IN FOCUS

Anticipating a next-generation census of stem-cell small RNA

Joseph B. Franklin

Yale University School of Medicine, New Haven, Connecticut

A new generation of technology promises a future of fast and cheap DNA and RNA sequencing by simultaneously analyzing millions of nucleic acid molecules. Graced with names such as “ultra high-throughput” or “next-generation” sequencing, this technological revolution actually consists of several competing technologies that allow large surveys of nucleic-acid samples — both genomic DNA and RNA transcripts. Fast sequencing of the RNA population in a cell has quickly gained traction as an efficient method for measuring gene expression levels within experimental organisms as well as human tissues [1]. With some improvements, next-generation sequencing should allow a high-resolution view of the RNA within single, differentiating stem cells.

Microarrays, which have been the standard for transcriptome analysis for several years, provide neither the flexibility nor the sequence-level data of the new technology [1]. Because microarray hybridization experiments can only query known reference sequence (such as published genomic sections), they ignore unidentified sequences from unassembled areas of the genome, or hybrid sequences that result from splicing. In contrast, a sequence-based RNA census (RNA-Seq) is, ideally, “hypothesis-free” and can identify and measure novel transcripts without such prejudice.

Like protein-coding mRNA, samples of small RNA, including microRNAs (miRNA), may be sequenced by the new technology after being purified and reverse-transcribed into complementary DNA (cDNA). Such small RNAs are interesting because they have important biological functions; indeed, mice lacking critical components of the miRNA pathway, such as Dicer or DGCR8, are not viable beyond the embryonic stage [2]. In vitro studies demonstrate that stem cells lacking Dicer also lack key proteins responsible for regulating differentiation [3,4]. A review of the piRNA pathway, an important regulator of stem cell differentiation that involves 26 to 32 nucleotide-long RNA molecules, appears in this issue [5].

However, while the importance of the miRNA and piRNA pathways in stem cells
is already established, a complete understanding of their regulatory function will require an exhaustive census of the diversity of small RNAs at work in these cells. Microarrays have provided measurements of previously identified small RNAs, including miRNA [6]. Now, next-generation sequencing promises to discover and measure the entire complement of small RNAs in stem cells, including those that have not been identified.

**ANALYZING SMALL RNAS DURING STEM-CELL DIFFERENTIATION**

Whether using microarrays or new sequencing technologies, taking a useful census of small RNAs during stem cell differentiation has been limited by the relatively large numbers of cells required to perform these analyses. To resolve differences in the small RNA contents of two asymmetrically derived daughter cells would require separate, extremely sensitive measurements of the RNA in each individual cell.

A team of researchers at Cambridge University and Applied Biosystems, which markets the SOLiD sequencing platform, recently published the first mRNA-Seq analysis of an individual cell (in this case, a mouse blastomere) [7]. The key to analyzing a single cell was obtaining sufficient blastomere cDNA, which, although a quite a feat, was aided by the large size of these early embryonic cells.

With the small sample provided by a single cell, the crucial limitation to overcome is one of sensitivity; low-abundance transcripts must be accurately represented, in spite of the small amount of starting material. Such accuracy would be crucial for a meaningful census of diverse small-RNA classes. The blastomere study compared the number of genes represented in the RNA-Seq analysis with those from the microarray, and the results suggested that RNA-Seq may be the more sensitive method, though it missed some genes detectable by array [7].

Though improvements in the yield and reproducibility of sample preparation should allow RNA-Seq to move to other single-cell applications, including small-RNA profiling, it may be the “next” next-generation technologies that provide the required sensitivity. Improvements to existing platforms are ongoing, promising increased sensitivity and moving toward single-molecule sequencing [8]. If such claims are realized, the accurate measurement of RNA in a single cell, including a stem cell, could become routine.

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