*Malays. Appl. Biol. (2015) 44(3): 29–32*

# A NON-INVASIVE TECHNIQUE TO DETERMINE THE EFFECTS OF PLUCKED FEATHER TYPE (SIZE) ON DNA YIELD IN PCR AMPLIFICATION

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## ABSTRACT

Non-invasive approach in genetic sampling was introduced in avian culture as to reduce the stress of handling technique especially in fragile individuals or endangered species. The conventional conservation method causes many unfavourable impacts mostly when gathering DNA sources which affect birds' behaviour. The ability to correctly determine the sex of bird is pivotal for conservation purpose in monomorphic bird. However, the problem arises when it comes to collecting the type of feather that could meet the requirement of PCR amplification. Each part of the birds feather contribute in functional morphology so, sampling larger feathers could disturb the flight performance. Therefore, there is a need to find which feather present the most reliable source of DNA that is sufficient for PCR amplification. DNA was extracted and quantified in five types of plucked feather from two species of monomorphic bird. The same five feather types were used in comparing PCR success through agarose gel electrophoresis visualization by exploiting the intron length differences of Chromo Helicase DNA-binding gene (CHD gene) on the Z and W sex chromosomes. The validity and effectiveness of using thoracic feather were tested with the aim to inflict the only potential feather that will be used for future sexing purposes at least reducing the impact from feather sampling.

**Key words:** monomorphic bird, non-invasive approach, polymerase chain reaction, intron length, CHD gene

## INTRODUCTION

Non-invasive technique might be the only possible choice when dealing with delicate individuals and endangered species. That is why it is crucial when selecting the source of sample for DNA extraction without placing the life of bird at risk such as invasively collecting blood as genetic source. There are numerous non-destructive sampling specimen types comprising buccal swab (Handel *et al.,* 2006) and feces (Idaghdour *et al.,* 2003), although so far feather is a well-established method of sample collection with DNA-sufficient assurance. Back to 1994, Morin and his colleague proved the relevancy of utilizing feather gathered from the nesting sites of Hornbill as satisfactory genetic source of DNA used in phylogenetic analyses (Morin *et al.,* 1994).

Since then, there were numbers of articles verified feather as the favourable DNA source (Hogan *et al.,* 2008) due to the minimal practise required, non-stressing and accessible sample collection (Harvey *et al.,* 2006). However, the availability of the feather would not give a credit to gather sample as much as we can. Experimental error notably involving Polymerase Chain Reaction (PCR) would demand for a several feathers that definitely compromise with the birds' flight ability. Nearly all earlier study utilized larger feather mostly on flight feather (remiges-wing and rectrices-tail) due to the chances of getting high DNA yield upon extraction. Furthermore, larger feather contains blood clot found in the superior umbilicus of the feather shaft that provide higher yield and better DNA quality apart from the basal tip of the calamus (Horvath *et al.,* 2005).

Therefore, various studies were simulated by feather clipping or plucking to give an idea on the serious impact on fitness and survival caused by feather sampling. Flight performance found to be decreasing when two primaries on each wing of European starlings were entirely clipped to the skin (McDonald and Griffith, 2011) concerning the flexibility and take-off trajectories when avoiding predators (Thompson *et al.,* 2010). In this study, DNA yields associated with amplification success

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were compared in five feather types (size) by exploiting the intron length differences of Chromo Helicase DNA-binding gene (CHD gene) on the Z and W sex chromosomes (Sacchi *et al.,* 2004). The advantages of using plucked feather over shed feather was highlighted as satisfactory amount of genomic DNA required can be only extracted from freshly plucked feather.

Otherwise, shed feather rely on the field conditions such as humidity, temperature and sunlight which cause some DNA destruction thus, providing an adequate amount of DNA yield (Murphy *et al.,* 2003; Li and Paulsson, 2002; Ravanath *et al.,* 2001). The validity and effectiveness of using thoracic feather were tested with the aim to inflict the only potential feather that will be used for future sexing purposes at least reducing the impact from feather sampling.

## MATERIALS AND METHODS

Sample collection and DNA extraction

Five types of feather were plucked from 4monomorphic avian species (*Acridotheres-javanicus and Streptopelia chinensis;* medium-sized bird*) and (Lonchura maja* and *Seicercus montis;* small-sized bird) using mist nets from Taman Negara, Johor. Feather samples included primary wing-1, tail-1, alular-2, and wing-covert-1 and thoracic feathers (2-6) were stored at –20°C before further analysis. The amount of each feather type varied depending on the species of bird and size of the feather.

Replicate from the same individual were included for the consistency of the results. Genomic DNA was extracted using the DNeasy Blood and Tissue Kit following a user-developed protocol (Purification of total DNA from hairs, nail or feather). Approximately 0.5-1.0 cm segment of feather was cut from calamus end and placed in a 1.5 ml Eppendorf tube. The incubation step was performed at 52°C for 4 hours. For maximized DNA concentration was obtained, longer lysis time was allowed. Five (5) µL of the extraction yield were used in the amplification process.

Quantifying DNA yield and PCR amplification

DNA yield (ng/µl) was quantified using the NanoPhotometer$^{TM}$ P-Class and was underwent electrophoretic visualization on 1.2% agarose gel for 45 minutes. DNA bands were compared using Quick - load 1Kb DNA ladder (New England Biolabs). A single set of primer pair was used for the amplification of CHD gene P2 (5'-TCTGCATCGCT AAATCCTTT-3') and P8 (5'CTCCCAGGATGA GRAAYTG-3') (Griffiths *et al.,* 1998). The amplification was carried out in 20 µL final volume using 4 µL of 5X Hot Firepol Blend Master Mix with BSA (Solis Biodyne), 0.4 µL of each primer, 10.2 µL sterile distilled water and 5 µL genomic DNA.

A positive and negative control were included in order to prove whether contamination or non-specific amplification of samples exist caused by exogenous DNA or PCR component itself. The amplification process began with an initial denaturing step at 95ºC for 13 minutes followed by 35 cycles of denaturation (95ºC for 20 seconds), annealing (40.2ºC for 30 seconds) for P2/P8 and then a final extension step at 72ºC for 10 minutes. The amplified products were visualized in 1.2% agarose gelstained with 1 µL ethidium bromide (50 µg /ml) using Quick - load 100 bp DNA ladder (New England Biolabs).

## RESULTS AND DISCUSSION

Table 1 shows the DNA concentration in every 3 µl sample loaded into Nanophotometer as reflected in the electrophoresis visualization of extracted DNA extraction in Fig. 1. As usual, larger feather (remiges and rectrices) yields higher DNA in all species of monomorphic bird relative to smaller feather. However, the amplification success from thoracic feather was just high as other feather (Fig. 2). This depends on the amount of thoracic feathers (3-8) based on size of the bird, used in extraction in order to provide high DNA yield. For medium-sized bird, the minimum amount of thoracic feathers used supposed to be 2-4 individual feathers otherwise; lower DNA yield would affect the amplification success.

**Table 1.** Nucleic acid concentration in five different feather types based on medium-sized and small-sized of monomorphic bird

| Feather type (ng/µl) Species | Primary wing | Tail | Wing-covert | Alular | Thoracic |
|---|---|---|---|---|---|
| *Acridotheres javanicus* | 115 | 120 | 55 | 50 | 26 |
| *Streptopelia chinensis* | 98 | 109 | 40 | 35 | 22 |
| *Lonchura maja* | 56 | 67 | 32 | 29 | 15 |
| *Seicercus montis* | 58 | 70 | 30 | 15 | 10 |

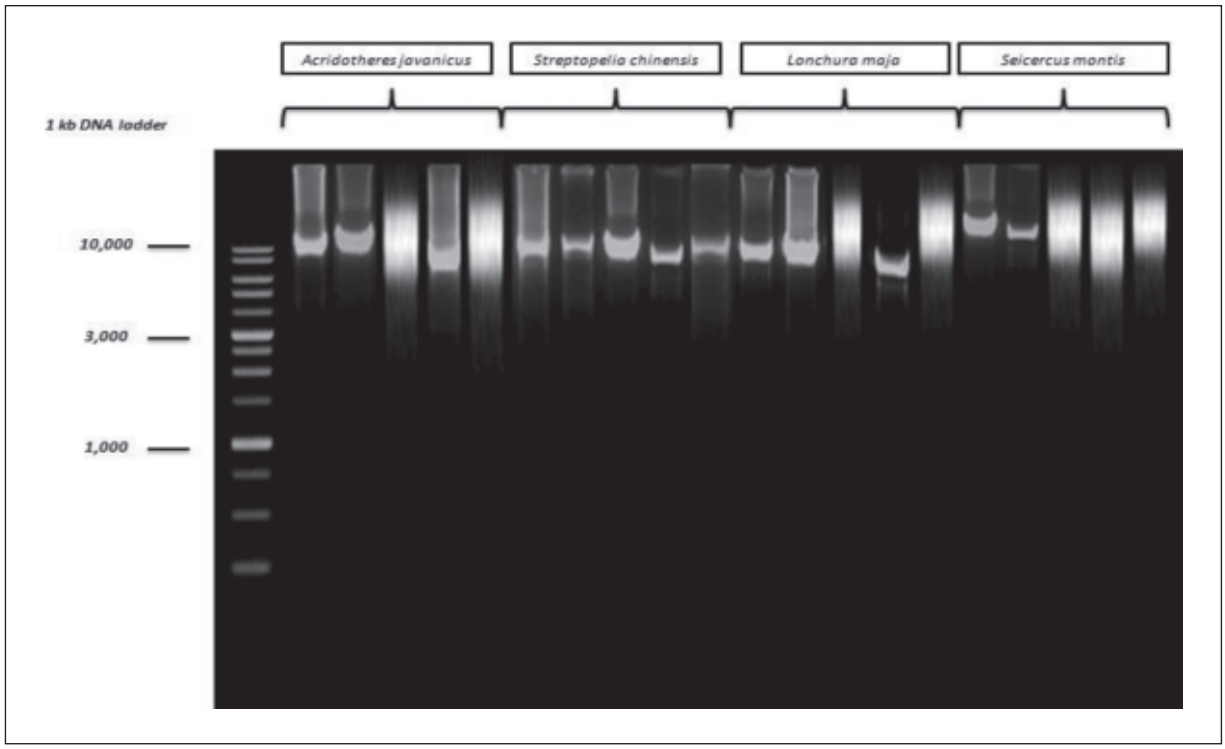


**Fig. 1.** Electrophoresis visualization of extracted DNA in five feather types in 4 species of monomorphic bird.

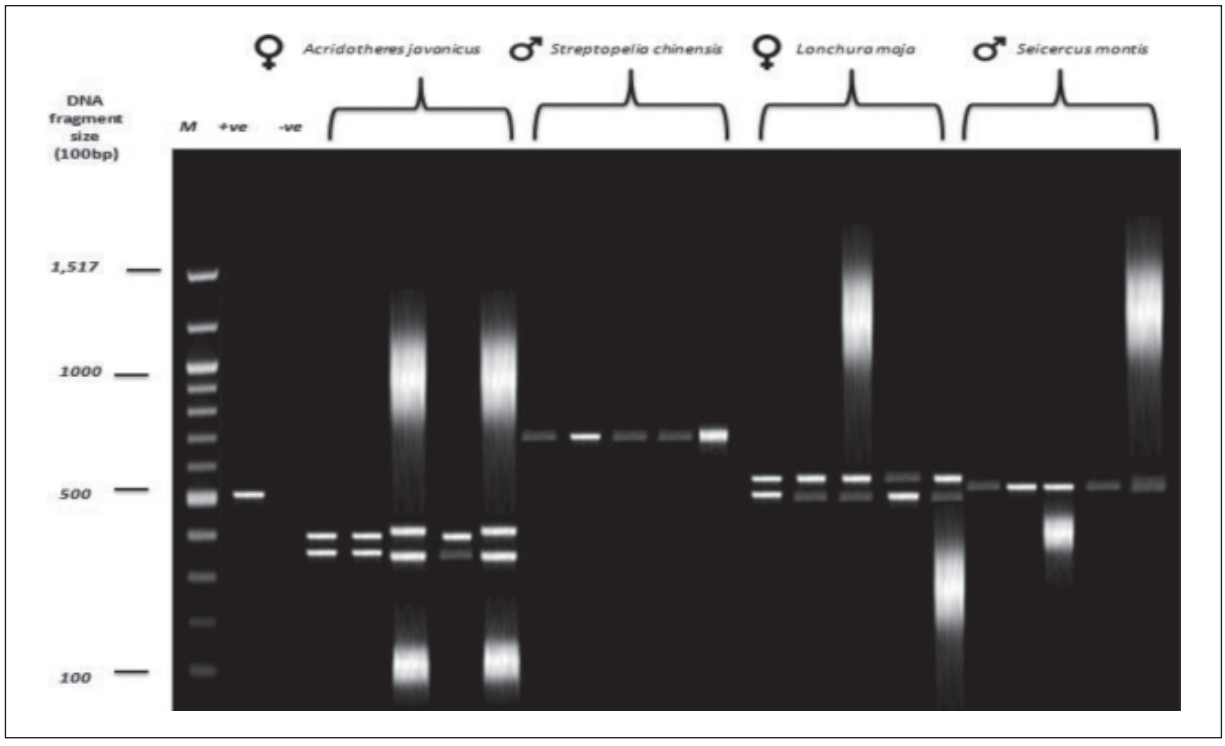


**Fig. 2.** Amplified CHD gene showing double band (ZW) in female, single band (ZZ) in male.

Furthermore, in small-sized bird, individual thoracic feathers required were up to 6 individual feathers. Similar study using thoracic feather was proven to be successfully effective in distinguishing sex in *Nymphicus hollandicus* (Cerit and Avanus, 2006). Lowered DNA yield (<10 ng/µl) from extracted thoracic feather resulted in the appearance of smear along the well or no band at all (not recorded). The high amplification success that was viewed in all species was likely influenced by the

fresh sample (plucked feather) that preserved the entire DNA inside feather shaft aided by a good storage condition at –20°C. This was supported by the significant of the initial condition of the feather sample regarding the outcome of the study in sexing Powerful Owl (*Ninoxstrenua*).

Based on their outcomes, the initial condition of the feathers is fundamental even though the types of feather have no effect on it (Hogan *et al.,* 2008). Furthermore, the use of plucked feather over shed feather reduced impurities in extracted DNA. Shed feather is collectively assessable compared to plucked feather as there is no trapping involves in order getting the fresh sample. It also has been applied in most studies as straightforward in sampling compared to hair, faeces and urine (Rudnick *et al.,* 2007).

However, feather that is exposed too long in the field tend to experience damaging DNA inside feather shaft due to high temperature, high relative humidity and sunlight (Nóra *et al.,* 2013). Thoracic feather might provide lower amount of DNA that used in amplification of CHD gene compared to large feather, however this study confirmed the effectiveness in providing satisfactory amount of DNA that contribute to the success in sexing monomorphic bird.

## CONCLUSIONS

Thoracic feather was proven to be valid and effective in sexing medium-sized and small-sized bird. Plucked feather provided good DNA yield with lower impurities, thus it can be used in sequencing analysis. Therefore, thoracic feather can be inflicted as potential feather that will be used for future sexing purposes at least reducing the impact from feather sampling.

## ACKNOWLEDGEMENT

The authors would like to thank the Ministry of Education for financing the project under the ERGS Fund (600-RMI/ERGS 5/3 (6/2012).

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