

DISRUPTIVE #7: FISSEQ - Fluorescent In Situ RNA Sequencing

Host Terrence McNally interviews George Church, Rich Terry, and Shawn Marcell. Podcast published on September 28, 2016.

Terrence:

Hello, I'm Terrence McNally and you're listening to DISRUPTIVE the podcast from Harvard's Wyss Institute for Biologically Inspired Engineering.

One of today's guests, George Church, has made the point that as medicine moves from very blunt instruments - where you had to open up a chest all the way, for example, or had to use molecules that would hit almost every part of your body - now molecules can find one base pair out of six billion and change it - He says we need observational tools that can deal with that high level of resolution and comprehensiveness.

And we're going to talk about one such tool. Fluorescent in situ RNA sequencing - F-I-S-S-E-Q - or FISSEQ.

Working copies of active genes — called messenger RNAs or mRNAs — are strategically positioned throughout living tissues, and their location often helps regulate how cells and tissues grow and develop. Until recently, to analyze many mRNAs simultaneously, scientists had to grind cells to a pulp, which left them unable to pinpoint where those mRNAs actually sat within the cell.

Now a team at the Wyss Institute and Harvard Medical School has developed a new method that allows scientists to pinpoint thousands of mRNAs and other types of RNAs at once - in intact cells.

FISSEQ could lead to earlier cancer diagnosis, help biologists better understand embryonic development, and even help map the neurons of the brain.

I'll talk with George Church, Wyss Core Faculty member and co-founder of ReadCoor, the startup that will bring FISSEQ to market; Wyss lead senior scientist, Rich Terry, President, Co-Founder, and CTO of ReadCoor; and Shawn Marcell, Wyss Entrepreneur-in-Residence and founding Chairman/CEO of ReadCoor.

The mission of the Wyss Institute is to: Transform healthcare, industry, and the environment by emulating the way nature builds.

Our bodies — and all living systems — accomplish tasks far more sophisticated and dynamic than any entity yet designed by humans.

By emulating nature's principles for self-organizing and self-regulating, Wyss

researchers develop innovative engineering solutions for healthcare, energy, architecture, robotics, and manufacturing. [02:06]

George Church is Professor of Genetics at Harvard Medical School and Professor of Health Sciences and Technology at Harvard and MIT. He's Director of the U.S. Department of Energy Center on Bioenergy at Harvard and MIT and director of the NIH Center for Excellence in Genomic Science at Harvard. He has co-founded a number of companies, including ReadCoor.

Church earned a bachelor's degree from Duke University in two years and a PhD from Harvard. Honors include election to the National Academy of Sciences and the National Academy of Engineering. He has coauthored hundreds of scientific papers, more than sixty patents, and the book, "Regenesis: How Synthetic Biology Will Reinvent Nature and Ourselves." [02:41]

To set the context for this episode, George Church offers an overview of the evolution of sequencing technology –

Church:

It dates back at least to the '60s when RNA sequencing and protein sequencing were the main ways of getting insight. In the mid-'70s, ways to do DNA sequencing based on electrophoresis came into play. Those were automated and made less radioactive, more fluorescent. In the '80s and '90s, it switched from slab electrophoresis, capillary electrophoresis. None of these scaled particularly well.

Then the breakthrough came around in the '90s. Recognizing that you could reprobe flat surfaces to get hybridization and other information led way to what's now called "next generation fluorescent sequencing", which originally in our group back in 1999 was called "fluorescent in situ sequencing".

There, "in situ" just referred to a flat surface. "In situ" today means literally in complex tissues with multiple cells and very high resolution.

McNally: [03:50]

Where does he see sequencing moving forward –

Church:

Sequencing technology in the future will, I think, take two major routes which are largely new. The obvious one is that the cost will decrease and accessibility will increase. Part of that I think will happen along with a new portability, that is to say we might be able to carry around real-time sequencing devices. My candidate with that would probably be nanopore sequencing.

Another major new avenue is this *in situ* methods where most of the information we want when we do microscopy or, for that matter,

visualization of imaging in general, is we'd like to know what all those little gray blips are that we see. We'd like to have the name of each of them, and that's what in situ sequencing is all about.

McNally: [04:36]

What is the importance of sequencing? What does it give us, and how important is this whole arena?

Church:

Sequencing, or being able to read DNA, has so many applications today that it's hard to even begin. It ranges from forensics, where you can identify the individuals involved in crimes or identify individuals that have been lost or lost track of. You can find trace amounts in environmental samples. You can determine whether a particular fish is what the chef is saying it is. There's famous cases of that.

You can use DNA sequencing for medical research, for medical diagnostics, use it for agricultural research, and veterinary diagnostics. You can use it for literally reading and writing information that has nothing to do with biology.

The list goes on and on of what you can do with the ability to read DNA. Our ability to write DNA is very intimately dependent upon our ability to read it as well.

McNally: [05:40]

Now there you were referring to DNA. What is the distinction between RNA and DNA, and - what's the similarity, what's the distinction?

Church:

Yeah. Myself and others will often use DNA and RNA somewhat interchangeably. You can turn RNA into DNA and vice versa with simple enzymes, and that occurs in nature.

But RNA is normally the conduit between DNA, which is the storehouse of information in cells and viruses, to actual functionality, either directly, where the RNAs have functions, physiological roles, catalytic or structural. RNA in the form of messenger-RNA is a way station on the way to proteins, which have the functionality of structure, catalysis, and regulation.

McNally:

One way that I've thought of it is that DNA is the blueprint and RNA is the more dynamic moment-to-moment interaction with the environment.

Church:

That's right. That's a good way of thinking about it, that RNA is more dynamic. It has many different roles in the cell. Some of those roles are even more prominent when the RNA is translated into protein, as some of them are, and then the proteins take on those dynamic roles of interacting with the environment and producing the body of the cell or the organism.

McNally: [07:04]

We narrow our focus to FISSEQ - “fluorescent in situ RNA sequencing” - and I ask for the story –

Church:

Like most science and engineering, there are goals and obstacles. In this case, you might date it back to my PhD thesis where we had the first direct DNA sequencing method, where we would re-probe a solid surface, in this case nylon membranes, and each time we'd re-probe, we'd get a new set of images. Those images would be new sequenced data. That was '83.

That led eventually to multiplexing and bar codes, which were a key component of modern next gen sequencing. The idea of re-probing and reimaging is really common to almost all of the next gen fluorescent high-throughput low-cost sequencing.

The first real barrier to turning those primitive 1980s genomic sequencing methods into in situ was miniaturization. The thing that made next gen sequencing so much more scalable and valuable than all the years and billions of dollars invested in the Genome Project, the thing that made next gen so much more valuable, was this miniaturization, where we could go from the millimeter scale of conventional sequencing, and even the genomic and multiplex sequencing in the '80s, down from millimeters to microns, and eventually even a submicron, nanometer scale.

That was the first barrier and also the first gigantic opportunity. We were coasting, drafting on the successes of the microfabrication in the electronics industry. Miniaturization was the key thing behind Moore's Law for electronics. [08:46]

The next big barrier came from ways to get the enzymes to behave so that you could get a large amount of information from a pixel and an image. You might mobilize cells or nucleic acids on a conventional microscope slide, but then you'd want each pixel, or voxel in three dimensions, to reveal what its identity is.

That meant that the enzymes had to be compatible with the fixatives that kept everything in place and with the complicated structure of the cell. There is a series, a path of discovery of all the nuances of getting those enzymes to behave themselves. [09:28]

There was also, in between the mobilization and the next step, which was miniaturization, there was also getting good fluorescence efficiency.

Then after dealing with real cellular material, real tissues and the complications they have for enzymatic systems or even hybridization systems that don't involve enzymes, were issues having to do with computational issues, representational issues, so you actually are getting all

the RNAs.

Most significantly, and the one that most recently we had a breakthrough, was being able to pack enough information into a cell. One of the advantages of conventional sequencing for RNA or DNA is that when you break open a cell and grind it to a pulp and spread it out over a slide, you now have lots of space for all of your sequence reads. [10:19]

McNally:

The thing that I said in the intro, which is that up till now you had to grind the cell, and you just mentioned it again. Why did you have to grind it? What allowed you to overcome that?

Church:

There were two reasons why we ground up cells in order to analyze the DNA and RNA. One of them was purification, to get rid of the barrier where the impurities - proteins and other molecules - would interfere with the enzymes getting access to the DNA or RNA.

The second was so that you could get that space that you need in between molecules, because in a cell, the molecules are more or less on top of each other. They're literally touching each other, and so one subnanometer molecule will be a subnanometer away from another one. That's really quite tight from a microscopy standpoint.

Third, was amplification - so you'd want it to be pure, you'd like it to be isolated, and you'd like to have more than one copy of each one, so that you could have good signal to noise. You'd have good detection competence. Those were the three main reasons. We've solved all three of them essentially in situ now. [11:32]

McNally:

As I mentioned in the intro where you said our observation has to catch up with our engineering, this is going to take our ability to observe, in some sense, a whole level down, correct?

Church:

That's right. When we want to engineer a biological system... For example, if we want to epigenetically create new tissues from stem cells for testing drugs or for transplants, we'd like to know whether those new tissues are really reflective of the old ones or have new properties that we want. Since the old tissues - the natural tissues - are part of this compact, complex structure, we want to really know what the differences are between our synthetic structures and the natural ones. That's one example.

Again and again, whenever you're doing biological or biotechnological engineering, you find yourself asking, "What is the cell thinking? What is the structure and function of this cell in this environment or with this genetic tweak?" This in situ sequencing is the tool that we really have needed all

along. [12:37]

McNally:

It allows you to see where the different RNAs and m-RNAs are in relationship to each other, and how they're interacting, who they're interacting with?

Church:

Exactly. The nucleic acids of the cell are not uniformly distributed. The cell is not a sphere with uniform distribution of molecules. It seems obvious once you state it that way. It's exquisitely important where the molecules of the cell are positioned. [13:05]

McNally:

Where before you had to grind, what was that specific thing that your team came up with that allowed you to do it without that step?

Church:

Right. (laughs) Ironically, one of the major breakthroughs in how we are able to do it without grinding up the cell is just, (a) realizing that it was desirable to not grind up the cell ...

McNally:

(laughs) I love it.

Church:

... and (b) then just doing it.

There were plenty of barriers once we made that leap, which had to do with making space for the reads, making sure the fluorescent intensity was high enough through various amplification methods, and making sure that the enzymes or hybridization probes had access to the sample.

But the key breakthrough was realizing that we could miniaturize, and in doing so, we would eventually miniaturize down to the size of a cell.

McNally: [13:54]

For another experience of the development of FISSEQ, I turn to Wyss lead senior scientist, Rich Terry, a member of Church's Lab and President, Co-Founder, and CTO of ReadCoor. Before coming to the Wyss, Terry designed, developed, and produced a cost-effective, high throughput, open source DNA sequencer, "The Polonator.", and prior to that was Senior Project Manager at (CERN) The Center of European Nuclear Research, in Geneva.

[14:18] First, I ask how he sees his path to the work he does today –

Terry:

I like to think of myself as a product of NASA's propaganda engine from the seventies and eighties. I really fell in love with aerodynamics and aerospace as a kid and always wanted to be an aerospace engineer. I followed that path

and got a master's degree in aerospace engineering from Boston University.

However, upon graduating Boston University, I really fell in love with mechanisms of biology and solving problems in biology, and initially through device development, then into implantable devices and neurology. At which point, I became more interested in really what's going on at the molecular level and had the good fortune to link up with George Church and really develop a lot of new and exciting tools across synthetic biology.

McNally:

Interesting, from outer space to inner space.

Terry:

Yes...yes. [15:24]

McNally:

Like Church, Rich Terry makes clear that their innovations are built on the work of many others.

Terry:

One of the largest challenges that we really faced was we were trying to develop a lot of these techniques prior to current next gen sequencing being a real robust tool that you could use.

If you think of FISSEQ, it really leverages all of the development that was done for DNA sequencing and for these high-throughput DNA sequencing methods. All of the techniques to do current DNA sequencing had to be developed in order to get to the point where you could do it then in situ. You had to leverage that entire field.

McNally: [16:07]

What are the major unmet needs that FISSEQ addresses?

Terry:

We think one of the major unmet needs that FISSEQ addresses is in the drug discovery pipeline. The tools we have and the tools pharmaceutical groups have to come up with new drugs and to test these new drugs is tremendous. They have really rich, detailed animal models. They have newer techniques that have come online like organs-on-chip. They're able to develop new compounds to test against these animal models or these new organ-on-chip-based platforms.

However, there's no good readout at the spatial molecular level to really tell how those drugs are affecting the cells and the organ platforms or the animal models that they have. This is a huge role that FISSEQ can play.

If you just look at an organ on-chip-model, we have lots of different cell types that you could deliver a drug to and look at the response. You don't have a great way of reading it out. But with FISSEQ you could potentially

read out all of those molecules as they sit in that organ-based chip platform.

McNally: [17:22]

How would you describe where it is you stand now in terms of where you want to go?

Terry:

From a technology standpoint, I think we're at an extremely exciting juncture where not only can we do this for one particular cell type in a very limited way, now we can do this across various tissues or organisms, organs, cells, and be able to start now really trying to answer exciting scientific questions related to disease or the connections within a brain. [17:56]

McNally:

Can you tell us the story so far of the translation process?

Terry:

Yes, so the translation process starts in the lab with particular individuals really trying to answer a very specific question or trying to come up with a new tool to meet a huge unmet need in the field. This is kind of how FISSEQ started.

As FISSEQ grew and as these other tools grew, they really incorporated and leveraged each other. Some of the new sequencing tools that came out were leveraged by FISSEQ and pulled in. As excitement grew internally within the lab for this technique, more and more people were attracted to the project. As that happens, we really take a hard look at what stage is it mature enough for a spin out or for a licensing agreement.

McNally:

What was the point that you reached that you said, "Okay, now's the time."

Terry:

I would say, after we released our science paper, we got enough interest, both internally and externally, that a switch really went off saying we have something here. There is a huge unmet need. What does the commercial avenue look like? [19:17]

McNally:

What happens at that moment?

Terry:

At that moment it really takes a team of individuals to both further the technology as well as further flush out the commercial endeavor.

It really takes a person to stand up and lead that effort. For me, I've been working on next gen sequencing technologies in general for about ten years now. I saw this as the most exciting thing really to come out of the space or come out of the Church lab on the diagnostic side in many years. I

immediately said, "I'd like to take this on and lead this project."

McNally:

Doing so means he's working with SHAWN MARCELL, founding Chairman and CEO of ReadCoor. Marcell joined the Wyss Institute in 2016 to work on start-up opportunities in the biotech area, after previously serving as CEO of several companies, including Metamark Genetics, SensiGen, and Redpoint Bio. He was also President of the Port of Technology, the largest life sciences and technology incubator in the US.

Marcell is a current member of the Monell Center's International Advisory Board, a fellow and advisory board member of the University of Pennsylvania's Weiss Tech House, and serves on the board of Sensana. He holds a B.A. in Economics from George Washington University and has been an adjunct faculty member at The Wharton School. [20:37]

Marcell admits that he majored in economics without being quite sure what he would do with that degree.

Marcell:

Along the way, I became very interested in biological sciences, and then the pharmaceutical and diagnostic industries, and so, when I graduated from school, I was really inspired to get into the healthcare industry, in the pharmaceutical and diagnostic sectors of the business. So I joined a very large, diversified healthcare company in the diagnostics division. That was absolutely fascinating.

Learning about how testing was the front end of diagnosis, and then treatment of disease and the entire care pathway really, really inspired me to jump in passionately in a career in research, tools, and diagnostics.

McNally: [21:37]

Why did you move from the commercial world to the Wyss Institute?

Marcell:

I've always been in the commercial world. Actually, the role at the Wyss, I consider a commercial role. You're probably familiar with the Wyss model where promising platform technologies are incubated and developed, and I like to say de-risked to a point where they become mature enough to become freestanding entities or companies on their own.

I'm working on the licensing, the financing of the company, and then the organizational build out of the company once it spins out. [22:20]

McNally:

I ask Marcell to talk a bit more about the value of de-risking in the Wyss model – where the aim is to turn your technologies into startups.

Marcell:

By undertaking the technology risk and the development risk or the majority of it, when the company becomes a freestanding entity, then those risks are not something that investors will need to fund much further. They can focus on say commercialization or growing the company into a viable enterprise.

McNally:

What's an example of where the Wyss would de-risk beyond where a mere academic institution might?

Marcell:

That's a great question. In an academic institution, you'll have a researcher will be addressing a problem, an experimental problem, and they will perform experiments, conduct research, and then publish their results. This is to build out the body of knowledge in a particular area and be a contributor to the body of scientific knowledge.

The Wyss will take it to the next level. For example, Dr. Church conceived of FISSEQ, and he conducted research and demonstrated the feasibility of FISSEQ, and papers were published in *Science* and *Nature*,

And then, the Wyss Institute takes it to the next level and works with individuals, gifted scientists like Rich Terry and his team, to reduce it to practice.

The Wyss has essentially financed that through the various grant process and funding process internally to build actual functioning sequencers to show that FISSEQ can work in a laboratory.

And then took it to another level and conducted actual experiments to show how FISSEQ could be used in various tissue types and various disease states.

Then, went to the further step of bringing in an entrepreneur-in-residence, someone like myself who can map out a pathway or help map out a pathway to take this technology that's now been reduced to practice, demonstrate it as useful, and show how it can be then delivered to the larger scientific community market. [24:55]

McNally:

Since Marcell is involved in bringing FISSEQ to market, I'm interested in how he talks about the science –

Marcell:

FISSEQ is, I like to say, where sequencing meets pathology. This is a once in a few decade development.

Pathologists diagnose most diseases today. Often, they do it by taking a specimen and looking at it under a microscope and making a determination. We also know that the genes embedded there, which the pathologist can't really see, also play a huge role.

Dr. Church and colleagues conceived of a way of merging those two. FISSEQ is the merger of morphology and sequencing, and by combining the data and delivering a spacial image of the sequences with the sequences, we can now have unprecedented clinical insight into diseases and conditions.

By understanding the processes going on down at that cellular and sub-cellular level, we can now really not only identify the mechanisms of disease, we can diagnose those diseases more effectively, and then, we can target treatments at them more effectively.

The ability to just take a specimen and throw it on a slide, and then sequence it in place has previously not been possible until the breakthrough with FISSEQ occurred. [26:47]

McNally:

You can now see things happening, if you will, sooner, faster, more specifically?

Marcell:

All of those things. All of those things.

Yes, sooner. The actual disease process can be observed essentially in real time. The diagnosis can theoretically be made more quickly. There's less preparation and handling, so it can be conducted more quickly.

Then, the output, the information that comes out the other end is vast, and far richer than what occurs today. Today, you get a sequencing read, but it doesn't tell you anything about where the RNA came from, where it was in the patient, what it was doing. It just, it's a sequence. Here, with FISSEQ, we can now identify the sequence, where it was in the cell, and even correlate it with cells next door, intercellular or with other elements within the cell, intracellular.

All of this is rich information that allows for far more effective diagnosis, targeted treatments, but also allows for unprecedented insight into the inner workings of cells.

McNally:

Understanding is also going to be richer...

Marcell:

Yeah. [28:17]

McNally:

Again - from his commercial perspective - I'm curious how Shawn Marcell views the development process for FISSEQ -

Marcell:

FISSEQ had to first deliver a proof of concept. That is "Can something as unconventional as in situ sequencing even be done?"

Conventionally, you take a sample, you pulverize, you extract, you amplify, and then sequence. Nobody ever took an intact specimen, ~~dropped~~, put it on a slide, and then tried to sequence it. That's very difficult, so that was the first big hurdle in my opinion was proving that sequencing could be done in situ.

The second part of it was then reducing it to practice and putting it on an engineering instrumentation and a platform that could do it in a realistic way. I mean, it's fine if you're a researcher and you do it one time on one thing, and it takes you weeks to do it. That's not the same as I got to put 32 samples on the machine and get the results out in three days.

McNally:

That's right. No one is going to buy that first one.

Marcell:

Right. No one is going to buy the first one.

So the next big hurdle was engineering. ~~and~~ How do we take this new capability, and then how do we deliver it in a way that's practical? [29:46]

McNally:

I return to George Church to talk about potential applications – which he believes are vast –

Church:

Some of them, I think, a little more obvious, which is like direct translation into the clinical settings - where you have diagnostics, replacing classical pathology slide analysis, many other imaging systems which are the cornerstones for a whole variety of decision processes in modern medicine.

But it's much bigger than that. We can not only visualize RNA and DNA, but also proteins. You can have antibodies that are labeled with nucleic acids that bind to the proteins, and then those nucleic acids can be immobilized the same way that the cells are immobilized. Then we can chew away all the proteins and then start visualizing these tags.

In principle, you could also detect small molecules by their impact on proteins, and hence, the tagging comes into play again by quantitating the amount of protein, the amount of molecule that binds the protein, that stabilizes it and so on.

Going beyond that, we know that we can encode information about time-related events in nucleic acids. Our lab has published a few papers on ways that you can encode ion concentrations for various molecules that are changing with time. You can turn that time series into a nucleic acid

molecule. Once it's a nucleic acid molecule, it again reduces down to a previously solved problem, which is fluorescent in situ sequencing.

Finally we can look into developmental biology and another time series, a specific time series, which is the branching that as each cell divides, the two daughter cells can get their own unique bar code, dynamic bar code, that we can produce with new versions of CRISPR. [31:41]

At each cell division, you get this ramification of developmental potential. We can encode and later read with FISSEQ exactly which cell begat which cell all the way from the single cell zygote all the way out to the trillions of cells in a mature plant or animal. [32:00]

McNally:

Staying with applications, Rich Terry echoes Shawn Marcell's point that FISSEQ is where sequencing meets pathology, as he envisions its potential in cancer diagnosis.

Terry:

If you look at the cancer space and how we evaluate patients based on the type of cancer they have, a lot of these techniques are either based on morphology or they're based on molecular analysis.

If you look in breast cancer, your analysis of the state of your breast cancer's really driven by morphometric features like malformed nuclei or tubular formation. These are done via canonical staining assays that have been around for a hundred years.

McNally:

Okay.

Terry:

You then have all these newer tools that have been developed in the RNA analysis space like Oncotype DX, which you can leverage to tell you how effective a chemo treatment will be. The problem is, no one's truly put the two of these techniques together in a parallelized, meaningful way.

We think in doing that with FISSEQ, which gives you the morphometric and it also gives you all of the molecular information overlaid in a single sample, we can get much better accuracy on delivering drugs to patients and coming up with their outcomes. [33:19]

McNally:

George Church sees another crucial aspect of the role of FISSEQ in cancer treatment –

Church:

In cancer, there's been growing acknowledgment in the field and interest in cancer heterogeneity, where you'll have either various different kinds of

cancer at a single site or in different sites of the body that either develop differently or during the metastasis process have evolved. They recruit cells from around them that are not cancerous but become part of the cancer ecosystem.

All this heterogeneity varies from person to person. It's not too far-fetched to say that each cancer is unique in a certain sense. Even different cancers in the same body can be quite different. The strategy you have for therapy should be reflective of that complexity, and we need software to help us deal with that level of complexity.

McNally:

How could FISSEQ ultimately impact the clinical care of patients?

Church:

Right, so not just cancer, but almost every pathological state has some personalization. You have different genetic and environmental components coming in with the patient, complicating and individualizing each... whether it's a renal failure or a liver disease that's not cancerous, and certainly especially so for neuropsychiatric disorders, degenerative diseases including cognitive decline, and so forth. Each of these has a generic component but also a very individual one.

Exactly where you want to place that drug, that scalpel, that laser will depend on the details of the imaging of the sample. [35:05]

McNally:

This is going to give us a great deal more ability to personalize, to individualize, both in our diagnosis and our treatment-

Church:

That's right. One of the big payoffs for a precision medicine initiative is going to be the personalization of, not just cancer, but a whole variety of diseases. Many of these diseases have an organic and organ-based analysis that would be either required or helpful. The ability to know the name of every molecule or certain subsets you've chosen in advance for a particular piece of tissue is a huge improvement, especially with the enormous lowering of costs that we've seen over the last few years. [35:45]

McNally:

Rich Terry explains the role FISSEQ will play in the exploration of the brain. a role recognized by President Obama's BRAIN Initiative –

Terry:

If you look at the way you track synapses in the brain, they're very slow, high resolution, microscopy techniques. These have their own challenges. One of the main ones is, it makes the pieces of the brain that you scan at very high resolution difficult to really align and track neuron synapses.

One thing FISSEQ gives you is the ability to, in a very high throughput manner, sequence large unit volumes of the brain and map those sequences back to synaptic bar codes. This really gives you a new tool that's much faster that will allow you to map all of the connections within a brain. Not only does it give you the ability to map those connections, but it will also give you all of the rich expressional information that will go with the mapping of those synapses. [36:53]

McNally:

Rich shares some of his hopes for the future –

Terry:

Looking forward in general, again, what I'm very excited about is developing new applications for diagnostics as well as drug discovery. We're at the point where we can really start pushing a lot of those applications and looking at not only the rich, complex interactions within some tissue types or within certain cells or co-culture of cells, but across whole organisms. Even from a developmental biology standpoint this is extremely exciting.

McNally:

Where would we be in the brain space ten years from now?

Terry:

The brain space... It would be phenomenal to be able to sequence even a whole small animal's brain and map every connection in the brain as well as read out the other richness of molecules that are present, whether they be small molecules or proteins or RNA or even DNA. [37:57]

McNally:

I ask Shawn Marcell how he's thinking about the commercial development of the various FISSEQ applications –

Marcell:

The FISSEQ platform is so useful in such a diverse number of research contexts. We've got a line around the block already of major research institutions with collaborators who are leaders in their fields with projects ready for us to sequence them for them and provide them data.

We're going to begin offering sequencing services to major research institutions around the world and also offering sequencing services to pharmaceutical companies.

Later, we will then supply working sequencers - working high-throughput sequencers that perform FISSEQ - to leading research institutions who have sequencing core facilities. [38:55]

McNally:

What would we be talking about a year from now?

Marcell:

A year from now, we're hopefully completing the projects we already have accumulated. Thanks to Dr. Church and the FISSEQ team, we have papers published in *Science* and *Nature*, and Dr. Church frequently speaks. As a result of that, just that visibility, we've had over 50 collaborators across more than 20 institutions approach us with projects. We've got plenty of work to do for the next year, so I'm hoping in a year, we can even finish all the projects that we already know of. [39:38]

McNally:

And then, five years from now, what would we be talking about?

Marcell:

Five years from now, this has all the ingredients of a major research tools and diagnostics company - and there's precedent for that. This is such a fundamental breakthrough in sequencing that the company in five years would be a fully integrated company providing sequencing services worldwide and providing sequencers, including bench-top versions that could be run in just about any research laboratory, and their attendant reagent chemistries to go with it, a whole range of software tools that enable users, both open-source and proprietary, and an entire range of data mining services.

All that data that will be accumulating in the cloud will be able to be mined and be a rich source of clinical and research data that will be highly useful and valuable across many sectors of healthcare. The company will be integrated along all of those lines and the amount of research being done in transcriptomics will accelerate concordantly. [40:55]

McNally:

Shawn, you started several companies. What is it in particular about FISSEQ and the Wyss that made you want to jump aboard?

Marcell:

I've been in the healthcare and diagnostics industry for 30 plus years now, and along the way, yes, I have been fortunate to be the founding CEO of a number of companies. I've had some experience now to look back and see where I've been at my best and where I've been the most excited and passionate, and it typically has been in an area where the technology has been very disruptive and has had wide applications and is doing something really new, innovative, and useful for society.

FISSEQ is such a fundamental technology and scientific platform that it's going to really change things in research, diagnostics, and ultimately healthcare arena, which will be useful and beneficial to society.

McNally:

Thank you very much, Shawn Marcell. It's been a pleasure.

Marcell:

Thank you, Terrence. [42:15]

McNally:

Finally I return to George Church. I'm curious how FISSEQ fits in with his other work –

Church:

My lab does cover a number of realms of innovative applications. This includes new ways of doing transplantation, for example, humanizing pigs. Clearly transplantation is something where the structure of the cells and the development of the organs is important. I'm sure FISSEQ will play a role there.

Resistance to environmental factors, including ways of eliminating malaria, Lyme disease, and so forth, there may be a role for it there to understand ecological and organismal subtleties and interactions.

We have a big commitment to the BRAIN Initiative. That is probably the most impactful future that we see for FISSEQ, among many of the others, - where some cells can be approximated by a sphere, for example, a lymphocyte may be floating around in the blood, but neurons very far away from that. The nervous system is probably the most complicated and impactful kilogram of material in the universe, at least our universe.

McNally: [43:25]

You know, one thing I hear is that because this is a tool, in some sense as we move forward 5, 10, whatever, we won't see it anymore. We'll just see the new things that have come from using it. Does that make sense?

Church:

Yeah, I think that would be the greatest measure of success is the thing that you invented disappears into ubiquitous use.

McNally:

Uh-huh.

Church:

I certainly hope that is true for this one. Credit is greatly overrated. What you want to see is impact. You want to go to the grocery store and the drugstore and the playground, and see it. That's the reward. [44:04]

McNally:

How has working within the Wyss structure, the Wyss culture, contributed to the development of FISSEQ?

Church:

The Wyss culture is amazing. It's just hard to describe. I've been involved in a number of different institutes and institutions all over the world, and the Wyss has a sense of not just collaboration, but playfulness, a disruptive, transformative, can-do attitude that has generated entrepreneurial opportunities, translational opportunities, but really the key thing is the out-of-the-box, interdisciplinary teamwork that is rarely seen.

Commercial and even many academic institutions that are at scale and focus on productivity, they're so focused on productivity that they're essentially turning cranks, and they're spending a lot of money producing something that was remarkable by current standards, but almost then immediately outdated by new technology.

I think Wyss on the other hand is the engine that produces those new technologies and brings fantasy into reality in remarkably short periods of time. [45:17]

McNally:

What difference does that make to you as a scientist, the difference between a discovery being a discovery and a discovery being translated into applications and products and so on? What difference does that make to you?

Church:

First of all, I'd characterize myself as a scientist-engineer. I may have started a little more on the science side, but I think making inventions and even discoveries seems at this point to be kind of a Pyrrhic victory without translation. It seems like it's ephemeral. You publish a paper. Maybe a few people cite it.

It's not nearly the same thing as getting the feedback from market forces and the feedback on impact that says, "Oh, if you just do a little bit more, you're going to have a much more positive impact," and the feedback on safety and security, all those sorts of things, which sometimes can provoke really very profound new basic science, which when it's all entangled with the engineering in my lab, it just makes it much more intellectually and socially gratifying.

McNally:

Very good. It's always a pleasure. Thank you very much, George Church.

Church:

Thank you. [46:29]

McNally:

You've been listening to DISRUPTIVE: FISSEQ - Fluorescent in situ RNA sequencing I'm Terrence McNally and my guests have been GEORGE CHURCH, RICH TERRY and SHAWN MARCELL,

You can learn more about their work with FISSEQ as well an exciting range of other projects at the Wyss website - wyss.harvard.edu – that’s W-Y-S-S dot Harvard dot edu – where you’ll find articles, videos, animations, and additional podcasts.

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My thanks to Seth Kroll and Mary Tol-ee-kas of the Wyss Institute and to JC Swiatek in production, and to you, our listeners. I look forward to being with you again soon. [47:11]