TIPS, TRICKS & TECHNIQUES

Using M&M's to Model Sanger's Dideoxy DNA Sequencing Method

NABT BioClub RECOMMENDATION

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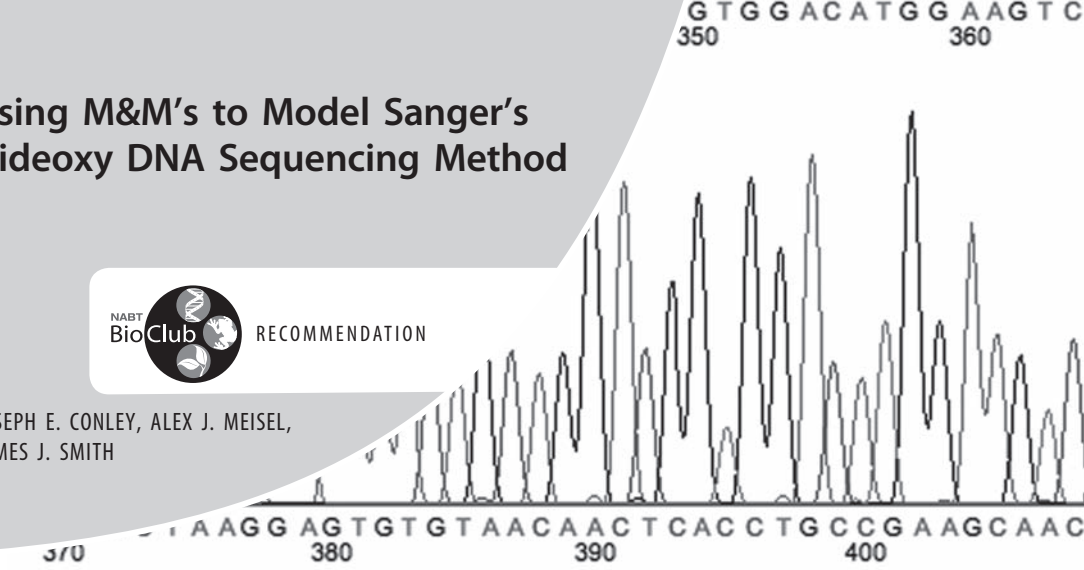

Abstract

This lesson is designed to facilitate student understanding of the molecular structure of DNA, the cellular processes involved in DNA replication, and how these principles were applied to develop a method to determine the nucleotide sequence of DNA. The lesson employs an active and cooperative learning approach accomplished via a modified jigsaw exercise. The specific replication/sequencing process in this lesson is Sanger's dideoxy method of DNA sequencing. In conjunction with related lessons in lecture and lab, students read the relevant section of an appropriate introductory biology textbook and/or view videos explaining how Sanger's dideoxy chain-termination sequencing method works. Students working in four teams (A, C, G, and T) then use green, blue, brown, and red M&M's as nucleotides to build a model of the process. Plain M&M's represent deoxynucleotide triphosphates (dNTPs), while peanut M&M's represent the "terminator" dideoxynucleotide triphosphates (ddNTPs). The lesson addresses Next Generation Science Standards and learning goals typically found in college biology courses at introductory and advanced levels.

Key Words: *DNA; models; Sanger sequencing; DNA replication; polymerase chain reaction; PCR.*

Introduction

DNA structure and nucleic acid structure in general are highly abstract concepts. As such, they can present novice learners with difficulties as they try to understand the various aspects of DNA structure (chains of four nucleotide building blocks) and its associated biological functions (transcription of DNA information to RNA information, and DNA replication to provide new cells with a complete set of chromosomes), which comprise core ideas within the *Next Generation Science Standards* (Standard HS-LS3; NGSS Lead States, 2013). An understanding of DNA structure also provides a necessary foundation for understanding biology applications such as the polymerase chain reaction (PCR) amplification of selected DNA fragments, and how the incorporation of modified nucleotides into the structure of DNA allows scientists to determine the base sequence of a DNA molecule.

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Physical models can be used to help students develop stronger mental models of abstract concepts such as DNA structure (Grosslight et al., 1991). As stated in the *Next Generation Science Standards*, developing and using models based on evidence to illustrate relationships within systems can be used as a tool to increase student understanding (Standard HS-LS1-4). The DNA double helix, perhaps the most iconic model in all of biology, is often modeled at the structural level in biology classrooms. Biological processes involving DNA can also be modeled, and lessons have been developed using paper cut-outs, candy, and other easily obtainable materials to improve student understanding (Latourelle & Seidel-Rogol, 1998; Altiparnak & Tezer, 2009). Activities such as these have been shown to increase the sophistication of students' mental models of complex biological phenomena (Mayer, 1989; Rotbain et al., 2006).

In the lesson described here, a DNA model constructed using plain and peanut M&M candies serves as a reference for students as they work to understand the structure of DNA and how DNA replicates in cells. (To accommodate students, faculty, and staff who might be allergic to peanuts, we suggest inquiring privately about whether there are people with nut allergies in the class. If so, instead of using M&M's, consider as an alternative using paper disks of four colors for the normal dNTPs, and disks of the same color but modified for the dideoxy NTPs.)

The goal of the lesson is to have student teams model the different DNA fragments that are produced in a set of DNA sequencing reactions by Sanger dideoxy chain termination (Sanger et al., 1977). Despite the emergence of next-generation DNA sequencing, Sanger sequencing remains important. It allows scientists to obtain DNA sequences that can be used to carry out phylogenetic analyses, targeted metagenomic analysis from environmental samples, diagnostic tests for defined pathogens, allele identification in genetic tests, and

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numerous laboratory applications involving genetic analyses and molecular cloning.

The lesson provides a way for students to visualize DNA fragments of different sizes and provides a starting point for discussions on how these fragments are produced. The lesson addresses and/or complements many learning objectives in the undergraduate biology curriculum, ranging from DNA structure and replication (5′ → 3′ polarity and the necessity of a free 3′–OH group for the addition of new nucleotides) to PCR (primed DNA synthesis) and electrophoretic separation of DNA fragments. In addition, the lesson touches on some interesting aspects of the history of biology (e.g., Sanger vs. Maxam-Gilbert sequencing; why one survived and the other did not).

This lesson was developed in the context of an inquiry-based Introductory Cell and Molecular Biology course in a residential science college on the Michigan State University (MSU) campus. This small-group cooperative learning exercise (Smith, 2000) complements a DNA lab "stream" (Luckie et al., 2013) in which students use agarose gel electrophoresis to purify 16S rRNA gene PCR products from naturally occurring bacterial isolates, which are then sequenced using Sanger's method at the Genomics Core of the MSU Research Technology Support Facility.

Here, we describe our experiences having students use M&M's to make a model of the products produced in Sanger's dideoxy DNA sequencing method. The core idea of Sanger's method is that the incorporation of a dideoxynucleotide into a growing DNA chain will "terminate" DNA polymerase-catalyzed synthesis of a DNA strand. By physically producing a model of the reaction products of the DNA sequencing reactions, students explore both DNA structure and how DNA sequences are determined by scientists.

○ Methods

To begin this modified jigsaw exercise (Aronson et al., 1978), students are placed into four teams (Teams "A", "C", "G", and "T") and each team is provided a sealable plastic bag containing all four deoxyribonucleotides (green, blue, brown, *and* red plain M&M's) as well as their appropriate dideoxyribonucleotide (green, blue, brown, *or* red peanut M&M's). To assemble team bags for this exercise, include in each bag 30 dNTP (plain) M&M's of each color as well as seven ddNTP (peanut) M&M's of the color corresponding to that team's designated base. Sufficient M&M candies for this exercise should be obtainable by purchasing three 12.6 ounce bags of plain M&M's as well as two 12.6 ounce bags of peanut M&M's. The M&M colors chosen to represent the different bases in this exercise correspond to the colors used to represent those same bases on electropherogram traces in .ab1 files obtained from Sanger sequencing runs.

Students are then introduced to Sanger sequencing through a short PowerPoint slide set (available at http://www.msu.edu/user/jimsmith/ABT_M&M_slideset.pptx). The slide set allows the instructor to introduce students to how DNA sequencing technology was developed and how the Sanger DNA sequencing process works. The slides include references to and excerpts from original articles describing the early methods of DNA sequencing as well as pictures of radiolabeled Sanger sequencing products visualized on x-ray film. Before the students begin building their M&M model, the instructor outlines the activity on the front board (Figure 1A), creating representations of the template DNA strand to be sequenced and the five-nucleotide DNA sequence to be used to prime DNA synthesis. A brief "chalk talk" ensues, showing how DNA sequencing chemistry would be applied and how the use of ddNTPs would lead to the synthesis of DNA fragments of varying lengths. Once the synthesis of two sample fragments has been outlined and written on the board, the student groups are given a "DNA Sequencing (w/ M&M's) Worksheet" (Appendix), which contains a number of leading questions; are reminded of their specific ddNTP; and are instructed to build an M&M model showing all the Sanger sequencing products possible given the template DNA sequence. Using the M&M's, the groups then construct models of the template DNA strand and all of the possible DNA fragments based on the specific ddNTP they were given (Figure 2). When the models have been completed by each of the four teams, each group contributes to a compiled data set by adding their data to the front board (Figure 1B, C). Each group lists the size of their fragments and draws where they would be visible on an electrophoresis gel. It is important for students to recognize that the shorter DNA fragments will travel farther than the longer fragments on the gel.

○ Learning Goals

- Explain the difference between deoxyribonucleotides (dNTPs) and dideoxyribonucleotides (ddNTPs), and explain why it is that ddNTPs terminate DNA synthesis reactions.
- Use an M&M model to apply knowledge of the structure and function of ddNTPs to predict and analyze the products resulting from Sanger dideoxy sequencing reactions.
- Interpret an electropherogram that is obtained from a DNA sequencing facility as a result of Sanger/dideoxy sequencing.
- Apply concepts of dNTP structure and function to explain DNA replication and DNA polymer extension.

○ Instructional Strategy

Prior to beginning the classroom activity, students should become familiar with the logic of Sanger's dideoxy sequencing method by reading an appropriate section in an introductory biology textbook (or similar material available online). Freeman's *Biological Science* (fifth edition, pp. 376–378) provides a good example of a short reading that students can do. Via this text reading, students will become familiar with the structure of ddNTPs, how they are different from dNTPs, and why they cause cessation of DNA elongation during synthesis.

This lesson uses a TOPE instructional strategy (C. A. Anderson, unpublished data). The TOPE strategy is an inquiry sequence in which students learn and use a specific technique (T) to make observations (O). With these observations, students find patterns (P), which they then use to develop explanations (E).

Technique. The technique in this exercise involves using M&M's to construct Sanger sequencing products. First, the instructor draws a representation of the non-template DNA strand and the template DNA strand on the board (Figure 1A) and then walks the class through constructing two-sample sequencing fragments. It should be made clear to the students that even though the sequence is represented on the board, we pretend not to know it, and the task is to use Sanger sequencing to figure it out. The primer from the worksheet is

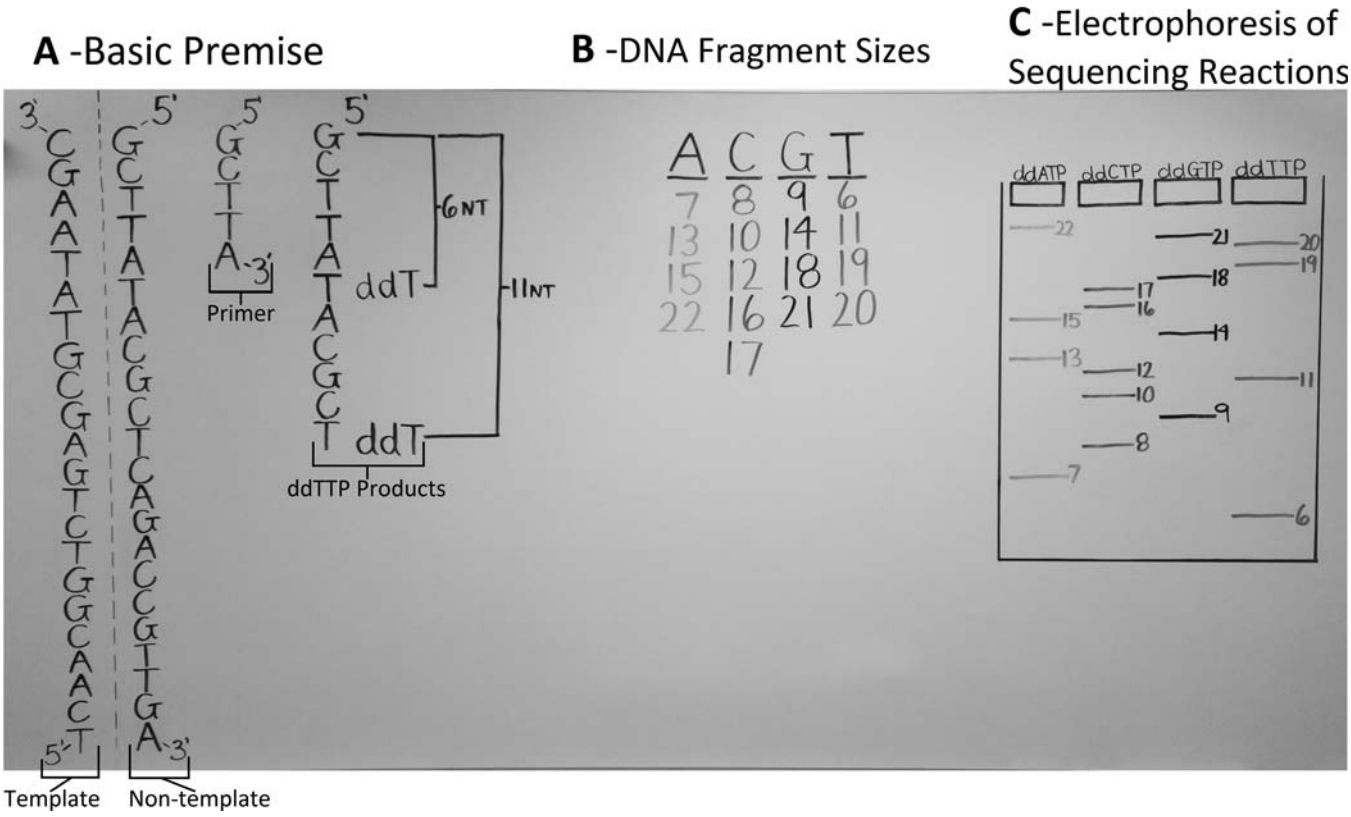

Figure 1. Sample whiteboard for instruction and discussion of the M&M exercise. (**A**) Visual representation of the non-template (right) and template strands (left) based on the worksheet accompanying the M&M exercise (Appendix). The primer from the worksheet is drawn next to the DNA strands. The fourth strand from the left in this figure represents the elongation process using the given primer. This elongation shows the students that incorporation of a ddTTP results in termination of the elongating strand. (**B**) Once the students have completed every possible strand length of their nucleotide, they will write the lengths (represented in number of nucleotides) on the board. The numbers represented in this figure are the resulting strand lengths using the shown template and non-template strands. (**C**) Representation of an agarose gel after electrophoresis of the students' fragments. Using the strand lengths calculated by the students, the instructor can "run" the class's gel. This allows demonstration of how gel electrophoresis by separation of DNA strands of different sizes can be used to allow determination of the sequence of the target strand.

drawn next to the non-template strand. The students should observe that the primer matches up to a specific location on the template strand. The primer attaches, and then the extension of the new DNA strand is shown, with the 5′ and 3′ ends of the strands labeled for reference. The instructor then shows how incorporation of a ddNTP stops synthesis of the growing strand.

With the technique now modeled by the instructor, the students are asked to transfer the two-dimensional model on the board to their M&M's. Each student team is asked to make the complete set of possible fragments given their ddNTP (ddATP, ddCTP, ddGTP, or ddTTP).

Observations. As students arrange their M&M's, they will observe the many fragments they form from the candy. Each group will have fragments of different lengths based on which ddNTP team they are on. It is important for students to observe that the fragments contain the same sequence of DNA but are different lengths. These different lengths are caused by the ddNTPs (peanut M&M's), which terminate the fragment extension (Figure 2). The teams should observe that they do not have a complete Sanger sequence because they have been using only one type of ddNTP.

Patterns. The first pattern students should notice is that whenever a ddNTP is added, the strand stops extending. Students will count the fragments as well as the length of each fragment and record them (Figure 1B). On the board will be a blank electrophoresis gel drawing with four wells labeled A, C, G, and T to represent the different types of ddNTP. Each group will mark their sequencing fragments on the gel as if they carried out agarose gel electrophoresis (Figure 1C). With the class data compiled on the board, the students will see that the longer sequencing fragments are marked closer to the wells than shorter fragments. They will also see the pattern that no two dashes (sequencing fragments) line up with each other on the gel. It is important that the students recognize that each group took the same DNA template strand and primer but used a unique ddNTP; together the teams developed a model of the complete set of Sanger sequencing products. The students should now be able to read the dashes from the bottom to the top of the "gel" and determine the sequence of the non-template strand. This reading provides the DNA sequence of the non-template strand they saw at the start of the lesson in a 5′ → 3′ direction.

Explanations. Having done the M&M activity and investigated how the structures of dNTPs differ from ddNTPs, students will be

Figure 2. Sample M&M model completed by a student team showing the DNA reaction products synthesized incorporating dideoxythymidine triphosphate (ddTTP) into the dNTP mix. (**A**) Representation of the template strand based on the provided DNA sequence (see Appendix). (**B**) Representation of the complementary non-template strand based on the provided sequence. (**C**) Representation of the reaction products of 6, 11, 19, and 20 nucleotides that are made during Sanger sequencing reactions run with the template DNA shown and ddTTP incorporated into the reaction mixture.

able to explain why ddNTPs are needed for Sanger sequencing. Students will show their understanding of ddNTPs and Sanger Sequencing through a series of hypothetical questions. These questions are provided for each team in the DNA Sequencing (w/ M&M's) Worksheet and can be completed either as a group in class or individually as homework.

○ Evaluation

Each student team photographs their set of DNA sequencing reaction products and turns this in along with their worksheet responses. The DNA Sequencing (w/ M&M's) Worksheet will test the students' knowledge of the structures of dideoxyribose and deoxyribose, as well as their knowledge of how this difference in structure affects DNA synthesis and, ultimately, leads to the ability to sequence DNA. The worksheet asks the students some basic questions about the Sanger sequencing technique and then asks them to apply this knowledge to determine the base sequence of a DNA fragment by using a picture of an autoradiograph showing the bands obtained from a set of Sanger sequencing reactions. Students are then asked to show on a gel template where they would expect to see these fragments after agarose gel electrophoresis.

○ Extensions

This lesson can be extended in several ways. For example, as an extension of the worksheet, students could be challenged to render the gel result as an electropherogram with colored peaks. They could then be asked, "What explains why the chain-terminating bases are represented as peaks rather than bands on a gel?"

The results of the M&M modeling exercise also could be used to compare and contrast Sanger DNA sequencing reactions with DNA replication processes in cells, and to explore the differences between the processes of DNA replication via PCR and DNA replication in vivo, and even aspects of transcription and translation. The exercise also can be extended to explore how mutations can occur when the incorrect nucleotide is incorporated into a growing DNA chain.

Although it is beyond the scope of our own instruction, the exercise could be used to lead nicely into longer discussions about the practical limits to the length of a DNA fragment that can be sequenced, how primers are designed and synthesized, and how many nucleotides long a primer needs to be. It should also be possible to have students model the actual 16S rRNA gene amplification with the actual primer pair that they are using in lab, but this would require the purchase of a lot more M&M's, and the class time investment that would be required to do this might prove counterproductive.

○ Conclusions

Once the lesson has been completed, students will have explored the structure of deoxy NTPs, dideoxy NTPs, and the way the two function during DNA polymer extension. The students will have constructed M&M models that represent dideoxy sequencing reaction products and the extension of DNA using both ddNTPs and dNTPs. The students will have applied this M&M model to create a drawing of an electrophoresis gel, giving them experience reading and constructing a DNA sequence using a gel. The students should

then be ready to extend these principles and apply them to the PCR amplification and sequencing of a 16S rRNA gene fragment.

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Appendix. Worksheet for Sanger's DNA Sequencing Method (w/ M & M's)

Set-Up

Four Teams of Students

- "A" Team
- "C" Team
- "G" Team
- "T" Team

M & M's

Plain: dNTPs
Peanut: ddNTPs
Color code: Brown = G, Blue = C, Green = A, Red *OR* Orange = T
Exercise based on the following DNA sequence:

3' – CGAATATGCGAGTCTGGCAACT – 5' (TEMPLATE STRAND)
||||||||||||||||||||
5' – GCTTATACGCTCAGACCGTTGA – 3'

Team Tasks

- Using the M & M's in the bag that your team is provided, represent the template strand with plain M & M's. See Figure 2 for a sample outcome showing the template strand.

Next, assume that you have a DNA PCR primer with the sequence:

5' – GCTTA – 3'.

- Given this PCR primer, represent the set of different DNA fragment(s) that you could synthesize if a ddNTP (Peanut M & M) was incorporated into your products at low frequency.
- Photograph your set of products.

  See Figure 2.
- On the white board, indicate the sizes of your fragments and show on the "gel" where they would appear.

Questions

As a team, write out your responses to the following questions and turn them in at the end of our session.

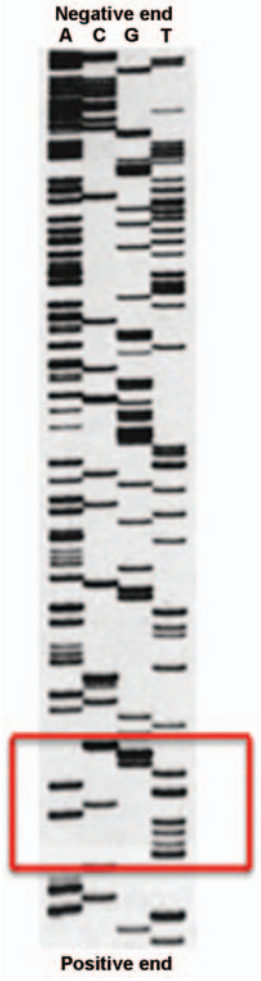
1. Draw the chemical structures of deoxyribose and dideoxyribose and circle the 3' ends of both structures.

   See slide #8 in online PowerPoint file, or consult a textbook or online resource for these structures.
2. Describe the significance of the 3' end of a growing DNA strand during PCR or DNA replication in a cell.

   A free 3' OH group is necessary for the next nucleotide to be added to the growing strand (chain extension).
3. Explain what would happen during a PCR elongation step if a dideoxyribonucleotide got incorporated into a growing nucleotide chain. Why would this happen?

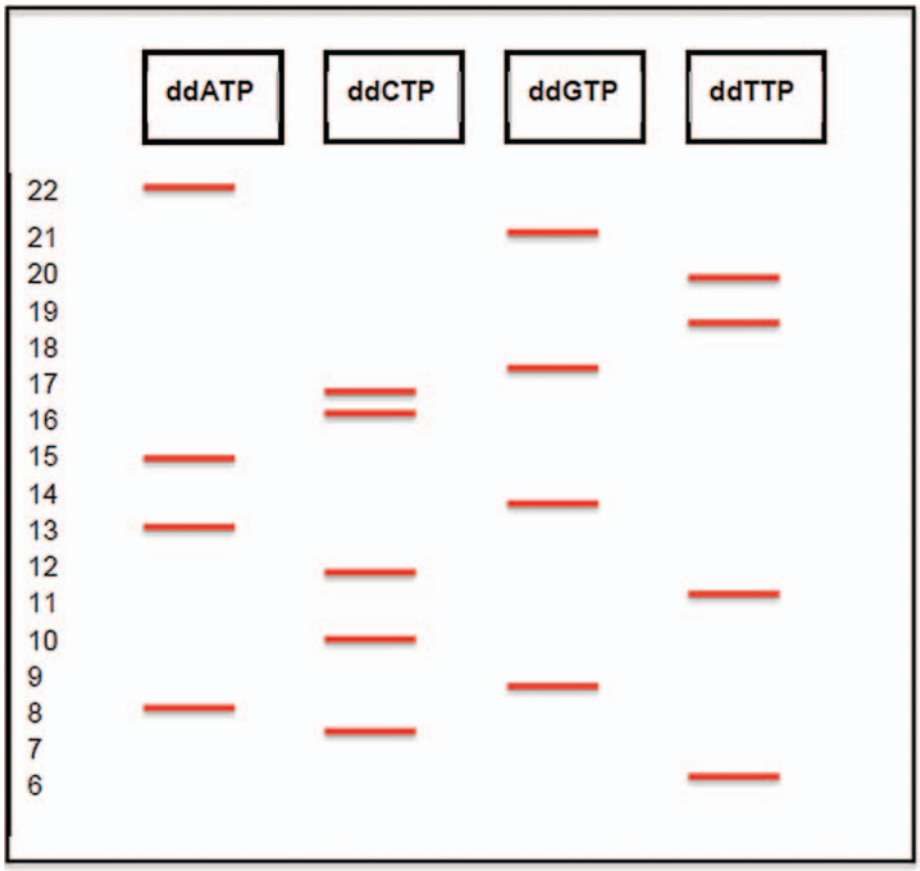
   Chain elongation would stop. The ddNTP does not have a free 3' OH group, which is necessary for elongation to continue.
4. A sequencing gel is shown below, with a group of twelve nucleotides highlighted with a box. Determine the correct sequence of this group of nucleotides (be sure to indicate the 5' and 3' ends).

   5'-TTTTACTATGGC-3'



Appendix Figure A1. DNA sequence provided to students.

5. Based on the DNA sequence you are working with (beginning of the exercise), indicate on the blank gel below where you would expect to find each of the bands that would tell you your DNA sequence after a set of Sanger sequencing reactions. The lanes are labeled with the reaction mixture that you have loaded into each well.



Appendix Figure A2. DNA Sequencing (w/ M&M's) Worksheet.