Nematode DNA Isolation using Qiagen DNAEasy Kit (cat #69506)

by Robyn Tanny July 2022

1. Chunk desired strain of nematode to three 10 cm NGMA plates (recipe below).

2. When the 10 cm plates are just about starved, wash the nematodes off the plates:
   a. Use a glass pipette to add 5 ml of M9 to one plate. After swirling around the M9, pour the M9 to the second plate. Add another 5 ml of M9 to the first plate.
   b. Swirl the M9 on the second plate. Pour the M9 to the third plate.
   c. Swirl and transfer the M9 on the first plate to the second plate.
   d. Swirl and pour the M9 from the third plate into a labeled 15 ml conical tube.
   e. Repeat steps b and d.

   NOTE: If the plates are too starved (ie - the worms have started to burrow), you can increase your yield by washing the worms off into the conical, allow the plate to sit for 5-10 minutes, wash the plate again into a second 15 ml conical. Waiting after some liquid is on the plate might help flood the burrowed worms back to the surface.

3. Allow the worms to settle in the conical. This will take about one hour.

4. Aspirate off the M9.

5. Add 5 ml of M9 to the 15 ml conical and allow the worms to settle again.

6. Aspirate off the M9. Use a Pasteur pipette to transfer the worms in the remaining liquid to a labeled **2 ml safe-lock tube (Eppendorf cat# 022363352)**. Wash the 15 ml conical with a little (less than 1 ml) M9 and transfer any remaining worms to the safe-lock tube.

7. Allow the worms to settle.

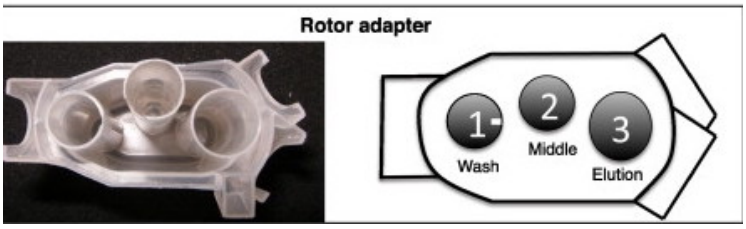
8. Aspirate off the majority of the M9 and use a micropipette to manually remove the remaining liquid. If you aspirate off all the liquid, you are also removing worms.

9. Optional: At this point, the worms can be stored at -80°C. This *might* help with cracking the cuticle, but it doesn't seem to make much difference. However, the worms can be stored at -80°C for several weeks until you are ready to isolate DNA.

10. Turn on a heat block to 56°C. Add 180 ul of Buffer ATL and 20 ul of Proteinase K (provided with the kit) to each sample. Incubate at 56°C with vortexing, rpm 1100 (or vortex a few times during incubation). Check the amount of lysis after 1 hr. If you still see worms, continue the incubation. If you see embryos or nothing, proceed to the next step.
    a. You can prep 12 samples at a time in the QiaCube, which takes a little over an hour to run the DNAEasy program. You can put ALL of the samples that you want to prep in a single day in the incubator at the same time. Take out 12 at a time as necessary.
    b. Buffers ATL may form precipitates upon storage. If necessary, heat the buffer at 37°C until it is fully dissolved.

11. While your samples are lysing:

a. Make sure the reagent bottles are filled with the appropriate reagents.
b. Fill both the wide-bore and regular 1000 μl filter tip holders in the QiaCube.
c. Label a rotor adaptor, DNAEasy column, and **1.5 ml elution tube (Sarstedt cat# 72.690.300/ Fisher cat #NC9972566)** for each strain being prepped.
d. Load the rotor adaptors with the column in position one and the elution tube in position 3. Make sure that the lids for the spin and elution columns are secured in the appropriate lid holders.



e. Load the filled rotor adaptors into the rotor of the QiaCube. Make sure to note which labeled rotor goes into which position (#1-12) of the rotor. You must load the sample for the particular rotor adaptor in the corresponding position of the shaker to ensure that the sample is eluted into the correctly labeled tube.
f. If you are not doing a full set of 12 samples, the loading chart a the end of this protocol demonstrates how to load the rotor and shaker.

12. Add 4 ul of RNAseA (100 mg/ml) (not provided with kit; use your favorite RNAseA). Incubate at room temperature for two minutes.

13. Place the lysed samples in the QiaCube shaker with the lids open. Make sure that the lids are pressed all the way down in the lid holder.

14. Start the DNAEasy program.
a. Note that this program does two 100 μl elutions, so you will have 200 μl at the end of the protocol.

15. Determine DNA concentration using Qubit Broad Range (cat# Q32850; follow Qubit-provided protocol).

•Label enough Assay Tubes for your samples, standard 1 and standard 2.
• Prepare the Qubit Working Solution by diluting the Qubit BR reagent 1:200 in Qubit buffer.
Prepare 200 µl of Working Solution for each standard and sample.
• For each **standard**, add 190 µl of of Working Solution to the appropriate tubes.
• For each **sample**, add 198 µl of Working Solution to the appropriate tubes.
• Add 10 µl of each **standard** to the appropriate tube.
• Add 2 µl of each **sample** to the appropriate tube.
• Briefly vortex all tubes.
• Incubate the tubes for 2 minutes at room temperature.
• Insert tubes in the Qubit Fluorometer to take readings.

16. If the concentration is very low, you can concentrate the sample using a speed-vac.

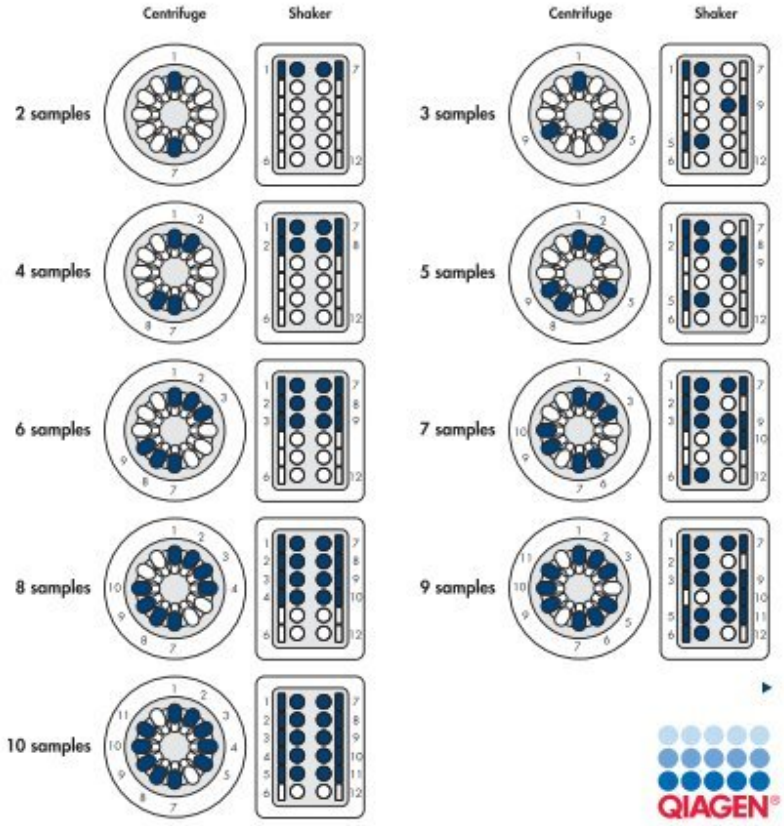
NGMA Recipe

	1 L	2 L
Peptone	2.5 g	5 g
NaCl	3 g	6 g
Agarose	7 g	14 g
Agar	10 g	20 g
Sterile water	975 mL	1950 mL

TechnicalInformation

QIAcube® loading chart

This Technical Information describes how to correctly load the QIAcube centrifuge and shaker if fewer than 12 samples are to be processed in a protocol run (see pictures below). When loading the shaker with samples in 2 ml microcentrifuge tubes, place tube lids into the slots at the edge of the shaker adapter. If the protocol requires the use of 2 ml screw-cap tubes, place Shaker Rack Plugs into the slots at the edge of the shaker adapter. Please note, 1 or 11 samples cannot be processed. For information about the type of sample and collection tubes that must be used with the QIAcube, see the other side.



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