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*Original Article*

# A simple and rapid method for the extraction of genomic DNA from piglet tails without using proteinase K

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***Abstract***

Genomic DNA (gDNA) is a prerequisite for genomic research. However, the collection of blood samples from young animals for gDNA extraction is not practical. Since piglet tail docking is routinely performed in pig farms to prevent injury from tail biting, docking tails served as a practical source for gDNA in piglets. However, extraction of gDNA from piglet tails is difficult because of inelasticity and cartilage. The purpose of this study was to develop a rapid and simple method of isolating gDNA from piglet tails for downstream molecular purpose. Chopped piglet tails with ceramic beads in lysis buffer were vortex-homogenized prior to DNA extraction with a general tissue gDNA extraction kit without a long incubation period in proteinase K. Obtained gDNA was used for polymerase chain reaction amplification of the microsatellite loci, reflecting the quality of gDNA. In conclusion, we reveal the practical tools for gDNA extraction from piglet tails for molecular analysis.

**Keywords:** ceramic beads; genomic DNA extraction; homogenization; piglet tails; vortex mixer

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## Introduction

Genomic DNA (gDNA) has been used for molecular diagnosis of genetic disease. gDNA can be obtained from several sources such as whole blood, serum, secretions and tissue. Since piglet tails are normally docked after birth to prevent tail biting and the spreading of infection in pig farms (Schrøder-Petersen and Simonsen, 2001), docked tails are a practical source for gDNA extraction in suckling piglets where blood aspiration is not suitable to perform. However, a piglet tail is composed of elastic tissue and cartilage, a major obstacle for gDNA extraction. Several methods have been used to extract the gDNA from tail tissues such as kits for the isolation of genomic DNA from animal tail tissues, tissue homogenizer and liquid nitrogen (Biase et al., 2002; Wang and Storm, 2006; Yue and Orban, 2005; Zangala, 2007). The standard DNA extraction is the conventional phenol-chloroform extraction (Sambrook and Russell, 2006). Most methods required a long incubation period of chopped or ground tissue in lysis buffer with proteinase K (h to overnight). Without using proteinase K, a tissue homogenizer such as an automated Precellys® 24 bead-based homogenizer (Bertin Instruments, Montigny-le-Bretonneux, France) has been effectively used for homogenizing tissue samples such as fish liver and pork meat (Redanz et al., 2015; Wu et al., 2008). However, an automated tissue homogenizer is quite expensive at approximately USD 13,500. Another nonproteinase K method that has been reported for gDNA extraction from mouse tails is the use of Chelex® 100 resin after tissues have been minced in lysis buffer to chelate polyvalent metal ions and inhibit real-time PCR inhibitors (Burkhart et al., 2002). In the present study, we propose a novel gDNA extraction method from piglet tail tissue samples based on ceramic beads and a vortex mixer. This method does not require proteinase K incubation, is less time-consuming and provides high DNA concentration. Furthermore, the obtained gDNA can be used for microsatellite amplification by polymerase chain reaction (PCR), reflecting the quality of the gDNA.

## Materials and Methods

***Animals:*** Twenty tails were collected from piglets at 1-2 days. Betadine and 70% alcohol were used to clean the cutting area and reduce infection. Tails were docked 0.5 inch from the tail heads. The sample collection protocol procedures were approved by the Chulalongkorn University Animal Care and Use Committee (CU-ACUC) (license no. 1631016), and care was taken to comply with the 3R concept. Samples were kept in absolute ethanol at -20 °C until used.

***Genomic DNA extraction:*** Piglet tails of 0.5 cm were chopped. Samples were transferred into 7 mL tissue grinding tubes containing ceramic (zirconium oxide) mix beads of 2.8 mm and 5.0 mm (Bertin Technologies, Montigny-le-Bretonneux, France) and 800 µL lysis buffer (0.1 M Tris pH 8.0, 0.4% SDS, 0.2 M NaCI and 5 mM EDTA) (https://nature.berkeley.edu/stahllab/resources.html). Samples were consecutively vortexed for 1 min and alternately placed on ice for 1 min for a total of 3 sets. Supernatant of 200 µL was utilized for gDNA extraction modified from ZR Genomic DNA-Tissue MiniPrep kit (Zymo Research, Irvine, CA). Briefly, Genomic Lysis Buffer of 800 µL was added and samples were vortexed twice for 5 min each at room temperature. Supernatant was transferred to a Zymo-Spin IIC Column and centrifuged at 10,000 *x g* for one minute. After DNA Pre-Wash Buffer of 200 uL was added, the column was centrifuged again. The gDNA Wash Buffer of 500 uL was then applied. After centrifugation, gDNA was eluted in 50 uL DNA Elution Buffer. DNA samples were stored at -20 °C. To evaluate the concentration and purity of genomic DNA, absorbance at 260 nm ($A_{260}$) and ratio of $A_{260}/A_{280}$ were measured by NanoDrop ND 1000 spectrophotometer V3.7 (Thermo Fisher Scientific, Waltham, MA), using a default $A_{230}$ as a reference absorbance.

***PCR of SW240 and S0090 microsatellites:*** The integrity of gDNA samples was evaluated by polymerase chain reaction (PCR) of SW240 and S0090 microsatellites. A 94-bp fragment of SW240 was amplified using forward (5′-AGAAATTAGTGCCTCAAATTGG-3′) and reverse primers (5′- AAACCATTAAGTCCCTAGCAAA-3′) (Nechtelberger et al., 2001; Putnova et al., 2003). A 246-bp fragment of SW240 was amplified using forward (5′-CCAAGACTGCCTTGTAGGTGAATA-3′) and reverse primers (5′-GCTATCAAGTATTGTACCATTA GG-3′) (Nechtelberger et al., 2001; Putnova et al., 2003). gDNA at 300 ng and primers at 200 nM each were mixed with the 2x KAPA 2GHS Ready mix (Kapa Biosystems, Wilmington, MA). Reactions started at 95 °C for 3 min followed by 35 cycles at 95 °C for 30 s, 55 °C for 30 s and 72 °C for 1 min. PCR products were evaluated by 1% agarose gel electrophoresis in 1× Tris-acetate-EDTA (TAE) buffer at 110 V for 40 min, stained with RedSafe Nucleic Acid Staining Solution (iNtRON Biotechnology, Gyeonggi, South Korea) and visualized on a UV transilluminator (Bio-Rad Laboratories, Hercules, CA).

## Results

The present study showed the genomic DNA extraction from piglet tails within 1 h. The concentration and purity of gDNA are exhibited in Table 1. In most samples, $A_{260}/A_{280}$ ratios were in the range of 1.8–2.0. The integrity of genomic DNA was demonstrated by 1% agarose gel electrophoresis. Most gDNA exhibited high molecular weight bands with no DNA fragmentation (Fig. 1). PCR of microsatellites from the gDNA samples is also demonstrated in Fig. 2.

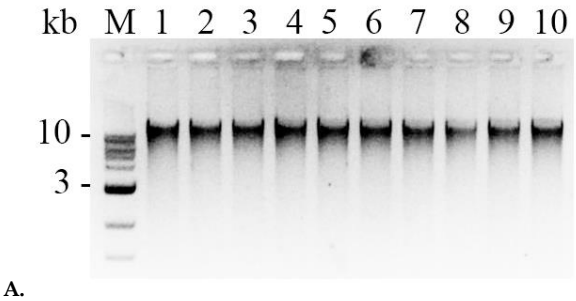
## Discussion

This study has shown a simple and rapid method for gDNA extraction from piglet tails which can be used for PCR amplification. The size of the tube played an important role in the successful genomic DNA extraction. We found that a mixture of 2.8 mm and 5.0 mm ceramic beads in 7 mL tube was suitable for the disruption of most animal and plant tissues in lysis buffer whereas a 2.8/5.0 mm beads in a 2 mL tube was not suitable for tissue homogenization which was

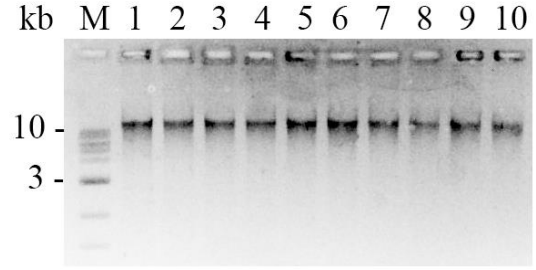
probably due to too small a space for the mechanical tissue disruption (data not shown). A combination of 2.8 mm and 5.0 mm ceramic beads is also suitable for grinding elastic tissues like skin, cartilage, and retina with a sample size ranging from 20 to 200 mg tissue (www.precellys.com). In general, gDNA can be extracted from hard tissues such as animal tails using the general gDNA extraction kit for tissue. However, in most protocols, an incubator and a long incubation time with proteinase K are required (at 55–65 °C for 3h to overnight) (Biase et al., 2002; Wang and Storm, 2006; Zangala, 2007). In the present study, the genomic DNA extraction process could be completed within 1 h without the requirement for an incubator. However, when comparing prices per reaction, using proteinase K (25 baht per reaction) was cheaper than using mixed ceramic beads in a 7 mL tube (240 baht per reaction).

**Table 1** DNA concentration and OD ratio ($A_{260}/A_{280}$) of DNA extracted from pigtail tissues.

| Sample no. | $A_{260}$ | $A_{280}$ | Ratio of $A_{260}/A_{280}$ | DNA concentration (ng/µL) |
|---|---|---|---|---|
| 1 | 0.573 | 0.297 | 1.93 | 130.00 |
| 2 | 0.556 | 0.292 | 1.90 | 139.00 |
| 3 | 0.554 | 0.282 | 1.97 | 138.50 |
| 4 | 0.451 | 0.238 | 1.89 | 112.50 |
| 5 | 0.547 | 0.279 | 1.96 | 137.00 |
| 6 | 0.590 | 0.307 | 1.92 | 147.50 |
| 7 | 0.535 | 0.262 | 2.04 | 133.50 |
| 8 | 0.548 | 0.297 | 1.84 | 137.00 |
| 9 | 0.536 | 0.299 | 1.79 | 134.00 |
| 10 | 0.564 | 0.303 | 1.86 | 141.00 |
| 11 | 0.485 | 0.257 | 1.89 | 121.00 |
| 12 | 0.512 | 0.271 | 1.89 | 128.09 |
| 13 | 0.516 | 0.470 | 1.91 | 129.00 |
| 14 | 0.475 | 0.239 | 1.99 | 119.00 |
| 15 | 0.438 | 0.249 | 1.76 | 109.50 |
| 16 | 0.616 | 0.347 | 1.78 | 153.90 |
| 17 | 0.616 | 0.347 | 1.78 | 153.90 |
| 18 | 0.610 | 0.330 | 1.85 | 152.40 |
| 19 | 0.519 | 0.277 | 1.88 | 129.90 |
| 20 | 0.508 | 0.287 | 1.77 | 127.30 |
| Mean | 0.537±0.05 | 0.296±0.05 | 1.88±0.08 | 133.70±12.60 |




**A.**




**B.**

**Figure 1** Genomic DNA quality analysis by 1% agarose gel electrophoresis. Genomic DNA (300 ng) was isolated from piglet tail samples no. 1-10 (A) and 11-20 (B) and analyzed on a 1% agarose gel electrophoresis. DNA was stained with Red Safe dye. Lane M: 1-kilobasepair (kb) DNA ladder marker 0.5 µg. (A) Lanes 1-10: Genomic DNA isolated from piglet tail samples no. 1-10. (B) Lanes 1-10: Genomic DNA isolated from piglet tail samples no. 11-20.

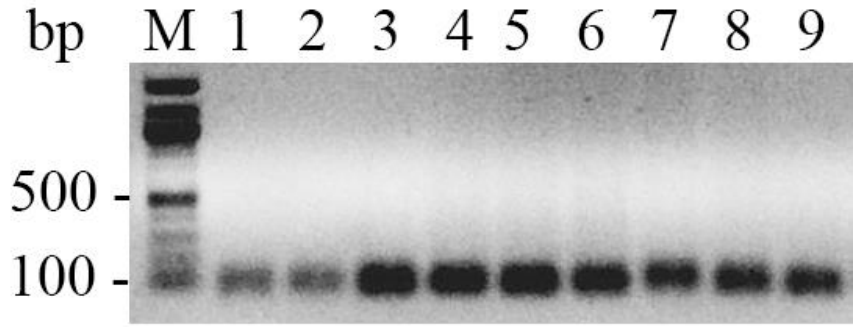



**Figure 2** Detection of SW240 microsatellites by PCR. Genomic DNA was isolated from piglet tails, subjected to PCR and electrophoresesed in 1% agarose gel. DNA was stained with Red Safe dye. Representative cases are shown. Lane M: 100-basepair (bp) ladder marker 0.5 µg. Lanes 1-9: PCR products from 9 piglets.

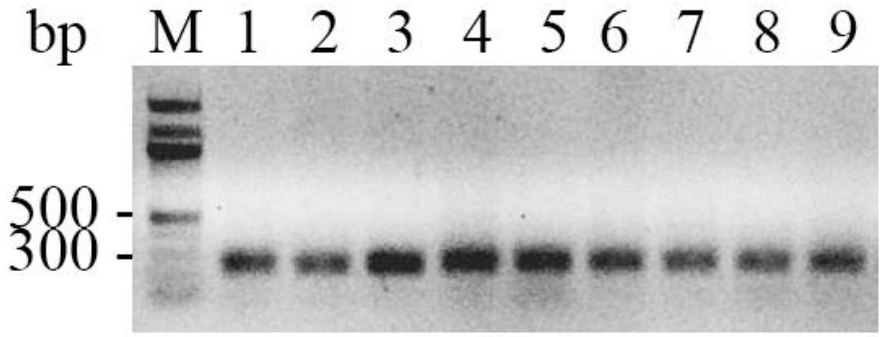



**Figure 3** Detection of S0090 microsatellites by PCR. Genomic DNA was isolated from piglet tails, subjected to PCR and electrophoresesed in 1% agarose gel. DNA was stained with Red Safe dye. Representative cases are shown. Lane M: 100-basepair (bp) ladder marker 0.5 µg. Lanes 1-9: PCR products from 9 piglets.

Absorbances at 230, 260 and 280 nm are some of the crucial parameters to determine gDNA yields and purity (Pereira et al., 2011). $A_{230}$ is useful for the identification of contaminants such as organic solvents, chaotropic salt or polysaccharide (Wilfinger and Chomczynski, 1997). In our case, $A_{230}$ was used as the reference wavelength. The purity of nucleic acids could also be assessed from the ratios of $A_{260}/A_{280}$. In our case, the overall ratios were approximately 1.8 - 2.0, indicating purified nucleic acid. If there has been protein contamination, the ratio would have been lower than 1.8 (Pereira et al., 2011). In our study, we used neither proteinase nor RNase treatment. However, the average ratios of 1.88 ± 0.08 revealed that the extraction method could get rid of protein contamination but not all RNA contamination. However, the DNA was functionality in the downstream application since it was suitable for PCR amplification as demonstrated by PCR of 2 microsatellites (Fig. 2).

## *Conclusion*

We reveal an effective protocol for the extraction of genomic DNA from piglet tails which can subsequently be used for PCR amplification, indicating that the extracted gDNA is suitable for other purposes following PCR amplification, such as parentage analysis, single-nucleotide polymorphism (SNP), genetic mutation and DNA sequencing.

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