Automation of Genomic DNA Isolation with VERSA 1100 NAP/PCR Setup Workstation using Aurora Biomed Inc's Blood DNA Isolation Kit

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I. Abstract

A high quality genomic DNA preparation is essential for downstream applications. This process was automated using the Versa 1100 Nucleic Acid Purification and PCR Setup Workstation from Aurora Biomed Inc. Aurora's AB96 Magnetic Bind Blood DNA Isolation kit (Cat # MB-7899), was used to isolate genomic DNA from pig's blood in a 96-well format. High, sharp molecular weight DNA bands of approximately 23 kbp in size were detected by agarose gel electrophoresis. Further qualification of the extracted samples was completed by using the purified samples as templates for the amplification of β-actin. Efficient recovery of DNA was indicated by the lack of detectable nucleic acids in both the wash fluids and a second elution. The concentration of isolated gDNA was consistently between 10.0-15.0 ng/µL.

II. Introduction

Many molecular-based applications including PCR, Southern blotting, DNA sequencing and microarrays require DNA of high purity, integrity and quantity. Increasingly higher throughput requirements for these applications have led to the development of new technologies and chemistries for DNA isolation[1-2]. Solid Phase Reversible Immobilization (SPRI) technology, using magnetic bead-based kits, has proven to be a cost effective means of isolating DNA, and is compatible with automated liquid handlers. This method of DNA isolation is based on the interaction between the ligand (i.e. carboxylic acid or silica) that coats the magnetic bead surface and the sample DNA[2]. Aurora Biomed Inc. has validated its VERSA 1100 Nucleic Acid Isolation and PCR setup Workstation with AB96 Magnetic Bind Blood DNA Isolation kit to offer a complete solution for this application.

III. Objectives

- Hands-free processing of blood samples
- Excellent gDNA yields
- Isolation of high quality gDNA that is:
  - ✓ Intact and of high molecular weight
  - ✓ High purity
  - ✓ Ready to use in PCR setup
  - ✓ Compatible with various other down-stream applications
  - ✓ Bankable

Figure 1: AB96 Magnetic Bind Blood DNA Isolation kit Cat # MB-7899

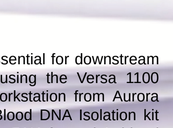

Figure 2: 96 Channel Aspirator

IV. Materials & Methods

The validation of the workstation and the DNA isolation kit chemistry was conducted as follows:

a. **DNA isolation kit**: AB96 Magnetic Bind Blood DNA Isolation kit (Figure 1) (Aurora Biomed Inc, Vancouver, Canada).
b. **Sample preparation**: The original samples of pig blood were placed on the deck in 48 wells of a 96-well-microplate (n=48), magnetic block, reagent reservoir, plate cooler, 96 Channel Aspirator (Figure 2) and loaded with tip boxes (Figure 3).
d. **Workflow and application of 96 Channel Aspirator :** The automation protocol was carried out as described in Figure 4. The 96 Channel Aspirator was employed in the aspiration steps to minimize the total duration of the isolation process.
e. **Programming**: VERSAware software was used to define the automation workflow (Figure 5).
f. **Amplification**: PCR setup was performed using the isolated gDNA for amplification of β-actin. Thermocycling was carried out off the deck, using a MiCycler Thermal Cycler (BioRad Labs, Canada).
g. **QC**: The quality of isolated gDNA ,in terms of its purity and recovery, was then analyzed using agarose gel electrophoresis (Figures 6 & 7) and $Abs_{260/280}$ (Figure 8) using a **Bio-Tek** HT Multi-Mode Microplate Reader (BioTek, Winooski, VT, USA).

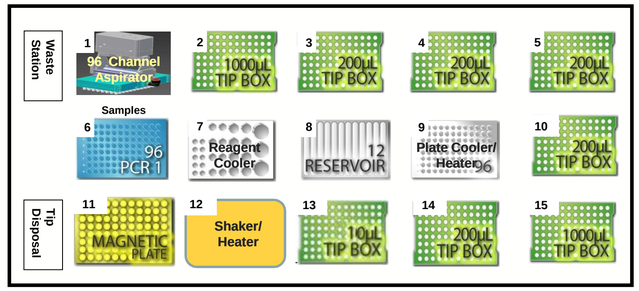

Figure 3: Deck of VERSA 1100 NAP/PCR Setup Workstation.

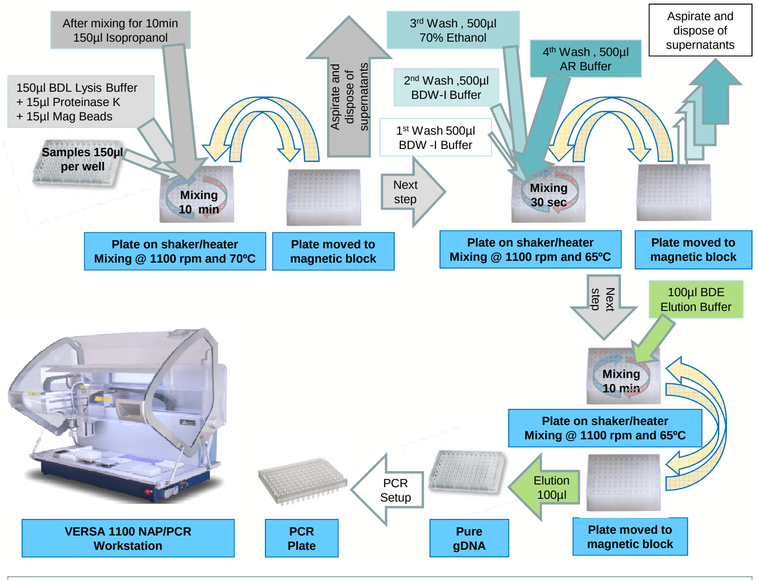

Figure 4: Automation protocol and workflow for gDNA isolation and PCR setup using Versa 1100 Nucleic Acid Purification and PCR Setup Workstation.

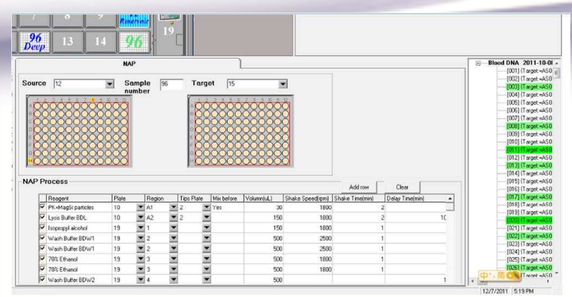

Figure 5: Screenshot of the VERSAware Software Interface

V. Results & Discussion

The data obtained indicates consistent and efficient liquid handling in the isolation process from the VERSA workstation which maximizes the DNA recovery as well as the uniformity of the DNA quality from the samples..

High molecular weight gDNA migrated close to 23kb standard molecular hyper DNA ladder, and smearing of gDNA in the lanes of the agarose gel was not detected with ethidium bromide staining (Figure 6). DNA bands were also equal in their brightness intensity suggesting consistency and reproducibility in liquid handling (Figure 6). This was further supported by Abs_{260} readings giving consistent sample concentrations among different samples in the range of 10.0-15.0 ng/µL, which is acceptable for most downstream applications.

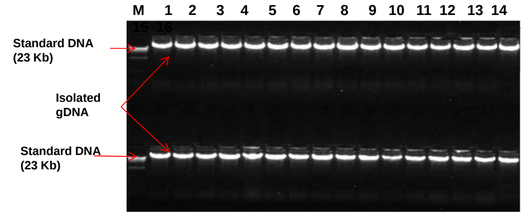

Figure 6: Gel electrophoresis of 32 randomly selected gDNA extracts obtained with the VERSA 1100 NAP/PCR Setup Workstation.

$Abs_{260/280}$ values were between 1.80-1.91 indicating that the isolated DNA was highly pure with insignificant presence of RNA and protein (Figure 7). Low variation in CV% among these values also suggests that all the blood samples were handled with uniformity during the automated process. Additionally, gDNA was not detected in either wash or second elution according to the readouts of Abs_{260}.

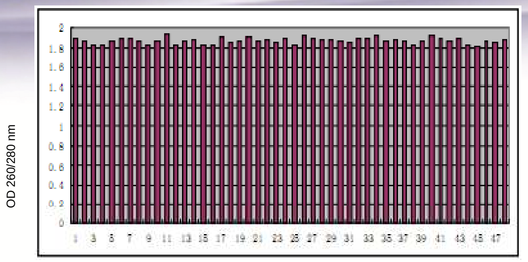

Figure 7: Abs $_{260/280}$ readings from 48 gDNA extracts obtained with the VERSA 1100 NAP/PCR Setup Workstation.

The isolated gDNA was highly representative, since an amplicon from the house-keeping gene β-actin was amplified from each of 16 randomly selected gDNA extracts (Figure 8). The amplicons were consistently represented by clear, intense bands. The absence of smears and additional bands indicates that lack of mis-priming and false positive amplification. The experiment was also validated by the absence of any amplicons from gDNA-deprived negative control (CK). The successful amplification also suggests that Aurora's newly designed buffer, the Alcohol Removing Wash Buffer (ARW) used as an alternative to alcohol drying in the post-alcohol wash step was also very effective.

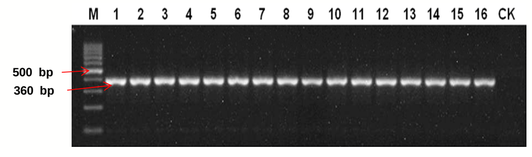

Figure 8: Gel electrophoresis of β-actin amplicons generated using 16 randomly selected gDNA extracts as PCR template.

VI. Conclusion

The VERSA 1100 Workstation is a cost-effective solution for efficient and reproducible automated isolation of high quality gDNA that is characterized by high molecular weight, high yield and suitability for downstream PCR applications as well as any other applications that require high quality gDNA.

VII. Acknowledgements

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VIII. References

1. Hukari et al. *J Lab Autom*. 2011;16(5):355-365.
2. Hellberg et al. *J Lab Autom*. 2011;16(4):308-321
3. Broutian et al. *J. Clin Virol*. 2011;50(4):270-275.

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