

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



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Abstract

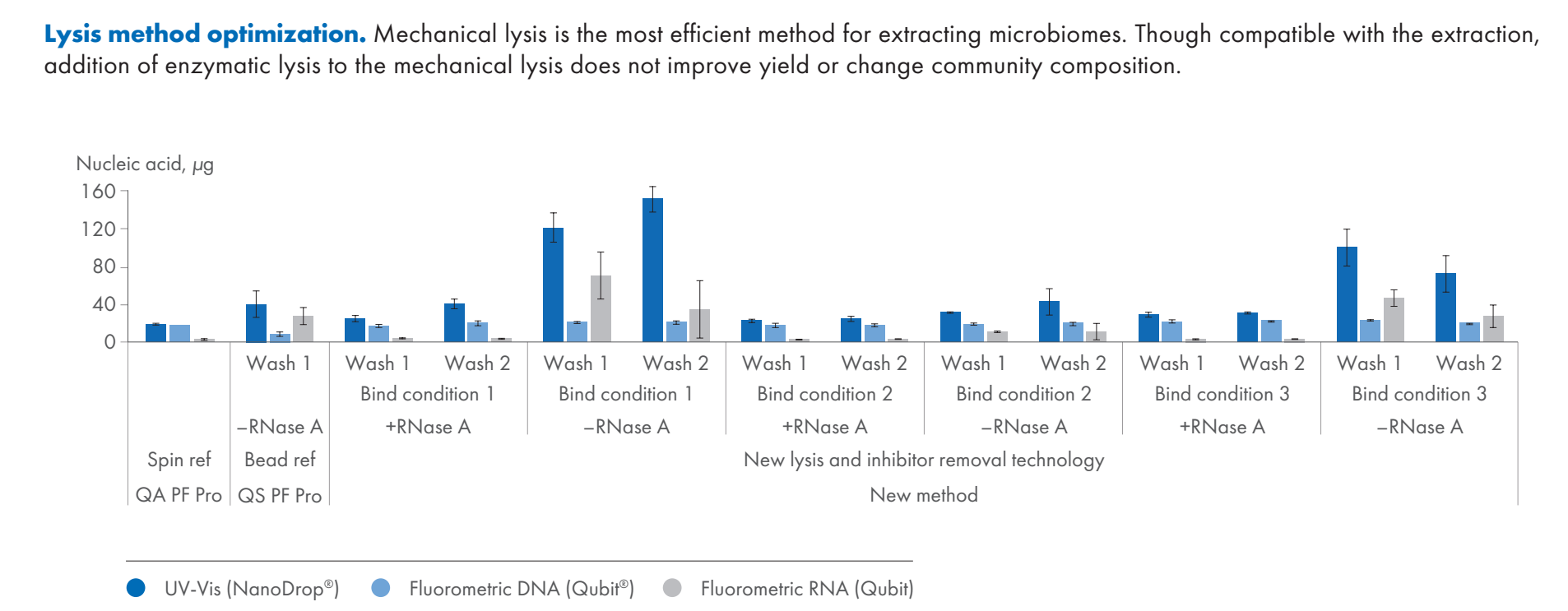
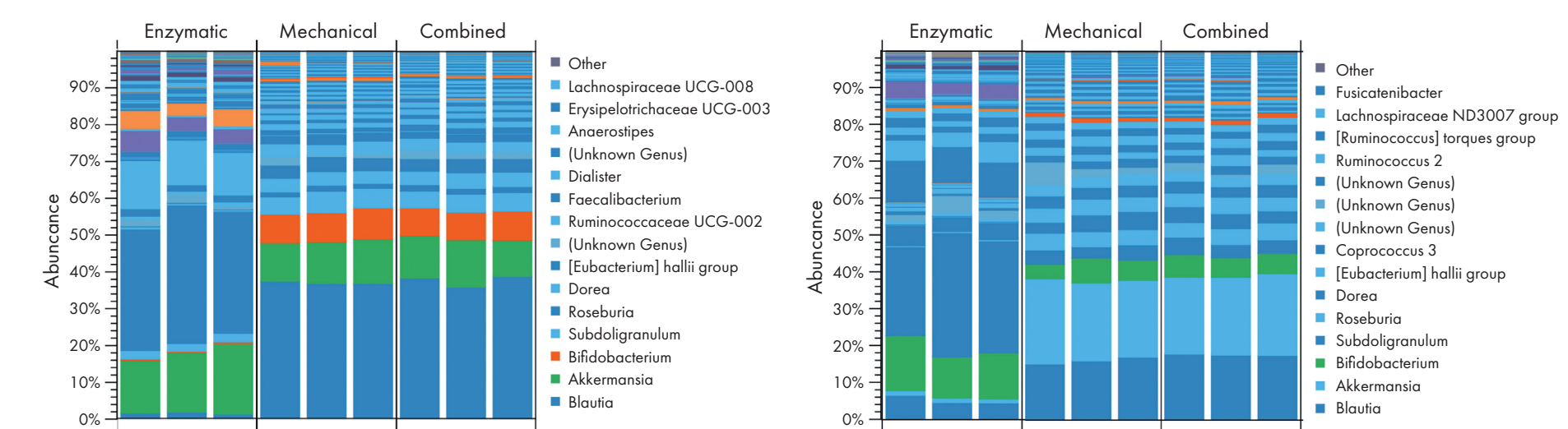
Improvements and standardization of nucleic acid extraction methods are needed to meet the needs of microbiome research, in order to provide the most unbiased representation of the sample. We investigated multiple methods to identify an automation-friendly improved protocol.

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.

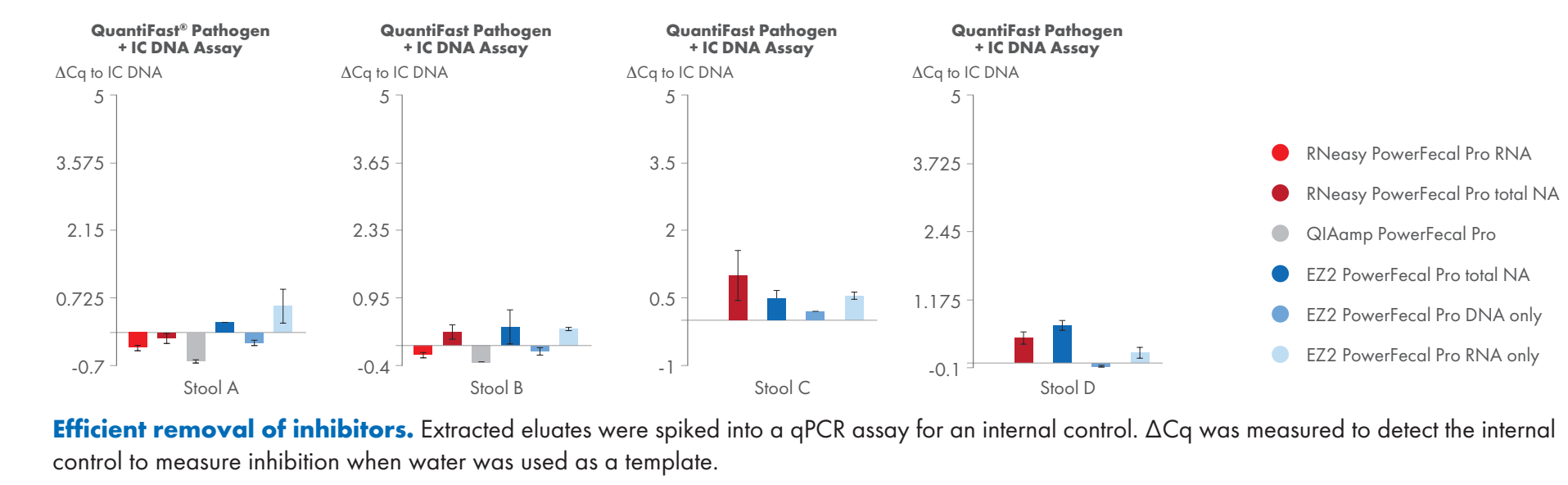
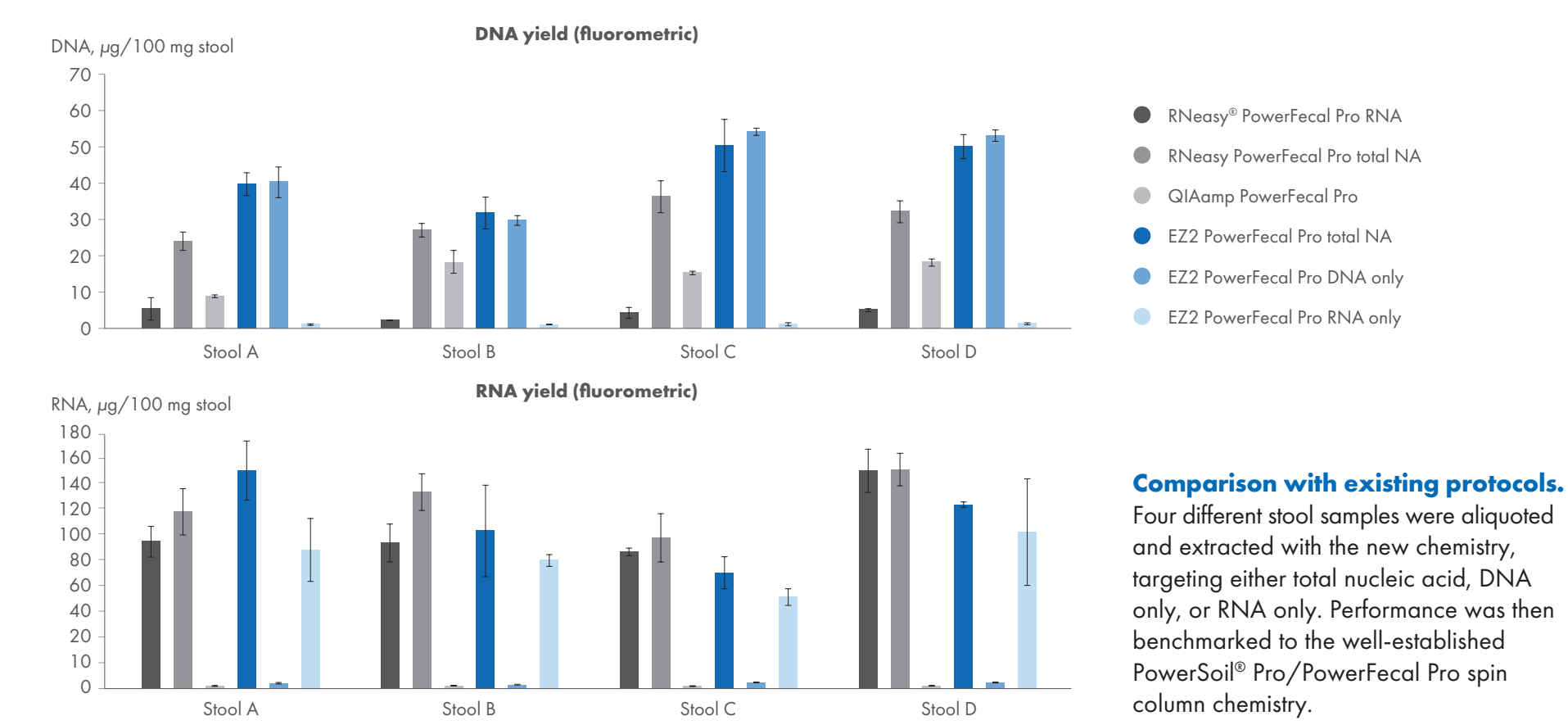
Lysis and chemistry optimization

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.

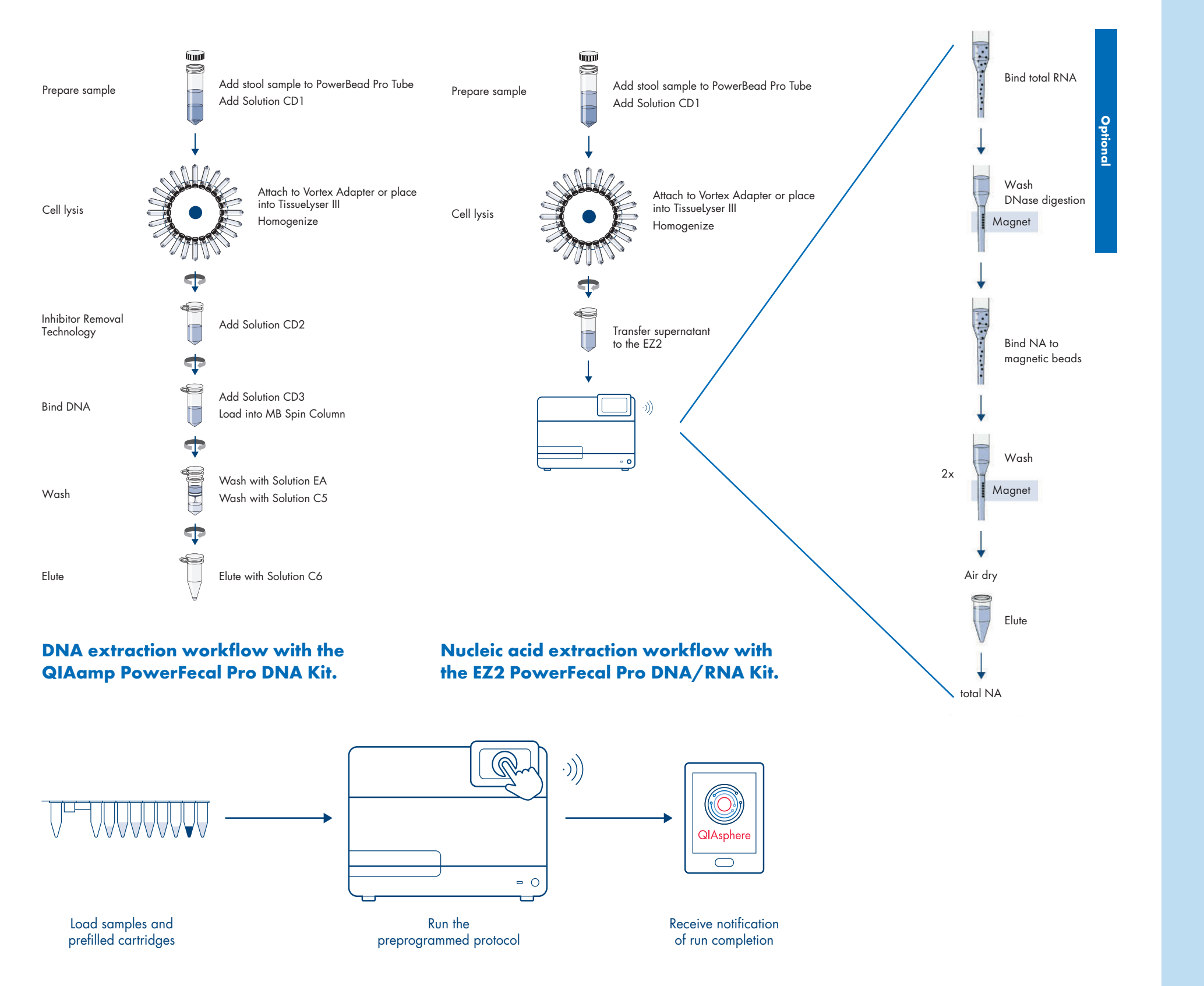


High and specific yields of DNA and RNA

Lysis and chemistry findings were used to create an optimized chemistry which was then benchmarked against established protocols.

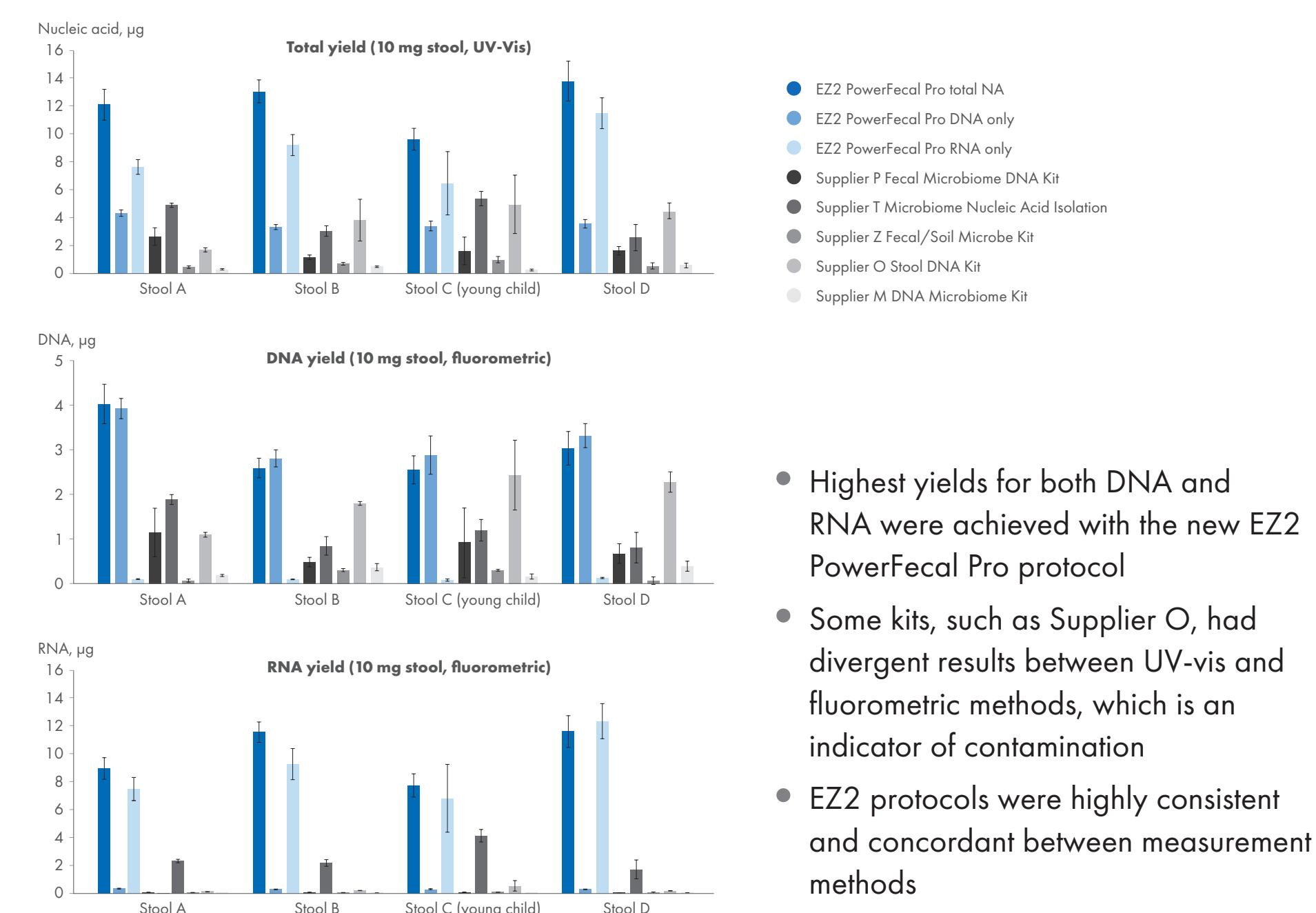


Chemistry improvements streamline workflow



Comparison with other methods: Yield

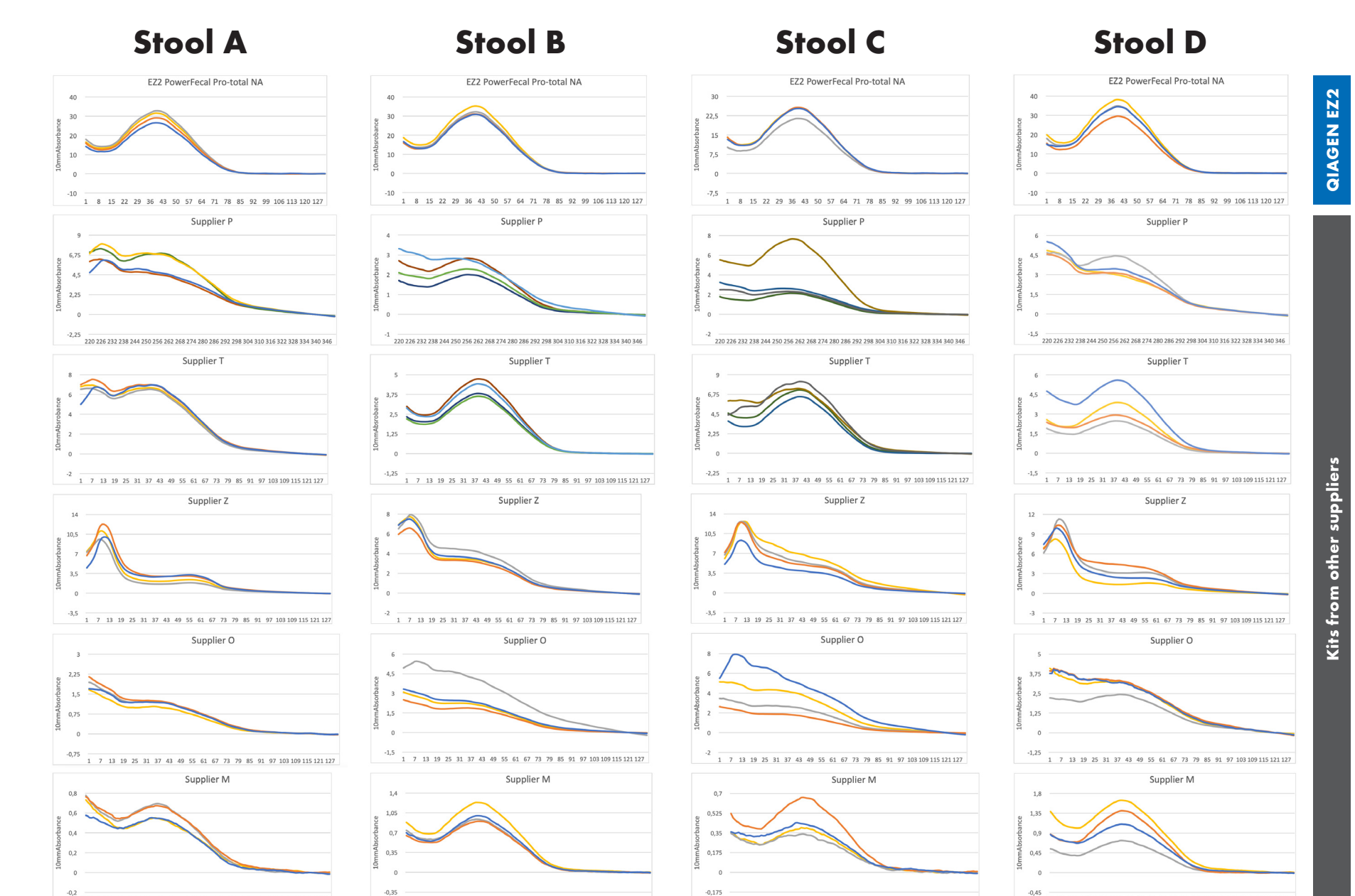
The newly developed method was benchmarked against other magnetic-bead based microbiome or fecal/stool protocols from other suppliers.



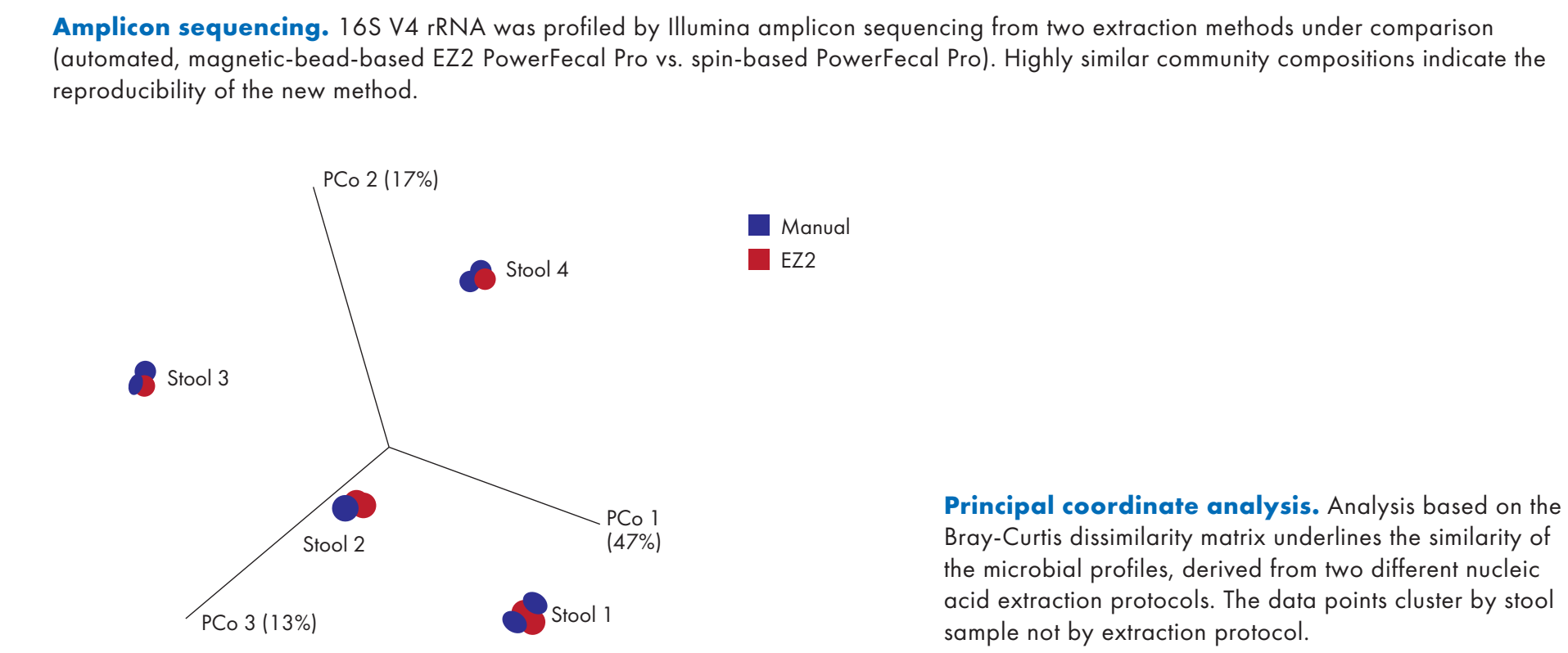
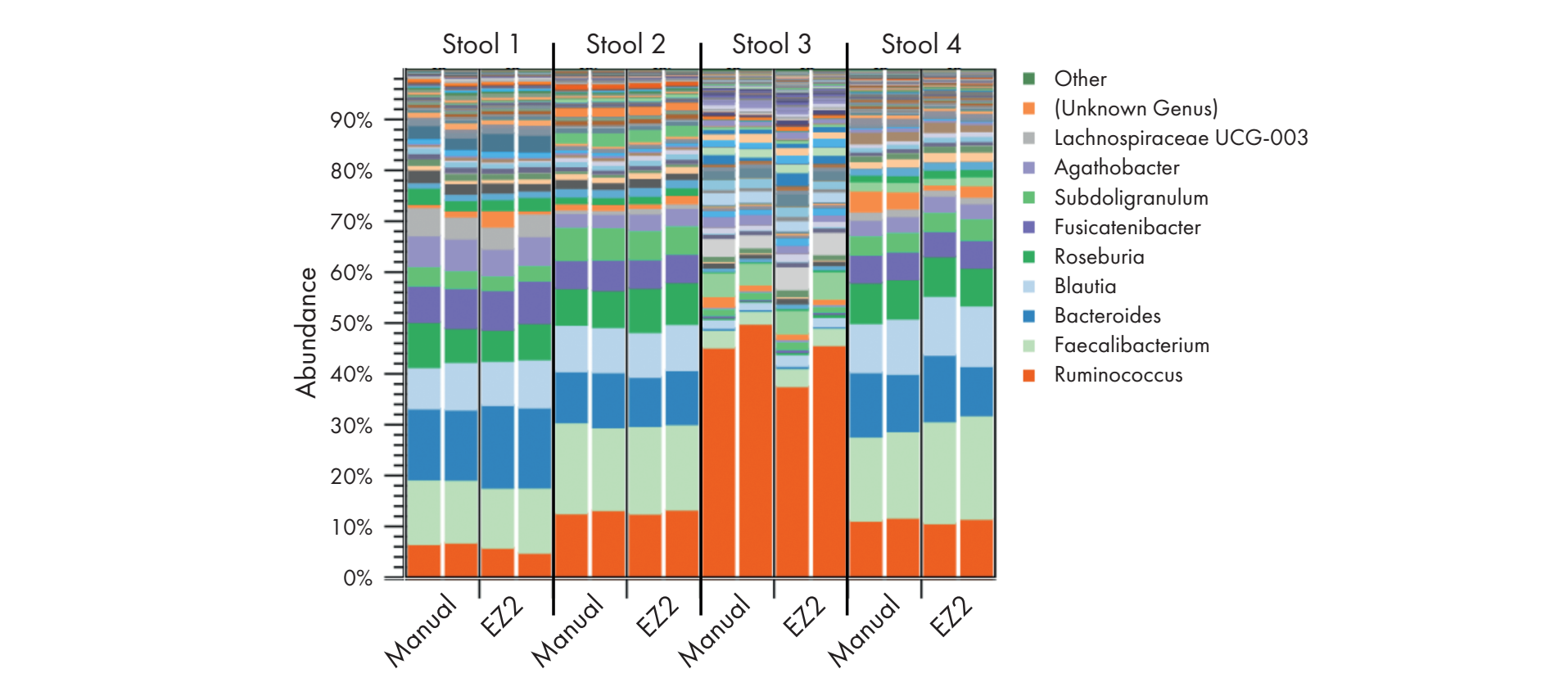
Higher extraction yields than with existing methods. The new and optimized EZ2 PowerFecal method presented here performs better than existing methods.

Comparison with other methods: Purity

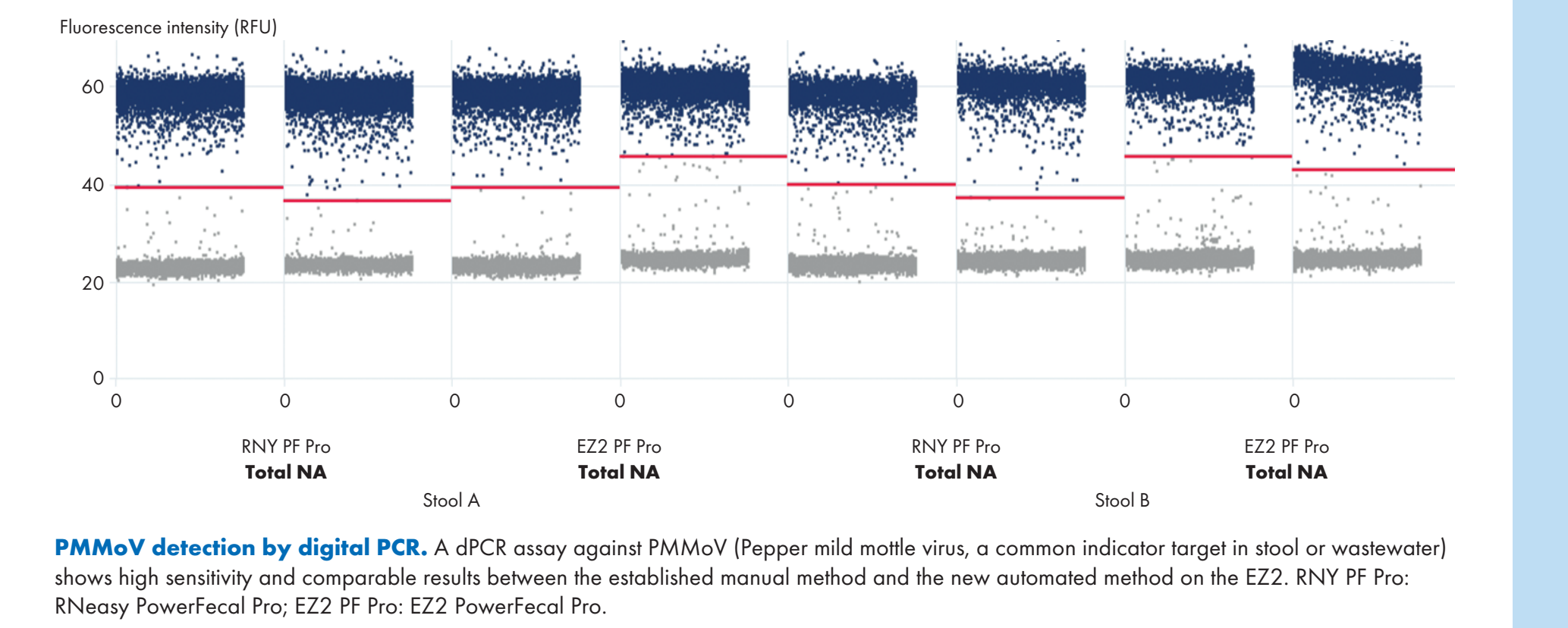
Stool samples are often inhibitory and difficult to extract pure nucleic acid from. This can be seen in the performance of many competing methods. The new EZ2 PowerFecal protocol efficiently removes inhibitors.



Highly consistent sequencing results



Viral extraction verified by dPCR



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.