Automated Isolation of Genomic DNA using the MACHEREY-NAGEL NucleoMag® Blood 200µL kit by Aurora Biomed's VERSA 1100

I. Summary

The isolation of high-quality genomic DNA is essential to numerous research and diagnostic workflows. DNA extraction from a large number of blood samples is labour intensive and is often a bottleneck in these workflows. To address this, MACHEREY-NAGEL and Aurora Biomed have teamed up to provide an efficient, flexible automation solution for extracting DNA from blood samples. MACHEREY-NAGEL's NucleoMag® Blood 200µL kit is engineered to provide rapid and reliable genomic DNA isolation from whole blood samples (fresh, stabilized with EDTA or with citrate). The optimized chemistry of the reagents ensures pure DNA is selectively bound to the paramagnetic bead particles, and impurities are efficiently removed by a series of quick wash steps. The resulting high quality DNA is eluted with elution buffer or water. Purified DNA is then ready for use in downstream applications such as PCR, enzymatic digestions, Next Generation Sequencing (NGS), etc..

Once 200µL aliquots of blood have been transferred to a 96-well plate, Aurora Biomed's VERSA 1100 automated liquid handling platform is capable of automating the entire DNA extraction workflow. The VERSA 1100 streamlines the workflow and increases sample throughput, while also freeing up staff to carry out more vital tasks. For the purpose of this validation, DNA was isolated from pigs' blood stabilized with EDTA. DNA yields of up to 21µg of pure, high quality DNA were achieved.

II. Materials and Methods

Equipment and DNA extraction kit

The NucleoMag® Blood 200µL kit (MACHEREY-NAGEL GmbH, Germany) has been optimized to isolate pure, high quality genomic DNA. This kit allows for variable sample numbers (1-96 samples) to be processed at a given time and has been designed with automation of the workflow in mind.

The VERSA 1100 automated liquid handing platform (Aurora Biomed Inc, Canada) is a flexible, open system that can be equipped with a 4-, 8- or 96-channel pipette head that include single-channel functionality (Figure 1).

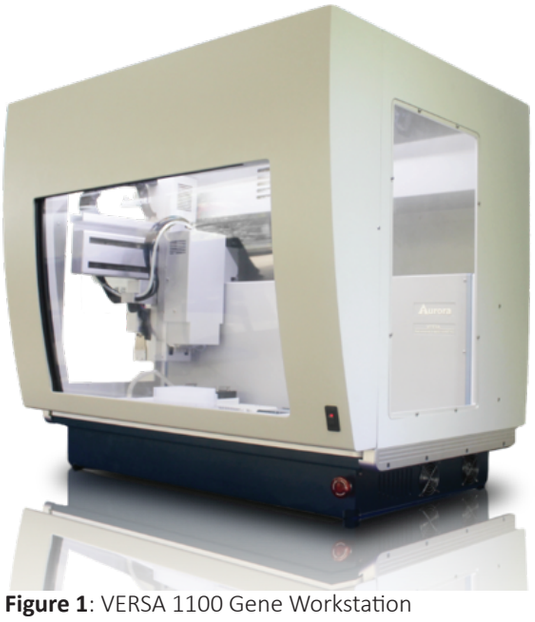

Figure 1: VERSA 1100 Gene Workstation

Automated workflow

The separation plate holding the 200µL blood samples is placed on the deck of the VERSA 1100 from which point the entire DNA extraction workflow is automated. Paramagenetic bead addition, DNA binding to the beads, wash steps and elution of the purified DNA were performed as recommended by the NucleoMag® Blood 200µL kit user manual. The VERSA 1100 deck configuration (Figure 2) and the developed automation program are optimized to maximize DNA yields and reduced the risk of cross-contamination.

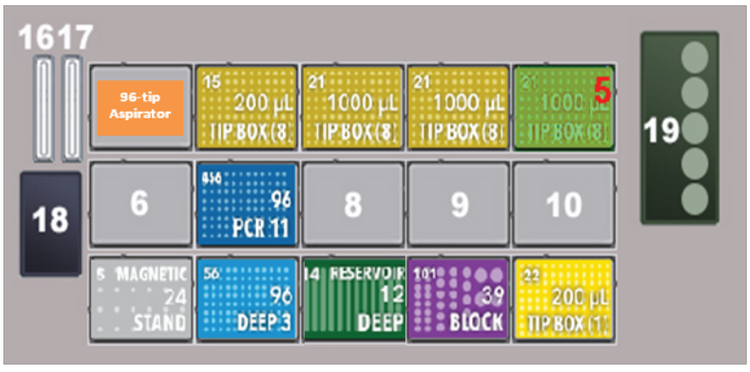

Figure 2: The deck layout of the VERSA 1100 used to automate the NucleoMag® Blood 200µL genomic DNA extraction workflow. Position #17 is the liquid waste disposal site, #18 is the tip disposal chute and #19 is the ReagentDrop bulk reagent dispensing system that is part of the robotic pipette head.

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III. Results

The NucleoMag® Blood 200µL kit is designed with automation in mind and compliments the VERSA 1100's powerful automation capabilities to reliably purify genomic DNA from a variety of blood sources. To validate the automation of the NucleoMag® Blood 200µl kit, genomic DNA was isolated from 48 pigs' blood samples containing EDTA.

The DNA yields averaged across the 48 samples was 11.4µg (Table 1). High purity of the isolated DNA was demonstrated by an average A260/A280 nm ratio of 1.75 across the 48 samples (Table 1).
The processing time for 48 samples is 120min.

Table 1: Summary of the DNA yields recovered from pig blood using the NucleoMag® Blood 200 µL kit. DNA was quantified using a Qubit 3.0. The final elution volume is 100 µL.

Sample	DNA Yield (µg)	DNA Conc. (ng/µL)	DNA Purity (260/280)	Sample	DNA Yield (µg)	DNA Conc. (ng/µL)	DNA Purity (260/280)
1	7.08	70.8	1.70	**25**	18.8	188	1.78
2	11	110	1.73	**26**	13.4	134	1.75
3	11.5	115	1.72	**27**	10.9	109	1.72
4	16.8	168	1.74	**28**	12.1	121	1.73
5	11.8	118	1.74	**29**	10.9	109	1.73
6	10.1	101	1.76	**30**	10.8	108	1.70
7	5.04	50.4	1.75	**31**	8.56	85.6	1.79
8	9	90	1.83	**32**	11.5	115	1.84
9	14.4	144	1.74	**33**	12.9	129	1.79
10	10	100	1.78	**34**	11	110	1.73
11	10.2	102	1.80	**35**	14	140	1.77
12	8.68	86.8	1.71	**36**	10.9	109	1.68
13	8.8	88	1.72	**37**	12.2	122	1.73
14	7.08	70.8	1.71	**38**	12.2	122	1.72
15	7.4	74	1.71	**39**	13.6	136	1.76
16	11.2	112	1.76	**40**	11.7	117	1.76
17	5.72	57.2	1.79	**41**	21.2	212	1.73
18	7.52	75.2	1.78	**42**	15.2	152	1.77
19	8.76	87.6	1.75	**43**	19.2	192	1.71
20	8.48	84.8	1.78	**44**	16.6	166	1.75
21	9.12	91.2	1.78	**45**	12.6	126	1.67
22	10.7	107	1.80	**46**	12.5	125	1.70
23	8.16	81.6	1.79	**47**	13.7	137	1.75
24	15.7	157	1.76	**48**	6.84	68.4	1.78
Average for 48 samples					**11.4**	**114**	**1.75**

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III. Results cont.

The quality of the isolated DNA was determined by resolving the extracted DNA samples in a 1% agarose gel. High molecular weight DNA was successfully recovered for all DNA extract irrespective of their origin, this is indicated by the single bright band observed in the agarose gel (Figure 3).
The DNA purified by automating the NucleoMag® Blood 200µL kit is suitable for use in various downstream applications.

To test for the presence of inhibitors in the eluted DNA samples, 16 of the eluted DNA samples were randomly selected and used as the templates for PCR reactions that amplified a 334bp fragment of the house keeping gene, encoding for β-Actin (Figure 4). The success of the PCR amplification of the β-Actin encoding gene fragment demonstrates the absence of inhibitors for downstream uses of the purified DNA.

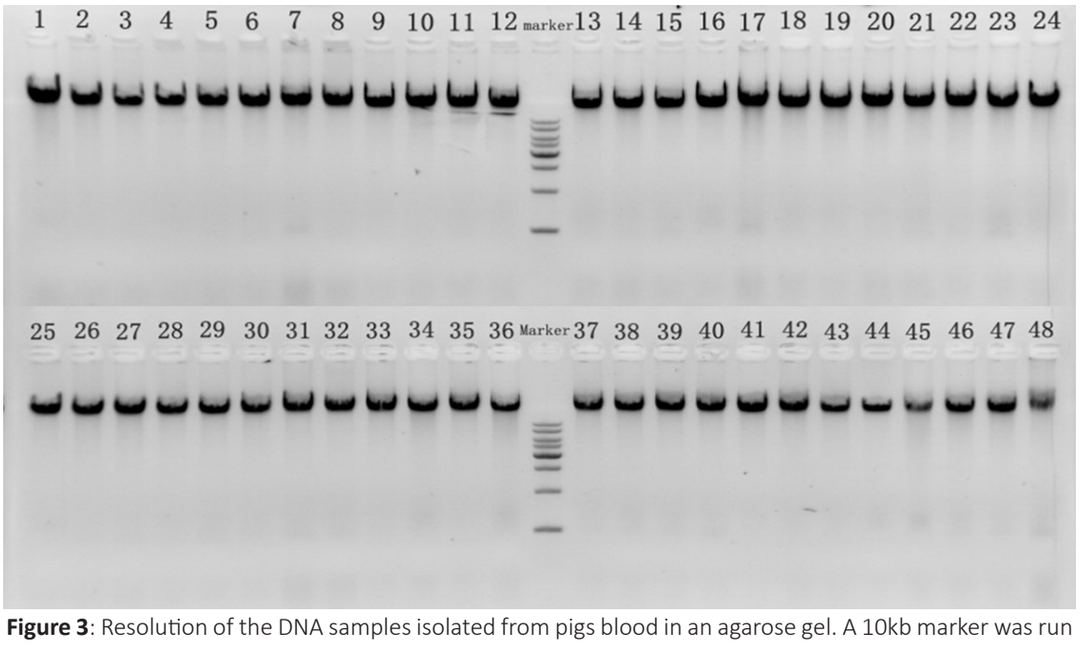

Figure 3: Resolution of the DNA samples isolated from pigs blood in an agarose gel. A 10kb marker was run with the samples.

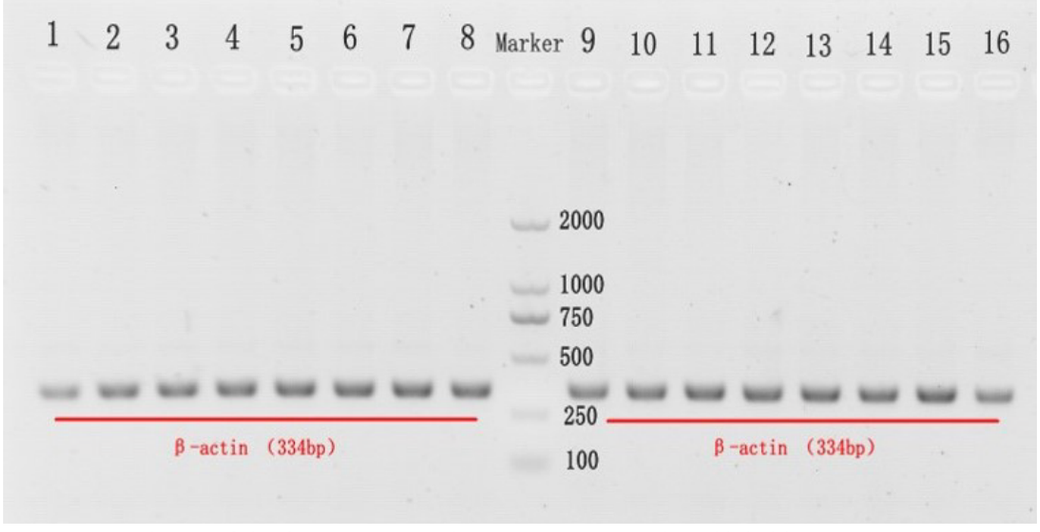

Figure 4: Resolution of the β-Actin PCR product in an agarose gel. A 2kb ladder was run with the PCR products

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IV. Conclusion

Combining the power of MACHEREY-NAGEL's NucleoMag® Blood 200µL kit with the precision of Aurora Biomed's VERSA 1100 automated liquid handling platform provides a walk away solution for the isolation of pure, high quality genomic DNA from blood samples. Moreover, the instrument minimizes the risk of manual errors and cross-contamination.

The VERSA 1100 increases reproduibilty and the number of samples that can be processed at any given time.

VERSA 1100's flexibility allows users to automate DNA extraction, PCR setup, sample normalization and general liquid handling applications on the same workstation.

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