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Translating Genome Sequencing to the Clinic

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New Single-Cell Transcriptome Method Allows Clinical Researchers to Zoom into Rare Cells

by *Julia Karow*

RESEARCHERS AT THE KAROLINSKA INSTITUTE and the Ludwig Institute for Cancer Research in Sweden, in collaboration with Illumina, have evaluated a new method for studying the transcriptome of a few or single cells, an approach they say will have important applications in clinical research.

In a proof-of-principle study published online in *Nature Biotechnology* this week, the team evaluated the performance of their mRNA-seq protocol, called Smart-Seq, and used it to study single circulating tumor cells from melanoma.

"It's an accessible method for single-cell transcriptome studies that can easily be used by other researchers to address any biological or clinical question that they are interested in," said Rickard Sandberg, an assistant professor in the cell and molecular biology department of the Karolinska Institute and the senior author of the study.

Smart-Seq, originally invented by a team at Illumina and commercialized as a kit by Clontech last year, converts polyadenylated RNA into cDNA, which is amplified and turned into sequencing libraries for Illumina sequencing.

It builds on the Switching Mechanism at 5' End of RNA Template, or SMART, template switching technology for generating cDNA, developed and patented by Clontech. While the technology, which enriches for full-length cDNAs, has been around for some time, it had not been used before to profile the transcriptomes of few or single cells, Sandberg said.

The main advantage of the template switching method is that it offers better coverage across the entire length of a transcript than other approaches, for example a method developed by researchers at the University of Cambridge in collaboration with Life Technologies. Good coverage is important for studying alternative splicing as well as for SNP genotyping, Sandberg said.

Last year, Clontech came come out with a commercial cDNA synthesis kit that is based on the SMART technology, called the SMARTer Ultra Low RNA Kit for Illumina Sequencing, which requires as little as 100 picograms of total input RNA. According to the company, the kit enables researchers to work with very small amounts of starting material, such as microdissected tissues, laser-captured cells, or biopsy samples.

Because of the availability of the kit, the protocol is "very easy to use," Sandberg said. In addition, it could be "easily adapted" to sequencers other than the Illumina platform because the early steps, up to the amplified cDNA, do not involve any platform-specific primers or adaptors.

To demonstrate the potential utility of Smart-Seq in clinical research, Sandberg and his team used it to analyze the transcriptome of single circulating tumor cells captured from the blood of a melanoma patient.

They isolated six potential circulating tumor cells based on the expression of a cell surface marker and generated transcriptome data for each of them, which they compared to the transcriptomes of single cells from a primary melanoma, two melanoma cell lines, human embryonic stem cells, and several types of blood cells.

Judged by their transcriptome, the circulating tumor cells

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were most closely related to the melanoma cells, and they expressed high levels of melanoma-associated genes.

"I think single-cell transcriptome analysis will play a very important role in the analysis of circulating tumor cells," Sandberg said. "Since we still know relatively little about them, it's important to use a global gene expression method to first verify their identity, to make sure you are actually investigating relevant circulating tumor cells, and to identify biomarkers." Later on, in more routine clinical applications, those biomarkers could then be assayed by methods other than sequencing.

Sandberg and colleagues are planning to use or are already using Smart-Seq in a number of projects, some of them with a clinical background. For example, they would like to study individual cells from primary tumors and metastases as well as circulating tumor cells from approximately 10 melanoma patients, a study that is still in the planning stage.

The method is also well suited to better understand tumor heterogeneity. "We still need to learn about tumor heterogeneity, what types of cells are in [tumors], and how the composition of cells affects the clinical outcome," Sandberg said.

While it's not yet well understood, cell heterogeneity may also play a role in diseases other than cancer, for example in atherosclerosis plaques or in the degeneration of neurons.

"Considering how little we know about what constitutes a real cell type, and how cell type identities change in pathologies in general, an unbiased quantitative global gene expression method in general is very useful," Sandberg said.

His team is currently using Smart-Seq to study cell differentiation in early embryonic mouse development by sequencing the transcriptomes of individual cells from preimplantation embryos of different stages.

Sandberg said he hopes core facilities or genome centers can offer the method to researchers as a service, which he said should be possible because it is very robust and easy to use.

The Genomics Core Facility at the European Molecular Biology Laboratory in Heidelberg is one place that has already adopted Smart-Seq and is offering it to its users for low-input transcriptome sequencing. Vladimir Benes, who heads the facility, told *Clinical Sequencing News* that he gets more and more requests for transcriptome sequencing from between 10 and 50 cells, and this protocol "seems to work really well." His lab uses the Clontech kit but employs a different enzyme, from Kapa Biosystems, to amplify the cDNA.

Benes said the method will allow researchers to study smaller samples, for example a few cells isolated from a tumor, which yields a better signal than larger heterogeneous samples that contain both tumor and normal cells. "This gives you the opportunity to zoom in and get a very clean signal," he said.