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A new method for analyzing gene expression in single cells opens a window into tumors and other tissues

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A team of researchers affiliated with Ludwig Cancer Research and the Karolinska Institutet in Sweden report in the current issue of *Nature Methods* a dramatically improved technique for analyzing the genes expressed within a single cell—a capability of relevance to everything from basic research to future cancer diagnostics.

"There are cells in tumors and in healthy tissues that are not present in sufficient numbers to permit analysis using anything but single-cell methods," explains senior author, Rickard Sandberg, PhD. "This method allows us to identify rare and important subpopulations of cells in all sorts of tissues. We can also use it to tease apart, more rigorously than ever before, how the expression of unique suites of genes transform cells from one state to another as, say, an embryo develops into an organism, or a tumor becomes metastatic."

Traditional approaches, which depend on the collective analysis of gene expression in millions of cells at once, tend to obscure biologically significant differences in the genes expressed by specialized cells within a particular kind of tissue. Single-cell analysis of gene expression overcomes this limitation. The leading method for such analysis—Smart-seq—was developed in 2012 by the biotechnology firm Illumina, together with Sandberg's laboratory.

To develop the new technique, named Smart-seq2, Sandberg's team

conducted more than 450 experiments to improve upon their initial method. The new procedure consistently captures three to four times as many RNA molecules, which often translates into 2,000 more genes per cell than current methods allow. It also captures far more full-length gene sequences, a steep challenge in such studies, which often capture only partial sequences of expressed genes. This will permit researchers to conduct a more granular analysis of how subtle differences between the same genes in different people—known as single nucleotide polymorphisms (or SNPs)—contribute to differences in biology and disease.

The new method is likely to be of great value to cancer research. Identifying rare sub-populations of cells in tumors and understanding their role in the survival and progression of cancers can provide invaluable information for the development of diagnostics and targeted therapies. A study recently published by Ludwig researchers described, for example, how certain subpopulations of cells in melanomas can be pushed into a drug-susceptible state and then destroyed by chemotherapy. More such strategies might be devised as researchers get a better handle on the cellular species found in different types of tumors, and the patterns of gene expression that define them.

Because Smart-seq2 relies on off-the-shelf reagents, it costs roughly a twentieth as much as the commercialized kit, which should allow researchers to conduct sophisticated analyses of single cells on a much larger scale. It can also be improved further by the scientific community, since its constituent components and rationale are both open to the public.

Armed with the more effective and affordable Smart-seq2, Sandberg's lab is now moving ahead on projects that require a large-scale, single-cell gene expression analysis. "Now all researchers can do their own single-cell gene expression analysis by buying the components of the process

described in this paper and assembling their own kits," says Sandberg.

More information: Smart-seq2 for sensitive full-length transcriptome profiling in single cells, DOI: 10.1038/nmeth.2639