

# Development of methods for detection and quantification of mRNA from single cells

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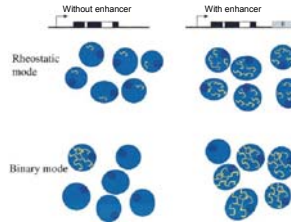
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## Introduction

Cells are in many aspects unique in their characteristics, even in a seemingly homogenous culture or tissue. Traditionally, gene expression analysis look at the average transcript levels of the studied cells. However, it is often interesting to distinguish a wide-spread response in the whole population from rare changes in expression taking place in a few cells. The natural variation in expression in individual cells is essentially an undiscovered area of research due to technical difficulties.

The most widespread theory of how to explain the nature of gene expression regulation in a population is known as the rheostatic model. When transcription is activated by an enhancer, the amount of transcript in individual cells is identical and proportional to the stimuli. A contrasting view is the binary model, in which enhancers act in a stochastic fashion to increase the probability that a regulated gene will be transcribed.

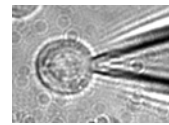
We have developed methodology to improve the fidelity and robustness of single cell gene expression analysis using reverse transcription real-time PCR. Special attention has been paid to adsorption phenomena, dilution effects, and inhibition of PCR by RT reaction component. Several genes were studied and their expressions were compared on the level of the individual cells.



Above: Rheostatic vs. Binary model for gene expression in a population. Yellow curves represent mRNA transcripts. The binary transcription model results in heterogeneous transcript levels, but the total mRNA amount are the same in the two populations. Stimulated (right) and unstimulated (left) cells.

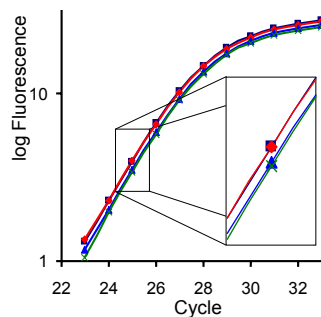
## Material and methods

MIN6-cells or primary cells of the Islets of Langerhans from mice were prepared according to standard protocols. Cells were collected using glass capillaries with a diameter of approximately 2-4  $\mu\text{m}$ . Pipettes were emptied in lysis buffer, immediately followed by freezing on dry ice awaiting RT-PCR. Reverse transcription was carried out using oligo(dT) and random hexamer priming. Real-time PCR was performed on Roche's LightCycler with SYBR Green I detection.



Left: Glass pipette in contact with cell surface. By applying negative pressure to the inside of the pipette, the cytoplasm of the cell is harvested.

## Low noise from RT-PCR



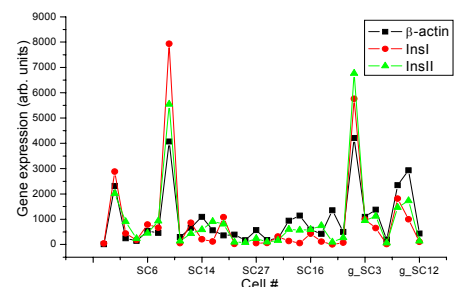
Above: Two MIN6 single cell samples run in duplicate reverse transcription reactions, followed by InsII PCR.

## Results

Primary cells from the Islet of Langerhans in mice and the pancreatic  $\beta$ -cell tumour cell line MIN6 were studied. The expression levels of  $\beta$ -actin, insulinI, insulinII, SUR1 and Kir6.2 were measured in about 50 cells. 70-95% of the samples resulted in a specific amplification signal. Relative expression levels were calculated assuming constant PCR efficiency. Some of the measured distributions are shown in the figures below.

The reproducibility of the method was tested by dividing single cell samples into aliquots after lysis and run reverse transcription and PCR in parallel (left figure). The low variability indicate sufficient lysis and mRNA accessibility.

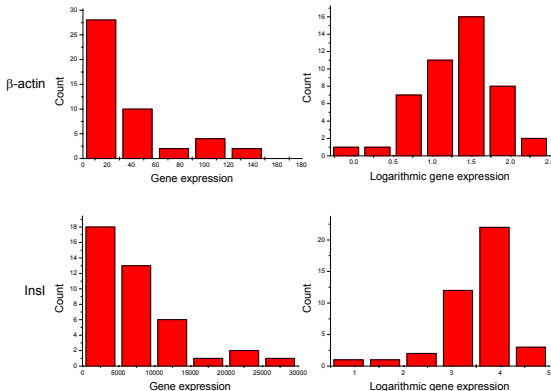
## Are some cells boosted?



Above: Gene expression (arbitrary units) of  $\beta$ -actin, insulinI and insulinII in 30 individual MIN6-cells.

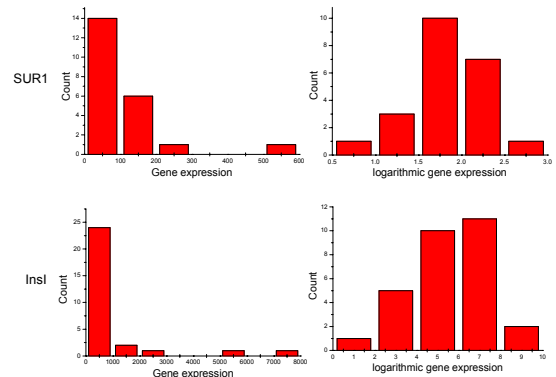
## Gene expression levels appear lognormally distributed

### Primary cells



Distributions of gene expression levels of primary cell population (left) and MIN6 cell line (right) in linear and logarithmic scale. 46 and 41 primary cells were used for  $\beta$ -actin and InsI respectively, 22 and 29 MIN6-cells for SUR1 and InsI respectively. Histograms of  $\beta$ -actin and InsI expression are shown for primary cells and InsI and SUR1 for MIN6 cells.

### MIN6-cells



## Conclusions

- Single cell mRNA harvest followed by quantitative RT-PCR is in our hands a robust method to measure the mRNA content of single cells.
- The single cell RT and PCR reactions show very little variability.
- The variation is mainly due to biological variation, not technical.
- Cell populations are heterogeneous at the mRNA level.
- The distribution of gene expression levels in a cell population are lognormally distributed.

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Figure reference: Fiering S, Whitelaw E, Martin D I K. BioEssays 22:381-387, 2000.