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Single-cell QUantitative In Situ RT-PCR (SQUIRT-PCR)

Quantitative real-time PCR (qRT-PCR) is a well-established method for amplifying DNA using fluorescent probes. Traditionally this is a step-wise process where an aliquot of the DNA to be amplified is deposited in a chamber and amplified/measured in the chamber. Current systems require significant time for sample preparation including tissue disaggregation, cell lysis and mRNA purification, sometimes on the order of days. Moreover because either disaggregated cells or cells lines are used, these systems are not in-situ techniques and do not serve to address complex biological problems.

Researchers at the Biodesign Institute of Arizona State University have developed a novel system and method to continuously sample, process and collect data from single cells. More specifically this system and method can analyze gene expression heterogeneity in-situ using single-cell mRNA expression analysis. Series of individual cells are serially lysed and run through a continuously flowing qRT-PCR system without the need to disaggregate the tissues beforehand.

This technology presents the first system capable of profiling gene expression without the requirement of prior disaggregation of live tissue. Moreover, because it is in-situ, it has the potential to address complex biological problems better than current systems.

Potential Applications

- qRT-PCR on single cells
  - Biomedical research and clinical applications
  - Assessing tumor cell population heterogeneity in single cell gene expressions
  - Perturbagens can be introduced to discover the effects of multiple doses on patient organ cells
  - May provide detailed transcriptional responses in cells to better understand how cell-cell communication takes place in-situ

Benefits and Advantages

- Indefinite sampling on the single cell level - does not require tissue disaggregation
- The final output of the system can be collected and output in real time allowing for on the fly modifications to parameters
- Information of the spatial location of each cell in the tissue is tracked for better understanding of cellular heterogeneity in tissues
- Cells go directly from live tissue to qRT-PCR in minimal time enabling a more accurate expression profile

- The total volume flux needed for sample processing is microliter scale
- The time interval between sample processing takes mere seconds, with complete completion of qRT-PCR within one hour
- Users can select specific cells for analysis and analyze at particular time points

For more information about the inventor(s) and their research, please see Dr. Meldrum's directory webpageDr. Meldrum's Biodesign directory webpage