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Mosquito DNA extraction using the Drábek-adapted method.

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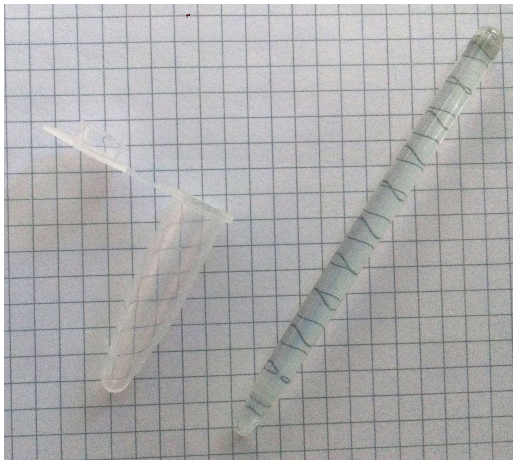
DNA extraction is a crucial and essential step for many molecular assays and applications such as genetic testing, polymorphism analysis, genomic studies, and molecular epidemiology. Current DNA extraction methods have several limitations for the high-throughput processing of samples: high-cost, low overall DNA yields and their use of molecular grade and toxic compounds. Phenol-chloroform extractions have long been regarded as the golden standard for DNA, RNA and Protein extractions but rely on proteolytic enzymes, carcinogens (Phenol). In the last couple of decades, commercially available column-based kits have demonstrated to be fast and allow for high-throughput processing at the cost of price. The method described herein is based on a technique developed in the Czech Republic by Drábek J (Immunology Department, Palacky University)[1] further optimized by Nasiri H (Medical Biotechnology Department of Tarbiat Modarres University of Irán[2] and others[3]. This method relies on the use of common household detergents for the molecular extraction of DNA from tissues and human blood. We have further optimized this method for DNA extraction from both vertebrate and viral DNA for use in several different molecular assays including end-point PCR, real-time PCR and DNA sequencing. This protocol describes our optimized approach for mosquito DNA extraction with special interest in applying said DNA in molecular taxonomy identification of genus and species.

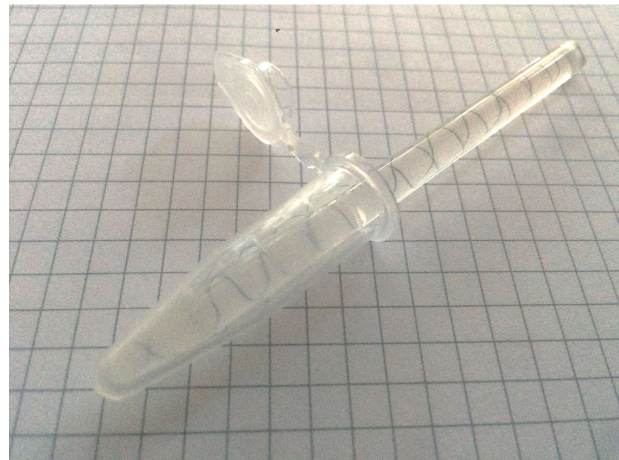
Procedure

1) Kill captured mosquitos by placing in BSL2 -80 ºC ultra-low freezer for 15 minutes.

2) Remove and discard mosquito abdomen and place remnants (thorax, head, legs and wings) in 1.5 mL Eppendorf microcentrifuge tube.

3) Add 25 µL of 1x PBS and homogenize mosquito using a glass pestle (see below).





NOTE: this pestle was improvised by our lab to allow for the processing of small

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samples against the inside conical end of a 1.5 mL Eppendorf tube. In our experience this method has shown to produce less aerosols than the use of tissue homogenizers and as such is amenable for use in procedures involving BSL3 discipline.

4) Top mosquito homogenate up to 200 µL with 1x PBS and add 200 µL of cell lysis buffer (see notes at the end of protocol) mix vigorously using a vortex and subsequently through inversion.

5) Spin at 15,000 G for 5 minutes and discard supernatant into 0.1% NaOCl.

6) Add 200 µL of cell lysis buffer and mix vigorously through vortex and subsequently through inversion until pellet is resuspended.

7) Spin at 15,000 G for 5 minutes and discard supernatant into 0.1% NaOCl.

8) Add 37.5 µL 10 mM pH 8.0 Tris-HCl and 37.5 µL the 40 mg/ml detergent solution followed by 3.125 µL de 5M NaCl (for a final concentration of 0.2 M) mix vigorously through vortex and subsequently through inversion.

9) Spin at 15,000 G for 10 minutes, transfer supernatant to a new 1.5 mL Eppendorf tube without disturbing the protein sediment.

10) Add 500 µL of 96% Ethanol at -20°C, mix gently and incubate at -20ºC overnight.

11) Spin at 16,000 G for 10 minutes, discard supernatant into 0.1% NaOCl.

12) Add 500 µL of 70% ethanol to pellet and wash by mixing / inverting. Do not vortex!

13) Spin at 16,000 G for 7 minutes, discard supernatant into 0.1% NaOCl.

14) Add 500 µL of 70% ethanol to pellet and wash by mixing / inverting. Do not vortex!

15) Spin at 16,000 G for 7 minutes, discard supernatant into 0.1% NaOCl.

16) Dry pellet by inverting microcentrifuge tube over paper towel, allow to air dry for 15 to 30 minutes.

17) Resuspend DNA in 50 µL of 8.0 pH Tris-HCl and incubate in thermomixer at 40ºC and 350 RPM for 30 minutes.

18) Proceed to assess DNA quality, yield and integrity or store at -20ºC until further use.

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Assessing DNA yield, quality, integrity and downstream application.

19) For DNA quantitation and quality assessment refer to our online multimedia protocol "Spectrophotometric evaluation of DNA using a Nanodrop ND-1000" available through YouTube (https://www.youtube.com/watch?v=k2tZTDDyxaw&feature=youtu.be).

    Note: Common spectrophotometric parameters for DNA extracted using this protocol are 29.68 ± 12.59 ng/μL for a total of 1.48 μg of total DNA extracted with 1.81 ± 0.21 A^{260}/A^{280} ratio and 1.16 ± 0.26 A^{260}/A^{230} ratio.

20) To assess DNA integrity load 25 μL of a mix including 20 μL extracted DNA with 5 μL of 6x orange loading buffer into a 1.5% agarose gel and run at 90 VDC (3.6 V/cm) for 80 minutes. Good integrity DNA should appear as a distinctive smear between 3kb and the gel wells. Fragmented DNA will either not show up on gel or appear as a smear spreading from 200 bp to 3 kb.

    Note: Typical good DNA integrity result of 11 of 12 mosquito samples as seen in 1.5% agarose gel after 80 minutes at 80 volts.

21) Final tests of DNA should involve any downstream procedure, either qPCR or endpoint PCR. At our Laboratory we usually employ mosquito DNA for molecular taxonomy applications and, as such, subject all extracted DNA's to this endpoint PCR technique.

Notes

1) All reagents used for cell lysis buffer, alcohol solutions and protein/DNA precipitation are ACS grade. However, the alcohol used should not be denatured.

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2) To prepare 1 litre of cell lysis buffer add 10 grs of Triton X-100 to a 500 ml flask and top up to 350 mL with distilled water and mix using a magnetic stirrer. Add 102.69 grams of sucrose (sacharose), 1.016 grs of Hexahydrated Magnesium Chloride and 10 mL of 1M Tris-HCl pH 7.6. Allow all reagents to dissolve completely then transfer to a 1 litre graduated cylinder and top up to 1 litre with distilled water. Dispense 50 mL aliquots and store under refrigeration (do not freeze).

3) Prepare 2 litres of saturated household detergent by dissolving 80 grs of commercial Foca® laundry detergent (or any other detergent not having whitening agents or bleach and/or any other additives) in 2 litres of distilled water, keep under agitation for at least 12 hours. Allow this saturated solution to sediment over 12 hours without further mixing. Without disturbing the sediment, retrieve the upper dissolved solution and filter through 2.5 cm 5-micron Luer-Lock syringe disc and then through Nalgene™ 500 ml filtration units (yellow, 0.22 µm). Aliquot working solutions by reducing concentration of solution to either 10 mg/mL for mosquito DNA extraction and human leukocyte concentrates or 20 mg/mL for whole blood samples.

References

1 Drabek, J. and Petrek, M., A sugar, laundry detergent, and salt method for extraction of deoxyribonucleic acid from blood. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub* **146** (2), 37 (2002).

2 Nasiri, H., Forouzandeh, M., Rasaee, M. J., and Rahbarizadeh, F., Modified salting-out method: high-yield, high-quality genomic DNA extraction from whole blood using laundry detergent. *J Clin Lab Anal* **19** (6), 229 (2005).

3 Bahl, A. and Pfenninger, M., A rapid method of DNA isolation using laundry detergent. *Nucleic Acids Res* **24** (8), 1587 (1996); Kotchoni, S. O. and Gachomo, E. W., A rapid and hazardous reagent free protocol for genomic DNA extraction suitable for genetic studies in plants. *Mol Biol Rep* (2008).

Revision history

1.0 Original document.

2.0 Protocol updated after change of detergent.

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