Steve Lewis 00:10
Welcome to Speaking of Mol Bio, a podcast series from Thermo Fisher Scientific about molecular biology and its trending applications in life sciences. I am Steve Lewis, and today we welcome a pair of co-authors from the University of North Carolina at Chapel Hill. Alejandro Gomez is a current PhD student in genetics and molecular biology. Jess McAfee recently completed her PhD from the same lab and began a new position at the National Institute of Environmental Health Sciences. Alejandro and Jess have each followed their fascination with CRISPR into amazing early career journeys. We discuss the molecular biology tools behind their work, including massively parallel reporter assays and CRISPRi and how they use these tools to explore pleiotropic variants tied to psychiatric disorders. We hope you enjoy.

Jess McAfee, PhD 01:06
Hey, it's so nice to be here.

Alejandro Gomez 01:08
Yes, thank you for welcoming us.

Steve Lewis 01:10
So very interesting stuff that you all have been working on, but I want to learn a little bit about how you came to where you are today. So why don't we start by talking to Jess first about what brought you into this area of research, and how did you get to this point in your career.

Jess McAfee, PhD 01:29
I think the chaos of life just kind of brought me to this spot. To be totally honest, I started in plant research and genetics. While I was a undergrad at UNC, I applied to every single lab I possibly could to just do something that had something to do with CRISPR work. And one lab took me, and they let me do CRISPR work on plants. And so I did that for two years. After I graduated, I applied to a bunch of jobs for a tech position, and I got something in plant research at NC State. Yeah, and then I moved to Japan for a year, and then I came back and I applied for a bunch of jobs, and Hyejung ended up hiring me as a lab manager and technician. So very much outside of my plant research. I was really interested in the research, but I really did not know anything about it, to be honest. So, a lot of that was learning about, you know, what all these terms like GWAS and eQTL's mean and human genetics and genomics, it's very different from plant genetics. So I was, you know, getting the tea on that. And I really, really liked it. I really enjoyed it. I found a lot of meaning in looking into something that meant a lot to me, like mental health disorders and things like that. So that's kind of how I ended up where I was.

Steve Lewis 02:48
I love it. And Alejandro, how about you?

Alejandro Gomez 02:50
Yeah. So, I also kind of had a little bit of a crazy, like, I don't know how I ended up here, kind of situation, like Jess. It basically like, I started my undergrad in St Peter's University, that's a small university in New Jersey, and originally, like, I came from high school thinking, I just want to do

something with CRISPR. Because at the time that I was graduating this big paper from Jennifer Doudna with the CRISPR technology being put out was around the same time that I was starting my undergrad. And I was like, “Wow I really want to do something with research, with gene editing, I want to be at the forefront of that research.” And unfortunately, my university actually didn't do much research work in general, just hands on. And actually, I started working in a lab that was related to chemistry. So my thesis project for my undergrad was actually related to more like organic chemistry and how we could harness water splitting reactions to depollute waters. And then after that, I applied to grad school because I knew I wanted to continue doing research work. And so my advisor told me, "Hey, you can do a PhD, and that will be something that I think you would find appealing." And so, I applied to multiple universities. That was around the time COVID happened. So it was, like, not possible to get into any universities with all the shutdowns that happened. So I took a year, and I worked in industry. After that, working in, like, manufacturing and things like that, relating to, you know, there was, like, a couple of jobs, one with was more quality control base, and another one was, like, related to working with placental derived materials. And then after that, I got into UNC, and so I came to UNC really excited, because I knew that there was a lot more kind of work with CRISPR research. And so with Hyejung's lab, was actually another student that was part of Hyejung’s lab that she's like, “You know, Hyejung is actually interested in starting some CRISPR work soon. So if you're interested in that you should probably join now, because it's an excellent time, since she's kind of branching off into that area.” And I'm like "Oh perfect, like, I'm going to rotate through her lab and I'm going to see what the project's about." And since then, that's kind of been, like, the big thing where I took over a lot of the CRISPR work that Hyejung was just starting to do in stem cell derived neurons. So that was a pretty cool thing.

Steve Lewis 05:25
I think a wonderful place to start, since you both shared this, is to ask what was it about CRISPR that drew you into science to the point that you wanted to study it and both pursue PhDs in gene editing?

Jess McAfee, PhD 05:44
I think it was just such a new technology with so much potential that I instantly just thought that, like, “What are the limits to this? Like, what could this be used for? And how can I get my hands in it and use it too and see what is, what we can do with it.” I actually didn't do my PhD with a lot of CRISPR work. That was Alejandro and his collaboration. I use a very different technology called MPRA which I don't know we'll probably end up talking about.

Alejandro Gomez 06:13
I think for me, like at that earlier time point, it was like you could do anything with it because it was just starting. And so actually, a lot of new technologies have been developed from the original paper. Like the original paper was like, just cut. It just cuts the DNA, double stranded break. And it's like, what, what can you do with that? You could program and you could guide the CRISPR to cut anywhere in the genome. And I thought that was cool. But now there have been new ways to use CRISPR. Many, many CRISPR technologies. And I think originally it was just like you could maybe fix any disease that has some sort of genetic basis. It could have some way to study it or target it. Now we know it's much more complicated than that, and it's not that like perfect of a technology. We're still perfecting it, but I still think, even to this day, has a lot of potential.

Steve Lewis 07:06
It's a really inspiring thing when a new technology can be so interesting to someone that they want to pursue the exploration with it, especially one that's a bit agnostic in terms of its ultimate targets that it can impact. So, really, really interesting. Now, you all are both published authors, but you also published a paper together. Can you talk about that?

Jess McAfee, PhD 07:35
This paper was really looking into these variants associated with many disorders and what they have in common versus variants that are associated with maybe just one disorder. So the variants that are associated with all these disorders come from something called GWAS, or genome wide association studies. Basically, they take a bunch of people with a disorder and they sequence their genome, and they compare that to a bunch of people without the disorder, and they find where those alleles differ in those two groups. So, I took these GWAS variants and I tested them out in human neural progenitor cells, HNPs, using an assay called MPRA or a massively parallel reporter assay. And basically what this whole assay reads out is, does one allele express more than the other allele? These are regulatory regions, so they're affecting the expression of a gene somehow, some gene that we don't know, because they're in the non-coding region of the genome. So I did these assays, and we got these lists of variants that affect gene expression more or less than the other one. And then we did a bunch of computational analysis that was done by Sool Lee in our lab, as well as a lot of other people too. And we found, you know, where these pleiotropic variants, variants that are associated with many disorders, differ from non-pleiotropic variants, the ones associated with just one disorder.

Alejandro Gomez 09:03
When Jessica started the MPRA was doing it in neural progenitor cells, and they were kind of following up on variants that were associated to many psychiatric disorders. And so when she published that paper, and they put it in the submission, the reviewers, one of the first things they ask is like, “You have to follow this up with a way that you can connect these variants to actual genes. Because, you know, MPRA gives you a readout where you know if a variant is doing some sort of regulation or not, but because it only tells you about the variant behavior and how that works in the real genome, you still don't have, like, a connection to a gene.” And so my part came in with actually leveraging CRISPR. And so we leveraged CRISPRi specifically, which is a form of CRISPR, where you, wherever you put it, it just inhibits gene expression. It closes off the chromatin around there. And so one of the things we can leverage about that is that if we want to actually know what gene a variant is regulating, we can just put the CRISPR where that variant is in the genome and then see what gene expression around it is decreasing in response. And so my part in the paper was kind of that. They had, kind of like many, multiple variants that could have followed up. So we kind of did a proof-of-concept study. We took, like, a few variants, and then we kind of just targeted them with CRISPR, and just saw what gene expression around those variants was changing

Steve Lewis 10:34
That must have been pretty new at the time, metagenome wide association, and then just the approach that you took for integrating, I guess, the pleiotropic hypothesis around it is. Did you have a lot of work that you could refer to before you undertook this?

Jess McAfee, PhD 10:56
So GWAS's have been around for quite some time. And in the past, I don't know, five-ish years like MPRAs, have been around to try to validate these GWAS variants. Because GWAS has an issue in which it can tell you the general region that these variants are associated with a disorder, but they can't tell you the like specific SNP because everything in that region is inherited together in these big chunks called like LD blocks, linkage disequilibrium blocks. So the MPRA comes in where it can break up these blocks and test them independently. And that has been done for quite some time, and I had done it previously in a different paper. So, by the time we got here, I kind of felt more confident, and we kind of knew what we were doing a little bit more. I mean, science is always kind of chaotic, right. Like you something happens, and you kind of figure out how to fix it and standardize your protocols and make things work, and, “Oh, I need more sequencing depth,” and all that kind of stuff, you know. But, yeah, it's, it eventually got done.

Steve Lewis 11:42
Really interesting, and there's a lot of techniques that I'd like to talk to surrounding that. And before I do, I wanted to mention because I had to look it up to refresh my memory. Jess, do you want to explain what pleiotropy is? Just a short definition for those who may not be familiar.

Jess McAfee, PhD 12:20
Yeah. So pleiotropy can actually mean a few different things, depending on the context. It can mean that multiple genes are working together in this pathway. But in our context, it means one variant is associated with and potentially causing multiple different disorders. For example, variant one allele A might be associated with schizophrenia and bipolar disorder, and that would be a pleiotropic variant.

Steve Lewis 12:51
And there are other conditions that are similarly impacted. And as I looked it up, sickle cell anemia, Marfan syndrome, just basically multi symptom genetic disorders can be caused from just this ability of a single gene to influence multiple phenotypic traits.

Jess McAfee, PhD 13:15
Yeah. So we know from the GWAS data that a lot of these disorders share a lot of genetics. So how are they being shared but then turn out as something different later in life. Like, if we are sharing a lot of the same genetics, how does one person end up being diagnosed with schizophrenia and another person end up being diagnosed with bipolar disorder? And that's something that we were kind of looking into. Like, do these variants that are shared between multiple disorders have something in common, some underlying pathway that's affecting what happens to these people throughout their development and later in life, and it's that different from the variants, the non-pleiotropic variants that only affects one disorder and not the others, or only associated with one disorder and not the others?

Steve Lewis 13:15
Do environmental factors impact in any way?

Jess McAfee, PhD 13:15
Oh, most certainly. We did not test that in this experiment, but many people have looked into environmental factors for all these different psychiatric disorders, and I'm sure that environment definitely plays a role.

Jordan Ruggieri 13:15
Hey there. Speaking of mobile listeners, if you're fascinated by the power of digital PCR and how it's being used to transform molecular biology, we've got a new podcast you won't want to miss.

Lisa Crawford 13:33
That's right. We're Lisa Crawford

Jordan Ruggieri 13:33
and Jordan Ruggeri

Lisa Crawford 13:33
Together, we're the hosts of Absolute Gene-ius, the podcast exploring digital PCR and how it's used alongside other technologies to support diverse applications and drive innovation.

Jordan Ruggieri 13:33
Whether you're into bioproduction, cell and gene therapy, oncology research, or just love geeking out on the science of absolute quantification, Absolute Gene-ius has something for you.

Lisa Crawford 13:33
So after this episode, come join us. You can find Absolute Gene-ius wherever you get your podcasts, like the app you're likely in right now, or you can visit thermofisher.com/absolutegeneius to learn more. That's Absolute Gene-ius, where genius is spelled G, E, N, E, I, U, S, with no spaces or dashes in the URL.

Jordan Ruggieri 15:15
Absolute Gene-ius, bringing you absolute quantification and absolutely great conversations.

Steve Lewis 15:25
Moving on a bit to talking about the techniques that are used in the lab. You know, we've spoken a bit about leveraging CRISPR, but what other molecular biology techniques are common in this area of research?

Alejandro Gomez 15:40
Yeah, so for our, my side of the story with the CRISPR targeting of variants, we usually have to begin by making library of guides. So, we start by making a list of variants that we're interested in targeting and then we have to design guide RNAs for them, for the CRISPRi library. And so we begin by basically designing those guides. You know, we do some QC to make sure they have good efficiency rates and everything. And then after that, we order the oligos in bulk, and then we kind of clone them

into plasmid backbone that contains, basically, it's like a standard CROP-seq backbone that we use that also has RFP fluorescence so that we can later leverage it to select for cells that took the guide plasmids. And so we basically put the guides into the plasmid. We do the cloning, bacterial transformation and selection. And after that, we do a maxi prep to get basically representation of the whole guide library. And then after that, we can basically package it into lentivirus. After that we deliver into cells and basically the only thing left over from there is, in my case, we do single cell RNA Seq, as I mentioned. We actually have a core at UNC that takes care of that for us, and they basically do library prep for the gene expression libraries for all the cells that we targeted, or the neurons, in my case. And then the only thing left over is doing the guide RNA enrichment, which just requires some steps of, you know, RT, because the core gives us the cDNA to process from the expression of the cells. And then from there, we process also guide RNA enrichment, which is basically some extra PCR steps to enrich the sequence of the guide RNAs in the transcriptome of the cells, so that we can get a better detection rate of what cells had which guides. After that, we just send it to sequencing after those libraries are finalized.

Steve Lewis 17:57
Impressive, just the breadth of techniques that you use. How about you, Jess?

Jess McAfee, PhD 18:02
Yeah, so MPRA is along the same line. We, you know, get these GWAS variants. We have to order them from, like, you know, some kind of company as oligos. I do PCR to add on these barcodes to them. I use restriction digestion to, like, add them into a plasmid. I'll, like, put those in bacteria and then extract the plasmids out. I'll do restriction enzyme digestion and ligation again, to put it in, like a minimal promoter and GFP, or like luciferase or whatever is going in there. And then again, back into the bacteria and then back out. And from there, I would put them into cells, and then I would extract the RNA and DNA from the cells using like kits of some kind. And then I would do RT-PCR on the RNA, and then PCR on the DNA, and then another round the PCR to add on our sequencing adapters for Illumina sequencing, and then lead, we would sequence that. So I did so many PCRs, I would be filling up like 50 mil conical tubes with my master mixes. So, yeah, we've done it all.

Steve Lewis 19:10
How has it been making a transition to a lab that's run by the federal government compared to academia?

Jess McAfee, PhD 19:21
It's very different in that the lab sizes are quite smaller. So you're interacting with the PI and maybe like one other person on a daily basis, where in Hyejung's lab, it was very, very large at certain times, where we'd have like 20 people, and we'd all be really chatty and running around everywhere, a bit chaotic sometimes, but in a really fun way. Yeah, the NIH has a lot of resources for us to use, a lot of core facilities that are not as busy because there's less people. So it's quite, quite easy and nice to use the core facilities. Yeah, a lot of structural things are quite different. Like in academia, the PI is busy with writing grants, getting securing funding for their research and for their students. At the NIH, they have allotted money based on how long they've been there. So they're not really writing grants as

much. They're more involved in doing the research and training and writing papers and analyzing data. Though they still have review processes they go through. It's just in a very different way.

Steve Lewis 20:31
Just that description alone, I think, paints a really nice picture of just how different it is right from writing grants. What keeps you motivated, Alejandro?

Alejandro Gomez 20:44
That's a good question. I think that you know, if you love what you do, you know, even if the uncertainty of the situation can become quite large. I mean, I always say the big, big reason why I think doing the PhD, especially from the beginning, seems so appealing to me, is because I felt like it is work, but it's like it's work I enjoy a lot and it feels almost like I'm playing and I'm just thinking and doing things and tinkering. And so I think that keeps it exciting, and especially when I'm doing new things, which you get a lot of opportunity to do new things, and especially academic labs. You, I think that's the biggest motivation to me that you know there can be this uncertainty in the background, but you come into the lab every day and you can do something cool and cutting edge and novel. I think that keeps me excited and keeps me going. So I think that's my take.

Jess McAfee, PhD 21:42
Yeah, like Alejandro, I do love playing around and being in lab and doing stuff. I also, I think all sciences have this, like, inherent hope to help someone, somehow, some way, and through the knowledge that they gain with their research. Like for me, I hope that the research I do ends up helping someone with a psychiatric disorder, you know, live a life that is better to them. And whatever way that is, I'll just be a rung on the ladder, you know, of knowledge that just gets built upon and built upon until, until that happens, and I think that's what keeps me going.

Steve Lewis 22:21
Adding to the body of human knowledge, I think is a really inspirational and motivating factor, for sure. Thank you both so much for your time today, I always close out the podcast by asking a couple of questions, and so we'll start the first one off with Jess. What have been the keys to your success?

Jess McAfee, PhD 22:45
My key to success has to be great mentorship and working with really good people. I couldn't have asked for a better lab in my PhD. Hyejung is amazing. And the people that I worked with, like Alejandro and Sool and Jiseok and everyone else in the lab are also really amazing people to work with. Yeah, that's my key.

Alejandro Gomez 23:06
Jess said it best. I think I always remember when I was starting the PhD, one of the things that there's three big things they emphasize when you're choosing a lab from your rotation. They say you need to look at whether the mentor works for you, the mentorship style. Whether the lab environment works for you, you get along with other people. And then just whether you like the science. And I think the science is easy to like, you know, once you're getting into these programs, you know what you're going to do, what you like, but it can be harder to find, I think, a good mentor and a good lab environment. So

I think that that's also similar to Jess, I mean, we come from the same lab, so it's hard not to think of it that way. But Hyejung is, I think, an excellent mentor. She's very well connected, which I think is also a huge plus, right. I think for us, that ability to have these, you know, through Hyejung's connections for after grad school to move on to other positions, just from people she knows, is a huge thing that I think can help with our success. And then also, you know, from the day-to-day experiments, like, I know, Hyejung is very good at, like, talking to people and seeing what's going on the field, and then going back to me and being like, "Hey, you know, I heard of this kind of technique, and you've been struggling with this. So maybe we can try that instead, and that can boost you on your way, right." So I think a good mentor is like that. It's like looking for ways to make your life easier in the lab and, and then, like, like she said, I think the people in the lab are also a huge plus. I think if there is, you know, kind of animosity or difficulty in relationship with your lab mates that can make everything harder or more stressful. So I think having a healthy lab environment is also a huge bonus.

Steve Lewis 24:50
For anybody who might be listening and maybe much earlier in their scientific careers, what advice would you give them?

Alejandro Gomez 24:56
My biggest advice is don't give up, even if you encounter initial obstacles. Like I know for me, when I was just applying to grad school for the first time, this kind of thing with COVID happened, and actually that was a difficulty even before graduation and going on to apply to grad school. I, like, couldn't do some internship opportunities. I kind of got missed. And, you know, with the world, environment and the way things change all the time, you never know, right. So you can run into these obstacles where something can be really out of your control, and it might seem, and I know, to me, it felt initially, at the time, like, “Oh, like maybe it's not meant for me, like I'm having these issues because, you know, it's just not meant to be.” But I think that, and especially for science, it's really good to be persistent, and it pays off. And I think that, in general, is a good thing, not just for science, but for life. So I think for me, that that's been the biggest thing is, like, be persistent, and eventually you will get the results you want.

Steve Lewis 26:05
Love it. Advice for younger scientists, Jess?

Jess McAfee, PhD 26:09
I echo Alejandro's, don't give up. You know, with your experiments, things aren't going to work all the time. You have to figure it out, it's going to be frustrating. Like, find people to help you. Reach out to mentors, to other people in the lab. Ask for help when you can, you know, use your resources. I think also, life is really chaotic, and you just got to do your best with what you got sometimes and make it work. And that's totally fine too. You know, I also applied to grad school three times in a row before I got in. Three years in a row. It just, it'd be like that, you know. Yeah, just stick with it, and just do your best. And whatever happens, happens. And you can, you can just choose to be happy with what you, what you got.

Steve Lewis 26:56

Just a marvelous attitude and really a great story from both of you about resilience and perseverance all the way through a lot of difficulty in your professional experience so far. So, thank you for sharing. We have a little bit of time so a bonus question. We'll start with Jess. What technology are you most excited about right now that may have an impact over the next 10 years?

Jess McAfee, PhD 27:31
Don't judge me, but AI is going to make such a difference. One person can only do so much. I say this all the time. One person can only have so much in their brain. Can only, can always read so many papers. But if we use AI, it can, you can combine so much data together. It can code so much faster than one person. It can, like, analyze data. It can explain things to you in a way that is just so more efficient. Obviously, you know, with all the check boxes, you need to make sure that it's correct information. You make sure the code is doing what it tells you it's doing. You know, you got to, you have to check your work, you can't just trust it blindly. But I think it's really going to revolutionize genetics, in general, because there's so much data out there that isn't combined together yet into one thing. And when that happens, and it's currently happening, it's going to be really revolutionary. And I think it's going to be very beneficial to us with, you know, all the caveats that come with AI usage as well.

Alejandro Gomez 28:36
I'm always excited about new CRISPR technologies whenever I see. In the in terms of the science side of things, or of what we do, I'm always excited about seeing new CRISPR technologies. I feel like there's always new things that I see. So I'm always very excited to keep up with that and any new methods really I like, I feel like I'm a little bit of a methods person that I like, when there's something new or like a new way, that even the current stuff we've been doing, like with MPRA or CRISPR, like somebody thinks of it and gives it a new twist. And so that can also be really exciting to see, because you're kind of, I mean, reminds you that you're at the forefront of the field, and you see these changing happening. I think that can be very exciting, exciting when you look into the future.

Steve Lewis 29:21
I love both of your outlook. Alejandro, Jess, thank you so much for your time today. We really appreciate it on the podcast.

Jess McAfee, PhD 29:29
Yeah, thank you.

Alejandro Gomez 29:30
Thank you.

Steve Lewis 29:31
That was Alejandro Gomez, a PhD student in genetics and molecular biology at UNC Chapel Hill. And Dr. Jess McAfee, a scientist at the National Institute of Environmental Health Sciences. A quick note that the views, thoughts, and opinions shared by Jess are solely her own and do not reflect the views or positions of the National Institutes of Health. Join us next time for more fascinating discussion about the

wide world of molecular biology. Until then, cheers and good science. Speaking of Mol Bio is produced by Matt Ferris, Sarah Briganti, and Matthew Stock.