（(DP349) RelaxGene Blood DNA System (0.1-20 ml)

——Small volume whole blood (＜ 600 μl)

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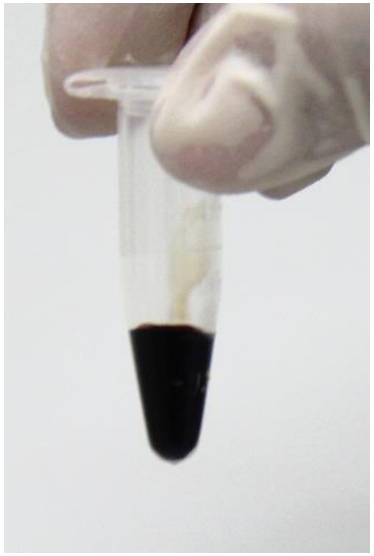
Experiment Preparation

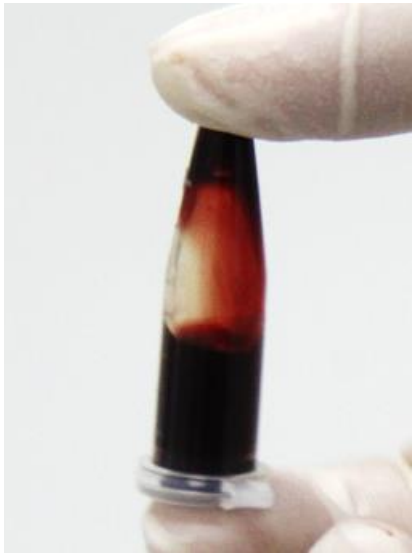
1. Anticoagulant whole blood (Take 300 μl human anticoagulant whole blood as example)
2. Pipette and matched sterile tips (2.5 μl, 200 μl, 1ml); 1.5 ml centrifuge tubes
3. 96-100% ethanol; 70% ethanol; Clean blotting paper
4. Vortex oscillator; Dry bath/water bath; Centrifuge

Note: This experiment takes human blood as an example. The whole blood of mammals can be extracted by this process. For anticoagulant blood of poultry, birds, amphibians or lower organisms, the red blood cells are nucleated cells, so the starting sample volume is 5-20 μl, and Buffer GS should be added to top up to 200 μl. For blood clot samples, the Liquefaction Columns CX1 (TIANGEN, RK165) (self-provided) can be selected to treat the blood clot.

Step 1



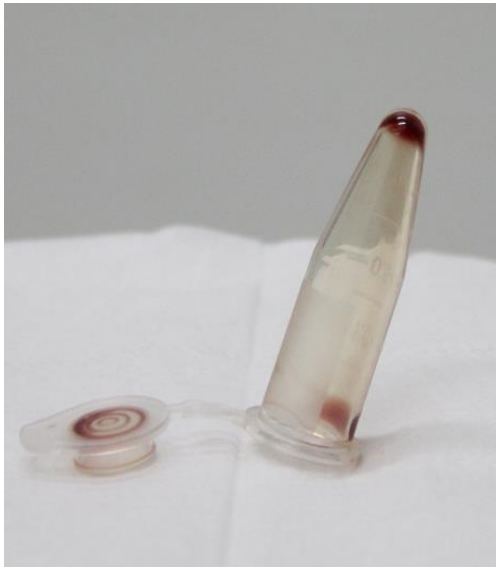
300 µl whole blood



Add 750 µl Buffer CLA, and mix well upside down for 20 times.

Step 2

Centrifuge at 12,000 rpm (~11,500×g) for 1 min.



Discard the supernatant, and invert the centrifuge tube onto a clean blotting paper for 2 min. Ensure the precipitation remains in the tube.

<u>This step should be handled carefully. To avoid the precipitation being poured out, it is recommended to use a pointed bottom centrifuge tube.</u>

Note: In rare cases, the sediment may be very loose, so slowly pour the supernatant. Invert the centrifuge tube onto a clean blotting paper is to reduce the return of the supernatant on the tube wall.

Step 3

Prepare the mixture of Buffer FGA and Proteinase K according to table 1. **The buffer should be prepared right before use, and be used up within 1 hour after preparation.**

Table 1 The amount of buffer needed for different volumes of blood (μl)

	Blood Volume (μl)						
	100	300	1000	3000	5000	10000	20000
Buffer CLA	250	750	2500	7500	12500	25000	50000
Buffer FGA	67	200	667	2000	3333	6667	13333
Proteinase K	0.5	1.5	5	15	25	50	100
100% Isopropanol	67	200	667	2000	3333	6667	13333
70% Ethanol	100	300	1000	3000	5000	10000	20000
Buffer TB	100	200	200	300	500	1000	1000
Top up with the Buffer FGA and Proteinase K mixture	10	30	100	300	500	1000	1000

Step 4

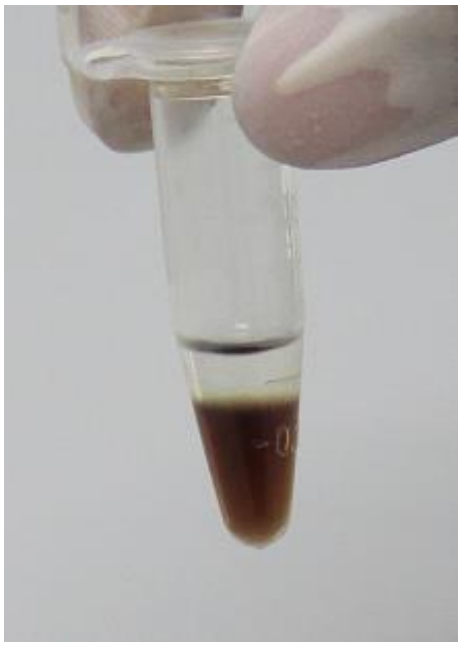
Add 200 μl Buffer FGA and Proteinase K mixture, and immediately mix well by shaking up and down violently or vortex until there are no obvious clumps in the solution.

Note: When dealing with multiple samples, shake up and down violently or vortex to mix well immediately after adding the mixture of Buffer FGA and Proteinase K for each sample. It is possible to have trace colloidal precipitates that are difficult to mix. At this time, additional Buffer FGA and Proteinase K mixture can be added (see table 1 for the specific additional amount) and vortex to mix well again.

Step 5

Incubate at 65℃ for 10 min, and mix well upside down for several times during the period.

Step 6



Add 200 μl isopropanol

Mix upside down for 50 times until the presence of filamentous or tufted genomic DNA appear. The pellet might be invisible.

Note: Complete mixing with isopropanol is important for pelleting DNA. Be sure to mix well.

Step 7

Centrifuge at 12,000 rpm (~13,400×g) for 5 min

Pour out supernatant, and invert the centrifuge tube onto a clean blotting paper.

If the precipitate is not visible (due to the high purity, the precipitate is transparent and difficult to observe), please carefully pour out the supernatant and continue the subsequent experiment.

Step 8

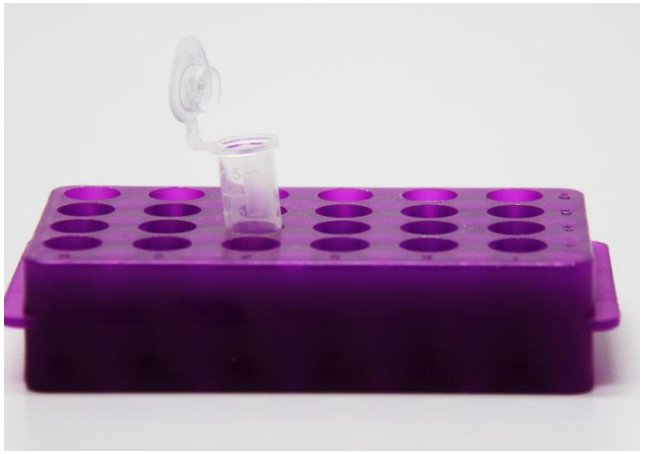
Add 300 μl 70% ethanol, and vortex for 5 sec.

Centrifuge at 12,000 rpm (~13,400×g) for 2 min.

Step 9

Invert the centrifuge tube onto a clean blotting paper for 5 min to ensure precipitation in the tube.

Step10

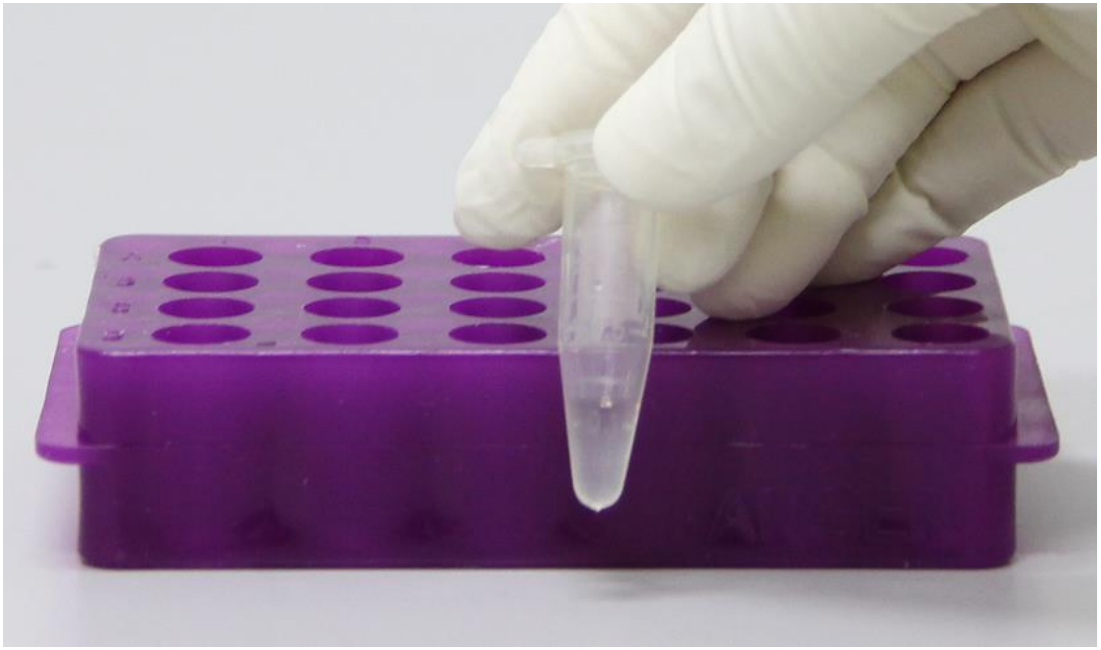


Air dry DNA precipitate until all liquid evaporates (at least for 5 min).

Note: Ethanol residues can inhibit subsequent enzymatic reactions (restriction enzyme digestion, PCR, etc.) experiment. But avoid over-dying, for too dry DNA is difficult to dissolve.

If the precipitate is not visible (due to the high purity, the precipitate is transparent and difficult to observe), please carefully pour out the supernatant and continue the subsequent experiment.

Step 11



Add 200 μl Buffer TB, and low vortex for 5 sec. Heat for 20 min at 65℃ to dissolve DNA, and flip for several times to help dissolve during the period.

Note: If insoluble substances exist, incubation time in 65℃ can be extended to 1 h.