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SNPscan protocol for genotyping

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Guangming Jiang[1], Meng Wu[1], Rui Liu[1], Mengya Chen[1], Renfang Han[1]

[1]Department of Epidemiology and Biostatistics, School of Public Health, Anhui Medical University, Hefei, Anhui, China

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Guangming Jiang

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MATERIALS TEXT

QIAamp DNA Mini and Blood Mini Kit	Qiagene
48-Plex SNPscanTMKit （custom-by-design)	Genesky

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1 Pipet 20 µl QIAGEN Protease (or proteinase K) into the bottom of a 1.5 ml microcentrifuge tube.

2 Add 200 µl whole-blood samples to the microcentrifuge tube.

3 Add 200 µl Buffer AL to the sample. Mix by pulse-vortexing for 15 s.

4 Incubate at 56 °C for 10 min.

5 Briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from the inside of the lid.

6 Add 200 µl ethanol (96–100%) to the sample, and mix again by pulse-vortexing for 15 s. After mixing, briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from the inside of the lid.

7 Carefully apply the mixture from step 6 to the QIAamp Mini spin column (in a 2 ml collection tube) without wetting the rim. Close the cap, and centrifuge at 6000 g (8000 rpm) for 1 min. Place the QIAamp Mini spin column in a clean 2 ml collection tube, and discard the tube containing the filtrate.

8 Carefully open the QIAamp Mini spin column and add 500 µl Buffer AW1 without wetting the rim. Close the cap and centrifuge at 6000 g (8000 rpm) for 1 min. Place the QIAamp Mini spin column in a clean 2 ml collection tube, and discard the collection tube containing the filtrate.

9 Carefully open the QIAamp Mini spin column and add 500 µl Buffer AW2 without wetting the rim. Close the cap and centrifuge at full speed (20,000 g; 14,000 rpm) for 3 min.

10 Place the QIAamp Mini spin column in a new 2 ml collection tube and discard the old collection tube with the filtrate. Centrifuge at full speed for 1 min.

11 Place the QIAamp Mini spin column in a clean 1.5 ml microcentrifuge tube, and discard the collection tube containing the filtrate. Carefully open the QIAamp Mini spin column and add 200 µl Buffer AE. Incubate at room temperature (15–25 °C) for 1 min, and then centrifuge at 6000 g (8000 rpm) for 1 min.

12 Determine the DNA yields by measuring the absorbance at 260 nm. Determine the DNA purity using A260/A280 ratio. Place the specimens with a total of more than 3 µg DNA in a -80 °C refrigerator until genotyping.

13 To evaluate the quality of dissolved DNA, pipet 1 µl DNA samples for 2% agarose gel electrophoresis.

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14 Adjust the DNA concentration to 5-10 ng/μl.

15 Add 4 μl DNA solution, 2.5 μl 4×DNA Lysis Buffer, and 3.5 μl ddH2O into 0.5 ml PCR tubes (or a multi-tube plate). Mix by pulse-vortexing for 15 s. Briefly centrifuge the tubes to collect all the drops.

16 Place the tubes into a Thermal Cycler 2720（ABI）, 98 °C for 5 min. Immediately insert the tubes into ice.

17 Add 2 μl 10×Ligase buffer, 0.5 μl Ligase, 1 μl, Probe mix, and 7.5 μl ddH2O to each of the tubes. Mix by pulse-vortexing for 15 s. Briefly centrifuge the tubes to collect all the drops.

18 Immediately place the tubes into the Thermal Cycler, and run the following program: (94 ºC（1 min→58 ºC（4 h) × 4 cycles → 94 ºC（2 min → 70 °C forever.

19 Immediately insert the tubes into ice.

20 Add 10 μl 2×PCR Master mix, 1μl Primer mix, 8 μl ddH2O, and 1μl ligation product into 0.5 ml new tubes (or a multi-tube plate), placed on ice in advance. Mix by pulse-vortexing for 15 s. Briefly centrifuge the tubes to collect all the drops.

21 Immediately place the tubes into the Thermal Cycler, and run the following program: 95 ºC, 2 min→ [94 ºC（20 s→62 ºC（40 s→72 ºC（-0.5 ºC/cycle（（1.5 min] × 9 cycles→（94 ºC（20 s→57 ºC（40 s→72 ºC（1.5 min（× 25 cycles → 60 ºC（60 min → 4 ºC forever.

22 Dilute the PCR products (1: 10) with ddH2O and add 1 μl of it into a new 96-tube plate. Add 0.5 μl Liz500 Size Standard and 8.5 μl Hi-Di. Mix by pulse-vortexing for 15 s. Briefly centrifuge the plate to collect all the drops.

23 Place the 96-tube plate into a Thermal Cycler, incubate at 95 °C for 5 min.

24 Place the 96-tube plate into a Genetic Analyze 3730 XL（ABI）and carry out DNA sequencing.

25 Analyze the raw sequencing data using GeneMapper 4.0 (ABI).

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