Development of a Platform for Massively Parallel Single-Cell Genomic and Expression Analysis

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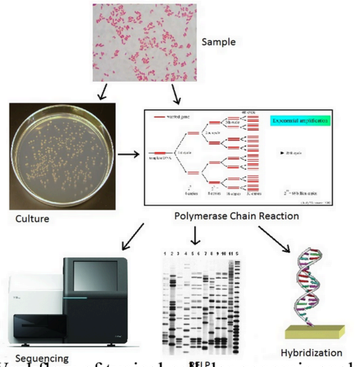
Background

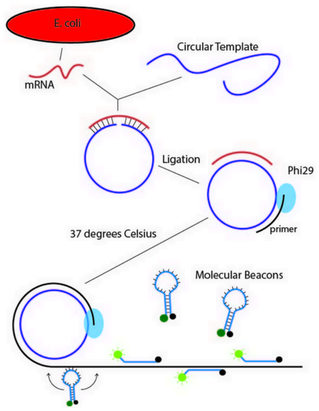
Analyzing cells for genomic and expression information is paramount to modern biological research and diagnostics. Typically, cellular analysis is limited to two options: gaining data from a limited number of cells, or averaging data from large numbers of cells. Techniques such as FACS which do offer high-throughput single cell analysis are not designed for DNA sequence analysis.



Workflow of typical cellular genomic analysis

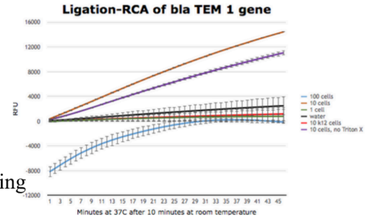
Research Goal: To develop and validate a microfluidic device capable of analyzing thousands of cells at a time for genomic and expression information. The device will be able to load, capture, and individually seal thousands of cells, for subsequent DNA amplification reactions that can identify specific sequences, and quantify the amount of nucleic acids present.

Rolling Circle Amplification

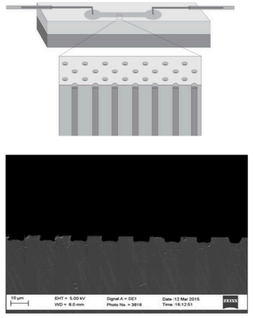


To the left is a diagram outlining the workflow of the ligation-RCA-detection reaction used to detect and quantify the presence of specific mRNAs. The mRNA acts as a template for the ligation of a padlock probe, which can then serve as the template for a RCA reaction. The product of this RCA is detected by molecular beacons.

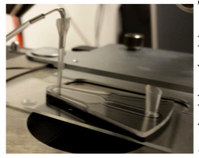
To the right is the ligation-RCA bulk control. which demonstrates the reaction work specifically and responds to increasing mRNA.



Microfluidic Devices



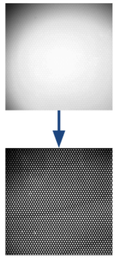
The device represented to the left is a fiber-optic array plate. The cores of the fiber-optics are etched to produce an array of microwells, the lower image is an SEM of a cross section of the wells.
1.2 pL well volume



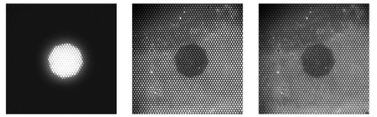
This device is a microfluidic channel with a microwell array, made of plastic rather than glass.
3.2 pL well volume

Cells can be captured in the wells such that there is no more than one cell per well, using a Poisson distribution. The wells then can be individually sealed by oil sealing, for the amplification reactions.

Oil Sealing



Aqueous Solution was sealed into the wells by flowing oil through the Devices. To the left, the trapping of fluorescent solution can be seen as the oil was pushed through the device.

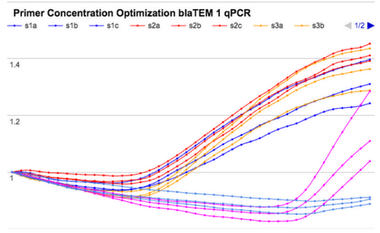


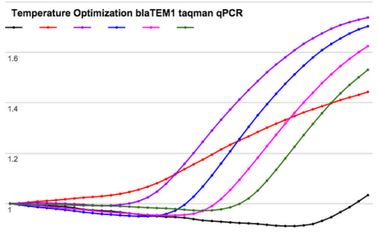
To the right, images of a specific area being photobleached can be seen, as well as images taken immediately, and 90 minutes after the photobleaching (left to right). This demonstrates that the oil is effectively sealing the wells from each other, as the difference in fluorescence between the bleached and unbleached wells remains for over a hour. If diffusion between the wells was possible, the darker area would regain the lost fluorescence.

Quantitative Polymerase Chain Reaction

A quantitative PCR reaction was designed using taqman probes to specifically detect DNA sequences. The Taqman probe is a section of DNA, complementary to the target DNA of the PCR, that contains a fluorescent dye and quencher, and will be degraded by the endonuclease activity of the polymerase. The released fluorophores allow the progression of the PCR to be visualized.

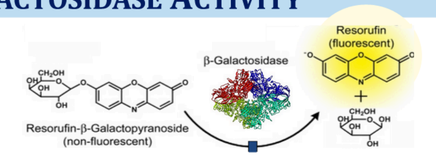
To the right are bulk qPCR reactions that are optimizing the reaction for primer concentration and temperature.

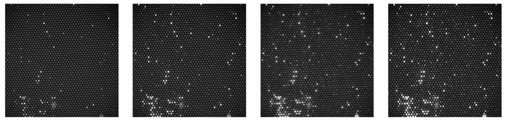




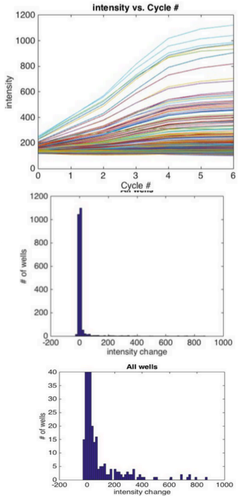
Beta-Galactosidase Activity

A final direction this project took was quantifying the activity of proteins in single cells. Here an assay was designed (right) that can quantify the activity of Beta-Galactosidase, an enzyme involved in bacterial metabolism





images taken every 6 minutes



Single cells were captured in the wells, along with substrate for the B-Gal that fluoresces upon enzyme activity, and the fluorescence from the wells was measured over time, as can be seen in the images above. Wells that appear bright contain bacteria with thousands to tens of thousands of enzymes. A Matlab program developed by the lab was used to analyze these images. The fluorescence over time is given by the trace to the upper left.
Histograms showing the number of cells that fall within each intensity change are also shown to the left. Here we can see a vast majority of the wells contain no cells and thus have no activity (as expected from the Poisson distribution. However there is a population of wells that do contain cells and have a variety of activities. This variety represents the heterogeneity within the cell populations.

References

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