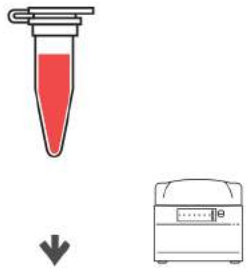
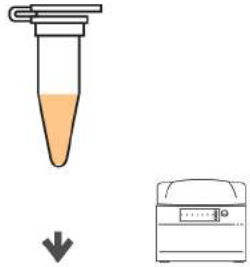
Step1:
RBC Lysis

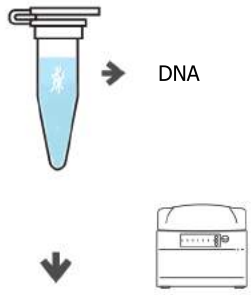


Step 2:
WBC Lysis

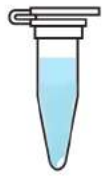


Step 3:
Protein Percipitate

Step 4:
DNA Percipitate



Step 5:
Wash



Step 6:
Rehydration

Contents

Protocol 1

Isolation of Genomic DNA (based on solution)

Type: MiniPrep

Some tips to know

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

Process

1. Add 1 ml RBC lysis buffer to 300 µl 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.

   Note: You will see a small pellet at the bottom of the microtube.
3. Discard supernatant. Aspirate the pellet in a way that about 20 µl of supernatant remains in the microtube, vortex for 10 s to resuspend the pellet, add 400 µl ROS to pellet and vortex for 20 s at high speed until the pellet is dissolved thoroughly.
4. Add 100 µl protein precipitation solution; shake vigorously for 5 s, vortex for 10-15 s at high speed then shake vigorously again for few s and centrifuge at 13000 rpm for 5 min.

   Note: The supernatant should be completely clear. If not add more 35 µl of protein precipitation solution to the microtube, shake vigorously and vortex the microtube at high speed then centrifuge at 13000 rpm for 2 min.

5. Pour supernatant to clean microtube; add 600 µl Isopropanol to supernatant. Invert 10 - 20 times rapidly. Centrifuge at 12000 rpm for 1 min.

   Note: Be sure the protein pellet is not dislodged during pouring.

6. Discard supernatant, aspirate the pellet. Add 600 µl ethanol 70% to the pellet; centrifuge at 10000 rpm for 2 min.
7. Discard supernatant, aspirate the pellet, and 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.
8. 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 the solvent immediately.

Protocol 2

Isolation of Genomic DNA (based on solution)

Type: MidiPrep

Some tips to know

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

Process

1. Add 6 ml RBC lysis buffer to 2 ml blood in tube, invert 5 times, vortex 10 s at high speed and centrifuge at 4000 rpm (2000 g) for 3 min.
2. Discard supernatant and add 2 ml RBC lysis buffer to the pellet, 5 times, vortex 10 s at high speed and centrifuge at 4000 rpm (2000 g) for 2 min.

   Note: You will see a small pellet at the bottom of the tube.

3. Discard supernatant. Vortex for 10 s to resuspend the pellet, add 1250 µl ROS to pellet and vortex for 20 s at high speed until the pellet is dissolved thoroughly.
4. Add 500 µl Protein precipitation solution; shake vigorously 5 s, vortex for 15-20 s at high speed then shake vigorously again for few seconds and centrifuge at 4000 rpm (2000 g) for 7 min.

   Note: The supernatant should be clear. If not add more 50 µl of Protein precipitation solution to the tube, shake vigorously and vortex tube at high speed then centrifuge at 4000 rpm (2000 g) for 2 min.

5. Pour supernatant to clean tube, add 3 ml Absolute Ethanol to supernatant. Invert 5 times slowly, put the tube in freezer or refrigerator for 2 min.

   Note: Be sure the protein pellet is not dislodged during pouring.

6. Separate the DNA by micropipettor 1000, which is set on 100 µl and transfer the DNA to a clean microtube. Add 1000 µl ethanol 70% to the pellet; centrifuge at 11000 rpm for 2 min.
7. Discard supernatant, aspirate the pellet and add 200 µl RRB. Mix by pipetting until the pellet is dissolved completely, continue pipetting the DNA for 1 min. **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.
8. 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 the solvent immediately.

Protocol 3

Isolation of Genomic DNA (based on solution)

Type: MaxiPrep

Some tips to know

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

Process

1. Add 20 ml RBC lysis buffer to 5 ml blood in tube, invert 5 times, vortex 10 s at high speed and centrifuge at 3200 g (4500 rpm) for 5 min.
2. Discard supernatant and add 4 ml RBC lysis buffer to the pellet, invert 5 times, vortex 10 s at high speed and centrifuge at 3200 g (4500 rpm) for 3 min.

   Note: You will see a small pellet at the bottom of the tube.

3. Discard supernatant. Vortex for 20 s to resuspend the pellet, add 2500 µl ROS to pellet and vortex for 20-60 s at high speed until the pellet is dissolved thoroughly, then incubate at room temperature for 5 min.
4. Add 1ml protein precipitation solution; shake vigorously 10 s, vortex for 15-20 s at high speed then shake vigorously again for few s and centrifuge at 3200 g (4500 rpm) for 12 min.

   Note: The supernatant should be clear. If not add more 50 µl of Protein precipitation solution to the tube, shake vigorously and vortex tube at high speed then centrifuge at 3200 g (4500 rpm) for 2 min.

5. Pour supernatant to clean tube, add 5 ml Absolute Ethanol to supernatant. Invert 5 times slowly, put the tube in freezer or refrigerator for 2 min.

   Note: Be sure the protein pellet is not dislodged during pouring.

6. Separate the DNA by micro pipettor 1000, which is set on 100 µl and transfer the DNA to a clean microtube. Add 1000 µl ethanol 70% to the pellet; centrifuge at 11000 rpm for 2 min.
7. Discard supernatant, aspirate the pellet and add 500 µl to 1ml RRB, based on your desired concentration. Mix by pipetting until the pellet is dissolved completely, continue pipetting the DNA for 1 min. **Alternatively**, you can vortex for 10 s after adding the RRB, then incubate at 37 °C for 10 min, afterward vortex for 10 seconds
8. The DNA is ready for further applications, you can use 25 µl of it for PCR reaction.

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

Protocol 4

Isolation of Genomic DNA (based on solution)

Sample type: Buffy coat

Some tips to know

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

Process

1. Add 1 ml RBC lysis buffer to 300 µl buffy coat 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.

   Note: You will see a small pellet at the bottom of the microtube.
3. Discard supernatant. Aspirate the pellet in a way that about 20 µl of supernatant remains in the microtube, vortex for 10 s to resuspend the pellet.
4. Add 400 µl ROS to pellet and then 20 µl proteinase K or RJ-Protease (order by Cat No. EB983018, EB983121). Mix thoroughly by pulse vortexing for 30 s, then incubate at 56°C for 15-30 min until the pellet is completely lysed. Pulse vortex every 5 min during incubation to intersperse the sample, or place it in a thermomixer or shaking water bath.
5. Add 200 µl protein precipitation solution; shake vigorously for 10 s, vortex for 10-15 s at high speed then shake vigorously again for few s and centrifuge at 13000 rpm for 5 min.

Note: The supernatant should be completely clear. If not add more 35 µl of protein precipitation solution to the microtube, shake vigorously and vortex the microtube at high speed then centrifuge at 13000 rpm for 2 min.

6. Pour supernatant to clean microtube; add 600 µl Isopropanol to supernatant. Invert 10 - 20 times rapidly. Centrifuge at 12000 rpm for 1 min.

   Note: Be sure the protein pellet is not dislodged during pouring.

7. Discard supernatant, aspirate the pellet. Add 600 µl ethanol 70% to the pellet; centrifuge at 10000 rpm for 2 min.

8. Discard supernatant, aspirate the pellet, and add 100-200 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.

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