



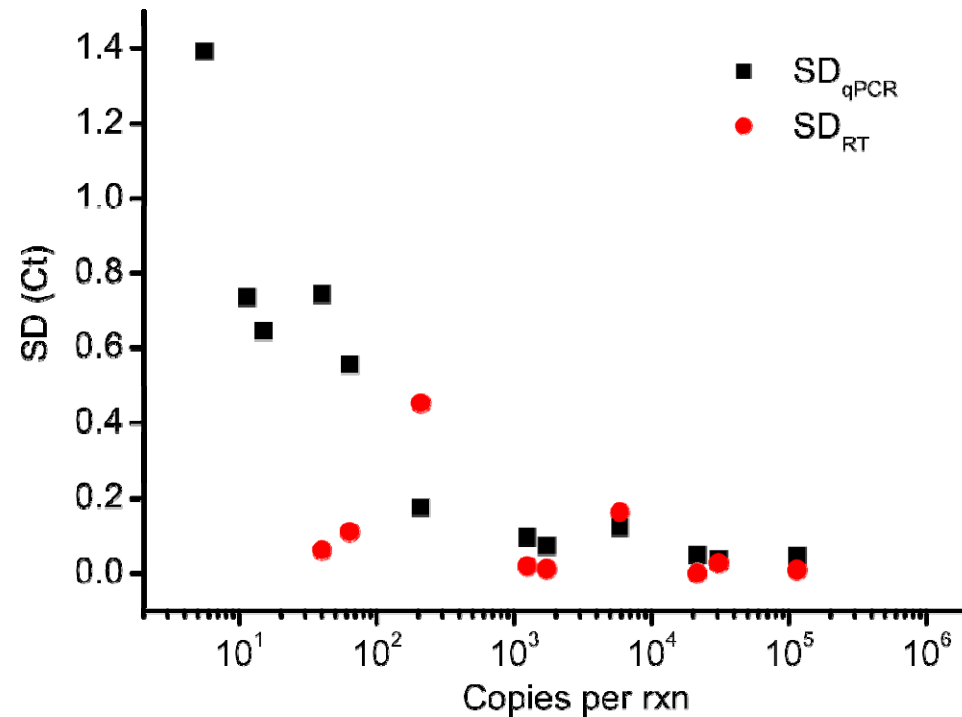
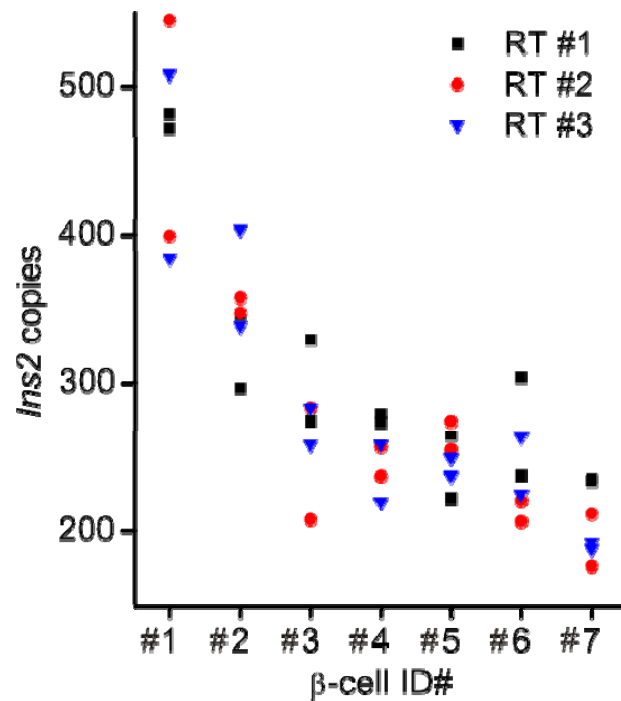
Quantitative PCR of heterogeneous tissue:  
Lessons from the islets of Langerhans

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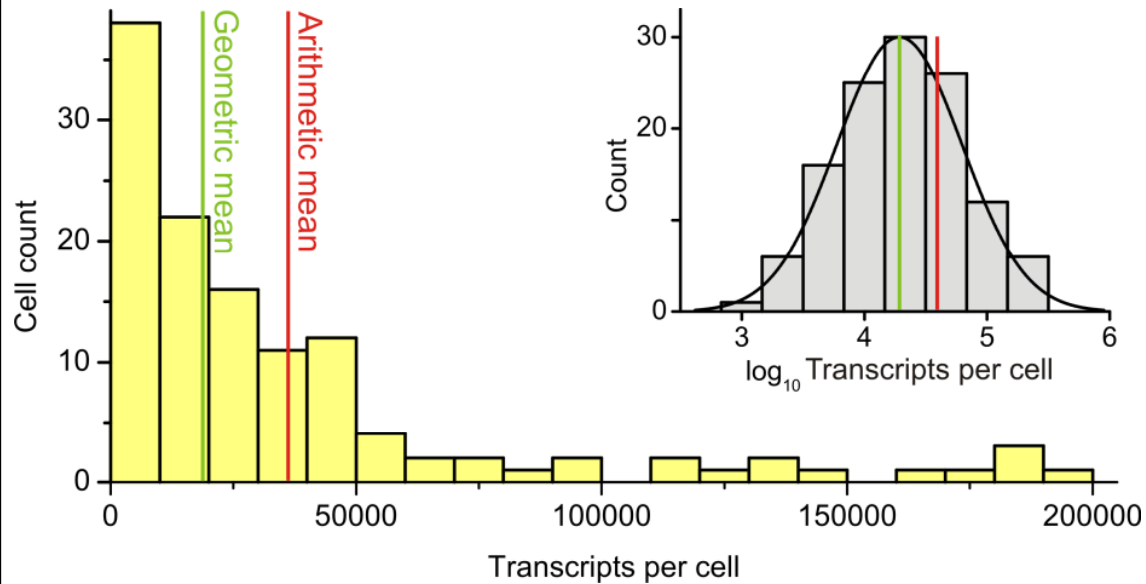
2007-03-27

# Technical reproducibility at low copy numbers

- Triplicate RT, triplicate qPCR
- Three genes, four dilutions of tot islet RNA
- $SD_{tot} = SD_{qPCR} + SD_{RT}$



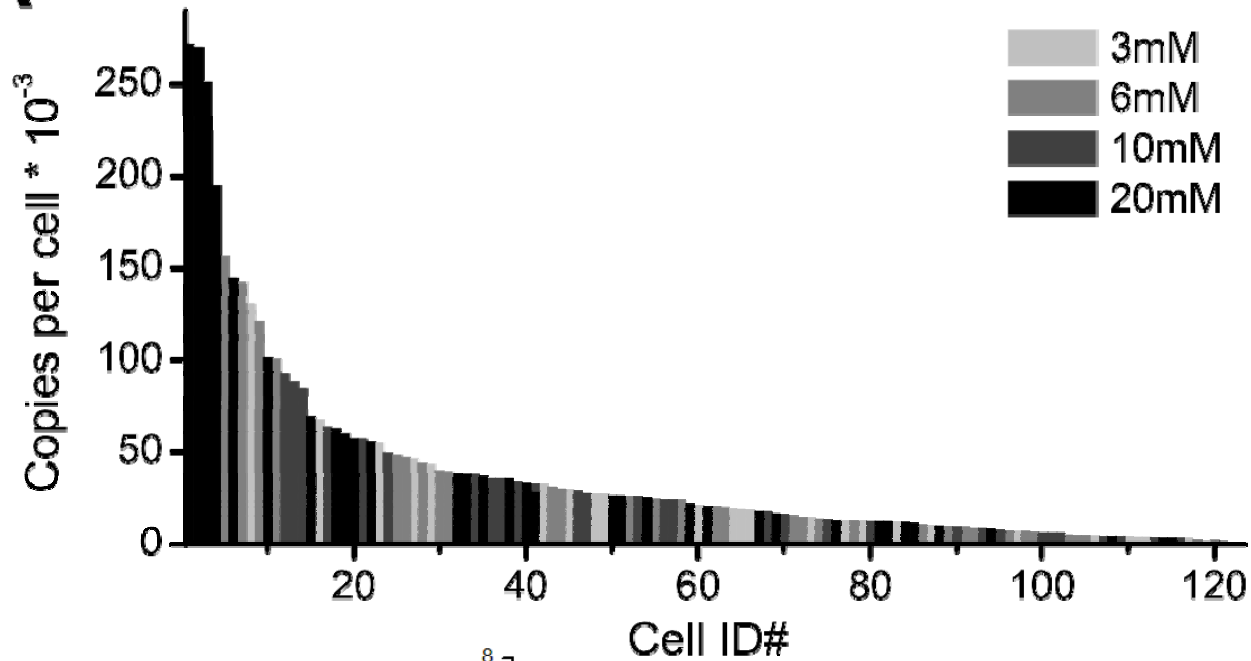
# Abundant transcripts are lognormally distributed



	Arith- metic	Geo- metric
ActB	3.3	4.9
Ins1	4.6	17
Ins2	3.0	9.5
Abcc8	1.4	1.5
Kcnj11	1.1	1.3

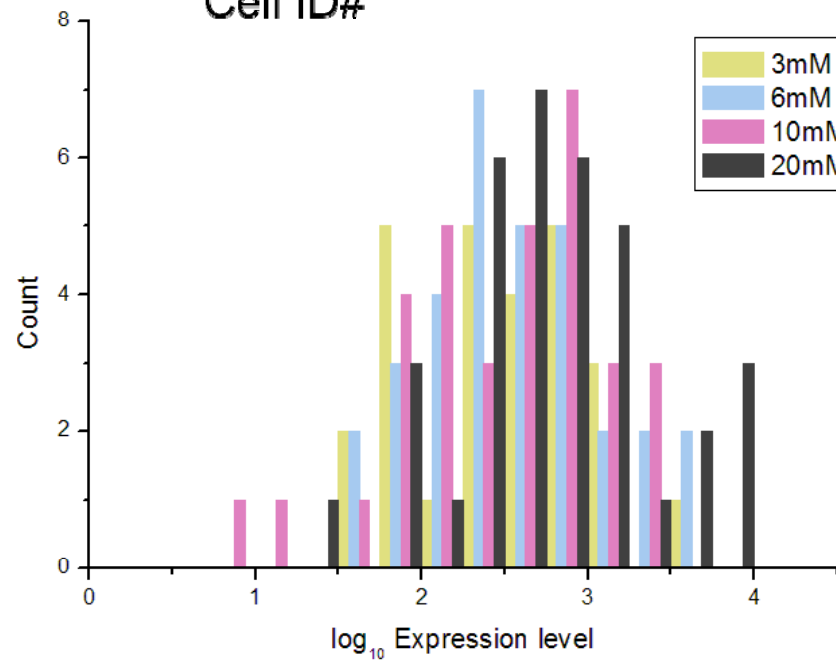
- 125  $\beta$ -cells
- Geom. Mean (Ins1): 19 000 copies/cell
- Arithmetic mean (Ins1): 37 000 copies/cell

# A



Effect on insulin gene expression of increasing glucose concentration

Most cells are unaffected



# Single cell correlations



**Table 2.** Pearson correlation coefficients based on logarithms of expression levels

	<i>ActB</i>	<i>Ins1</i>	<i>Ins2</i>	<i>Abcc8</i>	<i>Kcnj11</i>
<i>ActB</i>	1				
<i>Ins1</i>	0.15	1			
<i>Ins2</i>	0.12	0.90	1		
<i>Abcc8</i>	-0.02	-0.01	0.06	1	
<i>Kcnj11</i>	0.11	-0.02	0.24	-0.15	1

Pearson coefficient is calculated as

$$-1 \leq r = \frac{\sum(x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\sum(x_i - \bar{x})^2 \sum(y_i - \bar{y})^2}} \leq 1,$$

where  $x$  and  $y$  are the logarithms of expression levels of the two genes,  $i$  indexes the cell, and the bar indicates average.  $r$  close to 1 signifies strong positive correlation, while a value close to zero means no correlation. Negative  $r$  value would be anti-correlation.

~~Global extrinsic noise~~

Pathway specific extrinsic noise

..... Intrinsic noise .....

## Summary

- Reproducibility of traditional qPCR decreases rapidly at <100 initial copies
- Abundant transcripts are lognormally distributed
- Glucose affects insulin gene expression in  $\beta$ -cells in a heterogeneous fashion
- Global extrinsic noise is low
- Noise in insulin gene expression is dominated by pathway-specific extrinsic noise

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**LUND**  
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*Applied Biosystems*

Thesis defend titled "Quantifying gene expression in single cells"  
May 4th, at 13:15 in Lilla Aulan (MFC), University Hospital, Malmö, Sweden