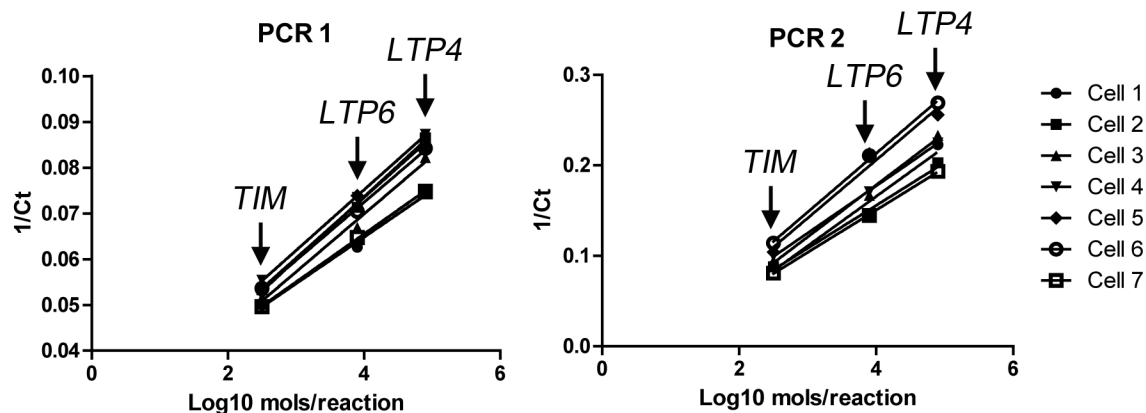


A



Demonstration of linear amplification of exogenous spike controls over two rounds of PCR. Freshly isolated mammary epithelial cells were single cell sorted directly into thin walled 96 well PCR plates containing the first strand synthesis buffer. This was immediately frozen on dry ice and stored at -20°C until the full amplification method could be performed. qrtPCR for spike controls LTP4, LTP6 and TIM was carried out in triplicate on the products of PCR 1 and PCR 2 from the single cell amplification of seven myoepithelial cells. The linearity of amplification was tested by converting the picogram quantities of each internal spike control to represent molecular amounts using the following formula $X \text{ g } \mu\text{l}^{-1} \text{ RNA} / (\text{transcript length} \times 340) \times 6.022 \times 10^{23}$. Therefore, LTP4 added at 10^{-2} pg was present at 8400 molecules, LTP6 added at 10^{-3} pg was present at 900 molecules, and TIM added at 10^{-4} pg was present at 90 molecules per reaction. These values were log transformed and plotted against 1/Ct qrtPCR expression levels.

B

