends, and then sequence which compounds were actually the ones that bound. So that's one way of doing it. But actually the what I really would like to talk more about is the newest invention we made for this, and that is that we are doing this in a living cell. And the living

cell we're using for doing that is an oocyte from *Xenopus*. They are quite easy to work with because they're quite large, so they're one millimeter in diameter. And that actually means that we have a reaction vessel that encompasses more or less one microliter. And what we do there is that if you come to me tomorrow, you say, “I have this exciting new target. I don't know so much about it, but I know the sequence.” And then you provide me with the with the sequence of that protein, and then I will just make the worst translation so I can so I can find out which DNA sequence could encode this protein. And then I'll have the DNA made synthetically. And they're fused together with another small protein that we call the prey. So in principle, you will make a fusion protein consisting of the target plus the prey. And that fusion protein will make messenger RNA out of out of the of the cDNA that was synthesized. And then we'll inject that messenger RNA into an oocyte. And these oocytes, they are protein factories. They are working real well for that, they can make a lot of protein. So we'll allow that to happen for a few days to express that fusion protein. And then after that, we can do what I talked about in the beginning, we can micro inject the library, those 500 million different compounds each with a piece of DNA into the oocyte and allow the binding to take place in sort of like a really, say, relevant, micro environment, where, in the end, talking about. I mean, the idea about all this is, in the end, is to develop some sort of pharmaceutical. And, I mean, in the end, it should work in a cell. So it's so for us, it seems very logical to do this initial binding and this initial screening, to do that in a cell. So we allow the binding to take place in the cell, and then together with the library, we inject a small piece of DNA, and that piece of DNA has a small molecule sitting on it, the bait that can bind to the prey part of the fusion protein. So now in the cell, what happens is that library molecules can bind the target and the bait can bind the prey part of the fusion protein, and now we have, sort of, it's like I told you before, we have two pieces of DNA bound to the same target protein, and then we break the cell, we'll dilute very much, we'll make an emulsion, and then we're back at what we talked about before. So we can just purify the DNA, or ligate, those two pieces of DNA in the emulsion, purify the DNA and PCR, and then it's just a matter of sequencing, and of course, a lot of computational work in decoding all these sequences.

Steve Lewis 10:56
It's an incredible process. And my understanding here is that you having the intracellular ability allows for a diversity of protein classes that you can screen for. So is it most of the cytoplasmic proteins?

Leif Kongskov Larsen, PhD 11:16
Yeah, so, cytoplasmic proteins is actually where we started, one could say, with sort of like some standard targets, a p38 alpha is one that was screened very much and good. It behaves really well on the system. So the system was made for that originally or made using that target for optimizations. But actually we have shown that we can also do transcription factors, for example. Transcription factors, this is special class of protein. They are very interesting cancer targets for many types of cancer, but they have proven to be very difficult to screen actually. They also have so when you're talking with DNA encoded library screening people, then they would say that they are very difficult to work with because they bind DNA sequence specifically. So maybe they will just bind some of the codons or some of the coding you have in your DNA on the library and give you some false positives. But we have found out ways to avoid that, actually, so, so, it's possible to work with transcription factors. One of the newest developments is that we have started working on membrane proteins that we can screen from the inside side of the cell. So that's also very exciting development, which is quite new and very exciting.

Steve Lewis 12:38
And you refer to this, this proprietary process as binder trap enrichment. Is that correct?

Leif Kongskov Larsen, PhD 12:46
Yes.

Steve Lewis 12:46
And some of the benefits from, from my understanding, is you often avoid the matrix effects that are often seen with solid phase binding. How does that work?

Leif Kongskov Larsen, PhD 12:58
It works in that way because we are working in solution all the time. I mean, we have these emulsions, and we don't normally, or most people, when they do DNA encoded library screening, is that they do the binding of the library to the target, and then they bind to some sort of matrix, nickel NTA beads or an antibody or streptavidin, and then wash away all the unbound library members. But there you have the problem with the matrix. So if you have any binders in the library that bind the matrix, a nickel NTA resin or something, then you will get those hits up all the time. Maybe the major advantages of working in the oocytes is that it's extremely fast, because normally if you want to start a project around some target, then the first thing you have to do is actually you have to clone that target, and you have to express the protein. Also you have to have it relatively pure in order to avoid false positives and so on. But for the screening in *Xenopus* oocytes, we actually just need the sequence, because the specificity is built in in the way that we can bind the DNA to the target. Also the bait DNA via the prey. So specificity is sort of built in in system, and we don't need to have sort of the part where the customer provide a very pure protein that is normally needed for doing DNA encoded library screening.

Steve Lewis 14:24
Compound question here. What was the inspiration for this approach, and did you view working in living cells as a critical component of the development?

Leif Kongskov Larsen, PhD 14:39
Well, maybe I'll turn it a bit upside down and maybe talk a bit more of the advantages of the oocytes. So one thing is, when we compare, we have targets where we have done both ways, either doing just the sort of the BTE. With pure, purified proteins, and also done the BTE in the cells. And what we can see is that for some targets, the cells simply work better, so we get more hits, and it just the process is just working better. Another thing is that for some proteins, it's actually not possible, or is very difficult, to sort of supply protein of the of a purity that can be used for the process. And for those proteins, it's really, is really critical that we can, that we have some other way of doing it, and that would be in in the oocytes.

Steve Lewis 15:37
Are there any limits to the size of the proteins that you can screen for?

Leif Kongskov Larsen, PhD 15:42

Well, that's a that's a good question. So, the limitations would be sort of how large a piece of synthetic DNA can you either synthesize directly or assemble. And also, of course, you need to have a capped messenger RNA to inject into the cell. And the capping processes might not be so efficient if you if you had a very long messenger RNA, and at least not when you're using the kit we're using. Normally, caps, puts on the cap at the same time as the transcription is made. So that might put limitations. But actually, in practice, we've been working with very large proteins. So we're talking single polypeptides around, I think, 400 or 500 kilodalton.

Steve Lewis 16:34
And I think it's very interesting doing it in physiologically relevant conditions as well. One of the things that I learned about as I was reading about Vipergen's technology is the turnaround time, because you can do everything kind of in a batch process is really accelerated?

Leif Kongskov Larsen, PhD 16:56
That is true. And so the turnaround time for doing the sort of classic BTE. For taking, getting a purified protein, and then, then, then get, do the screening and have the hits structures have found out what they are. That is, that is quite fast. I mean, it's like you can do that in maybe a month or so. Where I really think that we excel is, again, going back to the oocytes, because you don't need to have all the pre work with the protein. So actually, if you today told me, “Okay, I think this protein could be interesting to look at.” Then in like four weeks, I would say, “Okay, so my first step would be to have the DNA synthesized, inject the RNA into the oocyte, make a western blot to ensure that it is expressed actually at the right size and so on. That would be done in the first month, and then we can do the screening. That would maybe take a month, or maybe even a bit less.” So, it's incredibly fast from, say, from not from purified protein, but actually from idea of the project, to actually have some hits. So, and that's really exciting, I think.

Steve Lewis 18:08
And this is pretty applicable across many different disease areas, too, correct?

Leif Kongskov Larsen, PhD 18:15
In principle, I say that we are disease area agnostic, because, because it doesn't, the disease area doesn't really matter to us, as long as we can express the proteins in the oocytes. I mean, the only limitation is that if the protein we express is toxic to the cell. That might, in theory, at least, be a problem, but we almost never see it. So, yeah, almost any disease area, any protein, of course, it has to be accessible from the cytoplasm. And talking about, we talked about transcription factors before, and the reason we can screen transcription factors is the screening we're doing is in the cytoplasm of the cell. So a target has to be accessible from the sort of cytoplasmic side of a cell. But that seems to work really well for many targets. If you take, if you take receptors, for example, then there is an insight that you can access from cytoplasmic side. If you take transcription factors, even though they are working in the nucleus, then we have pretty good success with screening them just in the cytoplasm.

Steve Lewis 19:27
And still, again, benefit being the physiological relevance too, in inside the oocyte, which is just very fascinating as a concept for your company.

Leif Kongskov Larsen, PhD 19:40
Yeah, I think so. I think also, if I mean, the way I think about this is that if you so doing screening, I mean, normally you do it in a buffer, you have some, you have some HEPES, you have some TRIS, you have a bit of salt, you have maybe a bit of detergent, and so on. But the complex microenvironment of a cell, and the crowding effect of all the solutes that are present in the cell. It's simply it's not the same. So, it's much better to do it in in the cell I think.

Steve Lewis 20:14
Here at Speaking of Mol Bio, we're always excited to share the latest technology for use in your molecular biology workflows. Introducing the Applied Biosystems PowerFlex thermal cycler from Thermo Fisher Scientific. Built on decades of innovation, PowerFlex delivers the accuracy and flexibility your lab needs. With temperature accuracy within 0.15 degrees Celsius, seamless compatibility with full skirted plates, and the ability to run multiple protocols at once, PowerFlex helps you increase throughput without compromising performance. We continue to push the boundaries of innovation so your research and discovery can do the same. Learn more at Thermo Fisher.com/powerflex. The PowerFlex thermal cycler is for Research Use Only, not for use in diagnostic procedures. And now back to our conversation.

Steve Lewis 21:15
Now, let's pivot to talking a little bit more about the design and the molecular aspect of what maybe a customer might do with your company, or you might do yourself. What, what is the initiating process for, for working with Vipergen?

Leif Kongskov Larsen, PhD 21:32
So the initiating process, again, from, if we talking about working in oocytes, is that, of course, all the agreements have to be made that sometimes more us scientists, and I consider, I know I have a very nice director hat, but actually I see myself more of a scientist than one pushing the paperwork. So the paperwork has to be done, right. But that done, all we need is actually, again, this the sequence of the protein, and then we can more or less get started in doing the designs and so on. So that is actually all the customer has to provide us. So GenBank number, UniProt number is fine. And then we're, then we are going.

Steve Lewis 22:28
After a customer sends you a sequence, I assume there's some kind of de novo gene synthesis associated with that, and then your company probably focuses on the drug screening?

Leif Kongskov Larsen, PhD 22:40
Yes, that is true. So, we don't make DNA synthesis work here. But, I mean, there are really excellent providers that can, that can make large pieces of DNA at a reasonable price, and so on. I mean, Thermo is one of them, I can say.

Steve Lewis 22:56
That's right, that's our team out in Regensburg, Germany. We like them very much. And I'll just say that's very common today. Gene synthesis is becoming more of a manufacturing science, in a way, and a lot of companies are doing that approach, because the high impact work is the screening work that you're all doing. And I think the specificity aspect of what you're doing is really a tremendous benefit for your customers.

Leif Kongskov Larsen, PhD 23:28
So for us, I think what we try to concentrate and to focus on the things we're good at. And then, of course, if we need to do some stuff ourselves in order to have the speed that we need. That goes actually for DNA sequencing. So we have our own sequencer. We didn't have that in beginning, but we simply find out that we need in order to continuously improve the process we're working on, then we need the data from the next experiment. We need it actually, yesterday. So speed is really of importance for us. I think for the DNA synthesis part, speed is best provided for those that have it set up industrially. So that's really a cadeau to those people doing that. But for sequencing, we just need to be able to have the DNA in our hands and then not ship it anywhere but just go down and put it on the sequencer so we can add those data fast.

Steve Lewis 24:28
That makes sense. And as we're nearing the end of our conversation, I'd love to ask, as we've been speaking today, is there anything that I've missed that you might want to share with our listeners?

Leif Kongskov Larsen, PhD 24:40
No, I think, I actually think we have come quite well around the subject. So also, you were nicely prepared, Steve. So you could put the right questions at the right time. Thank you for that.

Steve Lewis 24:55
Well, thank you very much. Now I always end our podcast. Us with asking two questions. The first question is, if somebody wanted to follow in your footsteps, maybe that's a younger scientist, what advice would you give to them?

Leif Kongskov Larsen, PhD 25:13
That might be, I don't know how easy that is, and I don't know if my way to where I am today has been so straightforward. But I think it is to find something that you can find passion for. Because in the end, what you need to do in order to succeed in your career is to do well, and you do best with things that you really feel passion for.

Steve Lewis 25:44
I love that. And for our loyal listeners, I'm actually going to change our second question for season four, and that's, what technologies are you the most excited for over the next five to 10 years?

Leif Kongskov Larsen, PhD 25:59
For Vipergen and the way we're doing things is that I have seen that some efforts in SPR. SPR is surface plasmon resonance, and it's a way where you can, where can, it's a biophysical way of

measuring the interaction between, say, a protein and a small molecule. Actually load just a crude cell extract on the on the flow cell. And you can get that to work because it's pairs so well with the technology we're working on. But so that would be one thing to mention, but maybe I put another one also because it's so good a fit to say DNA encoded libraries, and that is targeted protein degradation, and especially talking about talking about DNA encoded libraries, then DNA encoded library screening is so well fitted to PROTACs. So PROTACs are molecules that consist of one part binding E3 ligase and another part binding your target. And since molecules coming out of DNA encoded libraries, they are selected for binding, not for our activity. Very often there's a there's really a great coincidence between those two things. But, for PROTACs, I mean, it's just a one to one. If a molecule from a DNA encode library screen can bind your target, then there's a good probability that you can actually make a PROTAC. And also already, because you have this DNA tag sitting on the compound in your library, then you also know the exit vector. You know where to put on the other end of the PROTAC. So that technology is really so well fitting to what we are doing here.

Steve Lewis 27:54
If you don't mind, what is exciting about that and what is PROTACs?

Leif Kongskov Larsen, PhD 28:00
So PROTACs are proteolysis targeting chimeras , and the chimera is because it's two functionalities in one molecule. So one functionality binds an E3 ligase that can ubiquitinate the target protein. And the other functionality is a binding functionality to the target you have. So you can, basically, you can bind the E3 ligase together with your target. Then you'll have it ubiquitinated and then it'll be degraded eventually in the cell.

Steve Lewis 28:33
And the implications in research, what do you see as being the benefit?

Leif Kongskov Larsen, PhD 28:39
So the benefit here is that if you're talking about sort of like drug discovery, or drugs in such as a general term. So what most drugs do is that they bind their target and then they inhibit it, but they don't remove it. But if you take like PROTACs, then the idea is that you'll actually direct your target for degradation, so you simply remove that protein from the cell. So, and that's a new modality, and it has some advantages, advantages for proteins that you cannot readily just inhibit it. So, if it's not an enzyme, what do you want to do with it. If you can just remove it, then that's actually a very good way of curing a disease. Could be.

Steve Lewis 29:34
I love it. Thank you so much Leif. I really appreciate you taking the time with us today. It's been very exciting getting to learn more about Vipergen and yourself, and we really appreciate you taking the time to speak with us today.

Leif Kongskov Larsen, PhD 29:49
Thank you so much. It's been fun.

Steve Lewis 29:53
That was Dr. Leif Larsen, Director of Biology at Vipergen in Copenhagen, Denmark. We've got more episodes dropping soon, so make sure to subscribe to get new content as soon as it's available. Until next time cheers and good science. Speaking of Mol Bio is produced by Matt Ferris, Sarah Briganti, and Matthew Stock.