The Future of High Throughput Assays: From Reporter Genes to qPCR Webinar 20 March 2013

[0:00:10]
Slide 1

Sean Sanders: Hello everyone and welcome to all of the viewers joining us for this live *Science*/AAAS webinar. I'm Sean Sanders, editor for custom publishing at *Science*.

In the webinar today, we'll be examining how a robust but historically low to medium throughput technique like quantitative PCR can be adapted to a high throughput screening and comparing this with traditional reporter gene assays particularly for applications of drugs discovery.

Slide 2

With me today in the studio, I have a wonderful panel of guests to talk about this topic. To my left is Mr. Daniel Sipes from the Genomics Institute of the Novartis Research Foundation in San Diego, California. Next, we have Dr. Andrea Weston from Bristol-Myers Squibb in Wallington Connecticut, sorry Wallingford, Connecticut and finally Dr. Patrick Faloon from the Broad Institute of MIT and Harvard in Cambridge, Massachusetts. Warm, welcome to all of you. Thanks for being with us.

Dr. Andrea Weston: Thank you.

Dr. Patrick Faloon: Thank you.

Sean Sanders: Before we get started, I have some important information for our audience. Note that you can resize or hide any of the windows in your viewing console. The widgets at the bottom of the console control what you see. Click on these to see the speaker bios, additional information about technologies related to today's discussion, or to download a PDF of the slides.

Each of our speakers will talk briefly about their work. After which we will have a Q&A session during which our guests will address the questions submitted by our live online viewers. So if you're joining us live, start thinking about some questions now and submit them at any time by typing them into the box on the bottom left of your viewing console and clicking the submit button. If you can't see this box, just click the red Q&A widget at the bottom of the screen. Please do remember to keep your questions short and concise as that will give them the best chance of being put to the panel.

You can also log in to your Facebook, Twitter, or LinkedIn accounts during the webinar to post updates or send tweets about the event, just click the relevant widgets at the bottom of the screen. For tweets, you can add the hash tag, #sciencewebinar.

Finally, thank you to Roche for sponsoring today's webinar.

Slide 3

Now, I'd like to introduce our first speaker Mr. Daniel Sipes. Mr. Sipes is currently the director of Advanced Automation Technologies at the Genomics Institute of the Novartis Research Foundation in San Diego, California. He holds a B.S. in molecular biology and a master's in immunology. Mr. Sipes has been involved in the development and implementation of high throughput screening systems throughout his career particularly in his current position where he develops and operates next-generation automation for high throughput screening and cell based profiling. Mr. Sipes recently served as vice president of the Board of Directors for the Society for Laboratory Automation and Screening. A very warm welcome to you, Mr. Sipes.

Mr. Daniel Sipes: Thanks, Sean, and thanks to AAAs for inviting us here and hosting webinar.

Slide 4

I'm going to be speaking about a couple of trends. The first trend is from simple to complex assay systems for high throughput screening. You know, high throughput screening being screening hundreds of thousands to millions of compounds. A second trend, which I hope will stimulate some discussion during the question and answer period, is a trend using HTS approaches for mechanism of action and other profiling applications.

Slide 5

But first, I'd like to tell you a little bit about the Genomics Institute of the Novartis Research Foundation. It's in San Diego and it's part of the Novartis Institutes for Biomedical Research. Our site has about 600 people mostly chemists and biologists but what is fairly unique to GNF is that we have a very powerful engineering group under the same roof with the scientists. What this allows us to do is push through any technical barriers that might otherwise impede the GNF discovery paradigm illustrated on this slide. That is we use novel assays and assay systems to study fundamental biology to discover innovative drugs and drug targets all powered by or leveraged by GNF technology.

Slide 6

We've been doing this at GNF for quite a number of years, about a dozen years. At first, we were quite progressive in the shifting from biochemical assays to cell-based assays in 1536-well format. This allowed to screen economically these millions of compounds and keep a low hurdle to running many high throughput screens every year.

We are following a shift to screen in more relevant systems. The disease relevant systems type of assays are iPSCs, hematopoietic stem cells, primary cells, patient derived cells for example. Readouts are getting much more complex such as high content imaging or even studying endogenous proteins and looking at profiles of activity. We're also dabbling with synergism where we're looking at combinations of compounds in this way.

[0:05:08]
Slide 7

For this presentation, I'm going to talk about three broad categories of HTS readouts. These aren't the only readouts possible for HTS but these three phenotypic readouts, gene or protein readouts, and what I think is somewhat the future and progressive are gene or protein profile readouts for HTS.

Slide 8

So some examples here are, you know, simple biochemical screens where you're running with isolated proteins, reporter genes, endogenous genes. We can study those in a facile way right now. Phenotypic screens are where we label the cells and some of the more progressive things like gene profiles and protein profiles I think that's the future. Sort of we're on the cusp of being able to screen with some of those and I just mentioned down here that some very

complex systems companies such as BioSeek actually have business models around this.

Slide 9

So I'm going to talk about phenotypic readouts right now.

Slide 10

So when I say phenotypic readouts, what I'm really talking about in this context are high content imaging where images can capture these morphological changes, which are otherwise not possible to interrogate if you're just screening for simply proteins or gene expression. With these high content approaches, you have flexibility between population based or whole well type of information than studying individual cellular information.

Slide 11

So what really makes these sorts of things possible are devices like this. This is the GNF washer/dispenser 2 relatively recently developed at GNF that has the same functionality of what we call are classic plate washer. That is it can wash cells, cell plates in 1536-well very gently and very precisely. So not just adherent cells but loosely adherent cells or even suspension cells can be processed in a machine like this.

Slide 12

Of course, what you also need for high throughput screening is to reduce this to practice and we've done this with our ultra-reliable robotics here. These allow both miniaturization to 1536 as well as scaling so we can run millions of compounds against these sorts of assays and do this many times a year so several screening campaigns a year.

Slide 13

Get the next slide. Next, I'm going to talk about some recent advancements in what I might consider more traditional HTS.

Slide 14

So that is this for instance cellular activity or protein detection. We've been screening like this for many years and it's basically been reduced to practice in part through vendors. Vendors have come up with very good reagents and these workflows are very optimal for HTS. Reporter gene assays, they're the old standby promoter

element driving your reporter of choice. Luciferase is very popular, very optimal HTS workflows.

For studying endogenous gene expression where you need to study one relevant gene against many samples, so think low number of targets, high number of samples, qPCR I think is the method of choice but historically this has been difficult to automate. Somewhat onerous but that is changing. New liquid handling techniques that Andrea is going to talk about and robotics are more advanced as well as kits are more homogenous.

There are some other new recent advancements such as SMARTflare that's marketed through EMD Millipor. These are gold nanoparticles functionalized to be sensitive to individual gene expression. You can put these in the media and they will cross the cell membrane without special transfection procedures and monitor in real time in live cells individual endogenous genes. So this is a new product. I don't have any experience with this. We're going to be trying that out but hopefully this will bring the study of gene expression to HTS in a high throughput fashion. It might be an alternative to qPCR.

Slide 15

Another way of looking at high throughput screening is to use it in different ways such for profiling. What I mean by profiling is instead of a single assay in a single target, you might have a single format be sensitive to multiple pathways. So what you can think of here is maybe one cellular background and different means to be sensitive to many different pathways. Compounds with similar mechanisms of actions should cluster in their activity or profile over these different assays and if there are unknown compounds and their profile of activity matches those known compounds you'll have an indication of the mechanism of action of that compound.

[0:10:18]
Slide 16

So at GNF fellow Fred King reduced this practice and what he did is set up a panel of reporter gene assays. Each reporter are promoter sensitive to different cellular pathways, trained this with some reference compounds and then we used this to determine mechanism of action. We get an idea of mechanism of action.

Slide 17

So here's how we put it to use. He actually did this on 41 reporter gene assays and published this a few year ago on the Journal JALA. He used ATK 293s transfection in 1536-well plates, trained this system with 14,000 compounds in eight-point dose response. Ran it on our ultra-high throughput screening platform. So there was no new technology development. It's very high throughput and fairly low cost to run this. The point here is we're using established UHTS technology for mechanism of action determination.

Slide 18

This is actually something we still run routinely at GNF where it's a service for all of Novartis. People submit their compounds. There's not much requirement, a couple of FTEs. It's very inexpensive to run and it gives you a very quick estimate of mechanism of activity. This can be used for triaging compounds from another HTS or an HTS and also from other programs.

Slide 19

So next, I'd like to look to the future a bit. I've presented up to this point technologies that were actually have implemented and now I'm going to talk a little bit about directions we are going, things we're looking into. That is endogenous gene or protein profiles.

Slide 20

So here are some profiling platforms: RNA-Seq, RASL-Seq from Illumina are looking quite promising for measuring endogenous gene expression. Luminex/Panomics is a system that we have at GNF. We just recently implemented a workstation around that. A couple other players here are Nanostring and Raindance microfluidic type of approaches for measuring either coding or noncoding RNA. Some of these technologies can actually measure hundreds to potentially thousands of RNAs on not only a per well but a per cell basis. That can be very powerful.

Next is protein profiling. There are a couple of providers that have been in existence for a while such as MSD and Luminex/Panomics. SOMAscan is starting to be deployed. I think they're up over a thousand plaques nowadays and Protein-Seq. So Protein-Seq is something pretty interesting from Olink Biosciences. Some of these can measure not only protein levels but posttranslational modifications as well as protein-protein interaction. They require a very low sample volume. Potentially dozens to hundreds of proteins can be measured in each microliter. Most of what I've just

mentioned leverage nucleic acid detection technologies so they can be highly multiplex, very sensitive and cheap.

I'll just mention a couple of other profiling technologies here that are beyond the scope of what I want to talk about in this presentation today.

Slide 21

So homogeneous proximity ligation assay is something very interesting I feel because it basically links protein expression, protein levels to the nucleic acid detection technologies. There have been a lot of recent advances through next-gen sequencing so I think this is something important to just go through quickly if you're not familiar with the PLA assay. It's essentially you have affinity reagents such as antibodies in this example linked to nucleotide probes. Your affinity reagents then bind a protein and if they bind at the same time, you can perform a ligation. You have an optional protease digestion and then you can detect that nucleic acid product, that ligation product through various means. Real-time PCR has been used quite a bit for PLA as a PLA readout but the next-gen sequencing technologies can also do it.

Slide 22

So PLA is this bridge between protein detection and next-gen sequencing. The recent advancements in next-gen sequencing I think pretty much all of you are aware they're tremendous. So linking protein expression and detection with next-gen sequencing is critical to really exploit protein profiling.

[0:15:14]

Also, to fully leverage this, the nucleic acid probes will need to be barcoded in some way. There's tremendous dynamic range here and enormous multiplexing and there's a reference I put in there to an assay. They can also be linked to traditional nucleic acid detection technologies such as qPCR.

Slide 23

I have a reference there for that. So in summary, I'd just like to state that GNF has maintained its engineering and HTS excellence for over a decade by doing our best to stay on top of these trends. Our engineering and automation HTS groups are meeting these challenges of more complex assay formats looking forward. High content imaging is something we do a lot of as well as synergistic profiling and mechanism of action determination. Next will be these multiplexed profiling applications.

Slide 24

So what I just presented very quickly involved a whole lot of people from GNF, far too many to list in acknowledgements so there's a picture of GNF with the employees there. So thanks to everyone at GNF and thanks to the viewers and AAAS for inviting me.

Sean Sanders: Okay. Thank you so much, Mr. Sipes.

Slide 25

Our second speaker today is Dr. Andrea Weston. Dr. Weston is currently a senior research investigator with the Lead Discovery Group at Bristol-Myers Squibb in Wallingford, Connecticut. She received her Ph.D. in physiology from the University of Western Ontario and completed postdoctoral training at the Institute for Systems Biology in Seattle, Washington and within the Investigative Toxicology Group at Pfizer in Groton, Connecticut. Her main areas of expertise are in transcription, epigenetics, development biology, and of course high throughput screening. Dr. Weston now leads a team of researchers in the design and implementation of high throughput, cell-based assays including high-content screening. Welcome, Dr. Weston.

Slide 26

Dr. Andrea Weston: Thank you, Sean. All right. So I'm going to shift gears a bit from Dan's broad approach and take a focused view to discuss a method or process that we've developed at Bristol-Myers Squibb to enable high throughput miniaturized 1536-well real-time PCR.

Slide 27

So here's an outline. I'll first go over the driver for enabling this technology and outline some of the key challenges for implementing this protocol and then discuss the development of capabilities at Bristol-Myers Squibb including some of our recent impact and move on to future perspectives.

Slide 28

So first off, what's the driver for enabling high throughput real-time PCR? The bottom line really is currently or until recently, there's been no cost effective high throughput solution for enabling endogenous expression analysis. So we like many other companies and certainly Dan alluded to, we typically run reporter gene assays and these have worked but they're not ideal. When you're using a

reporter gene assay, you're really not monitoring the gene expression in its normal chromatin context. You're often reliant on what is known about the promoter sequence for that gene. It's not often completely worked out and you know often it requires cell line development, which can be time consuming and of course you're limited to immortalized cell systems often, which gets us away from the sort of industry wide goal to leverage more relevant physiological models including primary cells and stem cells early at the lead identification stage.

Slide 29

So with that said, here's a brief schematic of how real-time PCR works. It's a technology that is not new. It's been around for almost 15 years and briefly on the left it involves isolating RNA from cells, reverse transcribing that RNA and then amplifying your gene of interest. However unlike conventional PCR where you have 5' and 3' primers, these flank of probe sequence, which has a reporter fluorophore on one end and a quencher on the other end. When this probe is intact, you have FRET. When PCR amplification is taking place, Taq polymerase will digest that probe and you'll have fluorescence. Of course, this is something we can measure in real time.

So if you look at the graph from the top right, this nicely shows PCR curves from a series of reactions and, you know, back in the day we used to stop these reactions at some arbitrary cycle, 35 or above often and run these reactions on a gel. That's endpoint PCR and you can see from this graph that's where the reaction is mot variable. Real-time PCR captures the inflection point known as CP or CT, which is a much more accurate point.

[0:20:24]
Slide 30

So as I indicated, real-time PCR has been around for about 15 years. We and many others have been screening in 1536-well formats for about half of that time and so it really begs the question why haven't we done this before. This slide really outlines that this whole process is not trivial. There are a number of challenges. RNA isolation is cumbersome. It's time consuming, prone to degradation problems. You know, our simple step and really to make this happen is to eliminate RNA isolation altogether and run these reactions directly from cell lysates.

It typically is a two-step reaction, however, there's been great advances in one-step products and we're leveraging those as well. These reagents tend to be expensive and of course like all high-throughput assays, one way to circumvent that is to miniaturize the reactions. It can be time consuming to thermocycle through multiple cycles but of course with the advent of 1536-welll amenable thermocyclers, this increases our throughput by at least fourfold over 384-well. Then finally, this technology is so exquisitely sensitive, which is one of the strengths of our qPCR, however, it means that any error in the process will be amplified exponentially. So it really demands precise state of the art liquid handling technology, which I'll talk about.

Slide 31

So this slide outlines three major platform advances that we have married together in partnership with different companies to really enable a solution for miniaturized high throughput RT-PCR. I talked about the cell reagents. Acoustic dispensing figures prominently in this protocol as well as of course the existence of thermocyclers that can handle 1536-well plates.

Slide 32

So first, I'm going to talk about on the reagent side of things. There have been advances since the first product cells to CT was put on the market and being able to conduct consistent reactions directly from cell lysates. Here's an example of the Roche Real-time Ready Lysis Buffer. We were an early beta tester of this product and here are some cells that have been lysed from that product. It doesn't lyse the nuclei right away so it's much less viscous. We found results are much less variable and it's very simple. It's a room temperature incubation with no stop reaction. Importantly it's compatible with some of these one-step products. So what we've been able to do is take a process that's multiple steps and really reduce it to just a few steps. Literally lyse the cells and then deliver those lysates to the one-step reaction mixture. Later on, I'll talk about how we've also been able to reduce the volume from a traditional 10 to 20 microliters to 300 to 600 nanoliters.

Slide 33

So this just quickly shows the very first experiment we ever did with this reagent. So in the green and the gray, you're looking at actual RNA. So this was a one-step reaction done on RNA from HepG2 cells looking at ApoA1. Whereas in the red and the blue, you're looking at each curve is a well of a column of wells containing either 8000 or

2000 cells per well of HepG2 cells. What we were excited by right away, this is a 10-microliter reaction by hand, was that although it didn't look as tight as the RNA, considering it was by hand it was our first ever reaction, we did get good reactions and these were fairly tight. So this gave us enough encouragement to move to the next step, which was really to leverage acoustic dispensing to gain the precision needed to really tighten up these reactions.

Slide 34

So for those who aren't familiar, acoustic dispensing technology uses sound energy to eject nanoliter droplets of fluid into in most cases a microtiter plate that's inverted over top of your source plate.

Slide 35

We at Bristol-Myers Squibb like many others have extensive experience with acoustic dispensing technology, but much of that experience when we embarked on this really resided on compounds in DMSO. When you're dealing with your tiny well formats and different fluids, they behave very differently in different wells and that's shown in this diagram here. So DMSO really has somewhat of a flat meniscus similar to if you were to put buffer into a well. However, when you're dealing with cell lysates, you have this effect where the lysates climb the walls of the well and do this differentially from one well to another and so you have different what's called angle of meniscus.

[0:25:17]

Around the time we were embarking on this, Labcyte was developing a platform known as the Omic software, which could actually on the fly survey well by well by well to identify that angle of meniscus and adjust the power to ensure that the trajectory and the precision of your droplet was compensated for any of this wall climbing effects. So that was exciting for us.

Slide 36

We partnered with Labcyte to compare reactions done by hand versus reactions done acoustically. So in this slide what you're looking at is on the left this is a 1-microliter reaction done by hand from direct cell lysates and on the right you're looking at the exact same reagents on the exact same day on the exact same plate delivered with the Labcyte echo. You can clearly see it's difficult to pipette these kinds of volumes into a 384-well plate but you can see that the acoustic transfer really tighten up the precision of these reactions. So that was exciting for us. At that point, we were still in

384, but through a lot of discussion and back and forth with Labcyte we released over the next several months push to move this into 1536.

Slide 37

What that entailed actually growing cells in echo source plates, so the plates that are amenable to acoustic transfer but have also been tissue cultured so we can grow our cells in them. Then working with Lab site to make sure we get the power right and the software right to really enable ideal droplets, you know, with morphology to ensure they hit the tiny wells of the 1536 RT-PCR plate. Here you can see these are droplets from samples that were spiked with fluorescent dyes and an image on the Lycore on to a film and you can see when you get the power right, you can avoid what we call satellite spots. You get very clean droplet morphology. This enables a very precise reaction. This is a 500-nanoliter reaction in 1536.

Slide 38

So we've made significant progress working with Labcyte and working with Roche on the reagent side in enabling these miniaturized reactions in these 1536-well plates. Just to give you a sense of the workflow here, so what we're talking about is actually culturing cells in 1536-well plates, lysing those cells in the same plates and then directly delivering that lysate acoustically to the final PCR plate, which has been preplated with your one-step master mix. Then sealing this plate, centrifuging and then reading it in or thermocycling and reading at the same time in the thermocycler. So it's a very streamlined process. We've really gotten away from the multi-step process that we're all familiar with and that really helps us reduce the error.

Slide 39

So here are some images showing the actual real-time PCR plate and I show these because even though we have a lot of experience with 1536-welll microtiter plates, these are very different than plates we're used to working with. First off, the wells are extremely tiny. They're less than a millimeter in diameter so really there's no room for error in dispensing. There's really limited options for dispensing. In our experience, acoustic dispensing really is the only method that fits the bill. These plates are also very low. They're not very high. So in terms of gripping them, there's limited options. It needs an overhead grip. They're not flat because the material composite that's used to make them and so that's posed some automation challenges. So it's a difficult plate to work with but we've worked

carefully with Roche to really test different materials and test different reagent protocols mostly over the past year I would say.

Slide 40

And we've been able to find a combination where we can get from the situation on the left where you see this heat maps of the CP and endpoint fluorescence on the bottom and very nonconsistent reactions across the plate to where we are today, which is we're seeing very consistent reactions across the plate able to generate CVs of less than 1%. These are 600-nanoliter reaction volumes and again it's one-step PCR directly from cell lysates. So it's gratifying to get to this point and especially to move to the point where we can actually start impacting programs at BMS.

[0:30:13]
Slide 41

Here, I'm showing a slide that centers around a target of interest. It's an epigenetic enzyme known to – we're looking for inhibitors and this enzyme has as its target a known oncogene. So here we're measuring an oncogene in cancer cells and you can see the second last column on the plate on the left has a control inhibitor and you can see very clearly the shift in CP value on the bottom in the green. Very tight reactions, Z' of about 0.5 in this case. On the right is basically a concentration response curve plates where we have control compound in ten concentrations starting from columns 111, 21 and 31 and able to generate IC50 curves. In the bottom, here in the black versus the red curves, you can see for a well-known reference compound for this target, we can generate IC50s that match between 384-well qPCR and 1536-well PCR.

Slide 42

So that's exciting. In this next slide, I just want to talk a little bit about dynamic range. It often comes up as a question going from cell lysates compared to RNA. On the right, you're seeing a titration of different numbers of cells and how the CP value varies. You can see we didn't actually go lower in this experiment but from 250 cells up to I would say about 5000 we can definitely see the expected CP value change. We do max out somewhere between 5000 and 20,000. This is likely a function of the volume of lysis buffer to the cells. At some point, you're going to have too many cells to be able to lyse. So you do lose some of your dynamic range but you do maintain linearity.

On the right these are experiments that were done by Rob Bertekap at BMS where basically across the top, he diluted the lysates twofold left to right and along the side top to bottom he altered the actual droplet volume that he was acoustically delivering to the plate. Everything is shown, the same data is shown graphically in the bottom graph and again you can see as you go through a twofold dilution you see your expected one CP difference, same if you reduce the actual volume you're dispensing but up to a point. It sort of tapers off around 40 to 50 nanoliters for this gene and of course it's going to vary from cell system to cell system and gene to gene.

Slide 43

So just to reiterate, we basically have developed this very streamlined protocol where we can actually enable 1536-welll real-time PCR directly from cell lysates. It's cost effective. What we're working through now is really putting the entire process together to automate this to be able to do multiple plates. When we first started working with Roche and the instrument three years ago, at that time it didn't have a programming interface.

Slide 44

So we've partnered with Thermo Fisher and I know Roche has partnered with them as well and other companies to develop the driver and to enable plate movement of these plates. So currently, we have the Orbiter from Thermo Fisher. Again, it's an overhead grip. It reliably can move these plates and it can now interact with the instrument and the next several months we're really going to be focusing on building the entire automated infrastructure to be able to ramp this up.

Slide 45

So I'll stop there and make a few acknowledgements. Especially from the Bristol-Myers Squibb side of things, I want to thank Rob Bertekap, Monique Anthony, and Sanjay also known as Adrian Saldanha. They've really done a lot of the work to develop these protocols and test to different conditions. I also like to thank the leaderships at Bristol-Myers Squibb including Andrew Alt, Jonathan O'Connell, and Martyn Banks for their support. We've partnered with many people at Labcyte and I've listed some of those names here, many, many people at Roche over the years so too many to name actually so I highlighted the three who we've been working with the closest, Jeff Holman, Rupal Cutting, and Matthias Hinzpeter. Finally Grace Mangialardi and Dean Mulyk from Thermo Fisher. Thank you.

Sean Sanders: Thank you so much, Dr. Weston.

Slide 46

We're going to move right on to our final speaker for this webinar, Dr. Patrick Faloon.

[0:35:06]
Slide 47

Dr. Faloon received his Ph.D. in molecular and cellular biology from the University of Maryland School of Medicine. During his postdoctoral fellowship, Dr. Faloon studied at Massachusetts General Hospital in the laboratory of Mark Fishman, and continued his fellowship Dr. Fishman at Novartis. Dr. Faloon is now a member of the Broad institute, sorry, Chemical Biology Program where he oversees the high-throughput screening automation group, and, along with his colleagues, executes projects to find small-molecule probes with specific and potent activity against a biological or disease mechanism. Welcome, Dr. Faloon.

Dr. Patrick Faloon: Thank you, Sean. So I sort of phrased my or framed my talk on thinking about what the current state of things in HTS are and things to think about in the future and potentially now thinking about some of the technologies I present and how they could be applied to things like qPCR.

Slide 48

So currently, when I run projects, I think about the relevance of the assay itself. In particular, we've previously mentioned things about the type of cells that you use and using immortalized cell lines versus primary cells and how relevant your assay or your biology that you're interested in is in a particular cell line. I mean we're very over reliant upon HeLas and HEX and these other cell lines that are sort of workhorses in the industry and so it will be great to move further and forward into primary cells and patient-derived populations and in addition iPS derived cells.

When you're thinking about biochemical projects, oftentimes we're working with purified protein and sometimes it's difficult to get a full-length protein and so you have to compromise and use smaller portions of protein or a particular domain that you're interested in targeting with a small molecule compound. We've talked about, you know, reliance upon these cells and reagents and trying to get costs and making sure you have availability of things for very large scale projects. Most of our projects tend to be screening about 100,000 to

400,000 compounds and then cost can escalate quite quickly when you're working with very important and expensive reagents.

Then also in HTS, we're always concerned about the timing for the assay, how long you incubate with compound, is it better to be doing a kinetic read depending upon the target or you know, we mostly are reliant upon endpoint assays and then thinking about automation and how everything works together. We're buying instrumentation from a variety of different vendors and how well do they work together. I think that also can be very time consuming trying to get things transferred from one instrumentation system to another. Then finally I just will touch about data analysis and sort of after you've done the HTS how do you filter through all the data and I think we need to be developing better tools to do that type of study or those types of studies.

Slide 49

So obviously, we're thinking about goals for the future and thinking about how to make things cheaper and faster and perhaps multiplexing and getting more information from a particular sample. I've already mentioned the reliance upon more relevant cell types and then just moving away from artificial reporter systems. I think Daniel mentioned luciferase and it is a very common reagent that we use, but we have had a number of issues with it and we'd like to move away from that. So if you can minimize tags or minimize tag sizes so that it doesn't impact the biology of your assay that would be great and moving things down. Obviously, we've talked about moving from 384 to 1536 formats but can you move that down further to single cell analysis. I think people are now starting to do some really interesting studies at the single cell level and thinking about how we can apply that to HTS.

Then expanding other parts of the HTS process. We use a combination of manual workflows with automation and we need to sort of maybe extend more automation to the earlier parts of the process to get things moving quicker and being open-minded about assay formats. We talk about the standard plates that we use, the 384s and the 1536s but thinking about arrays similar to microarrays that are used in sequencing and those type of research areas.

I talk about easing of automation. You know, having plate washers in extensive amounts of rinsing and removing of reagents and adding can be very cumbersome and increase noise in the assays so we need to think about technologies that can reduce these washing

steps. In particular with qPCR having one step reagents can help dramatically in that fashion. Also applying better industry standards and a good example is as SBS compliance with plate types and can we extend that to other parts that are common to lot of different instrumentations and I'll make an example later.

I talk about improving data analysis and something out of the scope of this is actually the chemical libraries that we screen and how we can really improve them to make our HTS more efficient.

[0:40:01]
Slide 50

So I have two workflows on this slide here and the upper one is sort of our traditional manner of running HTS where you're culturing cells manually in standard incubators, dispensing cells with Thermo Fisher combies or some other fluid handler into 384-wells. Transferring compound by pin tool and then reading on a PMT reader such as a PerkinElmer Vision where you're reading individual wells at a time.

The bottom workflow is something that we've been applying to a large scale project where we're using tissue culture robots to helps us get these cells up into the numbers that we need using 1536 cell format acoustic dispensing, which Andrea has done a great job explaining and then having very versatile robotic systems. The picture here we have a smaller nanocell system where we can actually move different instruments on and off of the system depending upon the needs for a particular assay. Then finally the plate readers that we use, CCD reader can read an individual plate in 30 seconds versus PMT readers, which can be several minutes long depending upon the assay type.

Slide 51

I mentioned moving into automated cell culture. We use the TAP Biosystems CompacT Tselect – SelecT excuse me and basically this is not replacing manual cell culture workflows but augmenting it so that we can continue cell culture through weekends and through the evenings and this allows us to culture and expand cells, generate condition media. But also you can measure confluency of flasks, it can plate cells for you and I mentioned this sort of integration of sort of compliance. A good example is the racks that we use. So this system has one type of rack but it's not the same type of rack that we have in our incubators on the HTS systems. So you then have to manually move plates from one rack type to another and it will be

great to have, you know, an alignment of a rack type for all different instruments.

Slide 52

This is, you know, kind of touching upon some of the points that Andrea made about 1536, fluid dispensers being good and accurate and reliable. But then also using good incubators with high humidity and having a large capacity so that you can do these larger scale projects. We've been reliant upon moat wells or evaporation barriers within the plates to help cut down on edge effects and things and then using the echo for compound delivery.

We just recently completed a large scale project where we're screening 900 cell lines against a library of compounds in the 1536 format. It was greatly made more efficient by using a Labcyte echo and ViewLux from PerkinElmer and we are processing essentially 40 cell lines per week over an eight-month period and then now can match the genetic relationships of these different cell lines to the targets of the compounds that are known and identify new vulnerabilities in these cancer cell lines. This is the work of Stuart Schreiber's group who I acknowledged at the bottom of the slide. We're now trying to do a larger scale project than this one in the future and that's going to take much more efficient workflows and processes.

Slide 53

Some of the examples of what we're trying to apply are something like the 3D bead-based cell culture. We have been using a global eukaryotic microcarrriers or GEMs from Global Cell Solutions. These are essentially allogeneic beads of about a hundred microns in diameter with little magnetic particles. They're coded with a variety of different substrates gelatin or laminin. You can essentially seed yourselves onto these beads and then now rather than having to wait for your cells to adhere to the bottom of the plate they're already adhered to the beads and you can use these beads as any other type of fluid to dispense into a particular assay. So we will load our compound into assay plates ahead of time and we call those assay ready plates or ARPs and store those in the freezer and then simply thaw them out to room temperature. Then dispense the cells or whatever biochemical reagents you need into the assay plates and you can trim off an entire day from your assay.

This image in the middle where I'm showing is jammed out for four hours of seeding and you can see individual cells sitting on the

exterior of the allogeneic beads. And then after six days these are HeLa cells, they now completely coat the beads and they actually remind me of embryonic bodies if anyone has done that, embryonic stem cell differentiation. The beauty is that with a magnetic particles where they have specialized conical tubes that use the magnets to spin and levitate the beads into the media so that allows for them to be exposed to fresh reagents. But you can also put the conical tubes on to a magnetic platform. The beads come down out of suspension and can be quickly fluid change and that can hopefully in the future be done in an automated fashion.

Slide 54

This is just a quick comparison of a variety of compounds. It's actually probably very hard to see but comparing the 384 adherent and 1536 adherent conditions to the 1536 GEMs. Essentially, almost all of the compounds that we've tested show very similar dose response curves in this cytotoxicity assay suggesting that the 1536 GEMs doesn't change any of the biology that we're investigating. We are further investigating GEMs to do freezing of cells down and then also exploring more cell types to study with this.

[0:45:24]
Slide 55

I'm also mentioning a few different formats that we are using like label free readers or kinetic impedance measurements like the ACEAs or the xCELLigence platform just because these allow us to do a broader range of targets and hopefully moving closer to the endogenous system that I list here. Not necessarily looking at particular proteins like I'll give an example of the EnSpire but you can think about potentially these can be applied in the future for higher throughput. For imaging, we've been working with some software where we can basically do a low magnification scan doing high content imaging and then do that low content read and then identify outliers and then go back and image them at a higher resolution. This speeds up imaging and it also cuts down on the amount of memory taken up in servers because you're using much less bandwidth on your servers.

Slide 56

For the label free, we use the PerkinElmer EnSpire, which is the Corning Epic technology in a 384-well format and we're not doing this at HTS but hopefully with reduction in costs and better availability of something like a 1536 format, we can use this for HTS. Essentially, what it does is it monitors changes in mass or dynamic

mass redistribution at the bottom of the cells in the well. You can actually add an antagonist in this case for GPCR and measure response and there's a little graph here showing a variety of antagonists and a nice dose-dependent relationship with that. You can actually use this same platform in a cell free system for a variety of different assays and we now have an analytical assay that Corning developed where we can look at compound aggregation in a cell-free environment to make sure that the compounds that you're interested in are actually not just clumping up in the well.

Slide 57 to Slide 58

I have a few different assay formats that we have been playing around with in the very early stage of development. One of them is the Curiox DropArray and this actually takes you away from a well-based plate and what you have are these little tiny beads on little hydrophilic spots surrounded by a hydrophobic plastic. Then you actually overlay these droplets with oil. It's actually a mixture of Teflon and some other reagents, which is gas permeable so you 're not losing any, you know, impact on cell growth or anything like that but you're reducing the well volume down to 1 to 2 microliters from a traditional 30 to 40 microliters for some assay plates in the 384 format. They're working on 1536 format that actually should be released fairly quickly and then they're actually investigating some qPCR related products in the future.

I also have an image just showing you that you can image these plates. They have good optical clarity. This is HUVEC cells scanned at 40x and stained with phallloidin and DAPI. Finally, I mentioned a reference here. Curiox has worked with a group at Genentech and they recently released a publication in Blood.

Slide 59

Then moving away to other formats thinking about array-based technologies, we have a small molecule microarray program at the Broad and essentially you can plate compounds on to a glass slide, a very small format and very high density. You can think about this type of technology being applied with either proteins being adhered to a glass slide or potentially even moving into qPCR with this type of technology.

In this particular case, the compounds are stamped on the slides and specific patterns with controls, etc., interwoven within your test compounds and you can add either a cell lysate or protein preps onto the glass slides and then detect binding with an antibody based

detection system. You can then scan these glass slides and get very quick information about a compound directly interacting with a particular protein.

Slide 60

Another platform that we have at the Broad, it's nicknamed L1000. It's a Luminex based technology and it's actually part of the LINCS project. It mediates – I'm sorry, it pairs ligation-meditated amplification or LMS with a Luminex detection system and you can typically measure about 1000 mRNA transcripts per well. Arvind Subramanian has done an extensive amount of work applying gene set enrichment analysis studies with the cell 1000 so that now we have a set of landmark genes of a thousand genes that can sort of predict the response of the other almost 20,000 genes. So it can dramatically cut down on the cost to do this. Typically right now, we run our mature candidates from the MLPCM probe program across a panel of 20 cell lines with a variety of different treatment times and it hopefully can give you an eye into mechanism of action and they actually are working on a manuscript on that. It should be coming out in the near future.

[0:50:32]
Slide 61

Then finally, I just, you know, we're always hoping for things to come down in cost and applying these technologies will require better readers and better instrumentation in everything from plate types and on will all impact what we can do with this. I think there's been some nice microfluidics technologies that are coming online where you can actually measure single cell level changes in expression. There's quite a few groups at Stanford and at MIT and Paul Blainey at the Broad where they actually take single cells either eukaryotic or prokaryotic and actually can isolate them with microfluidics and then do a variety of different assay types in microfluidic chambers.

I think I mentioned sort of applying microarray formats to other cell based or biochemical studies and I think Luminex type studies also as the cost come down will be applied to fuller sized HTS in the near future.

Slide 62

Then talking about data analysis, I think as part of the MLPNC network we share all of our HTS datasets in PubChem right now and I think people are becoming better about mining that data looking for how compounds behave in a variety of different assays. I think so the

next level is getting better tools to do cross-assay analysis so you can look at compound behavior across a whole panel of assays and then look for commonalities in that pattern to maybe identify compounds that impact a particular molecular pathway at different nodes of the pathway. Maybe you can find synergy in that way.

Then I think you know, just sort of a plea to the community is sort of releasing large-scale datasets that are not relevant in public or private domains. So again providing more opportunity to study things akin to what genetics research groups are doing and releasing these large-scale studies for public screening analysis. As part of that, there is the Bioassay Research Database or BARD, which is coming out in the fall and this is a supplement to PubChem and it basically provides a tool for looking at a variety of different HTS formats that have been studied primarily through the PubChem, sorry the MLPCN mechanism. But it also gives you very nice detail and a good user interface so you can really mine those databases for more information.

Slide 63

A large number of group in our institute that have helped a variety of different projects but I acknowledged most of them throughout the presentation. So thank you.

Slide 64

Sean Sanders: Great. Thanks so much, Dr. Faloon. Many thanks to all of our speakers for the fascinating presentations and we're coming close to the end of the hour so I'm just going to run through as many questions as we can.

Just a reminder to those watching us live, you can still submit questions by just typing them into the text box and clicking the submit button. So I'm just going to fire a few questions at you, all of you and I'm going to start with you, Dr. Faloon. You had mentioned cells in suspension, working with cells in suspension and I know Mr. Sipes mentioned a technology that you have that can handle cells in suspension as well. Could you both maybe just talk a little bit about some advice that you might give to people working with suspension cells? Dr. Faloon?

Dr. Patrick Faloon: I mean I think in our studies, you know, we're doing about 200 to 300, 384-well plates per day. So it's easy enough to just pass them through but I think you have to have good mixers and these type of things. You get even distribution. Obviously, you're having carryover

of older media so you want to minimize that as much as possible. So, you know, one thing that is hard on an HTS automation system is not having much for centrifugation and so you really have to kind of carry things throughout the whole process, but it's not a hindrance by any means. With our cell profiling project, we did a number of suspension lines and everything worked great.

Sean Sanders: Great.

Dr. Patrick Faloon: So I don't think it's a barrier.

Mr. Daniel Sipes: Yeah. For the simpler assays, I think, you know, it's not that bad working with the suspension cells and certainly processing them. We don't have automated tissue culture. At GNF, we actually do most of the offline before the assay tissue culture ourselves or manually so suspension cells are actually conducive to that.

Sean Sanders: Right.

Mr. Daniel Sipes: But for the online more advanced assays where there are many levels of staining and washing of cells and so on, they can be challenging and you know, not to promote our GNF washer/dispenser 2, but we actually run with them quite a bit with suspension cells and we read them out both sometimes high content. We could do some things like with an Acumen [from TTPLabtech] or something like that. We're also using flow cytometry quite a bit so we can process cells on the system do a number of wash steps and then take the place to flow cytometers. So yeah.

[0:55:29]

Sean Sanders: Great. I'm going to stay with you, Mr. Sipes. This viewer asked about the SMARTflare and I know you haven't really got into it but how do you think that might compare with qPCR as far as pros and cons?

Mr. Daniel Sipes: Yeah. I hate to speculate. We haven't even used it.

Sean Sanders: Okay.

Mr. Daniel Sipes: And it seems almost like a magic reagent. This came out of Chad Mirkin's lab is being sold through EMD Millipore. We're very excited about it. I would sort of expect it might not have the exquisite sensitivity and dynamic range that you get with qPCR, not sure. I imagine there'll be cell type specificity like it might not work so well with all the different cell types so that's just some speculation about

what the limitations might be. But given that it's something that you just add to the media and then migrates into the cells and that you can start doing kinetics studies potentially, it looks promising.

Sean Sanders: Good. Great. Dr. Weston, a question for you. What are the typical preparation and amplification times for a 1536-well plate and the qRT-PCR experiments that you're doing?

Dr. Andrea Weston: Preparation times.

Sean Sanders: Uh-hum.

Dr. Andrea Weston: I'm going to assume they're talking about assay preparation. So the one thing about qPCR that makes it attractive is once you've got this whole process nailed down, it really is sort of order your TaqMan reagents or whatever reagents you're using. For any given gene, you can pair it with any cell line and you can pretty much expedite the assay development time. It's very straightforward. You'll need to figure out things like cell density and where in the range you want to be with your gene expression but I think it's very agnostic to many different cells and many different genes. I think that answers the question.

In terms of thermocycling, you know it's a longer protocol than most, but still the value of the data you get I think is worth it.

Sean Sanders: Great. A question I'm going to pose this to you, Dr. Faloon. This viewer asks, they said they have a qPCR system right now actually from Applied Biosystems. How do they adapt it to high throughput? Can they adapt it or do they need to buy new system or what would they need to do?

Dr. Patrick Faloon: I think it depends. I mean some systems are sort of diffed where you might be able to buy an existing module that you just attach on to the sides so they can interface with the robotic arm. So I think that would be the first barrier is have it just interact. Most groups are pretty good about APIs and these types of things so on the software side you can integrate them with other instrumentation. So I think it depends on the generation of the instrument really in that respect.

Sean Sanders: Uh-hum.

Dr. Andrea Weston: I want to answer that. I talked a lot about 1536 well RT-PCR and really right now I only know of one instrument that can

accommodate 1536 plates. But it's important to point out that we have miniaturized these reactions in 384. So we've gone down to half a microliter in 384-well formats successfully. So you know if you don't need the 1536 and a super high throughput that's definitely something worth exploring.

Sean Sanders: Okay. Excellent. Let me shoot you another question, Dr. Weston. This asks about microRNAs and that we were talking about this off air whether it's possible to quantify microRNAs and how would this be implemented.

Dr. Andrea Weston: It's definitely possible. I've never actually done it so I don't have the details of how to implement, but I would imagine that the protocols were very similar to looking at normal gene expression. You know, we use this extensively to look at knock down in SiRNA or short hairpin RNA screens as well.

Sean Sanders: Uh-hum. Great. Mr. Sipes, I'm going to come back to you. In the PLA experiments that you talked about, you mentioned you could get your readout through qPCR or next-gen sequencing.

Mr. Daniel Sipes: Uh-hum.

Sean Sanders: Can you talk a bit about the comparison between those two and why you might choose one over the other?

Mr. Daniel Sipes: Sure. Yeah. I think most of the proof of principle and the applications I've seen, the readout actually has been qPCR.

Sean Sanders: Uh-hum.

Mr. Daniel Sipes: But Olink Bioscience had a publication last year with readout through next-generation sequencing and also the Fu lab at—oh no, I take that out, that's a slightly different application—

Sean Sanders: Okay.

Mr. Daniel Sipes: --for mRNA. But yeah, Olink had a publication and the real power there is basically the reduction in cost of next-gen sequencing. It just makes a lot – it opens up the practicality of applying this to high level multiplexing. Because I forgot what the cost is per data point in next-gen sequencing. I don't even know what it is. It's a dollar every million data points or something you know, like that. So if you can leverage those advancements, layer that on top of PLA and you're

talking about making it practical to screen at least from a cost point now every well in a cost effective way to give you that profile type of information on endogenous proteins, not only mRNAs but proteins.

[1:00:35]

Sean Sanders: Uh-hum.

Mr. Daniel Sipes: So it has the potential to be very powerful.

Sean Sanders: Okay, great. So we are unfortunately at the end of the webinar but I'm going to give you one more question and that's to think a little bit about where your work is going and what you would need on a technology side to really push it forward. So Dr. Faloon, we'll start with you if you can pull out your crystal ball and what would you need to really drive your research?

Dr. Patrick Faloon: I mean I think, you know, sometimes the limitation are plate readers and imagers and so obviously improvement and define physics maybe a little bit would help dramatically I think.

Sean Sanders: Okay. Dr. Weston?

Dr. Andrea Weston: I think you know, we've all expressed that we're looking to go label free endogenous more relevant cell models. I think many companies are grappling with how do we get access to these relevant tissues, patient tissues, you know, seeing the promise of induced pluripotent stem cells really pan out, things like that. You know and then on the technology side, real-time PCR is one example where it's somewhat label free. You're going in, you're measuring the endogenous gene. I think high throughput mass spec is going to be another big one where you can profile many different things in a reduced, miniaturized fashion. Then Patrick alluded to we're collecting more features per assay and so the data analysis gets more complex. How do we tap into that data and make sure we're leveraging the data that we're generating. So those are the three that I would highlight.

Sean Sanders: Final word to you.

Mr. Daniel Sipes: Sure. Yeah, I think the trend –we've all talked about studying endogenous activity, you know, the relevant cell model and measuring endogenous activity of either expression analysis or protein analysis and making that leap to high throughput screening is going to be challenging. There's a bit of a gap there right now. It will be challenging to see how that works out in a practical sense. I think

there have been some publications in papers that give us an indication of how that goes. But certainly applying these profiling techniques in an endogenous setting, in a disease relevant setting is the next step.

Sean Sanders: Uh-hum. Fantastic. Well unfortunately, we are out of time for this webinar so on behalf of myself and our viewing audience, I wanted to thank our speakers very much for being with us today, Mr. Daniel Sipes from GNF, Dr. Andrea Weston from Bristol-Myers Squibb, and Dr. Patrick Faloon from the Broad Institute.

Slide 65

Please go to the URL now at the bottom of your slide viewer to learn more about resources related to today's discussion and look out for more webinars from *Science* available at webinar.sciencemag.org. This particular webinar will be made available to view again as an on-demand presentation within approximately 24-48 hours from now.

We're interested to know what you thought of the webinar, send us an email at the address now up in your slide viewer, webinar@aaas.org.

So again thank you so much to our panel for being here and to Roche for their kind sponsorship of today's educational seminar. Goodbye.

[1:03:30] End of Audio