SOP 102: BLOOD DRAW PROTOCOL FOR TELOMERE LENGTH MEASUREMENT

1. **PURPOSE**: This protocol describes procedures for collection and storage for telomere length measurement (TL).
2. **BLOOD COLLECTION**

2.1. *Clinical criteria for collection of Blood*

Collecting blood for DNA should not be sensitive to time of day, fasting state, menstrual status etc. However, bulk telomere length in blood is dependent on the types of immune cells present at any one moment and site of blood draw. Therefore, since blood cell composition can change based on diurnal rhythm, and when someone has an active infection, it is best to follow these guidelines when possible.

2.2.1 Time of day:

Sample at the same time of day for all subjects, and especially for repeated measures for same subjects. We standardize our studies by taking a fasting morning blood draw, in the case that meal content affects distribution of leukocytes.

2.2.2. Health status:

Never sample blood if someone is showing signs of illness or has a major injury. It is prudent to call participants the night beforehand or to check by email to see if they have any symptoms including upper respiratory infections.

2.2. *Laboratory procedures for collecting blood*

2.2.1. Draw blood in EDTA tubes (lavender top, 4 ml, cat # 367844 from BD)

Invert the tube 30-40 times immediately after the blood draw. Keep the tubes on a rotating platform (e.g. Adams™ Nutator Mixer, Cat # 421105 from BD). Pipette 300 µl each into 5 of 2ml screw cap tubes (cat# SRS-72-694-006 from Sardstedt) within 4 hours of blood draw and store the blood at -80°C.

2.2.2. *Other blood collection details*

If you expect difficulties finding the vein: (ie, elderly sample, obese sample). Applying a warm heating pad 15 minutes prior to drawing can be helpful to prevent collapse of vein and encourage blood flow.

3. **STORAGE**

Store blood at -80°C before sending in batches. Please send us two tubes of whole blood and keep the remaining 3 tubes in case unforeseeable accidents happen during shipping.

Batching samples

If you are doing a study with longitudinal repeat measurements of telomere length, you should plan to assay samples from all time points together to avoid potential assay batch differences. Storing blood (not DNA) at -80°C and extract all samples together will be the best option. Our assay is done in batches of 96 samples from a 96-well source plate. We recommend assay all samples of different time points from the same participants on the same batch. Each batch will also contain equal numbers of your control group and

comparison groups to avoid potential plate batch effect. Please provide us a list of de-identified samples with the proper batching scheme.

4. GENOMIC DNA PREPARATION

If you are purifying genomic DNA on your site, we recommend using QIAamp® DNA Mini and Blood Mini kit (QIAGEN, Cat#51104 or cat# 51106). Store DNA samples at -80 °C.

4.1.RNase treatment

We recommend adding RNase A in genomic DNA purification. DNase-free RNase A can be purchased from QIAGEN (Cat #19101).

4.2. DNA quantification

We recommend using PicoGreen method (Quant-iT™ PicoGreen ® dsDNA Kits, Cat #P7589, P11596, P7581 or P11495, Invitrogen) to measure DNA concentration as Picogreen only measures double stranded DNA, not residual single stranded RNA. OD260/OD280 will measure both DNA and RNA and will potentially give higher readings. If you used RNase A in genomic DNA purification, it is unlikely you will have significant amount of RNA. If RNase A is not used in your genomic DNA preparation and the DNA is quantified by OD260/OD280 spectrophotometry, please provide DNA samples with a minimal concentration of 20ng/ul.

Pipette 20 ul of DNA at 30 ng/ul (DNA concentration between 10-30ng/ul is acceptable.) in 96 well plates (Biorad Hard-Shell® Thin-Wall 96-Well Skirted PCR Plates #HSP-9901). Seal the plates with foil seal (**Microseal® 'F' Foil MSF-100 1 from Biorad).** Spin the plates at 1000 rpm for 2 minutes in a tabletop centrifuge at 4 degree. Store the plates at -80°C until ready to ship. DNA samples in 96 wells plates should be placed inside ziplock bags and shipped on dry ice overnight to the address below.

5. SHIPPING

Before your shipment, call or email Jue Lin to schedule a date to make sure we will be in the lab to receive your samples.

Blood samples should be placed in boxes (cat# 82007-162,VWR), and shipped on dry ice overnight to the address below. DNA samples should be in 96-well plates. Place the 96-well plates in a ziplock plastic bag and ship in dry ice.

Please send an electronic file with the sample IDs to us at jue.lin@ucsf.edu.

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REVISION HISTORY

DATE	AUTHOR	REVISION #	REVISION REASON
July 2018	Jue Lin	A	Initial Release