*For TL comparison study, perform extractions on all 8 buccal swabs in four sets of 2 swabs. Each set of 2 swabs is extracted using the procedure and volumes described below and combined together before nanodropping (see Step 21)

1. Place two swabs in a 2 mL tube & add 600 uL Cell Lysis Solution.
2. Add 1.5 uL Proteinase K & vortex for 20 sec.
3. Incubate at 55.5°C for ~2 hrs.
4. Remove swab (use tweezers), scraping along the sides to recover as much liquid as possible
5. Allow sample to cool to room temp for about 30 min.
6. Add 100 uL Protein Precipitation Solution & vortex for 20 sec.
7. Incubate on ice for 5 min.
8. Centrifuge at 14,500 x g for 3 min.
9. Repeat steps 7 & 8
10. Pour out supernatant into a new 1.5 mL tube
11. Add 800 uL 100% Cold Ethanol & 1 uL Glycogen
12. Mix by inverting 50 times
13. Centrifuge at 14,500 x g for 5 min.
14. Discard supernatant & drain on clean paper towel
15. Add 300 uL 70% Ethanol, gently invert to clean pellet
16. Centrifuge at 14,500 x g for 1 min.
17. Discard supernatant & air dry for 5 min. (it is important not to over dry)
18. Add 25uL DNA hydration Solution & vortex for 5 sec.
19. Incubate at 65°C for 1 hr.
20. Incubate at room temp overnight with gentle shaking (speed 1; shaker/incubator at 200 rpm)
21. Combine 25uL elution from each 2 swab set into a single 1.5mL tube (4 x 25 uL; 100uL total per participant) and measure concentration on nanodrop.
Gentra Puregene DBS DNA Extraction (WJH 01/30/2020)

1. Clean the hole punch (standard 7mm diameter paper punch) before and after extractions using by punching regular filter paper (not FTA paper) wetted with 70% Ethanol four times followed by punching four times on dry area of regular filter paper.
2. Use punch to isolate six 7mm discs from DBS card directly into 2.0mL tube (aim for areas with lots of blood)
3. Add 600 uL Cell Lysis Solution and 3 uL Puregene Proteinase K (Cold) and vortex briefly
4. Incubate overnight at 55° C on shaker/incubator at 300 rpm
5. Vortex briefly and spin down any liquid on the lid, then incubate in ice for 1 min.
6. Add 200 uL Protein Precipitation Solution, vortex 20 sec. (speed 7)
7. Incubate on ice for 15 min.
8. Centrifuge at 14,500 x g for 3 min.
9. Repeat steps 8 and 9
10. Pour out supernatant into a new 1.5 mL tube and add 600 uL Isopropanol
11. Add 1 uL of Glycogen
12. Mix by inverting 50 times
13. Incubate on ice for 15 min.
14. Centrifuge at 14,500 x g for 5 min.
15. Discard supernatant and drain on a clean paper towel
16. Add 600 uL of Cold 70% Ethanol, invert 5 times
17. Centrifuge at 14,500 x g for 1 min.
18. Discard supernatant and drain on a clean paper towel
19. Add 600 uL of Cold 100% Ethanol, invert 5 times
20. Centrifuge at 14,500 x g for 2 min.
21. Pour out supernatant and drain on a clean paper towel, pipette out any remaining liquid carefully
22. Air dry 10-15 min. (it is important not to over dry)
23. Add 50 uL DNA Hydration
24. Incubate at 65°C for 1 hour
25. Incubate at room temperature overnight with gentle shaking (speed 1; shaker/incubator at 200 rpm)
26. Measure concentration on nanodrop
**Gentra Puregene Saliva DNA Extraction** (WJH 01/30/2020)

1. Pipette one cryovial worth of sample (~1.8mL) into a fresh 2mL tube and centrifuge at 2,000 x g for 10 min to pellet cells. Discard supernatant and resuspend cells in 500uL of PBS+EDTA.

2. Add .5 mL of saliva sample and 5.5 mL of Cell lysis Solution into a 15 mL tube, mix by pipetting

3. Add 30 uL Proteinase K, mix by inverting 25 times

4. Incubate at 55°C for 2 hours

5. Cool for 45 min. at room temp

6. Add 2 mL Protein Precipitation Solution, vortex for 20 sec.

7. Incubate on ice for 5 min.

8. Centrifuge at 2,000 x g for 10 min.

9. Repeat steps 7 & 8

10. Add supernatant to new 15 mL tube and add 6 mL isopropanol

11. Add 10 uL of Glycogen, mix by inverting 50 times

12. Incubate at room temperature for 5 min.

13. Centrifuge at 2,000 x g for 10 min.

14. Discard supernatant

15. Add 6 mL Cold 70% Ethanol, invert gently to avoid dislodging the pellet

16. Centrifuge at 2,000 x g for 2 min.

17. Discard supernatant and drain on a clean paper towel

18. Add 1 mL of Cold 100% Ethanol, invert gently to avoid dislodging the pellet

19. Centrifuge at 2,000 x g for 2 min.

20. Discard supernatant and drain on a clean paper towel, carefully pipette out any remaining liquid

21. Air dry 15 min. (it is important not to over dry)

22. Add 100 uL of DNA Hydration Solution, vortex for 5 sec.

23. Incubate at 65°C for 1 hour

24. Incubate at room temperature overnight with gently shaking (speed 1; shaker/incubator at 200 rpm)

25. Centrifuge 15mL tube at 100 x g for 1 min to aggregate residual liquid from sides of tube. Pipette up and down to mix and transfer sample to clean 1.5mL tube.

26. Measure concentration on nanodrop. If concentration is less than 100 ng/uL, repeat the extraction with another cryovial worth of sample. For the second extraction, instead of adding fresh DNA Hydration Solution in Step 22, add the final 100uL DNA Hydration elution from the initial saliva extraction of that participant to increase concentration.
1. Combine volume from two 1.5mL aliquots of the same participant into one 1.5mL tube for a total volume of 400uL. Centrifuge at 2,000 x g for 10 min to pellet cells. Discard supernatant and resuspend pellet in 200 uL PBS+EDTA

2. Add 200 uL of the resuspended sample and 3 mL of Cell Lysis Solution to 15 mL tube, vortex to mix

3. Incubate at 55°C for 1 hour

4. Cool to room temperature for 30 min.

5. Add 1 mL of Protein Precipitation Solution, vortex for 20 sec.

6. Incubate on ice for 5 min.

7. Centrifuge at 2,000 x g for 5 min.

8. Repeat steps 6 & 7. If solution is not completely pelleted after second iteration, centrifuge a third time at 2,500 x g for 5 min.

9. Pour out supernatant into a new 15 mL tube and add 8 mL of Cold 100% Ethanol and 13 uL of Glycogen

10. Mix by inverting 50 times

11. Spin at 2,000 x g for 4 min. discard supernatant and drain on a clean paper towel

12. Add 3 mL of Cold 70% Ethanol, invert gently to avoid dislodging the pellet

13. Centrifuge at 2,000 x g for 3 min.

14. Discard supernatant and drain on a clean paper towel, carefully pipette out any remaining liquid

15. Dry for 5-15 min.

16. Add 100 uL of DNA Hydration Solution, vortex for 5 sec.

17. Incubate at 65°C for 1 hour

18. Incubate at room temperature overnight with gentle shaking (speed 1; shaker/incubator at 200 rpm).

19. Centrifuge 15mL tube at 100 x g for 1 min to aggregate residual liquid from sides of tube. Pipette up and down to mix and transfer sample to clean 1.5mL tube.

20. Measure concentration on nanodrop. If concentration is less than 100 ng/uL, repeat the extraction with another 400uL of sample. For the second extraction, instead of adding fresh DNA Hydration Solution in Step 16, add the final 100uL DNA Hydration elution from the initial PBMC extraction of that participant to increase concentration.