Single cell gene expression profiling and quality control

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Abstract:

Gene expression profiling has become an exceedingly important tool for describing occurence of mRNA in tissue samples and even single cells. Most often we use it for characterization of cell types, degree of differentiation and pathology on a molecular level. In our newly established laboratory, we developed high resolution qPCR tomography to show distribution of tens of maternal mRNAs within a single oocyte. We demonstrated that distribution of mRNAs has an important role in further development of the organism. For high resolution qPCR tomography, where one oocyte is divided in tens of samples and about fifty genes are studied in each sample, we optimized dye based protocol for microfluidic high-throughput platform BioMark. Next step was complementing the molecular profile of tens most important genes with information about histology of each selected tissue section using laser microdissection. As a model we used embryonic development of mouse molar. Our goal was to describe interaction of up to one hundred genes in different stages of development and on the single cell level. This work also reviews development of molecular tools for testing samples for contamination, genomic background and RNA quality. Use of such tools enhances development of new analytical approaches and shows to be crucial quality control for challenging studies of gene expression in time and space not only on the single cell level. Such studies are expected to accelerate understanding of cell regulation and to find new molecular targets for therapeutic use.

Keywords: real time PCR, single-cell biology, single-cell gene expression, gene expression profiling, map of gene expression, qPCR tomography, high-throughput qPCR, quality control, RNA spike, DNA spike, genomic background, direct cell lysis