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DNA nanotechnology in the undergraduate laboratory: Analysis of molecular topology using DNA nanoswitches

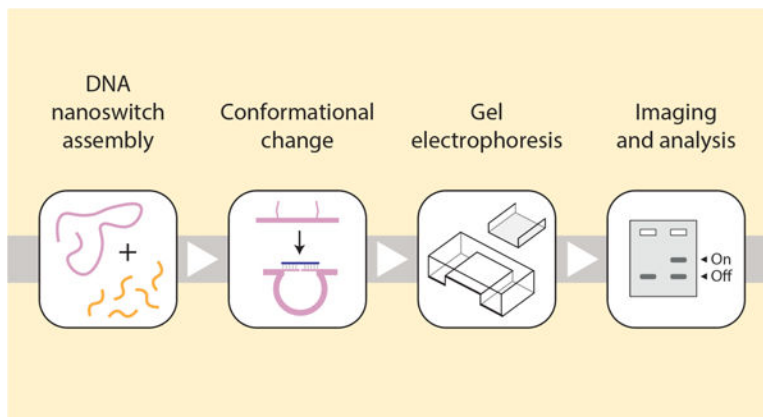
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Abstract

There is a disconnect between the cutting-edge research done in academic labs, such as nanotechnology, and what is taught in undergraduate labs. In the current undergraduate curriculum, very few students get a chance to do hands-on experiments in nanotechnology-related experiments most of which are through selective undergraduate research programs. In most cases, complicated synthesis procedures, expensive reagents, and requirement of specific instrumentation prevent broad adaptation of nanotechnology-based experiments to laboratory courses. DNA, being a nanoscale molecule, has recently been used in bottom-up nanotechnology with applications in sensing, nano-robotics, and computing. In this article, we propose a simple experiment involving the synthesis of a DNA nanoswitch that can change its shape from a linear “off” state to a looped “on” state in the presence of a target DNA molecule. The experiment also demonstrates the programmable topology of the looped state of the nanoswitch and its effect on gel migration. The experiment is easy to adapt in an undergraduate laboratory, requires only agarose gel electrophoresis, a minimal set-up cost for materials, and can be completed in a 3-hour time frame.

Graphical Abstract



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SUPPORTING INFORMATION

Online supporting information contains: (1) Reagent list, (2) detailed experimental procedures, (3) notes for instructors, (4) student handouts and (5) results from undergraduate students. For convenience, figures in instructor notes are labeled I#, and figures in students' handout are labeled S# in the SI.

CONFLICT OF INTEREST

A.R.C. and K.H. are inventors on patents and patent applications covering aspects of the DNA nanoswitch design and applications.

Keywords

Upper-Division Undergraduate; Biochemistry; Interdisciplinary/Multidisciplinary; Hands-On Learning/Manipulatives; Electrophoresis; Molecular Properties/Structure; Nanotechnology; Nucleic Acids/DNA/RNA; Undergraduate Research; Biophysical Chemistry

INTRODUCTION

As part of the undergraduate education program, laboratory courses have found as much importance as lectures and presentations.^{1,2} Laboratory courses complement existing lectures and enhance students' education beyond just theoretical knowledge. Emphasis on laboratory courses has seen steady rise over the past few decades,² with most basic courses in chemistry, biology or physics accompanied by a lab course. In some cases, already existing fields (forensic science, for example),^{3,4} have been updated with newly proposed laboratory experiments for students.⁵ This emphasis has only recently been devoted to newer fields such as nanotechnology, which are more research-based compared to standard curricula. Undergraduate students can benefit from engagement in research labs, and some of these skills can be developed by introducing new laboratory exercises to be part of existing courses.⁶ According to the National Academies and the American Association for the Advancement of Science, early student engagement in project-based laboratory exercises can improve the level of student participation in research.^{7,8} The need for new laboratory exercises that mirror current developments in research is also due to the increasingly interdisciplinary nature of research within the chemistry and other communities.⁹

The field of DNA nanotechnology involves the construction of nanoscale shapes and devices using DNA.¹⁰ While structural DNA nanotechnology deals with the construction of different types of architectures, dynamic DNA nanotechnology predominantly involves reconfigurable devices that can change shapes on sensing a biological (eg: nucleic acids) or chemical (eg: pH) stimulus.¹¹ There are many academic labs currently doing research in this field, but there are only very few courses designed on the topic for undergraduate students. The importance of discussing self-assembly and nanotechnology to students has been presented in this Journal before.¹² Nanotechnology-based experiments have previously been proposed for the General Chemistry lab courses.¹³ These proposals are typically materials science oriented with heavy focus on inorganic nanomaterials. Here, we propose a lab experiment based on a reconfigurable DNA nanoswitch, for third year biology or chemistry undergraduate students, specifically those in biochemistry and genetics lab courses where students already run gels (as in our own institution). This experiment will address two teaching concepts: (1) Experimental demonstration of dynamic DNA nanotechnology concept used for biosensing, and (2) Simple validation of topological changes in DNA using gel electrophoresis. Laboratory exercises designed to mimic research experience often fall under the "expository instruction" category where the outcome is predetermined or known to both the instructor and the students.¹⁴ However, these experiments not only help students understand what they have learned in theory,¹⁵ but also improve their hands-on skill set in the laboratory (for example, pipetting and electrophoresis).^{16,17} Some related experiments described in this journal include DNA topology analysis based on supercoiled DNA and

Topoisomerase I enzyme.¹⁸ Lab experiments based on gel electrophoresis have also been reported.^{19,20} Other DNA related experiments previously discussed in this Journal include observation of DNA molecules using fluorescence microscopy and atomic force microscopy (AFM).²¹ However, these are complicated techniques to be adapted for a larger chemistry or biology class and especially with instruments like AFM that are sensitive to noise and vibrations in the room.²² In this context, the experiment we propose will be an introductory peek into DNA nanotechnology, and help students understand characteristics of DNA devices. In addition, this exercise also helps understand gel electrophoretic patterns based on structure or topology in contrast to the typical migration based on molecular weight.²³ With some investment on component DNA strands, this experiment can be executed using equipment already available in a typical biology or biochemistry laboratory, and does not require expensive instrumentation or microscopy to readout and analyze results.

DNA NANOSWITCHES: DESIGN AND WORKING PRINCIPLES

Apart from being well-known as the genetic material, DNA is also an inherently nanoscale molecule that is used for bottom-up fabrication of nanostructures, where molecules are assembled together to form nanometer scale objects.¹⁰ DNA-based nanostructures can be planar or 3D objects, multi-dimensional arrays and programmable devices that can respond to external stimuli.²⁴ DNA nano-objects have found applications in drug delivery while periodic lattices and frameworks made from DNA are useful as scaffolds to align guest molecules such as proteins and nanoparticles.¹⁰ Devices made from DNA are useful in biosensing applications, where presence of a target molecule or biomarker causes a conformational change in the DNA device, leading to a signal readout.²⁵ One such example is the DNA nanoswitch,²⁶ constructed based on the principles of DNA origami, where a long strand of DNA scaffold is folded into any desired shape using short, complementary strands.²⁷ The DNA nanoswitch is a linear double stranded DNA consisting of a single-stranded M13 scaffold (7249 nt) and short complementary strands (49–60 nucleotides) called staples or in this case “backbone oligonucleotides” (Figure 1). Although any long single-stranded DNA can be used, M13 DNA is a popular choice as scaffold in the field of DNA origami since it is commercially available and cheap. Two of the backbone oligonucleotides can be modified to have single-stranded extensions (“detectors”) that are partly complementary to a target oligonucleotide. Once the target is added, it binds to both detectors of the nanoswitch and reconfigures it to form a loop. We call this linear form the “off state” and the looped configuration the “on state” of the DNA nanoswitch. The presence of the target can be identified by running the samples on an agarose gel as the linear (off) and looped (on) states of the nanoswitch migrate differently (Figure 1, inset). These DNA nanoswitches have previously been used for single-molecule studies,²⁸ biomolecular analysis,²⁹ in rewritable memory,^{30,31} and biosensing.^{26,32} Since the readout of the nanoswitch is topology-based, we use the programmable nature of the nanoswitch to create a laboratory experiment to analyze molecular topology and gel migration.

HAZARDS

1. Use mittens or tongs while handling hot flask of melted agarose as it may burn your skin.

2. Hot-plates have to be turned off and unplugged right after use to avoid fire hazard.
3. Care should be taken when plugging in gel boxes into power supplies to avoid electric shocks.

RESULTS AND DISCUSSION

The visual readout of the two states of the DNA nanoswitch is a simple system to study topological changes of DNA-based structures and the effect of topology on gel migration (instead of the “usual” molecular weight-based separation). The topology of the looped state can be further programmed by changing the detector positions on the scaffold DNA, and in turn changing the loop size (Figure 2a). To systematically study the effect of loop size on gel migration, we redesigned the nanoswitch to form 5 different loops of various sizes. In the design shown in Figure 2a, the nanoswitch has six detector strands that can be triggered by different target DNA strands. The detectors are spaced ~600 bp apart, and form 5 different loop sizes on binding specific target strands (Figure 2b). The first detector (D0) is complementary to one half of all 5 target DNA strands. The other half of the target strands are complementary to any of the other 5 detectors (D1–D5). Each loop has a unique migration pattern on the gel based on the loop size (Figure 2c). This topology-based gel migration can also be a discussion point when taught in an undergraduate laboratory. In routine gel shift experiments, band separation is based on molecular weight (as seen by the ladder on the right lane). The migration of the DNA ladder can be compared to migration of the different nanoswitches which are the same molecular weight but different loop sizes. Bigger loop sizes migrate slower in the gel due to larger molecular radius. The mobility of each looped species is characterized by their retardation factors (Rf) (Figure 2d). This experiment involves only a mixing step and incubation of nanoswitch with the target strands, followed by agarose gel electrophoresis to read out the results (Figure 2e).

The lab experiment can be extended by the instructor to include a Ferguson plot for calculating friction coefficients of the looped structures. The target-bound nanoswitches, despite having the same molecular weight, show unique migration patterns on a gel due to the difference in topology (loop size). The electrophoretic mobility for each of these looped nanoswitches can be analyzed using a Ferguson plot³³ (Figure 3a–b), where the migration of looped DNA is examined on agarose gels of varying percentage (0.4% to 1%). This plot is used to estimate the frictional coefficient of the looped species as given by the slope of the line (Figure 3c and Table S1). Bigger loop size indicates an increase in effective molecular radius, thus causing retardation in the gel. This trend is defined by the change in loop size between the different nanoswitches (roughly 600, 1200, 1800, 2400, 3000 base pairs). Students learn about the effect of molecular topology on gel migration and compare it to sized-based gel separation. This experiment also indicates how DNA nanostructures and DNA structures in general (such as circular versus linear DNA) are characterized.

ADAPTATION TO UNDERGRADUATE LABORATORY

Exposure of students to DNA nanotechnology research is predominantly through their involvement as interns in research labs in the field. As part of their regular curriculum,

undergraduates do not typically have a course in DNA nanotechnology except in a few universities. The nanoswitch-based topological analysis we propose is easily adaptable to a chemistry, biology or a biochemistry laboratory. Similar to typical lab experiments, the nanoswitch materials can be prepared beforehand by the teaching instructor and provided to students (Supplementary Note 1: Reagent preparation for instructor). Students can assemble the nanoswitch, add target strands, incubate and execute gel electrophoresis for the samples. Students can analyze the gels and measure Rf values as a post-lab exercise. We modified previously published experiments to fit a typical laboratory course timing (~ 3 hours) and provide a detailed step-by-step protocol in the supporting information file (Supplementary Note 2: Student instruction manual). We have used this protocol as a training experiment for undergraduate students in our research laboratory (typically sophomores, juniors and seniors). Students were provided with the protocol in Supplementary Note 2 and the materials, and all of the students completed the exercise within the proposed time. The Ferguson plots created using data from the gels showed expected trends, and these were consistent between different students. We have included representative results of the experiments performed by undergraduate students in Figure 4. These results suggest that the DNA nanoswitch system is a feasible platform for adaptation to undergraduate laboratories, not just in a topological context but also in potential biosensing³² and biophysical applications.²⁸ Instructors can adapt the nanoswitch for other nucleic acid targets or to explain different concepts using guidelines in our earlier work.^{32,34}

From the outcome of these experiments, we list here a few considerations for adaptation to undergraduate laboratory, as well as steps that might not be successful.

1. Importance of mixing step: In some cases, students may forget to add the target DNA which will result in no loop formation in that lane. Instructors can run a control gel from the material prepared to keep as a reference.
2. The experimental design allows combining data from different students. Thus, if one of the gels does not provide expected data (or other reasons), there will still be three data points that can be used to fit the trend.
3. Overheating of agarose solutions and evaporation can result in a higher gel percentage than prescribed, resulting in altered migration.

One additional parameter for adapting to undergraduate laboratories is the cost of the materials. The starting materials for the DNA nanoswitch are tabulated in Table S2. A start-up investment to buy the DNA scaffold and backbone strands at the lowest synthesis scale is ~\$450 (purchased from IDT). Combined with other reagents such as agarose and enzymes, the total set up cost is ~\$1000. These materials including the DNA can be used for more than 5 years, thus having an estimated average cost of \$200/year (or \$100/semester). To offset the initial trial phase for community colleges that cannot afford this, our lab is also open to providing aliquots of the backbone oligonucleotide mix to programs or universities on reasonable request. These materials can be used for many years if kept frozen, and the cost per lane for the experiments described here is only ~3 cents/lane, with the most expensive reagent being the agarose. Thus, the proposed experiment may suit the budget of most undergraduate laboratory courses. In addition, this experiment uses GelRed nucleic acid stain, which is non-toxic compared to ethidium bromide staining. The experiment is

safe for undergraduate students, with no requirements for specific waste disposal once the experiments are finished (gels discarded in regular trash and buffers can be discarded in sinks).

CONCLUSION

In this article, we described how a simple DNA nanostructure can be used to analyze molecular topology and gel migration in an undergraduate laboratory setting. The nanostructure is easy to assemble, and the mixtures can be frozen for long term use. Concepts discussed in this laboratory experiment can be extended to other DNA nanostructures as well as complement any existing courses or lectures on DNA nanotechnology.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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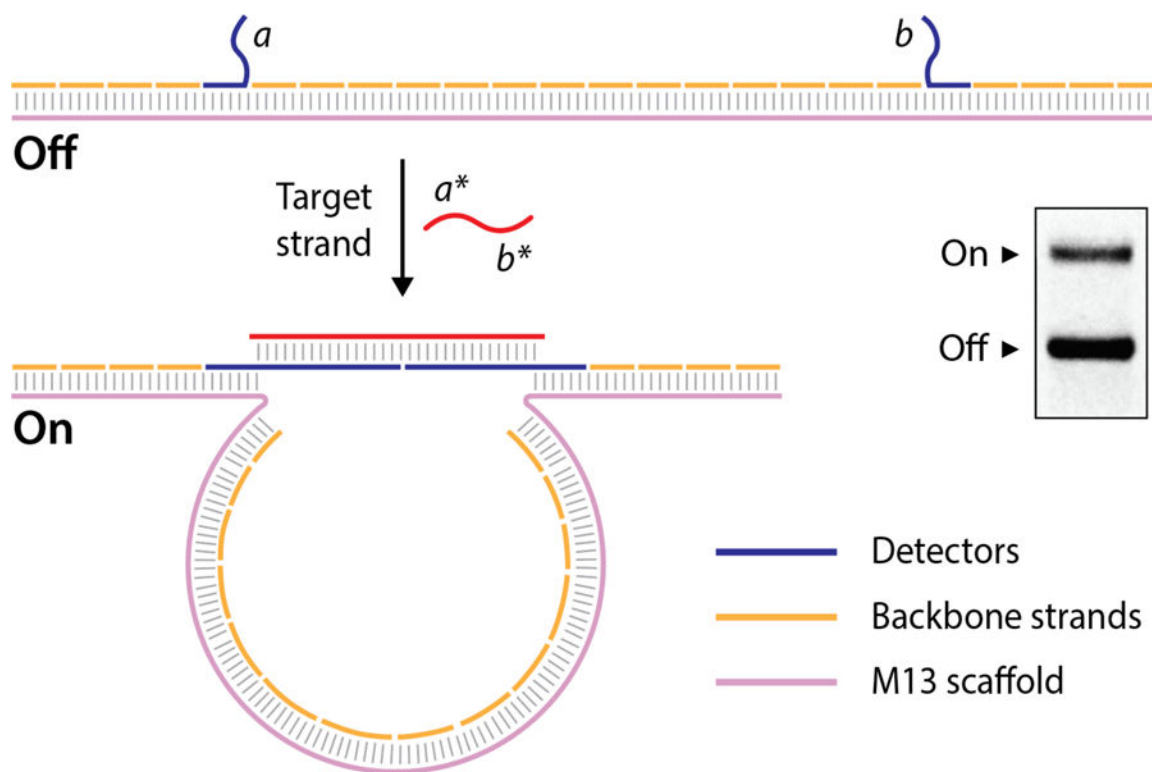


Figure 1. Design and working principle of the DNA nanoswitch.

The “off” state of the nanoswitch is a duplex, with two single-stranded extensions (detectors) that are partially complementary to parts of a target DNA strand. Target recognition and binding causes the formation of the looped “on” state. The off and on states of the nanoswitch can be easily identified on an agarose gel (inset).

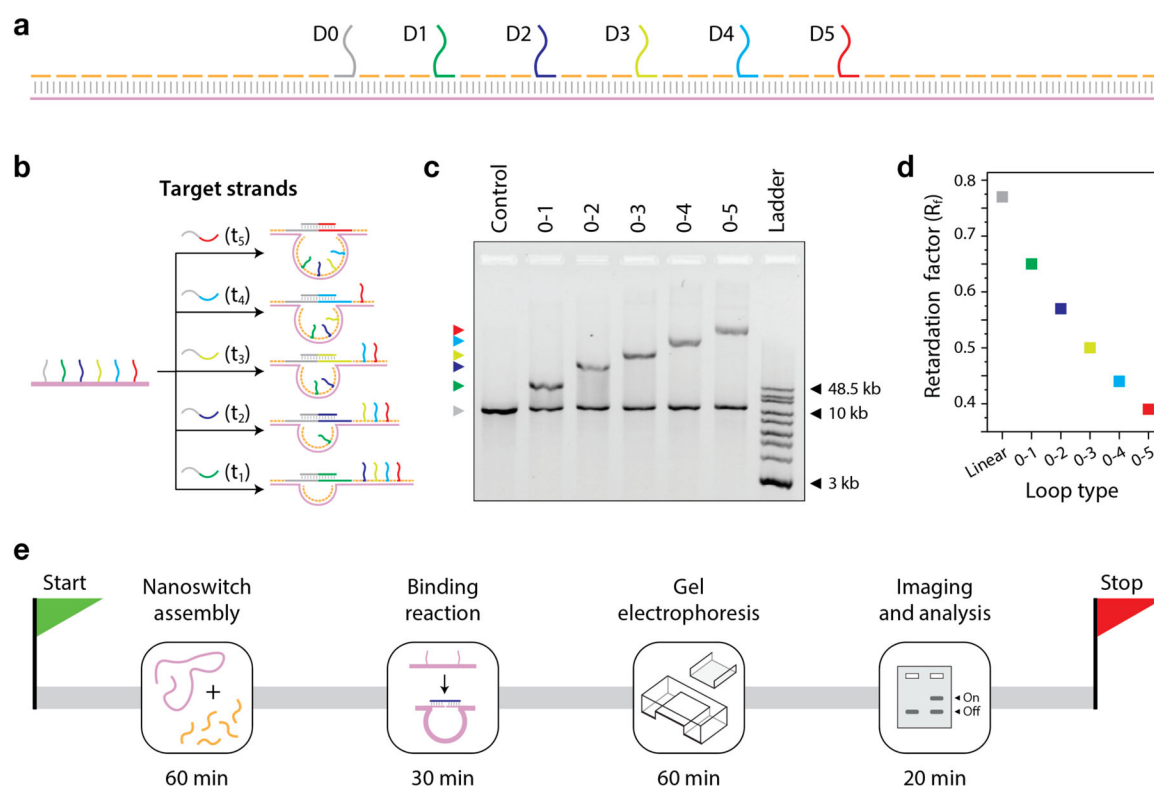


Figure 2. Analyzing reconfiguration of nanoswitch and loop size vs gel migration.

(a) Design of the multi-detector nanoswitch that can respond to five different targets. (b) Different target DNA strands activate the formation of different loops. (c) Nanoswitches forming different loops migrate differently on an agarose gel provide a unique signal. (d) Measured retardation factors (R_f) for different loop sizes. (e) Workflow for a lab-based experiment for an undergraduate course.

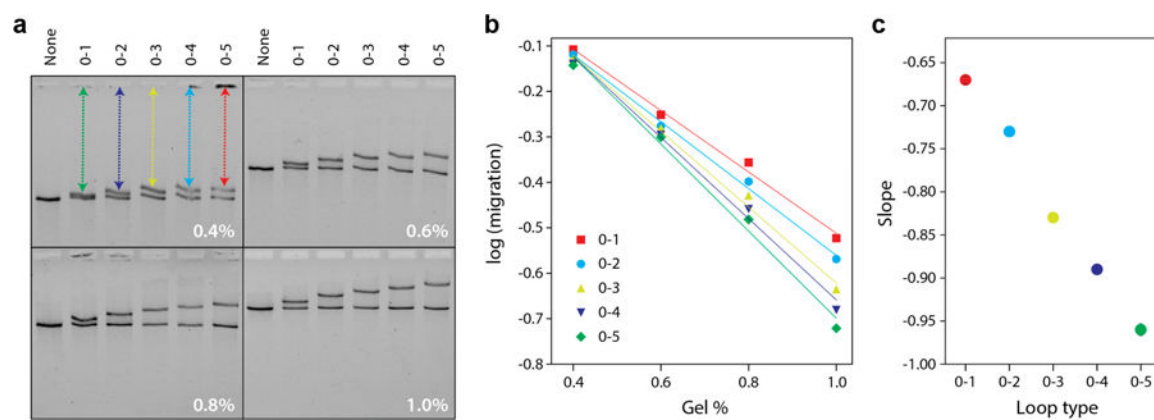


Figure 3. Ferguson plot analysis of looped DNA nanoswitches.

(a) Migration of different loop sizes in various agarose gel percentages. (b) Ferguson plot of the migration vs gel percentage. (c) Slope of the Ferguson plot provides the frictional coefficient for each looped species.

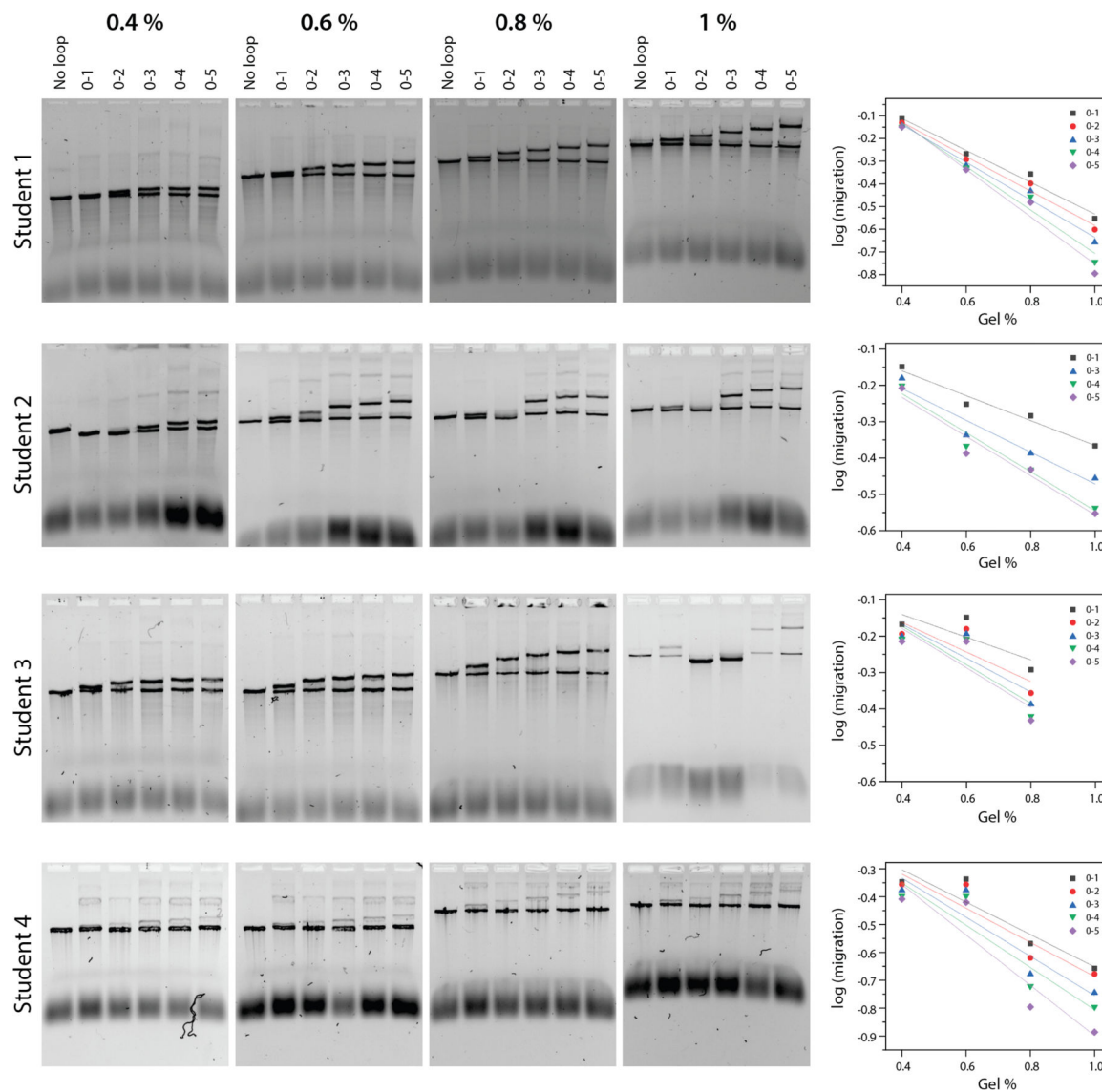


Figure 4. Representative results from experiments performed by undergraduate students. Students were given the manual in Supplementary Note 2. All students finished the lab work within 3 hours as proposed in our experiment. Gel analysis was done later using ImageJ and trends plotted using Origin. Students can use other software such as Microsoft Excel to plot the results. If a gel by a student is inconsistent, student groups can analyze other three gels to obtain the Ferguson plot (for example, results from Student 3).