Automated High-Throughput Extraction of Nucleic Acids from Inhibitor-Rich Samples

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Introduction

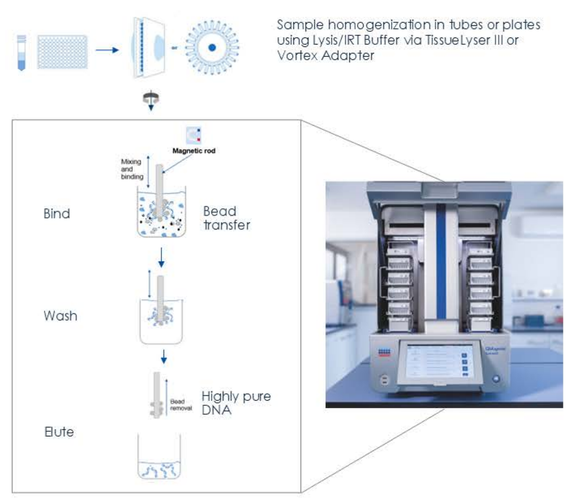
Nucleic acid isolation from stool samples is challenging due to the high abundance of amplification inhibitors, such as bile salts, polysaccharides, lipids and heme. These inhibitors can interfere with downstream molecular analyses when not effectively removed. Although many manual isolation workflows using optimized removal buffers are effective at reducing inhibitory substances, they are often labor-intensive, prone to variability and difficult to scale for high-throughput applications.

This study evaluated the automated, high-throughput platform – QIAsprint Connect – for isolating microbial DNA from inhibitor-rich stool samples. The performance of the automated workflow was assessed in terms of yield, purity, inhibitor removal and compatibility with downstream molecular analysis.

Materials and methods

Stool samples from adult and child donors were mechanically homogenized by bead beating in the presence of Lysis/Inhibitor Removal Technology (IRT) Buffer. Microbial nucleic acids were isolated using a magnetic bead-based purification workflow on the QIAsprint Connect platform. The automated protocol included nucleic acid binding, washing and elution steps and was designed to support high-throughput processing.

Performance of the QIAsprint workflow was compared with that of a manual workflow and a comparative automated high-throughput workflow. Nucleic acid yield and purity were assessed by spectrophotometric and fluorometric measurements. Inhibitor removal was evaluated using a qPCR internal control assay at two DNA input levels. Downstream compatibility was further assessed using digital PCR (dPCR) on the QIAcuity system.

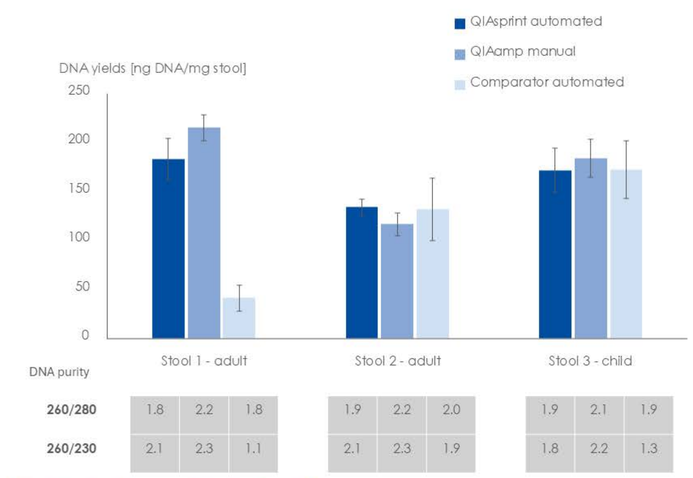


QIAsprint PowerExtract IRT workflow. Stool samples (50–100 mg) were homogenized in PowerBead Pro Tubes (containing zirconium beads) on the TissueLyser III and IRT buffer, followed by magnetic bead-based nucleic acid purification on the QIAsprint Connect instrument. Automated bind, wash and elution steps enabled efficient inhibitor removal and recovery of high-quality nucleic acids suitable for downstream molecular analyses.

High-yield, high-purity DNA was obtained from diverse stool samples

Automated extraction using the QIAsprint Connect platform generated consistently high DNA yields across samples from adult and child donors, with performance comparable to other methods. DNA yields were also reproducible across runs.

Purity metrics for QIAsprint workflow (A260/280 and A260/230 ratios) were within or close to ideal ranges, indicating effective removal of protein, salt and organic contaminants. In contrast, the comparator automated workflow showed reduced purity for some samples, reflected by lower A260/230 ratios.

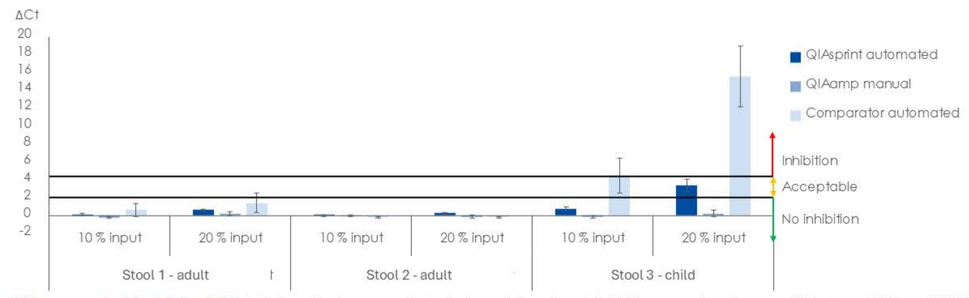


DNA purity									
260/280	1.8	2.2	1.8	1.9	2.2	2.0	1.9	2.1	1.9
260/230	2.1	2.3	1.1	2.1	2.3	1.9	1.8	2.2	1.3

DNA yield and purity across stool extraction workflows. DNA was extracted from three stool samples (n = 4) using the QIAsprint PowerExtract IRT PrepSet in combination with Essential Kit C on the QIAsprint Connect instrument, the manual QIAamp PowerFecal Pro Kit and a comparator high-throughput magnetic bead-based purification workflow with a kit that is recommended for stool samples. DNA yield (ng DNA/mg stool)assesses using Qubit is shown as bar charts for each sample and extraction method, and purity was assessed using A260/280 and A260/230 absorbance ratios.

Automated extraction on QIAsprint effectively removed PCR inhibitors from stool samples

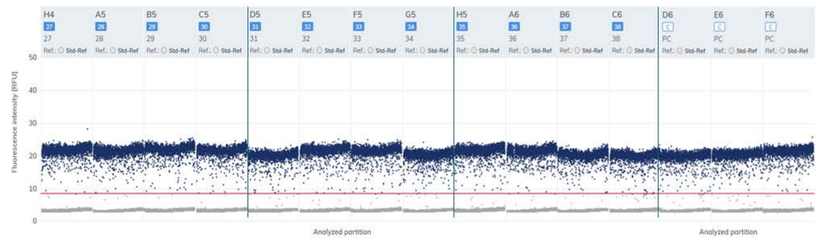
DNA extracted using the QIAsprint Connect platform demonstrated reliable performance, showing no appreciable inhibition in the inhibitor-sensitive qPCR internal control (IC) assay across stool samples. ΔCt values remained below the threshold for detectable inhibition for adult stool samples at both 10% and 20% input levels and for child stool at 10% input. At higher input levels, only mild inhibition was observed for the child stool sample. In contrast, the comparator automated workflow showed strong inhibition of child stool sample at 20% input, indicating reduced inhibitor removal under high-load conditions. These results indicated that the QIAsprint automated workflow consistently generated inhibitor-free DNA suitable for downstream amplification-based research applications.



qPCR assessment of stool-derived DNA. Inhibitory effects were evaluated using an internal control qPCR assay and are shown as ΔCt values at 10% and 20% DNA input levels for three stool samples. ΔCt represents the difference between reactions containing stool-derived eluate and water control reactions; values <2 indicate no detectable inhibition, values between 2 and 4 indicate mild inhibition, and values >4 indicate strong inhibition. DNA was extracted using the QIAsprint workflow, a QIAamp manual extraction workflow or a comparator high-throughput magnetic bead-based purification workflow with a kit that is recommended for stool samples.

DNA extracted using the QIAsprint automated workflow was fully compatible with dPCR

dPCR was performed on the QIAcuity system using an IC assay with up to 100 ng of stool-derived DNA per reaction extracted using the QIAsprint workflow. Consistent amplification of the IC was observed across adult and child stool samples, with fluorescence signal separation comparable to the positive control. The fluorescence intensity difference (ΔRFU) between the positive control and QIAsprint eluates remained below 1 across all conditions, indicating effective inhibitor removal. These results demonstrated that DNA extracted using the automated workflow supported high-sensitivity dPCR analysis across diverse stool matrices.



dPCR assessment of stool-derived DNA. 1D dPCR plots show IC amplification using up to 100 ng of DNA extracted from adult and child stool samples with the QIAsprint PowerExtract IRT workflow on the QIAsprint Connect instrument. dPCR was performed on the QIAcuity system using the QIAcuity UCP Probe PCR Kit and QN IC Probe Assay. Positive Control (PC) reactions are shown for comparison.

Conclusions

The QIAsprint PowerExtract IRT workflow addressed key challenges associated with nucleic acid isolation from inhibitor-rich stool samples by maintaining reproducibility, throughput and data quality across diverse sample types.

- High-throughput processing was achieved with minimal manual handling, supporting scalable microbiome research workflows
- DNA yields and purity were consistently high across adult and child stool samples
- Effective removal of inhibitory substances was demonstrated by inhibitor-sensitive qPCR assays, with minimal inhibition observed even at higher DNA input levels
- Stool-derived DNA extracted using the QIAsprint PowerExtract IRT workflow showed full compatibility with dPCR, supporting high-sensitivity downstream analyses

Overall, the high-throughput automated workflow on the QIAsprint provided a robust and reproducible approach for isolating high-quality microbial nucleic acids from stool with performance comparable to manual extraction and a comparator high-throughput magnetic bead-based purification workflow .

Sample to Insight