Analysis of the Quality of Isolated DNA in the Making of Guinea Pig DNA Test Standards

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Abstract
Analysis of the quality of DNA isolated from the manufacture of species DNA standards is one way to determine the quality of independently made species-specific DNA used in testing species DNA detection. This research was conducted to assess the quality of DNA isolated from the production of guinea pig DNA test standards. The benefit of this research is to provide an alternative reference for manufacturing species DNA standards. Moreover, this research is expected to enrich the methods of testing the quality of DNA isolated from different existing methods, using various test matrices. The DNA isolation method was carried out using the Dneasy Mericon Food kit. The quality of the isolated DNA was analyzed using a nanophotometer, measuring the parameters of concentration and purity values at the A260/A280 ratio. The results of DNA isolation indicated a concentration value of the isolated DNA ranging from 230.2 ng/µL to 238.5 ng/µL, with an average concentration value of 233.7 ng/µL. The purity value, read at the A260/A280 ratio, falls within the range of 2.04 – 2.11. Based on the study's results, it was found that the isolated DNA exhibited good DNA quality, making the DNA test standard suitable for use as a benchmark in species DNA testing.

INTRODUCTION
Analysis of the quality of isolated DNA obtained from the manufacturing process of species-specific DNA standards is one way to determine the quality of the independently produced species-specific DNA used in testing for species DNA detection. Various methods can be employed to achieve good quality DNA isolation, such as the centrifuge column isolation method or the magnetic beads isolation method. These extraction methods must, of course, meet the requirements for obtaining high-quality DNA from isolation.

The use of standard DNA tests in species DNA testing is most commonly done by employing organic standard DNA, which consists of DNA sequences designed to detect specific target species. Therefore, there is a need for a method to produce an efficient and cost-effective test by independently isolating the target DNA standard. Research conducted by Sophian & Syukur (2021) shows that good-quality DNA isolation can be achieved from the meat of the target species. During DNA isolation, the main parameters that must be fulfilled are the concentration and purity values of the isolated DNA (Sophian, 2021c; Sophian,
Based on the background above, this research was conducted to examine the quality of DNA isolated during the production of guinea pig DNA test standards. The purpose of this research is to offer an alternative reference for the manufacturing of species-specific DNA standards. The findings from this research are expected to provide valuable insights for similar studies, thus enriching the methods for testing the quality of DNA isolated using different test matrices.

METHOD

Materials

This study used guinea pig meat samples, each weighing 20 mg, for 10 replicates. The extraction kit utilized was Dneasy Mericon Food (Qiagen, 2020), and the reagents and binding solvents used were 96% ethanol and nucleotide-free water.

DNA isolation

Weigh the sample, which weighs 20 mg, and then put it in a 2 mL centrifuge tube. Add 700 µL of lysis buffer and 30 µl of proteinase K. Incubate in a Thermo shaker at 70°C for 60 minutes. After completing the incubation process, centrifuge it at 14,000 rpm for 5 minutes. Pipette 350 µl of the supernatant into a new 2 ml centrifuge tube and add 350 µl of 96% ethanol. Homogenize by vortexing for 10-15 seconds, then transfer 700 µL of the sample suspension into a spin column and centrifuge at 14,000 rpm for 1 minute. Discard the collection tube and transfer the spin column to a new collection tube. Add 600 µl of wash buffer AW2, then centrifuge at 14,000 rpm for 2 minutes. Discard the collection tube and transfer the spin column into a 1.5 mL centrifuge tube. Add 100 µl of NFW elution buffer and centrifuge at 14,000 rpm for 1 minute. Discard the spin column and store the sample in a 1.5 mL centrifuge tube. The next step is to analyze the quality of the isolated DNA by measuring it using a nanophotometer (Sophian et al., 2022).

DNA Quality Analysis

The analysis of DNA quality was conducted using a nanophotometer, with concentration and purity values measured at the A260/A280 wavelengths. The quality of the isolated DNA is well-assessed, provided that the DNA concentration value is greater than 20 ng/µl, and the purity value obtained falls within the range of 1.7-2.1 (Piskata et al., 2017; Sophian, 2021b; Qiagen, 2020; Sophian, 2021a; Sophian, 2021c; Sophian, 2021b).

RESULT AND DISCUSSION

DNA Isolation Results

DNA isolation data from the conducted research are presented in Table 1. From the table, it can be observed that the DNA concentration values fall within the range of 230.2 ng/µL to 238.5 ng/µL, with an average of 233.7 ng/µL, while the purity values range from 2.04 to 2.11, with an average of 2.07.

<table>
<thead>
<tr>
<th>No.</th>
<th>Concentration (ng/µL)</th>
<th>Purity (A260/A280)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>235.5</td>
<td>2.10</td>
</tr>
<tr>
<td>2</td>
<td>230.2</td>
<td>2.06</td>
</tr>
<tr>
<td>3</td>
<td>232.9</td>
<td>2.05</td>
</tr>
<tr>
<td>4</td>
<td>230.4</td>
<td>2.06</td>
</tr>
<tr>
<td>5</td>
<td>231.5</td>
<td>2.04</td>
</tr>
<tr>
<td>6</td>
<td>236.5</td>
<td>2.05</td>
</tr>
<tr>
<td>7</td>
<td>233.3</td>
<td>2.05</td>
</tr>
<tr>
<td>8</td>
<td>238.5</td>
<td>2.11</td>
</tr>
<tr>
<td>9</td>
<td>234.1</td>
<td>2.11</td>
</tr>
<tr>
<td>10</td>
<td>233.7</td>
<td>2.10</td>
</tr>
<tr>
<td>Average</td>
<td>233.7</td>
<td>2.07</td>
</tr>
</tbody>
</table>

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Discussion

In Table 1 above, the purity value of the isolated DNA is above 2, still falling within the range of isolated DNA purity values considered in the good category. These results are consistent with the requirements for DNA quality according to (Armbrecht, 2013; Qiagen, 2020; Ateş Sönmezoğlu & Terzi, 2019; Matlock, 2015). (Armbrecht, 2013; Ateş Sönmezoğlu & Terzi, 2019; Matlock, 2015; Qiagen, 2020) In the research conducted on several processed food products, it showed good DNA quality with a purity value ranging from 1.7 to 2.2 (Sophian, 2021a), as well as in the research conducted by Wulan et al. (2021), which compared several methods of DNA isolation with purity values ranging from 1.4 to 2.2. The isolated DNA concentration value showed a relatively high concentration, with an average value of 233.7 ng/µL.

In the DNA extraction process, the proteinase K enzyme plays an important role, as it works by destroying and digesting proteins. The use of this technique is considered more effective when compared to methods that use chemicals. This is because enzymes work quite effectively by directly targeting amino acid bonds in protein lysis. The proteinase K enzyme is active at a temperature of 65-70°C; thus, in some studies using this method, it is sometimes necessary to optimize the process before applying it (Utaminingsih et al., 2022; Sophian, et al., 2021b; Sophian, et al., 2021a; Sutanta et al., 2021). The purpose of this heating process is to activate the proteinase K enzyme so that it can efficiently perform lysis (Renshaw et al., 2015). The analysis of purity and concentration was performed using a nanophotometer by measuring the absorbance value at wavelength A260/A280. The use of the A260/A280 wavelength is a common method for detecting the concentration and purity of DNA (Sophian, 2021c); Bauer et al., 2004; Wilson, 1997). Matlock (2015) revealed that DNA or RNA is composed of 5 nucleotides, which yield varying absorbance values: guanine (1.15), adenine (4.50), cytosine (1.51), uracil (4.00), and thymine (1.47). The results of the purity analysis are obtained from the average absorbance values of these four or five nucleic acids. This forms the basis for determining the general purity value for DNA analysis in the range of 1.8-2.0. However, for RNA, the range value will be higher than this, as RNA includes uracil as one of its components, which has a higher absorbance value (4.00) compared to thymine found in DNA. Therefore, when averaged, the purity value for RNA will be higher than that of DNA.

CONCLUSION

Based on the results of the study, it was found that the isolated DNA exhibited good quality, indicating that the DNA test standard created can be utilized as a benchmark in species DNA testing. Suggestions for future research include investigating other species so that the findings from subsequent studies can enrich knowledge and serve as valuable sources of information for similar research.

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