Researchers Describe 'Digital PLA' Method to Quantify mRNA, Protein in Single Cells

Mar 28, 2016 | Madeleine Johnson

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NEW YORK (GenomeWeb) – Even clonal populations of cells can be heterogeneous relative to the central dogma of biology, with "noise" leading to variability in mRNA and protein copy numbers.

However, determining how many proteins are produced per mRNA in a given cell has long been a challenge. Now scientists at ETH Zurich, University of Bern, and the University of Chicago have developed a new method that uses digital PCR (dPCR) to quantify both mRNA and the DNA products of a proximity ligation assay (PLA) for proteins.

In a study published this month in *Molecular Cell*, the researchers showed the method has femtomolar sensitivity for protein quantification, and they used it to develop a stochastic model of gene expression in single cells.

As noted in an accompanying comment on the study, the technique joins other tools for simultaneous single-cell quantification of protein and mRNA, but is unique in its ability to provide absolute counts.

The big-picture purpose to develop the method "is really to understand, at the fundamental level, how cell biology works, and ultimately to be able to model it," said Savaş Tay, corresponding author on the study and a researcher at ETH Zurich who will be joining University of Chicago this summer.

The intrinsic noise in mRNA-protein correspondence between cells comes from cell size, differing time points in the cell cycle, and differing history of signals from a noisy environment, as well as regulation events, Tay said. "Their histories and the context that they went through determine what they will express at a given time point," he said. And, at the most fundamental level, there is also a stochastic nature to mRNA and protein existence, with thermal noise, diffusion, and other unavoidable variability.

To count proteins, the PLA method starts with two antibodies recognizing different epitopes. These have complementary DNA oligos attached, such that when they come in close proximity they hybridize and can be quantified. Traditionally, this is done with qPCR, with one protein yielding a lot

of amplified DNA signal.

"What we did was come up with an idea of quantifying PLA with digital PCR, instead of real-time PCR," Tay said. Developing the protocols then took about five years, he added.

The advantage of digital PCR is that a PLA signal is theoretically derived from one protein molecule. Hence the name "digital PLA," because "essentially we are counting single protein molecules, one by one."

And the benefits of digital compared to analog are numerous. With regular PCR, the baseline is unknown so quantitation is relative. Digital also gives "extremely high sensitivity," Tay said, as opposed to fold-change sensitivity in traditional PCR.

However, PLA relies on antibodies, so it is not perfectly sensitive. "We are not detecting every protein," Tay said, but perhaps every 50th protein, so the method does not have absolute single-molecule resolution. "But it is still better than fold-changes," Tay said, and it does not require a protein standard spike-in for quantitation.

Measuring mRNA using the method simply uses regular digital PCR. "Because of the extreme sensitivity of digital PLA, we were able to take single cells, lyse them, split their contents into two parts, and use one half for mRNA and the other half for protein quantitation," Tay explained.

In the *Molecular Cell* study, Tay and his colleagues looked at CD147, ICAM-1, and GFP. They used the data to infer a stochastic, two-state model for CD147 expression in single cells.

The next step will be to analyze many more proteins simultaneously, Tay said, with a goal of 100 proteins and 100 mRNAs in a given sample. The standard now for protein quantitation is CyTOF — a mass spec technique that uses heavy metal ions — which is powerful, but neither inexpensive nor robust, and it has a maximum of around 50 proteins, Tay said. A team at Stanford published a method earlier this year using CyTOF for proteins and PLA for RNA quantitation, rather than PLA for protein and PCR for RNA as in Tay's method. That technique allowed measurement in the range of 40 to 50 RNAs and proteins.

The correlation between mRNA transcript for a given gene and protein hasn't been measured in mammalian cells with good sensitivity before, Tay said. "Now that we can measure mRNA and proteins that the mRNA encodes for a given sample, this is really a powerful technique and a unique window to look into gene expression."

Using the method, the group found the correlation of abundances of mRNA and protein is "really poor," he said. "So, one cell might have really high mRNA but low protein, another cell might have high and high, another might have low mRNA but high protein, and this changes over time as well." Variation within cells for repeated dPCR or digital PLA results was small, meanwhile.

The group used Droplet Digital PCR from Bio-Rad in the study. It also found the method works using a digital PCR system from Fluidigm, but the Bio-Rad system provided the optimum cost per dynamic range, Tay said. The group usually develops its own microfluidic systems, but relied on a commercial platform in order to promote adoption of the technique by others.

The group now plans to patent parts of the method, and Tay envisions future clinical lab diagnostics applications that will take advantage of the femtomolar sensitivity, compared to the nanomolar

sensitivity of ELISA, for example. A digital ELISA technique from Quanterix called Simoa is already carving out a space in the market, Tay noted, suggesting there is already an interest in digital quantification of proteins in solutions or blood.

"Imagine that you can go and detect PSA in blood or serum let's say six months earlier than what the standard techniques can do now — that would give you a huge advantage in terms of treating prostate cancer," Tay said.

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