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Highly parallel and sensitive method for analyzing gene expression kinetics

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Synthetic biology is a bottom-up approach to a deeper understanding of biological phenomena by building synthetic gene circuits and studying their kinetics in cells. Analysis of kinetics of intricate synthetic gene circuits with multiple components plays a crucial role in elucidation of biomolecular networks and in their bioengineering applications. Since synthetic gene circuits are integrations of chemical reactions, it is eagerly expected to develop a method for monitoring temporal changes of gene expression not in terms of relative amounts of transcription products but in terms of their absolute amounts. Moreover, in the case of analyzing synthetic gene circuits introduced into living cells, a large number of originally-existing internal genes in the cells may interfere with external genes introduced from the synthetic circuits. Therefore, a highly multiplex method that can measure both the internal and external genes is required for investigating behaviors of such synthetic circuits.

Here, we describe a highly parallel and sensitive method for analyzing gene expression kinetics by measuring the absolute amounts of their transcripts without reverse transcription processes[1]. The method determines the absolute mRNA amounts by quantifying well-designed DNA tag sequences called DNA-Coded Numbers (DCNs) [2] generated by photo-chemical ligation of pairs of two chemically synthesized DNA oligonucleotides that specifically bind to the target mRNA. The converted DCNs are then amplified by PCR and finally detected on microarrays. The conversion to DCNs also provides advantages such as the use of target-independent microarrays and no need for sample labeling. The method was validated by using chemically synthesized RNA samples of known quantities and total RNA samples prepared from mouse liver and retina. The absolute amounts of 43-144 mRNA species were reproducibly quantified in parallel with high sensitivity (24-60 zmol) from a small amount of total RNA samples (20 ng) in 7 hours.

[1] Yokomori, M., Gotoh, O., Fujimoto, K., Suyama, A. submitted.

[2] Gotoh, O., Murakami, Y., Suyama, A. Multiplex cDNA quantification method that facilitates the standardization of gene expression data. *Nucleic Acids Res.* **39**, e70 (2011)