Systematic Review: Recent Development of Magnetic Nanoparticles for DNA Extraction

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Abstract

DNA is a research object for various molecular fields of study and DNA extraction is a basic procedure in a molecular biology laboratories. DNA extraction methods are evolving, from several steps using organic solvents to recent technology using magnetic nanoparticles as adsorbent for its purification step. Using magnetic nanoparticles for DNA extraction is magnetizing because it is simple without heavy machinery such as centrifugation and organic solvents. The application of this method is very wide and diverse. This systematic review summarises current developmental trends for some aspects of this DNA extraction system from nanoparticle synthesis method, extraction protocol, and different types of samples used. The main purpose of this review is to give a big picture of magnetic nanoparticle utilization in DNA extraction for the researcher to start their own exploration regarding this safe, simple, and rapid technique.

Keywords: *Magnetic nanoparticle, Nucleic acid, DNA isolation, Product development*

1. Introduction

Deoxyribonucleic Acid or DNA is a strand of polymer that carries genetic material stored in cells. DNA is an object of research for various technological fields, such as molecular diagnostic technologies such as PCR [1], bioinformatics [2] and genetic engineering [3]. The technique of obtaining DNA is an important factor in the development of this technology.

DNA extraction is a method for taking DNA from cells and separating it from other impurities. The principle of DNA extraction is cell lysis and DNA purification. Cell lysis is the stage where the cell is broken down so that the DNA comes out. In this stage, not only DNA comes out but also other molecules mix with the target DNA. Therefore, there is the next stage, DNA purification.

DNA purification techniques are very varied, starting from extraction with organic solvents such as phenol: chloroform extraction, DNA will be extracted in the water phase and impurities in the organic phase [4]; chromatography method using a column, DNA will be

bound to the column and impurities will flush out and then the DNA will be eluted with an appropriate solvent [5]; to modern methods that use adsorption using particles that can bind DNA [6]. One method of DNA adsorption is the use of magnetic nanoparticles.

Magnetic nanoparticles are nano-sized materials that have unique properties that can react to magnetic fields. This property is a typical property of the transition metal group, one of which is Fe or iron. Iron was chosen as one of the materials for making magnetic nanoparticles because of its non-toxic and biocompatible properties [7]. Apart from that, it is also very abundant in nature, in the form of iron oxide [8]. Magnetite or Fe_3O_4 is a form of iron oxide that exists in nature. Magnetite has the strongest magnetic properties among all other iron oxides [9].

The use and development of magnetic nanoparticles for DNA extraction is quite common. This review will collect information on the fabrication of magnetic nanoparticles that have been carried out and the DNA extraction protocol. We hope that this review can be used to broaden the horizon of knowledge regarding the use of magnetic nanoparticles in the DNA extraction process and can become a basis for further development and innovation. This review can also be a starting point for readers to start their in-house DNA extraction using magnetic nanoparticles.

2. Method

The search method uses Boolean operators and quotation marks to determine specific keywords. The keywords used “magnetic nanoparticles” AND “DNA extraction” OR “DNA isolation” were entered into the entry fields in four databases including JSTOR (https://www.jstor.org/); DOAJ (https://doaj.org/); Mendeley (https://www.mendeley.com/search/); and Scopus (https://www.scopus.com/search/form.uri?display=basic#basic). The article selection process is displayed in the PRISMA flow diagram to make it easier to observe the selection process at each stage so that the articles that will be reviewed are selected. Articles included in the inclusion are articles that contain how to make nanoparticles and their use in DNA extraction.

Search results with the keywords "magnetic nanoparticle" AND "DNA extraction" OR "DNA isolation" on JSTOR, DOAJ, Mendeley, and Scopus, display 1, 190, 10, and 166 articles respectively. Next, initial screening was carried out by reading the titles and abstracts of articles that discussed DNA isolation or extraction using magnetic nanoparticles, and then the articles were collected. Apart from screening the title and abstract, this stage also eliminates articles whose full text is not available. Obtained from the JSTOR, DOAJ, Mendeley, and Scopus databases respectively, leaving 1, 1, 8, and 62 articles.

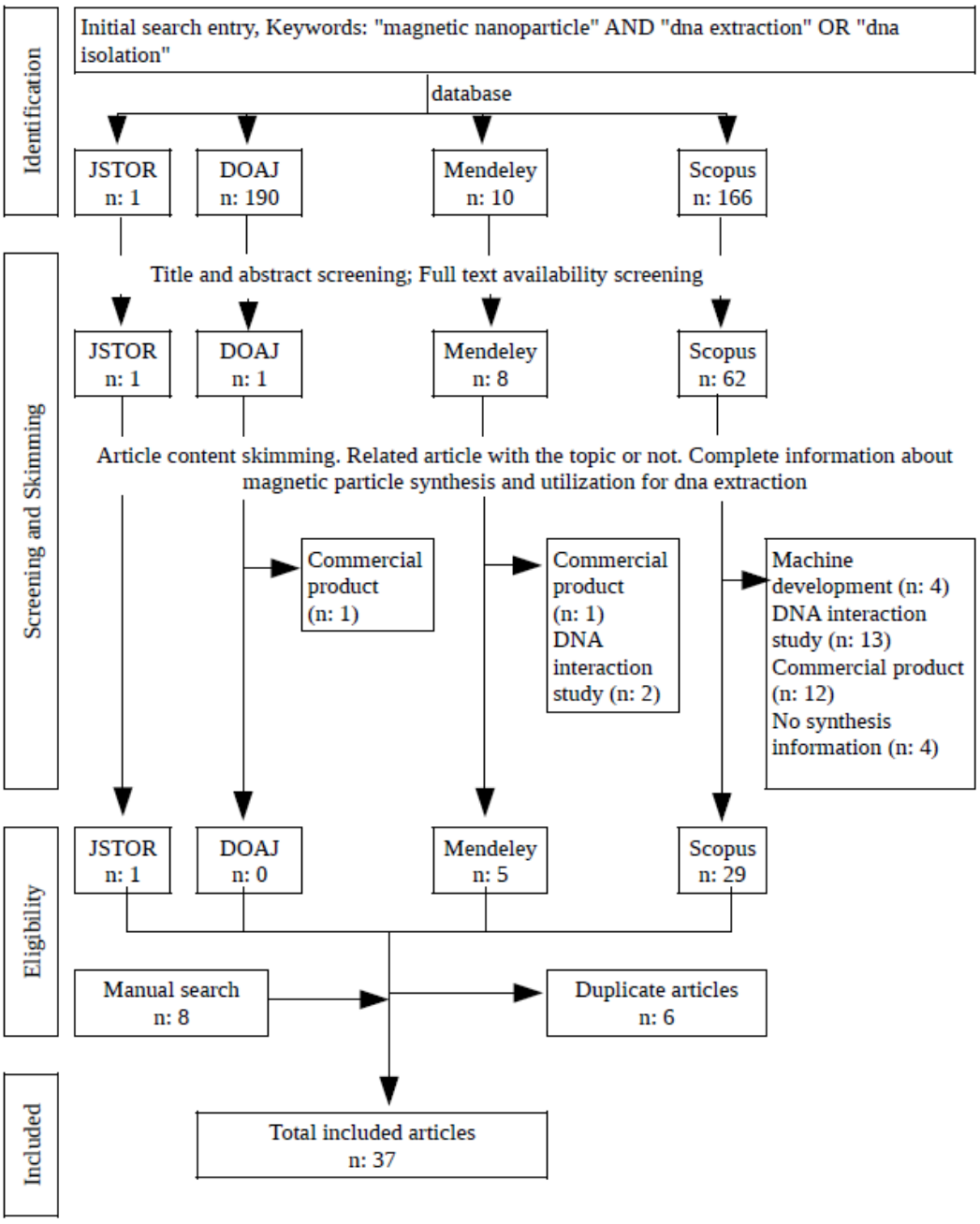
Then, the article skimming process is carried out to see the contents of the article at a glance. Articles that are eliminated from this stage are articles that do not explain how to make magnets and/or use magnets for DNA extraction.

In the DOAJ database, all articles were eliminated from the previous stage because DNA extraction was carried out using commercial magnetic nanoparticles, for the same reason one article was also eliminated from the Mendeley database and 12 articles from the Scopus database. There were two and 12 articles from Mendeley and Scopus that were eliminated because the studies were conducted using commercial DNA or DNA extracted using other

methods (not using magnetic nanoparticles) to conduct DNA interactions study. In the Scopus database, four articles were eliminated because they discussed the development of automated DNA extraction machines with magnetic nanoparticles, and four articles were eliminated because they did not include the magnetic nanoparticle synthesis process.

Next, the selection is carried out using the Mendeley reference manager to mark duplicate articles. There are eight additional articles from manual searches using Google Scholar regarding the use of magnetic nanoparticles for DNA extraction. In total, 37 articles will be reviewed.

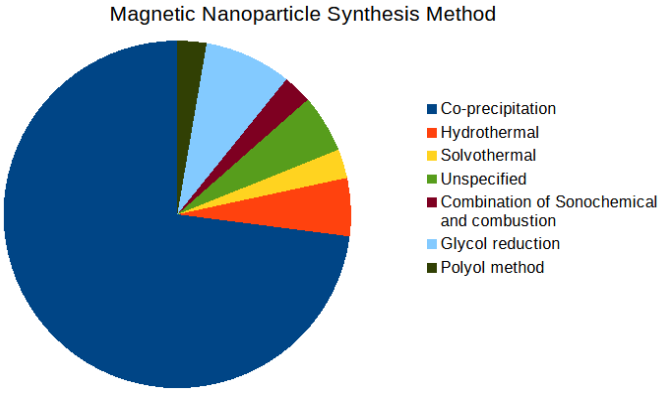
3. PRISMA Statement



4. Magnetic Nanoparticle Synthesis

Co-precipitation is the most common method used in making magnetic nanoparticles for DNA extraction. This is shown in Figure 1 (A). Co-precipitation is a very simple method. This method can be carried out at room temperature and does not produce toxic byproducts. The advantage of this method is that, with fixed parameters, the resulting product will always have similar quality. In addition, this method is very easy to scale up [10,11].

In producing magnetic nanoparticles, the precursors used are transition metals. For iron oxide, the precursor is Fe. In Figure 1 (B) it is shown that the precursors that are widely used are $FeCl_2$: $FeCl_3$ and $FeSO_4$: $FeCl_3$. It does not rule out the possibility of using other Fe salts such as $Fe(acac)_3$ [12]; $Fe(NO_3)_2$ [13]; $(NH_4)_2Fe(SO)_4)_2$; and $NH_4Fe(SO_4)_2$ [14,15]. The commonly used Fe^{2+} and Fe^{3+} ratio is 1:2, this is related to the amount of impurities or byproducts produced [16].



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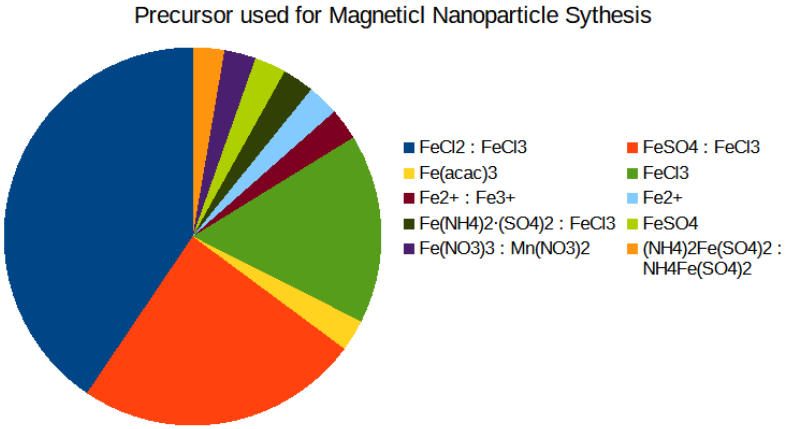

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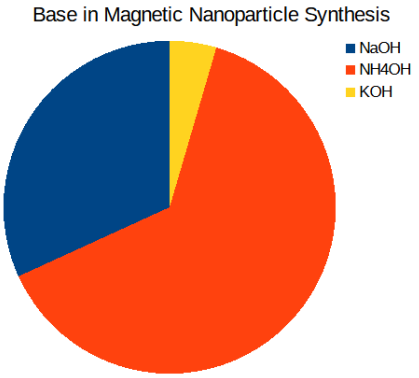
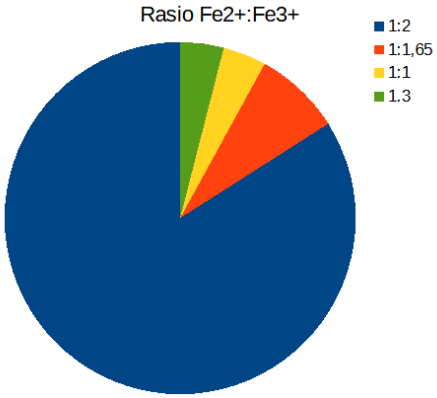


C.



D.

Figure 1. Recapitulation of Methods for Making Magnetic Nanoparticles Used for DNA Extraction. (A) Kind of Methods (B) Kind of Precursor (C) Kind of Base (D) Fe^{2+} and Fe^{3+} ratio used in co-precipitation

Iron oxide synthesis requires alkaline conditions, optimal at pH > 8 [17]. The base used is quite uniform. As seen in Figure 1 (C) there are 3 types of bases used, NaOH; NH_4OH, and KOH. According to research by Mascolo et al [18], the effect of the difference in base is the size of the nanoparticles produced. This could be related to the nature of the base. Strong base or weak base.

For their use in DNA extraction, magnetic nanoparticles can be modified or not. This can be seen in Figure 2. The most frequently used coating agent is tetraorthosilicate (TEOS). Magnetic nanoparticles are coated with the aim of protecting them from agglomeration [19,20]. Apart from that, it also protects it from oxidation [21]. However, the main goal is for magnetic nanoparticles to specifically bind DNA and separate it from impurities during DNA isolation [22].

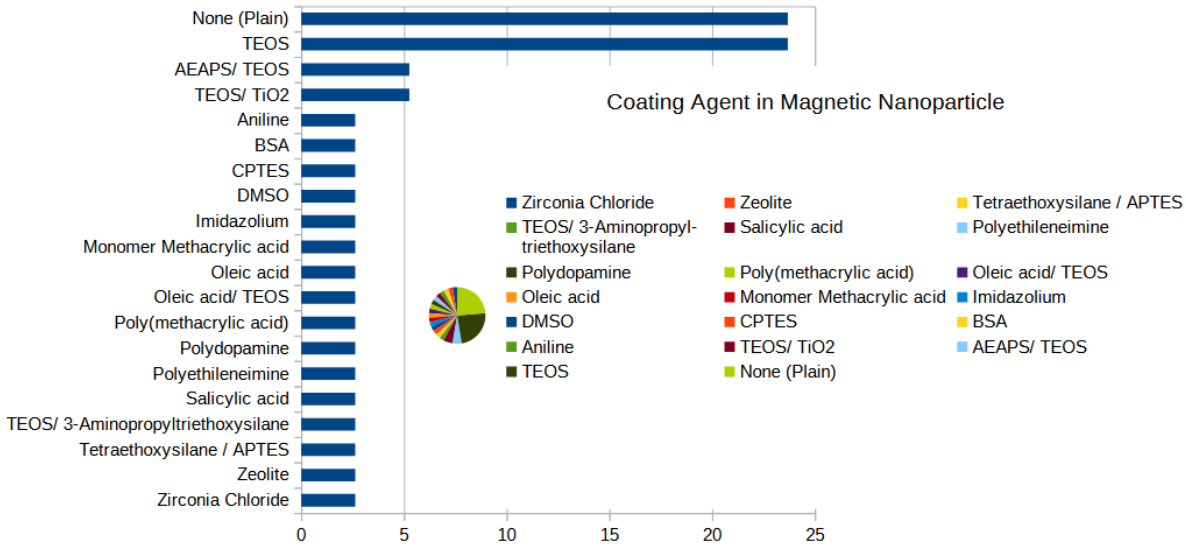


Figure 2. Coating Agent Used in Magnetic Nanoparticle Fabrication for DNA Extraction

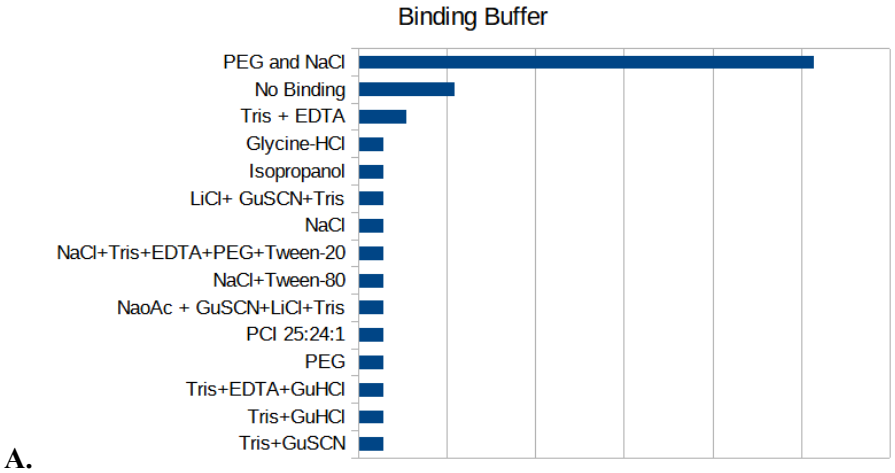
5. DNA Extraction

DNA extraction is divided into two steps. The first step, lysis, is the stage of breaking down cells. This step aims to make DNA come out of the cells or matrix that confines it. The second step is purification. This stage aims to purify DNA from impurities in the lysate mixture from the first step. In general, the DNA purification step with magnetic nanoparticles is divided into three. Binding, washing, and elution. These three steps are important because they relate to the interactions of DNA, impurities, and magnetic nanoparticles. ingredients that are often or commonly used in DNA extraction using magnetic nanoparticles can be seen in Figure 3.

Binding buffer is expected to provide conditions for DNA and magnetic nanoparticle materials to bind. From Figure 3 (A), it can be seen that generally, a combination of PEG and NaCl is used for the binding stage. This is possible because of the nature of PEG and NaCl which will cause DNA to precipitate and be adsorbed on the magnetic material. Other ingredients used will also have more or less the same purpose, such as isopropanol. Other impurity particles remain in the supernatant to be removed.

Washing buffer is used to dissolve impurities that are bound to the magnetic nanoparticles. The washing buffer that is often used is 70% ethanol, as seen in Figure 3 (B). Ethanol is used for impurity elimination, the same as the pellet washing stage in conventional DNA extraction methods [23]. The majority of other ingredients in the washing buffer are alcohol with some additional salt. This is unique because the function of 70% ethanol is to dissolve impurity salts and minimize DNA solubility, but here, these salts are instead added to the washing buffer.

Elution buffer is used to dissolve DNA that is still bound to the magnetic nanoparticles. The solution that is often used as an elution buffer is Tris EDTA. This can be seen in Figure 3 (C). The materials used as elution buffers are materials that can dissolve DNA. The nature of DNA is polar, so the solutions for elution are selected that are polar. The addition of salt, such as NaCl or potassium acetate is aimed to maintain the stability of the eluted DNA.



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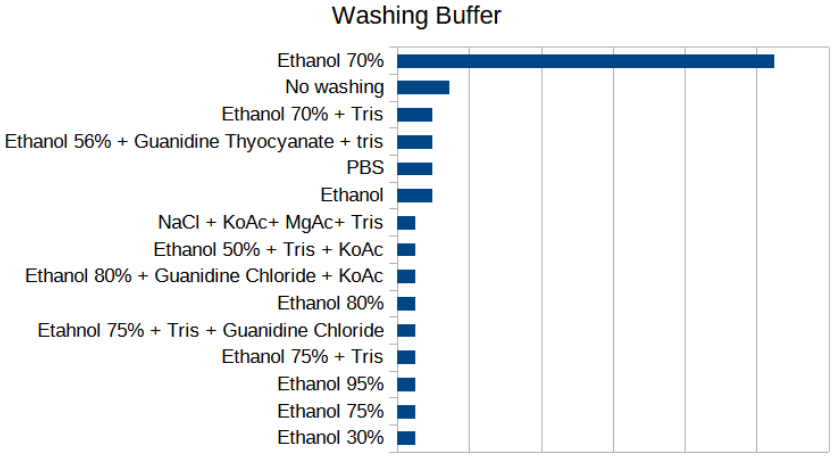

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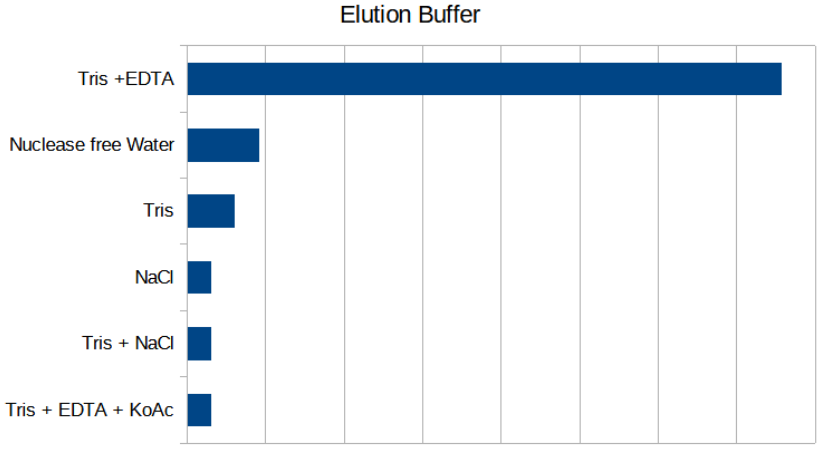


C.

Figure 3. Regular Reagent used in DNA Extraction Using Magnetic Nanoparticle. (A) Binding Buffer; (B) Washing Buffer; (C) Elution Buffer.

The types of samples used in extraction vary greatly. As seen in Table 1. sample types range from human specimens, animals, plants, bacteria, environmental samples, and food. Further development can be carried out for other types of species or other human specimens,

such as urine and sputum. These various sample types can be integrated into the next process of DNA research, this is discussed further in the next point, about DNA quality.

Table 1. Various Kind of Sample That Can Be Processed by Magnetic Nanoparticle DNA Extraction

Samples	Source	Samples	Source
Human specimen		**Animal**	
Saliva	[24,25]	Animal tissue	[49]
Blood	[12,25-31]	animal blood	[45]
Serum	[28]	Cattle	[43]
FFPE tissue	[32]	**Plant**	
Tissue	[31]	Cottonseed	[48]
Bacteria		Clover	[49]
Plasmid	[13,26,33-39,56]	Daisy	[49]
E. coli	[13,28,40-43,50]	Ryegrass	[49]
L. monocytogenes	[44]	Leaf (*A. littoralis*, Rice)	[43]
S. enteriditis	[44]	Petal *R. hybrida*	[43]
Arthrospira platensis	[45]	**Environment**	
P. viridiflava	[43]	Soil	[51]
S. aureus	[43]	Pond water	[52]
B. subtilis	[43]	**Food**	
X. campestris	[43]	Milk	[44,48,52]
O. anthropi	[46]	Juice	[52]
Fungi		**Any other Sample**	
R. oryzae	[14]	Virus	[28,53]
Aspergillus niger	[47]	Gel electrophoresis	[54]
Yeast	[28]	Cell culture	[49]
Fungus	[48]	HeLa cell	[55]
		Synthetic	[49]

6. DNA Quality

The quality of DNA extraction can be seen from the yield, purity, and downstream process. The yield value shows the amount of DNA that can be extracted. Purity is indicated by the absorbance value at a wavelength of 260 nm divided by absorbance at 280 nm. The third quality can be seen from the exploitation of DNA in advanced processes. The results of DNA extraction using magnetic nanoparticles can be seen in Table 2. The results of DNA extraction are quite varied. From a range of 0.03 µg to over 100 µg. This may be affected by many factors. From the type of sample, availability of the sample, and the total of cells extracted.

The purity of the extracted DNA can be seen in Figure 4. The purity ratio is categorized by a group, plant, animal, human, plasmid, bacteria, and any other sample. The purity range varies from 0.8 – 2.6 (in bacterial samples). DNA extract is said to be pure if it has a ratio in the range of 1.8. A value below 1.8 indicates that the isolate contains a lot of impurities in the form of protein. If the ratio gives a value above 2, it indicates that the DNA isolated may still

be contaminated by RNA [57]. This data may be further analyzed using meta-analysis to determine what factors influence the purity of the DNA extract.

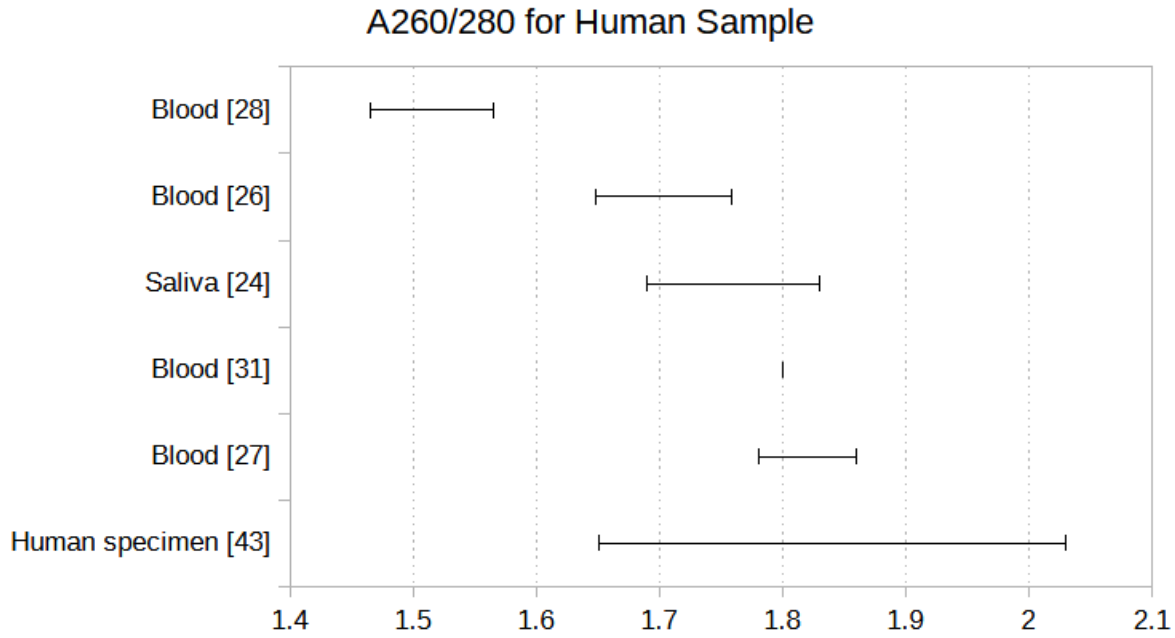
Table 2. DNA Yield for Various Sample

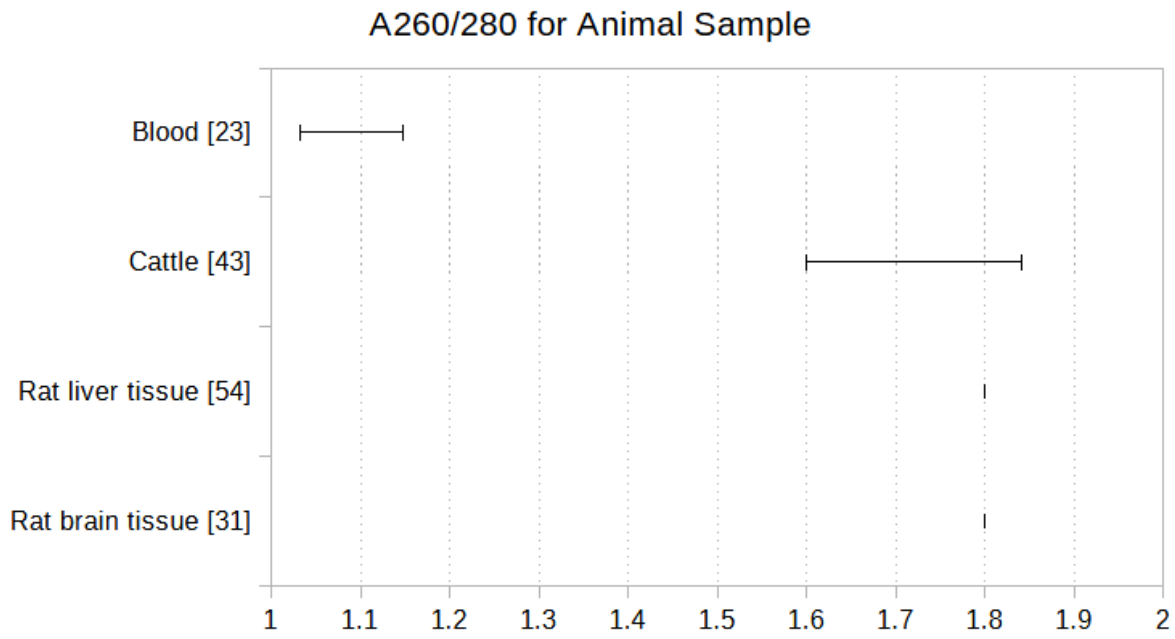
Study	Yield (µg)	Study	Yield (µg)
Animal sample		**Bacteria sample**	
Rat liver tissue [54]	1.8 ± 0	*X. campestris* [43]	0.58 ± 0.01
Cattle [43]	1.8 ± 0.14	*S. aureus* [43]	0.6 ± 0.01
Rat brain tissue [31]	2 ± 0	*P. viridiflava* [43]	0.62 ± 0.13
Blood [23]	3 ± 0.91	*E. coli* [43]	0.71 ± 0.11
Plant samples		*B. subtilis* [43]	0.73 ± 0.11
Plant sample [49]	0.03 ± 0.01	*E. coli* [28]	1.75 ± 0.08
Leaf of Rice (Nemat cultivars) [43]	2.37 ± 0.1	*A. platensis* [45]	2.38 ± 0.039
Aeluropus littoralis [43]	2.44 ± 0.12	*E. coli* [42]	3.27 ± 1.23
Petals of Rosa hybrid [43]	2.5 ± 0.16	Bacteria sample [49]	6.17 ± 1.75
Human specimen		Bacteria sample [41]	7.29 ± 2.98
Blood [31]	1.2 ± 0	*E. coli* [50]	19 ± 1.41
Blood [28]	1.31 ± 0.07	**Any other samples**	
Blood [26]	1.57 ± 0.37	Environment/ Soil [51]	0.49 ± 0
Human specimen [43]	1.95 ± 0.19	Virus [28]	0.69 ± 0.07
Saliva [24]	8.2 ± 2.71	Yeast [28]	0.95 ± 0.01
Blood [27]	62.93 ± 4.46	Gel Electrophoresis [54]	10 ± 0
Plasmid		Environment/ water [52]	16.5 ± 2.12
Plasmid [35]	6.9 ± 0	FFPE tissue [32]	17.5 ± 10.61
Plasmid [56]	8.57 ± 0.13	Food [52]	19 ± 1.41
Plasmid [33]	10.9 ± 0.49	Cell/ HeLa cell [55]	116.63 ± 45.36
Plasmid [38]	11.69 ± 6.67		
Plasmid [49]	14.92 ± 5.76		
Plasmid [26]	76.1 ± 32.2		
Plasmid [13]	91.1 ± 4.08		

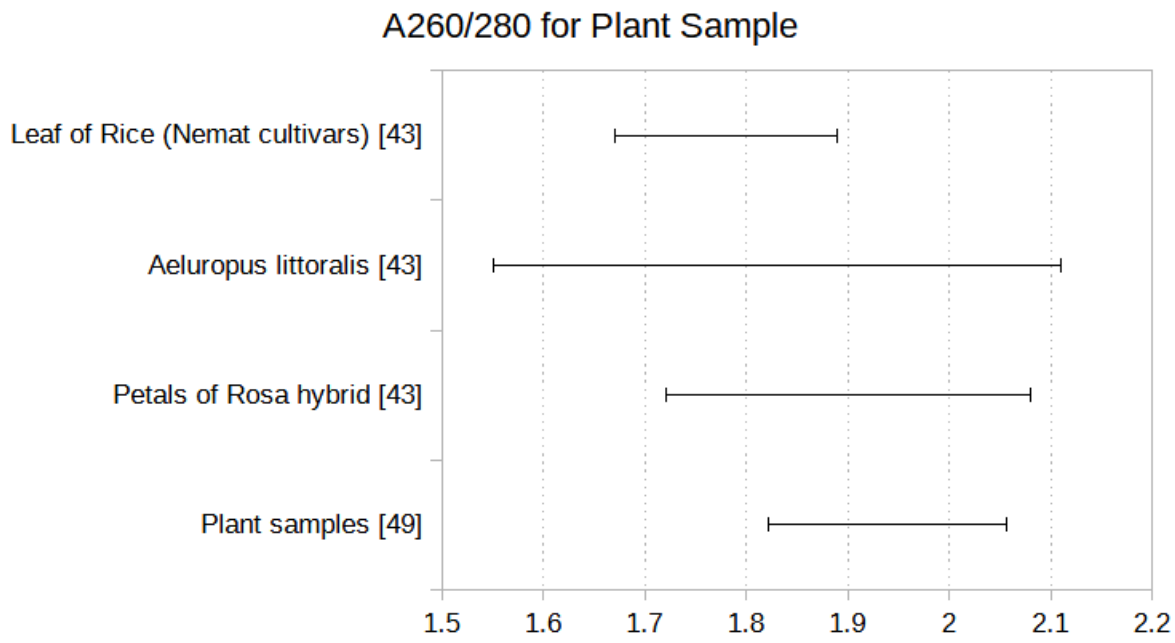
Table 3. Downstream Process Applying Extracted DNA Using Magnetic Nanoparticle

Downstream Process	Source
Electrophoresis	[24,28,29,33,31,38,39,42,45,47,50,51,54]
PCR (conventional, Multiplex, qPCR)	[12,14,24-28,30-32,34-35,38,40,44,47,49,50-53,55]
Restriction Analysis	[14,33,37,50,56]
Reverse Transcriptase	[43]
Sequencing	[25,46,49]
Cloning and Expression	[37,49]
Bisulphite conversion	[49]
Mass Spectrophotometry	[49]

The third quality is seen from the availability of the extracted DNA to be used in advanced stages of research. This list is shown in Table 3. Processes that exploit this DNA are very diverse, ranging from electrophoresis to mass spectrophotometry.






A260/280 for Plant Sample
Leaf of Rice (Nemat cultivars) [43]
Aeluropus littoralis [43]
Petals of Rosa hybrid [43]
Plant samples [49]
1.5
1.6
1.7
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1.9
2
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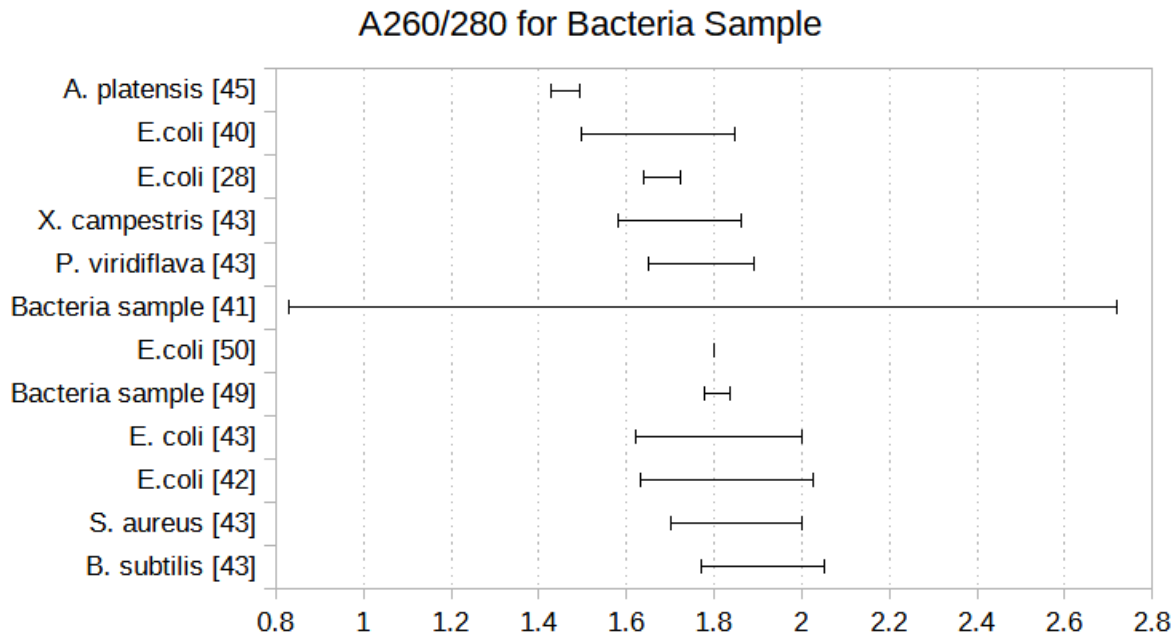
A260/280 for Bacteria Sample
A. platensis [45]
E.coli [40]
E.coli [28]
X. campestris [43]
P. viridiflava [43]
Bacteria sample [41]
E.coli [50]
Bacteria sample [49]
E. coli [43]
E.coli [42]
S. aureus [43]
B. subtilis [43]
0.8
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2.8

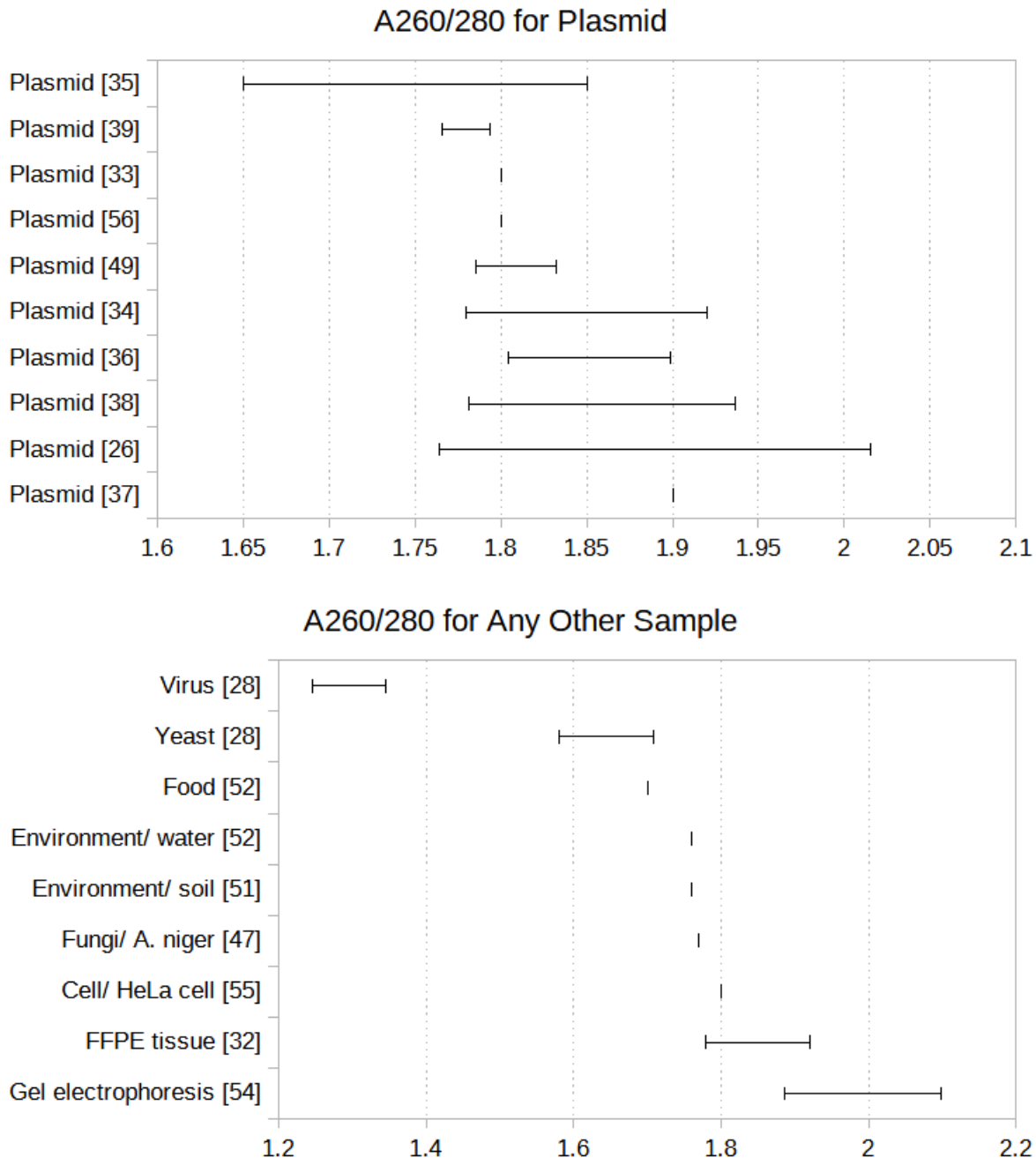

Figure 4. Forest Plot for A260/280 for Different Kind of Sample, Such as Human, Animal, Plant, Bacteria, Plasmid, and Any Other Sample

7. Conclusion

The adoption of magnetic nanoparticles as adsorbents for DNA extraction has become very common for various types of samples. From human, animal, plant, and bacterial samples. For diverse purposes such as health, environment, and food. The DNA results from the extraction can also be widely used for numerous molecular biology laboratory processes such as PCR,

cloning expression, and sequencing. Hopefully, this review can give a big picture as a starting point to carry out product development or further applications using magnetic nanoparticles for DNA extraction.

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