PORCINE RESEARCH
International Journal of the Bioflux Society
Research article

Two methods for isolating wild boar (*Sus scrofa ferus*) DNA

Teofil Oroian, Rareș G. Oroian, Augustin Vlaic, Vasile Cighi

University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca,
Faculty of Animal Husbandry and Biotechnologies, Cluj-Napoca, Romania.
Corresponding author: T. Oroian, teoroian@yahoo.com

Abstract. DNA isolation, one of the first steps in many molecular genetics studies, has to be efficient both in terms of yield and quality, but also in terms of costs. The present paper compares the DNA yield and purity of two inexpensive laboratory alkaline lysis protocols for wild boar DNA isolation, providing a comparison between two different starting materials: whole blood and hair bulbs. Both protocols yielded DNA quantities considered adequate for PCR-based analyses. Whole blood, as a starting material, was statistically superior to hair bulbs both in terms of yield and purity.
Key Words: wild boar, DNA isolation, whole blood, hair bulbs, laboratory techniques.

Introduction. DNA isolation is one of the first steps involved in any molecular genetic investigation. An ideal extraction technique should optimize DNA yield, minimize DNA degradation, and be efficient in terms of cost, labor, and supplies (Chen et al 2000).

The present paper compares the DNA yield and purity of two inexpensive laboratory protocols for DNA isolation, providing a comparison between two different starting materials: whole blood and hair bulbs. Both protocols rely on alkaline lysis: incubation at alkaline pH disrupts cell and nucleus membranes, denatures nucleases and dissolves the DNA, whose primary structure is relatively stable to such treatment (Felicello & Chinali 1993; Klintschar & Neuhuber 2000).

Study rationale:

- while commercial DNA extraction kits yield high quality DNA for subsequent analyses, their costs are higher compared to the protocols described in this paper;
- though DNA extracted from blood samples is of better quality, procurement of blood samples from wild boars is laborious (especially if dealing with live animals).

Materials and methods. For the present study we utilised two DNA isolation protocols previously described by Carsai et al (2009) and Balteanu (2010), to compare the efficacy of using different starting materials (whole blood *vs* hair bulbs) in terms of DNA yield and quality, when isolating wild boar (*Sus scrofa ferus*) DNA. The protocols originated from The Laboratory of Veterinary Genetics, University of California and were subsequently modified at INRA, Jouy-en-Josas (Balteanu 2010).

Hair samples (n=123) and blood samples (n=53) were collected as part of the previous activities of Project PN II 52105/2008 (Oroian et al 2012, 2012a, 2012b).

DNA extraction from wild boar hair bulbs

- 10 hair bulbs (roots) were cut and placed in an 1.5 mL sterile tube
- 50 µL solution A were added to the tube and the mix was incubated for 15 minutes at 97°C (alkaline lysis)
- 50 µL solution B were added to neutralize the pH.

Solution A: 200 mM NaOH (4 g + 500 mL ddH_2O).
Solution B: 200 mM HCl + 100 mM Tris-HCl, pH 8.5 (10 mL Tris-HCL 1M + 1.67 mL HClconc + ddH_2O [up to 100 mL]).

DNA extraction from wild boar blood

- 200 µL of whole blood were washed 3 times with 500 µL NE solution in an 1.5 mL tube (add NE solution, mix thoroughly, centrifuge at 14000 *g* for 10 seconds and discard the supernatant)
- 50 µL solution A were added to the tube and the mix was incubated for 15 minutes at 97°C (alkaline lysis)
- 50 µL solution B were added to neutralize the pH.

Solutions A and *B*: same as above.
NE solution: 10 mM NaCl, 10 mM EDTA, pH 7 (0.29 g NaCl + 1.86 g EDTA + ddH_2O [up to 500 mL]).
Notes: all solutions (NE, A, and B) and all the laboratory supplies (tips and tubes) were previously sterilized in an autoclave. NE can be successfully replaced with sterile PBS.
Recommendation: before starting, check if mixing 50 µL solution A with 50 µL solution B yields a neutral pH.

The data were processed statistically by estimating the mean and dispersion indices, using the formulae of Vlaic (2011). Statistical significance for each parameter was assessed using a two-tailed *t*-test.

Results and discussions. DNA quantities and purities were determined using a NanoDrop ND-100 spectrophotometer. The DNA samples extracted according to the protocols detailed above will be used for subsequent genetic analyses. DNA concentrations obtained using both starting materials (Table 1), are generally considered satisfactory for PCR-based techniques (Cosier, 2008). Higher DNA concentrations can be obtained by reducing the quantities of A and B solutions.

Table 1

Wild boar DNA yield, according to the starting material (ng/µL)

Starting material	n	$\overline{X} \pm s_x$	s	V%	Differences (±d)
Hair bulbs	123	264 ± 3.49	38.7	15	19**
Blood	57	283 ± 5.80	43.8	15	

Table 2 presents the purities obtained using the two different starting materials. While below the 1.8 value of "pure" DNA, the obtained purities are considered suitable for PCR-techniques (Cosier, 2008).

Table 2

Wild boar DNA purities, according to the starting material ($A_{260/280}$)

Starting material	n	$\overline{X} \pm s_x$	s	V%	Differences (±d)
Hair bulbs	123	1.53 ± 0.01	0.10	7	0.08***
Blood	57	1.61 ± 0.02	0.13	9	

Comparing the results obtained with the two different starting materials, both in terms of DNA yield and DNA quality, a statistically significant superiority ($p<0.01$ and $p<0.001$, respectively) was observed when whole blood was used as starting material. Relatively low V% values highlight the robustness of the described protocols.

When the starting material is blood, additional washes generally improve DNA quality. If higher purities are required these can be obtained via an alcohol precipitation of the samples (see Lamitina Lab Protocols 2007), another "low-cost" laboratory technique.

Conclusions. Both DNA isolation protocols presented here were satisfactory in isolating wild boar (*Sus scofa ferus*) DNA for subsequent analyses. Whole blood, as a starting material, yielded superior ($p<0.01$) DNA concentrations. The DNA purity was also higher (1.61 ± 0.02) for whole blood ($p<0.001$).

Acknowledgements. Financed by ANCS Romania trough Project PN II no. 52105/2008.

References

Balteanu V., 2010 Teza de doctorat, USAMV Cluj-Napoca. [In Romanian]

Carsai T. C., Vlaic A., Cosier V., Balteanu V. A., 2009 Cercetari privind polimorfismul la locusul genei leptinei in scopul aplicarii selectiei asistate de markeri genetici la taurine. Editura Bioflux, Cluj-Napoca. [In Romanian]

Chen H., Rangasamy M., Tan S. Y., Wang H., Siegfried B. D., 2010 Evaluation of five methods for total DNA extraction from western corn rootworm beetles. PLoS ONE 5(8):e11963.

Coșier V., 2008 Inginerie genetica. Editura Risoprint, Cluj-Napoca. [In Romanian]

Felicello I., Chinali G., 1993 A modified alkaline lysis method for the preparation of highly purified plasmid DNA from Escherichia coli. Anal Biochem 212:394–401.

Klintschar M., Neuhuber F., 2000 Evaluation of an alkaline lysis method for the extraction of DNA from whole blood and forensic stains for STR analysis. J Forensic Sci 45(3):669–673.

Lamitina Lab Protocols, 2007 Ethanol precipitation of DNA. Available online at http://www.med.upenn.edu/lamitinalab/documents/EthanolPrecipitationofDNA.pdf [last view: November 2012]

Oroian T., Oroian R., Covrig I., Pașcalău S., Cighi V., Chakirou O., Nistor S., 2012 Phenotypic characterization of the wild boar populations from the Sovata forest district, based on age and sex. Porc Res 2(1):27-34.

Oroian T., Oroian R., Cighi V., Pașcalău S., Covrig I., 2012a Biometric data in wild boar (*Sus scrofa ferus*) populations from Transylvania over 3 years of age. Porc Res 2(2):39-42.

Oroian T., Oroian R., Cighi V., Covrig I., Pașcalău S., 2012b Biometric data in wild boar (*Sus scrofa ferus*) populations from Transylvania, between 7 months and 2 years of age. Porc Res 2(2):43-45.

Vlaic A., 2011 Genetica animala. Editura AcademicPres, Cluj-Napoca. [In Romanian]

Received: 05 December 2011. Accepted: 15 November 2012. Published online: 01 April 2013.
Teofil Oroian, University of Agricultural Sciences and Veterinary Medicine, Faculty of Animal Husbandry and Biotechnologies, 3-5 Calea Manastur, Cluj-Napoca, 400372, Romania, e-mail: teoroian@yahoo.com
Rareș G. Oroian, University of Agricultural Sciences and Veterinary Medicine, Faculty of Animal Husbandry and Biotechnologies, 3-5 Calea Manastur, Cluj-Napoca, 400372, Romania, e-mail: oroianrg@yahoo.com
Augustin Vlaic, University of Agricultural Sciences and Veterinary Medicine, Faculty of Animal Husbandry and Biotechnologies, 3-5 Calea Manastur, Cluj-Napoca, 400372, Romania, e-mail: vlaic.augustin@gmail.com
Vasile Cighi, University of Agricultural Science and Veterinary Medicine, Faculty of Animal Husbandry and Biotechnologies, 3-5 Calea Manastur, Cluj-Napoca, 400372, Romania, e-mail: vasile_cighi@yahoo.com

How to cite this article:
Oroian T., Oroian R., Vlaic A., Cighi V., 2013 Two methods for isolating wild boar (*Sus scrofa ferus*) DNA. Porc Res 3(1):1-3.