Abstract

Recent breakthroughs in the RNA quantification of single cells are rapidly transforming the view on biology and medicine. Flexibility and sensitivity of reverse transcription quantitative PCR (RT-qPCR) make it an ideal method for quantification of single-cell material, but its limits had not been yet fully explored.

In this thesis, various factors influencing RT-qPCR performance in single-cell application have been assessed, including conditions of sample collection and processing, importance of quality control, performance of reverse transcription, preamplification and role of qPCR assays. We showed that prolonged time for single cell collection as well as repeated freeze-thaw cycles had negligible effect on RT-qPCR data quality. Direct lysis routinely applied for RNA extraction from single cells may be scaled up to 256 cells. The comprehensive comparison of 11 reverse transcriptases in low RNA input conditions identified 2 best-performing enzymes. Decrease in preamplification volume as well as poor primer design resulted in the loss of sensitivity. Finally, the established workflow has been applied to profile gene expression of astrocytes in mouse model of amyotrophic lateral sclerosis (ALS) identifying important components of ALS-induced changes to astrocyte transcriptome.

Altogether, the thesis represents a complete set of recommendations for performing single-cell RT-qPCR experiments based on the provided literature, experimental results and practical notes. It can serve as a guide to secure high experimental performance, analysis power and savings on experimental costs.

Keywords: single cell, reverse transcription, preamplification, quantitative PCR, amyotrophic lateral sclerosis, astrocytes