

Reduction of DNA contamination in RNA samples for reverse transcription-polymerase chain reaction using selective precipitation by compaction agents.

Añez-Lingerfelt M, Fox GE, Willson RC.

*Department of Chemical and Biomolecular Engineering, 4800 Calhoun Road, S222 Engineering Building 1, University of Houston, Houston, TX 77204-4004, USA.*

An important problem in measurement of messenger RNA (mRNA) levels by reverse transcription-polymerase chain reaction (RT-PCR) is DNA contamination, which can produce artifactually increased mRNA concentration. Current methods to eliminate contaminating DNA can compromise the integrity of the RNA, are time-consuming, and/or are hazardous. We present a rapid, nuclease-free, and cost-effective method of eliminating contaminating DNA in RNA samples using selective precipitation by compaction agents. Compaction agents are cationic molecules that bind to double-stranded nucleic acids, driven by electrostatic interactions and steric complementarity. The effectiveness and DNA selectivity of six compaction agents were investigated: trivalent spermidine, Triquat A, and Triquat 7; tetravalent spermine and Quatro-quat; and hexavalent Quatro-diquat. Effectiveness was measured initially by supernatant UV absorbance after precipitation of salmon sperm DNA. Effectiveness and selectivity were then investigated using differences in RT-PCR C(t) values with synthetic mixtures of human genomic DNA and total RNA and with total RNA isolated from cells. With 500 microM spermidine or Triquat A, the supernatant DNA could not be detected up to 40 cycles of PCR (C(t)12.6), whereas the C(t) for the mRNA was increased by only five cycles. Therefore, spermidine and Triquat A each show strong DNA selectivity and could be used to eliminate contaminating DNA in measurements of mRNA.