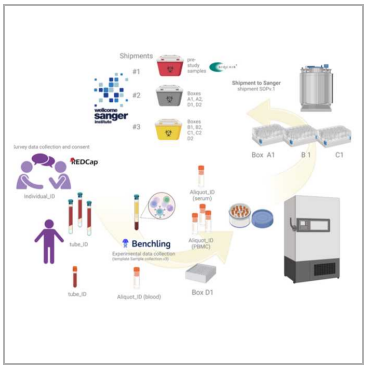

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https://www.sanger.ac.uk/news_item/researchers-will-map-immune-cells-across-latin-america-with-support-from-czi/

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Protocol status: Working
We use this protocol and it's working

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Blood Sample collection and PBMC isolation JAGUAR.v4

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Human Cell Atlas Method Development Community

Carla Jones
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ABSTRACT

AIM: This protocol aims to isolate and cryopreserve PBMC from healthy donors from JAGUAR sites for multimodal single cell analysis and blood for DNA extraction for genotyping.

IMAGE ATTRIBUTION

image created in Biorender

PROTOCOL integer ID:
82762

MATERIALS

Reagents:

- RPMI 1640 (Gibco, cat#22400-089)
- Washing media (RPMI 1% serum, 1% Lglu Lonza, Cat. #BE17-605E/U1, 1%Pen/Strep ThermoFisher, Cat. #15140122)
- Isopropanol (if using Mr.Frosty, Sigma-Aldrich, cat nº C1562-1EA) or Coolcell
- Serum (FBS) Sigma, Cat#F7524-500ml
- 2X Freezer media (each 1mL contains 800 µL RPMI and 200 µL DMSO (20%))
- Cryovial (V7634)
- BD Vacutainer CPT (CPT tube) Cat# 362753
- DMSO (Sigma)

Equipment:

- Mr. Frosty or CoolCell (CLS432001-1EA)
- Centrifuge (refrigerated swinging bucket centrifuge, e.g. Beckman Coulter Allegra X-12R)
- Ultra freezer (-80 oC freezer)
- Liquid Nitrogen tank (LN2)
- Sterile Pasteur pipette (E1414-0310)
- 15 mL Falcon tube (CLS430052)
- 2D barcode labels (Ziath)

BEFORE START INSTRUCTIONS

coolcell must be at room temperature

Blood Collection

15m

1 Collect blood (21g needle) into the tube using the standard technique for CPT tube (3 tubes per donor). After collection, store tubes upright at room temperature (RT, 18-25oC) for up to 2hr (if longer times are required keep tubes on ice until processing). 10m

Safety information

Work with human samples carries a risk of contamination. Needles and other sharps may expose workers to bloodborne pathogens. Consult healthy and safety personnel before using this protocol.

2 Collect an extra 1-2mL of blood in an EDTA blood collection tube and transfer blood to a cryotube labeled with a 2D barcode for DNA extraction (whole genome sequencing) and store at -80 until processing 5m

PBMC isolation

49m

3 After centrifugation, pipette to remove approximately half of the plasma (stored in a new tube at -80C) without disturbing the cell layer. Collect the cell layer with a Pasteur Pipette and transfer it to a 15 mL Falcon tube with a cap. *Collection of cells immediately following centrifugation will yield the best results. 5m

4 Centrifuge CPT tubes at 1500g RT for 30 minutes (break and acceleration at 5) 30m

5 Add 2mL washing media to the CPT tube to wash remaining cells and transfer to the same tube above, bring volume to 10 mL. Cap tube. Mix cells by inverting the tube slowly 5 times. 2m

6 Centrifuge for 5 min at 300 RCF. Tip off supernatant without disturbing the cell pellet. 5m

7 Add washing media to bring volume to 10 mL. Cap tube. Resuspend cells gently with a pipette 2m

8 Count cell numbers and viability using Trypan Blue (dilution 1:2) in a Neubauer chamber
CRITICAL: add 2D barcode stickers to the cryotubes while tube are at room temperature. 5m

PBMC cryopreservation

15m

9 Centrifuge for 5 min at 300 RCF. Aspirate as much supernatant as possible without disturbing the cell pellet. Resuspend pellets in 500 µl of 100% FCS and transfer all to a cryovial. 5m

10 Combine 500 µl 2X Freezer media (dropwise) with the cells in cryovial and gently pipette to mix. 5m

Place the cryovial into the Mr. Frosty (or CoolCell) and transfer to -80 oC as soon as possible.

11

CRITICAL: cell viability will be directly correlated to the time cells spend in the presence of DMSO - the quicker the better

12

After 24 hrs, move cryovials into liquid Nitrogen tanks until shipping, record date of liquid nitrogen transfer

5m