A Robust and Efficient Approach for High-Throughput DNA Extraction and Genotyping Across Various Animal Sample Types on Biomek i7 Automated Workstation

ABSTRACT

Animal genotyping is a crucial tool for scientific research that enables us to understand the genetic basis of various hereditary traits and diseases.[1] It plays a vital role in advancing our knowledge of genetics and biology and improving animal health and welfare.[2] By accurately identifying and analyzing genetic variations, animal genotyping has the potential to revolutionize animal breeding programs and conservation efforts.[3] It can guide breeding strategies for desired traits and help assess genetic diversity and population structure.1 With its significant applications in conservation biology, evolutionary biology, and animal breeding, animal genotyping is vital for achieving our research goals and improving the world around us.[4]

Efficient and high-throughput automation in DNA extraction is crucial for genotyping laboratories that handle diverse sample types from different species. The various characteristics of the samples demand a streamlined process that can handle them while maintaining accuracy and reliability. Additionally, automation offers cost efficiency by reducing labor costs and minimizing manual errors, enabling the processing of large sample volumes. Automated workflows significantly reduce processing time compared to manual methods, increasing productivity and throughput.

In this application note, we introduce an automated method for DNA extraction and genotyping across diverse sample types. The method involves using the DNAdvance extraction kit—which is based on SPRI technology—on a Biomek i7 Automated Workstation. After cell lysis, the DNA molecules are captured by magnetic beads, allowing them to be retained at the bottom of the wells through magnetization. Multiple washing steps are then carried out to remove impurities, and the captured DNA is efficiently eluted, providing a purified DNA sample that is ready for downstream genotyping analyses.

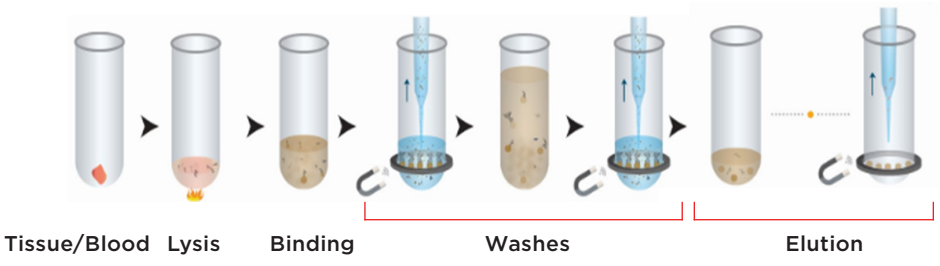

Figure 1. DNAdvance Extraction Workflow Overview.

MATERIALS and METHODS

Tissue samples are received in a 96-well, deepwell plate with a maximum size of 5 mm by 5 mm. The samples are either frozen or stored in ethanol (70%) until extraction. The Lysis Buffer (LBH) was manually aliquoted into each well, which takes around 10–15 minutes for 5 plates **(Figure 2, left side)**. The sample plate is then incubated at 55°C overnight. After incubation, the sample plate is centrifuged on an Avanti J-15 centrifuge (Beckman Coulter Life Sciences). The operator sets up the deck **(Figure 3)**, following the Guided Labware Setup using Biomek Method Launcher (BML). Once the deck is set up, the lysate is first transferred from the 96-well, deepwell plate into a new plate **(Figure 2, right side)**—up to 5 plates/run can be placed on Biomek i7 automated workstation. Then the DNA extraction process starts, which includes the binding step with the addition of magnetic beads (PBBA), ethanol washes and DNA elution **(Figure 2, right side)**.

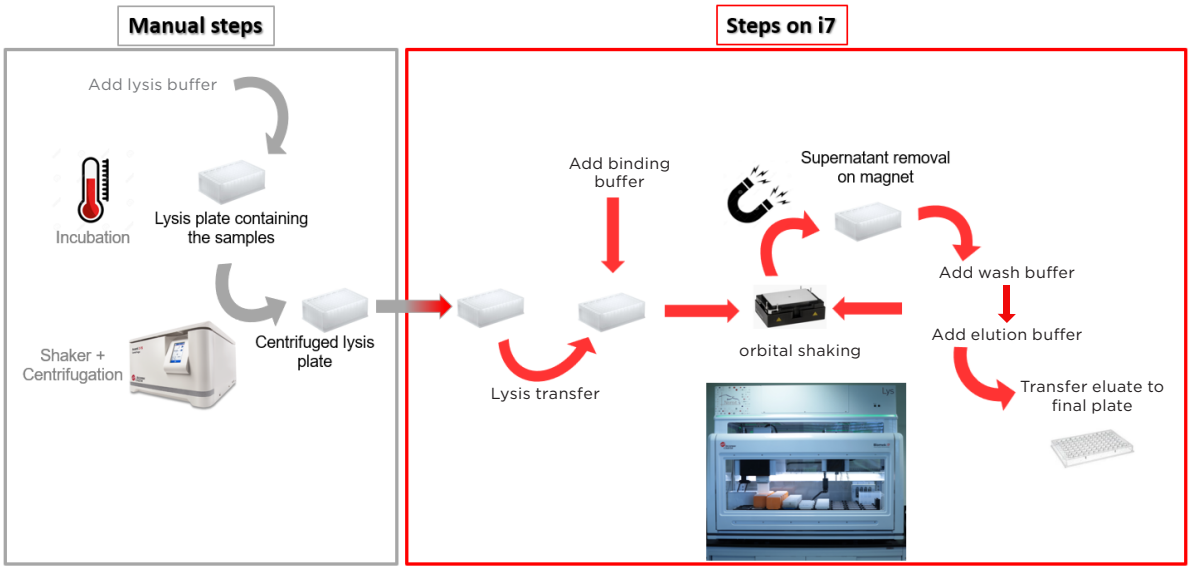

Figure 2. Automated DNAdvance Extraction On Biomek i7 automated workstation Workflow. Left: manual sample process. Right: automated extraction process on the Biomek i7 Automated Workstation.

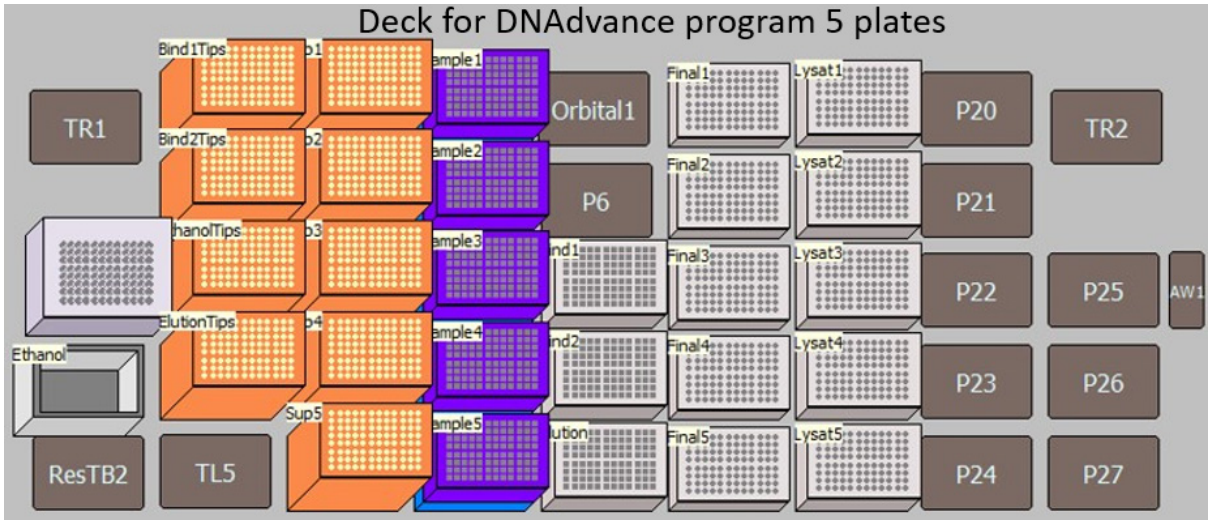

Figure 3. Overview Of The DNAdvance Deck Layout. The deck has a total of nine positions for tips (1 mL, orange), five positions for lysate plates (purple) with magnets (blue), five positions for elution plates Final 1-5 (gray), 1 position for elution buffer (gray), and two position for bind buffer (gray) and one shaking position.

Table 1. Automated DNA Extraction Run Time Using the DNAdvance Extraction Kit On a Biomek i7 Automated Workstation.

Plate Number	1	2	3	4	5
Run time	00h42	00h48	00h56	01h16	01h39

RESULTS and DISCUSSION

The automated process of transferring the lysate and extracting DNA takes different durations for different numbers of plates. Processing one, two, three, four and five plates takes 42 minutes, 48 minutes, 56 minutes, 1 hour 16 minutes and 1 hour 39 minutes, respectively **(Table 1)**. The deck setup requires only 10 minutes of hands-on time, with no additional intervention steps needed before the eluted DNA is ready for genotyping assay. It achieves 2000 samples/day extraction.

DNA was extracted from 5 different tissue types of various species. The average DNA yield from all tested samples exceeded the minimum yield cutoff of 20 ng/µL. Summary of yield and number/tissue type are listed in **Table 2**. The DNA concentration is measured by UV absorbance (excites at 350 nm and emits at 450 nm) with Hoechst 33258 (Thermo Fisher Scientific, MA, USA, Cat# H3569).

Table 2. Average DNA Yield From Tested Samples.

Species	Tissue type	Elution Vol. (µL)	Avg Yield (ng/µL)	No. Of Samples
Rabbit	Tail Biopsy	80	99	11
Lizard	Ear Biopsy	80	113	372
Oyster	Mantle Biopsy	80	120	1152
Trout	Egg	80	120	701
Sea bream	Fin (Punch)	80	90	1344

The size of extracted DNA is measured using a 1% agarose gel, and a 1 Kb Plus DNA ladder (Thermo Fisher Scientific, MA, USA, Cat# 10787018) is also used. The size of DNA from the same sample type is consistent **(Figure 4)**. We have observed that the DNA size from all the samples is above 15,000 bp (A. Rabbit ear biopsy, B. Lizard tail, C. Oyster mantle biopsy, D. Trout egg, E. Goose blood, and F. Duck blood). The uniformity in DNA size across all the samples indicates that high-quality DNA has been successfully extracted.

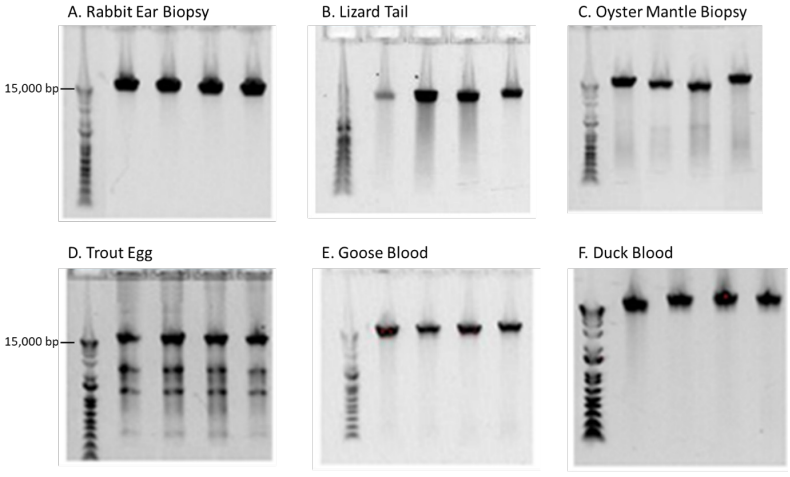

Figure 4. The DNA Size Extracted From Tested Samples. The size of DNA extracted from six types of animal tissues was tested in this demonstration. Four to five replicates were used for each sample type, and the DNA ladder indicates 15000 bp. Gel images of **(A)**. Rabbit Ear Biopsy, **(B)**. Lizard Tail, **(C)**. Oyster Mantle Biopsy, **(D)**. Trout Egg, **(E)**. Goose Blood, and **(F)**. Duck Blood

DNA integrity was evaluated using Femto Pulse Systems (Agilent Technologies in Santa Clara, CA). The quality of a lizard sample is demonstrated here (**Fig. 5**).

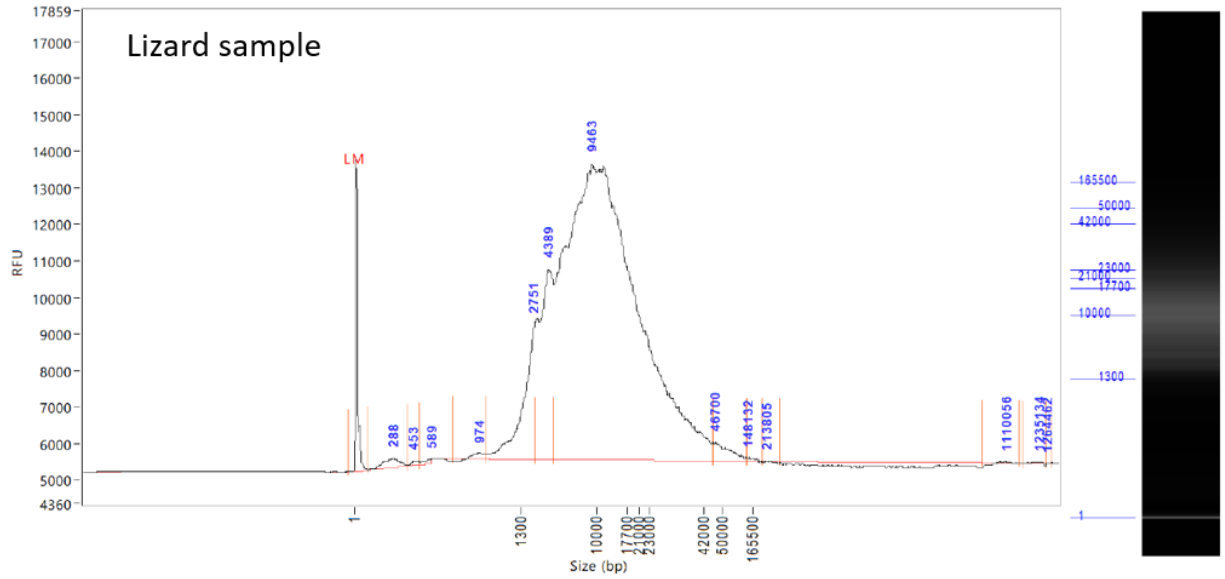

Figure 5. Femto Pulse Profiles Show The Integrity And Size Of gDNA. The size distribution of extracted genomic DNA from a lizard sample had a peak at 10,000 bp. LM = Lower Marker.

Following DNA extraction, genotyping and SNP data analysis were carried out on two different platforms to determine the compatibility of the extracted DNA **(Figure 6)**. The first platform was the Biomark EP1 System coupled with SNP Genotyping Analysis software (Standard BioTools, CA, USA). The second platform was the GeneTitan Multi-Channel Instrument paired with Axiom Analysis Suite Software (ThermoFisher, MA, USA). Each point on the scatter plot represents the genotype for one sample on that SNP. The data included the extracted gDNA results to produce reliable and high-quality genetic data.

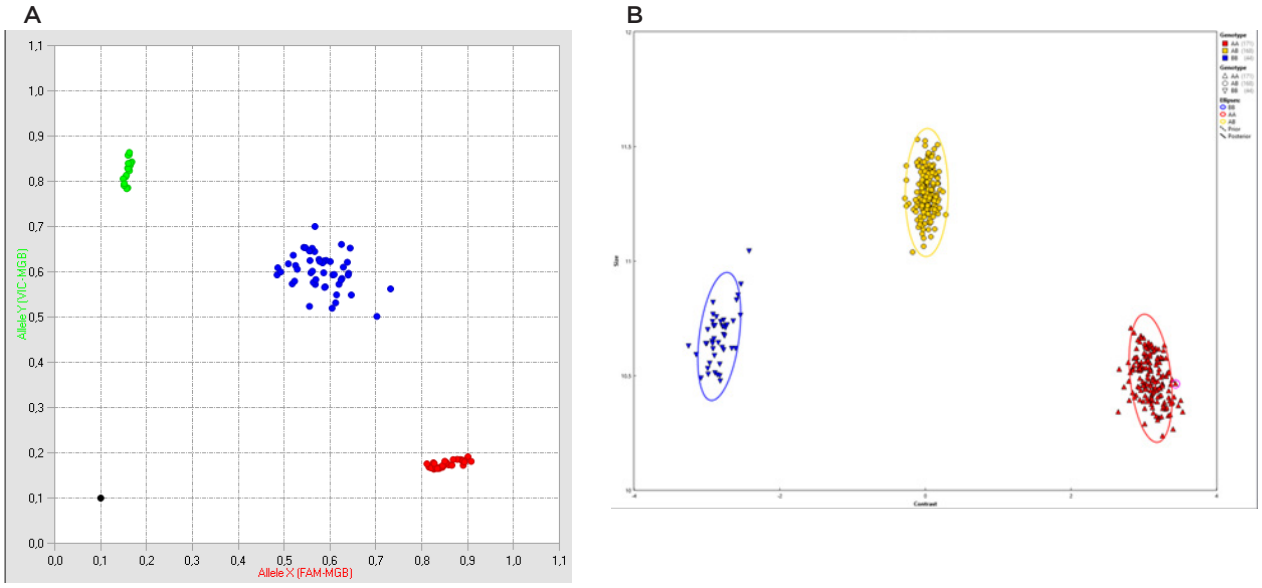

Figure 6. SNP Genotyping Data Analysis. Genotyping plots from the **(A)** SNP Genotyping Analysis software and **(A)** Fluorescence values obtained using Biomark EP1 system. Y-axis represents VIC fluorescence intensity, x-axis represents FAM fluorescence intensity. Both intensity values normalized by ROX fluorescence. Different color dots indicate different genotyping result: No Call (black), HOM A (green), HOM B (red), or HET (blue), enabling gender concordance to be established.[5] HOM = homozygous, HET = heterozygous. B. Polymorphic, high-resolution clusters.[6] **(B)** Axiom Analysis Suite Software.

Using the DNAdvance extraction kit on the Biomek i7 automated workstation instrument allows for automated extraction, significantly standardizing handling and reducing run time, making it possible to extract up to 2000 samples daily. Furthermore, this same automated extraction method has been tested on additional sample types with success **(Table 3)**.

Table 3. Automated Extraction Method Tested on Additional Sample Types

Species	Tissue
Fish: sea bream, sea bass, trout, sturgeon, meager, minnow, turbot, bleak, chub	Punch of flipper, egg, sperm
Poultry: duck, goose, chicken, quail, partridge	Inter-palmar, ergot, blood
Lizard	Biopsy of tail
Rabbit	Biopsy of the ear
Horse	Blood
Oyster, clam	Gills, coat
Shrimp	Whole Shrimp
Soldier fly	Larva, whole fly
Human	Saliva

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