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

I. Summary

DNA extraction from plant materials is routinely a pain point for researchers due to the laborious, inherit difficulties associated with processing plant samples. To address this, MACHEREY-NAGEL and Aurora Biomed have teamed up to provide an efficient, flexible automation solution for extracting DNA from plant tissues. MACHEREY-NAGEL's NucleoMag® Plant kit is engineered to provide rapid and reliable genomic DNA isolation from a variety of plant tissues. The paramagnetic beads efficiently bind DNA from 20-50mg of homogenized plant material. The optimized chemistry of the reagents ensure 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.

After the plant tissue has been homogenized, Aurora Biomed's VERSA 1100 automated liquid handling platform is capable of automating the entire DNA extraction workflow, apart from the single centrifugation step. The VERSA 1100 streamlines the workflow to increases sample throughput and free up staff to do technically demanding research. For the purpose of this validation, DNA was isolated from pumpkin, maize and wheat leaves. The average DNA yields from pumpkin, maize and wheat samples were 3.3µg, 2.3µg and 7.8µg, respectively.

II. Materials and Methods

Equipment and DNA extraction kit

The NucleoMag® Plant kit (MACHEREY-NAGEL GmbH, Germany) can process homogenized tissue samples of up to 50mg to isolate pure, high quality genomic DNA. This kit allows for variable number of samples (1-96) 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- or 8-channel pipette arm that include single-channel functionality (figure 1).

Manual workflow component

For effective DNA isolation from plant tissues, it is recommended that the samples be completely homogenized to a powder. This can be done using either a commercial homogenizer or a motor and pestle combined with liquid nitrogen.

Figure 1: VERSA 1100 Gene Workstation

The homogenized samples were then manually placed in the sample tube and lysed by incubating in MC1 buffer with RNase A added for 30 minutes. After which, the lysate was cleared by centrifugation and transferred to the separation plate.

Automated workflow

The separation plate holding the cleared lysate 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® Plant kit 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.

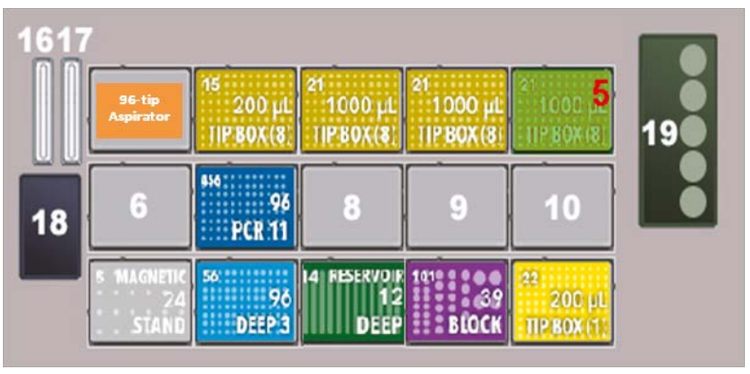

Figure 2: The deck layout of the VERSA 1100 used to automate the NucleoMag® Plant genomic DNA extraction workflow. Position #17 is the liquid waste disposal site, #18 is the tip disposal chute and #19 is the ReagentDrop bulk reagent dispensing system that is part of the robotic pipette head.

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

III. Results

Genomic DNA was isolated from pumpkin, maize and wheat leaf samples. Sample weights used for each of the eight pumpkin and maize leaf samples were 50mg, for the 48 wheat leaf samples 30mg of tissue was used.

The DNA yields from the three leaf sources reflected the inherit variation when isolating DNA from different plant species. The average DNA yields from pumpkin and maize samples were 3.3µg and 2.3µg (table 1a & b), while the average DNA yield from wheat samples was 7.8µg (table 2). The purity of the maize DNA samples were slightly lower, giving an average reading of 1.58 A260/A280 nm ratio (table 1b). The high purity of the DNA isolated was reflected in the A260/A280 nm ratios for the pumpkin and wheat samples for which the average readings were 1.75 and 1.76 (table 1a & 2) respectively.

Table 1a & b: Summary of the DNA yields recovered from pumpkin (a) and maize (b) leaves using the NucleoMag® Plant kit. DNA was quantified using a Qubit 3.0. The final elution volume is 100µl.

a

Sample	DNA Yield (µg)	DNA Conc. (ng/µL)	DNA Purity (A260/A280)
1	3.6	36.1	1.71
2	3.3	32.7	1.73
3	2.3	23.4	1.75
4	3.0	29.8	1.77
5	3.2	32.4	1.83
6	3.1	31.4	1.72
7	4.1	41.2	1.77
8	4.0	39.6	1.74
Average	**3.3**	**33.33**	**1.75**

b

Sample	DNA Yield (µg)	DNA Conc. (ng/µL)	DNA Purity (A260/A280)
1	2.3	23.2	1.60
2	2.2	21.8	1.58
3	2.2	22.2	1.60
4	2.4	23.5	1.57
5	2.3	22.6	1.60
6	2.3	23.4	1.58
7	2.5	25.2	1.56
8	2.5	25.1	1.57
Average	**2.3**	**23.38**	**1.58**

Table 2: Summary of the DNA yields recovered from wheat leaves using the NucleoMag® Plant kit. DNA was quantified using a Qubit 3.0. The final elution volume is 100µl.

Sample	DNA Yield (µg)	DNA Conc. (ng/µL)	DNA Purity (A260/A280)	Sample	DNA Yield (µg)	DNA Conc. (ng/µL)	DNA Purity (A260/A280)
1	6.1	61.2	1.82	25	7.9	79.2	1.74
2	6.1	60.6	1.77	26	7.8	78.4	1.72
3	6.5	65.4	1.82	27	6.6	66.4	1.76
4	6.4	64.2	1.79	28	8.4	84.4	1.72
5	7.3	73.2	1.82	29	9.2	91.6	1.72
6	7.9	78.6	1.76	30	8.0	79.6	1.74
7	7.9	79.2	1.79	31	7.5	75.2	1.79
8	8.6	86.4	1.77	32	8.0	80	1.67
9	7.3	72.6	1.78	33	8.6	86	1.79
10	8.1	81	1.71	34	7.3	72.8	1.74
11	7.4	74.4	1.80	35	7.2	71.6	1.80
12	7.6	75.6	1.81	36	6.7	67.2	1.73
13	7.1	70.8	1.78	37	7.5	74.8	1.73
14	8.9	89.4	1.76	38	6.8	68.4	1.79
15	8.6	86.4	1.83	39	9.2	91.6	1.73
16	8.8	87.6	1.82	40	8.8	87.6	1.76
17	9.5	94.8	1.74	41	9.6	96.4	1.72
18	7.3	73.2	1.74	42	9.0	90.4	1.71
19	7.3	72.6	1.79	43	7.0	70.4	1.77
20	9.5	95.4	1.76	44	5.4	54	1.70
21	12.2	122.4	1.74	45	5.2	52.4	1.73
22	8.6	85.8	1.79	46	5.8	58	1.73
23	9.8	98.4	1.71	47	6.0	59.6	1.72
24	9.1	91.2	1.82	48	5.5	54.8	1.75
Average for 48 samples					**7.8**	**77.73**	**1.76**

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

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 extracts irrespective of their origin. This is indicated by the single bright band observed in the agarose gel (figure 3a, 3b & 4).

The DNA purified by automating the NucleoMag® Plant kit is suitable for use in various downstream applications.

To test for the presence of inhibitors in the eluted DNA extracts, the samples were used as templates for PCR reactions that amplified a fragment of the house keeping gene, Actin. The success of Actin gene fragment PCR amplification demonstrates the absence of inhibitors for downstream uses of the purified DNA (figure 5a, 5b & 6).

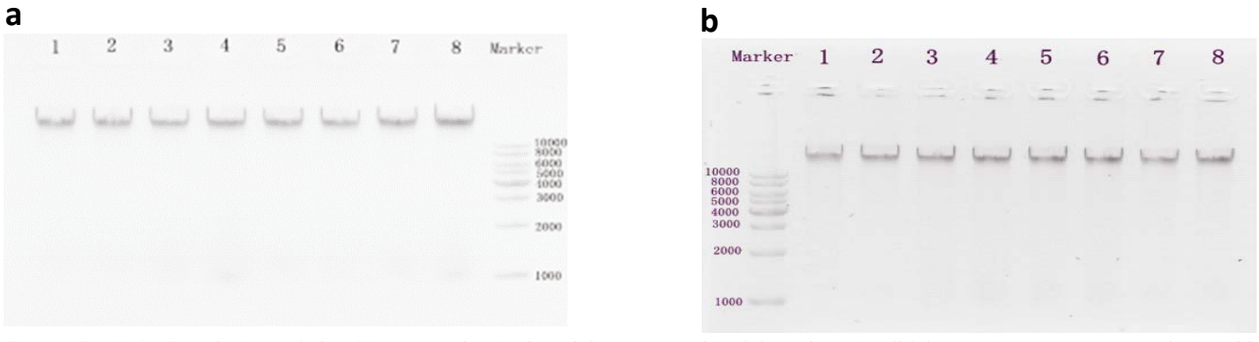

Figure 3a & b: Resolution of the DNA samples isolated from pumpkin (a) and maize (b) leaves in an agarose gel. A 10kb marker was run along side the samples

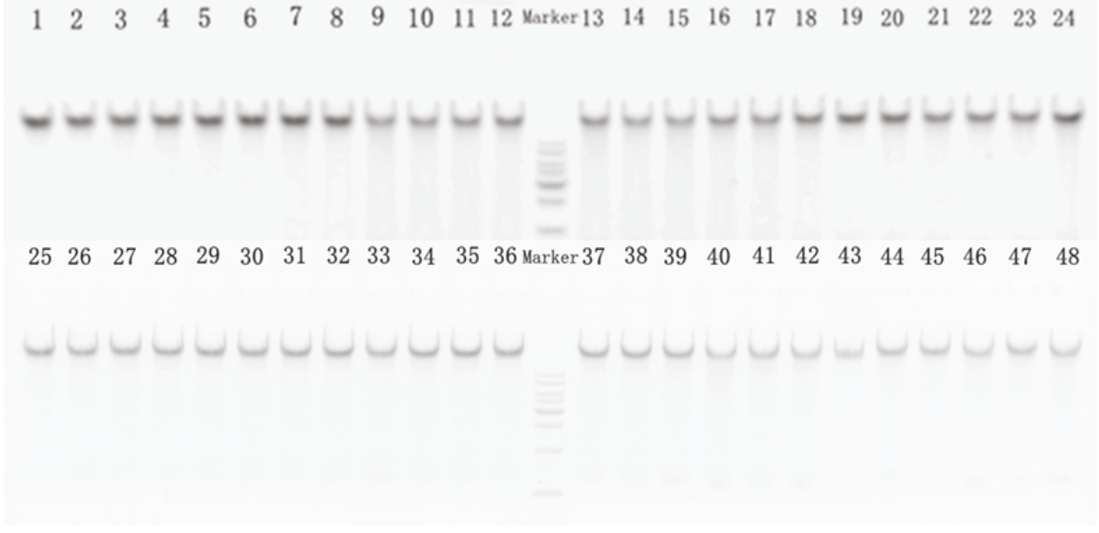

Figure 4: Resolution of the DNA samples isolated from wheat leaves in an agarose gel. A 10kb marker was run along side the samples

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

III. Results cont.

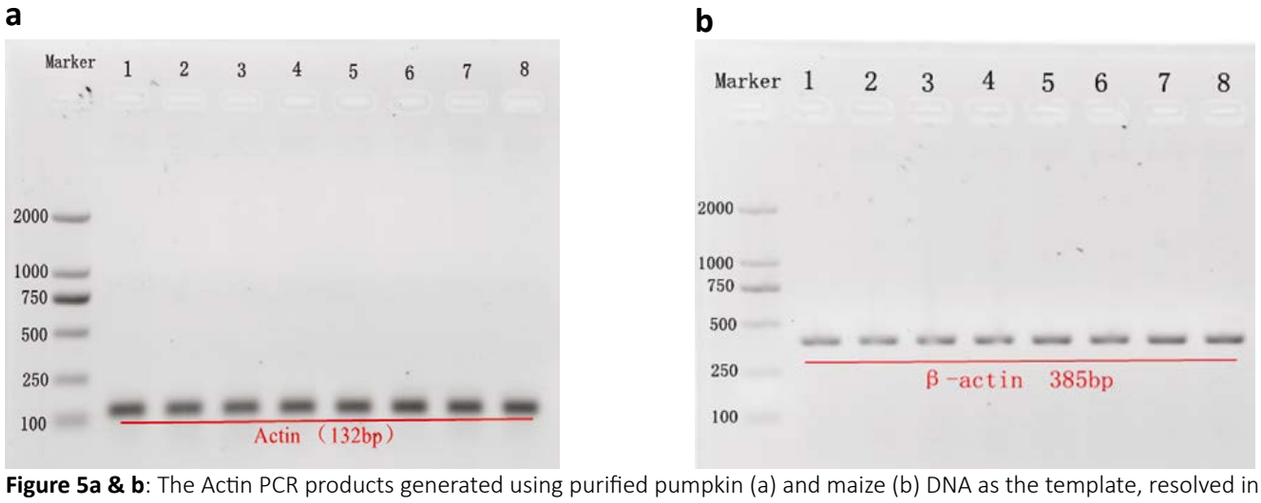

Figure 5a & b: The Actin PCR products generated using purified pumpkin (a) and maize (b) DNA as the template, resolved in an agarose gel. A 2kb marker was run along side the samples

Figure 6: The Actin PCR products generated using purified wheat DNA as the template, resolved in an agarose gel. A 2kb marker was run along side the samples

IV. Conclusion

Combining the power of MACHEREY-NAGEL's NucleoMag® Plant kit with the precision of Aurora Biomed's VERSA 1100 automated liquid handling platform provides a walkaway solution for the isolation of pure, high quality genomic DNA from various plant species. Furthermore, the VERSA 1100 reduces the risk of manual errors and cross-contamination, while also increasing reproducibility and sample throughput. The flexibly of the VERSA 1100 workstation allows users to automate DNA extractions, PCR, sample normalization and general liquid handling applications on the same instrument.

Aurora
ILLUMINATING SOLUTIONS
www.aurorabiomed.com

MACHEREY-NAGEL
www.mn-net.com