30th CONGRESS OF THE INTERNATIONAL SOCIETY FOR FORENSIC GENETICS
SANTIAGO DE COMPOSTELA
9-13 SEPTEMBER 2024
iSFG 2024

POSTER NR. 068

Highly Portable, Specific and Sensitive Platform for Fast-Track Wildlife Species Identification

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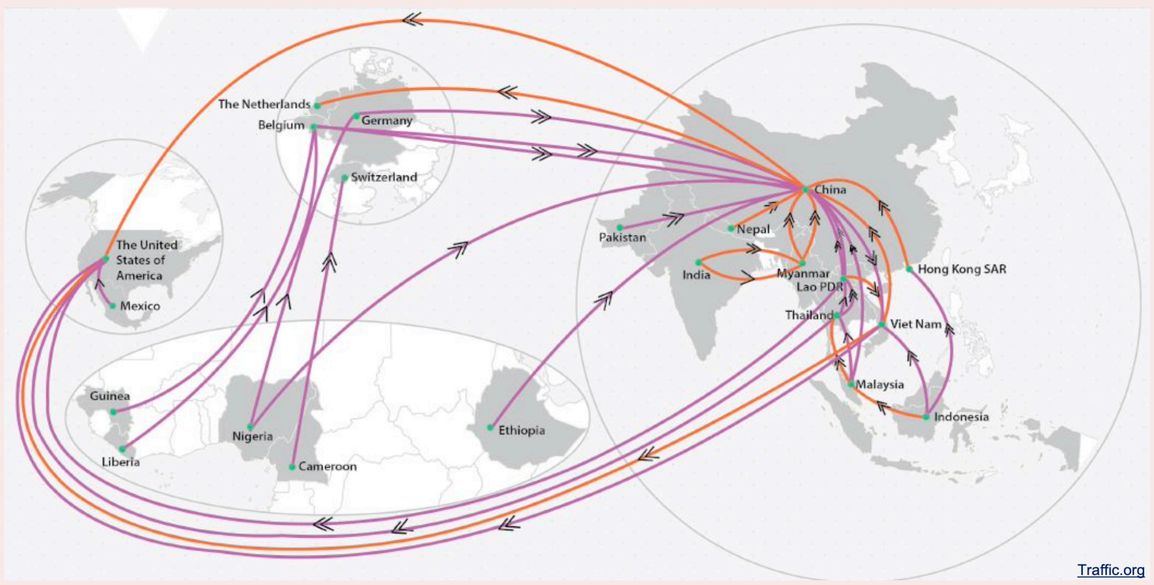
INTRODUCTION

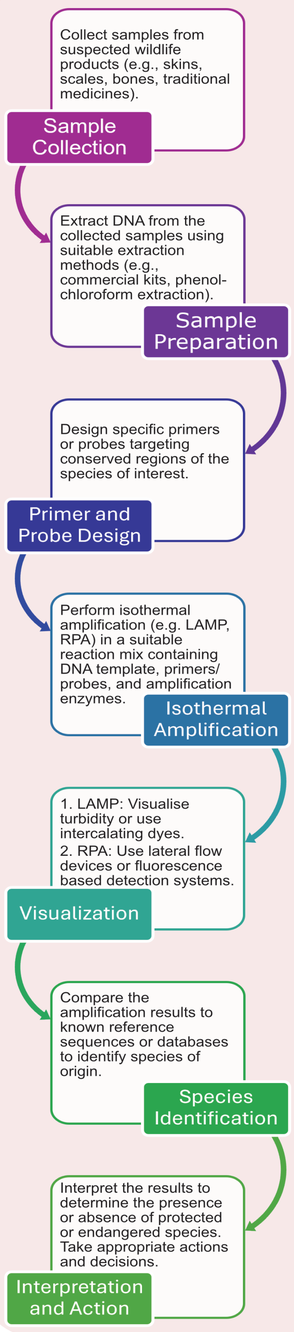
Wildlife trafficking poses numerous challenges due to the sophisticated and organised criminal networks complicating the law enforcement efforts due to varying legal systems and capacities among different countries. The trade is highly lucrative, incentivising the traffickers to persist despite the risks, driven by demand for rare species and their parts.



Major trafficking range states and major trade route of the most illegally traded mammal: Pangolin

One of the significant challenge in combating illegal wildlife trade is animal species identification. High diversity of species, complex morphological variations, processed animal tissues, evasive tactics by traffickers added with technological and methodological limitations complicate the process of proper and correct animal identification. While technologies like DNA barcoding, forensic analysis and high resolution imaging exists, they are not always available or used uniformly across different regions and enforcement bodies. Advances in isothermal amplification technologies have led to development of portable devices that can potentially be adapted for use by law enforcement agencies or customs officials to rapidly identify trafficked wildlife species at various checkpoints. Isothermal Amplification-based assays can amplify DNA rapidly and efficiently at constant temperature. These assays are simple to perform making them suitable for use in field conditions or resource-limited settings.

METHODOLOGY



Isothermal amplification assays can be used to identify species also from derivative products like powders, oils, or traditional medicines where the original morphology is no longer discernible. They enable on-site testing without need of extensive sample preparation or transport to specialised laboratories, thereby reducing delays and logistical challenges of traditional DNA analysis methods. Despite promising advantages, several challenges may arise in the process which need special attention:

Specificity	Sample Quality	Quality Control
Ensuring primers or probes are specific to target species can be challenging when closely related species share conserved regions, leading to false positives.	Wildlife samples often contain inhibitors that can interfere with amplification process. Overcoming these inhibitors without compromising assay sensitivity is crucial.	Extensive testing and establishing robust validation protocols across different laboratories and environments is essential for ensuring reliable results.

Addressing these challenges involves ongoing research to improve assay specificity, develop robust protocols for inhibitor removal, enhance accessibility to equipment and training, and reduce overall costs to facilitate broader adoption in wildlife forensics and conservation efforts.

References:

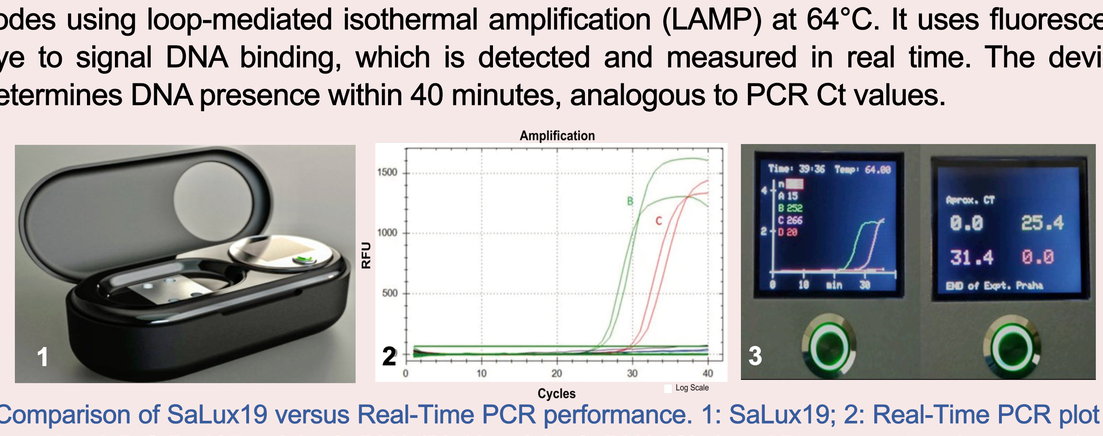
1. Mezzasalma, V., Ganopoulos, I., Galimberti, A. et al. Poisonous or non-poisonous plants? DNA-based tools and applications for accurate identification. Int J Legal Med 131, 1–19 (2017). https://doi.org/10.1007/s00414-016-1460-y

2. Hebenstreitova K, Salaba O, Trubac J, Kufnerova J, Vanek D. The Influence of Tanning Chemical Agents on DNA Degradation: A Robust Procedure for the Analysis of Tanned Animal Hide-A Pilot Study. Life (Basel). 2024 Jan 19;14(1):147. doi: 10.3390/life14010147. PMID: 38276276; PMCID: PMC10817434.

RESULTS

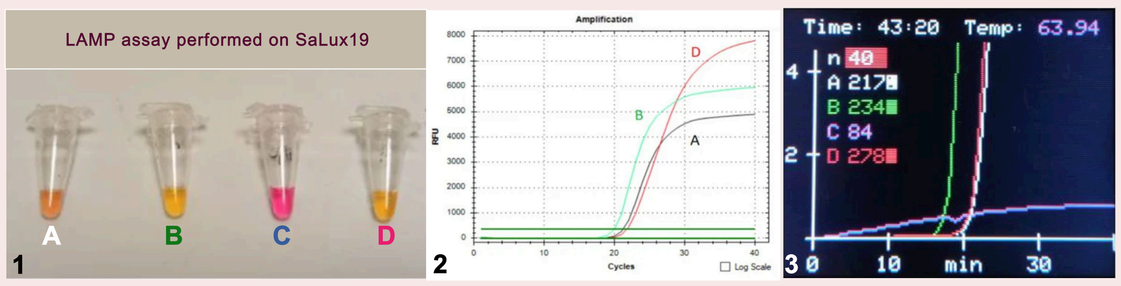
"Efficient Detection of *Sus scrofa* DNA Using Portable SaLux19 Device"

The pocket-sized SaLux19 device (14.5 cm × 9.5 cm × 4 cm) detects DNA/cDNA genetic codes using loop-mediated isothermal amplification (LAMP) at 64°C. It uses fluorescent dye to signal DNA binding, which is detected and measured in real time. The device determines DNA presence within 40 minutes, analogous to PCR Ct values.



Comparison of SaLux19 versus Real-Time PCR performance. 1: SaLux19; 2: Real-Time PCR plot of samples A,B,C,D. 127 pg/µL of nDNA (B),13 pg/µL of nDNA (C). A and D were no template controls.;3: The in-built display of SaLux19 device demonstrates real-time amplification of same samples.

The SaLux19 LAMP assay was validated with samples: lysed *Sus scrofa* blood (A), *Sus scrofa* DNA positive control (B), human DNA positive control (D), and a no template control (C). Results showed successful amplification for samples A, B, and D. Analogous tests with the CFX Connect Real-Time PCR system confirmed similar Ct values.



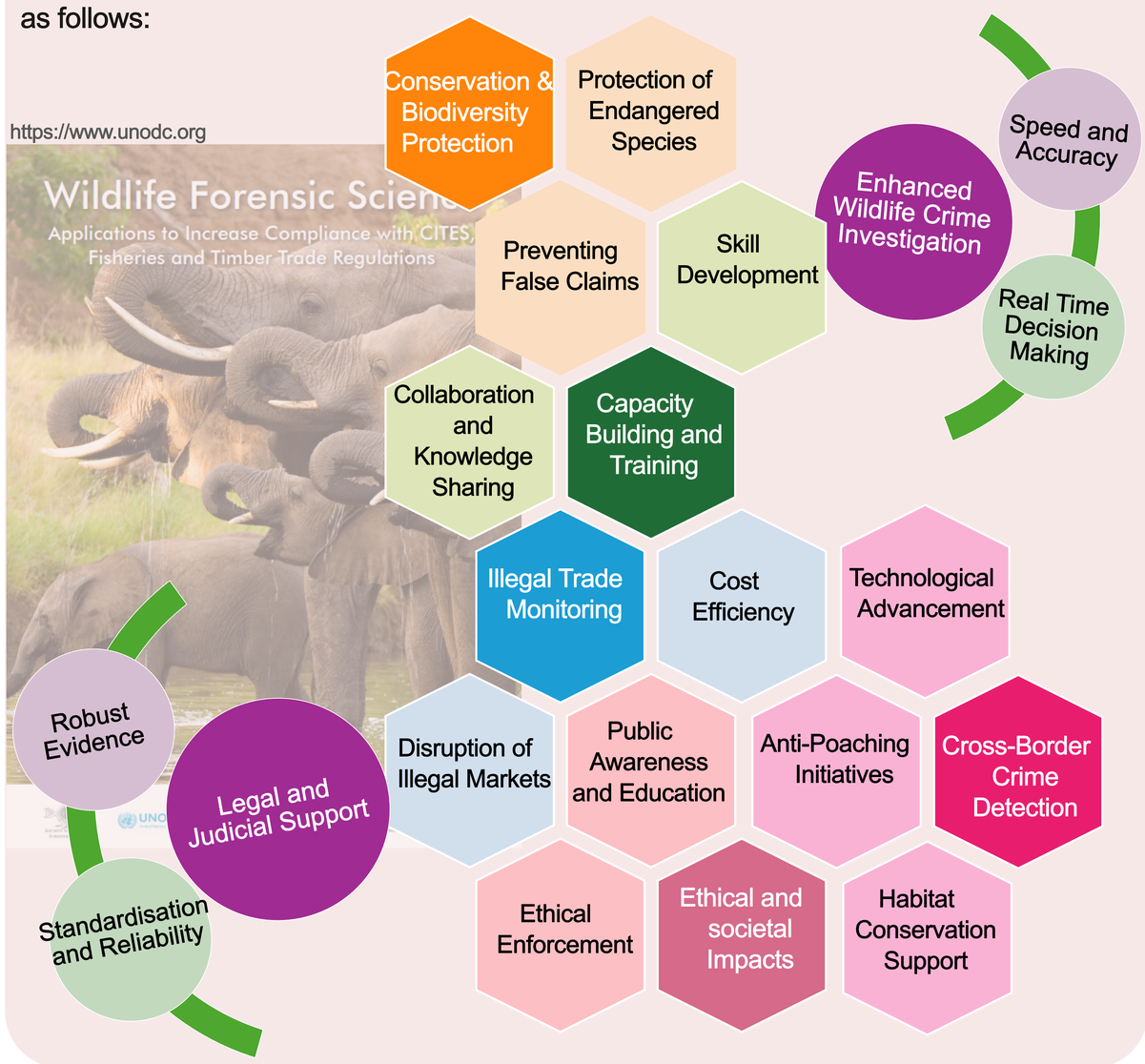
Validation of species identification assay on SaLux19 Device. 1: Colorimetric detection assay using SYBR Green; 2: Real-Time PCR of samples A,B,C,D.; 3: The in-built display of SaLux19 device demonstrates real-time amplification for same samples providing RFU values after end of each cycle.

Reference:

1. Ruszova, E.; Vanek, D.; Stühmer, W.; Khaznadar, Z.; Subhashini, N. The Utilization of the SaLux19-Based Loop-Mediated Isothermal Amplification (LAMP) Assay for the Rapid and Sensitive Identification of Minute Amounts of a Biological Specimen. Life 2024, 14, 579. https://doi.org/10.3390/life14050579

IMPACT

The rapid and efficient animal species identification assay developed on the portable SaLux19 device serves as a foundational tool that can be utilized as a building block for the challenges of the world of animal forensics. This assay significantly enhances the capabilities of the forensic community, making wildlife crime investigations more efficient and effective while contributing to the broader goals of conservation and legal enforcement as follows:



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