Optimization of stabilization and other preanalytical steps for efficient nucleic acid analysis from stool samples

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Abstract

Metagenomic and metatranscriptomic analyses provide valuable insights into microbial composition and activity but present several challenges, particularly from inhibitor-rich samples like stool. Effective stabilization and preanalytical processing are essential to ensure microbial composition remains unchanged during storage, achieve uniform lysis and remove inhibitors that could interfere with downstream analysis.

Here we describe a workflow for stabilizing and extracting high-quality nucleic acids from human stool samples. Samples were stabilized for up to 12 months at various temperatures and homogenized. Microbial cells, including gram-negative and gram-positive bacteria, fungi, archaea and viruses, were rapidly and efficiently lysed using bead beating and chemical lysis. Afterwards, inhibitory substances were removed, and high-quality DNA/RNA was purified via silica membrane binding, washing and elution. Microbial detection was carried out through PCR and sequencing.

This protocol enabled the extraction of microbial and viral nucleic acids with high yield and purity. Sequencing revealed diverse microbial communities, assessed by alpha diversity (Operational Taxonomic Units or OTUs) and functional profiling. This method is adaptable to other inhibitor-rich samples such as wastewater and sludge.

Preanalytical processing and nucleic acid isolation workflow

Bacterial nucleic acid stabilization in stool using PowerProtect® DNA/RNA is the first and crucial step of the workflow (Figure 1). The reagent penetrates cells, halts biological activity and preserves the microbial community and functional profile during collection, transport and storage.

Following post-stabilization handling, the QIAamp® PowerFecal® Pro Kit can be used for DNA isolation and the RNeasy® PowerFecal Pro Kit for RNA isolation (Figure 2) from inhibitor-rich samples like stool, gut, sludge and wastewater. The second-generation Inhibitor Removal Technology® (IRT) from QIAGEN® ensures high yields of pure nucleic acids for direct use in RT-PCR, qPCR and NGS. Proven bead beating and lysis chemistry complete the streamlined, proprietary protocol.

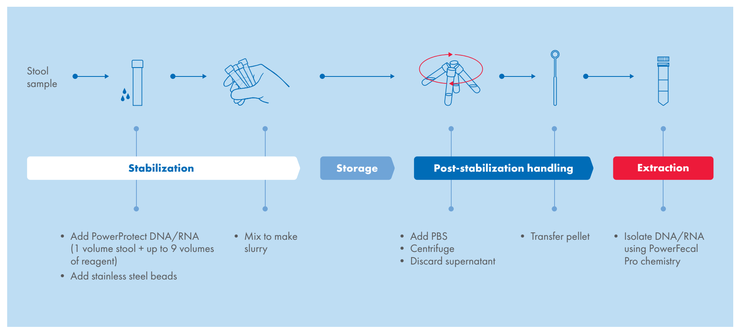

Figure 1. Sample stabilization with PowerProtect DNA/RNA Kit and RNA isolation with RNeasy PowerFecal Pro Kit

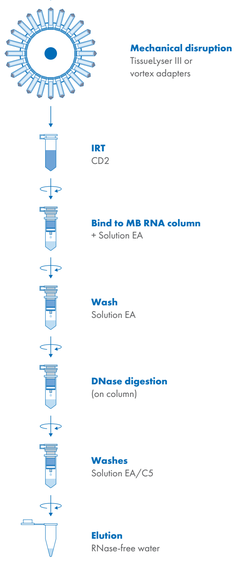

Figure 2. RNeasy PowerFecal Pro procedure

Stable DNA and RNA yields after storage

The yield and integrity of nucleic acids isolated from microbes in stool are influenced by the state of the digestive system, diet and the time between collection and preservation. For optimal quality, samples should be processed as soon as possible. If not, the PowerProtect DNA/RNA reagent can be used to stabilize stool samples for short-term storage at room temperature and for extended storage at 4°C.

Five separate stool samples were collected and aliquoted. Aliquots were stabilized with and stored in PowerProtect DNA/RNA at 2–8°C, room temperature or 35°C for various durations. DNA or RNA were extracted at the indicated storage durations using the QIAamp PowerFecal Pro or the RNeasy PowerFecal Pro, respectively. Bars marked "immediate extraction" were aliquots extracted at the time of collection. Error bars represent the standard deviation of four replicates per aliquot.

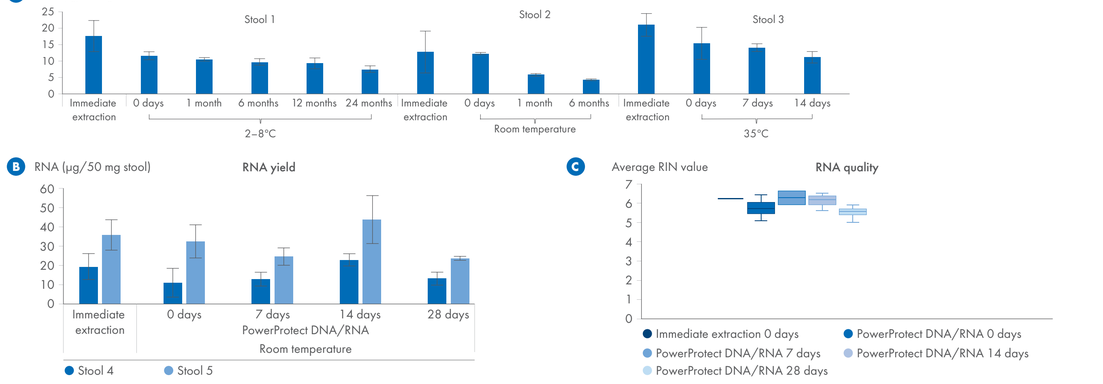

Figure 3. Consistent DNA and RNA yields from stool samples stored with PowerProtect DNA/RNA reagent. A Stool samples stored in PowerProtect DNA/RNA at 2–8°C maintain consistent DNA yields over 24 months. For shorter storage periods, room temperature or even 35°C is also viable (quantified using fluorometric technology). **B** Stool samples for RNA extraction remained stable for up to four weeks at room temperature, with comparable RNA yields across time points, showing only minor variations. **C** The average RNA Integrity Number (RIN) from two samples indicates stable RNA quality over four weeks of storage at room temperature.

Microbial diversity preserved after storage

DNA extracted with QIAamp PowerFecal Pro Kit was used for library construction with adapter-modified 515F-806R 16S primers, sequenced on a MiSeq® system, and analyzed with QIAGEN CLC Genomics Workbench and the CLC Microbial Genomics Module. Reads were mapped to the SILVA database at 99%, with OTU tables presented at the genus level.

As expected, microbial abundance differed among different stool samples (comparison of OTU tables A, B and C). However, length of storage in PowerProtect DNA/RNA did not affect the microbial diversity among aliquots of the same stool samples (comparison of bars within OTU tables A, B or C). In addition, Bray-Curtis dissimilarity showed no significant variation between time points (data not shown).

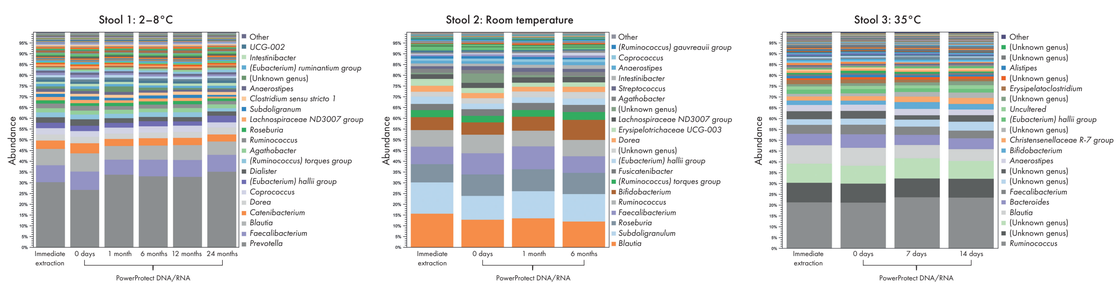

Figure 4. Microbial diversity remains stable in stool samples stored with PowerProtect DNA/RNA. A Stool 1 stored in PowerProtect DNA/RNA for 24 months at 2–8°C (recommended for long-term storage) maintained consistent microbial diversity. **B** Stool 2 was stored at room temperature in PowerProtect DNA/RNA for 6 months. Despite a decrease in DNA yield over time (Figure 3B), microbial diversity remained stable. **C** Stool 3 stored at 35°C for 14 days showed stable microbial diversity, showing that PowerProtect DNA/RNA preserves microbial abundance at higher temperatures, such as during transport.

Microbial detection using digital PCR after storage

The extracted DNA was additionally analyzed using dPCR Microbial DNA Detection Assays combined with the QIAcuity® Probe PCR Kit. The results confirm stable microbial composition of the stool samples over the storage period.

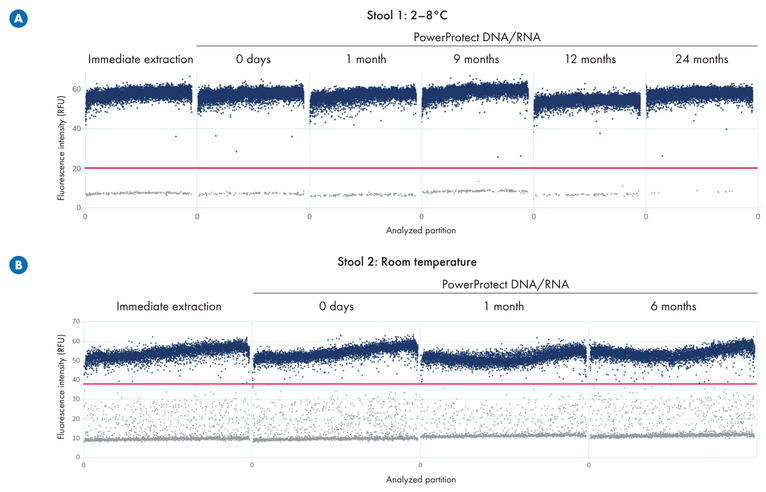

Figure 5. dPCR detection of microbial species in stool samples stored with PowerProtect DNA/RNA. DNA extracted from stool 1 stored at 2–8°C and stool 2 stored at room temperature was analyzed using dPCR. **A** *Faecalibacterium prausnitzii* (Gene Globe ID DMA00148; HEX), a key gut microbe, was analyzed in a stool sample stored at 2–8°C for up to 24 months. *F. prausnitzii* was consistently detected at comparable levels across time points, confirming stable preservation over two years. **B** *Bifidobacterium longum* (Gene Globe ID DMA00064; FAM), another common gut microbe, was analyzed in a stool sample stored at room temperature. dPCR analysis of 1 ng total DNA showed consistent detection of *B. longum* in the stool sample stored in PowerProtect DNA/RNA for up to six months, comparable to the reference stool sample which was immediately extracted.

Recommendations for reliable sample preparation

This optimized preanalytical and nucleic acid extraction workflow highlights the importance of refining key process steps, including sample storage, microbial disruption, nucleic acid isolation and analysis. To ensure the most efficient microbial DNA/RNA extraction from stool samples, we recommend:

- Fecal sample stabilization to prevent microbial shifts and nucleic acid degradation by using PowerProtect DNA/RNA.
- Effective cell disruption to ensure thorough lysis with bead beating on a vortex adapter or the TissueLyser III.
- Inhibitor removal for inhibitor-rich samples like stool; QIAamp PowerFecal Pro and RNeasy PowerFecal Pro Kits use patented Inhibitor Removal Technology for optimal purity.

Sample to Insight