Eddy Rubin

You are in Dallahass..? where?

I'm at University of Dalhousie in Halifax Nova Scotia

Have you ever heard of iGEM before?

Yeah, yeah…yeah

Awesome, it's a synthetic biology competition and Dalhousie its in its third year competing. We're pretty new on the scene considering its been going on for almost 13 years. I'll just give you a brief overview on our project, then I have some questions to ask you.

Sounds good.

Our project it is a little similar to your paper on the cow rumen where you were looking for biomass degrading genes. Similarly, we're targeting the porcupine microbiome for cellulose degrading enzyme in order to create a pathway in E.coli to create glucose from cellulose. We can then use yeast to produce ethanol, a biofuel.

Why are you targeting the porcupine?

We always try to put a local spin on things, so the softwood lumber industry is one of the top industries in Canada and Nova Scotia. The porcupine, a hind-gut fermenter, is a known eater of softwood bark, so as we know mammals aren't able to break down the beta 1-4 linkages so we target the microbiome of the porcupine assuming there would be bugs in there that can degrade cellulose.

Yeah. Ok. I'm a very critical person, but go on.

So that's the idea, that we would use waste from softwood lumber industry to create something useful, that being biofuel.

Then you're going to get the biomass degrading machinery from microbes in the porcupine, the metagenomic data and from that you will synthesise the genes and put them through assays? You have an assay for looking at, I dunno what the linkage is, or activities showing it has break down activities?

Yeah, were first targeting the more simple cellulose pathway which is just cellulose to glucose, not hemi cellulose and lignocellulose which are both, as you know, more complicated molecules.

And then you'll move those enzymes in yeast? Or what?

Into E. coli then utilize yeast for glucose to ethanol breakdown

Right right. In E. coli, I'm not that familiar with the functional literature so much. So then in E. coli they will secrete the enzyme?

Yeah, so were putting them in an expression vector that contains a pelB sequence which brings the enzymes into the periplasm. Then we're kind of hoping it just gets out through a porin, but that's kind of a more "hand-wavy" part of our project.

So we'll have a discussion about it, then we'll dive into what you need as far as expertise. You like the porcupine because its politically a good thing. But these are just chemical reactions, just because they eat a lot of softwood doesn't mean they're better at it. People have isolated a fair number of these enzymes already and with metagenomics frequently its hard to get full lengths and in fact we were quite lucky with the cow rumen because of the way we did. We put the stuff in the rumen, and we came back and were able to get material off the little bags we put. We just reached into the guts of the cows, they were fistulated cows, so we could stick our arms in it and we put these sacks with material in them. Were not sure, but we think that somehow the microbes that were adherent to them might be playing a role. Will you just stick a tube down [the porcupine] and extract gut microbiota from them?

Because iGEM is under its own umbrella, there are very specific ways you have to deal with animals. Most animals research is actually not allowed in iGEM unless you have very specific permissions. We're just taking it from their fecal samples.

Right so why not just go and take known enzymes, sequences of known enzymes that have been shown to be active?

That's a good question, the professor that started this project was thinking of finding more novel questions, enzymes that might be able to do a better job. We're doing both sequencing and functional metagenomics, as you know you get a certain percent identity with sequencing, but with function you can get an enzyme that does what you want it to do but doesn't look like an enzyme that's been described previously.

Its funny, our work was funded by British Petroleum (BP) and they were funding bio-energy research and eventually that said "hey we have enough of these enzymes". We did the experimental design and then we did the sequencing and then we synthesized some of the genes and then we worked with another group that was very good at doing the functional assays. We couldn't have done it. Anywas its just a thought, I know your professor is looking for novel enzymes but I think that is a romantic viewpoint. Its good, its science, anyways this is just a discussion. What year are you?

I'm in my final year of my undergrad

Right, what is your major

Micro and immu

Right, what do you want to do with your life

That's a really good question, I think I want to get a doctorate as of now, do my phd and in microbiology and immunology but I think I want to move into industry afterwords.

That sounds like a good area, microbiology's great. When is iGEM due?

The conference is November 8th-13th, so pretty soon

Wow. Yeah. That's like two years of work in a small amount of time.

Yeah, of course we won't get to the end of our idea

Yeah, even getting the sequence data and going through, you'll just have bits and pieces. Go through the sequence data and see if you can identify enzymes with these capabilities. You may at the same time synthesize known enzymes that have been shown to have activity.

Where we are right now we've already done the metagenomic sequencing and we've already ordered those enzymes with sequence of certain percent identity or catalytic domain homology from IDT. And we're currently just cloning most into expression vectors.

So you're already done. You've done a bunch of these things, you have some candidates. Now you want to be able…what's the next step. To show they have activity?

Yes. Currently right now we're troubleshooting a couple different assay. As you mentioned, they can be a little difficult, especially trying to get cellulose to dissolve nicely into plates. Were working with Avicel and cellobiose, kind of the normal things people are using in the field.

Okay so what can I help you with? What questions do you have?

In the last couple of years, you mostly publish on sequencing metagenomics rather than functional [metagenomics]. Have you have ever done it before; do you think there are any advantages?

Uh..[Long pause]..No. I'm interested in scalable things, I think functional is great but I'm a sequencing guy. I think you can really scale sequencing and you can scale synthesis. That's just a preference of mine, I'm more of a sequence person, and being able to extract info from sequence. There are people that work at a company that will have a particular target. You an spend your life developing an assay for one specific target but I didn't have a specific target. I think function is hard to do. Making giant shotgun libraries, functional libraries, is hard to really scale. Producing Terabytes of sequence and being able to computationally go through it, and pull out things that are candidates and then synthetizing it is more scalable. That's just my view point on it.

Where do you think the future of metagenomic sequencing lies? Do you think there will be many more advances in DNA sequencing technology?

I think Oxford Nanopore: these long read sequencers where you can get full assemblies. If you're looking for enzymes you can get by, you don't need to assemble full genomes, you're just pulling out enzymes. I think Oxford Nanopore which gives really long reads…

That's on the electrical output of the different bases right?

Yes! I think its going to scale. I think Pacbio doesn't have enough throughput but it gives you long reads. I think the massively parallel sequencing by polymerase… Illumina will not give you these long reads. You get enough depth where you get assembly. But anyways nanopre sequencing is going to come into it's own for metagenomics.

Both of our research targets the enzymes we want, rather than the organisms that produce it. Do you think there would be any advantages in knowing the organism that produces these enzymes?

I don't think so. You're just blowing apart a bunch of cars and looking for steering wheels, it doesn't matter that the steering wheel comes from a Volkswagen van…you just want a steering wheel.

I get that. One of my professors mentioned that it might be nice to know the whole organism because they might contain an operon that has many of the target enzymes.

True. You want to get it. Assemblies are good and certain enzymes do work better together. That is a reason for wanting to get the whole [operon]. Frequently you get an operon [through sequencing] you don't need the whole organism.

Awesome. I just have one last really science-y in-depth question. Do you have any thoughts where synthetic biology is going in the future? There was this big uproar earlier in the year when a Canadian scientist made horsepox by ordering synthetic DNA. Do you think there will be another moratorium, like there was in the 80s with cloning?

That's a really interesting question. I love synthetic biology, you want something to work and you build it. I am concerned about new technologies like CRISPR-Cas's ability to simply modify existing organisms. I'm not worried about human fetuses, but I'm worried about people messing with plant pathogens and things like that. Its suddenly an easy thing to do. Its really backyard science and I have concerns about that. I think a lot of this is connected with synthesis of DNA so there's one way you can regulate it is by really curtailing the ability to synthesize DNA. I think synthetic biology has a bright future. I think as far as doing nefarious things with it, it comes with new CRISPR-Cas tech making it easy for a backyard lab to do malicious things and I think its very hard to regulate. I think it's too big, it's already too easy.

Overall, it's a great area that will grow. The cost of synthesis will continue to come down, right now people work very hard on design but pretty soon you'll be able to synthesis a million different things and brute force function from synthesizing so many different variations, you wont have to think about it.

I'll leave you with one little nugget that I think. I just wrote an article in Nature on the future of DNA sequencing, it will come out in the next week or two, and I ended with saying you'll never know what these technologies will be used for. I think there will be a fair chance that sequencings biggest use will be for reading. People will synthesize information in the hard drives of computers and package it. We'll be using sequencing to read that out so the future maybe we're using DNA as an information molecule but not as a biological information molecule. It stores information, instead of having 0 and 1s you have A, T, C, Gt and it lasts for ever. Longer than anything we have now; longer than stone. It's incredibly compact. Sequencing is a trivial problem, every year it just gets so much better. Synthesis: there's not as much money in it but it's a trivial problem too. If people say, "hey we want to synthesize hard drives" people would get much better at synthesis and the technology would approve. That's what really drove sequencing technology. People want to sequence everybody's genome and that why money was put in to improve the technology. One of the narrow points of synthesis is there isn't enough demand, a bunch of academics is not enough demand. But if we decided we really wanted to use synthesized hard drives in the form of DNA, that would drive the demand and it would improve the technology. So you ever know about the future but I think these areas of synthesis is going to be a part of that. That sounds fun!

So iGEM is in Boston?

Yeah it is!

Drew Endy is the big priest or the guy?

Yeah haha he is the main guy.

I think iGEM is great, there's thousand of teams competing, aren't there?

In 2015 there were over 300 teams from 30 different countries but that equals thousands of students all over the world

That's a really cool thing, there aren't many things like it. Which ones won?

Imperial won last year

What for?

They made a better way to make co-cultures. So people can make better bioreactors

Oh…that's not very sexy

No, its doesn't… there were sexier sounding ones but this one did a really amazing job so that's why they won.

Drew Endy has this viewpoint that synthetic biology is engineering, and I don't think that. He has this view that we will figure out the pipes and therefore we'll be able to predict something. And I don't think it's like that. I think biology is so complicated you have to try a bunch of options to find the right one. I don't think you can just characterize them and put them together easily. What do you think about that?

I thought like you, I thought how could this be more like engineering then biology when I was in the lab doing this cloning. But I went and visited the UWaterloo iGEM team over the summer. Most of their students are actually math or engineering students that do all the modeling. They have a handful of biology student who help them with the nitty gritty biology but they [engineering and math students] model micro organisms and enzymatic processes. I think the math and engineering really helps with projection.

I don't disagree with that. Drew Endy has bioparts though: Part A and B and C and you'll have something that will work. I think that's a great vision, but I don't think its right. Every gene you put in there is going to require a different thing, it will effect the system. It's a whole system. Anyways, great chat.