Evaluation of PCR and Sequencing Outcomes After Automated Genomic DNA Extraction from Flesh Fly Pupa and Larva with the AllEx®64 Automated Nucleic Acid Extraction System

Introduction

Entomology is the scientific study of insects, including their morphology, physiology, development, and genetic characteristics, and is applied across various fields such as environmental and urban ecology, vector surveillance, and forensic science. Molecular biological approaches involving gene extraction and analysis are widely used to investigate insect development and phylogenetic relationships, particularly for accurate species identification when morphological discrimination is limited. DNA barcoding is a representative gene-based identification method and is commonly used in insect taxonomy and applied entomological research.

The AllEx®64 Automated Nucleic Acid Extraction System is a magnetic bead-based platform designed for high-throughput genomic DNA purification with high reproducibility. Optimized reagents and automated liquid-handling processes minimize user-dependent variability and enable reliable DNA recovery from diverse biological samples.

In this Application Note, genomic DNA was extracted from flesh fly samples using the AllEx®64 Automated Nucleic Acid Extraction System to evaluate its suitability for DNA barcoding-based species identification. Samples from pupal and larval stages were analyzed, followed by PCR amplification and DNA sequencing. This case study demonstrates the applicability of automated nucleic acid extraction systems to insect samples and provides a practical reference for molecular genetic studies in entomological research.

Materials and Methods

Materials

		
AllEx®64 Automated Nucleic Acid Extraction System [AEX064]	AllEx® Genomic DNA Kit [931-048, 931-096]	AllEx® Forensic DNA Kit [936-048, 936-096]

Sample Information

Sample	 Flesh fly pupa	 Flesh fly larva
Family	Sarcophagidae	
Genus	*Sarcophaga*	
Species	*S. crassipalpis*	*S. peregrina*

Extraction Conditions

Target	Genomic DNA (mitochondrial DNA)
Sample amount	1 specimen
Extraction system protocol	Forensic-P2
Operating time	21’ 53’’
Elution volume	Up to 100 µl

Methods

*** For more details and methods, please refer to the Instruction for use (IFU) of AllEx® Genomic DNA Kit or AllEx® Forensic DNA Kit.**

Preparation of Proteinase K Solution

To prepare a 20 mg/ml solution of Proteinase K, add PK-Storage Buffer to the lyophilized Proteinase K according to the instructions on the label. Gently invert the mixture until dissolved.

Protocol

1. Dry the samples at room temperature until all residual ethanol has completely evaporated.
2. Transfer each sample to a 1.5 ml microcentrifuge tube.
3. Add 25 µl of Proteinase K solution (20 mg/ml).
4. Pierce the outer surface of the sample with the tip to crush it. Then, add 300 µl of Buffer HL.
5. Briefly spin down the sample, then incubate it at 56 ℃ for 30 min using a heat block.
6. Centrifuge at 13,000 rpm for 5 min at room temperature.
7. Carefully transfer 200 µl of the supernatant to the 1st (or 7th) well of the cartridge.
8. Add 10 µl of RNase A Solution (20 mg/ml) to the 3rd (or 9th) well of the cartridge.
9. Load the fully prepared extraction cartridge and AllEx® Strip into the AllEx®64 Automated Nucleic Acid Extraction System and run the “Forensic-P2” protocol.
10. After extraction, collect the eluate from the 5th (or 11th) well.

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Results

Extraction Kit	Flesh fly							
	Pupa (n=2)				Larva (n=2)			
	Conc. (ng/µl)	Yield (µg)	A_{260}/A_{280}	A_{260}/A_{230}	Conc. (ng/µl)	Yield (µg)	A_{260}/A_{280}	A_{260}/A_{230}
AllEx® Genomic DNA Kit	221.0	22.1	2.17	2.18	99.9	10.0	2.09	2.02
AllEx® Forensic DNA Kit	459.5	45.9	2.29	2.16	1012.2	101.2	2.26	2.04

Table 1. Genomic DNA Quantification and Purity from Flesh Fly Pupa and Larva Using Different Extraction Kits
Genomic DNA was extracted from individual flesh fly pupa and larva specimens (n=2) using the AllEx®64 Automated Nucleic Acid Extraction System with the Forensic-P2 protocol. A comparative analysis was conducted on two extraction kits: AllEx® Genomic DNA Kit and AllEx® Forensic DNA Kit. The concentration and purity of the DNA were measured using a NanoDrop™ 2000 spectrophotometer, and the total yield was calculated based on the concentration and elution volume.
Both kits successfully extracted genomic DNA from flesh fly pupa and larva; however, the AllEx® Forensic DNA Kit demonstrated significantly higher yields, ranging from twofold to tenfold greater than that obtained with the AllEx® Genomic DNA Kit. These results indicate that the AllEx® Forensic DNA Kit is the more suitable option for genomic DNA extraction from flesh fly specimens.

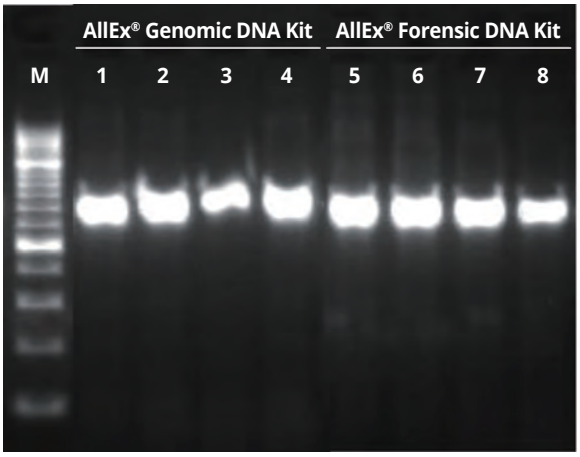

Figure 1. Agarose gel analysis of PCR products amplified from genomic DNA extracted using AllEx® Genomic DNA Kit and AllEx® Forensic DNA Kit
Genomic DNA was extracted from flesh fly pupa and larva using the AllEx® Genomic DNA Kit and the AllEx® Forensic DNA Kit, respectively, and used as a template for PCR amplification.
The PCR products were subsequently separated on a 2% agarose gel, with Lane M containing the GENESTA™ 100 bp DNA Ladder utilized as a molecular size marker.
Lanes 1–4 represent PCR products amplified from genomic DNA extracted using the AllEx® Genomic DNA Kit, while lanes 5–8 correspond to PCR products derived from genomic DNA extracted using the AllEx® Forensic DNA Kit.
Specifically, lanes 1, 2, 5, and 6 contain PCR products from pupa samples, whereas lanes 3, 4, 7, and 8 contain PCR products from larva samples.
A single, distinct band corresponding to the expected amplicon size of 582 bp was clearly observed in all samples, thereby confirming that both kits provide genomic DNA of sufficient quality and integrity for reliable PCR analysis.

Extraction Kit	No.	Sample	BLAST Result	Identity
AllEx® Genomic DNA Kit	1	gDNA from pupa	*Sarcophaga crassipalpis*	100%
	2	gDNA from pupa	*Sarcophaga crassipalpis*	100%
	3	gDNA from larva	*Sarcophaga peregrina*	100%
	4	gDNA from larva	*Sarcophaga peregrina*	100%
AllEx® Forensic DNA Kit	5	gDNA from pupa	*Sarcophaga crassipalpis*	100%
	6	gDNA from pupa	*Sarcophaga crassipalpis*	100%
	7	gDNA from larva	*Sarcophaga peregrina*	99.78%
	8	gDNA from larva	*Sarcophaga peregrina*	99.77%

Table 2. BLAST analysis of PCR amplicons amplified from genomic DNA extracted using AllEx® Genomic DNA Kit and AllEx® Forensic DNA Kit
The amplified PCR products from each sample were subjected to a sequence analysis, and the resulting sequences were compared against reference sequences in the NCBI database using the BLAST algorithm. The table provides a synopsis of the extraction Kit utilized, the sample identification number, the sample type, the top BLAST hit, and the corresponding sequence identity. The High sequence identity values confirm that the PCR amplicons were correctly amplified from flesh fly genomic DNA. This demonstrates that genomic DNA extracted using both kits is suitable for downstream sequence-based identification and molecular analysis.

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Conclusion

- **The AllEx® Genomic DNA Kit and the AllEx® Forensic DNA Kit were successfully employed to extract genomic DNA from flesh fly pupa and larva samples, with the extracted DNA exhibiting sufficient quality for molecular downstream analyses.**
  The purity of the DNA and the presence of clear PCR amplification products were indicative of the fact that both kits provided genomic DNA suitable for routine PCR-based applications.
- **The AllEx® Forensic DNA Kit consistently yielded higher concentrations of DNA and total DNA yield compared to the AllEx® Genomic DNA Kit in both pupa and larva samples.**
  This enhanced performance was particularly evident in limited or challenging insect samples.
- **PCR amplification employing genomic DNA extracted from the samples yielded a singular, specific amplicon of the anticipated size (582 bp) in all instances.**
  The absence of non-specific bands indicates high DNA integrity and compatibility with PCR workflows.
- **Subsequent sequence analysis of the PCR amplicons, in conjunction with BLAST analysis, substantiated a high degree of sequence identity with those of the flesh fly reference sequences.**
  The findings substantiate the precision of genomic DNA extraction and demonstrate its aptitude for facilitating sequence-based species identification.
- **In summary, the AllEx® Forensic DNA Kit is well-suited for the extraction of insect genomic DNA, particularly in cases where a higher yield and robust downstream performance are essential. Conversely, the AllEx® Genomic DNA Kit is designed to deliver reliable and reproducible results for standard genomic applications.**

Ordering Information

Cat. No.	Product	Size
AEX064	AllEx®64 Automated Nucleic Acid Extraction System	1 Unit
931-048	AllEx® Genomic DNA Kit (Single Type)	48T
931-096	AllEx® Genomic DNA Kit (Plate Type)	96T
936-048	AllEx® Forensic DNA Kit (Single Type)	48T
936-096	AllEx® Forensic DNA Kit (Plate Type)	96T