

Automation of Genomic DNA Isolation with Nucleic Acid Isolation Workstation

using Promega's Blood DNA Isolation Kit

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I. Abstract

To provide an automated solution to the bottleneck problem of having high quality genomic DNA for downstream applications, Aurora Biomed Inc has validated its Nucleic Acid Isolation Workstation. Using Promega's MagneSil® Blood Genomic kit, genomic DNA in 96-well format was isolated from human embryonic kidney 293 cell line, Chinese hamster ovary cell line, human cheek buccal cells, and human saliva. The high molecular weight isolated DNA was detected running close to 48.5 kb hyper DNA ladder in ethidium detection system. The fine DNA bands without any streaks indicated no shearing of the DNA molecules in the automated process. The isolated DNA presented a panel of genes for which amplification was carried with specific primers. The automated process was observed to be significantly efficient as no DNA was detected in the wash and extra elution steps except the actual elution step. The isolated DNA yield was 4.9µg/500µL of human saliva with an OD_{260/280} of 1.74-1.89.

II. Introduction

A high quality isolation of genomic DNA for sophisticated downstream applications has been a limiting and time-consuming factor. This technically laborious process has improved considerably in the recent years¹. However, higher throughput requirements for high quality DNA samples have led to the development of new technologies that provide faster DNA isolation with ease.

Magnetic bead-based solid phase reversible immobilization (SPRI) technology has also added success to the DNA quality. The isolation of DNA with microbeads is based upon the specific interaction of DNA with the ligand (silica, polystyrene, PVC, primary or secondary antibody, streptavidin, and protein A or G etc) on the bead surface². Aurora Biomed Inc has combined its expertise in automated liquid handling with Promega's MagneSil® Blood Genomic kit to offer an automated and fully validated application.

III. Objectives

- Validation of the workstation
- Validation of MagneSil® Blood Genomic kit
- 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 buccal or HEK-293 cell line or Chinese hamster ovary cell line or human saliva
- Excellent yield & purity

IV. Materials & Methods

The validation of Nucleic Acid Isolation Workstation was conducted as follows: deck positions were equipped as follows:

- a. DNA isolation kit:** MagneSil® Blood Genomic kit (Promega, USA)
- b. Sample preparation:** The original samples of the cells 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, 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.
- e. Amplification:** The single arm of the workstation was used to prepare plate for amplification of isolated DNA samples with thermocycler (BioRad Labs, Canada).

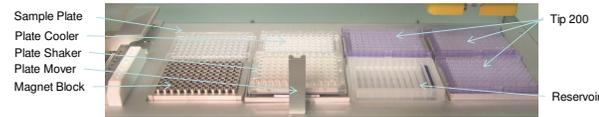


Figure 2. Deck equipment of Nucleic Acid Isolation Workstation.

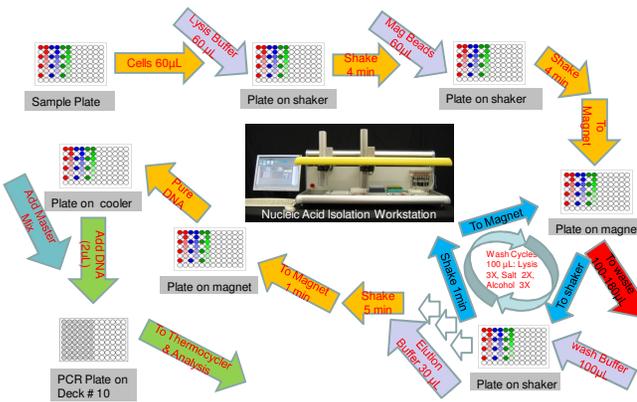


Figure 3. Automation protocol for DNA isolation on Nucleic Acid Isolation Workstation.

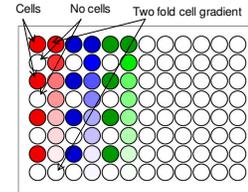
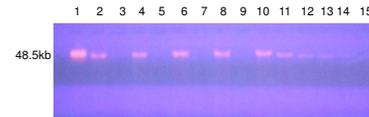


Figure 1. Map of the samples prepared in a 96-well plate containing human embryonic kidney 293 cell line (Red wells), Chinese hamster ovary cell line (blue wells), and human cheek buccal cells (green wells)

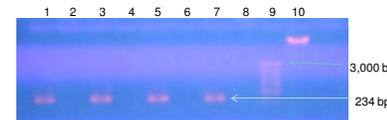
V. Results

- Q 1 (a). Was there any cross contamination of samples?**
(b). What was the DNA isolation profile of serially diluted cells?



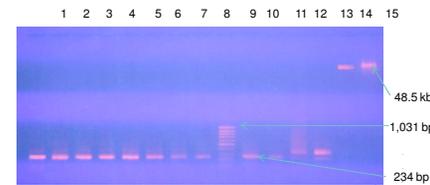
Answer: Agarose gel with genomic DNA from human embryonic kidney cell line showing no cross contamination of wells. The isolated DNA from two fold serially diluted cell samples was not visually detectable from the 5th to 8th dilution due to ethidium-gel detection limitations. Lane 1: Hyper DNA ladder; lanes 2, 4, 6, and 8: Sample aliquots of cells; lanes 3, 5, 7, and 9: No cells; and lane 10-15: Two fold diluted sample cells.

- Q 2: Was any cross contamination detected with PCR?**



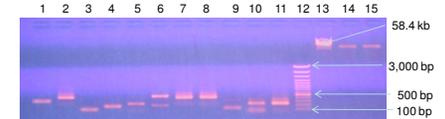
Answer: Cross contamination was also checked by amplifying β-actin gene amplicon from the isolated DNA samples from the wells having cell/no cells. The above picture shown no amplicon bands in lane 2, 4, 6, and 8 from the wells having no cells. Lane 9: 50 bp DNA ladder; lanes 10: Genomic DNA isolated from Human Embryonic Kidney Cell line cells.

- Q 3: Did gDNA get isolated in the serially diluted cell samples in the wells 5th to 8th as explained in answer 1 above?**



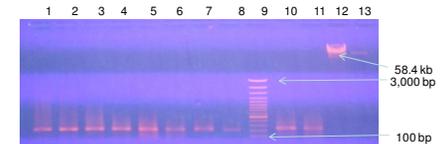
Answer: Yes. The amplification of β-actin gene from the isolated DNA samples from serially diluted wells from 1st to 8th shows that there was sufficient amount of gDNA present in these wells for amplification. The amplification of DNA is also shown from the gDNA isolated from human cheek buccal cells, and Chinese hamster ovary cell line (frozen) cell. Lanes 1-8: Amplicons from serially dilutes cells 1st to 8th; lane 9: 50 bp DNA ladder; lanes 10-11: amplicon from DNA isolated from human buccal cells; lanes 12-13: amplicon from DNA isolated from Chinese Hamster Ovary cell line (frozen); lane 14: Genomic DNA isolated from Human Embryonic Kidney Cell line cells; lane 15: Hyper DNA ladder.

- Q 4. How was the quality of the isolated gDNA?**



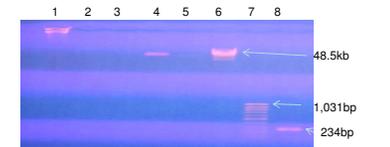
Answer: Quality of isolated gDNA is apparent from the high molecular weight DNA running close to hyper DNA ladder 58.4kb. The isolated gDNA was highly representative as a panel of genes were amplified from the same DNA sample. The gDNA showing no streaks in the lane indicated negligible shearing in the automation. Lanes: 1: Hypoxanthine Phosphoryl Transferase, 2: Transferrin Receptor, 3: β2-Microglobulin, 4: Isomerase Peptidylprolys, 5: glyceraldehyde-3-Phosphate Dehydrogenase, 6: Phosphoglycerate Kinase + Isomerase Peptidylprolys, 7: β-Glucuronidase, 8: TATA Box Binding Protein, 9: Ribosomal Protein, 10: β-actin + β2-Microglobulin, 11: β-actin, 12: 100bp DNA Ladder, 13: Hyper DNA Ladder, 14: gDNA HEK293 Cells, 15: gDNA HEK293 Cells.

- Q 5. Was it successful in isolating gDNA from human saliva?**



Answer: Isolation of gDNA from human saliva is shown as a high molecular weight DNA alongwith amplicons of β-actin gene. Lane s:1-8: β-actin amplicon from the gDNA isolated from two fold serially dilute saliva. Lane 9: 100 bp DNA ladder, lane 10: β-actin from (human buccal gDNA), lane 11: β-actin (human saliva gDNA, manual isolation), lane 12: Hyper DNA Ladder, and lane 13: gDNA from human saliva.

- Q 6. How efficient was the automated process of gDNA isolation?**



Answer: The above figure shows the 1st supernate from each of the steps of gDNA isolation where no DNA was detected except the elution step. Moreover, the repeat of the elution step did not show any DNA indicating the automated process significantly efficient. Lane 1: Supernate of lysis step, 2: Salt wash step-I, 3: Alcohol wash step-I, 4: Elution step-I (Main), 5: Elution step-II (optional), 6: Hyper DNA ladder, 7: The 50 bp DNA ladder, 8: PCR amplicon of β-actin gene.

VI. Conclusion

A high quality gDNA can be isolated with this Workstation.

VII. References

1. Berensmeier et al. Biotech International, 2007:6-8.
2. Grüttner et al. J Magnetism Magnetic Materials, 2001; 225:1-7