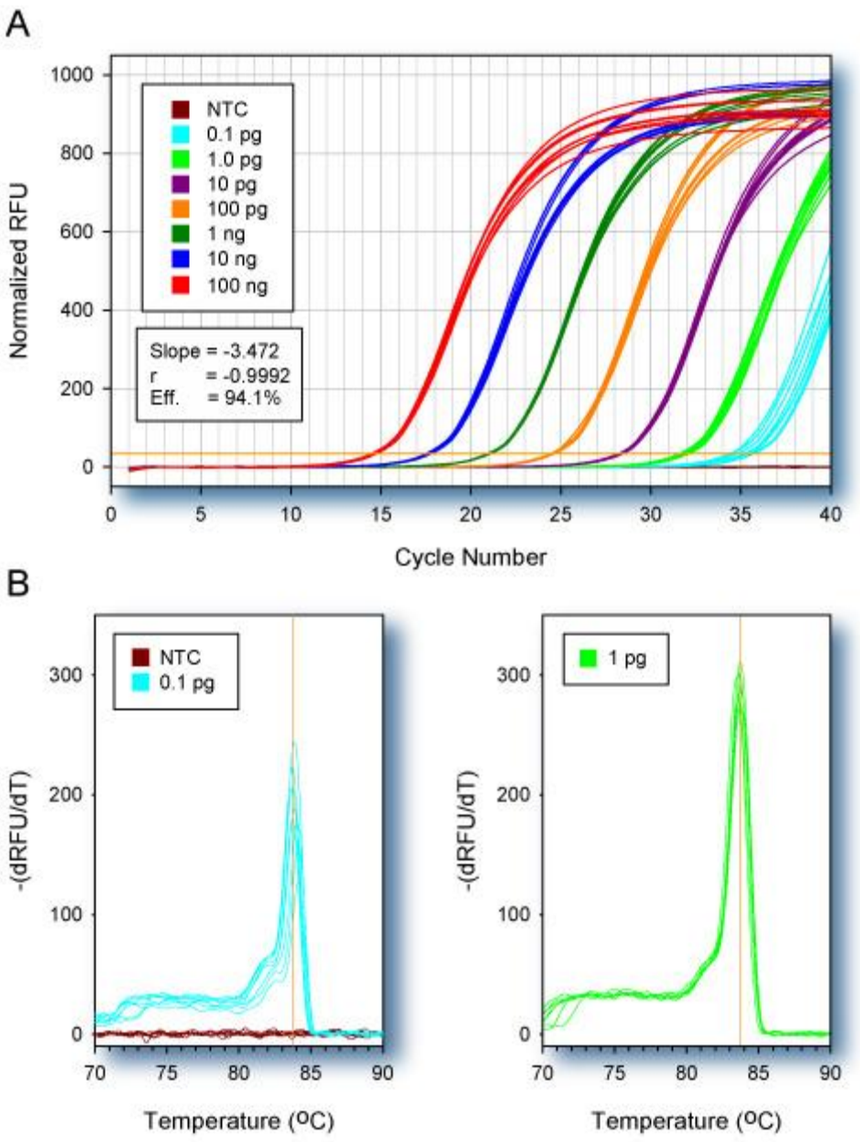
One-Step SYBR Green qRT-PCR



One-Step SYBR Green qRT-PCR with broad dynamic range, high sensitivity and high specificity.

A 202-bp fragment of glyceraldehyde-3-phosphate dehydrogenase (GAPD) mRNA was amplified from log-fold serial dilutions of HeLa cell total RNA (100 ng to 0.1 pg). Eight replicate reactions for each RNA quantity, and the no template control (NTC) were carried out in 25-µL volumes with the qScript™ One-Step SYBR® Green qRT-PCR Kit and 200 nM each GAPD specific primers (PrimerBank ID 7669492a2, Wang, X. and Seed, B. (2003) NAR 31(24): e154; pp.1-8). Reactions were assembled on ice, transferred to a MyiQ™ real-time detection system (Bio-Rad Laboratories), and incubated for 5 min at 50ºC followed by 2 min at 95ºC. PCR cycling was for 40 cycles of 3s, 95ºC; 30s, 60ºC. Immediately following PCR cycling the block temperature was ramped from 60ºC to 95ºC and melt curve data was collected. Panel A) Amplification plots and standard curve regression analysis. Panel B) Dissociation results (melt curve) for NTC, 0.1 pg and 1 pg reactions