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USC Scientists Developing Platform to Gauge Single-Cell Gene Expression in Tissue Samples

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By Ben Butkus

Scientists from the University of Southern California have been awarded $246,000 from the National Institutes of Health for the first year of a two-year project to develop a high-throughput, single-cell PCR method for analyzing genetic heterogeneity in tissue samples.

The technique, which combines attributes of tissue imaging, digital PCR, and real-time PCR, is expected to provide researchers with a tool to study low-frequency cancer-related mutations in morphologically intact tissue samples.

And, eventually, the method may be suitable for clinical applications, particularly elucidating and testing for drug resistance in colorectal, breast, lung, and other cancers, according to the researchers.

The research team, led by USC Keck School of Medicine assistant professor of pathology Emil Kartalov, blossomed from the increasingly popular notion that there may be more value to be gained from studying gene expression in single cells than in cell populations.

This is particularly true when studying gene expression in excised cancerous tissue samples, which often comprise a sea of wild-type cells riddled with a relatively small number of cells harboring mutations associated with cancer or resistance to anti-cancer drugs.

"For example, in colorectal cancer, cetuximab [marketed as Erbitux] is a great drug, very effective, but the problem is it's not effective on all patients," Kartalov told *PCR Insider* recently. "A significant percentage of the patients have drug resistance because they have a mutation [in the KRAS gene]. One [approach] is, then, before you give an expensive drug to the patient, [to] … do PCR on [your sample] and say here is the mutation, the patient has to get something else."

Kartalov noted that this approach is leaps and bounds better than traditional immunohistochemical methods because it provides a molecular snapshot, but it's still not very accurate "because first of all you have to figure out where you cut, and if you don't analyze the entire sample, then maybe you're missing something. And if you cut it with a scalpel, it's a pretty big piece."

A number of commercial molecular biology tool vendors such as Fluidigm and NanoString have in recent months introduced research-use-only kits and instrument platforms to better support such analyses by enabling molecular assays on the single-cell level.

In addition, digital PCR platforms from the likes of Bio-Rad, Life Technologies, and RainDance technologies can be used to detect small numbers of mutated genes — even down to a single copy — among thousands or millions of wild-type genes in a cell population.

Kartalov and colleagues are attempting to combine the best attributes of all of these approaches into a single, high-throughput, PCR-based method.

As part of their two-year project, which is being funded by the NIH's National Cancer Institute, the researchers will use photolithography to design a chip containing a matrix of millions of microwells that can be placed directly onto morphologically intact tissue slices.

Then, they perform individual allele-specific endpoint PCR reactions in each of the microwells, which are small enough that they contain only a handful of cells; and subsequently use a microarray scanner to detect fluorescence from amplification products to investigate genetic heterogeneity across entire tissue samples.

"One of the potential benefits of our method is [that] it analyzes the entire sample, and it doesn't have any bias to it," said Kartalov. "Since all the cells are going to be analyzed at the same time in a highly parallel fashion, you don't have to … commit to a particular section of the sample and nothing else, so we won't miss anything."

Kartalov noted that the method has parallels to digital PCR, and in fact he has "high hopes" that certain droplet-based digital PCR systems will be able to enable cancer studies by detecting small numbers of mutations in a large wild-type background.

"But the issue is that [digital PCR] completely loses the morphological information" because a sample must first be homogenized and then dispersed among thousands of individual droplet-based PCR reaction volumes, Kartalov said.

"Our system retains the morphological information, so we know which signal came from which part of the tissue," he added. "We have a picture of the tissue sample, and a picture of what mutations are present in which part of the tissue, and you can superimpose them and produce the equivalent of a biochemical microscope."

Kartalov also said that the method has the potential to increase the effectiveness of allele-specific PCR, which is often used to interrogate SNPs.

"If you do this kind of analysis just in an Eppendorf tube, with the entire sample being crushed down and stuck in the same tube, then you're fighting very large mathematical odds [that the] PCR method is specific," he said.

"But our method compartmentalizes the entire sample into … hundreds of thousands of tiny chambers, and each chamber is a separate PCR reaction," he said. "So now, you're fighting very different mathematical odds. Now, let's say you have 10 [mutant] cells … in only 100 wild-type cells, or let's say even 500, so your odds are 50 to 1. They're not 1 million or 100,000 to one. This greatly improves the capability of the allele-specific phase to work appropriately."

Currently, the group's prototype platform features matrices in which each individual well has a diameter of about 400 microns, which, when placed upon a typical tissue slice, will encapsulate several hundred cells.

However, Kartalov noted that since submitting its NIH grant proposal, the team has demonstrated that it can shrink individual reaction volumes down to about 50 microns — small enough to capture just a few cells — and may be able to go even smaller using "standard" lithography techniques.

Kartalov said that the method would initially be ideal for research use, in order to interrogate the cellular basis of diseases like cancer. But eventually it may be applicable for clinical use, as well, to detect mutations in cancer cells that confer resistance to certain treatments, and to pinpoint exactly where in a tumor those mutations are occurring.

The team is using KRAS mutations in colorectal cancer as its disease model "due to its immediate clinical usefulness in testing for drug resistance, as well as due to our local expertise and [access] to tissue samples" at the USC Keck School of Medicine, the researchers noted in their grant abstract.

In addition, the researchers are currently using TaqMan-type PCR with endpoint analysis on microarray scanners because no instrumentation platform currently exists to analyze their PCR chip in real time.

"One of the things I predict is that once we show this capability, people will produce the systems that can do both [amplification and analysis] at the same time," Kartalov said. "It's just engineering … for instance just fiber optics on one side and a heating element on other."

Kartalov noted that the group has not yet considered a commercialization timeline for its technology as the project is very early-stage.

Ben Butkus is senior editor of GenomeWeb's premium content and the editor of *PCR Insider*. He covers technologies and trends in PCR, qPCR, nucleic acid amplification, and sample prep. E-mail him here or follow his GenomeWeb Twitter account at @PCRInsider.

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