

DNA isolation from urine using the ReliaPrep™ Blood gDNA miniprep system

Isolate high quality, amplifiable DNA from urine using the ReliaPrep™ Blood gDNA miniprep system

Kit:	ReliaPrep™ Blood gDNA miniprep system (Cat. #A5081)
Analyses:	Absorbance, QuantiFluor® quantification, qPCR, gel
Sample Type(s):	Human urine
Materials Required:	▪ ReliaPrep™ Blood gDNA miniprep system (Cat. #A5081) ▪ 1X PBS ▪ Centrifuge (50ml conical tube capacity)

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

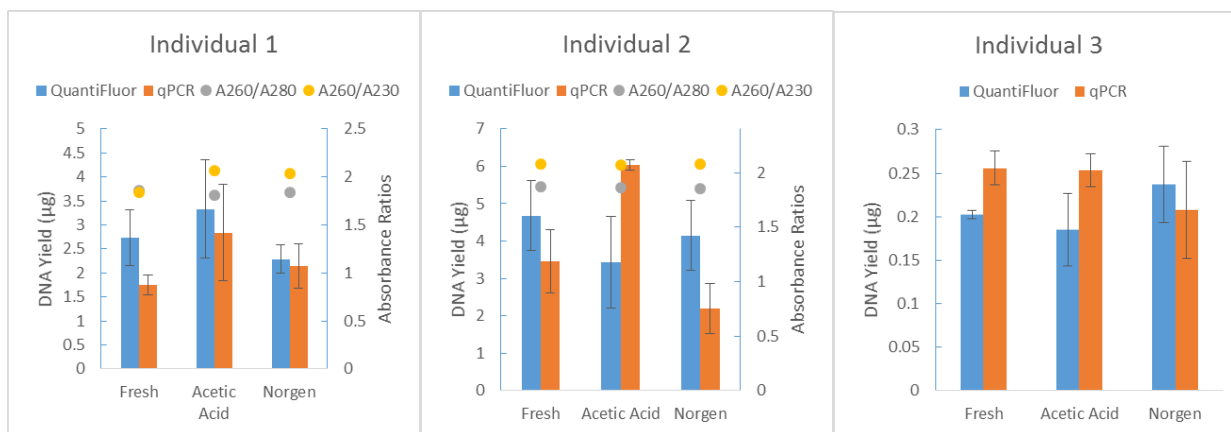
OR contact technical services at techserv@promega.com.

Protocol:

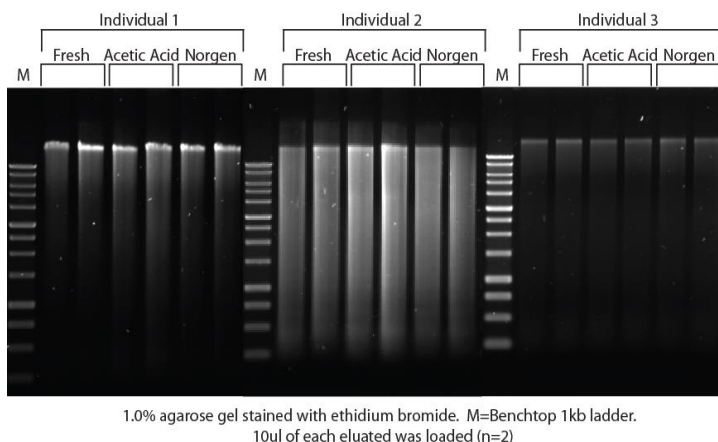
1. Collect urine in a sterile container. Either process urine immediately or add preservative for long term storage. Store urine at 4°C for short term storage.
2. Aliquot urine into 50ml conical tubes (Maximum of 50ml).
3. Spin samples in a centrifuge at 2000 x g for 20 minutes to collect cell pellet.
4. Remove supernatant and suspend pellet in 750µl of 1X PBS.
5. Transfer cell suspension into a 1.5ml microcentrifuge tube.
6. Centrifuge tube at 10,000 x g for 2 minutes to collect cell pellet.
7. Remove PBS and suspend pellet in 200µl 1X PBS and 20µl Proteinase K.
8. Add 200µl of Cell Lysis Buffer and vortex until pellet is suspended.
9. Incubate at 56°C for 10 minutes.
10. Add 250µl of Binding Buffer (BBA) and mix by vortexing for 10 seconds.
11. Transfer liquid portion of the sample onto the prepared binding column.
12. Proceed with the protocol in the technical manual (TM330) to purify the DNA using the ReliaPrep™ minicolumn.

Results: The above protocol was tested with 30ml of urine collected from 3 individuals. Each individual's urine was either processed immediately ("Fresh") or stored over-night in preservative. Preservation methods included 0.5% acetic acid (final concentration) and Norgen Biotek Urine Collection and Preservation Tubes (Cat. #18111). After isolating DNA using the ReliaPrep™ Blood gDNA miniprep system, DNA eluates were assayed for yield (QuantiFluor® ONE dsDNA system-Cat. #E4871 and qPCR), purity (A260/A280 and A260/A230 Absorbance ratios), amplifiability (qPCR), PCR inhibition (qPCR IPC standard), and quality/size (agarose gel electrophoresis). NOTE: Individual 2 had very yellow urine and was collected over 12 hours, Individual 3 had very light colored urine and collected over ~4hrs, Individual 1 had urine color between Individual 1 and 3 and was collected over 12 hours. Individual 1 was male and 2/3 were female. Pellet sizes formed after centrifugation of 30ml of urine were 2>1>3.

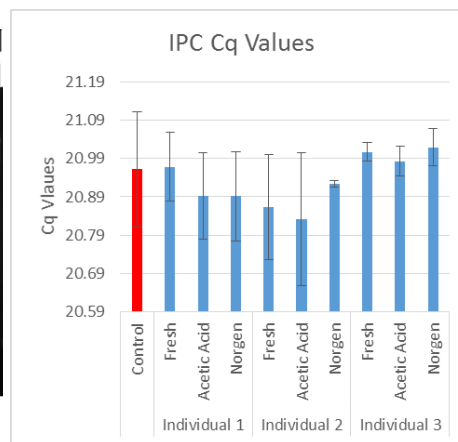
A.



B.



C.



Isolation of DNA from 30ml urine. A. DNA yield as determined by QuantiFluor® ONE dsDNA system and qPCR are displayed as bars (n=3). Absorbance ratios as determined by absorbance measurements are displayed as circles. The absorbance ratios for individual 3 were below instrument limits and therefore not displayed. **B.** Agarose gel analysis - 10µl DNA eluates (n=2). **C.** PCR inhibition testing. Cq values for an IPC (Internal PCR Control) with DNA from purifications show no significant shift compared to control.