DNA Extraction: Comparing DNA Using DNase, RNase & Electrophoresis

NAOKO KOSAKA, YOSHISUKE KUMANO

ABSTRACT
The Next Generation Science Standards (NGSS) were proposed in 2013 in the United States for K–12 learning to emphasize the importance of science and engineering practices in the three dimensions of science learning. In this study, we show that DNA extraction experiments, which are familiar in the United States, can be used not only as demonstration experiments but also as educational material for science practices. We could visualize extracted DNA using an enzyme treatment with DNase and RNase and agarose gel electrophoresis. We conducted a science practice in which a DNA extraction experiment was used as an extracurricular club activity in Japan. The students made predictions, conducted experiments, obtained results, and reflected on their findings. Although there are still points needing improvement, our results indicate that the DNA extraction experiment can be used as a new teaching material for NGSS science practices.

Key Words: DNA extraction; science practices; inquiry activity; NGSS; experimental material.

INTRODUCTION

It is expected that science and technology will become more sophisticated and incorporated into all industries and social life, and that the state of society will change dramatically. In the United States, the Next Generation Science Standards (NGSS) were proposed in 2013 for K–12 learning to emphasize the importance of science and engineering practices in the three dimensions of science learning (NGSS Lead States, 2013). In the United States, there is a need for improved inquiry activities, practices, that deepen student learning.

This research focuses on DNA extraction experiments and examines ways to use DNA extraction experiments for science practices rather than just for cooking labs or student enjoyment. DNA extraction experiments have been used in schools around the world for a long time (Madden, 2006). Students can extract and visualize DNA from familiar ingredients, resulting in an enjoyable experience. However, the experiments visualize DNA only at the beginning of the lesson and do not use visualizations for the science practices; in one study, one-third of the students reported that they couldn't believe the extract to be DNA after the experiment (Yatsu & Yamanoi, 2016). In the present study, the students not only obtained the extract from familiar ingredients but also performed enzymatic treatment and electrophoresis with the extract. We thought this would allow students to visualize and compare the amounts of DNA in the extracts. The project results represent new experimental teaching material for the NGSS's science practices in the United States.

PURPOSE OF THE STUDY

The purpose of this research was twofold. The first was to determine whether the extract obtained by the DNA extraction experiment could be enzymatically treated and electrophoresed to visualize the DNA. The second was to find out if students can engage in inquiry activities using established techniques and to share the method.

DEVELOPMENT OF TEACHING MATERIAL

Purpose
During electrophoresis, RNA, which shares a similar chemical structure with DNA, was stained. To confirm that DNA was present in the extract, we used DNase (deoxyribonuclease), RNase (ribonucleases), and electrophoresis.

Materials and Methods
DNA extraction method. First, 6% NaCl and 10% neutral detergent were dissolved in water. We called this solution the DNA extracting solution. Then, 20 g of the ingredient (broccoli or banana) was weighed and crushed in a mortar, and approximately 30 mL of the DNA extracting solution was poured into the mortar. After gentle mixing, we allowed the mixture to stand for 10 minutes.
mixture was then filtered with a quadruple layer of gauze. Finally, we poured cooled 100% ethanol onto the obtained filtrate.

**DNA confirmation method.** All extracts precipitated in ethanol were collected in 1.5 ml tubes (e.g., Eppendorf tubes from Sigma-Aldrich) using tweezers. A tube containing extract was filled with 100% ethanol and centrifuged at 15,000 rpm for 10 minutes (e.g., with a Microfuge 20/20 R from Beckman Coulter). Following this, the supernatant was discarded, and the tube was filled with 70% ethanol and centrifuged again under the same conditions. The supernatant was again discarded, and the tubes were held upside down with the lid open for one day to completely dry. We call the method of purifying DNA using ethanol in this way “ethanol precipitation.”

The products were suspended in 100 μL of a 10 mM Tris-HCl solution (pH 8.0) (e.g., from Nippon Gene or Thermo Fisher Scientific), referred to as an extract. To 10 μL of the extract, 1 μL of DNase or RNase was added and allowed to react at 37°C for one hour (which we refer to as the enzyme treatment). They were put together in a new 1.5 ml tube and left still in the incubator. The DNase used was DNase II from Sigma-Aldrich, and the RNase used was RNase A from the same company. After enzyme treatment, 1 μL of agarose gel loading buffer (e.g., from Sigma-Aldrich or Nippon Gene) was added to each sample. For electrophoresis, 1% agarose gel was made. TAE buffer and agarose (e.g., both from Sigma-Aldrich or Nippon Gene) were used for making it. To make a 100 mL gel, 1 g of agarose was dissolved in 100 mL of 1×TAE buffer, and heated by microwave until completely dissolved. After cooling to under 50°C, the gel was spread on a gel-maker set (e.g., from Mupid). The cooled and solidified agarose gel was placed in an electrophoresis tank (e.g., from Mupid) and filled with TAE buffer, and then the samples were injected into each well of the gel. Electrophoresis was performed at 100 V for 20 minutes. The DNA size marker used was 1 kb Plus DNA Ladder from Thermo Fisher Scientific. After electrophoresis, GelRed from Cosmo Bio was used for DNA staining, and a UV transilluminator (e.g., from AS ONE or Biocraft) was used for visualization. The agarose gel after electrophoresis was submerged in water in which GelRed was dissolved, and the mixture was shaken slowly for about 10 minutes. After that, the picture was taken using the UV transilluminator.

**Results & Discussion**

As shown in Figure 1A, a white suspension was obtained from the DNA extraction experiment with broccoli. Agarose gel electrophoresis was performed after enzyme treatment with DNase and RNase. A bright band was observed in the extract obtained from broccoli (Figure 2, lane 2). This band was not lost during RNase treatment (Figure 2, lane 4), but it disappeared after treatment with DNase (Figure 2, lane 3). This indicates that the extract from broccoli contained DNA. A band considered to be DNA is indicated by an arrow (←) in Figure 2.

Although a white suspension was obtained from the banana sample, as shown in Figure 1B, electrophoresis of the banana extract did not give bands that could be considered DNA (see Figure 3). A prior study that applied a method using the UV absorption spectrum concluded that extracts from broccoli contained DNA but extracts from banana did not (Baba et al. 2013). Our experimental results are consistent with those of the prior report. The extract from banana contained almost no DNA, and it is considered that most of the extract was made up of substances other

**Figure 1.** White suspension in (A) broccoli and (B) banana extracts.

**Figure 2.** Enzyme treatment and electrophoresis. Extract from broccoli.

The samples contained in each lane were as follows:
1. DNA size marker
2. Electrophoresis without enzyme treatment
3. Sample 2 treated with DNase
4. Sample 2 treated with RNase

**Figure 3.** Enzyme treatment and electrophoresis. Extract from banana.

The samples contained in each lane were as follows:
1. Electrophoresis without enzyme treatment
2. Sample 1 treated with DNase
3. Sample 1 treated with RNase
than nucleic acid, such as sugar. We think ingredients that we could not extract DNA from—probably ingredients with low cell density—had small numbers of cells even if the same amount of ingredient was used. For this reason, only a small amount of DNA could be obtained from banana, and it could not be confirmed. Our method of using enzymes and electrophoresis made it possible to compare the amounts of extracted DNA, making this experiment a promising option for science practices as teaching material, which was our first purpose.

### Practical Inquiry Activities

**Position & Goal of Teaching Material**

The aim of this educational material is to “engage in the science practices using DNA extraction experiments and develop the ability and attitude to scientifically explore.” We believe that this activity can be closely related to the NGSS’s practice 4: analyzing and interpreting data. Students can obtain data on the amount of DNA from the electrophoresis results, think about the reasons independently, and interpret the results.

**Sample of Activity Steps**

We propose using this activity involving DNA extraction experiments, especially in biology classes for grades 9–12. As shown in textbooks, students in these grades learn about biotechnology by associating NGSS’s HS-ETS1 (Engineering Design) and HS-LS3 (Heredity: Inheritance and Variation of Traits) (Miller & Levine, 2018; Nowicki, 2016). This activity works effectively when learning these contents. This activity should be conducted after going through the biotechnology unit and after conducting at least one DNA extraction experiment. Students can use the same methods as described in our Development of Teaching Material section and can use worksheets to engage in this activity (see the worksheet provided in the Supplemental Material available with the online version of this article).

1. **Setting of research theme and planning of experiments.** Students are free to choose familiar ingredients for their own DNA extraction experiment; they think about their expectations for when the DNA is extracted, and they establish experimental plans. This activity is related to the NGSS’s practice 1: asking questions; 2: developing and using models (if you treat the DNA extraction experiment as one model); and 3: planning investigations.

2. **DNA extraction experiment.** Students conduct DNA extraction experiments with their ingredients. They can compare the amount of extract that may contain DNA.

3. **Ethanol precipitation and enzyme treatment.**

The extracts obtained in the second step are precipitated with ethanol, and enzyme treatments are performed. As it takes one day to dry the DNA, this step should be divided into two lessons. Through this step, students can purify the DNA in the extract and determine the amount of DNA by distinguishing it from RNA.

4. **DNA electrophoresis.** Agarose gel electrophoresis is performed. If you don’t have tools for electrophoresis at your school, partnering with a local university may be a solution.

5. **Analysis and discussion of results.** By using the result of electrophoresis, students can determine the amount of DNA in their extracts. They can conclude how accurate their expectation was and discuss the results. This step is related to the NGSS’s practice 4: analyzing and interpreting data.

**Sample of a Student’s Activity**

We show here a sample case from Japan. Students engaged this activity after a “Genes and Their Functions” unit of their high school basic biology class (Ministry of Education, Culture, Sports, Science and Technology (2009)).

**Research theme.** The student compared amounts of extracted DNA from green kiwi and gold kiwi. She chose kiwis for her inquiry theme because she had often eaten them for breakfast.

**Predictions.** The student predicted that after extracting DNA from the same amount of green kiwi and gold kiwi, more DNA would be obtained from the green kiwi than from the gold kiwi.

**Prediction rationale.** The student conducted literature research and found that green kiwi and gold kiwi have different contents of actinidine, a protease, and that green kiwi contains more actinidine than gold kiwi does. In addition, it was reported that proteolysis contributes to the improvement of extraction efficiency for DNA experiments.

**Results.** She got two results.

1. Extracts were obtained from both green and gold kiwis, but there was no visual difference in the amount of extract. As shown in Figure 4 (indicated by the arrow), the extract appeared as a transparent object near the liquid’s surface.

2. According to the result of agarose gel electrophoresis, it showed that the green kiwi extract contained more DNA than the extract from the gold kiwi did (see Figure 5).

**Student considerations.** As shown in Figure 5, any band with more than 12,000 DNase-eliminated base pairs was DNA (indicated by an arrow). Band 1 is clearly brighter than band 2, and band 5 is clearly brighter than band 6. This indicates that DNA can be extracted from gold kiwi, but more DNA can be recovered from green kiwi. This is because green kiwi contains more of the proteolytic enzyme actinidine, likely because of the contribution of the kiwi proteinase to DNA extraction.

![Figure 4. Extracts from (A) green kiwi and (B) gold kiwi.](image-url)
Summary & Recommendations for Implementation

The purpose of this study was to introduce DNA extraction experiments as teaching material for science practices. Using DNase, RNase, and agarose gel electrophoresis, we successfully determined the amount of DNA extracted from the experimental material. In the DNA extraction experiments that are often performed in the classroom, DNA may not be obtained from some ingredients even if the same extraction method is used. Enzyme treatment and electrophoresis methods have also confirmed this by using broccoli and bananas. We demonstrated that DNA can be extracted from cauliflower, onions, and kiwis, in addition to broccoli. We also confirmed that DNA cannot be extracted from strawberries, tomatoes, pineapples, or leeks. Teachers must pay attention to type of ingredients when allowing students to choose which ones to study. In addition, if the experiment is to be performed as a demonstration and the extract is to be claimed to be DNA, then ingredients that can be said with certainty to include DNA in its extract, such as broccoli or onion, should be used.

During practical extracurricular activities, students were free to come up with ideas, research themes, and predictions. After the experiments, the students obtained their results on the amount of DNA extracted from the ingredients, and then they could analyze and consider their data. This activity especially corresponded with NGSS’s practice 4: analyzing and interpreting data.

As reference for implementation, we discuss here the cost of reagents and equipment (in U.S. dollars). Each 1.5 mL tube cost us about $0.01 and was sold as 200 or more tubes. The lowest price for a centrifuge was around $1,350 (e.g., Microspin 12 from Bio-Sun). As the reagent to be used after ethanol precipitation, DNase could be purchased for $192 and RNase for $56. DNase could cover about 300,000 samples and cost $0.064 per enzyme treatment (per sample). RNase was much cheaper. If you would like to make an electrophoresis tank, an easy method is reported by Ens and colleagues (2012).

The methods of DNA purification with ethanol and electrophoresis in this study can be simplified to make it easier to do in schools. Although there is room for improvement, it became clear that the use of enzyme treatment and electrophoresis in DNA extraction experiments allows students to learn exploratively and that this activity can serve as science practices.

Acknowledgments

This research received extensive support from Hiroyuki Araki, a professor at the National Institute of Genetics. In addition, Sachiko Sakamoto of the National Institute of Genetics provided experimental guidance. We would like to thank everyone at Kato Gakuen Gyoshu Junior and Senior High School for their cooperation in this study. We received valuable advice from Mr. Varun Ravindran and Masaharu Takemura, a professor at Tokyo University of Science, in writing this article. Associate Professor Subha R. Das of Carnegie Mellon University and DNAZone allowed us to participate in their outreach efforts and informed us about the status of DNA extraction experiments in the United States. We also thank all of you.

Other Student Research Themes

Other themes that students considered include:

- Differences in the amount of DNA extracted with or without kiwi seeds
- Differences in DNA extraction between the kiwi’s inside and outside
- The relationship between broccoli freshness and the amount of DNA
- Differences between cell size and the DNA extraction amount
- Examination of extraction conditions to increase DNA extraction efficiency
- Differences between cheese types and amounts of DNA

Students’ Attitudes

Based on the students’ responses, it can be stated that they enjoyed working with DNA extraction inquiry activities. They were able to think deeply, engage in extensive discussion, and come up with inferences or reasoning independently. They were able to try out various experiments. Even before these activities, they did not have the opportunity to plan experiments or lacked the skills to engage in such design. They were always waiting for the teachers’ advice or suggestions. Therefore, the new setup represented a big change. We were able to increase their motivation to learn more about the detailed nature of science. It is thought that this change occurred because the DNA extraction experiment was used not only as a demonstration experiment for fun but also as an inquiry activity for students to think scientifically. From these results we can say that students were able to engage in inquiry activities using established techniques, which was our second purpose.

Figure 5. Extracts from green kiwi and gold kiwi after enzyme treatment and electrophoresis. Lanes 1, 3, and 5 are extracts from green kiwi, and lanes 2, 4, and 6 are extracts from gold kiwi.

The sample contained in each lane was as follows:
1. Electrophoresed extract
2. Sample 1 treated with DNase
3. Sample 1 treated with RNase
4. Sample 2 treated with DNase
5. Sample 1 treated with RNase
6. Sample 2 treated with RNase

Other Student Research Themes

Other themes that students considered include:

- Differences in the amount of DNA extracted with or without kiwi seeds
- Differences in DNA extraction between the kiwi’s inside and outside
- The relationship between broccoli freshness and the amount of DNA
- Differences between cell size and the DNA extraction amount
- Examination of extraction conditions to increase DNA extraction efficiency
- Differences between cheese types and amounts of DNA

Students’ Attitudes

Based on the students’ responses, it can be stated that they enjoyed working with DNA extraction inquiry activities. They were able to think deeply, engage in extensive discussion, and come up with inferences or reasoning independently. They were able to try out various experiments. Even before these activities, they did not have the opportunity to plan experiments or lacked the skills to engage in such design. They were always waiting for the teachers’ advice or suggestions. Therefore, the new setup represented a big change. We were able to increase their motivation to learn more about the detailed nature of science. It is thought that this change occurred because the DNA extraction experiment was used not only as a demonstration experiment for fun but also as an inquiry activity for students to think scientifically. From these results we can say that students were able to engage in inquiry activities using established techniques, which was our second purpose.
References

Baba, N., Katayama, T., Kozai, T. & Yonezawa, Y. (2013). A revision of the laboratory work on extraction of DNA at lower secondary school level. Japanese with English abstract. *Japanese Journal of Biological Education, 53*(4), 168–75.

Ens, S., Olson, A.B., Dudley, C., Ross III, N.D., Siddiqi, A.A., Umoh K.M. & Schneegurt, M.A. (2012). Inexpensive and safe DNA gel electrophoresis using household materials. *Biochemistry Molecular Biology Education, 40*(3), 198–203.

Madden, D. (2006). Discovering DNA. *Science in School, 1*, 31–36. https://www.scienceinschool.org/sites/default/files/teaserPdf/issue1_discoveringdna.pdf

Miller, K.R. & Levine, J.S. (2018). *Miller & Levine Biology, Teacher Edition, TE31–TE52*. Pearson.

Ministry of Education, Culture, Sports, Science and Technology. (2009.) *Course of Study for Higher Secondary School*. Japan: MEXT.

NGSS Lead States. (2013). *Next Generation Science Standards: For States, By States*, Volume 1. National Academies Press.

Nowicki, S. (2016). *Biology, Teacher Edition, T28–T36*. Houghton Mifflin Harcourt.

Yatsu, J. & Yamanoi, T. (2016). The need for inspection of the control experiment to verify that an extract is DNA in high school biology classes. Japanese with English abstract. *Journal of Research in Science Education, 57*(1), 63–70.

NAOKO KOSAKA (kosaka.naoko.16@shizuoka.ac.jp) was a PhD candidate at the Graduate School of Science and Technology, Shizuoka University, Japan. YOSHISUKE KUMANO (kumano.yoshisuke@shizuoka.ac.jp) is a professor emeritus at the Faculty of Education, director of STEM Academy, and vice director of STEAM Education Institute, Shizuoka University.

Affiliate Members

**Biography Teachers Association of New Jersey (BTANJ)**

**Colorado Biology Teachers Association (CBTA)**

**Cleveland Regional Association of Biologists (CRABS)**

**Connecticut Association of Biology Teachers (CTABT)**

**Delaware Association of Biology Teachers (DABT)**

**Empire State Association of Two-Year College Biologists (ESATYCB)**

**Hong Kong Association of Biology Teachers (HKABT)**

**Illinois Association of Biology Teachers (IABT)**

**Illinois Association of Community College Biologists (IACCB)**

**Indiana Association of Biology Teachers (IABT)**

**Kansas Association of Biology Teachers (KABT)**

**Louisiana Association of Biology Teachers (LABT)**

**Massachusetts Association of Biology Teachers (MABT)**

**Michigan Association of Biology Teachers (MABT)**

**Mississippi Association of Biology Educators (MSABE)**

**Missouri Association of Biology Teachers (MOBioTA)**

**New York Biology Teachers Association (NYBTA)**

**South Carolina Association of Biology Teachers (SCABT)**

**Tennessee Association of Biology Teachers (TNABT)**

**Texas Association of Biology Teachers (TABT)**

**Virginia Association of Biology Teachers (VABT)**

**The National Association of Biology Teachers supports these affiliate organizations in their efforts to further biology & life science education.**