

## Review Form 1.6

Journal Name:	<a href="#">Asian Journal of Biotechnology and Genetic Engineering</a>
Manuscript Number:	Ms_AJBGE_83271
Title of the Manuscript:	A Simple and Effective Phenol-Chloroform method of DNA extraction from mammalian feces
Type of the Article	Short Research Article

### 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:

(<https://www.journalajbge.com/index.php/AJBGE/editorial-policy> )

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)																																							
Compulsory REVISION comments	<p>This manuscript writing is good. It has an interesting and new topic. But the results and discussion section is very little explained, and new references are unused and part of the discussion is incomplete that needs to be completed.</p>	<p>We have introduced the topic of DNA concentration in our study which was not dealt in the previous version of the manuscript. It is as follows:</p> <p>Hence, quantification of DNA from fecal samples is unreliable. We recommend quantification only for the purpose of finding the optimal DNA concentration for a workable PCR reaction.</p>																																							
Minor REVISION comments	<p>Well written Should be complete results and discussion section</p>	<p>we have elaborated the results and discussion section with the information below as instructed:</p> <p>DNA quantification showed that our modified method yielded more than adequate concentration in most of the samples subjected to experiment (Table 4). Likewise, it is commonly shown that a low amount of template DNA is inappropriate for successful amplification of target gene region [3], contradictorily, we used concentrations as low as 5ng/μL and achieved successful amplifications.</p> <p><b>Table 4: Quantification of concentration of fecal DNA samples</b></p> <table><tr><th>SPECIES NAME</th><th>SAMPLE COUNT</th><th>CONC (ng/ μL) (Range)</th></tr><tr><td>Elephant (<i>Elephas maximus</i>)</td><td>23</td><td>84 – 188</td></tr><tr><td>Indian gaur (<i>Bos gaurus</i>)</td><td>2</td><td>15 – 27</td></tr><tr><td>Samber deer (<i>Rusa unicolor</i>)</td><td>3</td><td>70 – 96</td></tr><tr><td>Black Naped Hare (<i>Lepus nigricollis</i>)</td><td>2</td><td>22 – 29</td></tr><tr><td>Wild pig (<i>Sus scrofa</i>)</td><td>2</td><td>210 - 456</td></tr><tr><td>Sloth bear (<i>Melursus ursinus</i>)</td><td>4</td><td>35 - 42</td></tr><tr><td>Slender loris (<i>Loris lydekkerianus</i>)</td><td>2</td><td>15 - 33</td></tr><tr><td>Jackal (<i>Canis aureus</i>)</td><td>7</td><td>23.8 - 30</td></tr><tr><td>Tiger (<i>Panthera tigris</i>)</td><td>13</td><td>56 - 318.3</td></tr><tr><td>Wild dog - Dhole (<i>Cuon alpinus</i>)</td><td>11</td><td>65 - 306</td></tr><tr><td>Mongoose (<i>Herpestes edwardsii</i>)</td><td>5</td><td>25 - 47</td></tr><tr><td>Hyaena (<i>Hyaena hyaena</i>)</td><td>7</td><td>450 - 870</td></tr></table> <p>The successful amplification was partly due to the modified preservation method [8] which is simple to follow, yet effective for quite a long duration, up till 1 to 2 years without the need for any sophisticated chemical treatment compared to the previous fecal DNA isolation methods [2, 10, 11, 12, 14]. The experiment was designed in a way to assess the applicability of the preservation and extraction protocol in mammalian scat samples of different dietary intake.</p> <p>Although there have been few successful studies on DNA being extracted from fecal samples by modified phenol-chloroform method [10, 14] both of them applied either chemical treatment or freezing for preservation of samples.</p>	SPECIES NAME	SAMPLE COUNT	CONC (ng/ μL) (Range)	Elephant ( <i>Elephas maximus</i> )	23	84 – 188	Indian gaur ( <i>Bos gaurus</i> )	2	15 – 27	Samber deer ( <i>Rusa unicolor</i> )	3	70 – 96	Black Naped Hare ( <i>Lepus nigricollis</i> )	2	22 – 29	Wild pig ( <i>Sus scrofa</i> )	2	210 - 456	Sloth bear ( <i>Melursus ursinus</i> )	4	35 - 42	Slender loris ( <i>Loris lydekkerianus</i> )	2	15 - 33	Jackal ( <i>Canis aureus</i> )	7	23.8 - 30	Tiger ( <i>Panthera tigris</i> )	13	56 - 318.3	Wild dog - Dhole ( <i>Cuon alpinus</i> )	11	65 - 306	Mongoose ( <i>Herpestes edwardsii</i> )	5	25 - 47	Hyaena ( <i>Hyaena hyaena</i> )	7	450 - 870
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Optional/General comments	<p>Include these references to your study</p> <p>Carvajal-Agudelo JD, Trujillo-Betancur MP, Velásquez-Guarín D, Ramírez-Chaves HE, Pérez-Cárdenas JE, Rivera-Páez FA. Field blood preservation and DNA extraction from wild mammals: methods and key factors for biodiversity studies. Revista UDCA Actualidad &amp; Divulgación Científica. 2021 Jun;24(1).</p> <p>Janabi AH, Kerkhof LJ, McGuinness LR, Biddle AS, McKeever KH. Comparison of a modified phenol/chloroform and commercial-kit methods for extracting DNA from horse fecal material. Journal of microbiological methods. 2016 Oct 1;129:14-9.</p>	<p>We have included both the references in our study reading the same in the result and discussion section.</p>
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PART 2:

	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)
Are there ethical issues in this manuscript?	(If yes, Kindly please write down the ethical issues here in details)	There are no ethical issues, as the sample type used in the study is faeces, which is a non-invasive sample type, generally considered as animal waste.