15	5. Total RNA	Extraction for	rom Insect	



RE-1

Total RNA Extraction from Chironomid

Protocol

Homogenization tube

← Chironomid : 1-20 pieces
← LRT (2-ME added) *1 : 500 μI

*1 Add 10 µl of 2-ME per 1 ml of LRT.

Homogenize:

Ball mill homogenizer Tomy Micro Smash MS-100

[zirconia ball (5 mmφ), 3,800 rpm 120 sec]

≥ 17,000×g (≥ 15,000 rpm), 3 min, RT

Transfer 385 µl of the supernatant to a new 1.5 ml micro tube

SRT : 175 μl

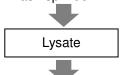
Vortexing (maximumspeed): 15 sec

Flash spin down

→ >99% ethanol: 140 μl

Vortex (maximum speed): 1 min

Flash spin down



Transfer all contents of the micro tube into the cartridge of QuickGene



Refer to the extraction protocol for each device written in the kit handbook. (from the step after transferring the lysate into the cartridge)



Results

The yield of total RNA / Protein contamination: A260/280

Number of chironomids	Yield (μg)	A260/280	
1 pieces	0.20	2.65	
20 pieces	2.05	2.22	

Common protocol is usable for the following

No Data

Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data. The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).



 $^{*}1\,$ Add 10 μI of 2-ME per 1 ml of LRT.



RE-2

Total RNA Extraction from Mosquito

Protocol

1.5 ml micro tube

Mosquito : 1 - 10 pieces (about 1-10 mg)
 LRT (2-ME added) *1 : 350 μI
 5mmφ One zirconia bead

Homogenaize with homogenizer 2 times

15,000 rpm, 3 min, RT

Transfer supernatant into a new tube

SRT : 175 μl

Vortexing (maximum speed): 15 sec

Flash spin down

→ >99% ethanol: 175 μl

Vortexing (maximum speed): 1 min

Flash spin down



Transfer all contents of the micro tube into the cartridge of QuickGene



Refer to the extraction protocol for each device written in the kit handbook. (from the step after transferring the lysate into the cartridge)



Results

The yield of total RNA / Protein contamination: A260/280 / Chaotropic salt contamination: A260/230

sample	Yield (μg)	A260/280	A260/230
No.1	1.5	1.95	0.66
No.5	16.2	2.16	1.96
No.10	24.0	2.17	2.07

Common protocol is usable for the following

No Data

Depending on sample and storage conditions, nucleic acid may not be extractable. Therefore, we cannot guarantee accurate data.

The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).





