

A Laboratory Class: Constructing DNA Molecular Circuits for Cancer Diagnosis

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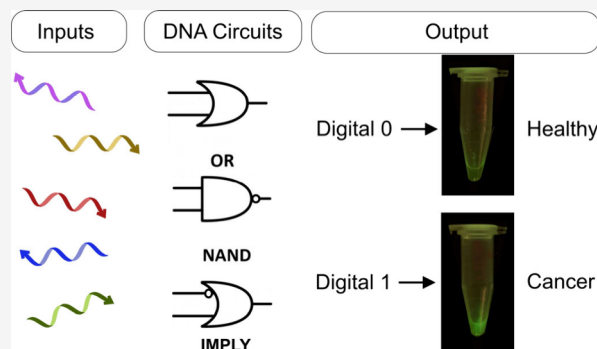


Supporting Information

ABSTRACT: It has been shown that active learning strategies are effective in teaching complex STEM concepts. In this study, we developed and implemented a laboratory experiment for teaching the concepts of Boolean logic gates, molecular beacon probes, molecular computing, DNA logic gates, microRNA, and molecular diagnosis of hepatocellular carcinoma, which are related to DNA molecular computing, an interdisciplinary cutting-edge research technology in biochemistry, synthetic biology, computer science, and medicine. The laboratory experience takes about 110–140 min and consists of a multiple-choice pretest (15 min), introductory lecture (20 min), wet laboratory experiment (60–90 min), and a post-test (15 min). Students are tasked to experimentally construct three molecular logic circuits made of DNA oligonucleotides and use them for the fluorescence-based

detection of microRNA markers related to diagnostics of hepatocellular carcinoma. The class was taught to undergraduate students from freshman to senior academic levels majoring in chemistry, biochemistry, biotechnology, and biomedical sciences. Students were engaged during the session and motivated to learn more about the research technology. A comparison of students' scores on the pretest and post-test demonstrated improvement in knowledge of the concepts taught. Visual observation of the fluorescence readout led to a straightforward interpretation of the results. The laboratory experiment is portable; it uses inexpensive nontoxic reagents and thus can be employed outside a laboratory room for outreach and science popularization purposes.

KEYWORDS: General Public, Undergraduate/Advanced High School Students, Public Understanding/Outreach, STEM, Biochemistry, Interdisciplinary/Multidisciplinary, Laboratory Instruction, Hands-On Learning/Manipulatives, Nanotechnology, Nucleic Acids/DNA/RNA, Molecular Biology, Molecular Computing



INTRODUCTION

Pioneering research, such as DNA nanotechnology, recombinant DNA, and CRISPR, is contributing to the transformation of biology-related disciplines.¹ Therefore, increased exposure of students to vanguard science is becoming a part of academic curricula in high school and undergraduate programs.^{1,2} Following this effort, forefront scientific approaches are framed for their introduction in teaching laboratory experiments, allowing students to learn competent concepts and technical skills.^{3–7} For biochemistry and molecular biology students, early engagement in understanding cutting-edge technologies can motivate and increase their preparation for later graduate studies and research.^{8,9}

Biotechnological tools oriented toward nucleic acid biomarkers have attracted the attention of the scientific community, thus leading to increasing efforts in their development.^{10–12} On the path toward personalized medicine, multiple nucleic acid sequences must be analyzed for precise diagnosis and proper treatment. Such nucleic acid sequences become inputs in the decision-making of molecular devices that produce a diagnostic and/or therapeutic output. These

inputs can undergo complex algorithms (similar to those executed by electronic computers) for their analysis. Although electronic computers are made from semiconductors, other materials can be used to build computers.^{13,14} Individual molecules and atoms have been proposed as building units for molecular computers.^{14–16} Efforts have been made to build molecular computers out of DNA since it is a compatible material for directly computing nucleic acid biomarkers.^{17–23} By using the principles of digital computing, DNA molecular computers are capable of multiplex parallel recognition of biomarkers (e.g., microRNA (miR)).¹⁹ The advantages of building molecular computers from DNA are their biocompatibility, affordability, and chemical stability. The success of manufacturing these DNA devices depends on qualified

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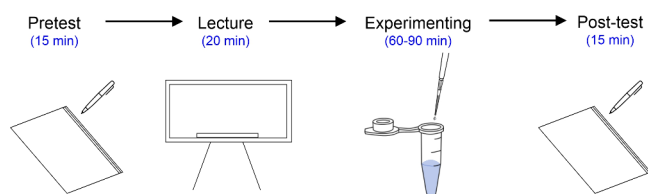
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personnel with interdisciplinary knowledge. Thus, it is essential to nurture an understanding of the molecular computing concepts associated with their application in personalized medicine. To achieve this goal, we aim to introduce an educational tool on DNA-based computation for molecular diagnostics and fill a current gap in academic curricula.

In this study, we developed a laboratory experiment that introduces basic concepts for building DNA molecular computers and their potential implications for cancer diagnostics. The laboratory experience consists of four activities (Scheme 1), which aim at constructing three Boolean

Scheme 1. Laboratory Class Layout Showing Four Different Pedagogical Components: (1) Pretesting Students' Knowledge, (2) Lecture, (3) Experimental Engagement, and (4) Use of a Post-Test to Monitor Student Learning Outcomes



logic circuits (OR, IMPLY, and NAND) and using them to classify mixtures of microRNA biomarkers as indicators of healthy or cancerous conditions. Students are exposed to the

following concepts: Boolean logic gates, molecular computing, DNA logic gates, molecular beacon probes, miR, and molecular diagnosis of hepatocellular carcinoma (HCC). This experiment can be integrated into chemistry, biology, biomedical sciences, and molecular biology courses for undergraduate students and advanced science classes for high school students.

KEY LEARNING CONCEPTS

Boolean Logic Gates

To teach the basis of DNA molecular computing, it is necessary to explain the foundation of modern computers. Computers are arrays of logic gates (transistors in electronic computers) connected in a particular order to complete specific logical tasks (Figure 1A). Therefore, a logic gate is the most basic unit of any computer. The different arrangements and connectivity of the logic gates allow computers to execute various tasks of arbitrary complexity. In digital computing, these logic gates follow Boolean algebra, a binary system where variables are defined with only two possible values: 0 (False, Low, OFF) or 1 (True, High, ON). Boolean logic is the most implemented in electronic and molecular computing because of its simplicity and robustness.^{24,25}

Boolean logic gates (e.g., YES, NOT, OR, AND, IMPLY, and NAND; Figure 1A) can accept and process the digital values of multiple inputs but produce only a single output. Each Boolean logic has a predetermined input(s), yielding a

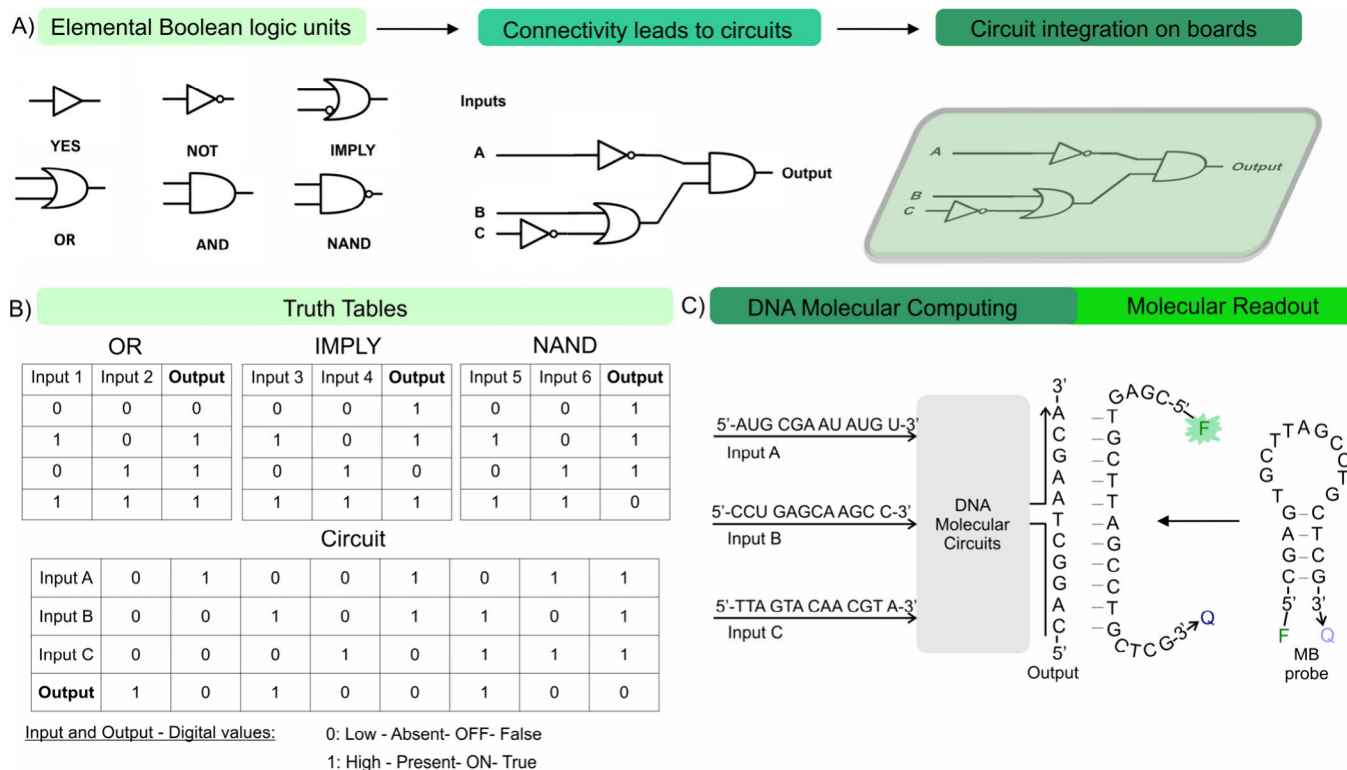


Figure 1. General concepts of computation. (A) Evolution pathway followed by electronic computers; starting from representatives of Boolean logic units (symbols indicated), their connectivity leads to circuits and circuit integration in boards (silicon-based motherboards). (B) Truth tables of OR, IMPLY, and NAND Boolean logic; the circuit made of NOT and OR gates connected to an AND gate is depicted in panel (A). (C) DNA molecular computing shows the intake of different nucleic acid sequences (DNA and/or RNA) as inputs to yield a new nucleic acid sequence as an output after inputs are processed by DNA molecular circuits. Molecular readout scheme shows a molecular beacon (MB) probe recognizing the output sequence via Watson–Crick interactions and producing a fluorescence readout due to the concomitant conformational change (unfolding of the MB native hairpin-like conformation).

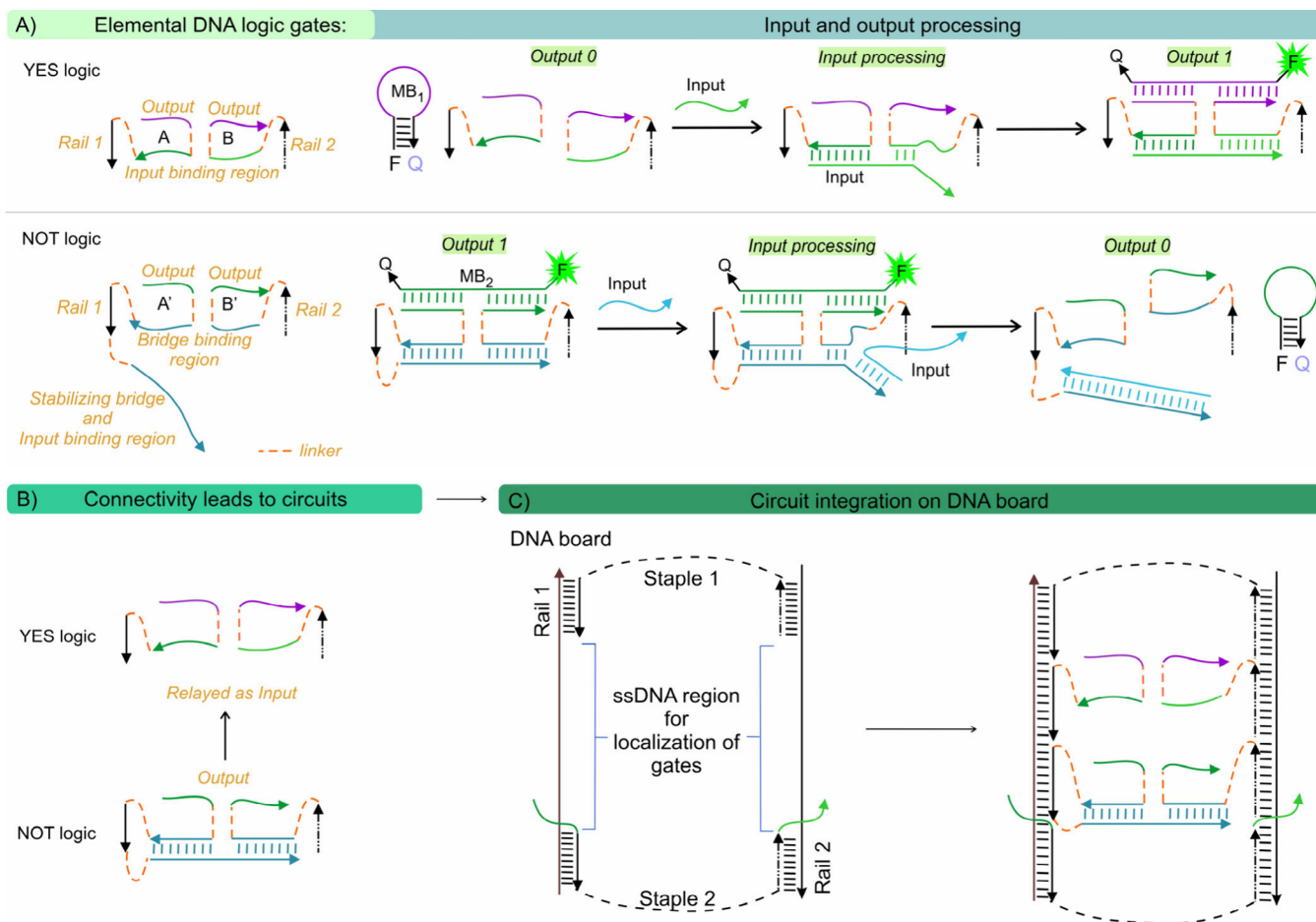


Figure 2. Molecular insight into the components of the DNA molecular circuits. (A) Elemental DNA YES and NOT logic gates.²⁹ In the YES gate, the input strand bridges strands A and B by binding to their green fragments, thus bringing the purple fragments in proximity to make an output sequence that is sensed by the MB probe (MB₁). In the NOT gate, the output sequence (green fragments) is formed in the absence of the input strand due to bridging of strands A' and B' via complementarity of their blue fragments with the stabilizing bridge fragment (cyan). The input strand sequesters the bridge fragment from the bridge-binding fragments of strands A' and B', thereby causing separation of A' and B' and decomposition of the output into two fragments (green). (B) The connectivity of YES and NOT gates is achieved by relaying the output sequence of the downstream NOT gate as an input for the upstream YES gate, which results in a DNA molecular circuit. (C) DNA board (left) is a four-stranded DNA nanostructure that allows spatial localization for connectivity of the individual YES and NOT logic gates (right).²⁹ Arrows represent single DNA strands, where arrowheads indicate the 3' ends. Stacked lines represent base pairs, and dashed lines indicate oligomeric linkers.

specific output set, defined by truth tables (Figure 1B). For example, the truth table of OR logic dictates output is digital 1 when either or both inputs are digital 1; in NAND's logic, output is digital 0 only when both inputs are digital 1, and for IMPLY's logic, the output digital 0 is obtained only in a specific input value combination (Figure 1B). In Boolean algebra, OR, IMPLY, and NAND logic are important to construct more complex computational circuits. A circuit is a set of logic gates purposely connected to achieve the desired output (Figure 1A). For example, in electronic computers, the circuits are realized by connecting each logic gate and integrating them on boards made out of semiconductor materials (Figure 1A), where the circuits direct the flow of electrons based on the programmed Boolean logic. The digital value of a circuit's output is dictated by the combination of values of the multiple inputs (Figure 1B), enabling output computing based on particular input combinations, a quality needed for personalized medicine.

Molecular Beacon Probes for Output Readout

In our DNA molecular computing designs,^{26,27} inputs and outputs correspond to nucleic acid sequences (Figure 1C).

The output is a new nucleic acid sequence generated after computing all inputs, and it can be detected using a complementary DNA oligonucleotide tagged with a fluorophore and a quencher at its opposite termini, known as a molecular beacon (MB) probe.²⁸ In the absence of a complementary output, MB is in a hairpin conformation, which keeps the fluorophore near the quencher, ensuring a low fluorescence. Upon the binding of MB to its complement, it stretches and distances the fluorophore from the quencher, enhancing the fluorescence signal (Figure 1C). Therefore, MB helps in transducing the nucleic acid output to a fluorescence signal and easily monitoring the molecular computing readout. In this laboratory experiment, high fluorescence intensity was interpreted as digital output 1 and low fluorescence as digital output 0.

Molecular Computing by DNA Logic Gates

Building DNA logic gates and circuits is possible due to the predictability of Watson–Crick base-pairing, where adenosine (A) pairs with thymidine (T), and guanosine (G) pairs with cytidine (C).³⁰ This predictable base-pairing is used to program Boolean logic functions that operate through the

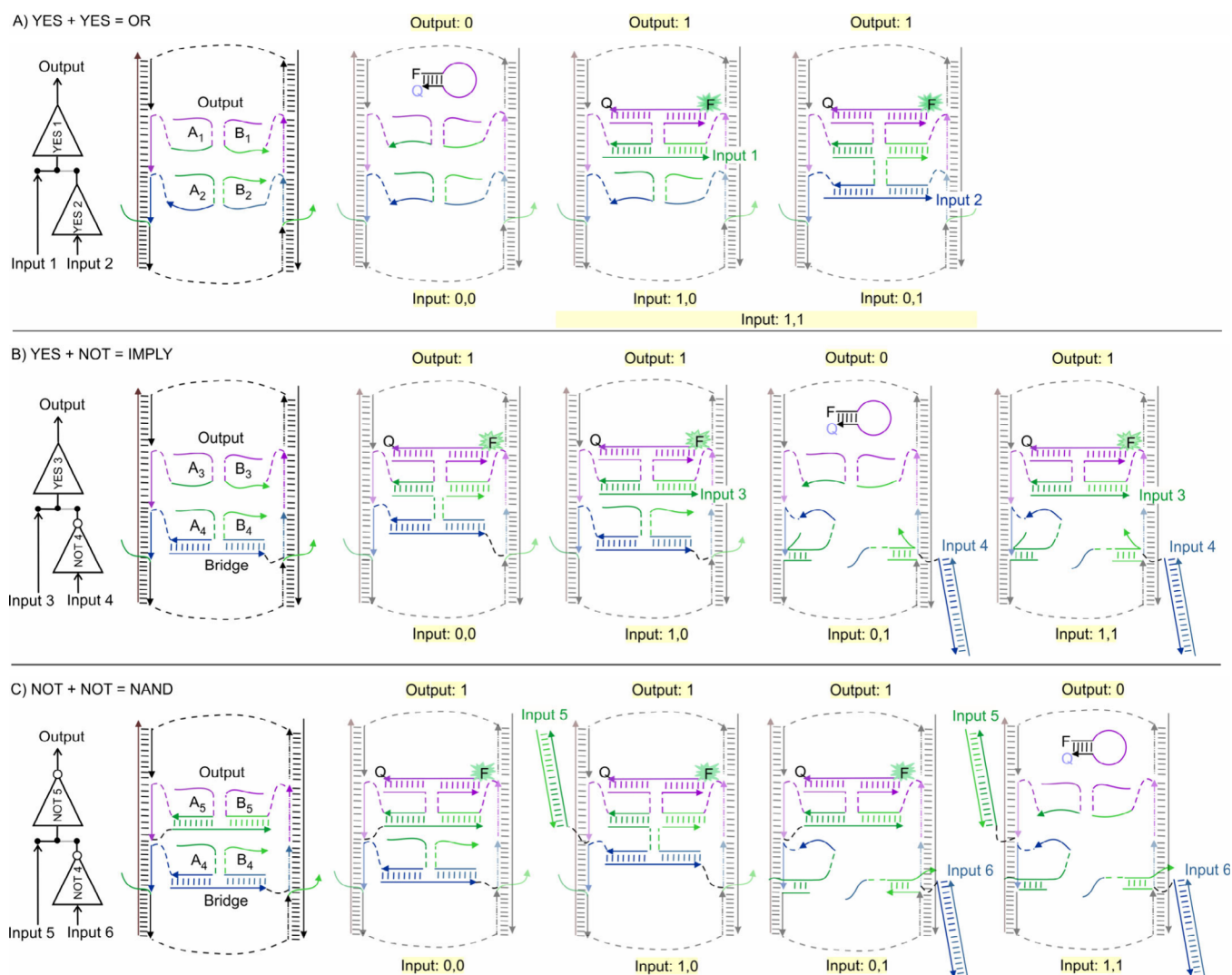


Figure 3. Constructing nonconventional DNA molecular circuits from YES and NOT gates with OR (A), IMPLY (B), and NAND (C) Boolean functions. (A) OR logic is achieved by connecting two DNA YES logic gates integrated into the DNA board. The output sequence made by proximal cooperativity of the purple fragments (A_1 and B_1) gives a digital 1 when the YES 1 gate is turned ON either directly by Input 1 or indirectly by the output of YES 2, which follows the truth table. (B) IMPLY logic is realized by connecting YES 3 with NOT 4 logic gates on the DNA board. The output sequence made out of the proximity of the green fragments of the (NOT 4 gate) A_4 and B_4 serves as another input for the YES 3 gate. Therefore, the IMPLY gate is OFF only when Input 4 is present alone, according to the truth table (Figure 1B).²⁹ (C) NAND logic is made by connecting two DNA NOT gates (NOT 4 and NOT 5) on the DNA board. As NOT 5 exhibits high output in the absence of one or both inputs (Input 5 or 6), NAND logic would turn OFF only when both inputs are present, as expected from the truth table.²⁹ Panels (A–C) show the connected YES and NOT logic symbols at the left. Black dots represent the connecting points of the logic gates for input uptake; gray lines represent the connectivity of the upstream and downstream gate that is turned on (turned black) in the absence of Input 1, Input 3, or Input 5. The oligonucleotide sequences of all DNA circuits, inputs, and MB probes are listed in the SI, Notes for Instructors, Table 1.

association and dissociation of DNA fragments.¹⁹ Modulating sequences of DNA molecules as DNA logic gates and circuits can yield higher-ordered DNA structures not found in nature (e.g., DNA board in Figure 2C). This laboratory activity introduces DNA as a building material of molecular computer hardware, a function different from the natural role of DNA as storage of genetic information.

In DNA molecular computing, the development of individual logic gates has been thoroughly addressed. However, achieving complex molecular circuits is still in its infancy. Here, we show the integration of DNA logic gates into circuits by their localization onto a DNA board, an approach that mimics Si-based circuits.²⁹ The DNA board is a molecular structure assembled in aqueous solution, where it provides spatial localization of two logic gates (YES and NOT) for their

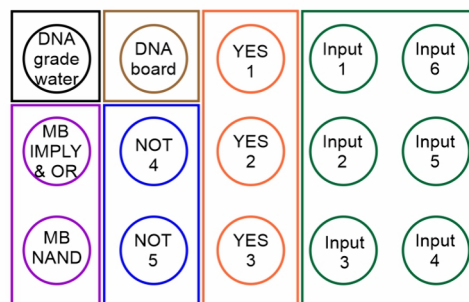
integration into DNA circuits (Figure 2C). The DNA YES gate is composed of two DNA strands (A and B) that are bridged by a nucleic acid input to make the output sequence (Figure 2A).^{29,31} In the NOT gate, a bridge fragment brings A' and B' together to form the output sequence (Figure 2A). The nucleic acid input sequesters the bridge fragment from a NOT gate, leading to the separation of the two output (green) fragments. The connectivity of the DNA YES and NOT logic gates can achieve NAND, IMPLY,²⁹ and OR Boolean logic circuits by rational designing the output sequence of the downstream gate as an alternative input of an upstream YES or NOT gate, as shown in Figure 3.

The DNA YES and NOT gates preserved their Boolean truth table by binding to only one input at a time, either an input externally added to the DNA circuit or an input relayed

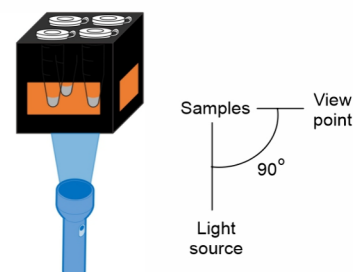
Table 1. Learning Objectives and Outcomes

Objectives	Outcomes
To learn the concepts of Boolean logic gates, molecular computing, DNA logic gates, MB probe, miR, and molecular diagnosis of HCC.	Students will be able to describe and apply the key concepts of Boolean logic gates, molecular computing, DNA logic gates, MB probe, miR, and molecular diagnosis of HCC.
To predict the output of DNA logic circuits based on their Boolean truth table.	Students will be able to interpret Boolean truth tables and predict their output.
To construct DNA logic circuits with OR, NAND, and IMPLY Boolean logic.	Students will gain the technical skills to construct, test, and report the readout of DNA logic circuits.
To use DNA logic circuits to classify mixtures of miR markers as indicators of cancerous or healthy conditions.	Students will be able to discriminate between a cancerous or healthy condition based on the response of DNA logic circuits to the mixtures of miR markers.
To understand the principles of fluorescence-based MB probes.	Students will be able to explain fluorescence phenomena based on the states of MB probes.

A) Solutions to prepare DNA logic circuits



B) Samples irradiation with flashlight



C) Setting as a portable kit

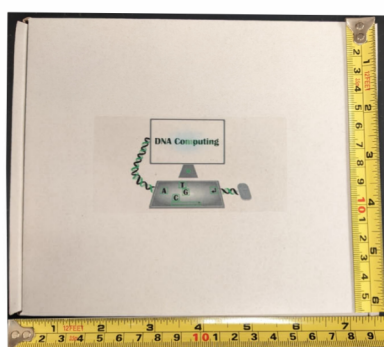


Figure 4. Setting up the materials and reagents. (A) Arrangement of 15 different microcentrifuge tubes (represented by circles) filled with solutions needed for constructing and testing the three DNA logic circuits: one tube with DNA grade water (black), one tube containing the assembled DNA board (brown), three tubes with different YES gates (orange), two tubes with different NOT gates (blue), two tubes with the MB probe solutions to report the output of the IMPLY/OR circuit and NAND circuit (purple), and six tubes containing individual inputs (green). (B) A tube-holder box made of black cardstock paper for holding samples containing four different input combinations of one DNA logic circuit. The samples are irradiated with a blue LED flashlight for output visualization at a 90° angle. (C) All necessary materials and reagents can be conveniently packaged into a 19 × 16 × 8 cm box as a portable education kit for the DNA molecular computing activity.

from the downstream gate (Figure 3). In Boolean algebra, achieving OR, IMPLY, and NAND logic by connecting YES and NOT logic gates has not been reported.^{24,32} However, computations made out of DNA can follow different connectivity paradigms. Therefore, this laboratory allows students to follow the progress of building DNA molecular circuits from individual DNA logic gates and to be familiar with new nonconventional avenues in constructing DNA molecular circuits.

Molecular Diagnosis of Hepatocellular Carcinoma (HCC) by microRNA Detection

To emphasize the relevance of DNA molecular computing as a biomedical tool, the DNA circuits were designed to recognize as inputs microRNAs (miRs) correlated with the diagnosis of

hepatocellular carcinoma (HCC), one of the most common types of liver cancer.³³ This practical relevance is an important component of the learning experience³⁴ that provides students with an outlook on the real-world application of the topic/skills learned.^{35,36} miRs are noncoding RNA of a short length (18–25 nucleotides), lacking secondary structures in their mature form.³⁷ miRs have been found to regulate processes like RNA gene expression/silencing and as signaling molecules of intercell communication. In their mature form, they can be found either in the cellular matrix or circulating in plasma.^{37,38} These features make them attractive candidates as biomarkers in the diagnosis of diseases. In cancer, more than one miR can be abnormally overexpressed and/or underexpressed. The precision of the diagnosis can be improved by considering as many miRs as possible.³⁸ However, analyzing multiple miRs

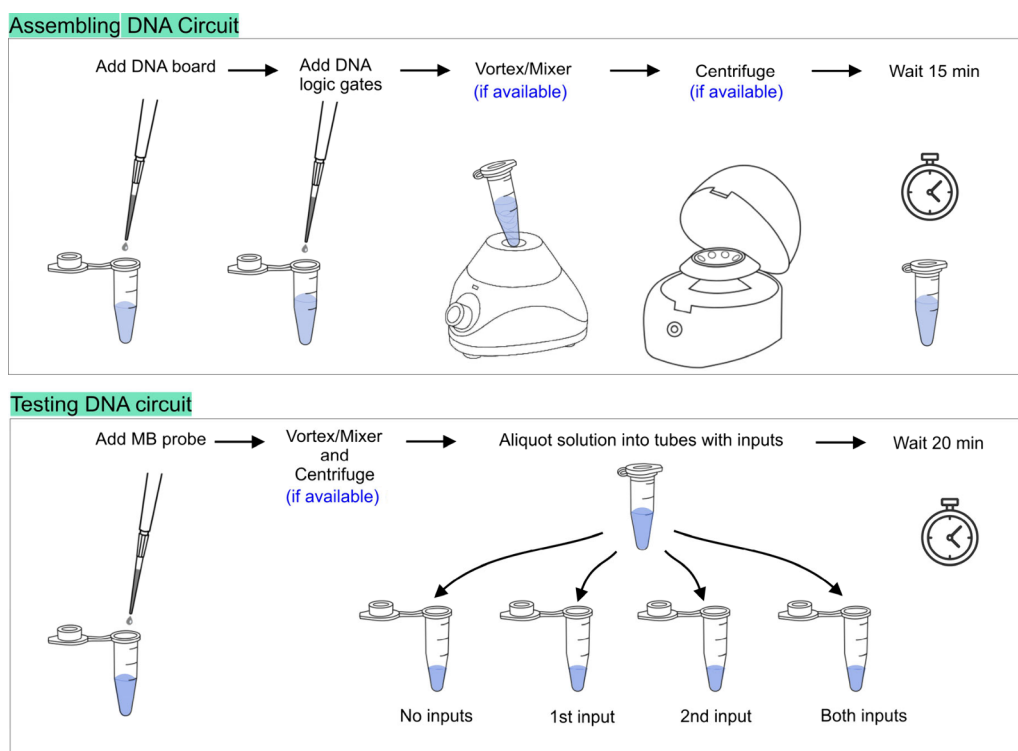


Figure 5. Outline of the two-step experimental procedure for the construction of the DNA circuits and their testing with different input combinations. Use of a vortex/mixer and centrifuge is considered optional. Alternatively, samples can be mixed by gently flicking the tubes, and the solutions can be forced to the bottom of the tube by tapping the tube against a surface.

(each with different aberrant expression levels) can be a challenging task. We selected miRs whose over- and under-expression have been correlated with the development, tumor growth, and metastasis of HCC.^{33,39,40}

Synthetic miR HCC markers were given to the students. An OR logic circuit was designed to recognize the selected overexpressed miR, while the NAND logic circuit could identify an underexpressed miR. The IMPLY logic circuit could bind to one overexpressed and one underexpressed miR. During the lecture ([Supporting Information \(SI\), Lecture Slides](#)), we introduced the biological background of miRs and how digital values could be assigned to their under- and overexpression during testing with each DNA logic circuit. To simplify interpretation of the DNA circuits' response, the sample was classified as "cancerous" if the miR input(s) triggered a high fluorescence signal. Conversely, low fluorescence indicated a "healthy" state.

CURRICULUM DESIGN

This laboratory activity provides students with a learning experience of DNA molecular computing, a multidisciplinary technology. [Table 1](#) lists the specific learning objectives and outcomes. The laboratory class can take place in one 110–140 min lab period ([Scheme 1](#)). The pre- and post-tests include multiple-choice questions about the key learning concepts ([SI, Pre- and Post-Test Questions](#)). These concepts can be delivered as a 20 min lecture ([SI, Lecture Slides](#)). Students are provided with all materials, reagents, and a manual ([SI, Notes for Instructors](#) and [Student Manual](#)). The [Notes for Instructors](#) document contains a checklist of materials, a glossary of technical terms, a list of all oligonucleotide sequences, instructions to set the experiment, additional suggested experiments, and a cost estimate. The [Student](#)

[Manual](#) includes the learning objectives, reinforcing information, experimental protocol, and conclusions.

Reagents can be prepared before the class or well in advance. The experimental work consists of preparing three DNA molecular circuits (OR, IMPLY, and NAND) and using them to classify mixtures of miR markers as healthy or cancerous. Their fluorescent readout can be visually observed using a flashlight and UV/blue light-blocking goggles or measured with a spectrofluorometer. Students can draw hypotheses based on the Boolean logic truth table of each circuit and predict the expected output response for each miR mixture. After testing and interpreting the fluorescence readout, they can draw conclusions based on their experimental results.

The learning objectives are oriented on the first levels of Bloom's taxonomy:⁴¹ (i) remembering, (ii) understanding, and (iii) applying DNA molecular computing principles. However, the experiment's complexity can be increased depending on the desired learning outcomes. For learning outcomes requiring higher-order thinking skills, students can be asked to analyze the output patterns of an unknown input mixture, to interpret if the level of specific miR inputs should be high (overexpressed) or low (underexpressed) to yield high output of each logic circuit, and to categorize the input combinations into the ones corresponding to a cancerous or healthy sample.

EXPERIMENTAL OVERVIEW

Materials and Reagents

Oligonucleotide sequences (including MB probes) can be ordered from Integrated DNA Technologies, Inc. (Coralville, IA, USA). Oligonucleotide sequences, extinction coefficients, and instructions to prepare all stock solutions and buffers can be found in the [SI, Notes for Instructors](#). Blue LED flashlights

of at least 200 lm and anti-UV/blue light (525 nm cutoff filter) safety goggles are needed for visual readout. The laboratory materials can be packed in individual kits, as shown in Figure 4C.

Methods

Figure 5 provides an overview of the experimental steps followed by the students. The detailed protocols are listed in the *SI, Notes for Instructors* and *Student Manual*. Students are asked to assemble three DNA logic circuits (OR, IMPLY, and NAND) by mixing premade stock solutions of the DNA board and DNA logic gates (see *SI, Notes for Instructors*). To build the three DNA logic circuits, a total of 15 different solutions are needed: (1) DNA grade water, (2) DNA board, (3–5) each YES gate, (6, 7) each NOT gate, (8, 9) each MB probe, and (10–15) each input (Figure 4A,C).

Step 1. Assembling the DNA logic circuits: In a clean microcentrifuge tube labeled with the name of the DNA circuit, students mixed 300.0 μL of DNA board solution and the volume of DNA logic gates (YES and/or NOT) as specified in the student manual and incubated the mixture for 15 min at room temperature (22–25 $^{\circ}\text{C}$) to allow molecular assembly.

Step 2. Testing the DNA logic circuits: After the 15 min incubation from Step 1, students proceeded to test the DNA circuits by adding 15.0 μL of the specified MB stock solution into the assembled DNA circuit, mixing, and then dispensing 40.0 μL of this mixture into four new microcentrifuge tubes, each holding one of the four possible input combinations. Students observed/measured the fluorescence signal after incubating the mixtures at room temperature (22–25 $^{\circ}\text{C}$) for 20 min.

Data Collection and Analysis

If choosing a spectrofluorometer, excitation and emission wavelengths must be set at 485 and 517 nm, respectively. Spectrofluorometers provide a quantitative value, where digital output 0 is at least 3-fold lower than digital output 1. If using a blue LED flashlight, anti-UV/blue light safety goggles are needed to block the blue light background and visually observe the emitted fluorescence from the samples. This option provides a qualitative measurement, where digital output 0 shows a distinguishably lower fluorescence intensity than digital output 1, as shown in Figure 6.

The readout corresponding to each input combination (no inputs, each of the two inputs, or both inputs together) allowed its interpretation as either cancerous or healthy states (Figure 6). Students analyze the output pattern for each circuit using the corresponding Boolean truth table to determine whether overexpression (digital 1) or underexpression (digital 0) of the specific miR input triggered the cancerous output (Figure 6). Students are expected to conclude that cancerous conditions are associated with overexpression of either Input 1 or 2 based on the OR output pattern. From the NAND output pattern, it can be concluded that underexpression of either Input 5 or 6 would indicate cancer. Correspondingly, simultaneous underexpression of Input 3 and overexpression of Input 4 are required to maintain a healthy condition (Figure 6, IMPLY circuit output).

HAZARDS

The chemicals used in these experiments are not listed as carcinogens in the National Toxicology Program's 15th Report

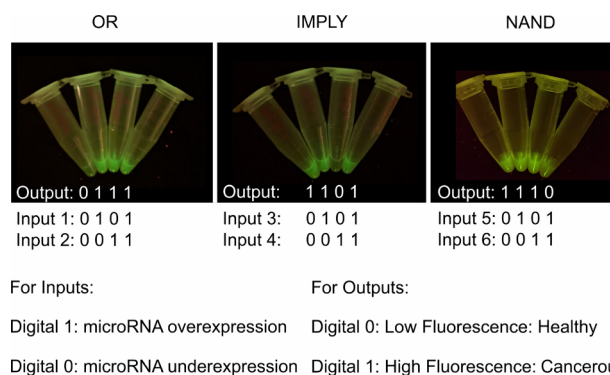


Figure 6. Data interpretation. Top: visual fluorescence readout of OR, IMPLY, or NAND logic circuits depends on the input combination. The sequences of Input 1 correspond to hsa-miR-21-5p, Inputs 2 and 3 to hsa-miR-221-3p, Inputs 4 and 6 to hsa-miR-409-3p, and Input 5 is the DNA complement of hsa-miR-221-3p. Bottom: key definitions of digital values for inputs and outputs.

on Carcinogens. For information about storage and handling, the reader can refer to the MSDS of chemical reagents used.

SAFETY

Students are advised to wear personal protective equipment (PPE): laboratory coat, gloves, and UV/blue light protective safety glasses. All containers should remain closed while not in current use and during mixing steps. It is recommended to clean the working space with soap and water before and after the experiment and that students wash their hands after finishing all experimental work.

Spills in the working space and nearby areas can be cleaned by covering the spill with a paper towel to remove the spill and then cleaning the surface with soap and water. Used paper towels were discarded with regular trash.

EXPERIMENTAL CONSIDERATIONS

Students are advised to obtain their fluorescence readouts immediately after the indicated incubation time. However, based on our experience, samples could be visualized up to 48 h later if they are stored at room temperature and covered from direct light. Instructors can construct each DNA circuit for demonstration to the class or have them as a backup for students who cannot complete all circuits. If choosing to use a spectrofluorometer, instrumental settings could require adjustment for optimal readout, and either a 45–50 μL quartz cuvette or 100 μL well-plates can be used for holding samples. Mechanical micropipettes are recommended for accurately measuring the listed volume; however, volume can be measured with disposable plastic micropipettes by drop counting (*SI, Student Manual* and *Notes for Instructors*).

RESULTS OF STUDENT ASSESSMENT

The student assessment in achieving the learning objectives was done under an IRB-approved protocol (IRB ID: 00006043). The laboratory experience was offered twice with a total of 9 volunteers (4–5 volunteers per session). The population of volunteers consisted of undergraduate students from chemistry, biochemistry, biotechnology, and biomedical sciences programs in their freshman to senior academic levels and current members of research laboratories from our institution. The recruitment was done by extending a formal invitation directly to the students or indirectly through

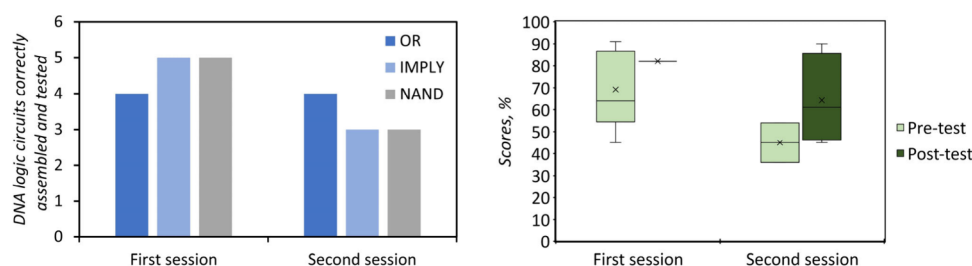


Figure 7. Students' experimental and theoretical outcomes of the laboratory activity. The left panel shows the number of DNA logic circuits correctly assembled and tested in the experiment by a total of 5 students in the first session and a total of 4 students in the second session. The right panel shows the pre- and post-test scores achieved by students in both sessions.

research laboratory advisors. Each session was scheduled based on the most voted meeting time by students who accepted the invitation. The volunteers participated as active learners and simultaneously shared their thoughts and recommendations for improving the learning experience.

Due to the two methods for fluorescence detection, students in the first session used a spectrofluorometer, and students in the second session used a hand-held lamp. We observed the spectrofluorometer challenged the students to numerically discriminate digital output 0 from digital output 1 since the fluorescence intensity values were variable. Therefore, we specified that digital output 0 would be at least 3-fold lower than digital output 1. The group that used a hand-held lamp had less struggle assigning the digital values to the fluorescence intensities. However, to ensure the effectiveness of this method, we designed a fluorometric box made of black cardstock as a tube holder to facilitate sample irradiation and simultaneously visualize all of the tubes under a dark environment/room (Figure 4B). Students acknowledged that this tool was helpful during the data collection and interpretation steps.

From the first session, 4 of 5 volunteers successfully constructed and tested all DNA logic circuits (Figure 7, left). The pre- and post-tests gave an average score of 69% and 82%, respectively (Figure 7, right), showing a 13% increase. The high average pretest score showed that students from this session had a degree of familiarity with the concepts. From the post-test scores, we observed that students' knowledge level was normalized after the laboratory class. Based on the students' comments, improvements were made to the lecture presentation, pre- and post-tests, and student manual (Table 2). All of these changes were incorporated to run the second session with another 4 volunteers. The final versions of these documents are available in the SI.

From the second session, 2 of 4 students successfully constructed and tested all DNA logic circuits, while at least one of the DNA logic circuits failed for the other 2 students (Figure

7, left). Nonetheless, the students with incorrectly responding DNA logic circuits readily identified or suggested an explanation for the corrupted readout, which gave insights into students' understanding of the taught concepts to discriminate erroneous data. The average scores for the pre- and post-tests were 45% and 64%, respectively (Figure 7, right), showing a 19% increase. The low average pretest score showed that students were less familiar with the concepts than students from the first session. Students achieved scores equal to or above the pretest average score and a highest score of 90% in the post-test. From this session, students mostly engaged in asking questions about challenges in developing the technology and its usage with actual patients. Overall, students determined which inputs would trigger a readout interpreted as "cancerous" or "healthy". However, students overlooked using the terms underexpression or overexpression when asked about the miR expression level based on the DNA logic circuit response.

Although it was not observed, one misconception could be the consideration of fluorescence as the output of the DNA logic circuits. It is important to emphasize that the DNA logic circuits used in this study (Figures 2 and 3) produce an output in the form of a nucleic acid sequence made of two fragments.^{26,27,29} The MB probe transduces the output sequence to an easily detectable fluorescence signal.

The achievement of the learning objectives and outcomes was assessed based on students' communication with the instructor, students' ability to correctly assemble and test the DNA logic circuits, students' conclusions, and students' performance on the pretest and post-test. Most students gained knowledge of the concepts taught and predicted the expected outputs for the DNA logic circuits based on their truth tables. Most students succeeded in experimentally constructing and testing the DNA logic circuits and correctly correlated the fluorescence readout with the diagnostic outcome. Regardless of the fluorescence detection method, students correctly described the principles of MB probes, such as MB probe conformational states based on fluorescence intensity, and were exposed to spectrofluorometry concepts such as incident light, wavelength selection, and light filters.

SUMMARY

Here, we report a laboratory experience of DNA molecular computing and its potential implication in biomedical practice. DNA molecular computing is a relatively recent interdisciplinary technology. Therefore, the reported laboratory activity not only introduces this topic into the educational curriculum but also contextualizes its relevance in molecular diagnostics. The experience covers the concepts of Boolean logic gates,

Table 2. Improvements after the First Session

Lecture presentation	- Addition of slides with detailed information about Boolean logic gates and connectivity. - Removal of slides about the history of DNA logic gates. - Simplification of the slides about miR and HCC.
Pre- and post-tests	- Correction of typos and addition of detailed test instructions.
Student manual	- Addition and highlighting of relevant information for data interpretation. - Reorganization of schemes and tables. - Addition/change of questions in the conclusion section.

molecular computing, MB probes, DNA logic gates, miR, and molecular diagnostics of HCC to introduce a multidisciplinary spectrum of concepts. The experiment is versatile and can be implemented in different classroom settings by offering visual observation or quantitative measurement of the fluorescence readout. It uses nontoxic and inexpensive reagents. Although the two-session pilot testing was performed with a few student volunteers, it yielded positive feedback and proved to be an engaging experiment, driving students' curiosity to know more about this novel technology.

Benefits of the Approach

As an interdisciplinary technology, DNA molecular computing integrates molecular biology, chemistry, and computer science/engineering fields. The laboratory activity reported here conveys the foundational concepts needed from these fields for the understanding and building of DNA molecular circuits for cancer diagnosis, such as Boolean logic, chemistry, and engineering of nucleic acids, fluorescence spectroscopy, and cancer biology. This experiment can be integrated into chemistry, biology, biomedical sciences, and molecular biology educational programs. Freshman to senior students can conduct the experiments individually or in groups in one class period of 110–140 min. All of the reagents and materials can be readily purchased from suppliers and are low cost. It can be offered as part of a laboratory course, conducted as a classroom demonstration, and used as an outreach activity since it does not require a special laboratory setup and is designed to be a portable self-contained kit.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available at <https://pubs.acs.org/doi/10.1021/acs.jchemed.4c00675>.

Lecture Slides (PDF)

Notes for Instructors (PDF, DOCX)

Student Manual (PDF, DOCX)

Pre- and Post-Test Questions (PDF, DOCX)

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Notes

The authors declare the following competing financial interest(s): A provisional patent was filed for the technology reported in this study.

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