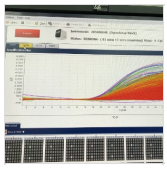

Working

Genotype with Quant Studio™ 12K Flex protocol

PLOS Neglected Tropical Diseases

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PROTOCOL STATUS

Working

Prepare the nucleic acid samples

1 Extract high-molecular-weight genomic DNA was from 2 ml venous blood using the QuickGene 610L Automatic DNA/RNA Extraction System (Fijifilm, Tokyo, Japan).

2 The quantitative detection of gDNA by NanoDrop 8000, the ratio of ultraviolet (UV) spectrophotometer readings at 260 nm and 280 nm wavelengths should be between 1.7 and 2.0 (i.e., A260/A280 = 1.7-2.0).

3 Normalize all gDNA samples so that each through-hole receives the same input quantity of sample. Standardization of the gDNA to 96-well sample plate with double-distilled water and the quantity of starting material is 10 μL at a concentration of 30 ng/μl.

Assay design and chip ordering by Thermo Fisher

4 30 independent SNPs derived from genome-wide association studies (S1 Table), were customized by Thermo Fisher Scientific company, USA. Custom TaqMan® Assay Design Tool was used for Custom Assays design, the following is the website: https://www.thermofisher.com/order/custom-genomic-products/ tools/genotyping/. Plate setup files (*.tpf or *.spf) contain the assay information for individual TaqMan®OpenArray® plates, including the gene symbol, gene name, assay ID, and location ofeach assay on the plate.

Perform the Quant Studio™ 12K Flex system

5 **Track the samples**

Create a sample information file (*.csv) to track where the samples are in the96-well sample plate. The OpenArray® Sample Tracker Software automatically maps the sample locations from the 96-well reaction plates to the appropriate locations in the 384-well sample plates and TaqMan® OpenArray® plates. Then export the sample information in table format (*.csv).

6 **Prepare the PCR mix and 384-well sample plate**

- a) Thaw the 96-well reaction plate containing prepared gDNA samples at room temperature. Mix the gDNA samples by vortexing, then spin for 1 minute @ 1000 rpm.
- b) Mix the 2×TaqMan® OpenArray® Genotyping Master Mix by gently invertingthe bottle 10 times.
- c) Add the master mix 2 µL to the 384-well sample plate.
- d) Using a 12-channel pipette, transfer the normalized gDNA samples 2 µL from the96-well reaction plate to the 384-well sample plate. Cover the sample plate with foil, then centrifuge for 1minute @ 1000 rpm to eliminate bubbles.

7 **Prepare the QuantStudio ™ 12K Flex OpenArray® Plate**

Transfer the samples from 384-well sample plate to TaqMan¨ OpenArray¨ plates using the QuantStudio™ OpenArray¨ AccuFill™ System.

- a) Confirm that the OpenArray® 384-well sample plate, OpenArray® AccuFill™ System Loader Tips, and plate holder are completely ready and put in the right place.
- b) Remove an OpenArray® plate from the freezer, and put in the room temperature for 10 mins.
- c) Prepare a syringe containing OpenArray® Immersion Fluid and attach the tip to the syringe.
- d) Close the enclosure door, then start the QuantStudio™ OpenArray® AccuFill™ Software. When the Remove OpenArray® Plate window appears, open the instrument door, carefully remove the indicated OpenArray® plate, then immediately seal the plate with OpenArray® Case Lid and Load the OpenArray® case with OpenArray® Immersion Fluid.

8 Load the OpenArray® plate into the instrument and run the OpenArray® plates

- a) Place the OpenArray® plate(s) on the plate adapter. Make sure that: Each plate is properly aligned in the adapter, and the plate barcode is facing up and toward the front of the instrument.
- b) Click 'Get Plate IDs' to import the barcode of the OpenArray® plates that wewant to run. The barcode of those OpenArray® plates will be populated and matched.
- c) Perform the Quant Studio 12K Flex platform instrument run. Temperature conditions were 10 min at 93 °C; cycling for 45 s at 95 °C, 13 s at 94 °C, and 2 min at 53.5 °C for 50 cycles, followed by incubation at 25 °C for 2 minutes.

Transfer and analyze experiment results

9 After an experiment run, close the run and re-open the *.eds file to display the Allelic Discrimination Plot screen. Then integrate the Sample names and Assay IDs into the resulting *.eds file.

10 Export the experiments results to anther computer. Analyze the experiment results with Taqman genotype analyzer v1.3.1.

11 Review the flags in the QC Summary, such as NOSIGNAL, NOAMP, OFFSCALE and et al. The flags BADROX and AMPSCORE are, by default, were not in use for the Genotyping experiment, need us manually remove these samples. View and, if necessary, change the analysis settings and Click Edit Default Settings, then specify the default call settings.

12 Export the analyzed data and select QuantStudio™ 12K Flex format (*.csv) for further analysis.