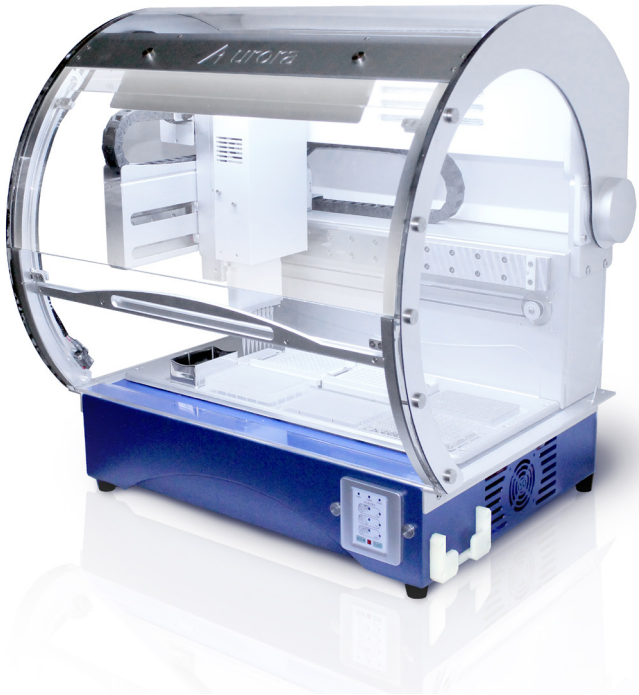
Aurora

VERSA 10

AUTOMATION OF THERMO FISHER SCIENTIFIC MAGMAXDNA USING VERSA10 LIQUID HANDLING WORKSTATION

UNIVERSITY OF WATERLOO



CIRTN Canadian Islet Research and Training Network
R2FIC Réseau de recherche et formation sur les îlots du Canada

- **Enhanced efficiency: Reduced hands-on time and increased throughput**
- **Precision and accuracy: Reduction in pipetting errors**
- **Consistency and reliability: Standardized workflow for improved**

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Automation of Thermo Fisher Scientific MagMAXDNA using VERSA 10 liquid handling workstation

The MagMAX DNA Multi-Sample Ultra 2.0 Kit (MMDNAT) uses magnetic beads to isolate genomic DNA (gDNA) from various biological samples. This produces high-quality DNA that can be used in various molecular biology applications, such as genotyping, sequencing, and PCR reactions. Automating the workflow requires protocol steps preparation on the software carefully and validating the instrument thoroughly in the laboratory. Aurora's VERSA 10 has been automated for a magnetic bead-based method of nucleic acid purification, which has been validated by the University of Waterloo for compatibility with the MMDNAT kit. This method can produce high-purity DNA yields and is a useful tool for laboratories that perform molecular biology research.

Aurora's VERSA series of automated liquid handling workstations are designed to automate different extraction protocols on a user friendly software known as VERSAware. VERSA 10 covers the low to medium throughput requirements with 6 deck positions (Figure 1). Utilizing air displacement pipetting technology, it offers precise and accurate handling of various liquid volumes. This application note provides a detailed guide on how to automate the MMDNAT kit using scripts specifically designed on the VERSA 10.

FIGURE 1. VERSA 10

The VERSA 10 deck layout enables the automation of every step involved in magnetic bead-based nucleic acid purification. It incorporates six positions for tip boxes, a 96-well PCR plate, a 96-deep well plate, a reservoir, a magnetic block, and a shaker, as well as a tip chute and liquid waste (Figure 2).

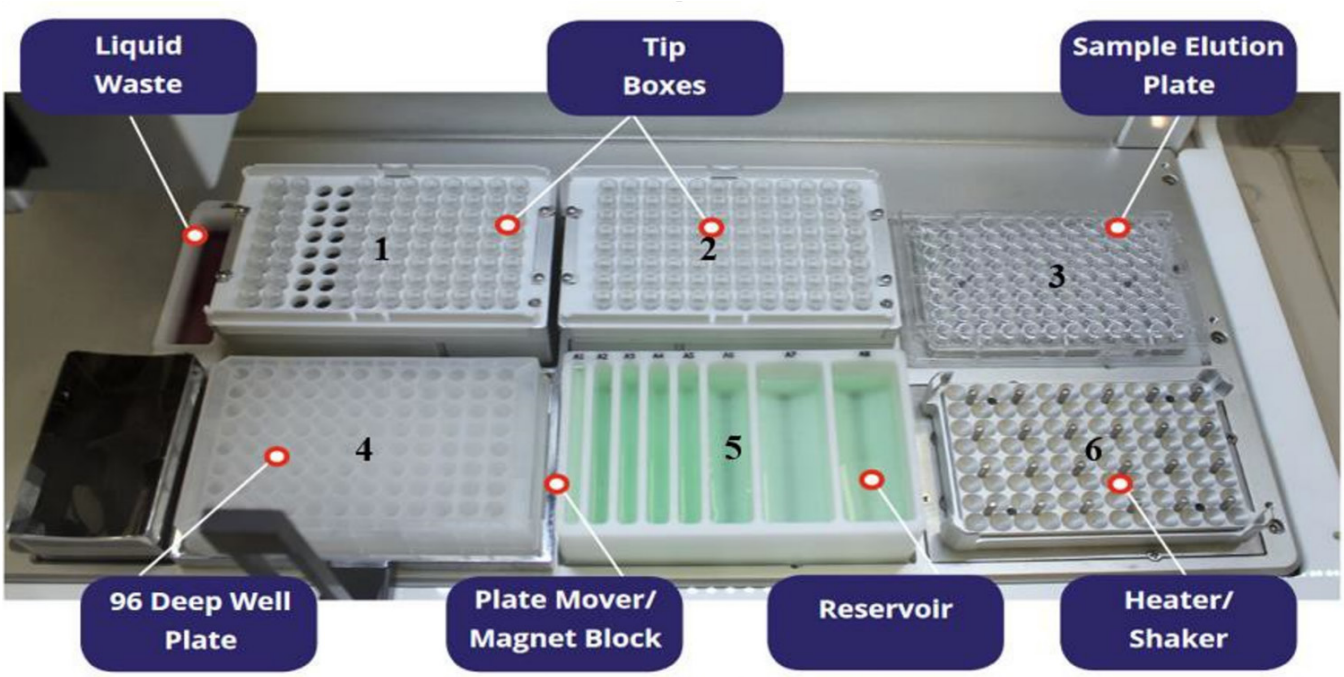

FIGURE 2. Deck layout of VERSA 10

MagMAX Magnetic bead–based DNA extraction steps:

1. Add the sample to a tube or well plate that contains the magnetic beads (sample preparation)
2. Mix the sample and beads together to allow the nucleic acids to bind to the beads
3. Place the tube or well plate in a magnet to separate the beads from the sample
4. Remove the supernatant and discard it
5. Wash the beads to remove any remaining contaminants
6. Elute the purified nucleic acids from the beads by adding a buffer solution and shaking
7. Collect the eluted nucleic acids for further analysis

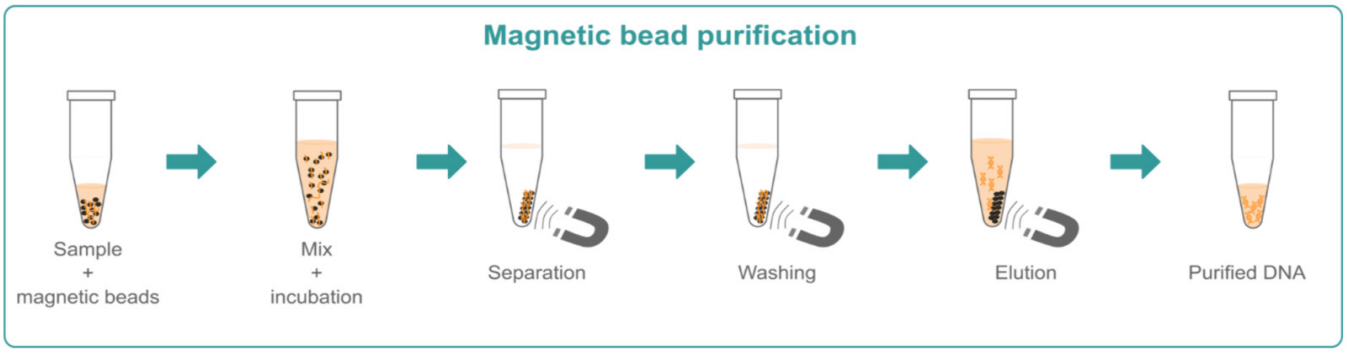

FIGURE 3. Magnetic bead based purification of nucleic acids

1. Automation protocol of MagMAX kit requires following steps:

- Ensure that all the modules are selected properly on the software
- DNA binding reagents/solutions are in the reservoir
- Consumables are on place
- Tip boxes are filled
- The tip chute is attached to the bio-hazard bag

2. Sample preparation

With reference to the overnight proteinase K digestion, transfer the reagents into the reservoir and start heating of Heater Shaker module (Deck position 6) (Figure 3)

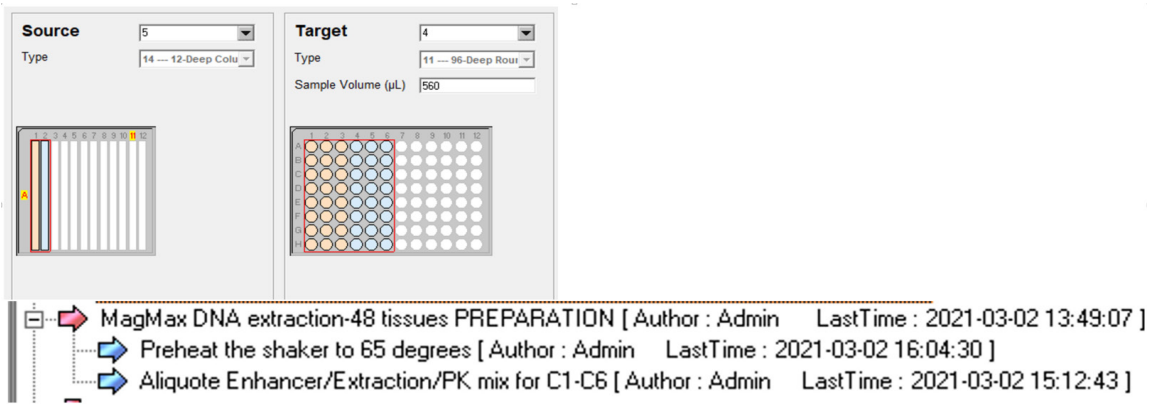

Figure 4. An overlook at the 'Assay' and 'Sequence' in VersaWare. A red arrow represents an 'Assay' while the blue arrow represents a 'Sequence'

- Load 528 µl of Enhancer Solution into well 1 and 2 of the reservoir.
- Load 1056 µl of Proteinase K into well 1 and 2 of the reservoir.
- Load 13.2 ml of Extraction Buffer into well 1 and 2 of the reservoir.
- The robotic arm will transfer the liquid based on the script from the reservoir to the specified wells of the deep well plate.
- Once the liquid has been transferred, placing the plate on the heating block.
- Incubate the plate at 65^{0}C overnight. Leave the instrument powered on and close the front hood before leaving.

3. DNA extraction on tissue samples

- Place the PCR plate on the adapter at deck sample elution plate.
- Manually transfer 400µl of the supernatant from each type of the tissue samples into the other half of the plate (Refer to page 6, part 4, step a of the MMDNAT kit manual).
- Then manually load following reagents:
  - o 66 µl of RNase A will be loaded into each well of column 1 on the qPCR plate of deck position 3

- 11.6 ml of Binding Bead Mix (refer to page 6 part 3 of the MMDNAT kit manual for details) will be loaded into well 1 & 2 of the 12-channel reservoir
- 17.6 ml of Wash Solution I will be loaded into well 3, 4 & 5 of the reservoir
- 17.6 ml of Wash Solution II will be loaded into well 6, 7 & 8 of the reservoir
- 13.2 ml of Wash Solution III will be loaded into well 9 & 10 of the reservoir
- 10.6 ml of Elution Solution will be loaded into well 11 of the reservoir

- Automate transfer of 10 µl of RNase A from PCR plate into the 96-well sample plate. Then transfer of plate to shaker and will shake the plate at 1800 rpm for 5 minutes. (Refer to page 6, part 4, Step b of the MMDNAT kit manual).
- Automate transfer of Binding Bead Mix from the reservoir to 96-well sample plate. The plate will be shaken at 2500 rpm for 5 minutes at shaker and then transfer to the magnetic plate for 5 minutes to pull down beads.
- After shaking, the robotic arm will remove the liquid while the magnetic beads are still attached to the bottom of the deep well plate. (Refer to page 4, steps data of the Flex_Protocol details).

4. Washing

- The robotic arm will dispense Wash Solution I from the reservoir to the 96-well sample plate.
- The plate will be shaken at 2500 rpm for 5 minutes and then stands on the magnet for 5 minutes to pull down beads. Then the robotic arm removes the liquid from previous step.
- The arm will add Wash buffer II from the reservoir to the 96-well sample plate.
- The plate will be shaken at 2500 rpm for 5 minutes and then stands on the magnet for 5 minutes to pull down beads. Then instrument removes the liquid from previous step.
- The arm will Wash Solution III from the reservoir to the 96-well sample plate.
- The plate will be shaken at 2500 rpm for 5 minutes and then stands on the magnet for 5 minutes to pull down beads. Then instrument removes the liquid from previous step.

5. Elution

- The shaker will be heated up to 65^0Cfirst. Then addition of Elution buffer from the reservoir to the 96-well plate. Finally, plate will be shaken under 65^0C at 2500 rpm for 5 minutes and then stands on the magnetic plate for 5 minutes to pull down the magnetic beads.
- Automated transfer of eluted samples from the 96-well plate to the qPCR plate. We only transfer 150 µl of the lysate, if needed, the volume can increase to 200 µl.
- Automated transfer of positive control and negative control into the qPCR plate.

After the automation of the whole protocol, transfer the final qPCR plate onto the thermocycler machine to proceed to the next workflow.

Conclusion:
Experience of Jamie

The Aurora VERSA 10 has allowed us to save time and improve efficiency with several commonly run assays in the laboratory, including ELISA assay setup and genotyping. The ability of the VERSA 10 to perform all steps of our genotyping protocol, including tissue digestion, DNA isolation, and PCR plate setup, has alone saved lab members a week of work per month, enabling them to focus on more essential tasks, such as data collection for their research. The utilization of the VERSA 10 as opposed to manual pipetting has resulted in a notable decrease in error rate and cross- contamination.

Acknowledgements

We extend our special gratitude to Associate Professor Jamie Joseph for his invaluable contributions to the internal validation process. His expertise and dedication have greatly enhanced the quality and accuracy of our instrument, and we are truly grateful for his involvement.