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| Journal Name: | [**Journal of Advances in Biology & Biotechnology**](https://journaljabb.com/index.php/JABB) |
| Manuscript Number: | **Ms\_JABB\_140819** |
| Title of the Manuscript: | **Recombinant Expression of Taq DNA Polymerase Using pBluescript SK(+) and Chromatography-Free Purification: A Cost-Effective Strategy for Low-Resource Laboratories** |
| Type of the Article | **Original Research Article** |

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| PART 1: Comments | | |
|  | Reviewer’s comment **Artificial Intelligence (AI) generated or assisted review comments are strictly prohibited during peer review.** | **Author’s Feedback** (It is mandatory that authors should write his/her feedback here) |
| **Please write a few sentences regarding the importance of this manuscript for the scientific community. A minimum of 3-4 sentences may be required for this part.** | **This study titled as "Recombinant Expression of Taq DNA Polymerase Using pBluescript SK(+) and Chromatography-Free Purification: A Cost-Effective Strategy for Low-Resource**  **Laboratories" holds potent importance as it demonstrate the first experimentally validated expression of thermostable Taq DNA polymerase using the widely available pBluescript SK(+) cloning vector traditionally used for blue-white screening—as a protein expression platform in E. coli. The authors mention that the protocol reduced the cost-per-reaction by 70–85%, depending on enzyme input and Their findings demonstrate that pBluescript SK(+) which is typically reserved for cloning purposes. On the otherhand, under-resourced research environments this study may act as open-access alternative to proprietary enzyme production. As it visualize fully open-access alternative to conventional Taq polymerase production by repurposing pBluescript SK(+), a widely available vector not previously validated for thermostable enzyme expression, it may be considered as a good finding for scientific community.** | We agree with the reviewer’s assessment and thank them for highlighting the importance of our work. In response, we have revised and expanded our response to read as follows: "This study presents the first experimentally validated use of the widely available pBluescript SK(+) vector for the expression of thermostable Taq DNA polymerase in *E. coli*. Unlike conventional strategies that require proprietary systems, our protocol relies solely on generic reagents and a chromatography-free purification method, making it highly accessible and cost-effective. The approach reduced the cost per reaction by 70–85% and preserved enzyme activity and thermostability, making it a viable and scalable alternative for teaching and research in under-resourced laboratories. This work thus addresses an important gap in enzyme production workflows for the academic and educational sectors." |
| **Is the title of the article suitable?**  **(If not please suggest an alternative title)** | **Yes, The title of the article is suitable.** | We thank the reviewer for confirming the suitability of the title. |
| Is the abstract of the article comprehensive? Do you suggest the addition (or deletion) of some points in this section? Please write your suggestions here. | **The Abstract of the article is comprehensive and also structured into a series-aim, methods, results and conclusions.** | We thank the reviewer for their positive evaluation of the abstract. |
| Is the manuscript scientifically, correct? Please write here. | The manuscript is scientifically correct | We appreciate the reviewer’s confirmation of the scientific soundness of the manuscript. |
| **Are the references sufficient and recent? If you have suggestions of additional references, please mention them in the review form.** | The references are sufficient. Most of the references are almost recent, however, the authors can consider replacing these below references if possible and only if they are not integral parts of the paper, as they are quite old.  Chien, A., Edgar, D. B., & Trela, J. M. (1976). Deoxyribonucleic acid polymerase from the  653 extreme thermophile Thermus aquaticus. Journal of Bacteriology, 127(3), 1550–1557.  Saiki, R. K., Gelfand, D. H., Stoffel, S., Scharf, S. J., Higuchi, R., Horn, G. T., Mullis, K. B., &   Erlich, H. A. (1988). Primer-directed enzymatic amplification of DNA with a thermostable DNA  polymerase. Science, 239(4839), 487–491.  Engelke, D. R., Krikos, A., Bruck, M. E., & Ginsburg, D. (1990). Purification of Thermus   aquaticus DNA polymerase expressed in Escherichia coli. Analytical Biochemistry, 191(2), 396– 400.  Lawyer, F. C., Stoffel, S., Saiki, R. K., Myambo, K., Drummond, R., & Gelfand, D. H. (1989).   Isolation, characterization, and expression in Escherichia coli of the DNA polymerase gene from  Thermus aquaticus. Journal of Biological Chemistry, 264(11), 6427–6437.  Pluthero, F. G. (1993). Rapid purification of high-activity Taq DNA polymerase. Nucleic Acids   Research, 21(20), 4850.  Brock, T. D., & Freeze, H. (1969). Thermus aquaticus gen. n. and sp. n., a nonsporulating   extreme thermophile. Journal of Bacteriology, 98(1), 289–297.  Gelfand, D. H. (1989). Taq DNA polymerase. In PCR Technology: Principles and Applications   for DNA Amplification (pp. 17–22). London: Palgrave Macmillan UK. | In response to the reviewer’s suggestion to consider replacing older references, we appreciate the opportunity to clarify that each of the cited works serves a foundational or contextually essential role that cannot be substituted by newer sources without compromising historical accuracy or scientific continuity. Chien et al. (1976) is the seminal study that first identified and characterized *Taq* DNA polymerase from *Thermus aquaticus*, demonstrating its thermostability and enzymatic activity—an essential citation in any work involving *Taq* due to its foundational nature. Brock and Freeze (1969) represents the original taxonomic description of *Thermus aquaticus*, and this reference was requested by our *T. aquaticus* strain supplier to ensure proper attribution. It also provides critical microbiological context for the source of the enzyme. Lawyer et al. (1989) was the first to clone and express the *Taq* polymerase gene in *E. coli*, which directly laid the groundwork for all recombinant production systems—including our simplified educational version—and is indispensable in tracing this methodological lineage.Engelke et al. (1990) pioneered a heat-denaturation purification approach for *Taq* polymerase, forming the conceptual foundation for our chromatography-free strategy. Our protocol refines this principle to enhance yield, cost-efficiency, and accessibility, and thus must cite the original strategy it builds upon. Pluthero (1993) is another key milestone that introduced a rapid, non-chromatographic method for purifying *Taq*, closely aligned with our resin-free workflow and thus necessary to establish methodological continuity and benchmark comparison. Lastly, Gelfand (1989) contextualizes the transformative impact of *Taq* polymerase in the PCR era from a biotechnological perspective. Citing this source reinforces our argument for broadening access to PCR-grade enzymes for educational and low-resource applications and supports the relevance of our strategy in a global, instructional context.Together, these references provide essential historical, methodological, and conceptual grounding. While more recent literature has been included where appropriate, these specific works have enduring relevance that justifies their retention. That said, we have replaced the Saiki et al., 1988 reference with a newer source that supports the same claim. |
| Is the language/English quality of the article suitable for scholarly communications? | The Language/English quality of the article suitable and fulfils criteria for scholarly communications. | We thank the reviewer for this affirmation. We have nonetheless performed a final round of proofreading to further improve clarity and consistency. |
| Optional/General comments |  |  |

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| **PART 2:** | | |
|  | **Reviewer’s comment** | **Author’s Feedback** (It is mandatory that authors should write his/her feedback here) |
| **Are there ethical issues in this manuscript?** | *(If yes, Kindly please write down the ethical issues here in details)* | We confirm that there are no ethical issues associated with this manuscript. No human or animal subjects were involved, and all procedures were conducted using microbial strains and publicly available plasmid systems. |