Telomere length measurement concerns: Cell sources, assay performance, sample handling

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Introductions

- What factors influence telomere length measurement?

- What matters?
- What could add “noise”?

- Goal
  - Standardization of methods
  - Knowledge of “do’s” and “don’ts”

Telomere Length

- Telomeres are on the ends of DNA
- Cell to cell variation
- Variation by chromosome within cells

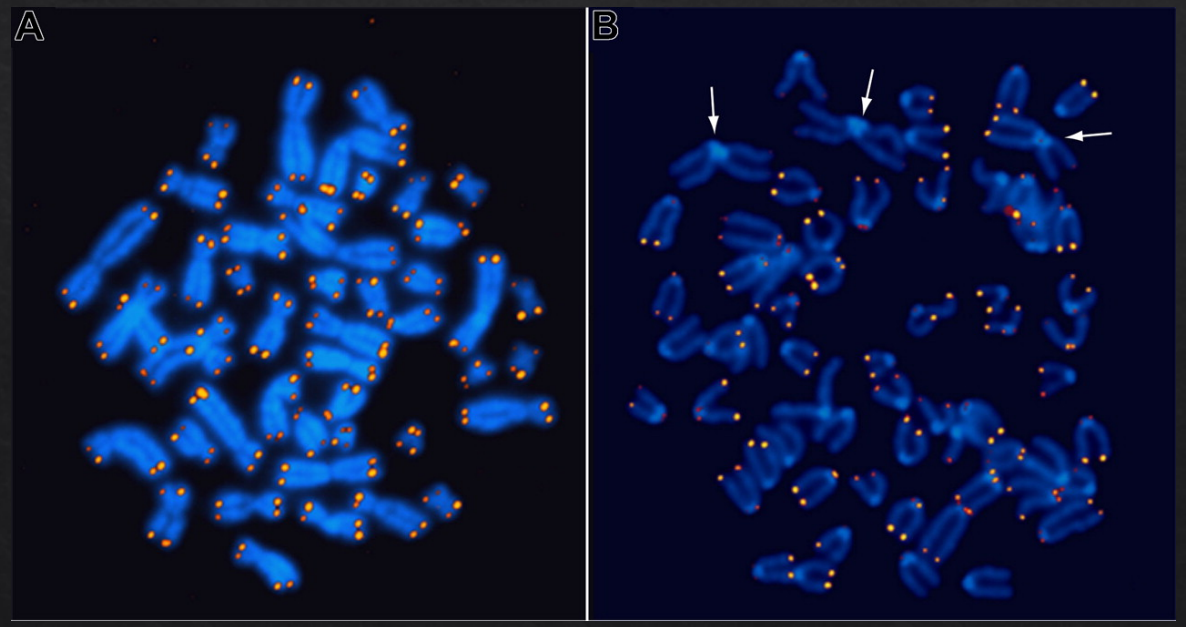
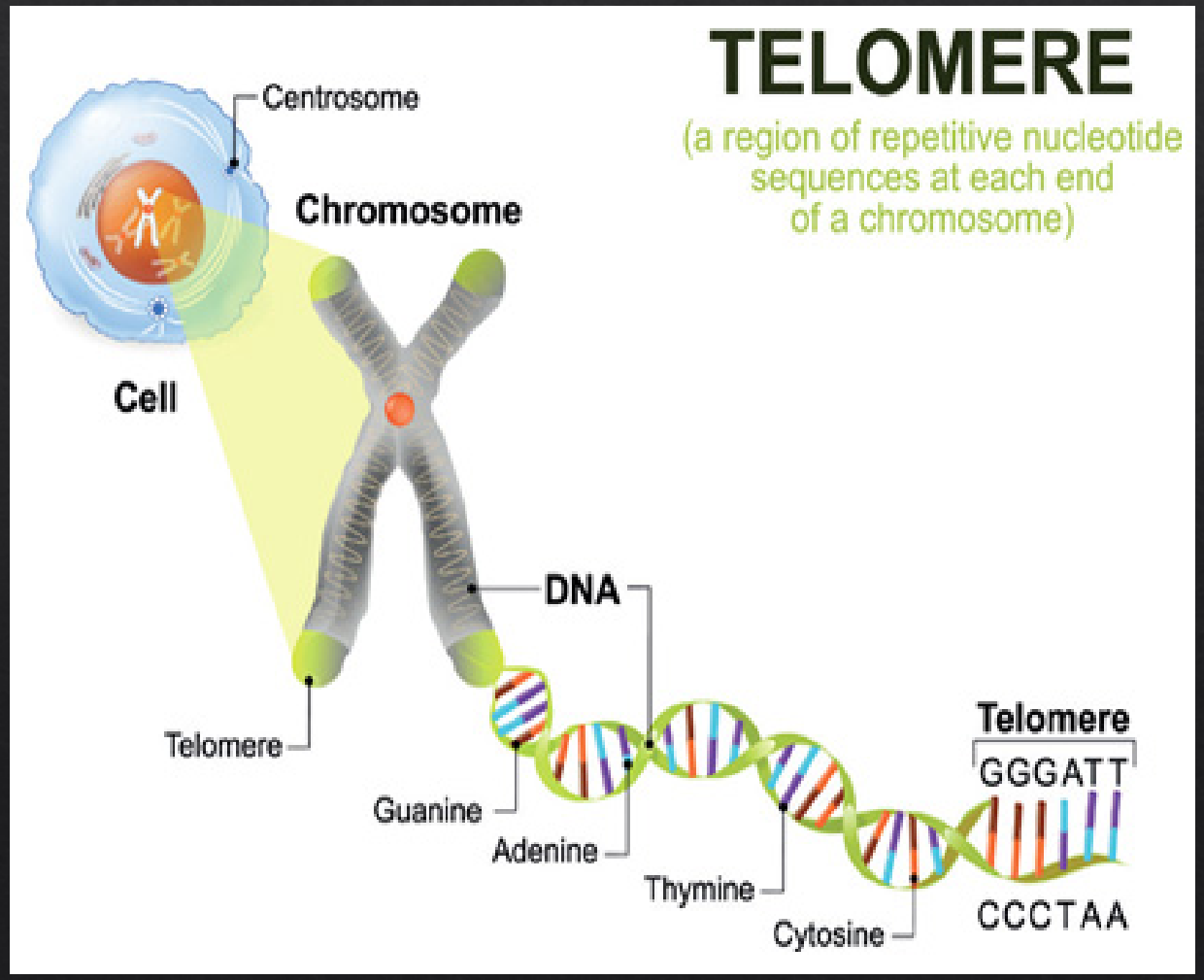


Figure shows fluorescence in situ hybridization to detect telomere repeats. From: Lansdorp, 2008, Blood



Common Cell Sources

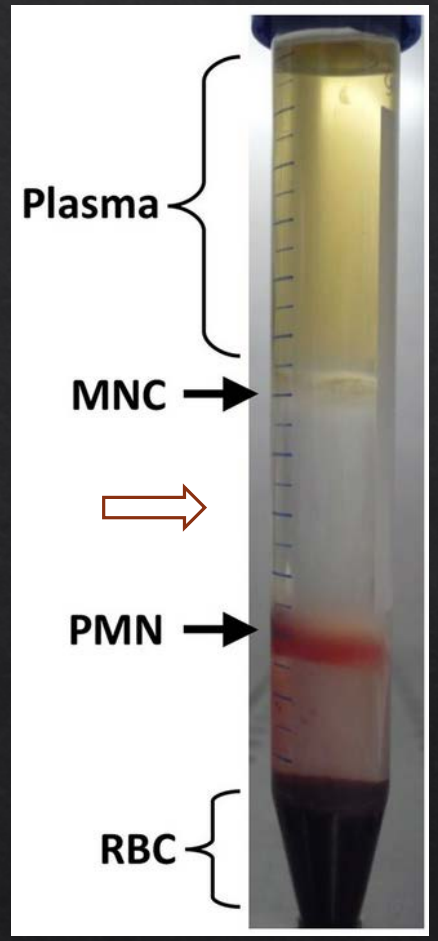
- Buccal cells

- Saliva

- Blood leukocytes

- Peripheral blood mononuclear cells

Peripheral blood mononuclear cells



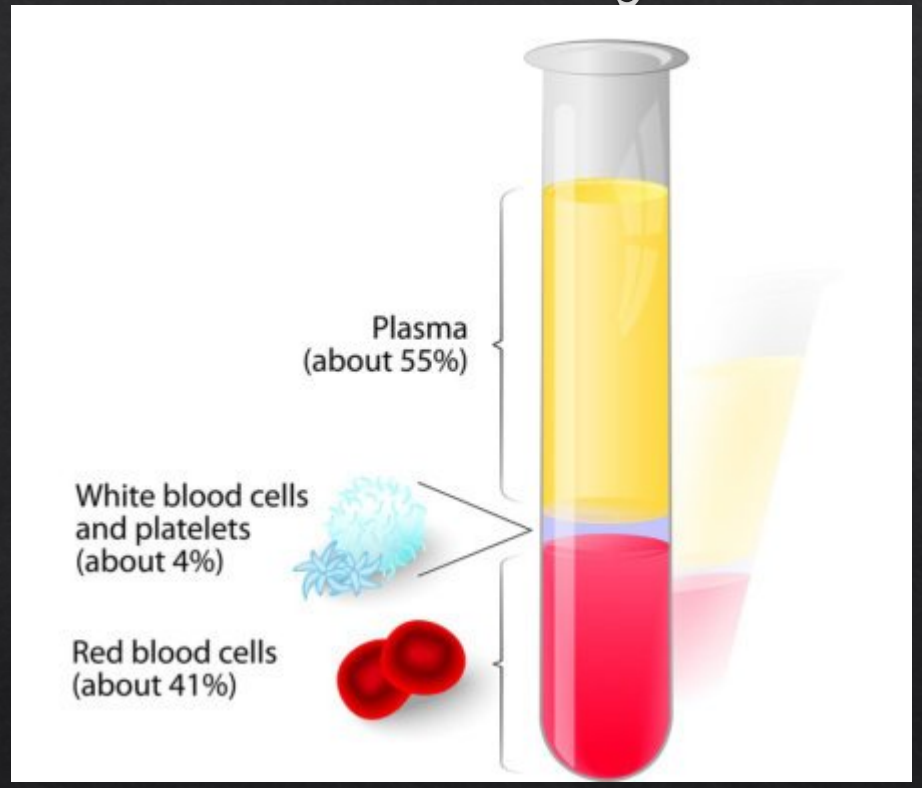
Density Gradient

Lymphocytes/PBMC

Granulocytes
60-80% of Leukocytes

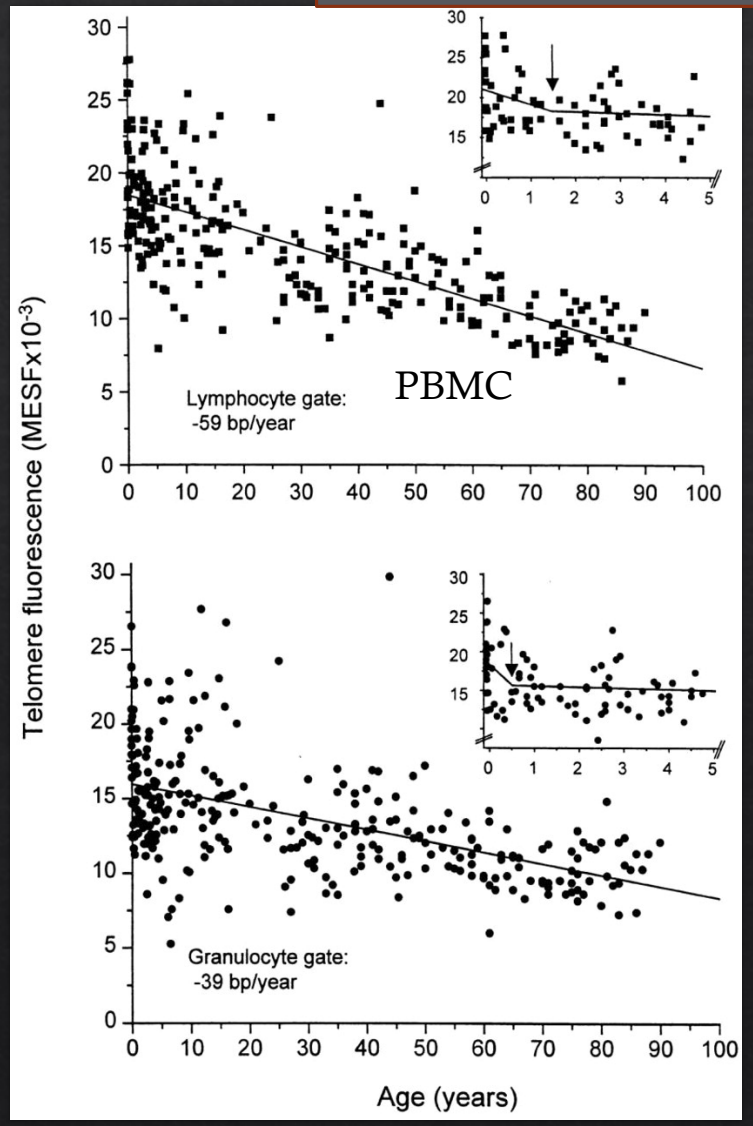
Red Blood Cells

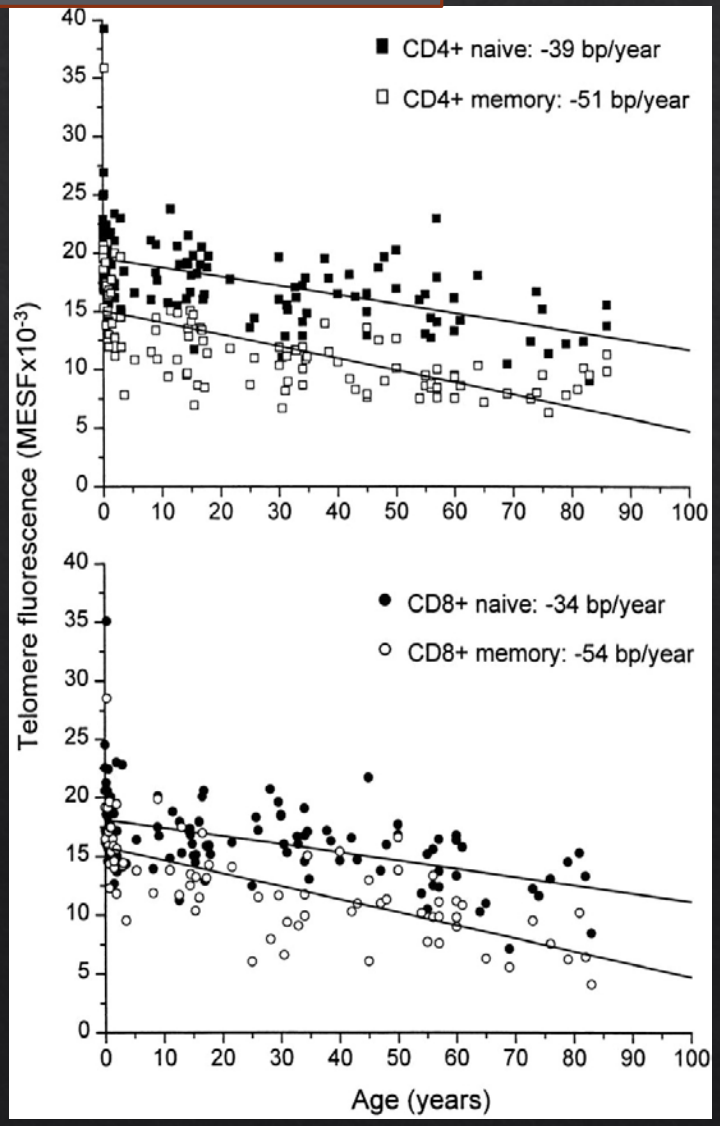
Whole blood Leukocytes



TL attrition with age by cell type

Need population based research to understand the value of cell type in predicting aging and disease.





Rufer et al., 1999. Journal of Experimental Medicine

Saliva vs Venous Blood, r=.56

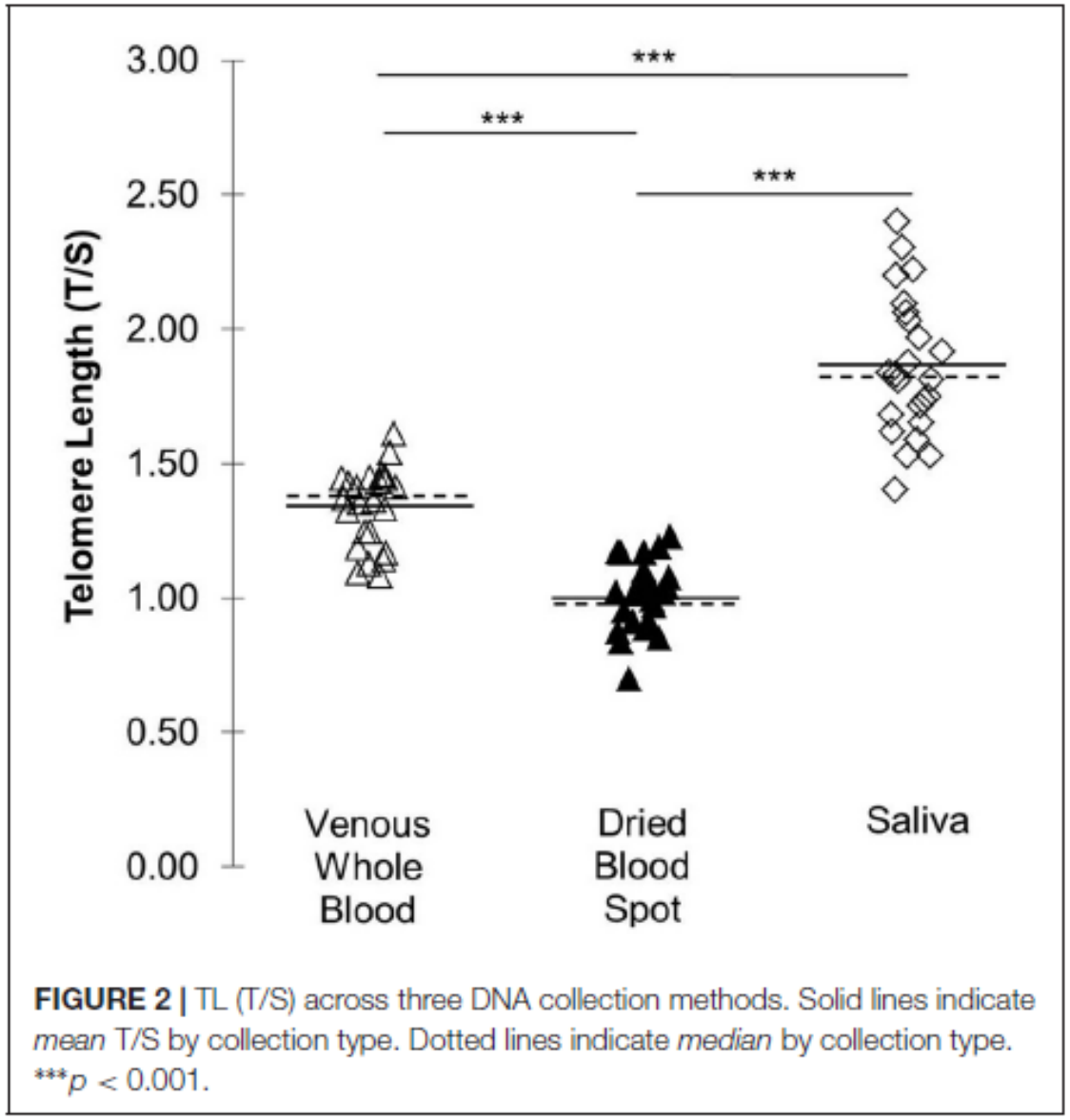
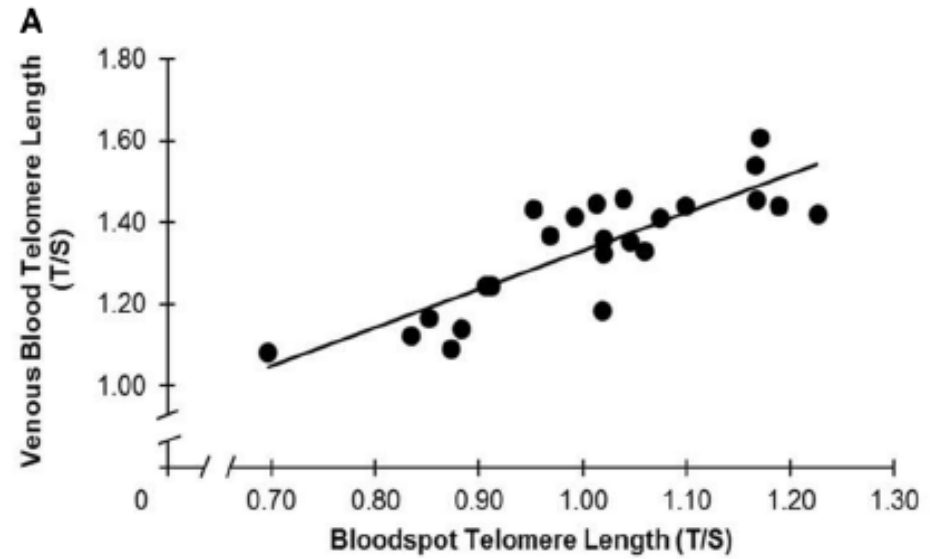
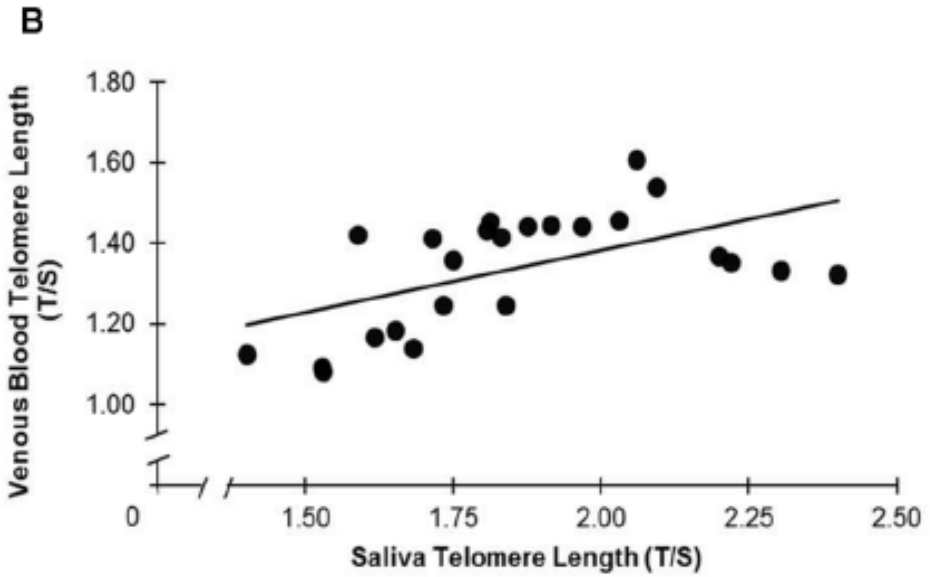


FIGURE 2 | TL (T/S) across three DNA collection methods. Solid lines indicate *mean* T/S by collection type. Dotted lines indicate *median* by collection type. ***$p < 0.001$.





Stout et al., 2017 Frontiers of Aging Neuroscience

Buccal Cells

- Easy to access
- Good for research in kids
- Buccal vs Venous Blood TL, highly correlated: r=.7-.9
  - Need more research establishing the utility of buccal cell telomere length as a predictor of aging

Sample Handling/Collection Procedures

From draw to freezer. Variations in sample handling could influence estimates of telomere length in blood.

- Type of blood tube used: ACD, EDTA, DNA tube
- Held at room temperature, overnight, frozen right away?
- Whole blood aliquots, Cell pellets, PaxGene tubes
- These are unknown factors

Variability in Telomere Estimates Introduced by Lab to Lab Variations in Assay Methods

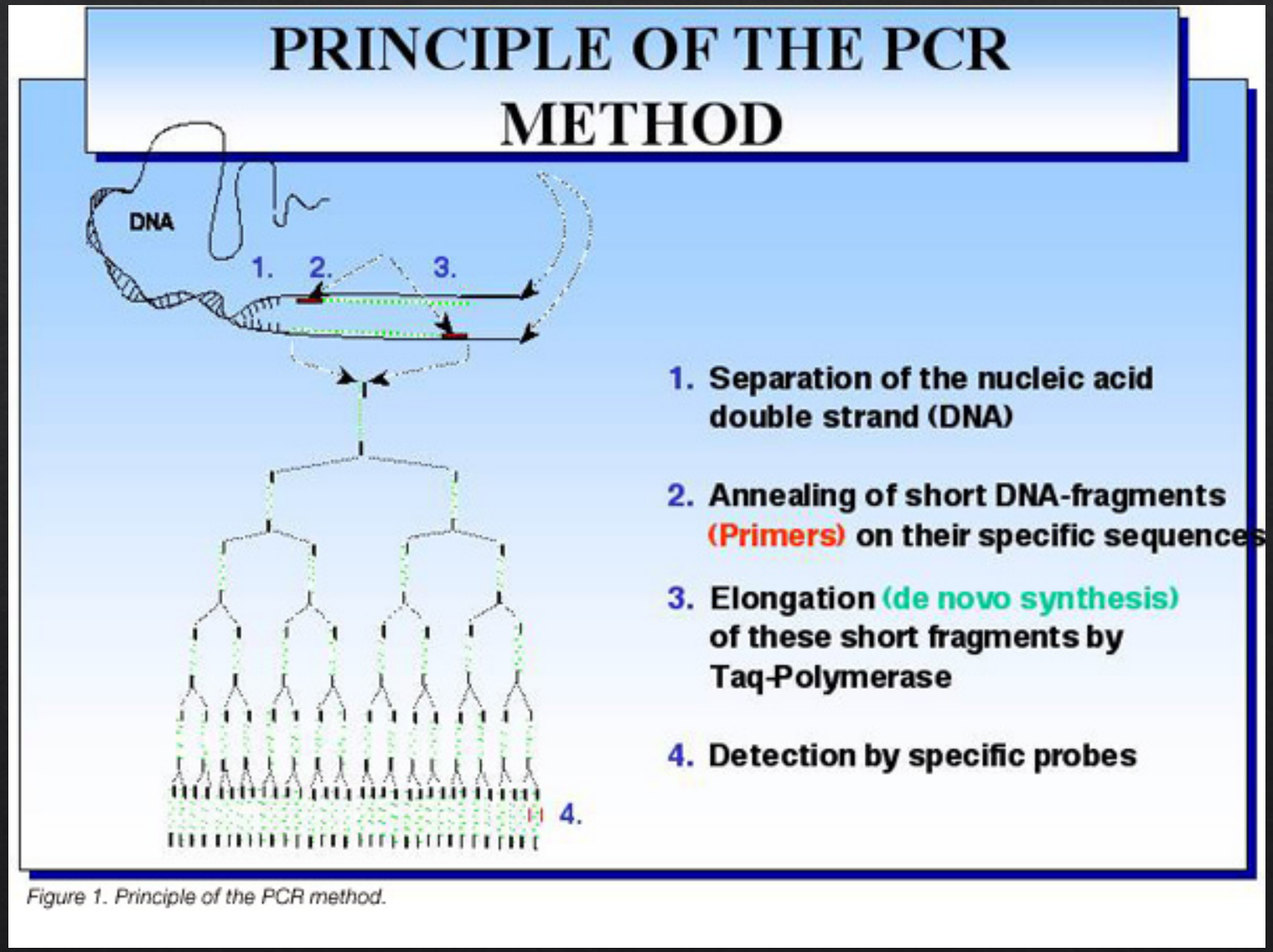


Figure 1. Principle of the PCR method.

Features of qPCR method for Telomere Length that could add noise:

- PCR efficiency not always evaluated: Ideal efficiency is 90-110%.
- Amount of Genomic DNA put in wells varies: 5ng-50ng
- Triplicate, duplicate, single wells
- Optimal way to adjust for plate to plate variability
- Should a standard curve to calculate estimates be required
- Coefficients of variation are not always reported. What are our standards for this?

Open Questions

1. Is PBMC telomere length, compared to leukocyte telomere length, a better predictor of aging and disease?
2. Do cell distributions within leukocytes and PBMCs influence telomere length estimates? Do we need to control for cell subsets? What predicts aging best?
3. Are buccal cells a good source for telomere length estimates? Do they predict aging and disease?

(continued)

4. Does sample handling of blood effect telomere length estimates?
5. Does storage time and storage type matter? Is it better to store whole cells or isolate DNA?
6. Does PCR efficiency alter telomere length estimates? Are there other assay components that matter (e.g., standard curve)? What are the best practices?

NIH National Institute on Aging
Turning Discovery Into Health

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