Isolation of Genomic DNA from Human Saliva with VERSA Mini NAP Workstation using Invitek Forensic Kit

Sikander Gill, Rajwant Gill, Richard Zuk and Dong Liang

Aurora Biomed Inc, Vancouver, BC, Canada V6A 1W2

I. Abstract

VERSA Mini Nucleic Acid Preparation / PCR set up workstation has provided an automated solution to the bottleneck problem of having high quality genomic DNA for downstream applications. Genomic DNA was isolated from human saliva using Invitek's InviMag Forensic Kit/KF96 in 96-well format. The gDNA isolation was carried both by automation, and by manual process. The quality of the isolated DNA, and efficiency of isolation process was compared. The high molecular weight isolated DNA was detected running close to 48.5 kb hyper DNA ladder in ethedium detection system. The high molecular band, and fine DNA bands without any streaks indicated no shearing of the molecules in the automation or manual process of the isolation. The automated process was observed to be significantly efficient as no detectable DNA was observed in the wash and extra elution steps except the actual elution steps. An excellent average yield of 18.3 ng/ul of the DNA was obtained with an average purity of ~1.8 based on $A_{260/280}$.

II. Introduction

It is a well known fact that a high quality isolation of genomic DNA for sophisticated downstream applications in the fields like forensic, bio-medical, food, and environmental has been a limiting and time-consuming factor[1]. The higher throughput requirements for high quality DNA samples have lead to the development of new technologies that provide not only the faster DNA isolation but also the ease.

The magnetic bead-based kits involving solid phase reversible immobilization (SPRI) technology have also added ease to the successful isolation of quality DNA. The isolation of DNA with micro magnetic beads is based upon the specific interaction with the ligand (silica, polystyrene, polyvinyl alcohols, primary or secondary antibody, streptavidin, and protein A or G etc) on the bead surface[2].

Aurora Biomed Inc has combined its expertise in automated liquid handling with Invitek's InviMag Forensic Kit/KF96 to offer an automated and fully validated application for forensic quality DNA.

III. Objectives

- Validation of the workstation
- Validation of Invitek's InviMag Forensic Kit/KF96
- Isolation of quality DNA
  - High quality, high molecular weight
  - Ready-to-use DNA for PCR set-up
  - Compatibility with downstream applications
  - Bankable DNA for long term storage
- Hands-free processing
- Capability of isolating genomic DNA from human saliva
- Excellent yield

IV. Materials & Methods

The validation of VERSA Mini Nucleic Acid Preparation / PCR set up Workstation was conducted by equipping the deck as follows:

a. **DNA isolation kit**: InviMag Forensic Kit/KF96 (Invitek, Germany)
b. **Sample preparation**: The original samples of the human saliva were prepared in a 96-well-microplate as outlined in Figure 1.
c. **Deck equipment:** The deck was equipped with tip boxes,, shaker, magnet block, reagent reservoir, sample plate, and plate cooler as shown in Figure 2.
d. **Automation protocol**: The DNA isolation was carried by the designing the protocol depicted in Figure 3.

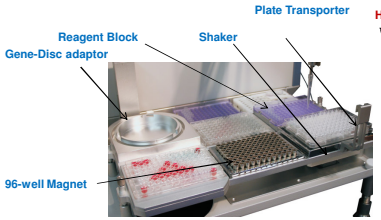


Figure 2. Deck equipment of Versa 1000 for DNA isolation.

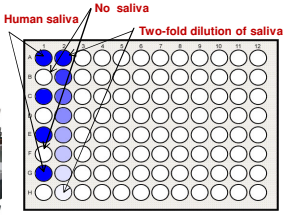


Figure 1. Plate map showing preparation of the human saliva samples

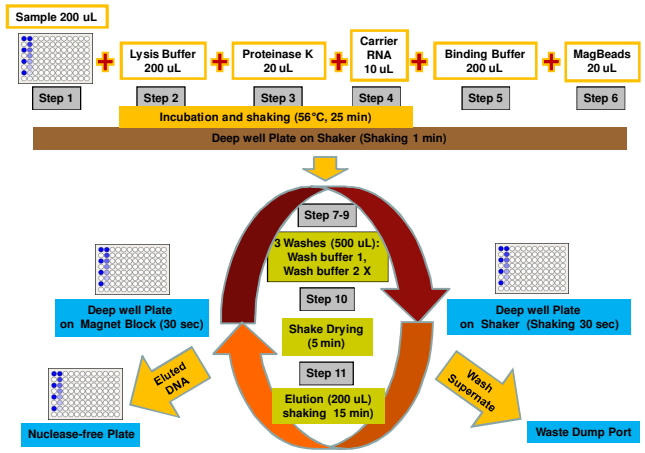


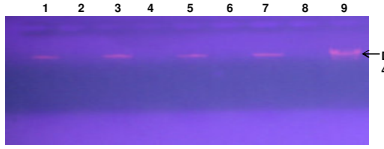
Figure 3. Automation protocol for DNA isolation on VERSA Mini Nucleic Acid Preparation / PCR set up Workstation using Invitek's InviMag Forensic Kit/KF96

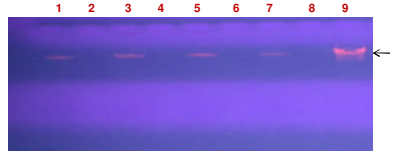
V. Results

Automated Extraction

Manual Extraction

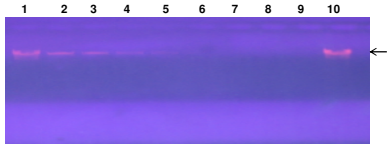
Q 1 What was the quality isolated DNA? Was any cross contamination detected in the process?

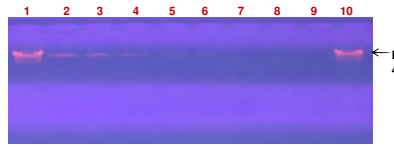




Answer: The high molecular weight DNA bands (lane 1, 3, 5, and 7) running close to the hyper band of 48.5 kb (lane 9) with absence of trailing in the gel in both the automated, and manual extractions indicate a high quality DNA indicating no shearing of the DNA strands during isolation. The appearance of the bands in the agarose gel, and the quantity of DNA in extraction ranged from 17.8 to 19.7 ng/uL with a SD of 0.9 in automated and from 17.2 to 19.8 ng with a SD of 1.0 in the manual process. The purity reflected by $OD_{260/280}$ 1.7 to 1.9 indicated an efficient removal of impurities. The isolated DNA bands in the agarose gel did not show any detectable cross contamination in both the automated and manual extractions processes.

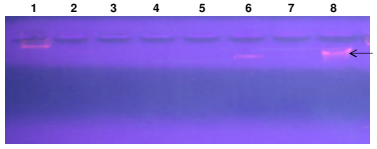
Q 2. What was the DNA isolation profile of serially diluted human saliva samples?

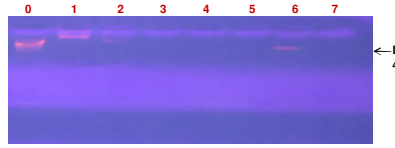




Answer: The two fold serially diluted saliva samples resluted in a similar pattern, and yield of DNA in both the automated ,a nd manual extrcations (lane 2 to 9). The ioslated DNA bands run close to the high molecular weight DNA band of 48.5 kb (lane 1, and 10).

Q 3. How efficient was the automated process of gDNA isolation?





Answer: The DNA bands from the supernate of saliva mixed with lysis buffer, proteinase K, and carrier RNA (lane 1), after binding with magnetic beads (lane 2), washes (lane 3, 4, 5), first elution (lane 6), and elution second (lane 7) show that DNA was efficiently bound to the magnetic beads which did not come off during salt / alcohol washes on the plate shaker, and get efficiently eluted in the first extraction leaving behind no-detectable DNA. Hyper DNA band of 48.5 kb in lane 8, and 0 in automation, and manual extrcation, respectively.

VI. Conclusion

A high quality gDNA can be isolated from human saliva using the kit with VERSA Mini Workstation

VII. Acknowledgements

Technical support from Tony Dong and Victor Navasero is acknowledged.

VIII. References

1. Berensmeier et al. Biotech International, 2007:6-8.
2. Grüttner et al. J Magnetism Magnetic Materials, 2001; 225:1-7
3. Caldarelli et al. Mol Pathol. 1999; 52(3):158–160.

*Correspondence should be addressed to Aurora Biomed Inc., 1001 E. Pender St., Vancouver, BC, Canada, V6A 1W2, Tel: 1-604-215-8700, Fax: 1-604-215-9700, info@aurorabiomed.com, www.aurorabiomed.com