Q&A: UCSD's Hardiman on an Aquatic Multispecies Array to Study the Effects of Endocrine Disruption

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By Justin Petrone

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Professional background: 2000-present, director, Biomedical Genomics Microarray Facility, University of California, San Diego; 1999-2000, senior scientist, Axys Pharmaceuticals, South San Francisco, Calif.; 1998-1999, senior scientist, NemaPharm, Cambridge, Mass.; 1993-1998, postdoc, DNAX Research Institute, Schering-Plough, Palo Alto, Calif.

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When researchers from the Southern California Wetlands Recovery Project approached researchers at the University of California in San Diego to adapt microarray technology to quantitatively measure the impact of endocrine disrupting chemicals on fish, it seemed like a logical step for improving environmental testing.

It also posed logistical challenges because limited genetic information exists about hornyhead turbot and other flatfish that have traditionally been used to characterize the presence of chemicals in the marine environment.

Enter Hardiman, who, working with UCSD colleague Michael Baker, eventually helped tackle the SWRP's goal of developing a multi-gene cross-species microarray, specifically fabricated to screen for responses to endocrine disruptors in Southern California coastal waters.

The array, which was discussed in an *Environmental Health Perspectives* paper last year, included fish genes that are involved in the actions of adrenal and sex steroids, thyroid hormone, and xenobiotic responses.

In that study, the researchers profiled gene expression in turbot and zebrafish that had been exposed to estradiol and 4-nonylphenol. Analyses of turbot collected from polluted areas revealed altered profiles compared with those from nonaffected areas, results that the authors say prove their array can be applied more broadly.

Hardiman discussed the ongoing project at Select Biosciences' Advances in Microarray Technology

Below is an edited transcript of that interview.

What is the root of this project?

I have always been interested in fish molecular biology. I did my PhD in Ireland on Atlantic salmon and seawater adaptation. And when I came to UCSD I had the opportunity to go back and revisit some of that work again. Michael Baker at UCSD was a professor that I had cited a lot when I wrote my thesis. I got to know him through the array facility at UCSD, and he and some of his collaborators were interested in the idea of developing an aquatic multispecies array. So I got drawn to that because it was something that I really wanted to be involved with. As time went by, we developed and tested the array with our collaborators at the Southern California Wetlands Recovery Project, between I guess 2005 and 2007, and in 2009 we published our first paper.

Why were you interested in studying endocrine disruptors using the multispecies array?

Endocrine disruption is of concern because of the effects on humans and aquatic species. We used the multispecies array because we didn't have genome sequence data available for many of the sentinel fish species of interest. We couldn't use a whole-genome array because to do something like this with the full transcriptome would have required the availability of sequence data, and an awful lot of work to generate this at huge expense. We had tried hybridizing turbot samples to zebrafish arrays and other fish arrays that were available, but we never had success. Initially I didn't believe that was going to work. Cross-species hybridization is kind of hit or miss and needs a lot of optimization.

I understand the interest in zebrafish but why did you choose hornyhead turbot, especially considering the issues it presented for you later?

Environmental monitoring agencies have used hornyhead turbot for years. They have been looking for the presence of toxins in the fish using chemical analysis, and that's good, but the one thing the array offers [that chemical analyses do not] is the ability to measure the downstream biological effects of contaminants on the organism itself using biomarkers associated with endocrine disruption. So with the array you are not just detecting the presence of chemicals, you are actually monitoring what happens to the fish, so it takes it a step further. Additionally, there was a lot of legacy data for this fish, the turbot, particularly in Southern California. It's a bottom dweller, a solitary fish living at the bottom of the sea, and is likely to be exposed to these chemicals, which sink and become associated with the sediment.

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Why did you originally develop a spotted array and then move to the Agilent platform?

Two things happened. First of all, we ordered oligos to print these arrays and that has a finite lifespan, about two or three years. So when that oligo DNA and [those] printed slides began to age, and we decided we wanted to add additional targets to the array, a rethink was in order. This was guided by the needs of collaborators at the Orange County Sanitation District, the City of Los Angeles Environmental Monitoring Division, the City of San Diego Ocean Monitoring Program, the Southern California Coastal Water Research Project, and university research groups in Long Beach, Riverside, and San Diego. We were then faced with the conundrum of, 'Do we add more oligos to these spotted arrays, or do we migrate to Agilent arrays?' We did a cost analysis on spotting arrays versus using Agilent, and it was less expensive to use Agilent as [fewer] replicates were required and the data quality was much higher. So that really was our motivation

We were just hoping to prove that a multispecies array would work and we present data to this effect. There are other incidences of multispecies arrays, particularly in the virology field, with probes that cover different viral strains. That had been done previously, but nothing like this had been done in the context of marine environmental toxicology. Arrays had focused on carp, zebrafish, to some extent salmon, but really nothing had been developed that could be successfully used across species. And that is what we wanted to do — develop a tool with global applicability that you could use to assess not only turbot but also salmon, halibut, all kinds of fish. We now have a couple of papers in the works with Oliana Carnevali and Cataldo Ribecco at the Università Politecnica delle Marche, in Ancona, Italy, using the array to assess the effects of marine pollution on sea bream and Dover sole, two species indigenous to Northern Italy. We are now also working with the groups of Shuk Han Cheng at City University of Hong Kong and Dazhi Wang at Xiamen University to assess the impacts of pollution on marine life in the Pearl River Delta in China.

How do you envision the array will be used in the future?

The array will be used in multiple ways. We have so far been focused on developing and validating the tool. We haven't really used the tool as an actual biomarker itself, where you take, say, 100 fish from a given marine environment, and get an assessment of the extent of the effects of pollution. Using this array as a diagnostic tool to better facilitate analysis of this nature is the next step. There is where we are headed.

Where does transcriptome sequencing fit into this project?

Right now we use Agilent inkjet technology, which is relatively inexpensive. We have working multispecies arrays, which work quite well, but as the probes are multispecies they are never a perfect match. It also takes a lot of time to empirically optimize probe efficacy for a given species. Whereas if you do transcriptome sequencing you rapidly identify sequence tags that quickly guide probe design for a given species of fish. A key issue right now facing the microarray world is the diminishing cost of digital gene expression, particularly when you multiplex samples using Illumina and other second-generation approaches. We are getting to a point where DGE will very soon be more cost effective than using multispecies or even species-specific arrays — the bottleneck at the moment is the requirement for many samples to mitigate the high costs of sequencing via barcoding strategies. As sequencing costs decrease, transcriptome sequencing will replace arrays.

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