Genomic DNA from Adult Female *Brugia pahangi*, Strain FR3

Catalog No. NR-46542

This reagent is the tangible property of the U.S. Government.

For research use only. Not for human use.

Contributor:

Steven A. Williams, Director of Filariasis Research Reagent Resource Center and Gates Professor, Department of Biological Science, Smith College, Northampton, Massachusetts, USA

Manufacturer:

Filariasis Research Reagent Resource Center supported by Contract HHSN272201000030I, NIH-NIAID Animal Models of Infectious Disease Program

Product Description:

NR-46542 is a preparation of genomic DNA extracted from adult female *Brugia pahangi* (*B. pahangi*), strain FR3. Filarial DNA was extracted using a protocol developed by the Williams Lab at Smith College. The protocol is available in Appendix I. *B. pahangi,* strain FR3 was originally obtained from researchers in Malaysia by Dr. John Schacher.[1,2]

B. pahangi is a thread-like filarial nematode with a life cycle consisting of a mosquito intermediate host and a wide variety of carnivorous definitive hosts including human and felines.[1,3] Mosquitos deposit infective third stage larvae (L3) on human skin. The larvae then penetrate and migrate to the lymphatic vessels where they develop into adult worms over several months. Development includes molting transitions into fourth stage larvae (L4) and juvenile adults to reach maturation. The matured female worms release large numbers of microfilariae into the host bloodstream. The microfilariae are ingested by a mosquito during a blood meal and penetrate the midgut and develop over a period of 10 to 14 days to L3.[4,5] L3 are developmentally arrested in the mosquito. The process repeats when the mosquito's proboscis penetrates the definitive host's skin.[4]

Material Provided:

Each vial of NR-46542 contains 0.5 µg to 2.0 µg of genomic DNA in TE buffer (1 mM Tris-HCl, 0.1 mM EDTA, pH ~ 8). The concentration is shown on the Certificate of Analysis. The vial should be centrifuged prior to opening.

Packaging/Storage:

NR-46542 was packaged in plastic vials. The product is provided frozen and should be stored at -20°C or colder upon arrival. Freeze-thaw cycles should be minimized.

Citation:

Acknowledgment for publications should read "The following reagent was provided by the NIH/NIAID Filariasis Research Reagent Resource Center for distribution by BEI Resources, NIAID, NIH: Genomic DNA from Adult Female *Brugia pahangi*, Strain FR3, NR-46542."

Biosafety Level: 1

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. Biosafety in Microbiological and Biomedical Laboratories. 5th ed. Washington, DC: U.S. Government Printing Office, 2009; see www.cdc.gov/biosafety/publications/bmbl5/index.htm.

Disclaimers:

You are authorized to use this product for research use only. It is not intended for human use.

Use of this product is subject to the terms and conditions of the BEI Resources Material Transfer Agreement (MTA). The MTA is available on our Web site at www.beiresources.org.

While BEI Resources uses reasonable efforts to include accurate and up-to-date information on this product sheet, neither ATCC® nor the U.S. Government makes any warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. Neither ATCC® nor the U.S. Government warrants that such information has been confirmed to be accurate.

This product is sent with the condition that you are responsible for its safe storage, handling, use and disposal. ATCC® and the U.S. Government are not liable for any damages or injuries arising from receipt and/or use of this product. While reasonable effort is made to ensure authenticity and reliability of materials on deposit, the U.S. Government, ATCC®, their suppliers and contributors to BEI Resources are not liable for damages arising from the misidentification or misrepresentation of products.

Use Restrictions:

This material is distributed for internal research, non-commercial purposes only. This material, its product or its derivatives may not be distributed to third parties. Except as performed under a U.S. Government contract, individuals contemplating commercial use of the material, its products or its derivatives must contact the contributor to determine if a license is required. U.S. Government contractors may need a license before first commercial sale.

References:

1. Schacher, J. F. "Morphology of the Microfilaria of *Brugia pahangi* and of the Larval Stages of the Mosquito." J. Parasitol. 48 (1962): 679-692. PubMed: 13976565.
2. Michalski, M. L., et al. "The NIH-NIAID Filariasis Research Reagent Resource Center." PLoS Negl. Trop.

Dis. 5 (2011): e1261. PubMed: 22140585.
3. Schacher, J. F. "Developmental Stages of *Brugia pahangi* in the Final Host." J. Parasitol. 48 (1962): 693-706. PubMed: 13976564.
4. Simonsen, P. E. and M. E. Mwakitalu. "Urban Lymphatic Filariasis." Parasitol. Res. 112 (2013): 35-44. PubMed: 23239094.
5. Li, B. W., et al. "Transcription Profiling Reveals Stage- and Function-Dependent Expression Patterns in the Filarial Nematode *Brugia malayi*." BMC Genomics 13 (2012): 184. PubMed: 22583769.

Appendix I: Genomic DNA Isolation from Filarial Parasites

1. Thaw worms over ice and transfer to a 2 mL round bottom tube. Add 100 µL lysis buffer (0.1M EDTA, 0.1M Tris- pH 7.5, and 0.2M NaCl; sterilized by autoclaving) per 250 µL worms in buffer.
2. Add one 5mm BB [5 mm stainless steel scientific beads from Qiagen® (catalog # 69989)] to the 2 mL round bottom tube and attach to vortex mixer with special adaptor [Vortex Genie Adaptor, MO Bio Laboratories Inc. (catalog #13000-V1), alternatively, you can tape the tube on its side to the flat portion of a regular vortex mixer platform.] Vortex on the highest speed for 45 minutes, stopping every 10 minutes to change the position of the tube to ensure all areas of the tube are equally mixed.
3. Centrifuge the tube quickly before opening. Add an additional 150 µL of lysis buffer and vortex briefly.
   Note: The total volume of lysis buffer should be equal to the starting volume of worms in buffer.
4. Add 30 µL 10% SDS.
5. Add 2 µL of β-mercaptoethanol and vortex briefly.
6. Add 60 µL of Qiagen® Proteinase K (20 mg/ml). Vortex gently. Centrifuge the tube briefly in a nanofuge.
7. Incubate at 65°C for 4 hours to overnight.
8. Add 1.5 µL RNase A (100 mg/mL) and vortex gently. Spin the tube briefly in nanofuge.
9. Incubate at 37°C for 1 hour.
10. Centrifuge the tube briefly. Add 1 volume Tris-buffered phenol (pH 7.9). Vortex to mix.
11. Centrifuge at 2,000 rpm for 5 minutes.
12. Carefully remove the top aqueous phase and transfer to a new 1.5 mL tube.
13. Add an additional 1 volume phenol and vortex to mix.
14. Centrifuge at 2,000 rpm for 5 minutes.
15. Carefully extract aqueous phase and transfer to new 1.5 mL tube.
16. Add 1 volume chisam (24:1 chloroform/isoamyl alcohol). Vortex to mix.
17. Centrifuge at 2,000 rpm for 5 minutes.
18. Carefully remove the top aqueous phase and transfer to a new 1.5 mL tube.
19. Add 1 volume cold isopropanol and 1/10 volume 3M sodium acetate. Vortex Briefly to mix.
    Note: At This point the DNA extraction can be stored at -20°C. If working with small amounts of starting materials you may add 3-6 µL of glycogen (5 mg/mL) to aid precipitation.
20. Centrifuge at 16,000 x g for 30 minutes at 4°C.
21. Carefully remove isopropanol without disturbing the pellet.
22. Wash the pellet with 1 mL cold ethanol (70-75%).
23. Centrifuge at 16,000 x g for 15 minutes at 4°C.
24. Carefully remove ethanol without disturbing the pellet. Resuspend in 50 µL 0.1X TE or nuclease-free water.
25. If necessary, incubate DNA for 10 minutes at 55°C to completely redissolve pellet.
26. Measure concentration using the nanodrop spectrophotometer. It is also a good idea to run your genomic DNA on a gel to check for the presence of RNA and the integrity of genomic DNA.
27. To remove residual organics dialyze genomic DNA against a beaker (approximately 250 mL) of 0.1X TE for 4 hours to overnight using a Millipore [47mm diameter 0.025 µm filters from Millipore (catalog number VSWP04700)] membrane.
    Note: If there are no residual organics, this step is not necessary.

BEI Resources
www.beiresources.org
E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898