Futures in Biotech, 48: Sequencing an Ocean's Genome

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Marc Pelletier
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[Music]

Marc Pelletier
I'll first start the show by introducing our co-host who's been on the show a number of times, too many to mention right now. So, it's – his name is Andre Nantel.

Andre Nantel
Yeah, three.

Marc Pelletier
Three? Well now four, right. He is a Senior Research Officer with the National Research Council of Canada and Adjunct Professor at McGill University, Montreal. Welcome Andre.

Andre Nantel
Greetings from Canada and I've learned this morning on the Google flu trends that Canada is now the Swine Flu capital of the world. We're very proud; we're trying to get this thing over and done with in time for the Vancouver Olympics.

Marc Pelletier
Wow. Well, everybody will be immune I suppose. By the way, the fact that you actually mention H1N1, I'd like to encourage people to go and talk to the doctors about the vaccine. Because there is a huge misconception about the safety and it's a CDC approved vaccine, it's safe, but rather than tell people to go get vaccinated, I'd rather they just talk to the doctors and see if it's right for them. Right? So go talk to your doctors about the vaccine. It's not so bad on adults, but it can be really harmful to pregnant women and to kids, so please talk to your doctor about it. So pretty scary stuff, though. I'm not scared of it but it's definitely worthwhile doing, and I'm going to be in line as soon as it's available to the people are at lesser risk.

Okay, so our guest, we're really, really lucky to have Ed Delong, who is a Professor in the Department of Biological Engineering and of the Department of Civil Environmental Engineering at a small school called MIT and he was on the show on episode 9 talking about his work and in episode 17 as a member of a panel talking about potential future of biotechnology. And it was really great and this was – it's this has been at least two years. So I thought we'd get him back and have him talk a little bit about his current work. Welcome, Ed.

Edward Delong
Great to be here, Marc. I am surprised you asked me back after those last two times but I am very delighted to be hearing you again and speaking with you.

Marc Pelletier

Well it was Linda Griffith that mentioned, that gave me your name as one of her favorite people, a scientist and she said that you could come into a classroom, throw a pile of dirt on the table and then make it the most fascinating thing in the world.

Edward Delong
I can definitely can throw the dirt around that's for sure, Marc.

Marc Pelletier
So we had a little bit of debate going on in our emails. I was thinking of calling the show Sequencing the Ocean's Genome but you were thinking going Ecosystem, Systems Biology. And that's a fairly complicated title. Even one that I sort of had to read three times to figure out.

Edward Delong
Yeah it's probably not so accessible but you know Sequencing the Ocean's Genome is so yesterday.

Marc Pelletier
So tell us a little bit of why you think Ecosystem, Systems Biology.

Edward Delong
Well so – yeah that's not the best title. I mean basically one way to simplify it is just say systems biology but think about what you mean when you say systems biology and to me it probably means something different than your average say molecular biologist, cell biologist, metabolic engineer. Because biological systems are properly viewed are extraordinarily complex and they go way beyond the test tube basically. Because organisms don't evolve as pure cultures the way we study them in a lab. They are present in really complex mixtures and in fact for any given species there is lots of variation basically, allelic diversity for each gene. So any given population of the same species out in the environment is composed of many, many, many variations on a theme. And then you bump it up a couple levels to the communities and the ecosystems now you are talking systems biology that really matters because that's where evolution and ecology happen which is what has created the biological world that we have around us.

So in a way I am kind of – in a way it foreshadows what we've seen with a lot of "-omic" technologies like when genomics got started and then transcriptomics and proteomics and metabolomics on up the molecular food chain if you will. That kind of happened in studying kind of model systems in the lab to understand complex biological regulatory networks and how they work in the cell. But I would argue with environment, ecology, we need to understand that we need to bump it up to the next level which is really studying systems biology in the context of ecosystems in all the biological hierarchies. That probably didn't make things that much clearer, Marc, but…

Marc Pelletier
Well if you are studying a system, right?

Edward Delong
Yeah

Marc Pelletier
If you think about the neuroscientists that are working on it, a brain has 100 billion cells.

Edward Delong
Yeah.

Marc Pelletier
Or is it a trillion? So they are trying to figure out things from a global perspective on the human brain with 100 billion and if you are going into the ocean or going into even a puddle and you are

looking at the number of organisms in a systems way, are you dealing with – well in the human brain there might be several hundred kinds of cells but you are looking at not only several thousand species or several hundreds of thousands of species but several hundreds of thousands of species plus their billion cells each, from a complete systems approach, how do you break this down? How do you even think of tacking it?

Edward Delong
[7:28] Yeah, that's a fair question, Marc and I think you have to approach it in different ways, so you can't be so naïve as to say we are going figure out all the variables in the system and understand every single regulatory network and feedback mechanism and environmental response and put it all together so that we can make absolutely deterministic predicted models. That would be naïve, but we can do is begin to study the elements of the system that make a difference and one can start simply from the ones that you can measure, the ones that are more abundant, the ones that seem to most heavily push on elements of the system that make a difference with respect to chemistry and biology and so on and model that part of it. That's a huge task in and of itself, but I think we can take some of the same principles that are being applied in systems biology in a more restricted fashion with very defined model systems and begin to apply them out in the environment. And why not? Because there is no reason why not to begin that process. You are right though that we are never going to be ultimately completely deterministic in terms of how we understand the operation of these systems.

Marc Pelletier
I think we will.

Edward Delong
We can certainly begin to model them better and now's the time to start.

Andre Nantel
So I am kind of dabbling in that same field essentially using modeling in systems biology in my own research and one of the things that's always challenging is getting sufficient data, enough data that represent all of the type of variability that you can have in your system. So, and in your studies what exactly are you modeling? Are you going to be modeling microbial population changes or metabolic pathways, their effect on the environment, what exactly is your objective here?

Edward Delong
Well, we'd like to work towards a number of those things. So let's – first of all you have to kind of start with what your knowledge base where you are starting out from and that really does start out from Marc's title, so we study the marine environment, let's just stick with the oceans for now, because that's a bit simpler than the dirt that I might throw on your desk. So in the oceans we're starting to get a pretty good feel for the dominant populations that exist in kind of different environmental contexts and some of the dynamics that goes on there. We could take, say the water column in Central Pacific is an example, the first 150 meters or so is the photic zone and we have a pretty good idea of how microbial populations of species are layered in that particular 150 meters. What particular key suites of organisms and their associated genes are responsible for many of the critical processes of primary production, photosynthesis, nitrogen metabolism and so on so that the inventory of organisms and genes and some of their variability in terms of gross abundance are pretty well nailed, okay. So that's the foundation and part of that foundation has been laid by sequencing the ocean's genome. So given that [phone rings] – sorry about this…

Marc Pelletier
I'll turn mine off too.

Edward Delong
Given that what we'd like to do from the system's perspective, it is pretty much walk the hierarchy in the same way that has historically developed in systems biology. So it's now becoming

possible to apply transcriptomics and proteomics to the same very complex communities. And so what we need to ask and this relates to your question a little bit on because it's quite complex, is what time and space domains, temporal and spatial elements do we want to take a lot at first and from our perspective because we can now start to apply transcriptomics to complex communities we kind of want to look on time scales that make a difference with respect to how we think gene expression and crosstalk is happening in this communities and what makes a difference with respect, for instance energy and carbon flux, through these communities.

Marc Pelletier
So let me ask you a very simple question, are you looking by taking a looking at the water column in the Pacific…

Edward Delong
Yes.

Marc Pelletier
Is that environment the largest unmodified system, not by its natural modification but say for example if you look at a large, large desert, the ecosystem that's found in this square meter is going to be the same as the one that's 500 miles away or just about identical. If you look at the – say for example the forest in Ohio versus the forest in Maine it's going to be extremely different. But is the Pacific where you're sampling the largest possible single or – system, is that making sense?

Edward Delong
[13:01] Yeah, I understand exactly what you're saying. It's – so it turns out you can quibble about what's the largest and if you want to just take an average of the global environment, this isn't necessarily intuitive but it turns out to the average depth of a sea is about 3,800 meters and it's about $4^{o}C$ down there and that's the biggest volume in the biosphere that really is encompassing the earth. So if you wanted to take a global average of the largest environment that we know of where life exists, it's more like 380 atmospheres and $4^{o}C$ at that median depth of the ocean. So that's the winner for the biggest.

But it is true, Marc, the reason why this site that we study with our colleagues at the University of Hawaii, Dave Karl in particular – why we study this patch of the ocean is that it is representative of a very big part of the central oceans and one of the questions you can asked is how is that patch, big patch, the Pacific, the same or different in terms of both the gene content, the organismal content and any responses we may see, biological response, to the Atlantic for instance.

Andre Nantel
Do you see in something as – in the Pacific any strong either seasonal or daily variations. You'd probably be able to detect daily variations during the changes in daylight over the day, right?

Edward Delong
Yeah and that's exactly right. So that's one temporal domain we really do want to watch because there are really marked changes in the diurnal patterns of gene expression, particularly in the phototrophs, so for us that's like our background. And so I'll tell you a story that's been really fun and this has been in collaboration with Penny Chisholm and other colleagues here in MIT is that we can take transcriptomic datasets from the Pacific, and I can tell you without knowing ahead of time, what time of day they were taken. So we can tell the time of transcriptomics because there are phototrophs there whose diurnal cycle has been very well studied in the laboratory and they are like clocks. So…

Marc Pelletier
Let me interject a little bit here because there is going to be – I understood that sort of…

Edward Delong
Okay.

Marc Pelletier
So transcriptomics, phototrophs and expression profiles.

Edward Delong
There's a lot of jargon flying around.

Marc Pelletier
It's good stuff though; I love it.

Edward Delong
Let's put it in English.

Marc Pelletier
Sure.

Edward Delong
Okay. So the microbes in the ocean, and particularly the little green ones that are able to use sunlight to fix CO_2.

Andre Nantel
Cyanobacteria

Edward Delong
Yeah. Time – time the way they turn their genes on and off for the particular jobs they have to do over the course of the day. So in the daytime we need to have our light antennas up and our photosynthetic machinery cranking so we can grab that sunlight and fix CO_2. And so also the enzymes that take CO_2 out of the atmosphere and make it into cell carbon, those are cranked up as well. And then at night time, sun goes down, the little green guys, those little green bugs say, okay, we are going to switch off our light antennas and we will express another suite of genes that has more to do with some of the carbon processing that we have to do and get some of our eating done at night, prepare ourselves for the sun coming up next morning.

And there is a rhythm of genes turning on and off over the course of the night and day that is quite regular, it's a circadian rhythm and it tells you what the organisms were doing. And because we've so well studied that pattern of some of the predominant organisms that live out there in the ocean, we can look at a suite of genes that are on and off and say, oh, well I know that must have been taken like about 3:00 in the afternoon. We have the system that will nail that one particular suite of organisms and genes. So it's almost like a ground truth.

So we can follow the rhythm now of gene expression, genes turning on and off and how that relates to the pathways that are being expressed, that processed carbon energy in these microbial communities and potentially, and we are not there yet but this is where it's headed, how the kind of metabolic cascade works. It's like a bucket brigade, right? The little green guys are fixing CO_2 but they are leaking carbon and then somebody else is eating that, there is somebody turning on their genes to take advantage of that. There is nitrogen metabolism. All these – all these complex processes that are like a bucket brigade that have to do with the flux and flow of the energy and matter from the phototrophs through the heterotrophes and as they sink down into the deep sea.

Andre Nantel
So are you only doing the Pacific or are you also doing other – or you or other groups – other environments in the oceans like the Arctic or places where rivers flow into the oceans, things like that?

Edward Delong
Absolutely. My colleagues here at MIT, and we are collaborating with them, are looking at the Atlantic. And without giving some unpublished stories away, I can tell you that if you compare the Central Atlantic to the Central Pacific, the genes that are present in one system, in the same organisms by the way, and absent in the other are actually telling you something about the chemistry that's going on there. So it turns out in some habitats – if you compare the Atlantic to the Pacific, there is a difference in the phosphorus composition. And that's absolutely reflected in the genomes of the same sorts of organisms that are in both the Atlantic and Pacific that they've accumulated genes to deal with, for instance, the lack of phosphate or the lack of nitrogen. So, in essence the content of the genome is diagnosing to some extent what's going on in the environment. And I don't want to talk about that too much because that's really work of my colleague Penny Chisholm and her students in collaboration with us, but what's really cool about that, what it's telling us is that the genomes are diagnostic of the environmental conditions in the environment once you dig deep enough.

Marc Pelletier
[19:36] You said the genome at one point and it was a – do you feel or it's – when you think about ecosystems, do you think of it as a genome or the genomes? And let me extend that question. When you are doing the samples, how – how do you pick – well, how many – in the samples you are taking, how many organisms are there and how many genes, different genes are there? And how do you detect them?

Edward Delong
That's a great question, Marc, and that gets at the underlying challenges associated with going out to the environment that are to some extent different than sticking with the model system in the lab, and it has to do really with sheer numbers. With these datasets that we take out in the real world size matters. And let me get into that. But let me address, let me address your first question. Do I think of it as a genome or as genomes? Okay, that's a key – and I think a really important question because this word metagenome is commonly used and thrown around as if the sample that you are studying and the organisms that represent it are some kind of singularity a metagenome and I think that that's a, it's an interesting concept in some ways but it leads you down the wrong path with respect to how to think about this system, and how can I describe this simply? When you look at a sample there are many layers of biological complexity that have to do with, even if it were a pure species what we know out in the environment is these populations are incredibly complex. There are lots – there is lots of diversity, genetic diversity in any given species population.

Andre Nantel
How may species, ballpark?

Marc Pelletier
That's my question.

Edward Delong
I'm not going to answer that one but I can…

Marc Pelletier
Okay.

Edward Delong
There is nice quantitative estimates that get you away from semantics into just what the data tell you, so let's look at a population in the ocean, what you typically see. This could be a population of the little green guys like Prochlorococcus, it could be a heterotrophe, name your favorite bug that lives out there. But if you look at a population what you'll see is compare one cell to its

neighbor, the range of genetic difference that you see in a population is something like about 10% average nucleotide identity.

What that means is that the population of organisms, species that is, the same species living in a population varies anywhere between being near 100% identical across parts of the genome to about 90% identical, and that's a lot of variation it turns out. And some of that is just point mutation and those sorts of difference but some of it is insertions and deletions rearrangements. And yet these things are cohesive as a population. Now, that's one species, okay. And then there is variations on each of those themes within different phyologenetic groups so that the complexity really is quite astounding.

Andre Nantel
So the stochastic definition of species which essentially was initially applied to large animals…

Edward Delong
Yes.

Andre Nantel
Probably doesn't really apply to the bacterial world.

Edward Delong
No. Because well typically it's the definition of species, it's the liaison, the ability to sexually recombine and that sort of thing and it's just not as useful a definition for prokaryotes. Although it is true the closer – the more closely related these small bugs are the more likely they are to undergo homologous recombination. So that relationship that is that genetic relationship just drives some of the dynamics for how genes move around, fix and exchange but, yeah the species definition doesn't work like I'm going to dance around that one, because it's…

Marc Pelletier
I have a dumb question, a really dumb question. Have you ever in your data analysis gone, oh, this is the data, this is looking normal, this is progressive effect of either day or weather changes and then all of a sudden, you know, look at the your gene expression profile and it's absolutely out of whack, and then you think about it, you think about it, and think about it and it was a whale that had just gone by and peed in your samples something, have you ever witnessed anything like that?

Edward Delong
So that could happen and we have seen some stuff, if you will, that we can't explain but typically…

Marc Pelletier
You don't have to answer it though.

Edward Delong
No, it has to do with the system that you are studying and you know we've joked about this before, right? You think I go to Hawaii, hell, I live in Boston and I'm trying to work on my tan and that's fair but actually…

Andre Nantel
I love Hawaii. I'm even wearing a Hawaii t-shirt right now,

Edward Delong
Good for you. I think that's great. You should come and sample with us. But…

Marc Pelletier
He'll work cheap, too.

Edward Delong
The reality is that we have a good – we're studying these systems because we have a pretty good handle. We know when the whales are coming by and peeing, you know and so we kind of take that into account. There is some stability to these systems, there is enough predictability that we can stay away from kind of anomalous fliers so that we can more look at just the pulse, the heart beat of the natural rhythm of the system and as we look at that over the right temporal and spatial scales we can start to put a picture together. So for instance we talked about the day/night cycle, that's almost like our control. We have to know what the natural variation is, with respect for instance that genes turning on and off so that then we can put more events on top of that.

[26:03] Let me give you an example, a bloom of phytoplankton that typically occurs in the ocean can happen over the course of a couple days or weeks. So one can imagine looking at the pulse of this fairly constant diurnal cycle genes turning on and off and then that's punctuated by some event, it might be the upwelling of a nutrient and then you could see the organisms responding to that nutrient. But listen to this because this is what's different about doing these kinds of studies in the communities as opposed to the lab, the changes in gene expression that happened are going to lead to some winners and some losers with respect to any environmental response and that plays out into changes in community structure so community succession or some species winning and some species losing.

And so this is a dynamic that's really hard to study in the lab but we have a chance to look at and feel and we have already seen examples of this where a particular environmental perturbation leads to some initial changes in gene expression. Those translate into changes in metabolic flux in species exchanges which finally are translated into winners and losers in terms of a community succession. So that you end up after that event with a different suite of microorganisms dominating than you did at the start that has to do with this flow of information from event to gene expression to community response to competition and succession.

Andre Nantel
So just a quick technical question on the profiling before I let Marc do his audible ads. You do them by pyrosequencing or you use microarrays?

Edward Delong
Yeah, this is by pyrosequencing. And this is where the downside is I mean you asked some really good questions initially. So the weakness of our approach is right now and this is why I said size matters, has to do with how deep we can really sequence. So what we can really observe right now and this is something that will improve over time is really…

Andre Nantel
An average gene response essentially, right?

Edward Delong
Yeah, the more abundant transcripts or genes that are being expressed is what we can see. That it turns out does tell us a lot but a lot of stuff slips under our radar right now and that's just the statistical price that we pay with this particular approach. Now the strength of the approach of course is we require no a priori knowledge. We can go in and look at every transcript that's being, that we – that's in our detection threshold and we don't have to have any prior information so we are learning lots of new things and in fact we recently…

Andre Nantel
How much coverage do you get compared to the general DNA population or if…

Edward Delong
That's a good question. If things are over a couple percent of the population or even more than that, we get good coverage across the genome. So for an organism that's maybe 10% of a

population we'll get on average maybe a couple x coverage of transcripts across the genome but of course it's uneven. The difference in the genomic DNA is that each gene is represented, or let's say, one-time across the genome and so it's kind of full coverage, stoichiometric. In the transcriptomic dataset some genes or transcripts are highly expressed.

Andre Nantel
All the time, yeah.

Edward Delong
And then others are low and so it's much more uneven and that adds to some complexity of data.

Marc Pelletier
Let me – I want to get a little bit back into the technology and the pyrosequencing. That's a whole new area. And it's a rather interesting shift that it's just simpler to sequence the genes in the ocean than to try and quantify each one using other approaches like RTPCR or whatnot with – I'd like to get into that a little bit. And then perhaps we can end with some of the important questions that – because you're basically learning about these various systems and attractions. One, are they predictable? And then have you seen – made predictions and they've come true? Are there major trends that you're seeing?

But before we get to that, we do have to thank our sponsors for Futures in Biotech. That is Audible.com. I think they have over 60,000 Audible books and radio shows and magazines and newspapers available. And – so we have a pick, and that pick is, Andre?

Andre Nantel
Pandora's Star by –

Marc Pelletier
Awesome.

Andre Nantel
I forget the first name, Hamilton.

Marc Pelletier
It's Peter Hamilton. Peter F. Hamilton.

Andre Nantel
Yes. It's essentially the first book of what is called the Commonwealth Saga. It's very interesting space opera science fiction book, where essentially you're several – initially, you're several hundred years in the future. And maybe I should spoil just a little bit the beginning of the book, which kind of tells you about the style, which can sometimes be very funny. Essentially one of the characters was an astronaut on the first mission to Mars, lands on Mars, and the first thing he sees after all these years of training and traveling, is a freaking hippie in a homemade spacesuit, because he and his friend essentially built a wormhole technology. And the first thing they decided to do was test it on live TV in front of billions of people. And these two guys essentially ended up – these are three main characters in the book. And I'm pretty sure that the guys who invented wormhole were inspired by Steve Jobs and Steve Wozniak of Apple. Except they're called Nigel and Ozzie but essentially it tells you a lot about the general humor in this book that is also very, very interesting.

There's a lot of separate timelines, that initially you go like, so what's the point of these separate stories that get really nicely wrapped up toward the end and it's a very satisfactory ending. And of course you get more because now there's five books in this saga, not only the ones that take place initially in the first two books of the Pandora's Star, and its follow-up whose name escapes me right now. And then there's a whole new series that's started that's taking place 1,200 years in the future, but because these characters live a very long time, some of your old favorites are still there as well their descendants.

Marc Pelletier
Well, you just have to go to Audible.com and check out Peter F. Hamilton, and you'll find Pandora's Star and all the books in that series. And so if you want to download it for free, you head over to Audible.com/Biotech and sign up for the Audible Listener Gold account, it gives you a free credit towards a free book. And if you don't like it – it's a 14 day trial. If you don't like – or if you don't feel like you're going to stay a subscriber, you cancel, but you get to keep the free book, so it's a win-win situation. So head over to Audible.com/Biotech if you want to download Pandora's Star. So I'd like to thank them for sponsoring Futures in Biotech.

So let's get to some of those – tell us a little bit about how you were – just technical, the experiment, on the bench, for doing pyrosequencing. But I'd really want to – we're running a little short on time, I'd really like to find out, what are the big questions? Like the global questions that can be answered by looking at small organisms in the ocean.

Edward Delong
[34:02] You bet. So technically – well, you spoke with Jeffrey Gordon I think previously on Futures in Biotech and his whole thing about pyrosequencing and I totally agree with, he calls it the democratization of sequencing, right? Because instead of having to go to a very large sequencing center and waiting for a year for your data to get back, it's now more responsive, it's like doing an experiment. So I don't – rather than getting into technical details, let me tell you how things have changed. You can now think of sequencing in the same way you might think of a gas chromatograph or any other laboratory instrument or device that's feeding you data back. That is you can simply use it.

Marc Pelletier
Tricorder?

Edward Delong
Yeah, well, I don't have one of those. I wish I did.

Marc Pelletier
I do. That's okay.

Andre Nantel
Do you have a pyrosequencer on the boat?

Edward Delong
Yeah, well, people have talked about that. How useful that is, if you could get it in real time it would be so cool. But unfortunately our – that is the technical issue. We have to process the samples quite a bit before we can feed them to the beast.

So right now the machines are still landlubbers, they are still land locked. But maybe some day they will be out there and – what real time information like this would be amazing. That's where we would love to head. And in fact there are some people working on sampling devices that can do some of that. So watching for instance phytoplankton blooms in real time, and harmful algal blooms, you can get a early warning system. That's a whole another story, maybe somebody else you could interview actually. But – so there are devices that you have out in the field, they give you real time data and send the information back by RF. We can't do that with sequencing yet. So we are still locked in the lab. But the process is pretty simple.

The challenges are really biological. If you want to look at gene expression, you know – or all experiments like that over the course of the development of microarrays and stuff even, the trick has been well how fast can I sample, right? Because you want to look at what the environment is doing to the organism not what I am doing to it while I am sampling. And so…

Marc Pelletier
Right, right.

Edward Delong
…it's always tough. So we worked really pretty hard on developing methods that we can take a very small amount of sea water and we are talking maybe a couple hundred mils, filter it, for what us is very fast, and that's a couple of minutes maybe. And then fix that sample, there are preservatives that can prevent RNase degradation and things like that. So that's the front end that we worked hard at. Out there in the field…

Andre Nantel
There's enough in 200 mils of sea water, there's enough organisms to do a pyrosequencing run?

Edward Delong
Yes. So what we have been able to do using linear amplification techniques, you need on the order of about 10^7 cells. And there's about 10^5 cells per milliliter in sea water out where we work. So if we can slam down 100 mils of see water very quickly, we have got enough sample for the RNA analysis oddly enough. We need less for the RNA because we do a linear amplification actually that was developed for looking at eukaryotic gene expression. We actually need more sample if we want to look at the DNA. But anyway once we get those samples we typically will transport them home on liquid nitrogen and run through the process of extracting either the RNA or DNA, make – getting that on to the pyrosequencing beads and then shooting that through the pyrosequencer. So I won't get the details of that, because…

Marc Pelletier
What about viruses?

Edward Delong
Well, viruses one can do too. And that's just a different…

Marc Pelletier
Oh, you don't get them in your samples? Oh, because you are filtering. There you go. All right.

Edward Delong
Yeah. We actually do though. Interestingly enough you asked about these weird events where a whale goes by and something like that. In some of our metagenomic samples even though we are sampling cells on a 0.2 micron filter, most of the viruses should go through. We have had on occasions samples that have been 10% or more of phage and in fact phage that we could identify in the photic zone where we're sampling, we've gotten 10% or more cyanophage in all the DNA and all we can figure is that we happen to grab a sample in which the cyanobacteria were basically undergoing a massive lytic cycle. That's when the little phage reproduces.

Marc Pelletier
Yeah.

Edward Delong
And so we cycled the intracellular phage associated with the cell fraction, which was 10% of the all the DNA.

Marc Pelletier
Wow!

Edward Delong

So we do see weird events like that and some of them are interpretable. And in fact …

Andre Nantel
So you….

Edward Delong
Go ahead.

Andre Nantel
Usually how many samples can you go through for one study?

Edward Delong
Okay so, it depends on the study and I've been telling you about kind of survey mode but since we are getting short on time I'll tell you where I think this stuff is headed. And that is looking at microbial communities, microcosms where we actually do a perturbation, because we want to get predictive. I mean, Marc asks the question, what are the big questions and can you be predictive and does it tell you something big?

Let me tell you a story about how we are doing stuff that relates to something big so that you can at least get a flavor of that. So, it turns out that dissolved organic matter in the ocean, that's just organic carbon in the ocean represents as much carbon on the earth as does CO_2 in the atmosphere. Okay, it's a huge pool of carbon.

Marc Pelletier
Wow! That's great.

Edward Delong
Hang on, once second here. The janitor's coming in.

Edward Delong
That's okay how is it going? Good.

Andre Nantel
He gave you the finger.

Edward Delong
Yeah. So that's a lot of carbon. And we're trying to figure out how that stuff turns over. And we actually don't know the details, and in fact the chemical nature of that carbon isn't very well defined either. So how do we get out?

Marc Pelletier
Could you repeat that again? This is where I'm – so, the carbon in that…

Edward Delong
Organic…

Marc Pelletier
… strata of the ocean is as large as the carbon in the atmosphere?

Edward Delong
There's as much carbon tied up in that organic carbon dissolved in the ocean as there is CO_2 in the atmosphere.

Marc Pelletier
All right.

Edward Delong
It's a big pool of carbon and so understanding the fate and the rates of degradation and the chemical nature of that is a big and significant factor in the overall carbon budget turnover on our planet. But we don't know a hell of a lot about it.

And so we've been trying some experiments to see if we can learn more and I am going to briefly walk you through those. So what we do is set up a 20-liter microcosm which is just a pot of sea water that we incubate under in situ temperature and light conditions. And ones a control; one, we actually add organic carbon to that we've collected on a site. So what we want to see is what's the effect of this carbon and what organisms are able to deal with it and how's it turning over. So then we can sample the control and we can sample the organic carbon addition experiment and we can look at the changes in gene expression over time, and we typically will sample rapidly at first on the order of every half hour or so. And then every hour or so maybe, over the course of 24 hours and ask what happened. Is the experiment where we added the organic carbon any different from the control, what are those differences, what does it tell us about organic carbon degradation.

And without getting into too much detail or – what I can tell you is, is that these experiments are actually really informative because they are taking us directly into particular organisms and pathways that are involved in this carbon turnover. So in the control that was just sitting there we didn't see a lot of changes at all, the same cells that were at the beginning of the experiment were at the end at 24 hours and it was kind of flat-lined with respect to unusual gene expression patterns. We just saw the diurnal, the day/night gene expression patterns, genes going on and off that we would have predicted we'd see.

In that experiment where we added the organic carbon, in two hours we saw particular groups of bugs coming up and then – that is, their genes being highly expressed relative to the rest, and that wasn't going on in the control and then another set of bugs in another two hours and so on. So we saw changes in suites of genes that were related to this organic carbon that eventually ended up with the population basically doubling or so and a completely different set of organisms coming up at the end than was there at the beginning.

In fact we know what those are. And they tell us something about the biochemistry of that organic carbon. I won't get into the specifics of that. But this small experiment we did relates to exactly what I was trying to tell you about, which is we can look at an environmental effect. We can watch the temporal changes in gene expression that tells which genes and pathways and organisms are lighting off and going off and on and then how that translates into some effect. In this case, it was basically the oxidation of this complex organic carbon and the organisms and pathways that came up tell us exactly what sorts of chemistry and carbon that we should be looking for. And now we can go back and test those hypotheses. So we are making…

Marc Pelletier
Let me ask you – sorry, I've got a question.

Edward Delong
Yeah.

Marc Pelletier
Were you – have you gone into areas where there is a lot of atmospheric carbon being emitted regionally? Are there ecosystems – I mean where you have matched the changes in gene profiles and organisms that have flourished similar to your model system, where you have added organic carbon and compared it to a control?

Edward Delong

We – yeah, but that's – Marc, you hit on one of the things that these technologies I think can really be applied for pretty darned quickly now that they are starting to become more easy to use and more accessible. It's still little pricey but it will get better.

And that's – so microbes are kind of the frontline when it comes to chemistry. They respond very quickly. Gene expression can change within a couple of minutes. And the sorts of genes that are expressed tell you something about what's going on in the environment.

So you can think about microbes as being sorts of canaries in the coal mine. They are exquisite biosensors. We don't need to deploy biosensors. They are all around us. And they are all out there. All we have to do is learn how to read their outputs. So with respect for instance on to an area that might be impacted by anthropogenic carbon as you are suggesting, Marc, I am certain that we could find particular gene proxies using these sorts of approaches that would allow us to use bugs as biosensors for particular chemical insults. That's one approach that I think should be explored pretty rigorously because I think it's going to work

Andres Nantel
Something I heard a lot in the press recently was this crazy hypothesis of seeding the ocean with iron to encourage carbon dioxide fixation by microorganisms. Have you tested whether that would work using your methods?

Edward Delong
[46:05] Well, we haven't done that. There're a lot of people, some of my colleagues here that have been involved in some of those iron fertilization experiments and let's get off technology for a second because I'll say a few words about that. There're many people who aren't considering that crazy right now. So there is an area that is being very seriously discussed that we should all be aware of as people on this earth, scientists that's now called geo-engineering. And this really reflects efforts to basically impact an environment more on a global scale. In the context of geo-engineering activities now it is to help mitigate climate change.

So there are many now geo-engineering schemes on the table that are aimed at either trying to draw down the CO_2 in the atmosphere, which is the biggest greenhouse gas which is leading to potential warming effects, or to actually just reflect more sunlight back out into space. Some of the schemes that people have come up are as wild as putting solar reflectors out in some out-of-earth orbit that could be manipulated robotically to reflect enough sun back that it will decrease the amount of solar radiation coming to the earth. So geo-engineering; it's wild but it's happening and people need to be aware of it.

And one type of geo-engineering that people have discussed is exactly what you say, Andre. It's dumping fertilizers, in this case, iron into the ocean, that would allow more plants, basically more of the algae, to fix CO_2 and in the hope and this is a hope right because this is what we can't control. We can control the input of iron into the ocean but we can't control what happens to those algae after they boom.

Marc Pelletier
Right, I like the earth the way it is. I don't want it to change. I want my kids to have the same ocean, at least no worse than we have. So leaving no trace might be the way to go.

Andre Nantel
Science never has bad effect. There're never any unforeseen consequences. We understand everything perfectly. Stop worrying.

Edward Delong
Yeah, let me worry. Well so you guys joke but listen you know what the argument is? We've already done experiments and we've been doing it. We've done geo-engineering, and that there is some validity to that argument in the sense that, yep, mankind is fixing more nitrogen for

instance than all the organisms on the planet combined which has only happened in the past 100 years with CO_2 and so on and so forth.

So these are – I am not weighing in one side or the other, although I do think we have a lot to learn before we should be messing about with the system because we don't know enough to make good predictions about many of the schemes that people are discussing. But it's seriously on the table now.

Marc Pelletier
Colleen's waving her hands. There's another show coming up.

Edward Delong
All right, that's it, I guess.

Marc Pelletier
No, I'd really like to thank you first of all for doing the work that you are doing. I think developing the technologies and databases of information and knowledge about how these ecosystems are working together and using it as the ecosystem itself and those as a reporter of what's going on is incredible. Just using pyrosequencing, it seems like one amazing way to get immediate data on the state of an ecosystem.

So thank you for your work and thanks for coming on today, for being on the show.

Edward Delong
Well, thank you, Marc. I guess, I'll just turn that around and say thank you for what you are doing in terms of being open source in getting information out to the people. So thank you for that.

Marc Pelletier
No problem. So that was Dr. Ed Delong who is professor in the Department of Biological Engineering and Department of Civil Environmental Engineering at MIT.

I'd also like to thank Andre Nantel, Senior Research Officer at the National Research Council of Canada and Adjunct Professor at McGill University in Montreal. Thanks, Andre.

Andre Nantel
Thank you.

Edward Delong
Thanks, guys; good fun.

Marc Pelletier
Thanks for coming on. I'd also like to thank the great folks who made this possible, our producer Colleen Kelly and Leo Laporte, Dane Golden, making Futures in Biotech possible.

If you want the transcripts of the show they are available, thanks to the folks at Pods in Print. If you need transcripts done they can easily handle even the most technical stuff, so if you need them head over to podsinprint.com. And you can get the transcripts at futuresinbiotech.com

Lastly I'd like to thank Phil Pelletier and Will Hall for their opening and closing themes. Futures in Biotech, I am Marc Pelletier. Thanks for listening.