Panthera tigris amoyensis Genomic DNA Extraction with a Simplified Method

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***Abstract* — Nowadays, eukaryotic genomic DNA is mainly extracted by the kits purchased from the biochemical companies. In this paper, a method for isolation of Panthera tigris amoyensis genomic DNA has been tried, which is really simple and quick.**

***Keywords* —Panthera tigris amoyensis; Genomic DNA extraction; Sheep; Cattle**

I. INTRODUCTION

Nowadays, the eukaryotic genomic DNA is mainly extracted by the kit purchased from the biochemical companies. One more fact is that DNA gel extraction kit is frequently used in most molecular laboratories. As researchers in the field of wildlife or livestock, we occasionally encounter cases of animal species identification, when the genomic DNA extraction of the animals is required. For a long time, we used to use the traditional phenol/chloroform extraction method to purify genomic DNA[1], which takes many hours due to the long Proteinase K (ProK) treatment[2,3]. Recently, to shorten the extraction time of *Panthera tigris amoyensis* (simplified as tiger) genomic DNA, we have tried a method, shortening ProK treatment combined with DNA affinity column, which works well really.

II. MATERIALS AND METHODS

Main Equipments and Materials

Eppendorf centrifuge (5415R, German), DNA gel extraction kit (Cat No. 2001250, SimGen, China), frozen heart of tiger stillbirth (Hangzhou Safari Park, China).

Methods

Step 1: DNA lysate

Fifty mg of sliced frozen heart of tiger stillbirth was grinded and homogenized in 1 mL 55℃ pre-heated DNA extraction solution [150 mM NaCl, 10 mM Tris-HCl (pH8.0), 10 mM EDTA, 0.1% SDS, 20 μg/mL ProK][3], which was then incubated for 10 min at 37℃ for the further ProK treatment. The supernatant was collected by centrifugation (16 100 *g* for 3 min at 4℃).

Step 2: DNA extraction with affinity column

According to Instructions of DNA gel extraction kit, 3 volumes of buffer G were added to the DNA lysate prepared on step 1, then the solution was mixed mildly and transferred to the DNA affinity column followed by a brief centrifugation (16 100 *g* for 0.5 min at 25℃). This operation could be repeated several times to increase the DNA concentration.

Step3: Column washing and DNA elution

According to Instructions, the column of step 2 was washed with 500 μL buffer WS once and 700 μL buffer WG twice by brief centrifugations. Then 1 min centrifugation (16 100 *g* at 25℃) was followed without addition

of any buffer to remove the ethanol as much as possible. Finally, DNA was eluted with proper volume of TE or sterilized pure H_2O.

III. RESULTS AND DISCUSSIONS

The experiments using the tiger genomic DNA extracted by the current method gave nice results, which were consistent with the results of our former experiments[4] (data not shown) indicating the good quality of DNA and the reliability of the current method.

In fact, our DNA extraction method has three points making itself much more effective than the traditional method[1-3]. One is to grind and homogenize the tissue slices with the DNA extraction solution pre-heated at 55℃. The second is 10 min incubation of the homogenized samples at 37℃ for further ProK digestion instead of several hours. The third is to extract DNA with affinity column instead of phenol/chloroform, which saves the time of the operation for collecting aqueous solution containing DNA (hard for new comers). In addition to the above, there are several other advantages, as described below.

1. Safety

As we all know, phenol is a kind of corrosive solvent, which has potential safety hazards. There is no such risk during the process of extracting DNA with affinity columns.

2. Environmental protection

Since phenol is not used, no phenol waste is produced and no special waste treatment is required.

3. Simple and quick

Compared with phenol/chloroform method, our genomic DNA extraction is simple and easy. New comers do not need special technical training. The whole process can be completed in half an hour.

4. No extra kits

DNA extraction by our method does not require additional reagent, except DNA gel extraction kit, a routine kit in the molecular laboratories. If a large amount of DNA is required, the affinity column provided by the plasmid extraction kit (Cat 1005250, SimGen, China) is suggested due to its greater DNA binding capacity than that provided by gel extraction kit.

5. No worrying of expiration

Usually, DNA extraction related kits have their own expiration dates. If the genomic DNA extraction is a routine work in your lab, the kit will be very helpful. If genomic DNA extraction is not a routine work, or our current method is adopted, it may not be necessary to purchase genomic DNA extraction kit, and we will not worry about the expiration date of the kit.

6. Suitable for other animals

In fact, we have also tried to extract genomic DNA from cattle and sheep. The concentration and purity of DNA are good enough for subsequent experiments such as PCR and qPCR (data not shown).

7. Cautions for choosing DNA gel extraction kit

In the process of studying the current method, we have tried several other DNA related kits or other DNA gel extraction kits. We do not recommend the DNA gel extraction kit, which requires the same volume of gel melting solution as DNA lysate instead of 3 volumes resulting in the low genomic DNA yield.

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