Protocol

Isolation of Genomic DNA (based on solution)

Sample type: Clotted Blood

Some tips to know:

- All centrifugation steps are carried out at room temperature (15–25°C).
- If any buffer (ROS or RBC Lysis Buffer) forms precipitate, please warm it to 56°C until the precipitate has fully dissolved.

Process

1. Add 1 ml RBC lysis buffer to 400 µl clotted blood in microtube, invert 5 times, vortex 10 s at high speed and centrifuge at 13000 rpm for 3 min.
2. Discard supernatant and add 1 ml RBC lysis buffer to the pellet, invert 5 times, vortex for 10 s at high speed and centrifuge at 10000 rpm for 2 min.
3. Discard supernatant. Add 500 µl ROS and then 20 µl RJ-Protease. Mix thoroughly by pulse vortexing for 30 s, then incubate at 56°C for 30-60 min until the sample is completely lysed. Pulse vortex every 10 min during incubation to intersperse the sample, or place it in a thermomixer or shaking water bath.
4. Add 200 µl phenol 50% and then 200 µl chloroform. Vigorously shake it for 30 s. Then pulse vortex for 15 s and centrifuge at 13000 rpm for 10 min.

5. Transfer the supernatant to a new tube. Add 5 µl Prime-RNase A to the isolated aqueous phase. Then pulse vortex for 5 s and incubate for 10 min at room temperature, 25 °C.
6. Add equal volume of isopropanol to the microcentrifuge tube. Invert 10-15 times rapidly. Centrifuge at 12000 rpm for 1 min.

Note: The DNA should be visible as a small white pellet.

7. Discard supernatant, aspirate the pellet. Add 600 µl ethanol 70% to the pellet; centrifuge at 10000 rpm for 2 min.
8. Discard supernatant and aspirate the pellet. Then, add 50 µl RRB. Mix by pipetting until the pellet is dissolved completely. Alternatively, you can vortex for 10 s after adding the RRB, then incubate at 37 °C for 10 min (or 20 min at room temperature, 25 °C); afterward vortex for 10 s, to dissolve the DNA. The DNA is ready for further applications; you can use 2-5 µl of it for PCR reaction.

Note: Do not dry the pellet and add RRB immediately.