

Molecular Identification of African Elephant (*Loxodonta africana*) via Target Gene (*cytb*) Quantitative PCR: A Case Study in Wildlife Conservation Genetics

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Abstract

Background: The African elephants (*Loxodonta africana*) are considered critically endangered due to poaching, habitat destruction and human-wildlife conflict. However, effective monitoring and protection of elephant populations require non-invasive and reliable methods for DNA detection.

Purpose: This study aimed at determining the sensitivity, specificity and robustness of a cytochrome b gene-based qPCR assay for detecting African elephant DNA in various sample such as feces, skin and hair.

Methods: qPCR assay was developed using the FAM and HEX channels for detecting African elephant DNA and an internal control. The assay was tested on 9 elephant DNA samples and non-target species that is Arabian Oryx. Serial dilutions of elephant DNA. Cq values and DNA concentrations were measured and analyzed to assess performance validation. CFX Maestro Software (BIO RAD) and QuantaSoft Software for the Qx200 Droplet Reader were referred to analyze qPCR assay. Cq was mainly referred as the key statistical indicator for the interpretation of data which represented cycle at which fluorescence exceeds a threshold of 30 Cq values.

Results: The results showed high sensitivity with Cq values ranging from 15.31 to 22.32 for African elephant samples whereas no amplification for non-target species Cq values >36 was reported. The assay detected DNA concentrations as low as 7.6 copies/ μ L in diluted samples. The internal control confirmed no amplification inhibition indicating successful PCR across all samples.

Conclusions: YouSeq African Elephant qPCR Test Kit is a reliable, sensitive and effective tool for detecting African elephant DNA from non-invasive samples. It provided a robust method for wildlife conservation and forensic applications especially for anti-poaching efforts and population monitoring.

Keywords: African elephant, *Loxodonta Africana*, DNA, qPCR, cytochrome b gene, non-invasive monitoring, anti-poaching.

Introduction

The African elephant binomially referred to as *Loxodonta Africana* is one of the critically endangered species. The specie is considered as crucial because of its prominent role in maintaining the balance of ecological system in the Sub-Saharan region of Africa. Since the specie is identified as the largest terrestrial mammal, the elephant plays an instrumental role in the dispersal of seed, regeneration of forest and comprehensively maintaining the diversity of entire ecosystem. Nevertheless, as per Gebrehiwet (2024), for many years, the specie has been threatened with the epidemic of poaching particularly for its ivory, habitat destruction and the increased human-wildlife conflicts. As a result of this, significant decline

in its population across the region has also been reported. According to International Union for Conservation of Nature (IUCN), significant decrease of approximately 62 % decline in the population of African elephants has been reported in the region for the last 30 years (Gobush et al., 2021). The prime reason behind this decline is amid enhanced poaching fueled to acquire ivories amid the expansion in the demand of illegal markets. Also, as per Wasser et al. (2015), more than 100,000 elephants have been killed mainly to acquire their ivories between the years 2010 to 2020. Specifically in the Sub-Saharan African, a decline of 10 of population each year is reported. Apart from this, the factors such as habitat loss along with the fragmentation caused by agricultural expansion,

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urbanization and the excessive concrete or infrastructure developments have emerged as a major threat for the survival of this endangered species.

Precise and accurate monitoring has now become essential for an effective conservation and wildlife protection. As per Wall et al. (2014), traditional field oriented methods such as direct observations and tracking lead to significant logistic constraints and these constraints are more evident in the case of remote areas. Moreover, it has also been discussed that traditional approaches for monitoring are not merely time consuming however, at the same time these approaches are exposed to errors. The reason behind this is the traditional approaches are dependent on visual sightings and signs such as dung or footprints that nevertheless are indirect signs and makes difficult to distinguish between species. In this regard, molecular identification techniques especially in the form of DNA based assays has proven to be as effective and critical tools in wild-life monitoring because of their prominence in offering non-invasive and precise approach for the species identification (Wall et al., 2014). The utilization of molecular tools such as environmental DNA (eDNA) and especially quantitative PCR (qPCR) has consequently revolutionized the approaches towards wild-life monitoring. Further, Broadhurst et al. (2025) suggested the molecular techniques such as eDNA and qPCR has revolutionized wild-life monitoring by means of detection of species from samples such as soil, water, feces and traces of DNA typically found in air. As a result of this, the reliability and accuracy of identification of species in terms of their presence and population sizes have been improvised even without getting exposure of direct animal contact. This ultimately has also reduced the risk of disturbance and stress to the concerned endangered species.

In addition, molecular identification facilitates to the detection of DNA with low concentration thereby allowing of monitoring of population in remote and protected areas (Li, 2023). These techniques has consequently provided an advantageous position especially in the conservation of African elephants that are usually existed in vast inaccessible regions. In addition, the capabilities of these techniques in obtaining genetic data from non-invasive samples such as dung and hair also provides an opportunity for the long term monitoring. This eventually contributes significantly to the biodiversity conservation along with enhanced eco-system management. Notably, when considering the molecular identification of African elephant, DNA has been most widely used by using

mitochondrial DNA (mtDNA) markers. As per Elyasigorji et al. (2023), mtDNA markers are considered to be as highly conserved and provides species-specific detection across various mammalian taxa. However, among different markers, cytochrome b (cytb) gene has attained significant attention because of its high mutation rate and specificity effective for the identification of elephant species. As per Johnson (2023), cytb gene has been effective for the conservation genetics because of these abilities of this gene to precisely distinguish between closely related species such as *Loxodonta africana* and *Loxodonta cyclotis*. Though recent studies such as Sekgobela et al. (2025) have indicated the effectiveness of qPCR-based assays in targeting this gene and providing sensitive, accurate and rapid detection of elephant DNA however, within the context of African elephants, the findings are rare. Moreover, very limited studies have incorporated the aspects such as feces, hair and ivory remnants which the current study has focused at.

Considering this, the present study investigated the efficacy of the YouSeq African Elephant qPCR Test Kit for detecting African elephant DNA by utilizing cytb gene as a target gene. The prime focus of the study was to assess the sensitivity and specificity in detecting African elephant DNA. The study also focused to establish the reliability of an assay in a forensic and conservation context especially in combating the illegal trade of ivory by facilitating to the monitoring of elephant populations in both protected and unprotected environment. With this focus, the study aimed to explore the broader ecological implications of the aforementioned molecular tool in conservation strategies, wildlife forensics and ecological monitoring.

Material and Methods

Research Design

The study adopted a descriptive and experimental research for assessing the efficacy of qPCR assay targeting cytb gene for the molecular identification concerned African elephant's samples. Firstly experimental set up was undertaken to determine the sensitivity and specificity of qPCR assay to detect elephant DNA from non-invasive samples, The experiment utilized serial dilutions of elephant DNA ranging from 1,000X to 10,000X in order to assess the limit detection under specific condition simulating degrading environmental samples. One *Oryx leucoryx* sample was also chosen as non-target to assess cross specie specificity of assay. Moreover, descriptive aspect of study allowed for detailed documentation of the performance of

PCR assay across different types of samples such as feces, skin, hair and their respective dilution levels. This also involved estimation of amplifications of DNA samples and their consistency for the results by referring Cq values as indicator of DA quantity.

Samples

The samples were collected in 2022 from a wildlife research facility housing African elephants. The total samples comprised 9 samples that is African elephants involving 8 female and 1 male. In addition, one Oryx leucoryx was included as non-target specie control. More specifically, samples were gathered from fecal matter, skin and hair collected from each sample. For assessing the sensitivity of qPCR, dilutions levels were set as 1,000X and 10,000X. For fecal matter, DNA was extracted by utilizing QIAamp DNA Stool Kit (Qiagen). Whereas for skin and hair samples, DNeasy Blood & Tissue Kit (Qiagen) was utilized.

Moreover, The YouSeq African Elephant qPCR Test Kit (Version 8.3) was referred to amplify the cytochrome b (cytb) gene. The assay was typically performed under the manufacturer protocols of reaction volume of 20 μ L (12 μ L MasterMix, 1 μ L primer/probe mix, 1 μ L internal control, 8 μ L template DNA/water. Also, cycling conditions of 95°C for 3 minutes, followed by 45 cycles of 95°C for 15 seconds and 60°C for 60 seconds were set. In addition, fluorescence was detected via the FAM (target gene) and VIC/HEX (internal control) channels. Additionally, in order to test the specificity of the assay, DNA from non-elephant species comprising the Arabian Oryx, was tested.

Inclusion and Exclusion Criteria

In terms of inclusion, samples only from healthy African elephants contained in controlled facility were taken in light of ethical guidelines of Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) and the IUCN guidelines for wildlife research. A non-target specie to control cross-reactivity was also included as Arabian Oryx. Only DNA samples taken from fecal matter, hair and skin that is non-invasive samples were part of the study.

With regards to exclusion, all elephants with any sort of medical conditions were avoided to include as samples. Also, samples with any symptoms of stress or related medical condition were also excluded in order to avoid DNA degradation and contamination. The main samples were only African elephant species with the exception

of one Arabia Oryx which was considered as target specie. Hence, all the other species were excluded from samples. Also, samples were also excluded that had any predictability of being compromised and degradation in order to ensure high quality DNA.

Data Analysis Technique

CFX Maestro Software (BIO RAD) and QuantaSoft Software for the Qx200 Droplet Reader were referred to analyze qPCR assay. Cq was mainly referred as the key statistical indicator for the interpretation of data which represented cycle at which fluorescence exceeds a threshold. Importantly, Cq values were inversely related against the quantity of DNA. This indicated a higher amount of DNA suggest lower Cq values and vice versa. Moreover, sensitivity of assay was examined by referring lowest DNA concentration producing positive amplification with a detection threshold of 100 copies per reaction. In order to ensure assay could detect minimal amounts of DNA, sensitivity was assess by using serial dilutions of elephant DNA ranging from 10^6 to 10.

Specificity analysis was performed mainly to assess the ability of assay to detect merely African elephant DNA and exclude cross reactivity with non-elephant specie. DNA of non-target specie was tested to evaluate false-positives rates. The cases were Cq values were observed ≤ 30 , positive results were considered thereby confirming reliable DNA amplification. However, when samples reported greater than the threshold of 30 Cq values, the samples were considered negative and considered low for accurate detection. Apart from this, inter-sample variability was also assessed by comparing results across different samples such as feces, skin and hair with positive control synthetic cytb fragments to confirm the performance of assay. Inter-sample variability tests were also performed within CFX Maestro Software for determining consistency of Cq values.

Results

Positive Control Amplification

Table 1 confirmed the positive control was successfully amplified in both 6-Carboxyfluorescei (FAM) and Hexachloro-6-carboxyfluorescein (HEX) channels. The Cq values of 17.71 (FAM) and 18.83 (HEX) were obtained. Since Cq values fall in the expected range for positive control therefore, this confirms the validity of assay. Moreover, it also ensures that the PCR reaction was functioning as expected. Moreover, the low Cq values

demonstrates that positive control was existed in ample quantity. This consequently provided a reliable benchmark

for determining the amplification of target and control genes across the samples.

Table 1: Positive Control Amplification (FAM and HEX Channels)

Well	Fluor	Content	Sample	Cq
A01	FAM	Pos Ctrl	Positive control	17.71
A01	HEX	Pos Ctrl	Positive control	18.83

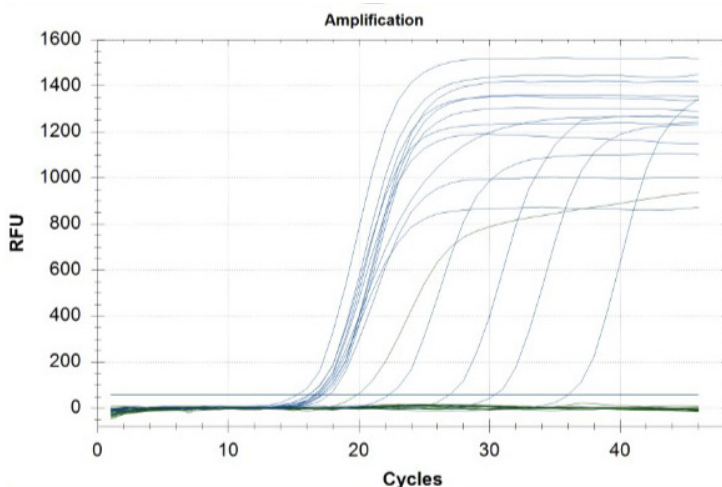


Figure 1: Amplification Curves for FAM and HEX Channels

African Elephant DNA Detection (FAM Channel)

Table 2 confirms detection of samples' DNA was also successfully done across multiple samples comprising feces, skin and hair. Specifically, Cq values for samples in the FAM channel ranged from 15.31 to 22.32. These values demonstrates varying DNA concentrations across the samples. The lowest Cq value of 15.31 indicated a higher DNA concentration in AF. Elephant 502393. On

the other hand, the highest Cq value of 22.32 indicated a lower concentration in AF. Elephant UNK. The successful amplification of DNA from these samples comprising those with higher Cq values confirms that sensitivity of the assay in detecting DNA even at low concentrations is critical in wildlife conservation efforts especially where non-invasive samples are often used for DNA analysis.

Table 2: Cq Values for African Elephant DNA Samples (FAM Channel)

Well	Fluor	Content	Sample	Cq
A02	FAM	Unkn	AF. Elephant 502395	16.87
B02	FAM	Unkn	AF. Elephant 502393	15.31
C01	FAM	Unkn	AF. Elephant 502398	17.16
C02	FAM	Unkn	AF. Elephant 502394	17.26
D01	FAM	Unkn	AF. Elephant 502397	16.22
D02	FAM	Unkn	AF. Elephant Jabali	16.81
E01	FAM	Unkn	AF. Elephant 502399	16.65
E02	FAM	Unkn	AF. Elephant UNK	22.32
F01	FAM	Unkn	AF. Elephant 502396	17.1
G01	FAM	Unkn	AF. Elephant 502400	16.6
H01	FAM	Unkn	AF. Elephant 1841620	16.2

Cross-Reactivity Testing (FAM and HEX Channels)

Table 3 demonstrates results for Cross-reactivity testing performed using sample A. Oryx AAZ3808 as a non-target species control. Cq values for the A. Oryx sample was much higher in both the FAM and HEX

channels that is 36.06 (FAM, Well F02) and 38.94 in HEX, Well F02. The aforementioned Cq values demonstrates that no significant amplification occurred for the A. Oryx sample in target gene (cytb). Therefore, this confirms the specificity of assay for African elephant DNA.

Table 3: Cq Values for Non-Target Species (A. Oryx) and Dilution (FAM Channel)

Well	Fluor	Content	Sample	Cq
F02	FAM	Unkn	A. Oryx AAZ3808	36.06
G02	FAM	Unkn	Dilution 1000	26.98
H02	FAM	Unkn	Dilution 10000	30.43

1D Amplitude

Threshold Value

All Wells: Ch 1: 2143 Ch 2:

Tilt Correction

No Wells

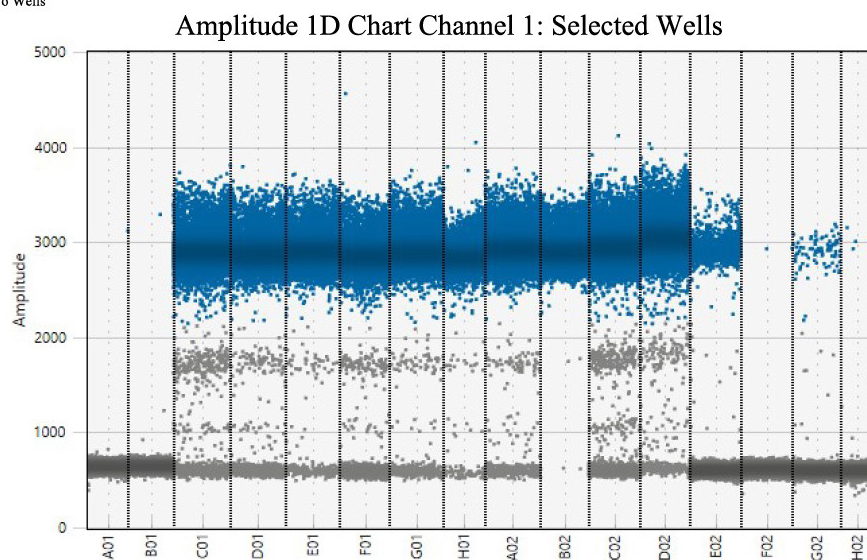


Figure 2: 1D Amplitude Chart

Sensitivity of the Assay (FAM Channel)

Table 4 demonstrates the sensitivity of the assay by performing serial dilutions of African elephant DNA. Specifically, the 1,000X dilution had the Cq values ranged from 26.98 to 30.43. However, for the 10,000X dilution, Cq values ranged from 30.43 to 32.98. This therefore indicated that the assay has the ability of detecting DNA even at very low concentrations. More specifically, these higher Cq values for diluted samples demonstrates that since the concentration of DNA decreases, the assay still detects the target gene.

However, increased Cq values are also reported. Therefore, gradual increase in Cq values with dilution as illustrated in Figure 3 also confirms the sensitivity of the assay in detecting even minute amounts of DNA. This therefore, make it suitable for detecting low-abundance DNA especially in light of degraded or trace samples.

Internal Control Analysis (HEX Channel)

Table 5 demonstrates the successful accomplishment of internal control as amplified in all samples. The samples confirms that PCR process was not inhibited hence confirming the effective DNA extraction

Table 4: Cq Values for African Elephant DNA Samples (HEX Channel)

Well	Fluor	Content	Sample	Cq
A02	HEX	Unkn	AF. Elephant 502395	36.02
B02	HEX	Unkn	AF. Elephant 502393	17.84
C01	HEX	Unkn	AF. Elephant 502398	18.07
C02	HEX	Unkn	AF. Elephant 502394	7.74
D01	HEX	Unkn	AF. Elephant 502397	18.43
D02	HEX	Unkn	AF. Elephant Jabali	9.57
E01	HEX	Unkn	AF. Elephant 502399	18.1
E02	HEX	Unkn	AF. Elephant UNK	23.9
F01	HEX	Unkn	AF. Elephant 502396	38.08
G01	HEX	Unkn	AF. Elephant 502400	18.11
H01	HEX	Unkn	AF. Elephant 1841620	18.55

across all samples. Cq values from 7.74 to 38.94 suggest internal control amplification validate the assay. This also ensures that all the reactions were performed optimally without considering inhibition. However, relatively low Cq values (7.74) indicated that internal control was also amplified efficient in samples even with high DNA

concentrations. Nevertheless, for non-target A. Oryx samples that is Cq values of 38.94, it can be said that there was a significant increase in the Cq values. This confirms that the absence of amplification for internal control especially in non-target species reinforce the specificity of assay for African elephant DNA.

Table 5: Cq Values for Negative Control and Dilution (HEX Channel)

Well	Fluor	Content	Sample	Cq
F02	HEX	Unkn	A. Oryx AAZ3808	38.94
G02	HEX	Unkn	Dilution 1000	3.25
H02	HEX	Unkn	Dilution 10000	32.98

The sample AF. Elephant 502395 with the Cq value of 16.87 reported as the highest DNA concentration that is of 10,310 copies/ μ L. This indicated that there is a substantial DNA available for amplification as also show in Figure 3. However, the sample AF. Elephant 1841620 with the Cq value of 32.98 demonstrated a significantly lower DNA concentration that is 7.6 copies/ μ L. Hence this is also consistent with the hypothesis that higher Cq values reflects a lower DNA concentration. In this sense, the results demonstrates the relationship between Cq values and DNA concentration where it was confirmed that assay does not merely detect DNA however, at the same time it is also used to quantify DNA concentrations across the samples.

Discussion

Notably recent advances in molecular forensics have demonstrated the importance of high throughput

and accurate DNA testing in order to trace the origin of ivories leading to support law enforcement efforts to combat poaching. The advancements in light of eDNA and qPCR has further elaborated the importance of molecular tools and techniques for assessing the presence of species without direct exposure with species. This consequently has revolutionized the approach of population monitoring with the more effective and less invasive method of monitor population. As also suggested by Lopez et al. (2023), the potential of advanced molecular techniques such as qPCR for detecting DNA from environmental samples have opened new avenues from monitoring elephant populations across large geographic areas especially in the situations where traditional methods are considered costlier and less effective at the same time.

The findings of the present also demonstrates the successful implementation cytb gene based qPCR essay for detecting DNA for *Loxodonta Africana* across varied samples.



Datafile Name: C:\Users\USER\Desktop\African Elephant_20250929_132332_608-2.ddpcr
Report Generated By: QX User
Report Generation Date and Time: 10/7/2025 1:48:40 PM

Concentration

Error Model

Poisson
Total

Confidence Model: 95%

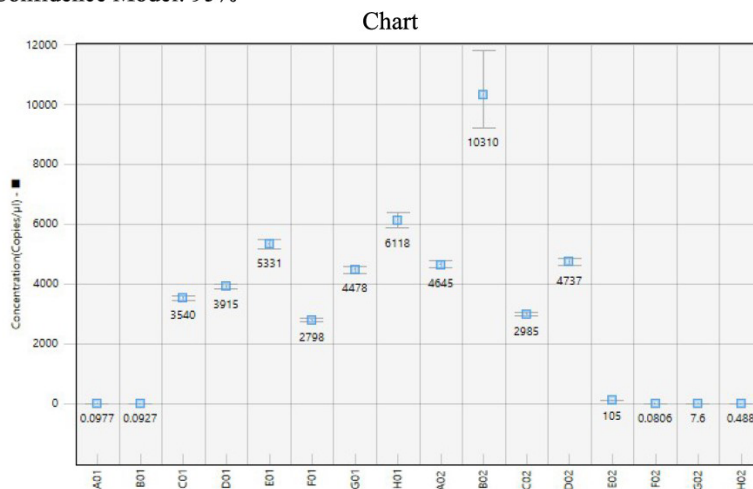


Figure 3: DNA Concentration Analysis

Based on this, it can be confirmed that the applied method is of significant prominence particularly in wild-life conservation. Therefore, this offers sensitive and specific tool for non-invasive purpose strategically beneficial for challenging field conditions. The findings such as LeClair et al. (2025), also confirmed that utility of molecular tools in the detection and monitoring of endangered species has become of significant importance. The findings of LeClair et al. (2025), also signified the importance of molecular techniques particularly for qPCR specifically for identifying and tracking wildlife DNA which has become vitally importance in the contemporary wildlife protection and forensic investigations.

Referring to current study, qPCR assay successful detected samples of African elephant in varied types of samples such as feces, skin and hair. The Cq values ranging from 15.31 (AF. Elephant 502393, Well B02) to 22.32 (AF. Elephant UNK, Well E02) for FAM channel confirmed this detection. Moreover, the results also validate the sensitivity of assay in detecting low DNA concentrations. Based on the obtained results, it was confirmed the sensitivity factor was also satisfied considering its criticality in the real world conservation efforts (Hays et al., 2024). More specifically, the ranges of Cq values reflected varied DNA

concentrations across samples with even lower DNA concentration. This therefore confirmed that Cq values are negatively correlated with DNA concentration (Whale et al., 2022).

One of the other key strength of the performed experiment and assay was the specificity. Arabian Oryx, a non-target species was also tested where the obtained Cq valued suggested that there was no cross-reactivity with the cytb target gene of concerned samples. The findings are aligned with the research of McDonald et al. (2024), suggesting cross species contamination often leads to false positives outcomes that therefore, signify their importance in forensic applications. This is also aligned with Kahila Bar-Gal (2025), indicating the prominence of assay in wildlife forensic and the need for accurate tools to misidentification of samples.

Moreover, the sensitivity of assay was also tested by means of serial dilutions of samples DNA. The obtained Cq values for the for the 1,000X dilution ranging from 26.98 to 30.43 and for 10,000X dilution ranging from 30.43 to 32.98 suggested that assay remained effective in detecting even low concentration of DNA. This therefore signify the importance of assay in specific forensic investigations where DNA degradation becomes a concern (Bhojar et al.,

2025). This is also aligned with the findings of Isham et al. (2024), suggesting high dilutions validates the robustness of assay. This also make it appropriate for the use of in such fields where DNA is largely fragmented or trace in negligible amounts.

Furthermore, integration of internal control for both FAM and HEX channels ensured the integrity of PCR amplification process. The study presented successful amplification of internal control across all samples as the Cq values ranged from 7.74 to 38.94. This therefore confirmed that PCR reactions did no inhibited and the DNA extraction was resulted in successful across all the samples. Also as per Giglioti et al. (2022), the high Cq values for non-target samples confirms that there is a lack of amplification for non-target samples. As a result of this, it confirmed the specificity and reliability of assay.

Conclusion

The efficacy of YouSeq African Elephant qPCR Test Kit for molecular identification of African Elephant (*Loxodonta africana*) through the target gen. *cytb* across varied biological was successfully tested. Assay demonstrated high sensitivity capable of detecting DNA at low concentrations and specificity. Moreover, no cross reactivity was reported when considered non-African sample. In the presence of internal control, the robust performance of the assay was reported thereby confirming its potential and prominence in the wildlife conservation and challenging forensic monitoring. Also, the ability of assay in quantifying DNA concentration along with the detection strengthen the applicability of assay for anti-poaching efforts, population monitoring and conservation genetics.

One of the considerable strength of the study was it demonstrated exceptionally sensitive approach for detecting African elephant DNA in a variety of sample types which was rare in the existing body of literature. Also, no cross specificity was also confirmed indicating the robustness and sensitiveness of the approach. The approach was also non-invasive which ultimately enhances its ability to detect low concentrations DNA thereby making it suitable for large scale and field monitoring. Nevertheless, the limited samples of the study that is only 9 lower the generalizability of the current approach which the future researchers can effectively enhance its generalizability to wider population that is not specific to African Elephants only.

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Availability of data and materials

All data utilized or analyzed during this study is available to share when asked.

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Conflict of Interest

The authors declare no competing interests.

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