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

——Medium volume whole blood (10 ml)

TIANGEN BIOTECH(BEIJING)CO., LTD

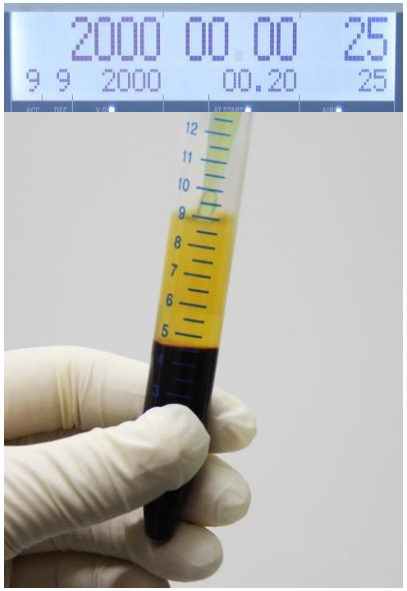
Ver. No. 20170331

Experiment Preparation

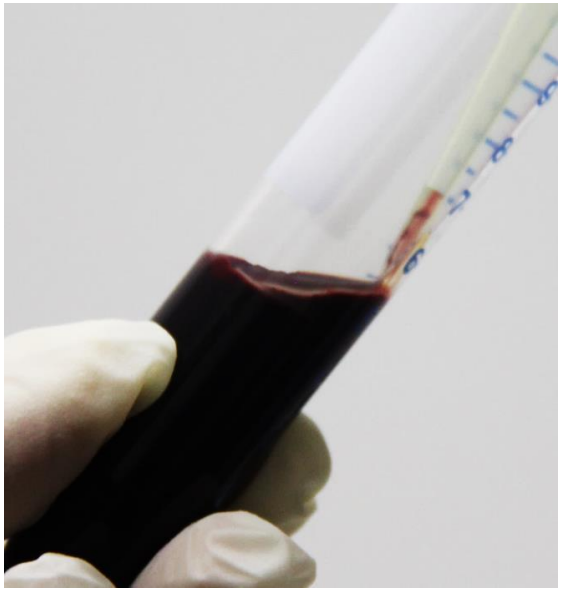
1. Anticoagulant or fresh blood (Take 10 ml human fresh (a)/anticoagulant (b) blood as example)
2. Pipette and matched sterile tips (2.5 μl, 10 μl, 200 μl, 1ml); 15 ml centrifuge tubes
3. 96-100% ethanol; 70% ethanol; Clean blotting paper
4. Vortex oscillator; Dry bath/water bath; Centrifuge

Step 1 (a-1)

10 ml fresh whole blood



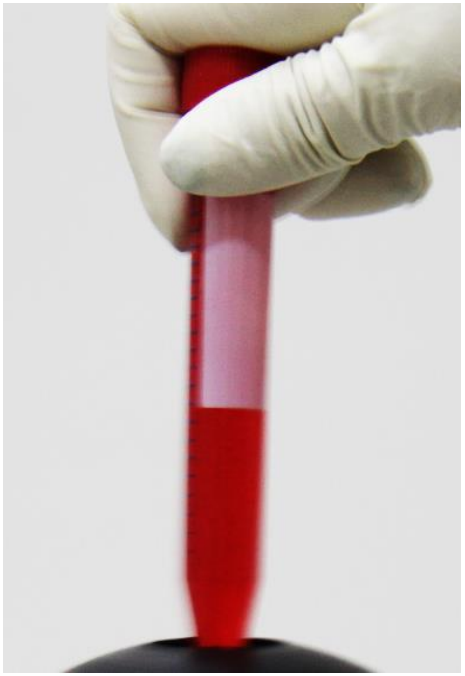
Centrifuge at 3,600 rpm (~2,000 g) for 15-20 min, discard the plasma supernatant



Transfer the intermediate buffy coat into a 15 ml centrifuge tube

Step 1 (a-2)

Add 10 ml Buffer CLA

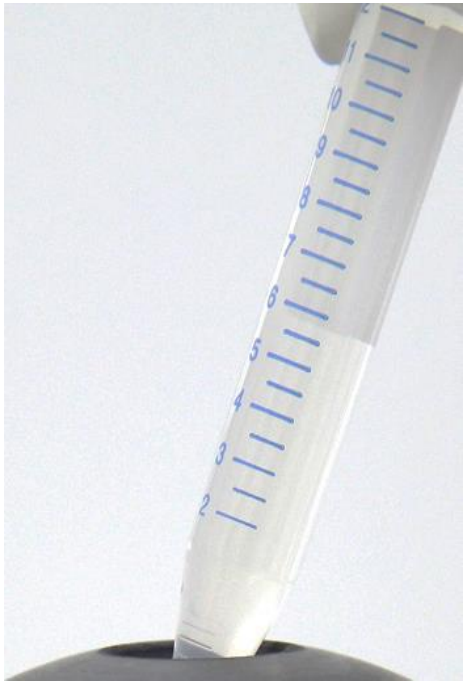


Vortex for 10 sec to mix

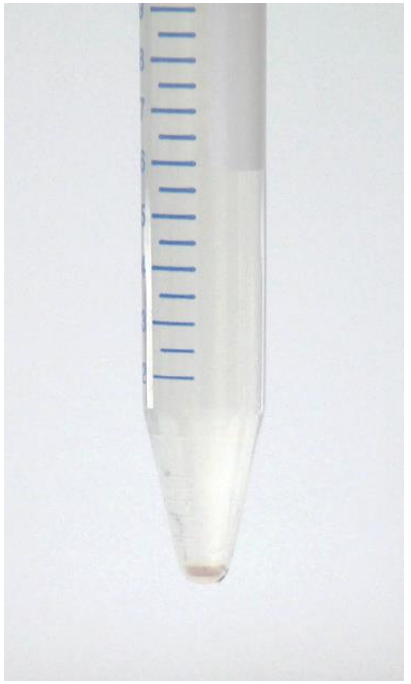
Centrifuge at 3,600 rpm (~2,000×g) for 2 min. Pour out supernatant.

Step 1 (a-3)

Add 15 ml Buffer CLA



Vortex for 10 sec to mix



Centrifuge at 3,600 rpm (~2,000×g) for 2 min. Pour out supernatant.

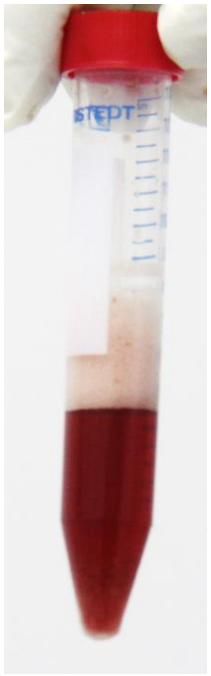
Step 1 (b-1)

Add 5 ml whole blood and 5 ml Buffer CLA into two 15 ml centrifuge tubes

Mix well upside down for 5 times

Centrifuge at 3,600 rpm (~2,000 g) for 3 min, and discard the supernatant

Step 1 (b-2)



Add 2.5 ml Buffer CLA, and mix well by vortex for 10 sec to a centrifuge tube.

Centrifuge at 3,600 rpm (~2,000×g) for 3 min.

Pour out supernatant.

Step 2

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)

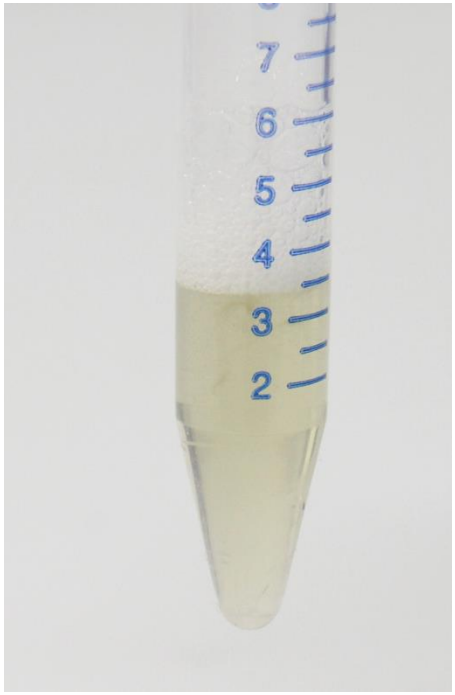
	Blood Volume (μl)						
	100	300	1000	3000	5000	10000	20000
Buffer CLA	250	750	2500	7500	12500	25000	50000
Buffer FGA	67	200	667	2000	3333	6667	13333
Proteinase K	0.5	1.5	5	15	25	50	100
100% Isopropanol	67	200	667	2000	3333	6667	13333
70% Ethanol	100	300	1000	3000	5000	10000	20000
Buffer TB	100	200	200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

Step 3

Add 6.7 ml 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.

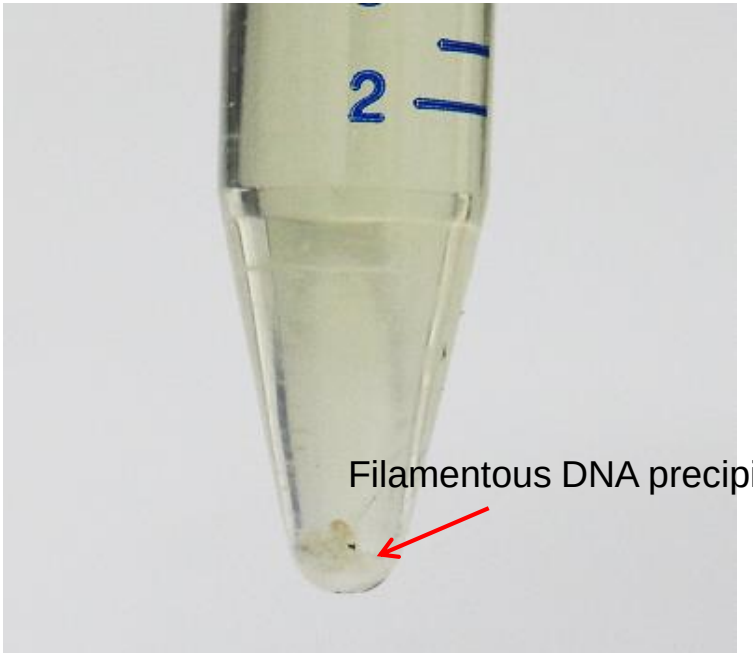
Step 4



Incubate at 65℃ for 30 min, and mix well upside down for several times and the solution became clear.

Step 5

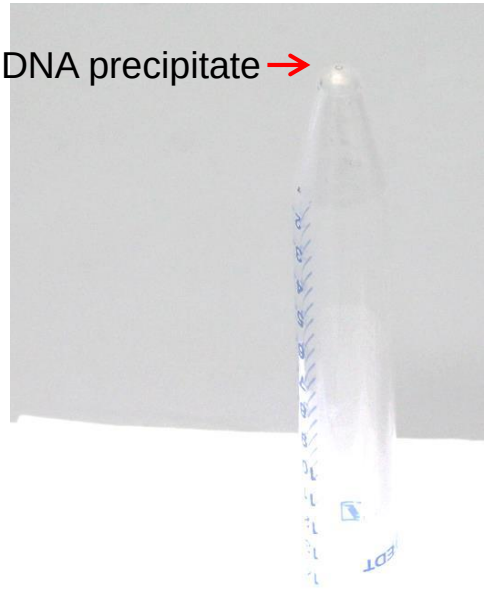
Add 6.7 ml isopropanol



Mix upside down for 50 times until the presence of filamentous or tufted genomic DNA appear.

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

Step 6



Centrifuge at 3,600 rpm(~2,000×g) for 10 min

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

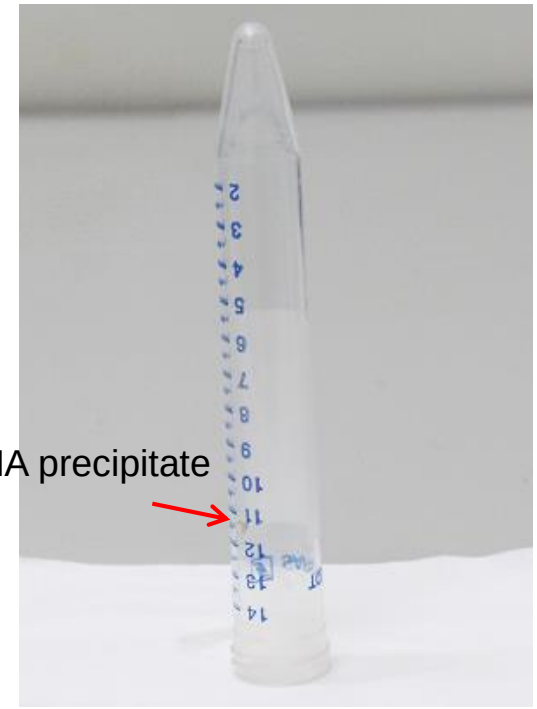
Note: In rare cases, the sediment may be very loose, so slowly pour the supernatant. If the sample has enough white blood cells, you can see the white DNA precipitate.

Step 7

Add 10 ml of 70% ethanol, and vortex for 5 sec.

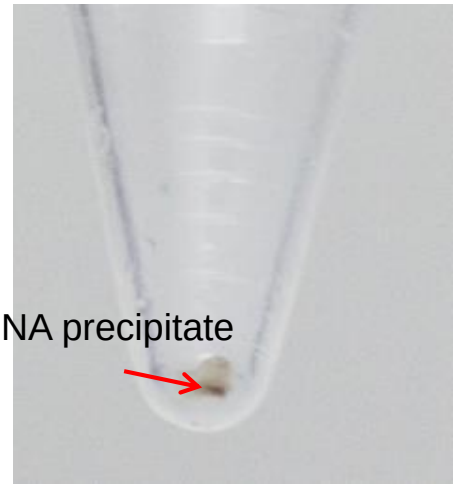
Vortex at 3,600 rpm (~2,000×g) for 3 min, discard the supernatant

Step 8



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

Step 9



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.

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 10

Add 1 ml Buffer TB, and low vortex for 5 sec. Heat for 30 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.