Promega

Product Application

DNA purification from human feces with the ReliaPrep™ gDNA MiniPrep System

Isolation of pure, sequencing quality DNA from human feces using the ReliaPrep™ gDNA MiniPrep System.

Kit: ReliaPrep™ gDNA Miniprep System

Analyses: PCR, qPCR, 16s Metagenomic Sequencing

Sample Type(s): Human Feces

Input: Up to 100mg of sample

This protocol was developed by Promega Applications Scientists and is intended for research use only.

Users are responsible for determining suitability of the protocol for their application.

Further information can be found in Technical Manual #TM330, available at: www.promega.com/protocols

or by e-mailing technical services at techserv@promega.com

Materials Required:

- CTAB Buffer, homemade or MC1411
- 100% Isopropanol
- Heat block capable of holding temperatures of up to 95°C
- Disposable pestle (i.e.: 12-141-363 from Fisher Scientific) or other homogenization method such as bead beating (optional)

Protocol:

**Important, pre-warm elution buffer to 70°C.

1. Place up to 250mg of sample (50-100mg is a good starting amount) into a 2ml microcentrifuge tube (click fit or screw top is preferred).
2. Add 1ml of CTAB Buffer and vortex for 30 seconds
3. Heat sample at 95°C for 5 minutes
4. Vortex thoroughly for 30 seconds-1 minute, until large solids are mostly dispersed.
5. If necessary, manually homogenize with disposable pestle or other preferred technique (For example, bead beating 30 sec). Very dry or frozen samples may need this step. Some small intact pieces are OK especially with high fecal input.
6. Add 40µl of Proteinase K and vortex to mix.
7. Incubate sample at 70°C 10 minutes.
8. Spin 5 minutes at max speed.
9. Carefully remove 300µl of supernatant and transfer to a clean 1.5ml microcentrifuge tube.
10. Add 10µl of RNase A and incubate 5 minutes at room temperature. (optional)
11. Add 300µl of Lysis Buffer to supernatant. Gently mix by inversion.
12. Add 600µl of 100% isopropanol and gently invert to mix.
13. Load 600µl of sample to a ReliaPrep™ Binding Column placed in a collection tube, spin for 1 minute at max speed. Discard flow through.
14. Load the rest of sample to the ReliaPrep™ Binding Column, spin for 1 minute at max speed. Place Binding Coulmn into a new collection tube.
15. Add 500µl of Column Wash Buffer (CWD).

16. Spin 2 minutes at max speed and then discard flow through.
17. Repeat steps 15 and 16 twice, for a total of three washes.
18. Place Binding Column in a labeled elution tube. Add 100-200µl of pre-warmed Elution Buffer to Binding Column. Spin 1 minute at max speed.

Example Results:

DNA was isolated from 100mg of human fecal material using the ReliaPrep™ gDNA Miniprep System with the protocol above. DNA concentration was measured by fluorescent dye using the QuantiFluor® ONE dsDNA System (Cat. #E4871) (Figure 1). Purity was calculated by measuring absorbance at 230, 260, and 280nm (Figure 2). Amplification of gram positive and negative bacterial DNA was measured by qPCR amplification (Figure 3). Human DNA from feces was also detected by qPCR with human specific primers (Figure 4). Finally, 16s metagenomic sequencing of the V3-V4 region was performed with DNA isolated from human fecal samples (Figure 5). Pure, amplifiable DNA was isolated from human feces across a variety of biological replicates using the ReliaPrep™ gDNA Miniprep System with the method described above. DNA isolated using this method can be used successfully for a variety of downstream applications.

1.

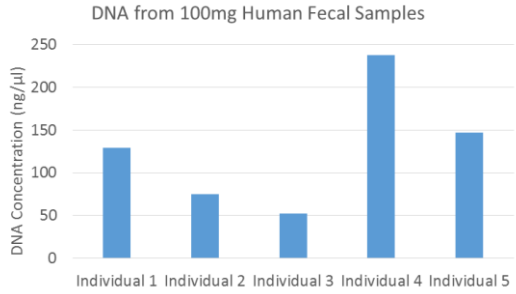
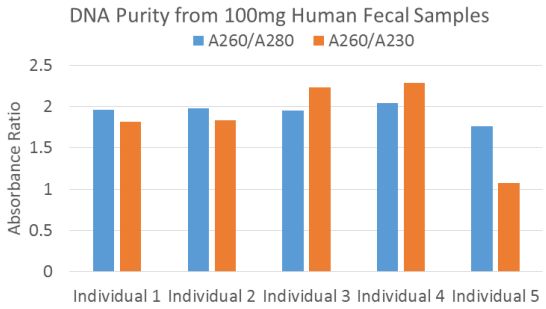


Figure 1. Concentration of DNA isolated from human feces.
DNA concentration was measured using QuantiFluor® ONE dsDNA System (Cat. #E4871). n=5 for all conditions.

Figure 2. Purity of DNA isolated from human feces.
Absorbance at 260, 280, and 230nm and purity ratios were calculated by dividing A260/A280 and A260/230. n=5 for all conditions.

2.



Example Results Continued:

Figure 3. Amplification of bacterial DNA isolated from human feces.
Samples were amplified by qPCR with a gram negative or gram positive specific primer set. Bacterial DNA standard curves were used to calculate concentration by linear regression.

3.

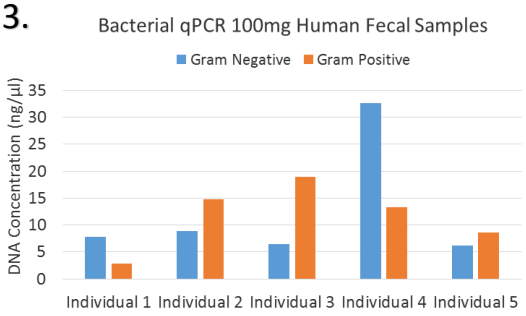


Figure 4. Amplification of human genomic DNA isolated from human feces.
Samples were amplified by qPCR with human DNA specific primer set. A human DNA standard curve was used to calculate concentration by linear regression.

4.

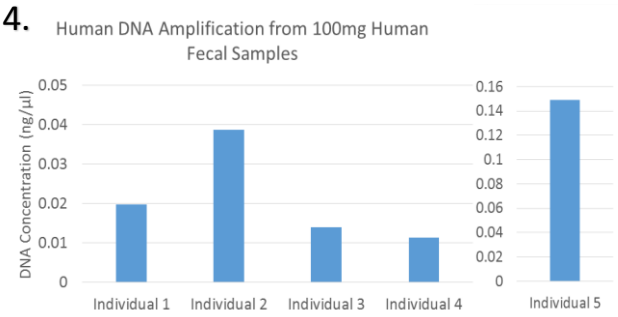


Figure 5. 16s metagenomic sequencing results with DNA from human feces.
Phyla detected by 16s metagenomic sequencing (V3-V4 region of 16s rRNA on an Illumina miSeq) with a relative abundance of greater than 2% are listed for DNA isolations from five individuals.

5.