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1. Purpose

"National Biobank Consortium of Taiwan" (hereinafter referred to as the "Integration Platform") primarily integrates domestic human biobanks — their data, biospecimens, and related information (hereinafter referred to as "Integration Platform biospecimen data") — through a cloud-based system, making them available for application and use by medical, clinical, industry, and related biomedical research investigators nationwide. To achieve cross-institutional collaboration, it is necessary to establish consistent quality standards for dispensed biospecimens and consistent clinical data content. To maintain good specimen quality and to ensure consistency in the steps for extracting DNA from blood specimens, so as to facilitate future related research, this Standard Operating Procedure is hereby established.

2. Scope of Application

This SOP applies to the process of extracting DNA from blood specimens through completion of quality testing.

3.Responsibilities

All human biobanks that have joined the Integration Platform, when extracting DNA from human blood they have collected,are recommended to follow this Standard Operating Procedure, or must otherwise achieve the same quality and yield.

4. Description

This SOP can be divided into three stages

1. Buffer Preparation
2. Blood DNA Extraction
3. Blood Specimen DNA Quality Testing

5. Operating Procedures

5.1 Reagent Preparation

Equipment:

1. Micro analytical balance
2. Magnetic stirrer
3. Stir bar
4. pH meter (pH meter)
5. 4℃ freezer
6. Water purification system

Consumables:

1. Weighing paper, spatula
2. Graduated cylinders (50ml, 500ml, 1000ml)
3. 3ml dropper (dropper)
4. Serum bottle (1L)

Materials:

1. Sterilized secondary distilled water (ddH_2O), abbreviated as secondary water
2. Tris(hydroxymethyl)aminomethane (Tris-HCl)
3. Potassium chloride (KCl)
4. Magnesium chloride (MgCl2)
5. Ethylenediaminetetraacetic acid (EDTA)
6. Sodium dodecyl sulfate (SDS)
7. Sodium chloride (NaCl)
8. Nonyl phenoxypolyethoxylethanol (NP40) surfactant
9. Hydrochloric acid (HCl) (Note: used to adjust pH)
10. Sodium hydroxide (NaOH) (Note: used to adjust pH)

Procedure:

1. **Prepare a 500ml stock solution of 1M Tris(hydroxymethyl)aminomethane (Tris-HCl) solution:**

   Using the micro analytical balance, weigh out 78.82g of Tris(hydroxymethyl) aminomethane powder, pour it into a serum bottle, and add 400ml of secondary water and a sterilized stir bar. Move the serum bottle onto the magnetic stirrer and stir until thoroughly mixed, while simultaneously using the pH meter (pH meter) to adjust the pH to 7.6. Finally, adjust the solution volume to 500ml with secondary water. Store in the 4℃ freezer.

2. **Prepare a 500ml stock solution of 1M potassium chloride (KCl) solution**

   Using the micro analytical balance, weigh out 37.275g of potassium chloride powder, pour it into a serum bottle, and add 500ml of secondary water and a sterilized stir bar. Move the serum bottle onto the magnetic stirrer and stir until thoroughly mixed. Store in the 4℃ freezer.

3. **Prepare a 500ml stock solution of 0.1M magnesium chloride (MgCl2) Solution**

   Using the micro analytical balance, weigh out 10.165g of magnesium chloride powder, pour it into a serum bottle, and add 500ml of secondary water and a sterilized stir bar. Move the serum bottle onto the magnetic stirrer and stir until thoroughly mixed. Store in the 4℃ freezer.

4. **Prepare a 500ml stock solution of 0.1M ethylenediaminetetraacetic acid (EDTA) solution:**

   Using the micro analytical balance, weigh out 18.612g of ethylenediaminetetraacetic acid powder, pour it into a serum bottle, and add 400ml of secondary sterilized water and a sterilized stir bar. Move the serum bottle onto the magnetic stirrer and stir until thoroughly mixed, while simultaneously using the pH meter (pH meter) to adjust the pH to 8. Finally, adjust the solution volume to 500ml with secondary water. Store in the 4℃ freezer.

5. **Prepare a stock solution of 20% sodium dodecyl sulfate (SDS), 200ml:**
   Using the micro analytical balance, weigh out 40g of sodium dodecyl sulfate powder, pour it into a serum bottle, and add 200ml of secondary water and a sterilized stir bar. Move the serum bottle onto the magnetic stirrer and stir until thoroughly mixed. Store at room temperature.
6. **Prepare a 500ml stock solution of 5M sodium chloride (NaCl) solution :**
   Using the micro analytical balance, weigh out 146.1g of sodium chloride powder, pour it into a serum bottle, and add 500ml of secondary water and a sterilized stir bar. Move the serum bottle onto the magnetic stirrer and stir until thoroughly mixed. Store in the 4℃ freezer.
7. **Aliquot nonyl phenoxypolyethoxylethanol (NP40):**
   Pour nonyl phenoxypolyethoxylethanol (NP40) into a serum bottle, and wrap the outside of the serum bottle in a layer of aluminum foil to shield it from light. Store at room temperature.
8. **Prepare 1,000 ml of TKM1:**
   Take the following solutions from the 7 solutions prepared above, and mix them in the stated proportions to obtain :
   1,000 ml of TKM1 solution. The formulation ratios are as follows:
   10ml Tris(hydroxymethyl)aminomethane (Tris-HCl)10ml Potassium chloride (KCl)
   100ml Magnesium chloride (MgCl2)
   20ml Ethylenediaminetetraacetic acid (EDTA)
   860ml secondary water

9. **Prepare 1000ml TKM2:**
   Take the following solutions from the 7 solutions prepared above, and mix them in the stated proportions to obtain1,000 ml TKM2 solution. The formulation ratios are as follows:
   10ml Tris(hydroxymethyl)aminomethane (Tris-HCl)
   10ml Potassium chloride (KCl)
   100ml Magnesium chloride (MgCl2) 20ml Ethylenediaminetetraacetic acid (EDTA)
   25ml Nonyl phenoxypolyethoxylethanol (NP40)
   835ml secondary water
10. **Prepare 250ml TKM3:**
    Take the following solutions from the 7 solutions

prepared above, and mix them in the stated proportions to obtain250 ml TKM3 solution. The formulation ratios are as follows:

2.5ml Tris(hydroxymethyl)aminomethane (Tris-HCl)

2.5ml Potassium chloride (KCl)

25ml Magnesium chloride (MgCl2)

5ml Ethylenediaminetetraacetic acid (EDTA)

40ml Sodium chloride (NaCl) solution

175ml secondary water

Precautions:

When preparing reagents, the pH value must be correct, or it will affect blood DNA extraction.If a reagent is left standing overnight, the pH must be reconfirmed as correct before use.

5.2 Blood DNA Extraction

Equipment:

1. Water bath
2. –80°C freezer
3. Microprocessor-controlled large-capacity high-speed centrifuge
4. Benchtop high-speed refrigerated microcentrifuge (eppendorf centrifuge 5415 R or equivalent product)
5. Benchtop high-speed microcentrifuge
6. Ultra-micro spectrophotometer (Thermo NANODROP 2000 or equivalent product)
7. Pipetman (pipetman) (1000ul, 200ul, 10ul)
8. Water purification system
9. Chemical fume hood (chemical hood)
10. Ice machine

Reagents:

1. TKM1
2. TKM2
3. TKM3
4. 20% sodium dodecyl sulfate (SDS) solution
5. 5M sodium chloride (NaCl) solution

6. 75% ethanol (75% EtOH)
7. 100% ethanol (100% EtOH)
8. Sterilized secondary distilled water (ddH_2O), abbreviated as secondary water.

Note: Before starting the experiment, first prepare eight 50ml conical centrifuge tubes, aliquoting 50ml of each of the above eight reagents into them, and write the buffer name on the lid and side of each.

Consumables:

1. 15ml conical centrifuge tube
2. 50ml conical centrifuge tube
3. 3ml dropper (dropper)
4. Sterilized 1.5ml microcentrifuge tube (eppendorf)
5. 10ml graduated pipette (pipette)
6. Filter tip (1,000ul, 200ul, 10ul)
7. Ice bucket
8. Specimen storage box (9x9 grid)
9. Microcentrifuge tube rack (for holding 1.5ml microcentrifuge tubes)
10. Stainless steel tube rack (5x10, for holding 15ml conical centrifuge tubes)
11. Stainless steel tube rack (4x4, for holding 50ml conical centrifuge tubes)

Procedure: (the entire process must be performed in the chemical fume hood)

1. Turn on the water bath and set the temperature to 55℃.
2. Take two 50ml conical centrifuge tubes, and write the specimen number on the lid and side of each.
3. Thaw the frozen buffy coat layer (buffy coat) 1.5ml microcentrifuge tube along with the frozen buffy coat (buffy coat)-containing CBC tube (which still contains the red blood cell layer). Using a dropper, transfer the thawed red blood cell layer and buffy coat (buffy coat) separately into the same 50ml conical centrifuge tube. Do not discard this dropper yet.
4. Take a second new dropper and draw up 3ml of TKM1, add it to the CBC tube, then use the dropper from step 3 to rinse the CBC tube and the 1.5ml microcentrifuge tube. Transfer the rinsed residual specimen into the same 50ml conical centrifuge tube from step 3 as well.
5. Using a dropper, draw up 10 ml of TKM2 and add it to the 50ml conical centrifuge

tube from step 3. Then, using the dropper from step 3, gently draw and release the liquid up and down approximately 100 times, to break apart the specimen's cells.

6. Place this 50ml conical centrifuge tube into the microprocessor-controlled large-capacity high-speed centrifuge, secure the centrifuge's dedicated rotor lid, and centrifuge at 3,200 rpm for 15 minutes.
7. Remove the supernatant, retain the pellet, and use a dropper to add 10ml of TKM1, shaking to disperse the pellet.
8. Place this 50ml conical centrifuge tube into the microprocessor-controlled large-capacity high-speed centrifuge, secure the centrifuge's dedicated rotor lid, and centrifuge at 3,200 rpm for 10 minutes.
9. Remove the supernatant from the 50ml conical centrifuge tube, retaining the pellet.
10. Using a dropper, transfer the centrifuged pellet from the 50ml conical centrifuge tube into a new 15ml conical centrifuge tube.
11. Take a new dropper and draw up 1ml of TKM1, add it to the 15ml conical centrifuge tube containing the pellet, and shake to disperse the pellet.
12. Place this 15ml conical centrifuge tube into the microprocessor-controlled large-capacity high-speed centrifuge, secure the centrifuge's dedicated rotor lid, and centrifuge at 3,200 rpm for 3 minutes.
13. Remove the supernatant from the 15ml conical centrifuge tube, retaining the pellet. Repeat the pellet washing steps 11 through 12 until the supernatant appears clear and transparent, retaining the pellet each time.
14. To the washed pellet, use a 1000ul pipetman to add 0.4ml of TKM1, and shake to disperse it.
15. Using a 1,000ul pipetman, add a further 0.4ml of TKM3, and shake to disperse it again.
16. Using a 200ul pipetman, add 50µl of the 20% sodium dodecyl sulfate solution, and shake to disperse it again.
17. Place this 15ml conical centrifuge tube in the 55℃ water bath until the supernatant appears clear and transparent (this step requires at least 30 minutes). Because the specimen will settle at the bottom of the conical tube, the tube may be shaken periodically to accelerate dissolution of the specimen.
18. Remove this 15ml conical centrifuge tube from the 55℃ water bath, let it stand at room temperature to cool, then use a 1,000ul pipetman to add 0.4ml of 5M sodium chloride (NaCl) solution, and mix thoroughly.

19. Place it into the microprocessor-controlled large-capacity high-speed centrifuge, secure the centrifuge's dedicated rotor lid, and centrifuge at 3,200 rpm for 25 minutes.
20. Using a 1,000ul pipetman, draw the centrifuged supernatant and transfer it into a second new 15ml conical centrifuge tube.
21. Place the 15ml conical centrifuge tube from which the supernatant has been drawn once more into the microprocessor-controlled large-capacity high-speed centrifuge, secure the centrifuge's dedicated rotor lid, and centrifuge at 3,200 rpm for 1 minute. Using a 200ul pipetman, transfer the remaining supernatant into the second new 15ml conical centrifuge tube.
22. Add 2.5ml of 100% ETOH to the second 15ml conical centrifuge tube (containing only supernatant), and mix gently and thoroughly.
23. Place it in the -20℃ freezer for 30 minutes to allow the reaction to go to completion.
24. Remove the 15ml conical centrifuge tube from the -20℃ freezer, and let it stand at room temperature to return to room temperature.
25. Using a 1,000ul pipetman, draw up the white, thread-like precipitate in the 15ml conical centrifuge tube, transfer it into a new 1.5 ml microcentrifuge tube, and add 1ml of 75% ETOH, mixing gently and thoroughly.
26. Place the 1.5 ml microcentrifuge tube into the pre-chilled 4℃ benchtop high-speed refrigerated microcentrifuge (eppendorf Centrifuge 5415 R), and centrifuge at 13,200 rpm for 10 minutes.
27. Discard the supernatant, retaining the pellet, then centrifuge using the benchtop high-speed microcentrifuge for 30 seconds. Finally, use a 200ul pipetman to remove the remaining supernatant, and air-dry at room temperature.
28. At the same time, visually estimate the size of the pellet, in order to assess the volume of water needed for resuspension.
29. Using a 200ul pipetman, add the required amount of secondary water.
30. Once the pellet has fully dissolved, gently tap the bottom of the 1.5 ml microcentrifuge tube to mix the blood DNA within thoroughly. Centrifuge using the benchtop high-speed microcentrifuge for 30 seconds.
31. Place the 1.5 ml microcentrifuge tube in an ice bucket containing crushed ice; using a 10ul pipetman, draw 2ul of blood DNA, and use the ultra-micro spectrophotometer to measure the DNA concentration and O.D. value.

32. Once the blood DNA concentration has been measured, place it in numerical order into a specimen storage box (9x9 grid) (with the specimen number and specimen type noted on the lid and side of the box), and store it in the -80℃ freezer.

Note: Although manually extracting blood DNA is a more complex procedure, the yield per 1ml of buffy coat is approximately 50-100 ug DNA, far higher than the yield from machine extraction, and at a much lower cost. Therefore, as a biobank (biobank), in order to store the greatest possible quantity of blood DNA, manual extraction of blood DNA is recommended.

5.3 Blood DNA Quality Testing (as needed, by horizontal electrophoresis)

Equipment:

1. High-sensitivity gel imaging system (SYNGENE In Genius 3 or equivalent product)
2. Microwave oven
3. Mini electrophoresis tank
4. Micro analytical balance
5. Benchtop horizontal shaker (DSR-2800D-N or equivalent product)

Reagents:

1. UltraPure™ TAE Buffer, 10X (Thermo Cat no.15558042)
2. Agarose gel powder, Agarose-Molecular Biology Grade (Invitrogen Cat no.75510-019)
3. Ethidium bromide (EtBr)

Consumables:

1. Weighing paper (12x12cm), spatula
2. Plastic wrap
3. 500ml sterilized Erlenmeyer flask
4. 1L sterilized serum bottle
5. Graduated cylinders (50ml and 1L)

Procedure:

1. Preparing the Agarose Gel (0.8% gel)

1. Prepare 1X TAE Buffer: using a 1L graduated cylinder, pour 100ml of UltraPure™ TAE Buffer (10X) into a 1L serum bottle, then add 900ml of secondary water (ddH_2O), shake up and down to mix thoroughly, and store at room temperature.
2. Using a 50ml graduated cylinder, pour 40ml of the 1X TAE Buffer into the sterilized Erlenmeyer flask.
3. Zero the microbalance with weighing paper on it, then use a spatula to scoop 0.32g of agarose gel powder (Agarose), and add it into the Erlenmeyer flask containing the 1X TAE Buffer, mixing gently.
4. Cover the Erlenmeyer flask with plastic wrap, and use the tip of a 10ul filter tip to poke several small holes in the plastic wrap, place it in the microwave, and

microwave for 30 seconds, then shake to mix thoroughly. This step must be repeated until the agarose gel powder (Agarose) is completely dissolved in the 1X TAE Buffer and appears completely transparent. (Repeat at least three times.).

5. Take the fully dissolved, transparent agarose gel solution out of the microwave, and place it on the benchtop horizontal shaker, shaking slowly for 5 minutes.
6. Using a 2ul pipetman (pipetman), draw up 2ul of EtBr and add it to the Erlenmeyer flask, mixing thoroughly.
7. Pour into the casting tray, and let cool for 1 hour to solidify into the agarose gel (gel).

2. Performing Horizontal Electrophoresis

1. Place the solidified agarose gel (gel) into the mini electrophoresis tank, and pour in 450ml of TAE Buffer (1X).
2. Mix 1.2ug of DNA with 2ul of 6X Loading dye, then add sterilized secondary water (ddH_2O) to bring the total volume to 10ul.
3. Using a 10ul pipetman (pipetman), inject the entire volume of the above thoroughly mixed liquid into a well of the agarose gel (gel) in the mini electrophoresis tank.
4. Perform horizontal electrophoresis for 35 minutes at a voltage of 85 volts (current of 500 mA).
5. Remove the agarose gel (gel) from the mini electrophoresis tank, and take it to the high-sensitivity gel imaging system to confirm whether a major band is present (see Attachment 2). Save the file and keep a photograph on record.

Note: Because blood DNA is very precious and the extracted quantity is limited, quality is usually good, so in principle horizontal electrophoresis testing may be omitted. Alternatively, a few tubes of blood DNA may be randomly selected for testing as circumstances warrant.

6. Quality Standards for Dispensing

Blood DNA dispensed by the Integration Platform shall be accompanied by the following information:

1. The blood DNA's optical density (O.D) value:
   --The 260/280 ratio should fall between 1.6-2.2
   --The 260/230 ratio should fall between 1.6-2.5

Note: (1)Because some specimens are difficult to obtain or very scarce, the extracted quantity of blood DNA may be very small and unable to be purified. This may be discussed with the applicant; if the applicant is able to accept it, the specimen may be dispensed even if the O.D. value is not ideal, but this must be noted and the applicant must be fully informed.

(2)It is recommended that the 260/280 ratio and the 260/230 ratio preferably both be >1.8, to confirm that the DNA does not contain significant protein contamination.

2. Whether the horizontal electrophoresis gel image shows a major band (as needed)
   --Blood DNA usually has good quality, and a major band is generally present, so a gel run is not performed for every case. It is provided only when specifically requested by the applicant.

7. References

1. SAJJA SUGUNA, et al. GENOMIC DNA ISOLATION FROM HUMAN WHOLE BLOOD SAMPLES BY NON ENZYMATIC SALTING OUT METHOD. International Journal of Pharmacy and Pharmaceutical Sciences 2014;6(6): 198-199. https://innovareacademics.in/journal/ijpps/Vol6Issue6/9478.pdf

2. Samuel Asamoah Sakyi, et al. Modified DNA Extraction Technique for Use in Resource-Limited Settings: Comparison of Salting out Methods versus QIAamp Blood Mini Kit, Annals of Medical and Health Sciences Research, 2017; 7(3):131-5.

8. Attachments

Attachment 1: Example of Measuring Blood DNA Concentration and O.D. Value Using an Ultra-Micro Spectrophotometer

Table 1: Optical density (O.D) values are good, all >1.8, with concentrations maintained below 1500ng/μl.

Blood DNA O.D. Value				
No.	Concentration (ng/μl)	O.D.260/280	O.D.260/230	Volume of DNA to Load for Gel Run (μl)
1	1105.9	1.86	2.21	1.80
2	872.4	1.83	2.07	2.29
3	1176.3	1.85	2.03	1.70
4	1094.6	1.85	2.24	1.82
5	1195	1.85	2.18	1.67
6	951.5	1.86	2.05	2.10
7	1238.6	1.85	1.84	1.61
8	1162.5	1.85	2.1	1.72
9	1054.3	1.85	2.1	1.89
10	1206.3	1.87	2.14	1.65

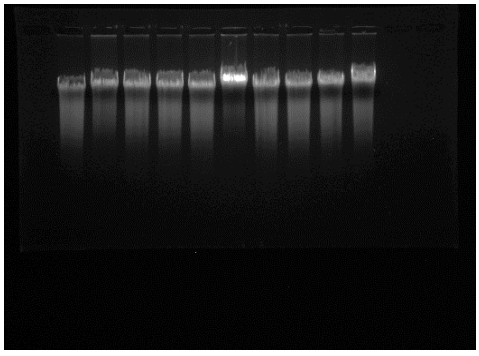
Note: Concentration and O.D. detected by Thermo NANODROP 2000

Attachment 2: Example of Blood Specimen Extracted DNA Concentration, O.D. Value, and Horizontal Electrophoresis Gel Image Example

Fresh Blood Extracted DNA Concentration, O.D. Value, and

Blood DNA O.D Value

No.	Concentration (ng/(μl)	OD 260/280	OD 260/230	Volume of DNA to Load for Gel Run ((μl)
1	1105.9	1.86	2.21	1.8
2	872.4	1.83	2.07	2.3
3	1176.3	1.85	2.03	1.7
4	1094.6	1.85	2.24	1.8
5	1195	1.85	2.18	1.7
6	951.5	1.86	2.05	2.1
7	1238.6	1.85	1.84	1.6
8	1162.5	1.85	2.1	1.7
9	1054.3	1.85	2.1	1.9
10	1206.3	1.87	2.14	1.7



1 2 3 4 5 6 7 8 9 10

Note: Optical density (O.D) values are good, and all specimens show a major