



Telomere Length Measurement: Sample Collection & Storage Checklist

The Telomere Research Network (TRN) (trn.tulane.edu) was funded by the NIA/NIEHS to establish best practices for the measurement of telomere length in population-based studies. The TRN is currently investigating, [in a systematic and rigorous set of experiments](#), the importance of multiple pre-analytic factors on telomere length measured using different assays. Over the next two years we expect to have specific guidance for these factors based on our data and comments from our colleagues and experts around the world. These recommendations are offered as initial guidelines for parameters that have the potential to impact the reproducibility, repeatability, and accuracy of telomere length measurement that should be recorded and evaluated for their impact on telomere length measurement. To make comments and/or request clarification please contact Stacy Drury, MD, PhD, Director of the TRN at telomerenetwork@gmail.com.

Sample:

- Specimen type^{1,2}
- Collection procedure
- Lot number and expiration date of collection tubes or kit
- Sample storage temperature and buffer^{3,4}
- Sample storage duration until processing and/or DNA extraction⁴
- Number of freeze-thaw cycles

Considerations for specific sample types:

- Blood: buffer/anticoagulant used in collection
- Buccal: method of stabilization (desiccant or stabilization buffer)
- Buffy coat, PBMCs, and other blood components: isolation procedure
- Saliva: volume of saliva collected and method of stabilization
- Organ tissues: fresh or frozen, stabilization matrix or storage media
- Cell lines: name of cell line, culture conditions (media and supplements), passage,

DNA:

- DNA extraction kit & reagent lot numbers, including mechanical vs manual extraction
- DNA extraction batches (e.g. lot number, batch)
- DNA storage temperature, duration, and concentration^{5,6}
- DNA storage buffer/solution
- Method of measuring DNA concentration
- Number of freeze-thaw cycles⁷

Key Points

1. Sample storage and handling should be uniform within a study
2. Use same DNA extraction method/kit for all samples
3. If storage, handling, or DNA extraction differs within a study, assess this as an independent variable in all analyses and in relation to ICCs

**REFERENCES:**

1. Dlouha D, Maluskova J, Kralova Lesna I, Lanska V, Hubacek JA. Comparison of the relative telomere length measured in leukocytes and eleven different human tissues. *Physiol Res*. 2014;63 Suppl 3:S343-S350.
2. Lin J, Smith DL, Esteves K, Drury S. Telomere length measurement by qPCR - Summary of critical factors and recommendations for assay design. *Psychoneuroendocrinology*. 2019;99:271-278. doi:10.1016/j.psyneuen.2018.10.005
3. Zanet DL, Saberi S, Oliveira L, Sattha B, Gadawski I, Côté HC. Blood and dried blood spot telomere length measurement by qPCR: assay considerations. *PLoS One*. 2013;8(2):e57787. doi:10.1371/journal.pone.0057787
4. Kong PL, Looi LM, Lau TP, Cheah PL. Assessment of Telomere Length in Archived Formalin-Fixed, Paraffinized Human Tissue Is Confounded by Chronological Age and Storage Duration [published correction appears in PLoS One. 2016 Dec 9;11(12):e0168238]. *PLoS One*. 2016;11(9):e0161720. Published 2016 Sep 6. doi:10.1371/journal.pone.0161720
5. Dagnall CL, Hicks B, Teshome K, Hutchinson AA, Gadalla SM, et al. (2017) Effect of pre-analytic variables on the reproducibility of qPCR relative telomere length measurement. *PLOS ONE* 12(9): e0184098. <https://doi.org/10.1371/journal.pone.0184098>
6. Röder B, Frühwirth K, Vogl C, Wagner M, Rossmanith P. Impact of long-term storage on stability of standard DNA for nucleic acid-based methods. *J Clin Microbiol*. 2010;48(11):4260-4262. doi:10.1128/JCM.01230-10
7. Shao W, Khin S, Kopp WC. Characterization of effect of repeated freeze and thaw cycles on stability of genomic DNA using pulsed field gel electrophoresis. *Biopreserv Biobank*. 2012;10(1):4-11. doi:10.1089/bio.2011.0016