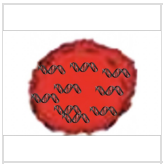

Aug 25, 2020

Optimized HT gDNA extraction from Dried Blood Spot using QIAcube HT for Malaria Genomic Epidemiology Studies

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1 Works for me dx.doi.org/10.17504/protocols.io.bh69j9h6

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ABSTRACT

Genomic DNA (gDNA) extracts from human dried blood spots (DBS) are commonly used for infectious diseases surveillance, genomic epidemiology studies of targeted pathogens, and Biobanking. In particular, DBS samples are routinely collected during cross-sectional and large-scale epidemiological studies (i.e. Malaria Indicator Survey) in malaria-endemic countries for malaria surveillance and genomic studies. DBS samples have multiple advantages over venous blood, including less invasive sample blood collection (finger prick) and the recommended short- and long-term storage at room temperature, which facilitates sample transportation (domestically or internationally). Here we present an optimized high throughput gDNA extraction protocol to recover *Plasmodium* gDNA from human DBS (collected on Whatman filter paper) using field samples from Zambia and stored at room temperature with desiccant pellet (up to 2 years).

Using the QIAcube HT instrument and QIAmp DNA 96-well kit, this protocol allows for gDNA extraction from human DBS samples in a manner that is automated, reproducible, and fast when compared to other methods. After gDNA extraction is completed, genomic DNA quantity and integrity is assessed using Qubit 1x dsDNA High Sensitivity Assay and Genomic DNA ScreenTape on the Agilent TapeStation 4150, respectively. In this case, gDNA samples that were obtained from field collected human DBS samples and extracted using this optimized protocol were then used for *Plasmodium falciparum* whole-genome sequencing by hybrid capture.

Using this protocol, we first compared gDNA concentration between 2X6mm and 3X3mm Uni Core full punch (100% blood punch) and found a statistically significant difference in DNA concentration obtained from 2x6mm (ng/µL) vs 3x3mm DBS punches (range =0.44-2.01ng/µL) (*P-value= 0.0003*) with a median increased of ~4-fold in the gDNA yield when using 2x6mm hole punches. *See the attached Comparison of DNA concentration 2X6mm vs 3X3mm Uni Core Punch documents*. Furthermore, we tested and demostrated that using this protocol with 2X6mm punches, it is feasible to successfully extract sufficient gDNA for *P. falciparum* whole genome capture and deep sequencing from DBS samples (n=93) that had been stored at RT for up to 2 years with variable level of parasitemia. While generally concentrations were outside the recommended range for the Genomic DNA ScreenTape for the TapeStation DIN calculation, from those samples DIN was estimated, values ranged from 6.2 - 8.7 with average fragment peaks of 35,325 bp, indicating that DNA integrity is suitable for downstream analysis. Becuase these were field DBS samples, we reported high variation in gDNA concentration (ranges= 0.50-4.88ng/µL) which reflected the variation in blood volume and adsorbance across DBS samples. *See the attached gDNA concentration for 93 DBS field samples documents*.

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

1. QIAamp® DNA Mini Kit and QIAamp DNA Blood Mini Kit Handbook.
2. QIAcube HT User Manual 07/2013.
3. Stangegaard M, et al. Evaluation of Four Automated Protocols for Extraction of DNA from FTA Cards. Journal of Laboratory Automation 2013 Oct;18(5):404-10. doi: 10.1177/2211068213484472.
4. Kumar A et al. Optimization of extraction of gDNA from DBS: Potential application in epidemiological research & biobanking. Gates Open Research . 2019 Nov 14;2:57. doi: 10.12688/gatesopenres.12855.3.

ATTACHMENTS

Citation: Abebe A. Fola , Jack Dorman, Maria Levy, Ilinca Ciubotariu, Giovanna Carpi (08/25/2020). Optimized HT gDNA extraction from Dried Blood Spot using QIAcube HT for Malaria Genomic Epidemiology Studies. https://dx.doi.org/10.17504/protocols.io.bh69j9h6

Comparison of DNA concentration 2X6mm vs 3X3mm Uni Core Punch.docx | Comparison of DNA concentration 2X6mm vs 3X3mm Uni Core Punch.csv | Examples_gDNA_Integrity_Tapestation.pdf | gDNA-concentration-for-93-DBS-field-samples.html | gDNA concentration for 93 DBS field samples.csv

DOI

dx.doi.org/10.17504/protocols.io.bh69j9h6

PROTOCOL CITATION

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MANUSCRIPT CITATION please remember to cite the following publication along with this protocol

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KEYWORDS

Dried blood spot (DBS), genomic DNA, laboratory automation, malaria genomics

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PROTOCOL INTEGER ID

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GUIDELINES

Follow standard molecular biology techniques including:

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

MATERIALS

NAME	CATALOG #	VENDOR
Ethanol 200 Proof	2716	Decon Labs
Qubit dsDNA HS Assay Kit	Q32854	Thermo Fisher Scientific
QIAamp 96 DNA QIAcube HT Kit	51331	Qiagen
QIAcube HT	9001793	Qiagen
QIAcube HT Plasticware	950067	Qiagen
Reagent Trough with lid Vf=70mL	990554	Qiagen

NAME	CATALOG #	VENDOR
Reagent Trough with lid Vf=170mL	990556	Qiagen
Uni-Core 6.00mm	WB100040	Ge Life Sciences
Genomic DNA ScreenTape®	5067-5366	Agilent Technologies

MATERIALS TEXT

- BioSafety Cabinet Class II
- Centrifuge (Eppendorf 5424R)
- Plate centrifuge (Sigma 4-16)
- Thermomixer C with Smartblock (Eppendorf 5382000023, 5363000039)
- Water bath (only for dissolving Qiagen ATL reagents ifp ecipitates are visible)
- Vortex mixer
- Pipette (P20, P200, P1000)
- 8-channel pipette (20-300 µL)
- Pipette filter tips (20 µL , 200 µL, 1000 µL)
- Serological Pipettes (25mL, 50 mL)
- Reagent reservoir (50 mL)
- Conical centrifuge tubes (50 mL)

SAFETY WARNINGS

- Refer to the QIAcube HT manual for further safety warnings concerning instrument operation and cleaning.
- Refer to the Safety Data Sheets (SDS) for health and environmental hazards for QIAGEN reagents.
- Operate in a BSL-2 lab setting

DISCLAIMER:

BEFORE STARTING

Clean all relevant surfaces with 10% bleach followed by 70% ethanol and wipe using Kimwipes. If any boxes of filter tips were used previously, UV them for 30 minutes. Select the samples (Dried Blood Spots) to be extarcted and include 1 negative control every 47 samples. For a full plate that means 94 samples and 2 negative controls. The first negative control should contain punches from clean filter paper and all downstream components, while the second should contain lysis buffer in addition to downstream components.

DBS Pretreatment 20h

1 Label a new S-Block with important information such as date, project name, sample type, etc. Use the same information to generate .csv file that contains sample ID and well position which will be imported to QIAcube HT robot.

2 Using clean tweezers, remove a DBS sample from its storage plastic bag and place face-up (the side with the most visible blood) on a cutting mat. Use a clean 6mm Uni-Core punch to remove a circular section and eject the punched section directly over its respective well in the S-block. Repeat so that each well has two punched 6mm circles total per sample in order to obtain as much blood as possible.

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3 Clean a Uni-Core punch after each use by immersing it in 10% bleach followed by 70% ethanol (for **00:00:10** each) and then letting it air dry. Use a rotation of multiple Uni-Cores (at least 4, to allow Uni-Cores to dry without impeding workflow). After each DBS sample, also spray the cutting mat with 70% ethanol and wipe dry. Repeat Step 2 until all selected samples have been processed.

4 Check Buffer ATL for precipitates. If precipitates are visible, incubate buffer in water bath at **56 °C** for **00:05:00** , or until precipitates are fully dissolved.

5 Create a working solution of Lysis Buffer in a 50 mL Falcon tube by preparing **360 µl** **Buffer ATL** and **20 µl** **Proteinase K** per sample. Include an extra 10% volume to account for buffer loss resulting from the transfer of buffers. Vortex at 2,000 rpm for at least **00:00:10** and transfer solution to a 50 mL reservoir via serological pipette.

6 Use a multichannel pipette to dispense **380 µl** **Lysis Buffer** into each well in the S-Block containing the dried blood punches. Seal the S-Block with Tape Pad. **Caution:** use the Lysis Buffer **immediately** after preparation, as the mix will precipitate within minutes.

7 Load the S-Block into the Thermomixer set to **56 °C** at 900 rpm and cover with lid. Incubate overnight (defined here as **16:00:00**).

QIAcube HT Extraction 3h

8 Open the QIAcube control software (version 1.6.61) and select the **Buccals_350ul_QCHT_withALEtOHmix_min_V5_dk** protocol. Follow instructions provided by the software and manual. Change the final elution volume to **120 µl** , but otherwise use default parameters. In addition, consider the following notes as needed:

- Fill the water bath with deionized water. Turn on to preheat at **56 °C** .
- Start with a clean QIAcube HT. If left idle for longer than 1 week, run the UV light for **00:30:00** .
- Check Buffer AL for precipitates. If present, incubate in the water bath at **56 °C** for at least **00:05:00** .
- Remember to import sample IDs using pre-prepared .csv file. See the HT manual on how to prepare and import a .csv file.
- Reagents volumes are provided by the QIAcube software based on the number of samples to be included in the run, but it is important to add an extra 10% volume for the AL/Ethanol mix.
- Fill the reservoir reagents with the appropriate volumes under a clean and UVed biological safety cabinet (BSC) using sterile serological pipettes.
- Thoroughly mix AL/Ethanol solution in a 50 mL Falcon tube by vortexing at least 1 minute (or until the solution is homogeneous).
- If running less than 96 samples, make sure to hermetically seal unused columns/wells with a Tape Pad to avoid vacuum errors.
- 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 within QIAcube HT robot.

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9 After overnight incubation is complete, centrifuge S-Block containing the samples at 2,400 RCF for **00:01:00** using a plate centrifuge, properly balanced.

10 Gently remove Tape Pad seal on the incubating S-Block and use a multichannel pipette to transfer a total of **350 µl** (**175 µl** X 2 - using 200 µL multichannel pipette) of lysate to a new, properly labelled S-Block. Avoid taking debris from filter paper. Note that the gDNA can be re-extracted from the filter paper at a later time, so seal the S-block containing the remaining filter paper and store it at **-20 °C** for future re-extraction.

11 Place the new S-Block that contains only the lysate in its QIAcube carriage and follow software instructions for starting the run. Save the Pre-Run Report in case troubleshooting is needed.

12 Once the run is complete, save the Post-Run Report, apply Elution Cap Strips to the Elution Plate, and transfer to storage (at **-20 °C** for short-term storage, and **-80 °C** for long-term storage) unless doing immediate quality assessment or downstream analysis.

13 After each run, clean the QIAcube instrument by following software and manual instructions.

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