



**SDI Review Form 1.6**

Journal Name:	<a href="#">Asian Journal of Research in Biochemistry</a>
Manuscript Number:	<b>Ms_AJRB_43243</b>
Title of the Manuscript:	<b>High-level expression and purification of DNA and DNase free Taq DNA polymerase</b>
Type of the Article	<b>Original Research Article</b>

**General guideline for Peer Review process:**

This journal's peer review policy states that **NO** manuscript should be rejected only on the basis of '**lack of Novelty**', provided the manuscript is scientifically robust and technically sound. To know the complete guideline for Peer Review process, reviewers are requested to visit this link:

(<http://www.sciencedomain.org/page.php?id=sdi-general-editorial-policy#Peer-Review-Guideline>)



SDI Review Form 1.6

**PART 1: Review Comments**

	Reviewer's comment	Author's comment (if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)
<b>Compulsory</b> REVISION comments	<p><b>Major concerns:</b> To overproduce the polymerase <i>E. coli</i> DH5<math>\alpha</math> strain was used. However, a type of expression vector has been not given. I have found only such information (lanes 44-45): "The Taq DNA polymerase was transformed by heat shock method into <i>E. coli</i> DH5<math>\alpha</math> competent cells that were prepared by CaCl<sub>2</sub> method." It is impossible! I am sure that an appropriate recombinant plasmid carrying the Taq DNA polymerase gene was introduced to <i>E. coli</i> cells. Which expression vector was used to obtain the recombinant protein? How was the recombinant vector created? Lanes: 46-47 and lanes 118-119: What is the sequence of specific primers that were used to confirm the presence of the Taq DNA polymerase gene in any vector? Description of the method used to determine DNase activity (lane 95) is incomplete and there occurred mistakes.</p> <p>Image descriptions are unreadable. Fig. 2 contains four panels (not two) and each of them should be described in the figure legend, because each panel presents something different; proteins separated by gel electrophoresis were visualized using different staining procedures.</p> <p>Clinical samples should be described (lanes 110-115). <b>ethical issues:</b> In this study, different clinical samples were used to test DNA polymerase activity. Unfortunately, the origin of these samples has been not given. I am afraid that author(s) should obtain research ethical approval before beginning the study from local ethics committees to use biological/clinical material</p>	<ol style="list-style-type: none"> <li>1. The taq DNA polymerase gene was cloned to plasmid pLoxGentrc and the cloned plasmid was transformed to <i>E.coli</i> DH5<math>\alpha</math> by calcium chloride method.</li> <li>2. The sequence of the gene specific primers has been included in the appropriate place in the manuscript and highlighted.</li> <li>3. The DNase activity assay has been rewritten for more clarity.</li> <li>4. The image descriptions have been edited to improve the clarity and the edited images are included in the manuscript.</li> <li>5. Fig. 2 has been now divided into four panels as suggested and explanations have been given.</li> <li>6. We have ethical clearance for the use of clinical samples. The <i>E.coli</i> and <i>Acinetobacter</i> samples were got from SDM College of Medical Sciences, Dharwad. The tuberculosis sample is got from Rajiv Gandhi Institute of Chest Diseases (RGICD), Bangalore.</li> </ol>
<b>Minor</b> REVISION comments	<p>Lane 111: It should be <i>Acinetobacter</i> spp. instead of <i>Acinetobacter</i> sp. Lane 114: There occurred a mistake in a name of bacterium ; it should be <i>Tubercle bacilli</i> instead of tubercular bacilli)</p>	<ol style="list-style-type: none"> <li>1. <i>Acinetobacter</i> sp. is changed to <i>Acinetobacter</i> spp.</li> <li>2. Tubercular bacilli is changed to tubercle bacilli</li> </ol> <p>The changes are highlighted in the text.</p>
<b>Optional/General</b> comments	<p>The manuscript describes a good purification strategy that can be used to obtain high-quality Taq DNA polymerase. The manuscript requires extensive editing of English language and style.</p>	<ol style="list-style-type: none"> <li>1. Thank you for the feedback.</li> </ol>