An optimized RNA extraction workflow for stool and gut samples

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Abstract

Metatranscriptomics is an effective tool for understanding the composition and in vivo activity of the gut microbiome. However, challenges in sample preparation, such as maintaining microbial composition during storage, ensuring uniform cell disruption and effectively removing inhibitory substances, could interfere with downstream analysis. Here, we present an optimized RNA extraction workflow for stool and gut samples and validate the extracted RNA in downstream PCR and NGS-based metatranscriptomic analysis.

Human and animal stool samples were stabilized for up to two months in various solutions at different temperatures and homogenized. Microbial cells, including gram-negative and gram-positive bacteria, fungi and archaea, were lysed using bead beating and chemical lysis. Inhibitory substances were removed and inhibitor-free RNA was captured on a silica membrane, washed and eluted. RNA quality was assessed for purity, yield and integrity. The purified RNA was validated in downstream applications such as RT-qPCR and RNA sequencing.

This optimized RNA extraction method efficiently recovered microbial and viral RNA with high yield, quality and purity, showing no RT-qPCR inhibition. RNA sequencing revealed highly diverse communities based on alpha diversity and functional profiling. The method is easily adaptable to other inhibitor-rich samples like wastewater or sludge.

Sample stabilization and RNA extraction workflow

The RNeasy® PowerFecal® Pro Kit efficiently isolates total RNA from inhibitor-rich samples, such as stool, gut, sludge and wastewater. It utilizes the second-generation Inhibitor Removal Technology® (IRT®) to deliver high-yield, pure RNA for immediate use in RT-PCR, qPCR and NGS. Proven bead beating and lysis chemistry ensure effective and straightforward extraction.

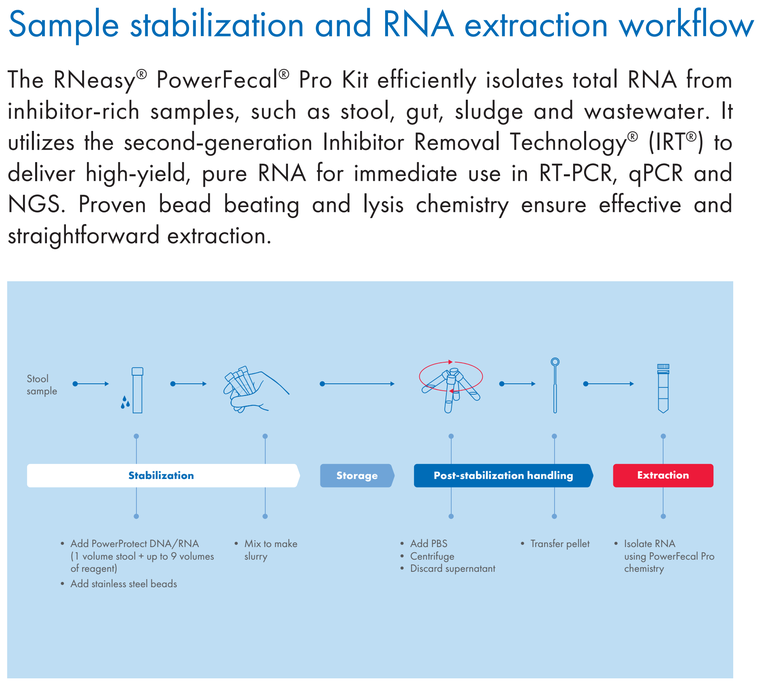

Figure 1. Sample stabilization with PowerProtect® DNA/RNA Kit and RNA isolation with RNeasy PowerFecal Pro Kit

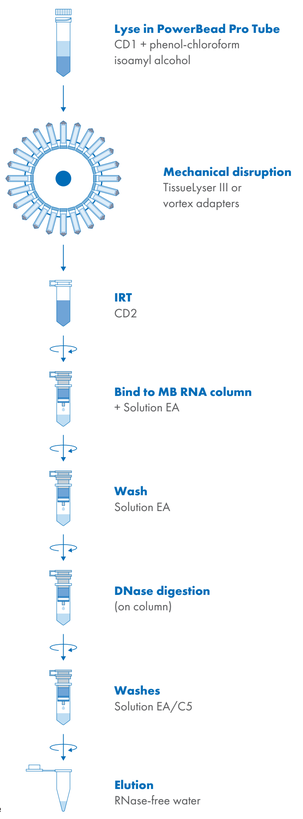

Figure 2. RNeasy PowerFecal Pro procedure

Consistently high RNA yield and integrity after sample storage

The yield and integrity of nucleic acids isolated from stool microbes are influenced by the state of the digestive system, diet and time between sample collection and preservation. For optimal quality, process samples as quickly as possible after collection.If sample storage is required, the PowerProtect DNA/RNA reagent can be used to preserve RNA integrity during storage at room temperature. We show that the QIAGEN workflow using PowerProtect DNA/RNA reagent and RNeasy PowerFecal Pro delivers higher RNA yield and integrity with minimal degradation for up to 28 days, outperforming a kit from another supplier.

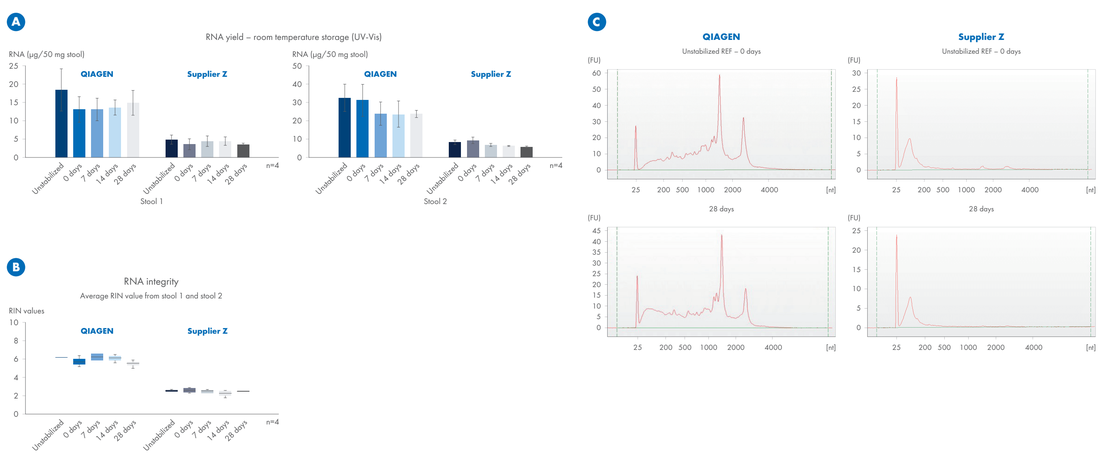

Figure 3. Two stool samples, with four replicates each, were processed at day 0 and after storage and stabilization at 7, 14 and 28 days using QIAGEN or Supplier Z workflows. Unstabilized samples served as references. **A.** The QIAGEN workflow maintained consistent RNA yields for up to 28 days compared to Supplier Z. **B.** RNA integrity (RIN value) was higher with the QIAGEN workflow compared to the workflow from Supplier Z. **C.** Bioanalyzer® electropherograms confirmed these results, showing well-defined 16S and 23S ribosomal peaks with minimal RNA degradation in the QIAGEN workflow, unlike the workflow from Supplier Z.

Efficient removal of PCR inhibitors

The QIAGEN RNeasy PowerFecal Pro workflow resulted in the highest RNA yields across different stool samples compared to other suppliers. The kit isolated RNA with high integrity and effectively removed PCR inhibitors, ensuring reliable downstream analysis.

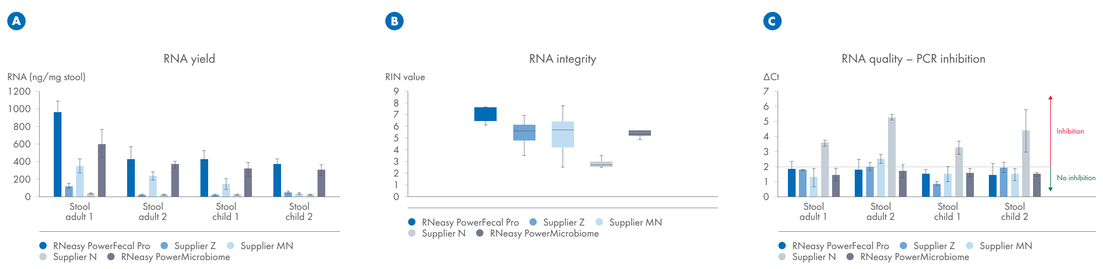

Figure 4. Four frozen stool samples in triplicates were used for RNA isolation using RNeasy PowerFecal Pro Kit, a legacy QIAGEN kit (RNeasy PowerMicrobiome®) and kits from other suppliers. **A.** RNeasy PowerFecal Pro Kit achieved the highest RNA yields. **B.** RNeasy PowerFecal Pro Kit also achieved the highest RIN value and 23S/16S ratio (data not shown; MW 0.9) **C.** Efficient removal of PCR inhibitors by RNeasy PowerFecal Pro kit was demonstrated using the QuantiNova® SYBR® Green RT-PCR Mastermix + QuantiNova IC Probe Assay in comparison to of all of the above kits.

Successful detection of norovirus and SARS-CoV-2 by quantitative and digital PCR

The optimized RNA extraction method enabled the successful detection of norovirus and SARS-CoV-2 from wastewater samples using both RT-qPCR and dPCR. The kit's high efficiency and reliability make it a valuable tool for monitoring viral pathogens in environmental samples such as wastewater.

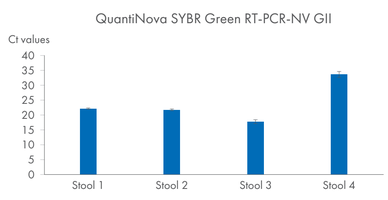

Figure 5. Different amounts of norovirus variant GII RNA were detected by RT-qPCR using RNA isolated via RNeasy PowerFecal Pro workflow from norovirus-positive patients' stool samples (tested in triplicates).

Figure 6. RNA was extracted from wastewater samples using the RNeasy PowerFecal Pro Kit and analyzed via dPCR. Various factors affecting wastewater pellet concentration were assessed, including bead beating for SARS-CoV-2 release and the use of phenol-chloroform-isoamyl (PCI) during RNA isolation. dPCR analysis confirmed that bead beating is essential for efficient viral particle release, while PCI appears to be less suitable for viral RNA extraction.

Recommendations for efficient microbial RNA isolation from stool samples

- Stabilize stool samples using PowerProtect DNA/RNA to prevent microbial abundance changes and RNA degradation.
- Ensure effective cell disruption with bead beating using a vortex adapter or TissueLyser III.
- Remove inhibitors in inhibitor-rich samples like stool using the RNeasy PowerFecal Pro Kit, which incorporates patented Inhibitor Removal Technology.
- Obtain pure RNA without contaminants using the long-proven silica-based purification method.
- For wastewater samples, pellet 40 mL of wastewater by centrifugation (4500 × g, 10 min – 2 h) to achieve optimal RNA yields.

Sample to Insight