lithium chloride purification, a rapid and efficient technique to purify RNA from DSS-treated tissus

Emilie Viennois

Abstract

Dextran sodium sulfate (DSS) is commonly used in mouse studies to induce a very reproducible colitis that effectively mimics the clinical and histological features of human inflammatory bowel disease (IBD)
patients, especially ulcerative colitis. However, the mechanisms of action of DSS remain poorly understood, and
observations by our laboratory and other groups indicate that DSS contamination of colonic tissues from DSStreated
mice potently inhibits the quantitative reverse-transcription polymerase chain reaction (qRT-PCR)
amplification of mRNA. A prior study used poly-A-mediated mRNA purification to remove DSS from RNA extracts, but we herein
report a second efficient and cost-effective approach to counteract this inhibition, using lithium chloride
precipitation to entirely remove DSS from RNAs. We also explored how DSS interferes with qRT-PCR process, and
we report for the first time that DSS can alter the binding of reverse transcriptase to previously primed RNA and
specifically inhibits the enzymatic activities of reverse transcriptase and Taq polymerase in vitro. This likely explains
why DSS-treated colonic RNA is not suitable to qRT-PCR amplification without a previous purification step.
In summary, we provide a simple method to remove DSS from colonic RNAs, and we demonstrate for
the first time that DSS can inhibit the activities of both polymerase and reverse transcriptase. In order to reliably
analyze gene expression in the colonic mucosa of DSS-treated mice, the efficiency rate of qRT-PCR must be the
same between all the different experimental groups, including the water-treated control group, suggesting that
whatever the duration and the percentage of the DSS treatment, RNAs must be purified.

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Materials

DSS by Contributed by users

Protocol