Improved extraction of total nucleic acid from microbiome samples with a new EZ2® PowerFecal® Pro DNA/RNA method



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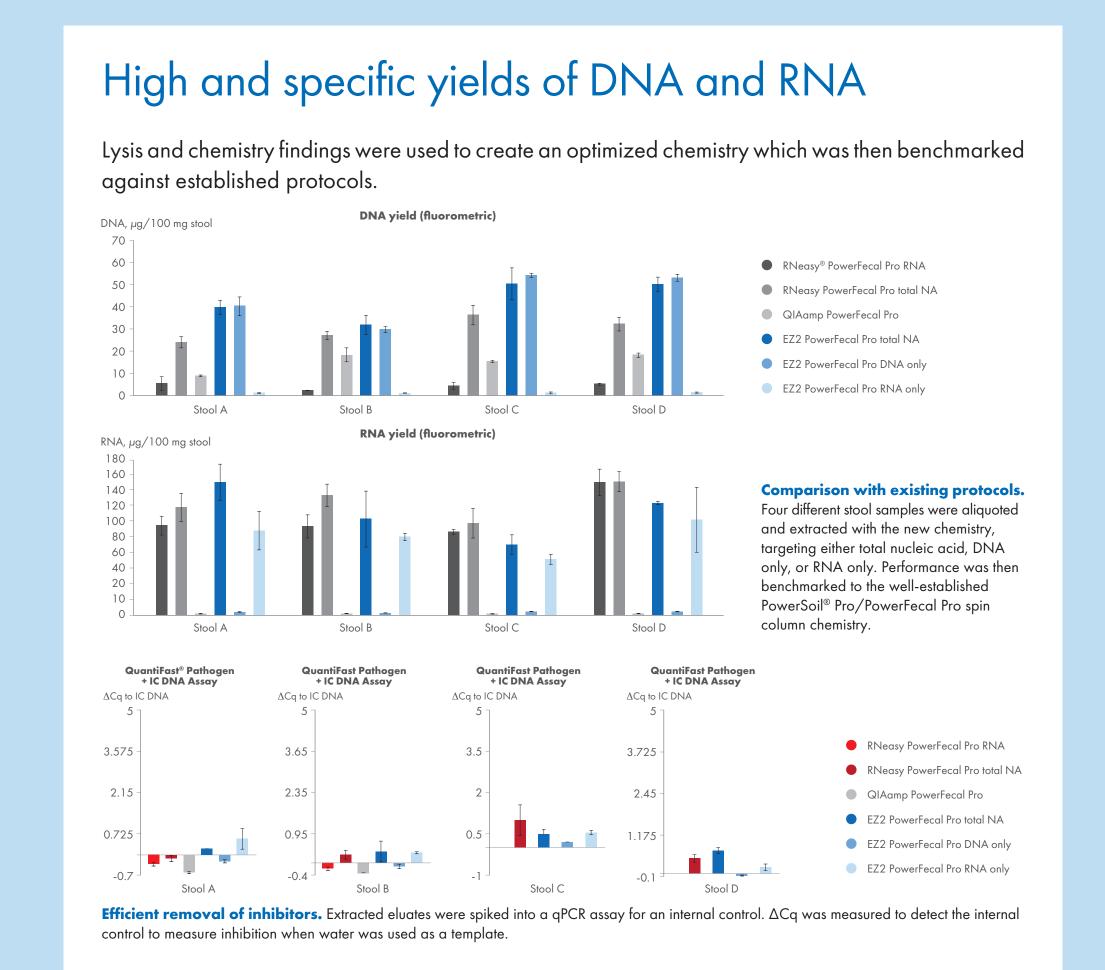
Abstract

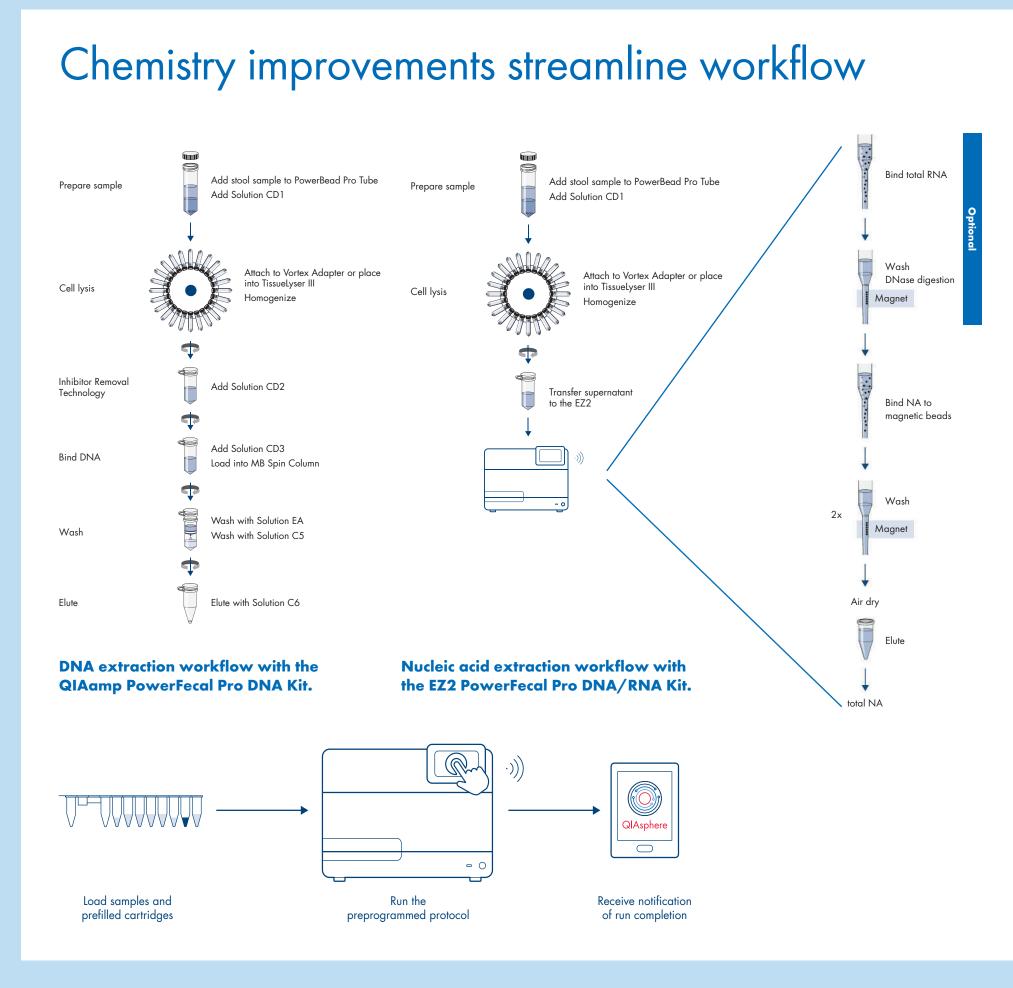
Improvements and standardization of nucleic acid extraction methods are needed to meet the needs of microbiome research, in order to provide the most unbiased representation of the sample. We investigated multiple methods to identify an automation-friendly improved protocol.

We extracted inhibitor-free DNA and RNA simultaneously from human microbiome samples. Yield and purity were evaluated using spectrophotometric and fluorometric assays, bacterial content was analyzed with PCR and sequencing-based assays, and inhibitor removal was investigated using specialized PCR assays. The most promising approaches were evaluated for their suitability for automation on a liquid handling device.

The resulting method provides a useful microbiome extraction technology which is suitable for a wide spectrum of human microbiome samples and is equally efficient at isolating DNA and RNA. Having an unbiased, high-efficiency, widely applicable nucleic acid extraction method will aid standardization and comparability across microbiome samples.

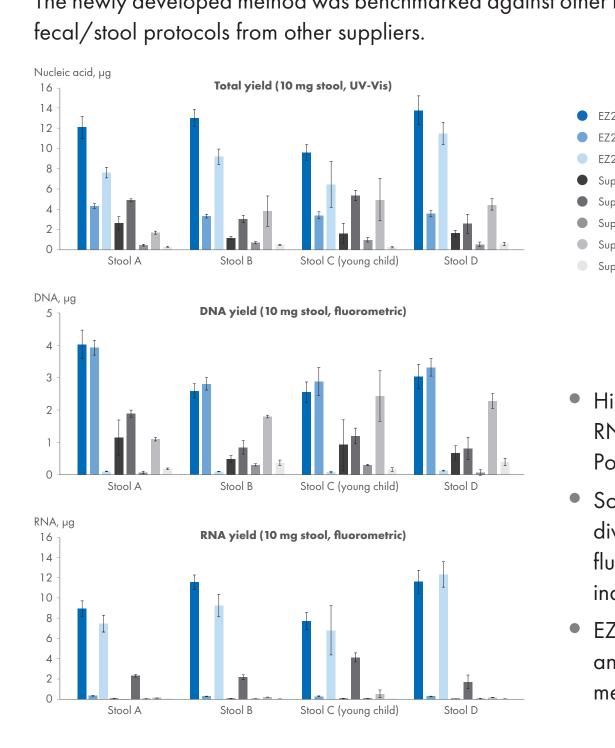
Lysis and chemistry optimization We investigated every aspect of the extraction process in large experimental matrices to find improvements in performance, comparing lysis methods and chemistries to determine the combinations that enable both RNA and DNA extraction at improved efficiencies. Lysis method optimization. Mechanical lysis is the most efficient method for extracting microbiomes. Though compatible with the extraction, addition of enzymatic lysis to the mechanical lysis does not improve yield or change community composition. Spin ref Bead re QA PF Pro QS PF Pro UV-Vis (NanoDrop®) Fluorometric DNA (Qubit®) Fluorometric RNA (Qubit) Chemistry optimization. Multiple combinations of lysis, inhibitor removal technology, bind, and wash chemistry were investigated to optimize the binding on magnetic beads. QA PF Pro: QIAamp® PowerFecal Pro; QS PF Pro: QIAsymphony PowerFecal Pro.





Comparison with other methods: Yield

The newly developed method was benchmarked against other magnetic-bead based microbiome or

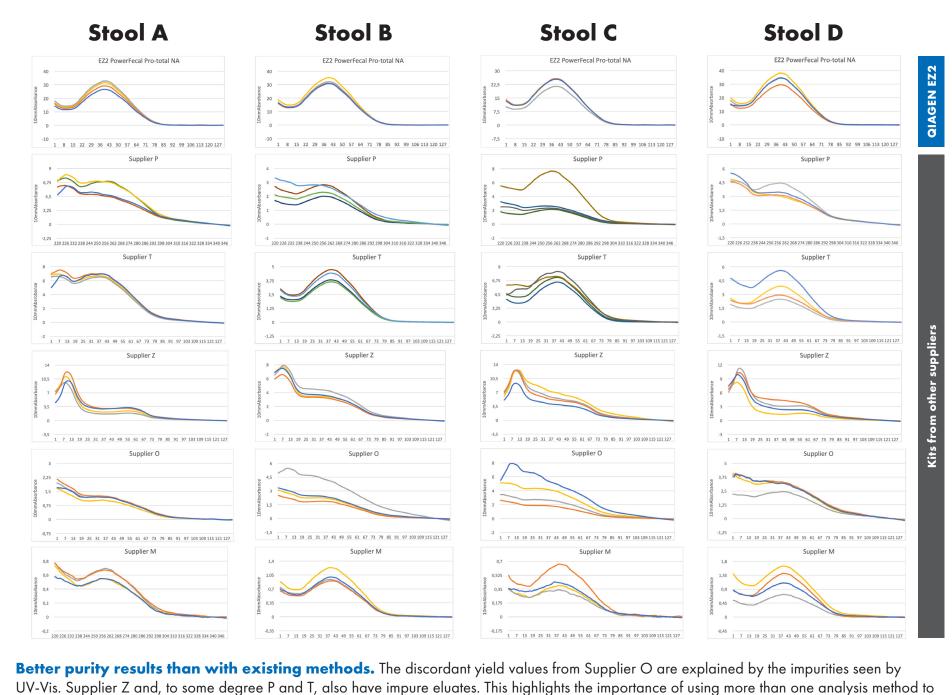


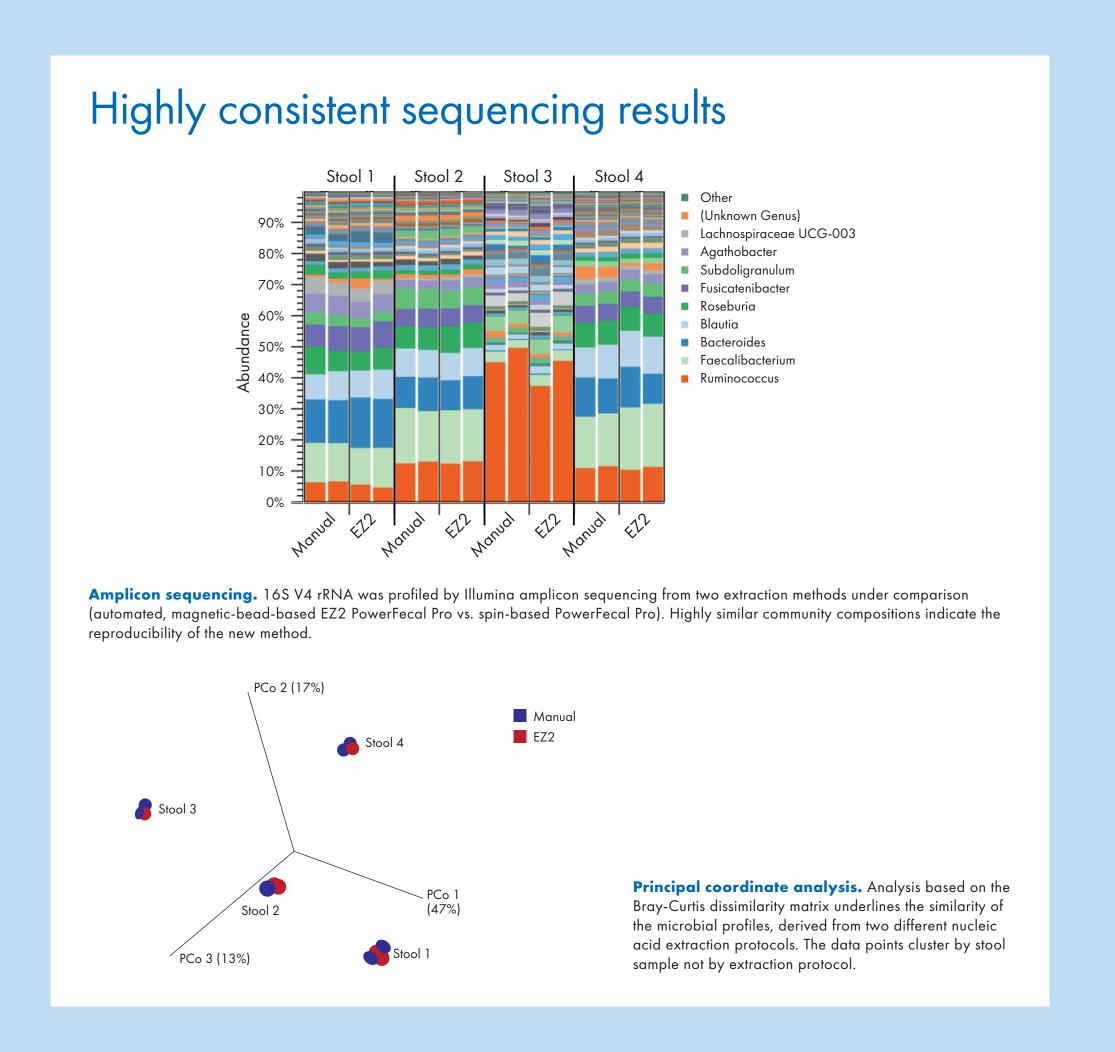
- EZ2 PowerFecal Pro total NA EZ2 PowerFecal Pro DNA only EZ2 PowerFecal Pro RNA only Supplier P Fecal Microbiome DNA Kit
- Supplier T Microbiome Nucleic Acid Isolation Supplier Z Fecal/Soil Microbe Kit Supplier O Stool DNA Kit Supplier M DNA Microbiome Kit
- RNA were achieved with the new EZ2 PowerFecal Pro protocol
- Some kits, such as Supplier O, had divergent results between UV-vis and fluorometric methods, which is an indicator of contamination
- EZ2 protocols were highly consistent and concordant between measurement

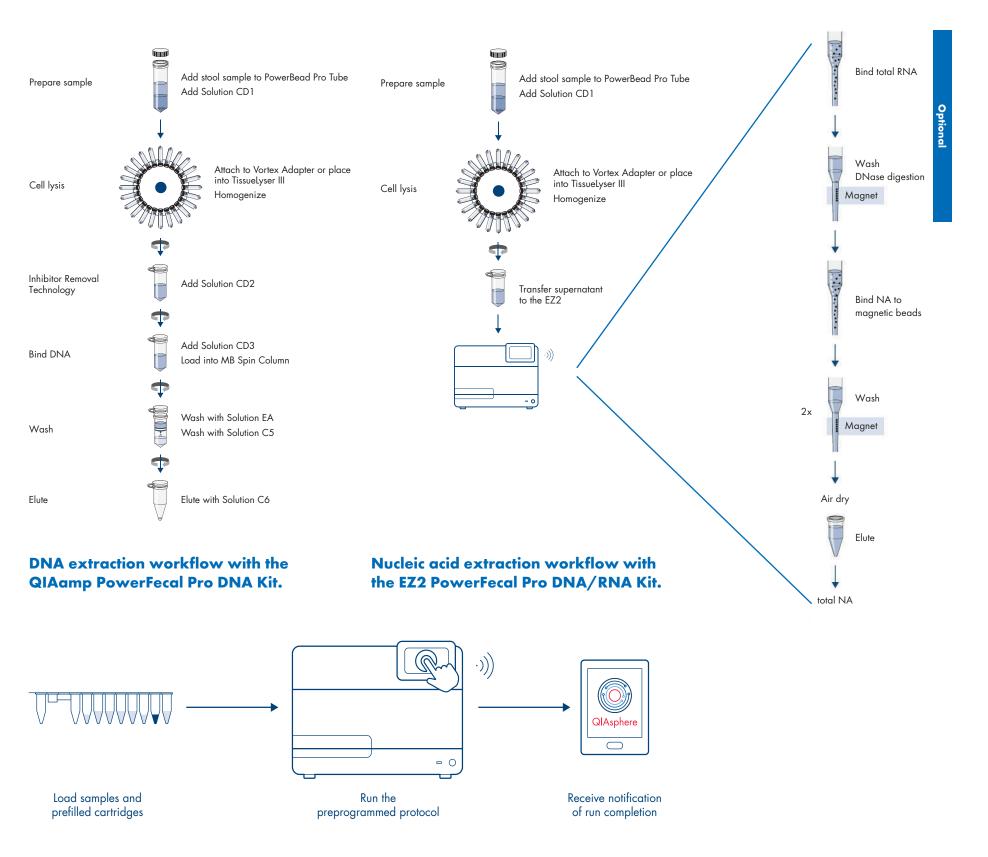
Higher extraction yields than with existing methods. The new and optimized EZ2 PowerFecal method presented here performs better than existing methods.

Comparison with other methods: Purity

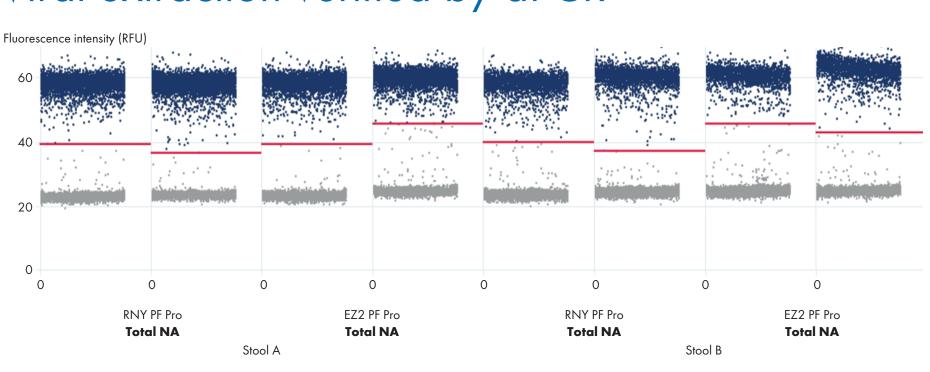
Stool samples are often inhibitory and difficult to extract pure nucleic acid from. This can be seen in the performance of many competing methods. The new EZ2 PowerFecal protocol efficiently removes







Viral extraction verified by dPCR



PMMoV detection by digital PCR. A dPCR assay against PMMoV (Pepper mild mottle virus, a common indicator target in stool or wastewater) shows high sensitivity and comparable results between the established manual method and the new automated method on the EZ2. RNY PF Pro: RNeasy PowerFecal Pro; EZ2 PF Pro: EZ2 PowerFecal Pro.

Conclusions

Optimizing an automated nucleic extraction workflow allowed us to both streamline the workflow and improve performance in terms of yield and purity, while maintaining the community composition seen by the widely used PowerSoil Pro/PowerFecal Pro manual extraction procedures. We believe this improved method using the EZ2 instrument will assist researchers investigating the microbiome:

- High and specific yields of DNA and/or RNA using the same kit
- Automated, streamlined workflow
- Isolation of bacterial, archaeal, fungal and viral nucleic acids
- Optimized for inhibitory substances like stool. External testers have successfully used the method on skin, genital, and oral swabs, and other human microbiome samples.