UCSD to Use Single-Cell Sequencing to Create 3D Transcriptional Map of Brain

November 15, 2012

UCSD to Use Single-Cell Sequencing to Create 3D Transcriptional Map of Brain

By Monica Heger

Researchers at the University of California, San Diego, plan to use single-cell transcriptome sequencing techniques to map gene activity in the human brain.

The team recently won a five-year $9.3 million grant from the National Institutes of Health for the project, which involves sequencing 10,000 single brain-cell transcriptomes and generating a 3D map of gene activity in the human brain.

The project is being led by Kun Zhang, an associate professor at UCSD's Department of Bioengineering and Institute for Genomic Medicine. Other collaborators include Wei Wang, within UCSD's chemistry and biochemistry division; Jerold Chun, a professor of molecular biology at the Scripps Research Institute; Jian-Bing Fan and Mostafa Ronaghi with Illumina; and George Church's lab at Harvard University.

The project has two main components, Zhang told *In Sequence*. The first part involves using microdissection or flow sorting techniques to grab individual cells from brain tissue and perform RNA-seq on each of the cells. For this, the team plans to do 10,000 single-cell transcriptomes by the end of the five years.

The second part of the project involves developing an *in situ* RNA-seq protocol to act as a "fingerprint" for transcriptional location, so the transcriptomes of the individual cells can be placed within the context of the brain. For this part, the plan is to assemble at least 500 transcripts.

"Generating a large dataset from single cells extracted from a tissue section without having a good record of where the individual cells come from is not enough," Zhang said. The *in situ* sequencing technique will "allow us to directly visualize, count, and identify the individual transcripts while maintaining all the spatial information, so we know how many transcripts there are in a certain location, how the cells interact, and in what position" of the brain they reside.

The datasets from the single cell RNA-seq and the *in situ* RNA-seq steps can then be

combined, Zhang said, "so we can align [the single cell transcriptome data] to the brain anatomy."

"For any whole transcriptome dataset we're getting from an isolated cell, we can ask, 'What happened to these 500 genes and can we use those 500 genes to find a matched pattern, so that we can align the dataset to a specific location in the brain?'"

In the project's first year, the team plans to sequence the transcriptomes of 500 cells and publish its method. It then plans to scale up in the second year, incorporating as much automation as possible.

The actual sequencing itself will be done at Illumina, Zhang said, so it will be done on whatever platform the company determines is appropriate — currently the HiSeq 2000 platform.

For the single-cell transcriptome sequencing, the team will build on a protocol published recently in *Genome Research* that makes use of multiplexing. However, said Zhang, that method "only allows you to sequence polyA mRNA, and we're trying to sequence also all the non-coding RNAs that might not have a polyA tail."

Nevertheless, the new method will incorporate many of its ideas, such as doing multiplexing early in the process. "The earlier you can label your samples with barcodes, the sooner you can combine them and then process 96 samples in one shot, so cut down on the downstream processing and labor," Zhang said.

Zhang said the team is also working on improving accuracy and coverage — challenges for single-cell sequencing techniques due to amplification biases that are compounded by starting with DNA from a single cell.

Zhang said that they are aiming to do 10,000 cells because the human brain is so diverse. Not only are there many different cell types, but even within the same type of cell, there is a huge amount of variability. "To do a comprehensive characterization, we need to sample a large enough amount … We decided to do 10,000, because only when we get to that scale might we be able to get to an accurate picture of what's going on in the human adult brain."

For the *in situ* sequencing method, the team is collaborating with George Church's group at Harvard, which is working on a method in which sequencing can be done within brain tissue itself.

First, a chemical reaction will create pores on the cells, enabling a customized microfluidic device to deposit sequencing reagents into the cell. Rather than sequencing on a flow cell, the sequencing will take place within the tissue, Zhang said, and the signal will be read out with a microscope.

He said that Church's group has already been able to get the protocol to work on cultured cells on a Petri dish. The important part is keeping the molecules immobilized. The remaining aspect is developing an imaging system, Zhang said.

Zhang anticipated that the group would spend the first two to three years developing and optimizing this protocol. The basic technique would be imported from Church's lab, he said, but modified for UCSD's specific samples.

Zhang said that by the end of five years, the project should "establish a baseline diversity of the normal human brain," which would then enable researchers to study differences in gene activity

between normal brains and the brains of patients with Alzheimer's, Parkinson's, autism, or other disease populations.

Additionally, he said, the same mapping technique could be applied to other types of cells, such as stem cells and cancer cells. "There are many other contexts that we can apply this strategy," Zhang said.

Monica Heger tracks trends in next-generation sequencing for research and clinical applications for GenomeWeb's *In Sequence* and *Clinical Sequencing News*. E-mail her here or follow her GenomeWeb Twitter accounts at @InSequence and @ClinSeqNews.

Related Stories

- New Single-Cell Transcriptome Method Allows Clinical Researchers to Zoom into Rare Cells
  July 25, 2012 / Clinical Sequencing News
- Single-Cell Sequencing Can Isolate Individual Bacterial Species from Metagenomic Samples
  March 13, 2012 / In Sequence
- Illumina Plans to Submit MiSeqDx Assays for Cystic Fibrosis to FDA this Year
  November 1, 2012 / Clinical Sequencing News
- Paired Ends: Rebecca Chambers, Ross Fitzgerald
  October 29, 2012 / In Sequence
- MiSeq Customers, WGS Service, and T21 Test Developers Drive Illumina's Clinical Business
  October 24, 2012 / Clinical Sequencing News

footer