Speaking of Mol Bio Episode 6 - S1E6-Global-EXT4959

Transcript
Dr. Gabriel Alves 00:09
Welcome to *Speaking of Mol Bio*, a new podcast series about molecular biology and its trending applications in life sciences. I am Dr. Gabriel Alves.

Steve Lewis 00:20
And I'm Steve Lewis.

Dr. Gabriel Alves 00:22
In our first season of *Speaking of Mol Bio*, we are focusing our conversations on four exciting application areas: CRISPR cell engineering, multi-omics, exosomes, and single cell analysis. And today, we are returning to the world of CRISPR cell engineering with our conversation with Dr. Doris Beylkin.

Steve Lewis 00:43
Doris holds a PhD in molecular, cellular, and developmental biology, and boasts over 10 years' experience as a technical support specialist at Thermo Fisher Scientific, GE Healthcare, and other companies. She rejoined Thermo Fisher last spring, and now serves as the company's global market development manager. We hope you enjoy our conversation.

Doris Beylkin, PhD 01:07
CRISPR really is a game changer, I think it's really the first time that we can really manipulate DNA within a living cell and change it randomly to knock it out, or very specifically to make a specific change in the genome. It's really enabled a lot of research for whether it's loss of function, or basically changing the function of a gene so that you can really see what mutations and manipulation of that gene can create.

Steve Lewis 01:45
Yeah, CRISPR, when it when it first came out, and some of the seminal papers were written, I believe published around the 2014, 2015 timeline, I do remember that one of the concerns for it was designing synthetic guide RNA, as well as off-target effects. And we've come a long way, in even less than 10 years. Can you speak about some of those developments?

Doris Beylkin, PhD 02:14
Yeah, absolutely. So, there was a lot of talk quite early on, you mentioned off-target effects, and I think there was quite a bit of debate actually in the field of whether that was a big deal or not. And I think a lot of folks were really comparing it to RNAi, which at the time was really the best way for us to be able to manipulate or do loss-of-function studies. And off-target effects are really a known issue with that methodology. And certainly, we've developed ways to deal with that. But I think with CRISPR, for the most part, it seems a lot cleaner. And you see that it's really clear when you take a look at high-throughput screening data, and you're looking at a large number of what would be considered negative controls, there's a lot less noise with CRISPR than with, with siRNA, shRNA. And so, I think that really did cause a lot of excitement. There were some experiments where they did show quite a bit of off-targeting. And I think a lot of the ways that we deal with that is really just kind of minimizing the amount of time that each of the active components are present. And that tends to help minimize the accumulation of those types of off-targets.

Dr. Gabriel Alves 03:39
And for people using CRISPR, what are one of the most common problems they face besides off-target effects?

Doris Beylkin, PhD 03:47
Well, I think it all starts with delivery, because the experiments are all done in cell culture. And so, you need to be able to get all of the components into the cells. And that consists of an RNA guide, and that can come in various forms, and then the Cas-9. And the Cas-9 really has to be either a large piece of DNA, plasmid, for example. You can use mRNA, you can use protein, but those are pretty large molecules, and you need to get them into the cells. I think that's probably the first place I would look for troubleshooting if anyone was having difficulty with getting their knockouts.

Dr. Gabriel Alves 04:33
Doris, what are some mol bio methods and techniques that you think that are most fundamental and people that are performing CRISPR should be familiar with?

Doris Beylkin, PhD 04:45
Yeah, so, anytime you're talking about manipulating DNA, of course, you're thinking molecular biology. So, you know, one of the major things that you'll need to have some experience with, or you will gain experience with and CRISPR experiment is, you know, PCR and sequencing. Because one of the first things that you'll want to do is really kind of assess what kind of changes did you get in the cell in your gene where you're targeting to understand anything else that you're measuring downstream in terms of phenotype. On the flip side of that, certainly looking at the protein as well is going to be important to really understand if you have a functional knockout. Usually, you'll want to do some kind of a functional assay as well. So, really understanding your target and what you're expecting from a knockout will help you with that.

Dr. Gabriel Alves 05:40
I heard you mentioning RNA guide a couple of times. And there's some troubles involving RNA guides and their ability to provide their function during the CRISPR workflow. What are some of the troubles that you see, or problems that you see, with RNA guides?

Doris Beylkin, PhD 06:04
Yeah, there's a few different ways that you can design guides. It, there's a few different applications, and I haven't really mentioned them yet. So, certainly knockout is the major way that researchers are using CRISPR guides. I think there are some pretty useful algorithms out there for designing guides to ensure that you're getting a knockout. There are other applications using sort of modified Cas-9 proteins where you can either activate or interfere with transcription of the genes. So, CRISPRa and CRISPRi. Design rules, of course, for those are quite different because they're more targeted to the promoter regions, or the very early parts of the coding region. Whereas the knockouts really are looking in the in the coding region, specifically, to create a premature stop codon. So, there's a number of different strategies that you might want to use, depending on what it is you're trying to do and the type of system that you're using. Getting back to knockout again, you know, there are some things that can happen, because really, the way CRISPR knockout works is you direct the Cas-9 to make a double strand cleavage in the DNA at a specific location. And the repair, or I should say imperfect repair, of that double strand break is what causes random insertions and deletions. And those random insertion deletions in a percentage of your cells will create a premature stop codon. And that's really what we're aiming for to create the knockout. Now, biology is, of course, complicated. And

that made my job as a technical support agent, you know, interesting, for sure. But there's a lot of different things that can happen. The cell can, or the gene can, sort of adjust to the changes that were made in the sequence, you can change splicing, you can kind of skip that particular exon potentially through splicing, alternative splicing, and kind of get around the knockout. And so, there's a lot of different things, you know, truncations at either end, that can sort of get around that knockout. So, generally, it's a good idea to test multiple different guides in different locations of the gene that you're interested in. It's also important when you're doing the analysis to use a number of different antibodies, or potentially a number of different assays, to make sure that you know what you're looking at. So, you know that you're not creating some kind of protein that can get around the whole knockout or the functional knockout aspect.

Steve Lewis 09:16
There's a lot of aspects of that where you have to design some of your studies in a really complicated way, it sounds like, because there can be a lot of downstream impacts. What are some of the tools that are used for design? Is there something that people can go online and play around with from like the NIH, for example?

Doris Beylkin, PhD 09:44
Yeah, there's a lot of different tools available. Many of them are quite good. There's also commercially available predesigned guides as well that customers can choose if they, you know, if they just want to get something off the shelf that can work as well. It really just depends on what they want to do, or if they have a very specific targeting mechanism in mind. That typically comes into play more when you're doing very specific changes to the sequence. And that requires, you know, another component, which is a template that can help with homology directed repair. So, if you have, you know, either an oligo, a DNA oligo, or even a plasmid that has homology arms to your gene in the region that you want to target, and then that change sequence that you want in the middle, you can basically replace the existing endogenous sequence with your template sequence. And so, when you get into complicated experiments like that, then you definitely will be looking for guides that are within a specific region, and you're having to design your template so that it can function in an efficient way.

Steve Lewis 11:08
In terms of being technical support, I think that that's a really interesting aspect for the life sciences industry. Having the PhD really focused on something so advanced and so technical. Do you have any maybe memorable customers who have called in? Maybe who would be tapping into that, right? That's a resource that is tremendous, right? Your mind being able to actually support when these new technologies are coming out. I'm curious to hear about some of your experience there.

Doris Beylkin, PhD 11:45
Yeah, it was definitely exciting. You know, I started right as RNAi was starting to become really popular. And then through the age of CRISPR and how explosive that was. I think, actually, one of the challenges with CRISPR was that it was out in the general public. And we would actually get calls from people in the general public who wanted to know about CRISPR, which was kind of interesting. But it's always nice to be able to talk with scientists about something exciting and engaging for them as well. And you know, I was in that, in that arena for such a long time, because it was different every day, we had the whole gambit, from customers who were kind of regular, our regulars who would call us and we'd kind of develop a relationship with, or you just have the one-off. And it could be anyone from a graduate student who's just learning to, you know, someone who's really advanced in their career, maybe they're an MD/PhD, and they're just kind of delving into a new area for them. And so, it's really quite exciting to get to talk with

all of them about what they're working on, what their goals are, and really help them through whether they're having trouble or they just wanted to know how this all works and how to get started.

Steve Lewis 13:20
Really a testament to how new technology can force an industry almost into the public zeitgeist. It's really interesting to see how CRISPR has reinvigorated interest there.

Doris Beylkin, PhD 13:34
Yeah, definitely.

Dr. Gabriel Alves 13:38
We hope you are enjoying this episode of *Speaking of Mol Bio*. We want to take a quick moment to tell you about our School of Molecular Biology. It is a great educational hub for molecular biology, with rich and reliable content designed for new and experienced molecular biologists alike. Check it out today at thermofisher.com/molbioschool. And now, back to our conversation. Doris, I ask this question to almost all of our guests, then I would like to know from you as well, where do you see CRISPR research going in the next five years?

Doris Beylkin, PhD 14:18
As I mentioned, there are a few different applications that kind of cropped up within the first few years. Obviously, the knockout was the first. Knock-in and that homology-directed repair was kind of the second thing hot on their heels. And then CRISPRa and CRISPRi really are made possible by fusion proteins with Cas-9 that have activator or repressor domains. More recently, researchers have been looking into other types of options for changing the function of CRISPR Cas-9. One of the neat things about Cas-9 is that in combination with CRISPR, it's kind of a new way to bring any sort of activity to a specific location in the genome. And so, base editing is one of the areas where I think researchers have really started to make up some ground in terms of being able to make a specific base change at a specific location where that wasn't possible before. So, I think there's a lot of different possibilities with bringing in different functions to the Cas-9 and using those really towards therapeutics eventually.

Dr. Gabriel Alves 15:36
One of the things we've seen throughout the years was the evolving of DNA polymerases, for example. And they are becoming more the higher technology with for example, with hot start, increased fidelity, and such, and we see the same trend with Cas-9. So, I would like to get your insight on the development of Cas-9. What do you see where the most opportunities are? Or where do you see, how do you see the enzyme performing better?

Doris Beylkin, PhD 16:07
I mean, there's a lot of different ways, you know, there's a lot of different actually Cas enzymes that are already available and/or being developed. And, you know, looking at improving specificity, improving efficiency. And, you know, all of that can be done through either evolution studies, or really just looking within the Cas enzymes that are that are already existing, I know that the Cas-13 enzymes have been an area of focus. But yeah, I think with the mechanisms that we have to really change and evolve and develop proteins to better serve the needs that we have, you know, I think the possibilities are pretty much endless.

Steve Lewis 16:57

So many variations, of course, are possible when it comes to protein design, and a lot of that starts with *de novo* gene synthesis. I'm curious, how might CRISPR customers, you mentioned double stranded breaks already and then targeted insertions of oligos, I'm curious for somebody who might be looking at both CRISPR and *de novo* gene synthesis, what might be your guidance in that area? Which one should you look at first? Or should you do it in concert with one another?

Doris Beylkin, PhD 17:41
Yeah, I guess it depends somewhat on what it is you, where you would like your protein to be active, right? CRISPR is really a way to modify the gene within the cellular context, whereas, you know, gene synthesis, certainly you can create stable cell lines from that, but you're basically adding to the cell rather than changing the endogenous gene. So, it really kind of depends on whether you're focused on keeping the cellular context the same or if adding a new gene to it would suit your needs.

Steve Lewis 18:27
Mill out, if you will, exactly what you're trying to get out of it so in context of an existing cell, whereas with *de novo* synthesis, you could achieve the same thing. However, it might take a long time if you're working with technologies like Gibson Assembly to make that full working cell line. So, you can ultimately get, likely, to the same product, maybe 10 years down the road from both avenues. But right now, this is a really kind of the state of the art and looking at cells almost like with an engineering approach.

Doris Beylkin, PhD 19:04
Yeah, absolutely. And thank you for bringing up *de novo* gene synthesis, because it's just another great example of how far we've come in these 70 years of really being able to make the DNA just how we want it. And it just blows my mind that within one person's lifetime, we've come from barely knowing what DNA was, or what it looked like, or what its function was, to being able to completely create something new out of DNA or manipulate it within the cell.

Dr. Gabriel Alves 19:40
Since the discovery of DNA, we've had such a progress and we have the DNA and molecular biology technology being used in all different applications. And I wanted to hear more from Doris how the progressing field of DNA, molecular biology, has been enabling these technologies and the progressing each one of those technologies.

Doris Beylkin, PhD 20:11
You know, everything builds on top of one another, right? Nothing happens in a vacuum. So, certainly having more flexibility, you know, it used to be all about, you know, how you're going to get at a certain question because you don't have a lot of options, but now that we can completely manipulate, in almost an unlimited way, the gene sequences for study, it allows us to make specific perturbations that you can then, you know, investigate in a number of different ways, right? You can look at the protein, you can look at the exosomes, see how those change, you know, look at expression levels at the mRNA or the protein level, you know, all these things that you we've been doing, but now with that specific change that you've made for a specific reason because you have a hypothesis about what that might do.

Dr. Gabriel Alves 21:18
There was one guest in the show that mentioned that in the future medication won't be more like medication x, that is to treat y disease. There'll be medications that will be individualized or

customized to fit that one individual. Do you think that CRISPR technology can collaborate to the future development of those medications?

Doris Beylkin, PhD 21:43
I'm sure there is an application. I'm not specifically aware of how CRISPR is used for that. But I know that there's a lot more to CRISPR technology than I've necessarily cracked the surface of. But yeah, individual therapeutics, that is a pretty amazing area of focus. You know, just the idea that you can within a quick timespan address or assess what, you know, specific needs, genotype, whatever, a specific patient has, and how you can target therapy directly to their needs and get that all done in a short period of time so that, you know, you can get the therapeutic developed for that specific patient. It's just mind-boggling.

Steve Lewis 22:42
And it's becoming more accessible, too. I know that personalized treatments, we're looking in the millions of dollars today. Over time, the costs come down, and you're seeing a lot more accessibility to technology. Specifically, I liked your example of just general, maybe nonscientists calling in and trying to learn more about the technology. Of course, you know, there's always dual use research concerns with that, biosafety and biosecurity implications. And I think around the world, regulatory agencies are trying to figure out how to keep up with the pace of technology. It's a really interesting challenge that I don't envy. And as we see more and more news stories break through, and it almost seems like CRISPR is the de facto something to point a finger to and say this scientist was trying to do XYZ because of CRISPR, and it is a lot more complicated than simply like, you know, "I'm gonna create some kind of either existing or nonexistent plasmid that that might be of some kind of concern." And I think that's important to say on podcasts like this, because science, while it can be dual use, in a lot of cases, you have the price considerations, it's very expensive to do all of the applications that Doris was just expressing, and also it takes a lot of minds and access to the equipment as well. So, you know, the idea of the rogue bad actor, I think oftentimes kind of comes up and draws out a lot of fears. But I'm curious, Doris and Gabriel, if you wanted to in your bedroom go develop something, it's mind-bogglingly difficult as well, right?

Doris Beylkin, PhD 24:57
I think just as with, you know, biological systems, nothing happens in a vacuum and research either, right? It all, like I said, builds upon itself and requires a huge, heavy lift. I was just at a conference last week and looking at some of the therapies. You know, all of my career has really been in the Research Use Only space, but, you know, a lot of people are bringing what we learned from that into the clinic. And just looking at the acknowledgement slides for some of these things that are going into clinical trials, it's just like, there was one person who had three whole slides filled with all the different teams that they had for these clinical trials. So yeah, it's definitely not something that you can easily just do in your bedroom, like you said.

Steve Lewis 25:56
And not to dismiss the danger, but also, I think with, you know, the science communication that we're trying our hardest with this podcast is also kind of bringing the realities of some of the concerns to light and addressing them. And I think that's one aspect that really comes up around CRISPR, is what is it enabling?

Dr. Gabriel Alves 26:20
Absolutely. It reminds me, Steve and Doris, do you remember when Dolly the sheep was cloned the amount of news report that came with the fear, with the new technology. So, similarly with CRISPR now. But these technologies, though, eventually, a lot of bioethics comes into it, but

these new technologies that will enable the cure and treatment for diseases, and the bioethics field has to be strong and prevent that those technologies are used improperly.

Doris Beylkin, PhD 27:00
Making sure all, yeah, all of the safety protocols are in place. And, you know, it just takes time really, to get to a place where you can say, “yes, this is this is safe to use in a human”, right? You know, it's not, I think that was probably the hardest thing when you speak with people who would call in from the general public really thinking, you know, just from seeing, like a NOVA special or something on CRISPR and saying, “hey, can you help me?” Well, science, for better or worse, moves slower than that, you know, it takes quite a lot of time to build upon what you learn, make sure that you're looking at all of the right things, and that when you're changing something here, you're not having unintended consequences somewhere else. And, you know, that's definitely a huge concern. So, that's why we have all the mechanisms in place to make sure that's safe.

Dr. Gabriel Alves 28:07
Yes, and one of these mechanisms are the regulatory agencies from any country. The FDA being the main one here in the United States, ANVISA in Brazil, all these regulatory agencies that Steve mentioned. They now will have to start looking into adapting to the new reality, to the faster pace the technology and science are moving towards to, and we saw an example, perfect example, during COVID with the speeding up the process on getting the vaccine approved. So, yeah, there's a perfect example that teaches us and those regulatory agencies that there's a need, and we need to maybe go back and look into the safety protocols. And those are some of my thoughts.

Steve Lewis 29:01
And fortunately for I guess everyone, industry is also self-policing. An example would be the International Gene Synthesis Consortium, of which Thermo Fisher is a part, where sequences of concern are essentially compared against different databases to really know, not just that one vendor, but across all of the vendors, who might be ordering what and what those sequences are. So, it's interesting. Industry will play a role advising the regulations as they come out too, and I think we're very fortunate in this space that's kind of nebulously being called synthetic biology is really, has some really great thought leaders in place, including within our own GeneArt business where we make synthetic DNA as well.

Dr. Gabriel Alves 30:04
Doris, I'd like to ask you another question that I ask all of our guests is, what would you say that is your secret ingredient for your success?

Doris Beylkin, PhD 30:15
I'd say always being curious. Because, you know, that was really always helpful for me in getting to the bottom of troubleshooting issues, first of all, and, you know, being curious is really the best way to learn more about what you don't know, which is probably a lot more than you do know, at any given point in time. So, yeah, just stay curious and make sure you're asking questions, because you can go with the first answer, or you can kind of dig deeper and really understand what's going on.

Steve Lewis 30:54
That was Dr. Doris Beylkin, global market development manager at Thermo Fisher Scientific. If you'd like to hear more of today's conversation, you can view the extended video version of this interview by visiting the URL in the Episode Notes. And, if you enjoyed our interview with Doris,

please consider sharing it with a friend or colleague who might be interested. This episode was produced by Matt Ferris, Sarah Briganti, and Matthew Stock. Thanks for listening.