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Peer Review

Review of: "Optimization of Pre-Sampling Conditions for Salivary Genomic DNA Extraction: A Single-Donor Analysis"

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I must say that this work represents a thoughtful contribution to the important task of standardizing pre-analytical protocols for saliva-based genomic research. The authors have clearly invested considerable effort into developing a rigorous methodological approach, and this careful attention to detail is evident throughout the work.

The choice of a repeated measures design with a single donor is particularly well-designed and methodologically sound. By focusing on one individual across multiple sampling conditions, the authors have successfully eliminated the noise of inter-individual variability and created a controlled environment to truly isolate the effects of behavioral variables on DNA recovery. The achievement of a coefficient of variation as low as 1.71% is genuinely impressive and speaks volumes about the precision of both the collection protocol and laboratory procedures. This level of reproducibility is exactly what the field needs as we move toward larger-scale genomic studies.

I also appreciate the authors' transparent and scientifically mature approach to discussing their findings. Rather than overclaiming or extrapolating beyond what a pilot study can support, they correctly position this work as a proof-of-concept that demonstrates technical feasibility. Their recognition that consistent, moderate yields are often more valuable than higher but variable yields shows a pragmatic understanding of real-world genomic workflows that will resonate with readers working in this space.

The manuscript is well-written, and the results are clearly presented, making it accessible and useful for researchers who may want to implement similar protocols in their own laboratories.

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I have a few suggestions that might strengthen the methodological transparency and replicability of the protocol. These are relatively minor enhancements that don't diminish the quality of the work already done.

First, it would be helpful if the authors could provide some additional details about the toothpaste used in the brushing intervention. Different oral care products contain various chemical agents—surfactants like sodium lauryl sulfate, antimicrobials, abrasives, and whitening compounds—that could potentially interact with oral epithelial cells in different ways. For example, the study conducted by Yang et al. (2021) showed that fluoride toothpaste containing emulsifier 30 and sodium lauryl sulfate surfactant can cause a degree of oral soft tissue exfoliation when compared to fluoride toothpaste containing only emulsifier 30, which caused less damage. While this may not have been a primary consideration in the current pilot phase, including information about the specific formulation used (brand name and key ingredients) would make the protocol more reproducible for others and could help researchers anticipate potential variables in future validation studies. This is simply a matter of adding methodological detail rather than any concern about the current findings.

Second, while the authors appropriately mention in the limitations section that the donor did not have conditions known to affect salivary composition, I wonder if it might be worth including more explicit inclusion criteria for the participant. For instance, specifying the donor's age range would be useful, as we know that salivary characteristics and epithelial cell turnover can vary across different life stages. Similarly, clarifying whether the saliva collected was unstimulated (resting) or stimulated would be valuable information for anyone attempting to replicate this protocol. These additions would simply enhance the completeness of the methodological description.

Furthermore, including a brief participant characterization section might be beneficial—such as general health status, medication use, smoking status, or time since last meal. Again, this is more about providing readers with a complete picture of the baseline conditions rather than suggesting any deficiency in the current approach.

Overall, I believe this is a well-crafted pilot study that successfully achieves its stated aims. The demonstration that simple, modifiable variables—particularly brushing—can significantly impact gDNA yield consistency is both practical and important. The work provides a solid foundation that others can build upon as the field moves toward more standardized saliva collection protocols for genomic applications.

My recommendation is acceptance with minor revisions to incorporate the methodological details suggested above.

Article mentioned: Yang M, Zhao HP, Yang J. *Shanghai Kou Qiang Yi Xue*. 2021;30(3):312-315.

Declarations

Potential competing interests: No potential competing interests to declare.