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Race-Ethnicity, Poverty, Urban Stressors and Telomere Length in a Detroit Community-Based Sample

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Appendix A: Laboratory Procedures used for Measuring Telomere Length

Regarding the PBMC purification, we used the BD Vacutainer® CPT™ Cell Preparation Tube with Sodium Citrate. Specifically, we spun the cells in a horizontal rotor (swing-out head) for 30 minutes at 1500 to 1800 RCF (Relative Centrifugal Force) according the manufacturer's instruction. According the manufacturer, this should yield mononuclear cells (https://www.bd.com/vacutainer/products/molecular/citrate/).

We used the telomere length measurement assay that was adapted from the published original method by Cawthon (Cawthon 2002, Lin et al. 2010). The telomere thermal cycling profile consisted of:

> *Cycling for T (telomic) PCR: denature at 96°C for 1 second, anneal/extend at 54°C for 60 seconds, with fluorescence data collection, 30 cycles. Cycling for S (single copy gene) PCR: denature at 95°C for 15 seconds, anneal at 58°C for 1 second, extend at 72°C for 20 seconds, 8 cycles; followed by denature at 96°C for 1 second, anneal at 58°C for 1 second, extend at 72°C for 20 seconds, hold at 83°C for 5 seconds with data collection, 35 cycles.*

The primers for the telomere PCR are *tel1b* [5'-CGGTTT$(GTTTGG)_5$GTT-3'], used at a final concentration of 100 nM, and *tel2b* [5'-GGCTTG$(CCTTAC)_5$CCT-3'], used at a final concentration of 900 nM. The primers for the single-copy gene (human beta-globin) PCR are *hbg1* [5' GCTTCTGACACAACTGTGTTCACTAGC-3'], used at a final concentration of 300 nM, and *hbg2* [5'-CACCAACTTCATCCACGTTCACC-3'], used at a final concentration of 700 nM. The final reaction mix contains 20 mM Tris-HCl, pH 8.4; 50 mM KCl; 200 □M each dNTP; 1% DMSO; .4x Syber Green I; 22 ng E. coli DNA per reaction; .4 Units of Platinum Taq DNA polymerase (Invitrogen Inc.) per 11 microliter reaction; .5 - 10 ng of genomic DNA. Tubes containing 26, 8.75, 2.9, .97, .324 and .108ng of a reference DNA (from Hela cancer cells) are

included in each PCR run so that the quantity of targeted templates in each research sample can be determined relative to the reference DNA sample by the standard curve method. The same reference DNA was used for all PCR runs.

To control for inter-assay variability, eight control DNA samples are included in each run. In each batch, the T/S ratio of each control DNA is divided by the average T/S for the same DNA from 10 runs to get a normalizing factor. This is done for all eight samples and the average normalizing factor for all eight samples is used to correct the participant DNA samples to get the final T/S ratio. The T/S ratio for each sample will be measured twice. When the duplicate T/S value and the initial value vary by more than 7%, the sample is run the third time and the two closest values are reported. To estimate the LTLs in terms of base pairs, a standard conversion formula of 3274+2413*(T/S) was established by examining a set of control DNA and comparing its T/S ratio to its number of telomere base pairs measured via Southern blot.