Worley: Hi! Welcome to another Boyce Thompson Speaks podcast. I’m your host Jay Worley and I’m joined today by Noe Fernandez-Pozo, a scientist here at the Boyce Thompson Institute for Plant Research, and the developer of the Sol Genomics Network virus-induced gene silencing tool, an online resource for designing gene silencing constructs. We’ll link to a paper recently published in Molecular Plant, a Cell Press Partner journal. It’s open access and the online tool is freely accessible.

Worley: Welcome Noe!

Fernandez-Pozo: Thank you.

Worley: So first let’s just get to know you a little bit, where are you from?

Fernandez-Pozo: I’m from Málaga. This is a city in the south of Spain on the Mediterranean Sea.

Worley: Sandy beaches sound really nice right now; it’s about 3 degrees outside ...Fahrenheit.

Fernandez-Pozo: Yes, much better in Málaga. We have like 60 degrees more than here probably.

Worley: So, did you attend school there as well?

Fernandez-Pozo: Yes, I went to the Málaga University.

Worley: Is that also where you got your PhD?

Fernandez-Pozo: Yes, that’s pretty common in Spain.

Worley: Okay so it’s less common over here in the States where you go and get your PhD almost always somewhere else than where you studied as an undergraduate.

Fernandez-Pozo: Yes.

Worley: And so was that all in plant biology?

Fernandez-Pozo: Yes, I studied biology but I got the subjects that would be like the majors here I guess that are based on molecular plant biology, biotechnology—these kind of subjects.

Worley: Ok, so did your interest in plant science come as a result of your college studies or was it something that happened a little bit earlier in high school or even growing up?
Fernandez-Pozo: I think that was in college. In high school, I was very interested in science but I think Málaga University is much stronger in plant science I think than other kinds of biology. So all of the professors were better in plant science and out of all the subjects the best ones were in plant science.

Worley: Your paper is on a computer program that can help you design gene-silencing constructs. So let’s start from the very basic here—so gene silencing in plants is sort of taking advantage of a plant defense. What is it defending against?

Fernandez-Pozo: For example, in that case, it would be defense against viruses because when a virus infects the plant it tries to replicate so it tries to use all the infrastructure of the plant to get all the genes of the virus replicated in the plant. So when the plant detects that, it tries to destroy all of the genes from the virus so in the same way, we can use that in the lab to silence one gene.

Worley: Okay, but I’m sure this was actually discovered probably somewhere else. That’s sort of a tricky thing so you actually have a little bit of a neat story about where this came from.

Fernandez-Pozo: Yes, when I was writing the paper I found another paper that talked about the discovery of that process in the petunia. When they were studying the color of the petunia flowers, their receptors were trying to overexpress the anthocyanin in petunia flowers to get very deep purple flowers.

Worley: Okay so, what was it, anthocyanin?

Fernandez-Pozo: Yes.

Worley: Is what gives it a very deep purple color?

Fernandez-Pozo: Yes, but when they got that it’s actually the opposite thing, they got white flowers. So they realize that when you try to overexpress that, it is the same process like when the virus tries to overexpress all these genes to replicate. So the plant understood that that was like an attack from a virus so it tries to silence these genes from the anthocyanin and the flowers were white.

Worley: Can you give us a brief description of what gene silencing is and how it works?

Fernandez-Pozo: Yes, usually genes are expressed in the plants to be translated later into a protein that will do something in the plant. So if the plant doesn’t want to express that gene that could be silencing, that will not be translated to proteins.

Worley: Then what are the general principles that sort of drive gene silencing? Is it very specific? Is it very general? What’s going on?
Fernandez-Pozo: Yes, it is very specific because when a virus attacks a plant, the plant wants to silence all of the genes from the virus and not other genes that can be expressed in the plant. So the plant tries to detect the most specific parts of this gene and tries to compare that with all of the genes that are expressed in the cell and digest these genes.

Worley: So it is very specific just for the genes, which are detected, or the transcripts that are detected as foreign?

Fernandez-Pozo: Yes, that’s correct.

Worley: Is this detecting viral RNA or viral DNA or viral proteins or what exactly is there about the virus that it’s detecting?

Fernandez-Pozo: The virus introduced in the plant is double-stranded RNA, so that is very specific and in the same way we have to introduce in the plant the same thing. We can use also viruses and we can introduce inside this virus vector a construct for a gene.

Worley: So really we are just highjacking a virus to silence one of the plant’s own genes here? (5:01)

Fernandez-Pozo: Yes.

Worley: Okay, so what makes a well-designed construct then? So, we have something I’m guessing we have to express as a double-stranded RNA?

Fernandez-Pozo: You have to set a construct that this is specific only to the genes you want to silence because if you have a part of a sequence that could be also in other genes, you will also silence your target genes and also other off-target genes.

Worley: But if we wanted to, you could actually, in theory, design constructs to multiple—or an entire family of—genes, so many genes that are related.

Fernandez-Pozo: Yes, sometimes it’s even impossible to silence just one gene because when you have two genes that are very, very similar because they are from the same gene family. For example, when you try to silence a tomato gene in *Nicotiana benthamiana*—like tomatoes are diploid and *benthamiana* is polyploid—you will get two copies of the same gene. They are very, very similar and you have to silence both of them. It’s impossible to differentiate.

Worley: So sometimes it’s even desirable? So in actually designing these constructs, that’s where computers really come into play because there’s way too much genetic information out there to actually go through and scan with your two eyes what’s
going on here. What are the important things a computer actually looks at to determine a best match?

Fernandez-Pozo: Yes, actually it’s supposed that the plant is checking for all the genes that are expressing in the cells. You have to do the same thing with the computer. You have to compare if your construct is as specific to all the transcripts in the plant. So, for example, in tomatoes you have like 30 thousand genes. You cannot do that manually and with a computer you just can map all the small regions, like the standard interference RNA, and map all of them against the genes and check where they are.

Worley: So when your program is finished running, what does it actually spit back out at you? What’s the output that you actually see?

Fernandez-Pozo: Basically what you get is a graph where you can see from left to right the whole length of your gene. And from the top to the bottom, you can see the list of the target and of target genes that the program found. Then, you will see in blue all the target genes and in red the off-target genes. The program will highlight in yellow the region that is the best for your construct. The region that has the most coverage of the target, the genes you want to silence, and the less coverage of the off targets.

Worley: So basically if you wanted to silence multiple genes, you could also tell the computer that you wanted it to do that?

Fernandez-Pozo: Yes, after you get the output you can play with the parameters, you can change the target genes you want to silence, the length of the region you want to get for the construct, the size of the small interference RNA, the number of mismatches, and you can set a custom region. You can play with that with a mouse to get the windows of the construct you want to select.

Worley: Are you using the same computer language for all of these different steps? It sounds like it’s one of these new sorts of dynamic web interfaces.

Fernandez-Pozo: Yes, we have different languages. We have in the front end, in the part that is very interactive that occurs in the web browsers. Mainly we use JavaScript and Ajax, jQuery and now, html5 canvas to draw the graph on the fly so we can expand the graph, zoom in on the graph, redraw the graph on the fly all the time very fast. And also in the back end, usually we have Perl that is there, the language we use in the SOL Genomics network.

Worley: You’ve got a chosen language for each specific step going on here. So is this tool available just for Nicotiana benthamiana species? Nicotiana benthamiana, I should mention, is sort of the workhorse for VIGS and the one that we use the most commonly because it is the most amenable to using virus induced gene silencing.
But are there other species that you can try VIGS with? Does this program work with other genomes?

Fernandez-Pozo: Yes, we have a long list of species of plant species you can use and also it’s very easy to add new plants. If somebody wants to add a new plant, they can just tell us and we can add it. We have also tomato, corn, soy, rice, grape, eggplant, cotton, Arabidopsis thaliana, there are many plants.

Worley: You’ve got a whole salad over here. Where can people find this tool that you’ve developed?

Fernandez-Pozo: This tool is available at vigs.solgenomics.net. This is in the SOL Genomics network, it is a resource for all the solanaceous plants, hosted at the Boyce Thompson Institute.

Worley: So that’s vigs.solgenomics.net. It can be a little bit hard to find there, like 'sol' stands for solanaceous...

Fernandez-Pozo: Yes, also if you go to the SOL Genomics site and just look in the top menu, in the top menu bar go to tools and the second one is the VIGS tool.

Worley: So has this tool been well received? Have you been popular at conferences when you go and you tell people about this?

Fernandez-Pozo: Yeah, I think people really like it. Also, because it’s very interactive as you can play a lot with the results and many people told me they are interested in using that.

Worley: So before I let you go Noe, I just wanted to know is there anybody you wanted to thank in particular before we leave?

Fernandez-Pozo: Yes, I would like to thank the other authors in the paper, Hernan Rosli, Professor Greg Martin, and Lukas Mueller from Boyce Thompson Institute.

Worley: Well thanks for joining me today, Noe.

Fernandez-Pozo: Thank you.

Worley: And thank you for listening. The tool you heard about today is funded in part by the National Science Foundation. You can find this resource at the SOL Genomics Network website, that’s solgenomics.net. Under the Tool heading it should be the second option down. The paper is titled, "The SGN VIGS Tool: User-Friendly Software to Design Virus-Induced Gene Silencing (VIGS) Constructs for Functional Genomics", published online in Molecular Plant, it’s open access, you can go and read it right now no matter who you are. I’m Jay Worley and this has been another Boyce Thompson Speaks podcast.