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Sanger Activity Guide

Exploring Sanger:
This activity guide for the Sanger (dideoxy) method of DNA sequencing will help you consider various ways you can use these materials. We encourage you to modify these lessons and activities to meet the needs of your students and the learning objectives of your curriculum.

Targeted Subject(s):
The following courses would benefit from this activity

- Introductory level biology - CP/Honors
- AP Biology
- Biotechnology

Intended Grade Level(s):
This activity has been written at the high school level. However, this activity can be tailored to your student population and differentiated as needed.

Prior Student Knowledge Required:
It is expected that students understand the following concepts prior to working through this model:

- Hydrogen and covalent bonding
- DNA structure
- Deoxyribose structure compared to ribose structure
- Chromosomes, genes, and alleles
- Base pairing rules
- DNA replication
- PCR Reactions

Key Words:

- Sanger Sequencing
- DNA
- Gene
- Nucleotides (dNTP)
- Base pairing
- Complementarity
- DNA polymerase
- Hydrogen bonds
- Covalent bonds
- Taq polymerase
- Buffer
- Thermal cycling
- Primers
- Denaturation
- Annealing
- Extension
- Electrophoresis
- Next Generation Sequencing

Learning Objectives:
Students will

- **Identify** the steps of the Sanger Method
- **Model** the Sanger Method process, including the directionality (5' and 3' ends) of the primers and single-stranded PCR products.
- **Compare** and **Contrast** all of the sugar molecules associated with genetics material and biotechnological processes (ribose, deoxyribose, and dideoxyribose)
- **Describe** how the resulting strands will appear on an agarose gel.
- **Describe** how the resulting phosphorescent strands will appear on a chromatogram.
- **Extend** their model of first-generation sequencing to applications involved in next-generation sequencing.

Materials Needed:

- 3 Gray Dideoxy A Nucleotides
- 4 Gray Dideoxy C Nucleotides
- 4 Gray Dideoxy T Nucleotides
- 2 Gray Dideoxy G Nucleotides
- 26 Gray A Nucleotides
- 19 Gray T Nucleotides
- 17 Gray G Nucleotides
- 35 Gray C Nucleotides
- 1 Sticker Sheet
- 14 Primer Pieces

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Other Materials Suggested:

- Dynamic DNA Kit© (available from 3D Molecular Designs)
- Flow of Genetic Information Kit© (available from 3D Molecular Designs)

Possible Extensions:

- Connection to Gel Electrophoresis and Extrapolation to appearance on a Chromatograms
- Next Gen Sequencing and use of technology in drug discoveries

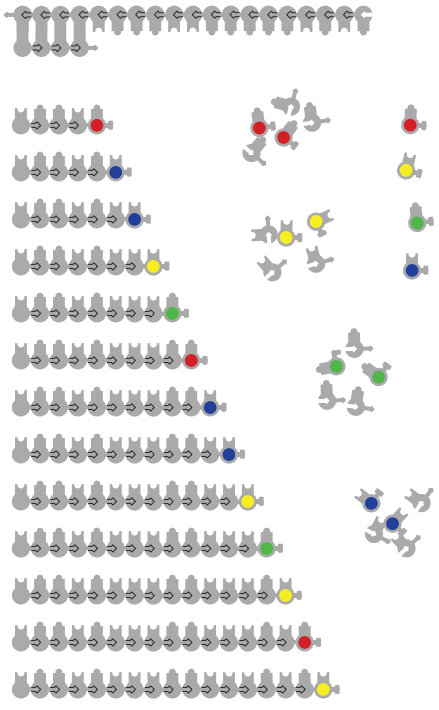
Instructions for the Activity:

Pre-Lesson Set Up

- **Assemble sets of nucleotide bags**. These bags should have the four nucleotides in separate bags. This pre-assembly greatly aids in student completion of the task, as well as increases the processing time for students to create base pairs through the model process. It is suggested that you place your colored deoxynucleotides and the dideoxynucleotides in a brown paper bag. This stops the students from purposefully pulling specific nucleotides.
- **Assemble primers**. The primer sequence that is used is the same as the Primer #1 from Exploring PCR. It will work better to highlight the importance of the colored stickers on the ddNTPs if you use the primers without the stickers on them.
    - Primer: **TGAC**
- **Assemble ddNTPs**. The dideoxynucleotides need to have stickers placed on top of them. This will represent the color that will be expressed through fluorescent signals attached to the ddNTPs

Activity Steps

It is suggested that students work in groups of no more than 5 students. This section of the activity will introduce students to the methods of Sanger sequencing and how this type of protocol will flow directly into DNA electrophoresis.



1. Grab needed materials
    - Nucleotide bags
    - Primer Sequences; placed to the side in a stack
2. Using the Unknown Sequence as a guide and following nucleotide base pairing rules:
    - First add the primer to the 3' end of the unknown sequence.
    - Then, use the nucleotide bags to add free nucleotides to the 5' end of the primer.
3. When you pull a dideoxynucleotide, ddNTP, where N stands for any one of the nucleotide bases, then that strand is complete. Separate that single strand from the Unknown Sequence.
4. Repeat this process, starting first with adding a primer to the 3' end of the Unknown Sequence and then add nucleotides as you did in step three.

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Teacher Tips or Notes for Successful Implementation:

Generalized flow of the activity

- The activity is set up so that there is a time for front-loading of the information, kinesthetic manipulative time where the students work with the model, and finally an extension to understand how Sanger sequencing has both been historically utilized and where research tools have progressed. Timing of each area is dependent on the student population and what background knowledge the students are progressing from. This lesson, in total, should take approximately two days.
- It is suggested that the teacher form groups of five students and allow them 15 minutes to conduct this activity. After that point, pool students generated single stranded DNA (ssDNA) sequences together for analysis and discussion. It is also suggested that the teacher encourages a "game-like" atmosphere - creating a competition between groups to see which group is the most effective "Taq polymerase" and creates the most ssDNA segments. This will help to keep students on task and the process working in a timely fashion.
    - At the end of the 15 minutes, all of the sequences can be joined together to model electrophoresis of the class results. This allows for multiple sequences to be obtained in the 15 minute window.
- To also increase the speed/flow of the lesson, the teacher can opt to pre-assemble the Unknown Sequence. For re-use of the same sequence, the teacher can place a strip of tape along the back of the nucleotide sequence.

Resources:

- "Sanger Sequencing." Sigma-Aldrich, 1999, www.sigmaaldrich.com/technical-documents/articles/biology/sanger-sequencing.html .
- Sikkema-Raddatz, Birgit, et al. "Targeted next-generation sequencing can replace Sanger sequencing in clinical diagnostics." Human mutation 34.7 (2013): 1035-1042.
- Sanger, F et al. "DNA sequencing with chain-terminating inhibitors." Proceedings of the National Academy of Sciences of the United States of America vol. 74,12 (1977): 5463-7. doi:10.1073/pnas.74.12.5463

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