

Role of Tilted Angle and Electrical Field Magnitude in an Electrokinetic Cell of Cylindrical Geometry

Systems biology research requires methods that are both quantitative and high-throughput to characterize cellular networks. To achieve this goal, methods such as quantitative PCR (qPCR) hold great promise, as they are able to accurately quantify a wide range of mRNA concentrations. However, qPCR's throughput, cost, and relative quantification need further improvement to meet the demands of modern systems biology. Designing a successful qPCR assay typically requires careful primer and probe design as well as significant optimization. Multiplexing in qPCR, while increasing throughput and content of qPCR assays, increases effort required in design and optimization while also increasing cost since multiplexing typically relies on costly specific detection chemistries such as TaqMan probes or Molecular Beacons. Quantification of gene expression in qPCR experiments is usually either relative to a reference gene or based on a standard curve, making quantification either semi-quantitative or time-intensive and laborious.

To increase the throughput and content of qPCR assays while decreasing their cost, we have developed a novel assay utilizing differences in melting temperature of PCR products to multiplex with the nonspecific detection chemistry, SYBR Green. In order to automate quantification of gene expression levels in qPCR, we have developed a differential equation model of qPCR, enabling us to: 1) extract sufficient information to quantify the concentration of an mRNA species from a single qPCR assay by fitting our model to the data, and 2) quantify the concentration of multiple mRNA species simultaneously (i.e., multiplex) using a nonspecific qPCR assay by fitting a model for multiple species to the data. The result of our efforts is a cheaper, higher content, more quantitative, and faster multiplexed qPCR assay, more suited to the demands of modern systems biology research.