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xMAP® Insights

Connecting the xMAP® Community to Innovative Applications & Resources

January 2019

Cancer research & precision oncology

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xMAP® Insights

A Magazine for the Multiplexing Community

Greetings,

Welcome to xMAP® Insights — the magazine created to connect you to the multiplexing community by sharing innovative applications of xMAP Technology, while also providing valuable resources to catalyze your next experiment.

This issue focuses on custom multiplex assay development for cancer research and precision oncology, including conversations with:

- Christian Regenbrecht on his use of DigiWest® to inform personalized cancer medicine
- Tim Waterboer on the development of serological fingerprints to reveal the link between infections and cancer
- Evi Lianidou on the development of a 14 gene assay to interrogate circulating tumor cells
- Bryan Severyn and Jannik Andersen on the development of a 26-gene signature to measure RAS activity
- Ankit Patel on QuantiGene™ assays that use branched DNA technology

If you have suggestions for future articles, please contact me directly at **hgraham@luminexcorp.com**. I'm grateful to have you as a reader and look forward to hearing from you.

If you ever find yourself in Austin, please reach out, as we'd be happy to host you on a tour of our campus. Warm Regards,

Hilary Graham

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Adopting DigiWest®: How a Multiplex Immunoassay is Making a Difference in Precision Oncology

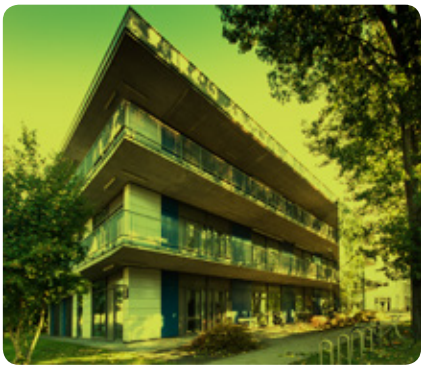
Christian Regenbrecht,
CEO, Cellular Phenomics & Oncology

At **CPO** (Cellular Phenomics & Oncology), a Berlin-based company specializing in cellular phenomics and oncology, scientists are making significant progress in precision oncology by combining patient-derived, three-dimensional cell culture models with Luminex-based **DigiWest® technology.** The resulting data from this approach can inform the development of targeted therapies and treatment selection, and delivers real-world clinical benefit.

According to **Christian Regenbrecht**, CEO of the company, CPO's PD3D® (Patient-derived 3D) cell culture models allow comprehensive drug screening of individual drugs, as well as combination therapies. His team has generated more than 400 of these cell culture models from a wide variety of different tumor entities for pharmaceutical and biotech companies. PD3D® models are similar to organoid models, making it possible to recreate the tumor's native architecture for the best approximation to its *in situ* form and function. They can be used as tools for preclinical and translational research, such as drug screening in the preclinical context as well as in CRISPR experiments, but increasingly PD3D® models are also making their way as avatars for predicting patient response in ongoing clinical trials.

The CPO team was eager to include proteomic **results** for its models very early in the developmental process, and adding the DigiWest® technology made that happen. The team had previously considered mass spectrometry, but it wasn't a fit because the results took too long to be useful for clinical work. DigiWest®, on the other hand, is a multiplex immunoassay technique that can quantify as many as 800 analytes in a single reaction. This approach allows Regenbrecht and his colleagues to deliver results in 7-10 days, complete with information about post-translational protein modifications such as phosphorylation and acetylation.

In addition, DigiWest® works well with the precious tissue samples with which CPO typically works. "We have only little amounts of tissues, cells, and therefore protein," Regenbrecht says, noting that they must generate a great deal of



Christoph Sachse (left), Christian Regenbrecht (center), Markus Templin (right)

information from each small sample. "DigiWest® is of benefit to us because it needs little amounts of input material, and we gather a high-density set of output information."

Regenbrecht adds that DigiWest® "is a robust assay that is highly reproducible, and so the data would also be reliable for clinical utilization. But for the moment, for our customers in the pharmaceutical field, it is very important that we can provide data they can rely on for future experiments." For example, a recent analysis of samples from a patient with colorectal cancer revealed drug responses that varied by as much as 30-fold, indicating that a combination therapy might be the best treatment course. By using DigiWest® on these models, CPO scientists identified the mechanism responsible for aberrant activity in the MAP kinase pathway.

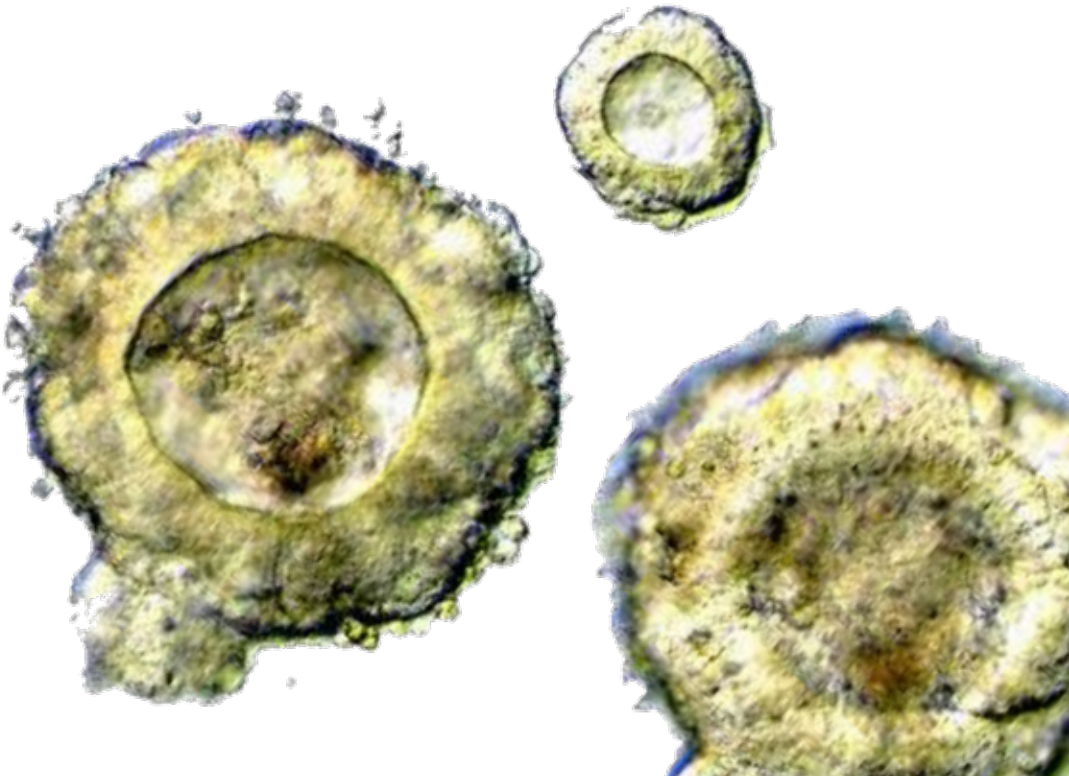
Looking ahead, Regenbrecht believes that cell modeling will need to represent even more complexity, such as incorporating immune components or vasculature. He also knows that in these early days of precision oncology, stakeholders like CPO have to carefully balance the promise of what can be done while still being appropriately cautious about a novel approach. "We are presenting data that was unthinkable two years ago," he says. "We are at the forefront of medicine." Still, though, work needs to be done on how to deliver this information in a way that makes the most sense for the physicians, oncologists, and pathologists responsible for dealing with this kind of data at the bedside, he adds.

For more examples of DigiWest® in action, check out these publications:

DigiWest® for biomarker discovery
DigiWest® for characterization of compound actions
DigiWest® for deciphering of Wnt signaling
DigiWest® for targeted proteomics of tumor organoids

Learn more about DigiWest® from the inventors at NMI

CELLULAR PHENOMICS & ONCOLOGY



PD3D cell models

Five Things We Learned from an xMAP® Power User

At the fourth annual **xMAP® Connect** user group meeting held in Amsterdam late last year, we had the good fortune of sitting down with **Tim Waterboer, PhD,** leader of the Infections and Cancer Epidemiology Group at DKFZ, the German Cancer Research Center. Waterboer has the distinction of publishing more papers citing xMAP Technology than any other user, so we were eager to learn about his work. His lab, which uses xMAP for a multiplex serology assay designed to detect signs of long-ago infections, tests about 100,000 samples each year from a portfolio of 500 different antigens. Here are five surprising highlights from our chat.

Tim Waterboer, PhD

1: Infection history is like a fingerprint

Waterboer's team uses multiplex serology testing to generate what they call serological fingerprints. "It's a very individual signature of infection exposure," he says. "Antibodies tell us about the person's history, the cumulative exposure a person has had, long after the acute infection is gone." He believes this information will feed into the delivery of personalized medicine by offering insight into a person's risk of developing diseases caused by chronic infection.

2: Infections cause more cancer than you think

According to Waterboer, infections are the third leading cause of cancer overall, behind tobacco exposure and obesity and other dietary factors. "There is too little public awareness" of the link between infectious disease and cancer, he says. "It's important for people to know that you can prevent cancer through vaccination." Figuring out which infections are associated with which forms of cancer requires a deep dive into those serological fingerprints, particularly because it's usually the long-ago infections that eventually lead to cancer. "If we ask what cancer can be caused by an infection, then usually we have to go back 10, 20, or 30 years in time to find the answer," he adds.

3: Even high-capacity labs choose manual pipettes

Though Waterboer's team runs 100,000 samples a year through multiplex serology testing, they still pipette each and every sample with a single-channel pipette. Serum is notoriously difficult to handle, and Waterboer's lab faces the added challenge of working with samples sent in from all over the world, processed with many different methods and in various states of quality. For optimal results, they avoid automation for this step and stick with the tried-and-true manual approach.

The Infections and Cancer Epidemiology Group at DKFZ

4: Multiplexing pairs well with microarrays

Waterboer turned to multiplexing technology some 15 years ago when he realized that the protein microarrays he was using at the time lacked the necessary sample throughput. Today, though, his lab has embraced arrays once more, this time choosing whole proteome microarrays to perform biomarker discovery in bacteria. These results inform the selection of antibody panels the team uses next to study potential biomarkers across a lot more samples with the higher throughput of multiplexing technology.

5: Epidemiology sneaked up on him

Though he runs an epidemiology group now, Waterboer started his multiplexing efforts in basic research. "When I developed the multiplex serology system, I had nothing about epidemiology in my mind," he recalls. It was the epidemiologists who approached him after realizing that the small sample volumes and high-throughput capacity involved in the multiplex workflow were so well suited to that field. "To my surprise, I liked it very much," he says. Since then, his lab has expanded into translational applications and public health research as well.

Read more about Waterboer's multiplex serology method:

- Kinetics of the Human Papillomavirus Type 16E6. Antibody Response Prior to Oropharyngeal Cancer
- Sensitivity and Specificity of Antibodies Against HPV16E6 and other Early Proteins for the Detection of HPV16-driven Oropharyngeal Squamous Cell Carcinoma
- Human Papillomavirus Antibodies and Future Risk of Anogenital Cancer

xMAP®-powered Assay Provides Critical Clues from Liquid Biopsy Samples

Liquid biopsies are opening new avenues for cancer research and diagnosis, but even experts in the field continue to nail down the best protocols for analyzing the cell-free DNA and circulating tumor cells (CTCs) contained in these peripheral blood samples.

Evi Lianidou, professor of Analytical Chemistry and Clinical Chemistry at the University of Athens, is one of those scientific pioneers. As early as 2005, she geared up to investigate the utility of gene expression analysis in CTCs. Her group, in collaboration with Clinical Oncologists in Greece, were the first to show the prognostic significance of CTC detection in early breast cancer. However, beyond detection and enumeration of CTCs, she was mostly interested in characterizing these rare cells at the molecular level. Using RT-qPCR, she evaluated the expression of a few cancer genes in these cells, but the number of important gene markers to be analyzed in these cells was continuously growing. But she quickly encountered a problem: the classic microarrays she expected to use for her project were not sensitive enough to be applied in such a low number of cells. Casting about for an alternative, she encountered xMAP® Technology from Luminex. She adopted the bead-based platform for multiplex gene expression analysis and has been deploying it for game-changing studies ever since. For instance, the scientists at the Analysis of Circulating Tumor Cells Lab applied xMAP Technology to simultaneously **interrogate the expression of six genes in CTCs**, as well as DNA methylation markers in these cells.

Moreover, CTCs are highly heterogeneous, so bulk population analysis could not achieve what Lianidou needed. Today, Lianidou and her team have developed a **14-gene assay to interrogate CTCs**. Unlike existing FDA-cleared CTC assays which enumerate cells but fail to offer a molecular characterization, Lianidou's xMAP-powered assay generates a wealth of data about gene activity even at the single-cell level. One of the reasons she built this assay on xMAP Technology is the ease with which assays can be refined or expanded later. "We want to have a lot more than 14 genes," she says, noting that new information about cancer biology, cell therapy biomarkers, drug targets, and more can all be quickly added to the multiplex panel as it becomes available. Her team is currently evaluating the assay's performance on clinical samples.

Those clinical samples are one of the reasons that Lianidou has been such a champion of xMAP Technology. With this type of research, she says, scientists don't have the luxury of running more than one test on a sample because the input amounts are so small. With the Luminex platform, "we can test many targets in the sample at the same time," Lianidou says. "It's very important for high-throughput analysis to get a lot of information from these precious samples."

As a longtime developer of xMAP assays, Lianidou offers a simple tip for novice users: don't skip the *in silico* step. "We always start from the *in silico* analysis of primers," she says. Before her team even thinks about moving into chemistry, they carefully check to make sure all target sequences are unique in the genome and that primers will not interact with anything unexpected. "Specificity is a very important issue," she says. "Make sure you ask, 'Do we detect only this target and nothing else?' And make sure no target is missing."

Analysis of Circulating Tumor Cells (ACTC) Lab, University of Athens

"We can test many targets in the sample at the same time,"

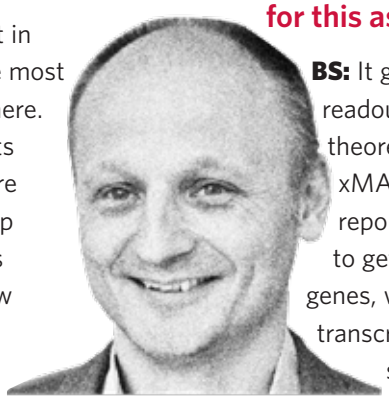
— Evi Lianidou, Professor, University of Athens

Querying RAS: How a Multiplex Assay Became a Robust Transcriptional Readout for HTS

A few years ago, scientists at Merck & Co were looking for a better way to evaluate the activity of the RAS-MAPK pathway, which is particularly important for cancer development and can also signal susceptibility or resistance to emerging therapies. They used the Thermo Fisher QuantiGene™ Plex Gene Expression multiplex assay, based on xMAP® Technology, to develop a 26-gene signature to measure RAS activity. We caught up with two key members of that team, Bryan Severyn and Jannik N. Andersen, to learn more about how multiplexing changed the questions they could ask.

Why did you develop this screening assay?

Jannik N. Andersen: There is huge interest in drugging RAS, which is probably one of the most hypervalidated, bona fide oncogenes out there. Merck had an interest in finding new targets and new approaches to drugging RAS. There are two conceptual ways to do that: develop inhibitors of known MAPK-pathway targets (e.g., SHP2, RAS, MEK, ERK) or identify new mechanisms/compounds that reverse the transcriptional signature linked to RAS. We set up this assay so we could have a biomarker readout for RAS activity in cells and then screen a library of primarily clinical-stage compounds to see if we could reverse that activity. It was an innovative approach to use transcriptional readouts as your primary assay for screening compounds.



Jannik N. Andersen

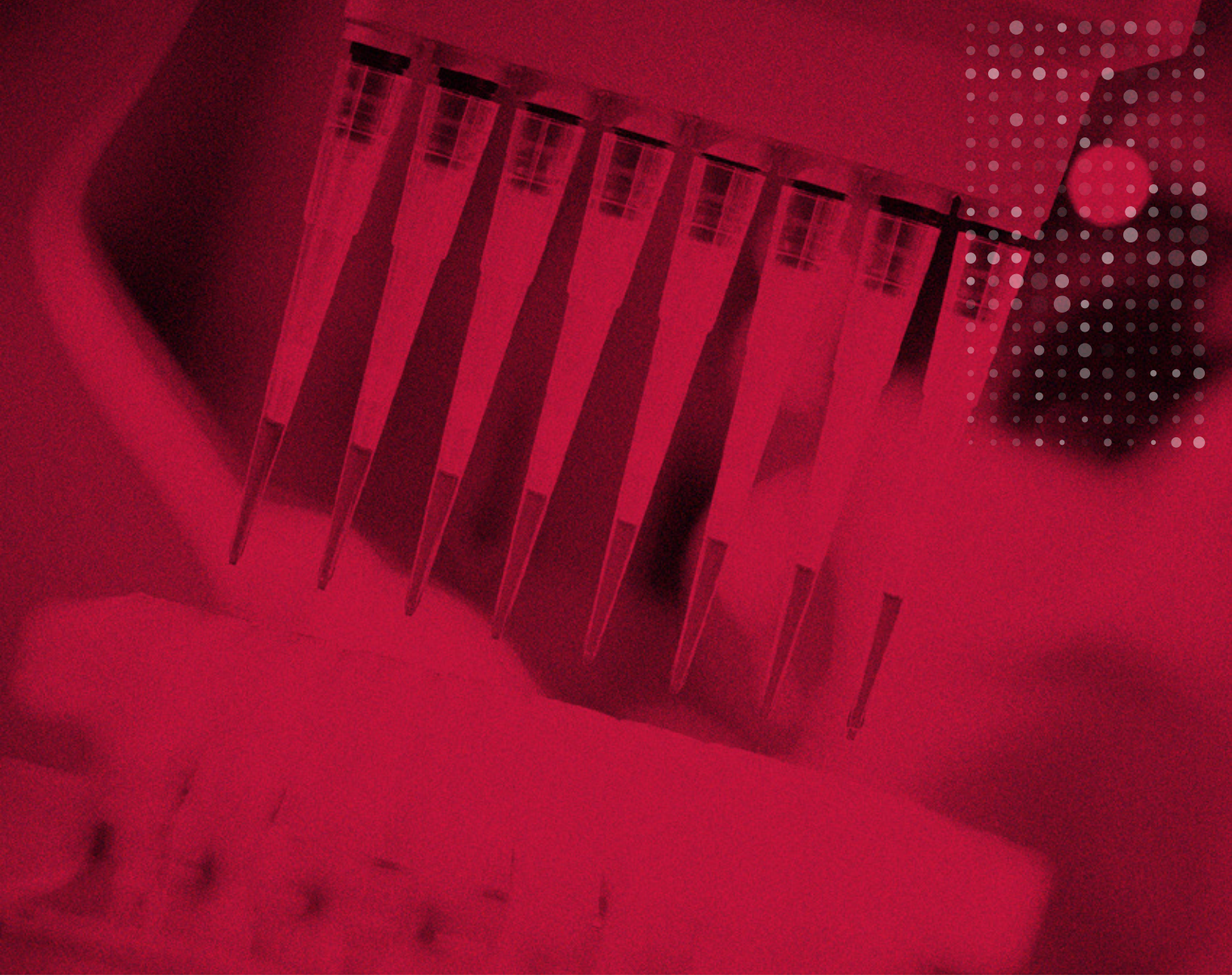
Bryan Severyn: The bioinformatics group developed a large gene signature, which was validated in several hundred patient tumor samples and cell lines. It was really nice to be able to translate this information into a strong smaller sized multiplexed readout using the xMAP system. In doing that, we selected several of the most robust genes to create an up-signature and a down-signature. We screened a library of 725 'high-value' compounds that included both known pathway inhibitors, as well as clinical stage compounds developed for other therapeutic areas, to see if the compounds reversed the RAS signature.

Bryan Severyn

Why did you choose QuantiGene™ for this assay?

BS: It goes back to the fact that you can have multiplexed readouts — for transcriptional readouts you can theoretically have up to 80 genes when using the xMAP Technology. If you just had a single plex system reporting on the RAS pathway, you might not be able to get a strong assay window. With this signature of 26 genes, which reports movement across a broad range of transcriptional readouts, you can really generate a robust signal. This helps confirm that you have a robust assay as well as providing information on which compounds are significantly reversing the RAS pathway. Also with this approach, you can perform correlation statistics because you have the power of a multiplexed readout to compare to a control compound that is known to down regulate the RAS pathway. It is a very robust system, extremely reproducible from day to day and even from lab to lab. That gave us peace of mind that this was going to work really well.

JNA: That's an important point because we were a small group focused on finding rational drug combinations to advance the Merck AKT inhibitor (MK-2206). We had proof of concept that compounds could inhibit the RAS gene signature and be combined favorably with our AKT inhibitor, which resulted in potent anti-tumor activity. The transfer to Bryan and the high-throughput screening group was possible because QuantiGene is such a robust platform.



BS: It was almost seamless. The workflow from Jannik's lab to our lab was really straightforward and very quick. We were able to get the whole screen up and running in a very short amount of time, and the results were fantastic.

Do you have any advice for people who are just getting started with QuantiGene™?

BS: The most important thing is doing the up-front legwork to identify really robust genes that are modulated by whatever compound or treatment you're looking at. That will translate into a great QuantiGene™ assay.

JK: The dynamic range of modulation is important for a successful screen. Otherwise, you have too many bystander genes that may not change much in response to pathway modulation.

Want to learn more?

Read more about the RAS-MAPK High-Throughput Gene Expression Screen

Learn more about Thermo Fisher QuantiGene™ Plex Gene Expression multiplex assay

Watch how QuantiGene™ is used for the molecular classification of breast cancer tumors

Meet Ankit Patel

Ankit Patel helps develop QuantiGene assays using branched DNA technology

When you want an assay that can detect nucleic acids, you'll likely choose the high-quality Invitrogen™ ProcartaPlex™ and QuantiGene™ Plex assays from Thermo Fisher Scientific, a Luminex partner. The QuantiGene assay uses branched DNA (bDNA) technology, relying on signal amplification instead of target amplification for optimal quantitation of transcripts. One of the scientists behind the design and development of the QuantiGene assay is Ankit Patel, a scientist in bioinformatics services at Thermo Fisher. We caught up with him to learn more about his work and the assays he develops.

Q: What do you do at Thermo Fisher?

Ankit Patel: I would describe my primary role as bioinformatics services, where we work directly with distributors, sales, marketing, and/or customers to help design new assays. Clients will approach us with their projects, and we work with them to develop the best assay possible to answer their experimental questions, including designing DNA oligo probes to be used in the assay. The oligos are the dynamic portion of the assay, and the oligos are designed to specifically detect the target genes of interest. I have been working with the bDNA technology for 15 years.

Q: Tell me about your professional journey. Have you always supported QuantiGene assays? I know Affymetrix and Panomics were the originators of this product.

AP: This was my first job out of college. I was hired by Genospectra in Fremont, California, the city where I grew up. Genospectra merged with Panomics, which then merged with Affymetrix, which then bought eBioscience … and then we were acquired by Thermo Fisher Scientific. Almost 15 years later, here I am, still in bioinformatics services.

Q: What do you like best about your current role?

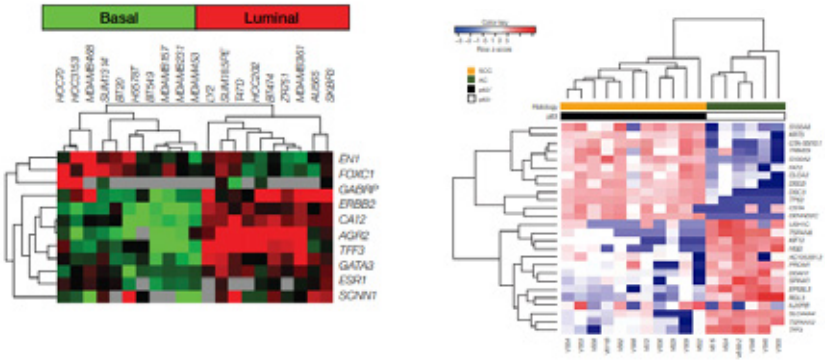
AP: Every day is a different day. There is a lot of variety in my role, which keeps things fresh. I also get to work directly with customers, so I get to hear and see a lot of cutting-edge ideas in our industry.

Q: What is an initiative or project you're especially proud of?

AP: It's hard to narrow it down to just one! Monsanto used our assay during their testing of a double-stranded RNA that is being introduced into corn to help save honey bees.

Q: What is something most people don't know about a QuantiGene product, instrument, or service?

AP: The true beauty of the assay is that there is no need to purify the target RNA or DNA, and it works with classically difficult samples such as FFPE tissue samples. Additionally, it is a very robust and highly accurate assay. We also have solutions for high-throughput screening, and QuantiGene is the ideal solution for secondary screening for compounds that target genes.



Q: What current research or clinical trends in the industry are you excited about?

AP: Hepatitis B is intensely studied these days, and we have created solutions for the research world to better understand how to treat—or even, hopefully, one day cure—a patient with this virus, which is epidemic in many parts of the world.

Q: If you could solve any scientific challenge or human health crisis, what would it be?

AP: For human health, I would say better therapies for diabetics. I was born here in the US, but my ethnicity is Indian, and there are many members of our community and of my family that struggle with this disease as well as other metabolic syndromes.

Q: What's your favorite thing to do outside of work?

AP: As the father of two little toddlers, free time is hard to come by. Whenever I can, I just enjoy putting my feet up and relaxing a bit, and watching football or attending games.

6 Tips for QuantiGene™ Assays

We asked Ankit Patel, a scientist in bioinformatics services at Thermo Fisher, to share his tips for working with QuantiGene Plex assays. Here's what he had to offer.

1. The customer will have a direct line to the bioinformatics team to work on the panel list and special design requests. We handle all of the oligo designs. It's a full service.
2. It helps to use multiple reference/housekeeping genes in your panel for more robust data normalization, in case one of them is being affected by a treatment or condition.
3. Don't worry about purifying any RNA. QuantiGene assays work directly from lysates or homogenates and work particularly well with difficult samples, such as FFPE tissue sections.
4. Make sure you have the right equipment: Luminex instrument, hybridization oven, magnetic plate washer, etc.
5. Be prepared for all of the data. You can measure up to 80 genes simultaneously, so there are lots of insights to be gained.
6. If you don't know which targets you want to include in your custom panel, we have a pathway search tool in our online panel configurator to help you. Alternatively, you can start with one of our predesigned panels that are composed of carefully curated gene targets specific to a particular disease, pathway, or research area.

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