Supplementary Information

Methods

Control experiments verified that RNA degradation and inhibition of reverse transcription and PCR reactions did not affect our results. To test for extensive RNA degradation during the pre-RT steps, external RNA was added to the lysis buffer together with the cell. No degradation was seen compared to control. Furthermore, if these problems were extensive, we would see a cut-off expression level that would be similar for all genes. Instead, we observe minimum values for a high expression gene that are larger than the maximum values of a low expression gene. This evidences that that RNA degradation does not affect our results. Increasing the number of collected cells in a single tube did not inhibit the reverse transcription or PCR until at least 10 cells were used. We have previously investigated the properties of the reverse transcription reaction and use optimum protocols (Stahlberg et al. 2004a; Stahlberg et al. 2004b). It is also known that the RT mixture at high concentrations may inhibit PCR (Liss 2002). In our hands, up to 2 µl of the RT mixture could be used in 10 µl PCR-reactions without detectable inhibition. These results indicate that the technical variation due to practical handling and inhibition is very low, i.e. high technical precision (see Supplementary Information Figure 1).

Figure texts

Figure 1 Demonstration of technical reproducibility. Two samples of single MIN6 cells were split in two and analysed separately in duplicate reverse transcription reactions, followed by real-time PCR using *Ins2* primers. The figure shows the resulting amplification curves, with fluorescent measurement in logarithmic scale. Note that the spread between the two cells is small in this case, yet larger than the minimal variation seen between the duplicate RT-PCR reactions.

Table

Glucose	*Gene*	*N*[1]	*Arithmetic mean*[2]	*Geometric mean*[3]	*log_{10} Geom. mean (SD)*[4]	*Shapiro-Wilk p-value*[5]	*Skew-ness*[6]
Islet cells							
5 mM	ActB	51	130	61	1.79 (0.51)	0.56	-0.02
	Ins1	70	1700	190	2.29 (0.94)	0.32	0.06
	Ins2	44	5200	1100	3.03 (1.03)	0.005	-0.12
	Abcc8	28	130	98	1.99 (0.34)	0.97	-0.05
	Kcnj11	15	30	24	1.38 (0.22)	0.77	0.31
Islet cells							
20 mM	ActB	45	420	300	2.47 (0.37)	0.53	0.01
	Ins1	49	7700	3200	3.51 (0.67)	0.09	-0.70
	Ins2	47	16000	10000	4.01 (0.53)	0.05	-0.70
	Abcc8	23	180	150	2.16 (0.33)	0.21	-0.01
	Kcnj11	18	34	31	1.49 (0.23)	0.48	-0.19
MIN6 cells							
10 mM	ActB	21	6400	474	2.68 (0.36)	0.62	0.08
	Ins1	20	1300	47	1.67 (0.64)	0.46	0.21
	Ins2	21	28000	1588	3.20 (0.51)	0.27	-0.05
	Abcc8	14	1100	74	1.87 (0.52)	0.05	-0.42
	Kcnj11	9	130	11	1.04 (0.35)	0.43	0.17

Table 1 Summary of statistics on primary cells and MIN6 cells.
[1] The total number of cells analysed was 60, 61 (84 and 85 for *Ins1* and *Ins2*) and 22 respectively for primary cells in 5 mM glucose, primary cells in 20 mM glucose and MIN6 cells in 10 mM glucose.
[2] The arithmetic means are calculated as $(\Sigma X_n)/N$.
[3] The geometric means are derived from the formula $(\Pi X_n)^{1/N}$.
[4] Logarithm of the geometric mean with corresponding standard deviations.
[5] If $p \geq 0.05$ then the null hypothesis that data are lognormal cannot be rejected.
[6] The skewness is calculated using the formula $\Sigma(X_n-\mu)^3/(N-1)\sigma^3$.

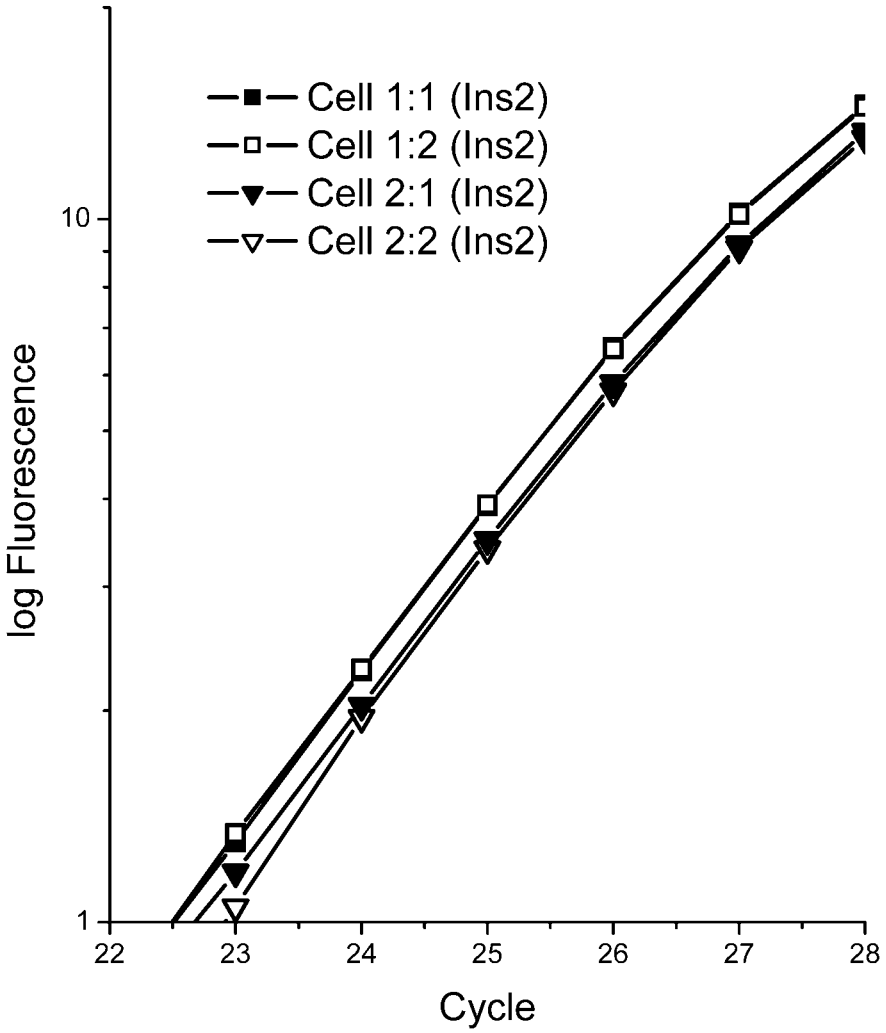
References

Liss, B. 2002. Improved quantitative real-time RT-PCR for expression profiling of individual cells. *Nucleic Acids Res* **30:** e89.

Stahlberg, A., J. Hakansson, X. Xian, H. Semb, and M. Kubista. 2004a. Properties of the reverse transcription reaction in mRNA quantification. *Clin Chem* **50:** 509-515.

Stahlberg, A., M. Kubista, and M. Pfaffl. 2004b. Comparison of reverse transcriptases in gene expression analysis. *Clin Chem* **50:** 1678-1680.

Figure 1


Cell 1:1 (Ins2)
Cell 1:2 (Ins2)
Cell 2:1 (Ins2)
Cell 2:2 (Ins2)
log Fluorescence
10
1
22
23
24
25
26
27
28
Cycle