Lineage Segregation in Embryonic Stem Cells: A Novel Transcriptomics Method for Identifying the Parts Lists

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Abstract

ABSTRACT

Quantification of low abundant transcripts from limited amounts of starting material has remained a challenge for RNA-seq at current sequencing depths. Here, we describe a Designed Primer-based RNA-sequencing strategy (DP-seq) that uses a defined set of heptamer primers to amplify the majority of transcripts while preserving their relative abundance. Our strategy reproducibly yields the high levels of amplification necessary for sequencing-library generation and offers a dynamic range of over five orders of magnitude in RNA concentrations. Sequencing libraries made from the serial dilutions of mRNA reveal that DP-seq is highly reproducible with at least 50pg of mRNA. We further observed expression of more than 6000 mouse transcripts from libraries made from 1pg of mRNA, which is equivalent to mRNA from 1-10 cells. We applied this approach to study cell lineage segregation in embryonic stem cell cultures, which models early mammalian embryogenesis. The amplification strategy revealed novel sets of low abundance transcripts, some corresponding to the identity of cellular progeny before they arise, reflecting the specification of cell fate prior to actual germ layer segregation.