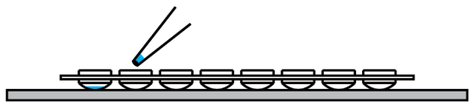
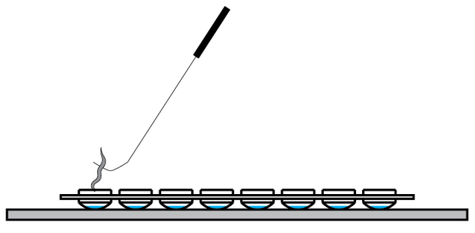
Worm-to-CT Protocol Overview

LYSIS

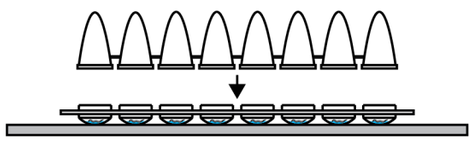


1. Place the lid of a PCR strip upside-down on the platform of the dissecting scope and add 10 µL of lysis solution (with DNase) to each slot.

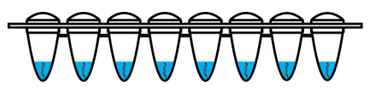


2. Pick single nematodes into each slot of the lid containing the lysis solution.

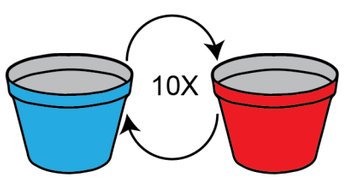
Recommended: To avoid contaminating the lysis mixture with a large amount of bacteria, first let the nematode crawl on an agar plate without bacteria for 5 min.



3. Carefully lock the PCR strip on its lid.

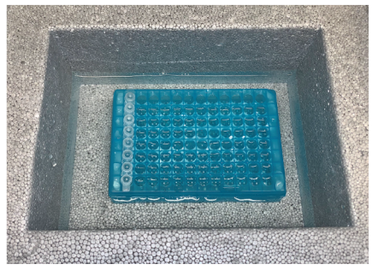


4. Spin down the lysis mixture, PCR strip upside-up.

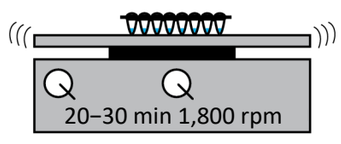


5. Freeze-thaw the lysis mixture 10x using liquid nitrogen and a hot water bath (at ~40 °C). This step takes 5–10 min.

Recommended: Keep the PCR strip upside-up in a 96 well plate holder during step 5, so that the lid does not come into contact with the outside liquids.



6. Mix the lysis mixture for 20–30 min at 4 °C on a thermal mixer. The nematode should have disolved complettely in the lysate.



7. Add 1 µL of stop solution to each of the samples.

Potential stopping point: Store the lysate at 80 °C for up to a week.

Reverse Transcription Option 1 - bulk worm samples

RT master mix (1 RXN)

2x RT buffer: 12.5 µL
20x RT Enzyme Mix: 1.25 µL
Nuclease-free water: 0.25 µL
(RT mastermix final volume: 14 µL)

1. Assemble the RT master mix on ice.

2. Add 14 µL of RT mater mix to each lysate, mix thoroughly, and place in a PCR machine with the cDNA synthesis program.

cDNA synthesis

Step 1. 37 °C, 60 min
Step 2. 95 °C, 5 min
Step 3. 4 °C, ∞

Potential stopping point: Store the cDNA at -20 °C.

Reverse Transcription Option 2 - single worm samples

1. Add 1.25 µL of reverse transcription mix to 5 µL of each sample in a PCR tube.

Reverse transcription

Step 1. 25 °C, 5 min
Step 2. 42 °C, 30 min
Step 3. 85 °C, 5 min
Step 4. 4 °C, ∞

2. Place the tubes in the thermocycler and run the reverse transcription program.