MINDS-ON 4: DNA Sequencing

Suggested Timing: 30 minutes

In this hands-on activity, students will create a paper-based model of the process of DNA sequencing using the method established by Frederick Sanger in 1977 to understand how DNA is sequenced for procedures such as DNA barcoding.

Prior Knowledge and Skills

- Understanding of basic mechanics of DNA replication (i.e., strand separation, annealing of primer, extension of DNA strand using DNA polymerase and termination)
- Understanding of complementary base pairing (C-G, A-T)
- Familiarity with process of gel electrophoresis (migration of DNA fragments in a gel exposed to an electrical current)

Success Criteria

- Quality responses are provided during group discussions
- Sequences correctly match base pair template strand and complementary strand

For each group of 4 students

BLM M5: DNA Sequencing

1 copy of DNA Sequencing – G (Guanine)
1 copy of DNA Sequencing – C (Cytosine)
1 copy of DNA Sequencing – T (Thymine)
1 copy of DNA Sequencing – A (Adenine)
1 copy of DNA Sequencing – Group Consolidation

For each group of 4 students

4 pairs of scissors
1 stapler
1 **pink** highlighter pen or marker
1 **yellow** highlighter pen or marker
1 **blue** highlighter pen or marker
1 **green** highlighter pen or marker
1 roll transparent tape

Did you know?

Frederick Sanger won his second Nobel Prize for Chemistry in 1980, sharing it with Walter Gilbert, for their contributions concerning the determination of base sequences in nucleic acids, and Paul Berg for his work on recombinant DNA.

1. Explain to the students that they will be determining the base sequence of a piece of DNA by modelling the process of DNA sequencing through the chain termination method developed by Fred Sanger in 1977.
2. Photocopy and distribute the **BLMs** to the students. Each group of four students will also need one of each of the four colours of highlighters or markers, as well as a stapler (or they could all share one stapler if necessary) and a roll of transparent tape.
3. Each group member will need to choose one of the colours (each colour corresponds to one of the four ***nucleotides***).
4. Have each student colour in the boxes of his/her letters in his/her colour (e.g., colour the Gs green as shown in Figure 1). This colouring represents a fluorescent dye-labelled ***dideoxynucleotide*** (ddNTP) which has been incorporated into the DNA strand as the strand is replicated (through ***polymerase chain Reaction (PCR)***). As the DNA strand is replicated many times, each of the bases in a DNA strand can be identified.
5. Next, each student should cut around the outside of the chart, and then cut out each row along the dotted lines (see Figure 2). This will result in each student having a number of strips of paper. These strips represent the copies of DNA strands of different lengths present in a reaction mixture.
6. For each strip, the student should cut the paper to the right of the letter which has been coloured in (see Figure 3). This is to model the termination of strand extension as a result of the addition of a ddNTP. The ddNTP stops strand extension because its molecular structure does not allow another nucleotide to attach next to it. The ddNTP, therefore, identifies the base at the position where the strand extension stopped.

Continued...

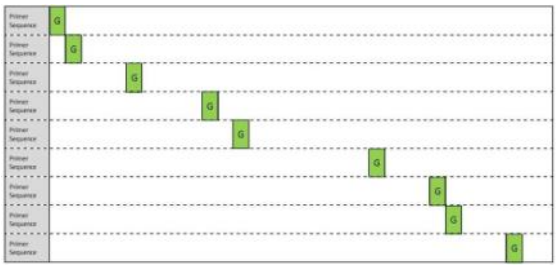

Figure 1: *Colour in the boxes with the letters.*

Figure 2: *Cut out each strip along the dotted lines.*

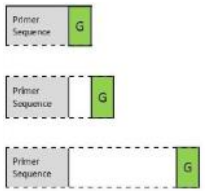

Figure 3: *Cut out each strip to the right of the letter.*

7. Next, each student should stack his/her strips with the longest at the bottom and the shortest at the top, then staple the stack together at the end marked "primer sequence" (see Figure 4). He/she needs to makes sure that the primer sequence end is lined up carefully or the bases will not line up accurately. This models the arrangement of fluorescent nucleotides as they would appear after ***gel electrophoresis***. Shorter DNA strands migrate through the gel material faster than longer strands, hence becoming arranged in relative length order.

Figure 4: *Stack the strips from longest to shortest and staple.*

8. Then each student will carefully lay his/her stapled strips in the rows as marked the sheet marked **DNA Sequencing – Group Consolidation** (see Figure 5) and tape in place.
9. In order to determine the complementary strand sequence, the students will read down the columns, identify which letter is in each column.

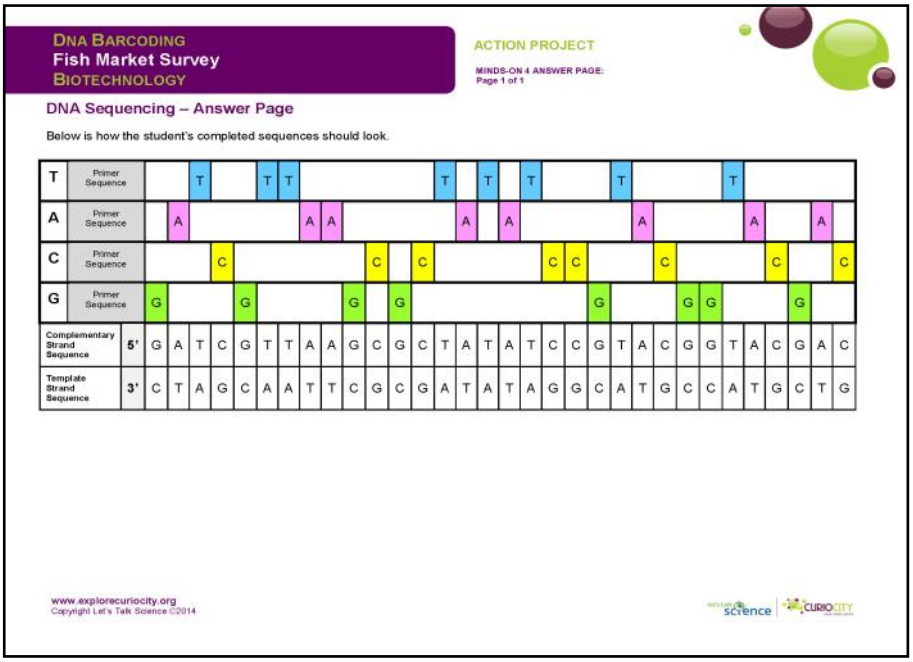

Figure 5: *Lay the strips on the sheet and tape in place. Read down the columns to determine the complementary and template base sequences.*

Students may want to use a ruler or the edge of a piece of paper to keep the rows straight as they read. They will record the letters in the row marked **complementary strand sequence** (see Figure 5). Using complementary base pairing rules (A-T, C-G), they will also determine the **template strand sequence**.

Extensions

- Students could watch one of the animations below and compare it to what they did in the activity.
- Students could watch more than one of the animation below and rate them according to how easy they are to understand, how comprehensive, etc.

CONNECTING TO CONTENT ON CURIOCITY

- Sanger Sequencing Backgrounder (**Backgrounder** 2012)
- Polymerase Chain Reaction (PCR) Backgrounder (**Backgrounder** 2012)
- Polymerase Chain Reaction (PCR) (**Video** 2012)

ADDITIONAL INFORMATION

Animations about DNA (Sanger) Sequencing

- http://highered.mheducation.com/sites/0072556781/student_view0/chapter15/animation_quiz_1.html (Accessed Sept. 9, 2016)
  After watching this animation from McGraw-Hill, students can complete a short quiz (located below the animation).

- http://www.wiley.com/college/pratt/0471393878/student/animations/dna_sequencing/index.html (Accessed Sept. 9, 2016)
  This DNA Sequencing animation from Wiley provides a comprehensive look at Automated DNA sequencing. It includes interactive elements and quizzes.

- http://www.dnalc.org/resources/animations/cycseq.html (Accessed Sept. 9, 2016)
  This animation from the Biology Sequencing Library in the DNA Learning Centre is provided by the Cold Spring Harbour Laboratory. It can also be downloaded for PCs or Macs.

- http://www.dnalc.org/view/16036-Fred-Sanger-1975.html (Accessed Sept. 9, 2016)
  This is a companion animation to the one above in which Fred Sanger describes in first person how he developed his method.

- http://www.dnalc.org/view/15531-DNA-sequencing-method-as-devised-by-Sanger-3D-animation-with-narration.htm (Accessed Sept. 9, 2016)
  This short animation closely resembles what the students do through the hands-on modelling. This animation is also from the Biology Sequencing Library in the DNA Learning Centre.

- http://www.phgfoundation.org/tutorials/dna/5.html (Accessed Sept. 9, 2016)
  This DNA Sequencing Tutorial is produced by the phg foundation, which is part of the Foundation for Genomics and Population Health. This animation is also available on YouTube.

Other Resources

- https://commons.wikimedia.org/wiki/File:Sequencing.jpg (Accessed Sept. 9, 2016)
  This image of a sequencing gel on Wikimedia Commons shows what an actual sequencing gel looks like.

- http://www.dnaftb.org/23/index.html (Accessed Sept. 9, 2016)
  This section from DNA from the Beginning focuses on the big idea "A gene is a discrete sequence of DNA nucleotides." It includes a page about the concept, an animation narrated by Fred Sanger, a gallery, videos, Fred Sanger's biography, and a "Problem" which is a concept-based online activity for the students to do.

Videos on YouTube

- https://www.youtube.com/watch?v=e2G5zx-OJIw - How does Sanger Sequencing Work? – Seq It Out #1 (2015, 3:27 min.)
- https://www.youtube.com/watch?v=ry4HLAJMIuw - Sanger Sequencing of DNA [HD Animation] (2016, 3:39 min.)
- https://www.youtube.com/watch?v=oYpIlbI0qF8 - DNA Test Methods - DNA Sanger Sequencing (2008, 1:07 min.)
- https://www.youtube.com/watch?v=jFCD8Q6qSTM - Next Generation Sequencing (NGS) - An Introduction (2015, 9:29 min.)