GenSolve™ DNA COMPLETE performances

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This study was designed to compare the yield and quality of human genomic DNA extracted from blood and saliva samples collected on GenSaver™ 2.0 and GenSaver™ Color 2.0 cards with GenSolve™ DNA COMPLETE kit and a commercial column-based DNA extraction kit.

Introduction

Nucleic acids and other biomolecules extracted from biological liquids dried and stored on Specimen Collection cards are used for analyses in a variety of applications from Human identification, Genomics, Animal identification to Plasmid screening.

GenSaver™ 2.0 and GenSaver™ Color 2.0 cards are designed for the collection, transportation and long-term preservation of biological materials such as blood, buccal cells, plasma, serum, urine etc., at ambient temperature. Successful downstream analysis of these samples is dependent on the purification of genomic DNA. The use of an appropriate extraction kit ensures that the purified genomic DNA is of good quality and sufficient in quantity for multiple downstream applications such as PCR, sequencing, genotyping and gel analysis.

In this study, we compare the yield and overall quality of genomic DNA extracted by GenSolve™ and a commercial extraction kit from dried blood and saliva spots collected and stored on GenSaver™ 2.0 and GenSaver™ Color 2.0 cards.

Materials and methods

Sample preparation

A volume of 7µl of whole human blood and saliva were spotted on GenSaver™ 2.0 and GenSaver™ Color 2.0 cards respectively and then dried at room temperature for at least 3 hours.

DNA extraction

DNA was extracted using both a column-based DNA extraction kit and the GenSolve™ DNA COMPLETE kit.

The dried blood and dried saliva samples were processed according to the manufacturers' protocols for each
kit. Briefly,

Column based DNA extraction kit Protocol:

1. Place one, two or three, 6mm punches from a DBS or DSS into a RNase/DNase-free 1.5ml microcentrifuge tube and add 180µl of Buffer ATL.
2. Incubate at 85°C for 10min.
3. Add 20µl Proteinase K stock solution. Mix by vortexing and incubate at 56°C for 1h.
4. Add 200µl Buffer AL to the sample. Mix by vortexing and incubate at 70°C for 10min.
5. Add 200µl ethanol (96%-100%) to the sample and mix thoroughly by vortexing.
6. Carefully apply the mixture from step 5 to the spin column (in a 2ml collection tube). Centrifuge at 6000xg for 1min. Place the spin column in a clean 2ml collection tube and discard the tube containing the filtrate.

7. Carefully open the spin column and add 500µl Buffer AW1. Centrifuge at 6000xg for 1min. Place spin column in a clean 2ml collection tube and discard the tube containing the filtrate.
8. Carefully open the spin column and add 500µl Buffer AW2. Centrifuge at full speed 20000xg for 3min.
9. Place spin column in a new 2ml collection tube and discard the tube containing the filtrate. Centrifuge at full speed for 1min.
10. Place the spin column in a new 1.5ml collection tube and discard the tube containing the filtrate. Carefully open the column and add 100µl Buffer AE. Incubate at room temperature for 1min and then centrifuge at 6000xg for 1min.

GenSolve™ DNA COMPLETE, GSC-100
Protocol:

1. Pre-heat Incubator/Shaker to 56°C.
2. For each reaction combine 609µl of Lysis solution with 11µl Proteinase K in 1.5ml screw top tube.
3. Close cap and vortex briefly to mix the solution.
4. Punch up 6mm DBS or DSS elements into a 1.5ml screw top tube containing Lysis solution + Proteinase K.
5. Close cap and place in Incubator/Shaker and shake at 1400RPM for 1.5hours at 56°C.
6. After removing the samples from shaker, place 50µl of elution buffer per sample at 56°C for later use in DNA purification at Step 10.
7. Remove samples from shaker, vortex samples for 5sec then briefly centrifuge.
8. Place spin baskets in 2ml dolphin tubes.
9. Decant DBS and Lysis Solution mixture into spin basket.
10. Close cap and centrifuge at 16000xg or greater for 2min (Discard spin basket and element).
11. To filtrate in 2.0ml dolphin tubes add 20µl of Recovery Solution B per sample.
12. Add 600µl of 100% Ethanol per sample.
13. Close cap and vortex for 5sec then briefly centrifuge.
14. The DNA solution is now ready for the DNA purification protocol.
15. Place DNA column in dolphin tube.
16. Transfer 600µl of DNA solution from step 13 on the previous page to the DNA column.
17. Close cap and spin down at 6000xg for 30sec, discard filtrate.
18. Repeat steps 15 & 16 until all sample has been loaded on the column.
19. Combine 250µl of WS1 and 250µl of WS2 per sample and mix. Add 500µl of Wash 1+2 to DNA column.
20. Close cap and spin down at 6000xg for 30sec, discard filtrate.
21. Place DNA column in new collection tube.
22. Add 500µl of WS3 to DNA column.
23. Close cap and spin tube and column at 6000xg for 3min, discard filtrate. Place tube and column in centrifuge again and spin 16000xg for 1min to dry column.
24. Place spin column in new 2ml collection tube and add 10µl of 56°C Elution buffer.
25. Incubate for 1min at room temperature (25°C).

26. Spin columns at 6000xg for 1min, discard column.

27. Transfer eluent to RNase/DNase free screw cap tube.

DNA quantitation

Extracted DNA was quantified using quantitative PCR performed on a LightCycler 96 from Roche. Reactions were prepared using PerfeCta Fast Mix II from Quantabio with Universal ProbeLibrary Human G6PD Assay according to the manufacturer's instructions. Standard curves for DNA quantification were done using Human Genomic DNA (0.2mg/ml) supplied by Roche.

DNA quality

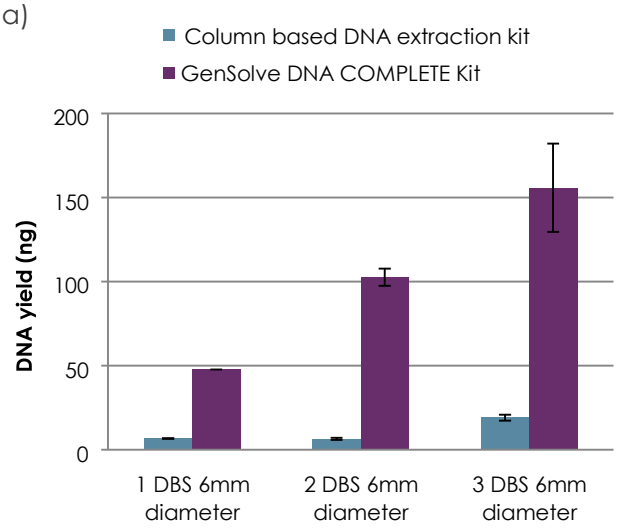
The quality of the DNA was determined by amplification of a 3.8kb fragment of human beta-globin gene using FastStart High Fidelity PCR system from Roche according to the manufacturer's instructions.

Data Analysis

DNA quantification

The total yield of DNA from one, two or three 6mm punches of DBS (Figure 1a) and DSS (Figure 1b) was on an average seven times greater with the GenSolve™ DNA COMPLETE compared to the commercial extraction kit.

1a)



1b)

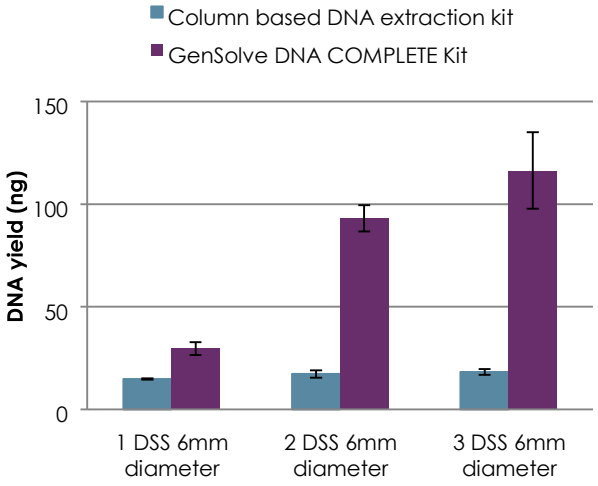


Figure 1: Total DNA yield extracted and purified from three replicates blood spots collected on GenSaver™ 2.0 card (a) or from saliva collected on GenSaver™ Color 2.0 card (b) using commercial extraction kit (light blue bars) *is on average seven-fold less than using GenSolve™* DNA COMPLETE *kits* (purple bars).

Quality assessment by agarose gel electrophoresis of the amplified 3.8kb human beta-globin DNA fragment from DBS samples (Figure 2a) and DSS samples (Figure 2b) indicates more full-length DNA yielded by the GenSolve™ DNA COMPLETE kit compared to the commercial kit.

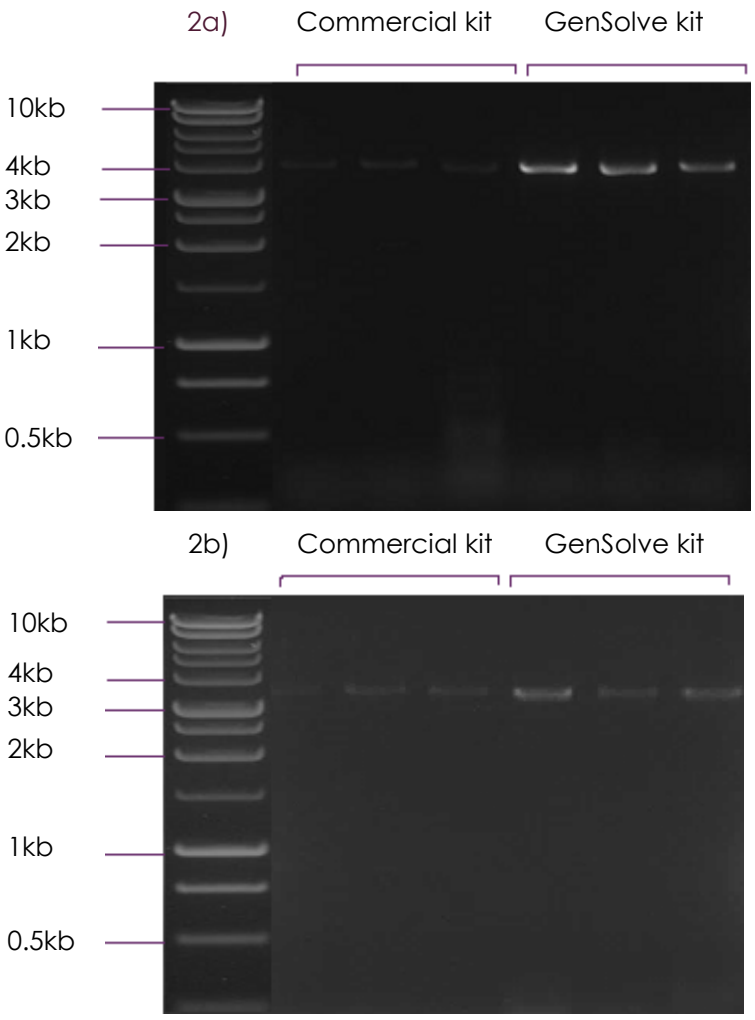


Figure 2: Agarose Gel Electrophoresis (1% TAE 1X) of 3.8kb human beta-globin gene PCR amplification product from dried blood spot stored on GenSaver™ 2.0 card (a) and dried saliva spot stored on GenSaver™ Color 2.0 card (b) using commercial extraction kit and GenSolve™ DNA COMPLETE kit. M: DNA ladder Perfect Plus 1kb

Conclusion

From the data presented in this report, the following can be concluded:

- GenSolve™ protocol provides seven times higher recovery of purified double stranded DNA.
- GenTegra's GenSolve™ DNA COMPLETE Kit yields better quality double stranded DNA.

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