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High-Throughput gDNA Extraction of Mosquito Tissues using QIAcube HT

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We use this protocol and it's working

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Abstract

Genomic DNA (gDNA) is crucial to the study of many important aspects of vector-parasite interactions of vector-borne diseases. Many studies operate at the population level of those diseases and as such, it is of immense value to have a high-throughput method of gDNA extraction for field collected mosquitoes that maintains sample integrity. Here we present such a protocol that has presently been optimized for the study of *Anopheles gambiae*, the primary vector of the malaria parasite *Plasmodium falciparum.* All mosquitoes used in optimization were of the KEELE strain, insectary-raised, unfed, and stored in desiccant to mimic the preservation method generally used for entomological samples in malaria endemic areas.

This protocol provides an automated DNA extraction method from dried mosquito thorax and/or abdomen using the QIAcube HT instrument. The QIAcube HT is intended to perform automated, medium- to high-throughput purification of nucleic acids for molecular biology applications such as gDNA sequencing, qPCR of malaria parasite, target capture, etc. The QIAcube HT delivers high performance and reliability, enabling purification of high-quality nucleic acids from 8–96 samples per run. Yield is further maximized and efficiency increased through homogenization via TissueLyser II (QIAGEN) as compared to manual homogenization with pestles.

Genomic DNA quantity and quality was assessed via Qubit 1x dsDNA High Sensitivity quantification and Genomic DNA ScreenTape for the Agilent TapeStation 4150. For thoraxes (n=12), the average gDNA concentration was ~2.4 ng/uL (range: 1.13-3.89 ng/uL) while for abdomens (n=20) it was ~4.0 ng/uL (range: 1.28-8.14) in an elution volume of 100 uL. While concentrations were outside the recommended ranges for the Genomic DNA ScreenTape (or sometimes outside the functional range) for the TapeStation DIN calculation, values ranged from 4-6 with fragment peaks around 7000-9000 bp for TissueLyser-homogenized samples. Attached are TapeStation, and Rmarkdown reports, and an Excel sheet containing the IDs and conditions of the samples in the run.

Attachments

MosDNAExt_Abdomen_Ho...
1.4MB

MosDNAExt_HomogTime_...
151KB

Abdomen_Homog_Time_M...
16KB

Guidelines

Follow standard molecular biology techniques including:

- Use only filter tips
- Change gloves frequently and especially when there is suspicion of any contamination
- Have solutions of freshly prepared 10% bleach and 70% ethanol available for decontamination of surfaces/equipment

Materials

MATERIALS

- Ethanol 200 Proof **Decon Labs** **Catalog #**2716
- QIAamp 96 DNA QIAcube HT Kit **Qiagen** **Catalog #**51331
- Reagent DX **Qiagen** **Catalog #**19088
- TissueLyser II **Qiagen** **Catalog #**85300
- QIAcube HT **Qiagen** **Catalog #**9001793
- TissueLyser Adapter Set 2×24 **Qiagen** **Catalog #**69982
- Eppendorf Repeater E3X **Fisher Scientific** **Catalog #**13683553
- SafeLock 2.0 mL RB Tubes **Qiagen** **Catalog #**990381
- QIAcube HT Plasticware **Qiagen** **Catalog #**950067
- Reagent Trough with lid Vf=170mL **Qiagen** **Catalog #**990556
- Reagent Trough with lid Vf=70mL **Qiagen** **Catalog #**990554
- Eppendorf Combitips Advanced Biopure 1mL **Fisher Scientific** **Catalog #**13683721
- Stainless Steel Beads 5 mm **Qiagen** **Catalog #**69989

Additional Equipment and Consumable

- Centrifuge (Eppendorf 5424R)
- Water Bath
- Vortex mixer
- Plate centrifuge or spinner
- Pipette (P20, P200, P1000)
- Pipette filter tips (20 µL , 200 µL, 1000 µL)
- Magnetic rack

Other materials such as petri dishes, tweezers, pipettes, pipette filter tips, centrifuge, etc. are general use and the exact brand selected should not impact results.

Safety warnings

Refer to the QIAcube HT manual for further safety warnings concerning instrument operation and cleaning.

Before start

Clean surfaces with 10% bleach followed by wiping with 70% ethanol. Place previously opened boxes of tips under UV light for at least 15 minutes. Autoclave and label 3, 2 mL Round Bottom (RB) tubes per mosquito. Make sure 1 of those tubes has a 5 mm stainless steel bead inside. Also include a tube with a bead for a negative control (1 every 47 samples). Time estimates are based on a full plate of 94 samples and 2 negative controls.

Sample Homogenization

5h

1 Using clean tweezers, separate the head and abdomen of a mosquito from the thorax on a sterile petri dish. Move either the thorax or abdomen (depending on which will be used for extraction) to the 2 mL RB tube with a 5mm stainless steel bead in it. Store the other 2 segments in the other 2 RB tubes and place in -20 or -80 C.

3h

2 Set a water bath to 56 °C and check Buffer ATL for precipitates. If there are precipitates visible, incubate buffer for 00:05:00 minutes.

3 Create a working solution (in a 15-50 mL Falcon tube depending on number of samples) of Lysis Buffer which is composed of 180 µL **Buffer ATL** and 1.3 µL **Reagent DX** per mosquito. Vortex well (2000 rpm for at least 00:00:15 seconds). Consider including an extra 10% volume to account for buffer loss.

4 Using an Eppendorf Repeater, dispense 180 µL of well-vortexed **Lysis Buffer** into each tube containing a steel bead and the body segment to be extracted.

5 Visually balance samples in a TissueLyser adapter set and load into the TissueLyser II.

- Lyse *Abdomens* at **30 Hz for** 00:00:30 **seconds.**
- Lyse *Thoraxes* at **30 Hz for** 00:01:40 **minute.**

Spin down tubes for 00:00:30 seconds at 15,000 rpm.

10m

6 Add 20 µL **Proteinase K** to each sample tube**.** Vortex for 00:00:10 seconds at 2000 rpm and spin down for 00:00:30 seconds at 15,000 rpm.

7 Load samples into floating racks and place in 56 °C water bath. Incubate for 01:00:00 hour.

1h

QIAcube Extraction

2h 30m

8 While the samples are incubating, start the QIAcube instrument and select the **DNeasy InsectLysates200ul AL-EtoH_tv** file. Use default parameters provided by instrument.

20m

Follow software (version 1.6.61) and manual instructions for setting up the run in addition to the following notes (as relevant):

- Check **Buffer AL** for precipitate and incubate at 56 °C for around 00:05:00 minutes if precipitate is present
- Dispense buffers (volumes provided by QIAcube software based on the number of samples) in reagent troughs under a biosafety hood
- Consider including an extra 10% of AL/Ethanol to account for loss in transfer
- **Thoroughly** mix AL/Ethanol solution in 50 mL Falcon tube by inverting the solution at least 5 times (or until solution is homogenous)
- If running less than 96 samples, make sure to hermetically seal unused columns/wells with a Tape Pad
- Make sure all components (Elution plate, QIAamp plate, and when ready the S-Bock) are pushed firmly to the upper left portion of their carriages

9 After incubation of mosquito solution is complete, spin down the tubes for 00:00:30 seconds at 15,000 rpm.

10 Move tubes to a magnetic rack to immobilize 5mm beads. Using a 200 uL pipette, immediately transfer as much of the 200 µL supernatant as possible to the S-Block. [Some loss may be observed on occasion (5-10 uL) but it should not have a significant impact on DNA yield]. 20m

11 Place the properly labelled S-Block on to the QIAcube carriage. Labels should include information such as date of extraction, sample type, project name, etc. After placing the S-Block in its carriage in the instrument, follow the QIAcube software and manual instructions for starting the DNA extraction run. It is advisable to save the Pre-Run report in the event that troubleshooting is needed. 1h 30m

12 Once the run is complete, save the Post-Run report and transfer the properly labelled Elution Plate (now containing the extracted DNA) to storage (at -20 °C for short-term storage, and -80 °C for long-term storage) unless doing immediate quality assessment.

13 Follow software and manual for cleanup instructions. 20m