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Gene Therapy Research & Methods: A Novel Course that Provides a Structured yet Independent Research Experience for Undergraduates

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Abstract

Like many other professors, I receive an excess of requests from undergraduate students looking for research opportunities, but I am limited by the amount of time and resources available to me. I have also attended advisory board meetings in which the members have stressed the importance of giving students hands-on research or lab experience with practical methods to prepare them for the workforce. I sought to address both of these issues by developing a novel course that combines the structured elements of a traditional lecture course with the independence of a research project to give a class of undergraduate students a meaningful and practical learning experience within a single semester. The desired outcome of this course is a well-trained and semi-independent group of undergraduate researchers with an appreciation of experimental design, specific laboratory methods, and data analysis. The achievement of this outcome was measured via both traditional student surveys and data from real experiments in which the students were able to successfully address a real-world problem.

The new course, entitled "Gene Therapy Research & Methods", is a novel and interdisciplinary hybrid lecture + lab course that is designed to give undergraduate students hands-on experience with cutting edge molecular biology and cell culture techniques. The weekly lectures focus on the gene delivery methods that are used for gene and cell therapies in the clinic, while the lab periods challenge the students to develop a strategy to improve non-viral gene delivery.

This paper describes the content developed and lessons learned over the four offerings of this course from the past 6 years. For example, the experiments conducted in the course each year have ranged from using RNA interference or Cas9 knockout of host cell proteins that inhibit gene delivery to engineering the sequence of a promoter to optimize transcription and prevent epigenetic silencing, but this paper will focus on the promoter design experiments. In either case, students were allowed to select their own targets for inhibition or design their own promoter sequences, which provides them with the opportunity to be creative, analyze recent literature, and formulate their own hypotheses (instead of performing routine experiments in a lab manual). Furthermore, mastery of each experiment is a core tenet of this course. If an experiment fails, the students are required to repeat it until it works before they move on to the next experiment, thereby lending a degree of credibility to the claim that this course is a genuine semi-independent research experience. The probability of the students' success in each experiment is bolstered by a suite of training videos and quizzes that the students must complete before attempting the experiment. I am available to help the students during each scheduled lab session, but I do not guide them through the individual steps of each experiment in a synchronized fashion.

This paper also includes the results of student surveys and comments, along with actual data that show the groups were able to design minimal promoter sequences that achieved transfection efficiencies and transgene expression levels comparable to commonly used promoters.

Introduction

Undergraduate research experiences (UREs) provide several known benefits for students, including an enhanced knowledge of science and experimental design, increased interest in post-graduate education/research, and improved resilience [1], [2], [3]. Furthermore, at least one study has shown that research experience leads to higher employment rates for undergraduate students [4]. However, while most universities strive to support a variety of programs that enable undergraduates to conduct research on or off campus, the demand for UREs typically outpaces supply due to constraints on PI availability, funding, and lab space.

Many schools already have traditional lab courses alongside independent study and senior design classes that are designed to give students some experience in the lab, but each of these course types has its own advantages and disadvantages (Table 1). For example, most senior design classes tend to be limited to upper classmen, which prevents underclassmen from getting research or design experience that could help them secure a summer internship. Younger students may be eligible for independent study courses, but the availability of those positions is limited. Traditional lab courses are available to every student, but they are typically not regarded as genuine research experiences.

These issues have motivated several programs to develop **course-based undergraduate research experiences (CUREs),** in which a class of several students is tasked with addressing a single research question that has unknown or multiple possible outcomes [5]. In this way, several students can simultaneously gain research experience (instead of the more common approach of hiring a single student or group of students to investigate a research question as an independent study). CUREs have been shown to yield several benefits for students, including enhanced self-confidence in critical thinking and application of the scientific method [6], [7], increased retention of course material [8], and increased involvement of first-generation students and other demographics in research [9]. Instructors may also benefit from CUREs by choosing a research question that is directly relevant to their own research program or lab. Overall, the goal of most CUREs is to enhance undergraduate participation in research by providing new opportunities that give a larger number of students experience in experimental design, analysis of novel data, and exposure to a variety of lab practices.

Table 1 – General characteristics of lab-based courses.

Type of Class	Open to:	Lecture?	Independence	Novelty	Creativity
Course-based UG Research Experience (CURE)	FR-SR	Yes	Medium	High	High
Traditional lab	FR-SR	Yes	Low	Low	Low
Independent study	Variable	No	High	High	Variable
Senior design	SR only	Variable	Medium	Variable	Variable

This paper describes a CURE that I developed with a single weekly 1.5 hour lecture that covers materials that help students learn fundamental concepts that are relevant to the experiments that they will do in the lab. **Each of these lectures is prepared at a sophomore level to allow underclassmen to confidently take the class.** The lectures are also recorded to allow students to revisit them on demand. In addition to recorded lectures, I have also prepared training videos for

each experiment (available upon request). Students are required to watch these videos and then earn a 100% grade on corresponding quizzes (after up to 3 attempts) before conducting an experiment. This approach prepares the students to work independently during lab. As the instructor, I am present in the lab to provide raw materials and limited troubleshooting assistance, but the students are otherwise expected to complete each experiment on their own with minimal intervention from the instructor.

The lab portion of the course has a scheduled meeting time (3 hrs/week), but students are welcome to come to lab any time during normal working hours to complete experiments at their own pace (only after completing the corresponding quizzes and getting approval to work in the lab from the instructor or a TA). This flexible schedule allows me to require that students repeat an experiment if it fails. Unlike some other lab courses that follow a set routine of experiments from week to week, which forces students to move on from an experiment even if it fails, **students in this mastery-based course are required to repeat an experiment if it fails**. Furthermore, 35% of the overall grade is dependent upon students successfully completing all the experiments. Another benefit of this flexible schedule is that it allows the students to complete the assigned tasks at their own pace, which more closely mimics a genuine research experience than a lab class with a rigid weekly schedule. Indeed, I am pleased to report that multiple ambitious students have taken the initiative to work ahead and complete most of their experiments before midterms!

Finally, **this course also requires students to apply their creativity and critical thinking skills to a specific research problem.** The course could be adjusted to address a variety of biomedical engineering problems, but this paper will describe the most recent offerings of this course, which have focused on **Gene Therapy Research & Methods**. Since the approval of the first gene therapy in 2017 (Kymriah, [10]) by the U.S. F.D.A., the market for gene therapies has shown strong, exponential growth (Figure 1) [11]. Furthermore, using employment data from the city of Philadelphia as an example, the number of jobs in the cell and gene therapy industry doubled from 2019 to 2022 [12]. Therefore, there is a strong current and future demand for students that can work in this field, which requires advanced knowledge and experience with applied genetics, cell culture, and associated techniques.

While gene and cell therapies have successfully saved many lives, they do have some significant disadvantages. For example, most of the FDA-approved therapies use either a lentivirus (LV) or adeno-associated virus (AAV) to deliver therapeutic genes. Each of these viruses has its own disadvantages, ranging from the oncogenesis induced by non-specific integration of genes by LV or the eventual silencing of the episomes delivered by AAVs [13]. In addition, both viruses share one common disadvantage - their genomic capacities are relatively limited. An AAV capsid can only hold 4.5 kbp of DNA [14], while lentiviruses are limited to 6-9 kbp [15]. This may be sufficient to treat some monogenic disorders caused by the mutation of smaller genes, but it disqualifies these viruses from treating disorders caused by larger genes or a set of multiple genes. In this course, **students are challenged to address this issue of cargo capacity by designing their own compact promoter sequence**, which could potentially be used in real viral or non-viral gene therapies to free up space for large therapeutic genes. For example, while most of the promoters that are commonly used for gene therapy are over 1 kbp (e.g., EF1α = 1.2 kbp, CAG = 1.8 kbp), students are required to limit the size of their promoter to 500 base pairs or less. The students then have a company (e.g., Life Technologies) synthesize their promoter sequence, which they clone into a GFP expression plasmid that is evaluated by delivering it to HEK-293T cells with Lipofectamine LTX. This non-viral gene delivery approach is different from the viral methods

used in the clinic, but it is less time-consuming, more affordable, and safer. Students subsequently use fluorescent microscopy, qPCR, and flow cytometry to analyze the effectiveness of their promoters in terms of transgene delivery and expression. Since students start this process by designing their own promoter sequences and then performing the cloning and transfection experiments at their own pace (and multiple times, if necessary) with minimal assistance, it is appropriate to call this course a genuine research experience that prepares them for a career in the gene and cell therapy industry or graduate studies. Finally, if this course is adopted at other schools, it could allow students to analyze the sequences and results of previous students to iteratively design better compact promoters that could have a significant impact on the field of gene therapy.

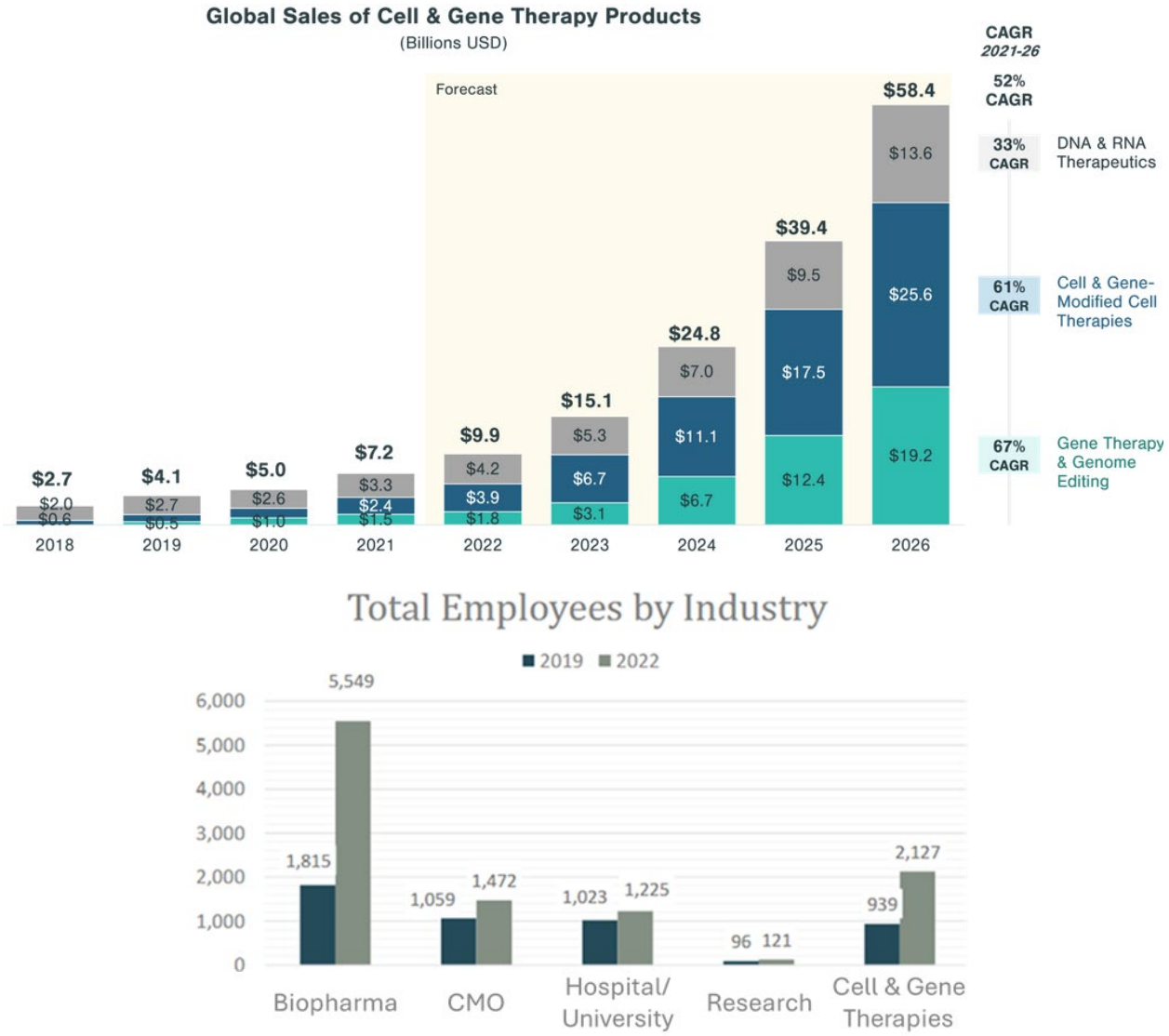


Figure 1 – Recent growth of the gene and cell therapy industry. Top: Growth of market for gene and cell therapies[11]. Bottom: Employees hired to various fields in Philadelphia from 2019-22 [12]

Learning Objectives

The following sections will describe the learning objectives and outcomes for the lecture and lab portions of the course, along with the specific workflow of the experiments conducted in the lab. As previously mentioned, the course includes a weekly 1.5 hour lecture. Each lecture is designed to prepare students for the experiments they conduct in lab and/or introduce concepts that are relevant to the field of gene and cell therapy. A list of the specific learning objectives for this course is shown below, while the weekly schedule of lecture topics is shown in Table 2. Copies of the lecture slides are available upon request from the corresponding author. There are no prerequisites required for this course, but the students are given access to a variety of review videos prepared by the instructor that help them to get up to speed on biological concepts as necessary.

- **Objective 1: Students will understand how transcription is regulated in cells.**
  - **Outcome 1:** Students will use this knowledge to construct a novel promoter that delivers high level gene expression in HEK-293T cells.
  - **Outcome 2:** Students will appreciate the importance of epigenetic mechanisms and how they can eventually silence a therapeutic gene.
- **Objective 2: Students will understand how translation in regulated cells.**
  - **Outcome 1:** Students will be able to design a gene sequence with an optimal sequence and structure that is not inhibited by 2' structures or host cell codon bias.
  - **Outcome 2:** Students will know how to enhance translation by flanking their gene with sequences that increase nuclear export or stabilize the mRNA transcript.
- **Objective 3: Students will learn the physics and biochemistry concepts that are necessary to understand common biotechnology methods (e.g., microscopy)**
  - **Outcome 1:** Students will be able to conduct and troubleshoot the experiments required to measure transfection efficiency.
  - **Outcome 2:** Students will be able to apply the methods used in this class to applications outside of the scope of this class (e.g., we only use sequencing to confirm plasmid sequences, but they could use it to identify genetic disorders)
- **Objective 4: Students will be familiar with the benefits and risks of gene therapies.**
  - **Outcome 1:** Students will be able to design viral gene therapies that provide durable, high-level transgene expression without adverse side effects
  - **Outcome 2:** Students will be able to effectively explain and weigh the risks and benefits of gene and cell therapies to patients or other lay people
- **Objective 5: Students will construct eukaryotic gene expression cassettes.**
  - **Outcome 1:** Students will use Gibson Assembly to insert a promoter into a GFP expression plasmid in a lab setting.
  - **Outcome 2:** Students will learn how to build sequences for lentiviruses and AAVs in the lecture portion of the course.
- **Objective 6: Students will learn how to culture and transfect cells.**
  - **Outcome 1:** Students will be able to aseptically culture human cells.
  - **Outcome 2:** Students will be able to deliver genes to human cells with a variety of non-viral techniques.
  - **Outcome 3:** Students will be able to qualitatively and quantitatively measure transgene expression via microscopy and flow cytometry (respectively).
  - **Outcome 4:** Students will be able to design qPCR experiments to measure the concentration of target genes or plasmids within eukaryotic cells.

Table 2 – Schedule of lecture and lab topics

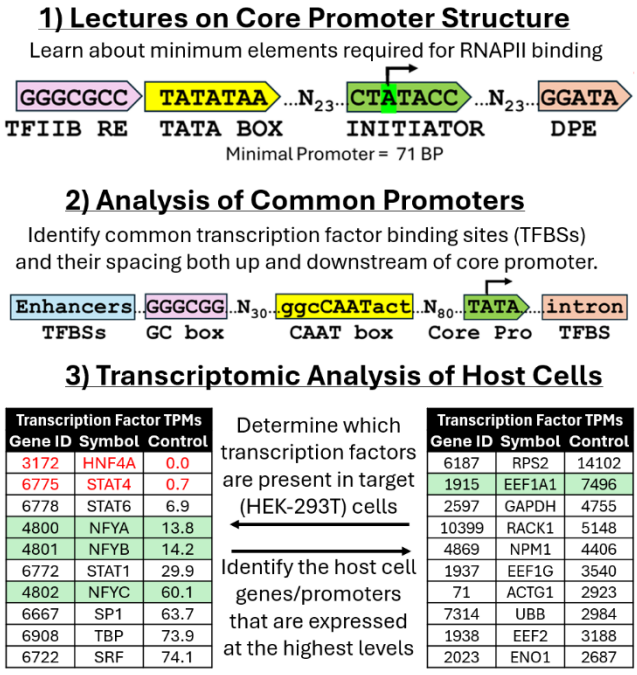
Week	Lecture	Labs/Tasks
8/25	Course Overview	**Task 1: Design a novel compact promoter** **Workshop:** Promoter design/analysis tools + literature **Workshop:** Identify TFs + strong promoters (mRNA-seq) **Workshop:** Cloning methods(Gibson Assembly)
9/1	Labor Day	
9/8	Non-Viral Gene Delivery (NVGD) Methods I	
9/15	Non-Viral Gene Delivery (NVGD) Methods II	**Task 2: Clone new promoters into expression plasmid** Lab 1a: Digestion of pEF-GFP with SalI-HF (1 hr) Lab 1b: Agarose gel electrophoresis (45 min) Lab 1c: Purification of plasmid backbone (1 hr) Lab 2a: Gibson Assembly (1 hr) Lab 2b: Transformation of DH5a E. coli (2 hrs) Lab 3a: Small scale *E. coli* culture Lab 3b: Plasmid DNA isolation/miniprep (1 hr) Lab 3c: Nanopore DNA sequencing
9/22	Cas9-Mediated Genomic Editing (Knock out/in)	
9/29	Optimizing mRNA Transcripts	
10/6	Technical Writing Seminar	
10/13	**Fall Break**	
10/20	Midterm Exam	**Task 3: Compare transfection methods** Lab 4: Transfection of HEK-293T cells with PEI Lab 5: Transfection of HEK-293T cells w/Lipofectamine Lab 6: Electroporation of HEK-293T cells **Task 4: Compare novel promoters to EF1α + MinPro** Lab 7: Lipofection to compare promoters *(repeat lab 7 a total of three times)* Lab 8: Measure plasmid copy number via qPCR
10/27	qPCR and rt^{2}PCR: Plasmid Copy Number	
11/3	Next-Gen DNA Sequencing	
11/10	Fluorescent Microscopy & Flow Cytometry	
11/17	Adeno-Associated Virus Therapies in the Clinic	
11/24	Lentiviral Gene Therapies in the Clinic	**Thanksgiving Break**
12/1	Adenoviral Gene Therapies in the Clinic	Finish transfections and flow cytometry, as necessary Prepare final lab reports
12/8	Herpes Simplex Viruses for Cancer Therapy	

Lab Structure: Tasks

The lab portion of the course includes experiments that allow the students to design and evaluate their novel promoters. These experiments were also selected because they utilize methods that are commonly used in other fields and industries. Experiments are also grouped together into "Tasks" that the students must successfully complete to pass the course (e.g., each Task accounts for 5-10% of their grade). Each task is described on the following pages and listed in Table 2. A complete copy of the lab manual with the protocols used for each experiment is available upon request.

Task 1: Design a compact promoter that expresses a gene at high levels in eukaryotic cells

No experiments are conducted in the first 3 weeks of the course. Instead, each scheduled lab period is used to hold interactive workshops that teach the students about the structure of core or minimal promoters, manipulation of epigenetic mechanisms to enhance transcription, and how to construct effective gene expression cassettes (Figure 2). These lectures give students the tools they need to design a 500 bp promoter from scratch, while also showing them how to use different programs to guide their designs. For example, students use Transfac GTRD [16] to identify transcription factors present in commonly used promoters like EF1α, which they can then utilize in their own sequences. They are also required to analyze transcriptomic data from the target cells used in lab (HEK-293T) to determine which transcription factors are present in the host cells. This exercise helps students identify transcription factors with known DNA binding sites that they can incorporate into their own promoter (e.g., NFY). The transcriptomic data can also be used to identify the host cell genes with the highest expression levels. For example, Figure 2 shows that the EEF1A1 gene has one of the highest expression levels (TPM = transcripts per million sequencing reads) in HEK-293T cells, which is one of the reasons that the promoter for this gene (EF1α) is commonly used in gene delivery experiments (and as a positive control in our project!). In our workshops, I show the students how to locate and retrieve the promoters for host cell genes like EEF1A1 using NCBI Entrez so they can analyze them with Transfac and use them as a scaffold to design their own promoters. At the conclusion of these workshops, students are required to submit a proposal with their promoter sequence and a rationale for how it was designed. The students have the freedom to design their sequences however they wish, but I make myself available to discuss any ideas they may have.



Transcription Factor TPMs		
Gene ID	Symbol	Control
3172	HNF4A	0.0
6775	STAT4	0.7
6778	STAT6	6.9
4800	NFYA	13.8
4801	NFYB	14.2
6772	STAT1	29.9
4802	NFYC	60.1
6667	SP1	63.7
6908	TBP	73.9
6722	SRF	74.1

Transcription Factor TPMs		
Gene ID	Symbol	Control
6187	RPS2	14102
1915	EEF1A1	7496
2597	GAPDH	4755
10399	RACK1	5148
4869	NPM1	4406
1937	EEF1G	3540
71	ACTG1	2923
7314	UBB	2984
1938	EEF2	3188
2023	ENO1	2687

Figure 2 – Schedule of interactive workshops that show students how to design promoters.

Task 2: Use molecular biology techniques to construct a plasmid with a synthetic promoter

Students begin their lab work by constructing a eukaryotic expression plasmid for green fluorescent protein (GFP) that is driven by their synthetic promoter (Figure 3). The process starts with a restriction enzyme digest that excises a weak minimal promoter out of a GFP expression plasmid (pMinPro, which is later used as a negative control that provides low expression levels).

The digested plasmid is then run alongside a ladder and a sample of uncut plasmid on an agarose gel to confirm digestion and separate the plasmid backbone from any undigested parental plasmid. For example, Figure 3 shows an example of an agarose gel from the class in which the digest was only partially complete. Indeed, the uncut plasmid sample in lane 3 shows two bands (a lower band representing supercoiled plasmid and an upper band that represents nicked or closed circular DNA), one of which aligns with the top band in the digested samples. The students

carefully cut the digested plasmid (i.e., the lower of the two bands in the digest sample lanes) out of the gel and then use a gel extraction kit (Zymo) to extract the purified plasmid fragment from the gel for subsequent cloning.

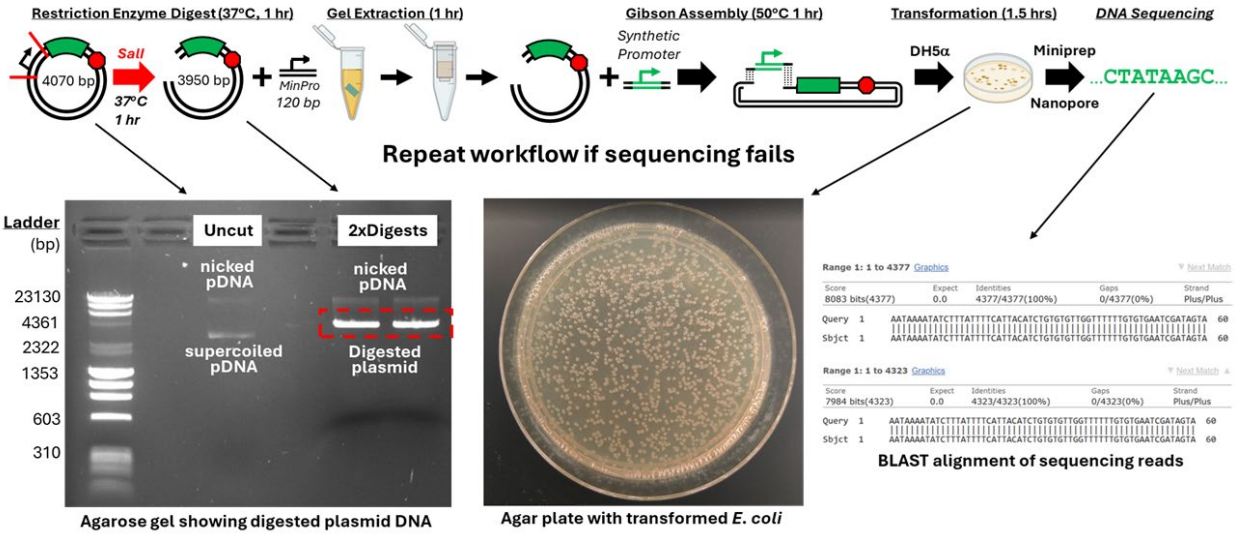


Figure 3 – Task 2: Construction of a GFP expression plasmid with a compact synthetic promoter.

In the next experiment, students mix the purified plasmid fragment with their synthetic promoter sequence. The promoters are synthesized by Life Technologies ($75/each) with extensions that allow the promoters to be ligated to the digested plasmid fragment to create the desired GFP expression plasmid via Gibson Assembly [17]. Specifically, the students mix the two types of DNA fragments with a HiFi DNA Assembly master mix (NEB) that contains a 5' exonuclease that resects the 5' ends of each double stranded DNA fragment to produce complementary single stranded DNA extensions that can then anneal to each other to form the plasmid. However, the temperature of the reaction is quickly increased to 50°C to denature the 5' exonuclease before it degrades the entire sequence. This is also the optimum temperature of the DNA polymerase and ligase enzymes in the mixture, which replace any excess bases removed by the 5' exonuclease and then permanently ligate the fragments together, respectively.

After the Gibson Assembly is complete, the students transform the new plasmids into competent DH5α *E. coli* using the heat shock method. Since the plasmids also contain a gene for ampicillin resistance (β-lactamase), the cells are spread on LB agar plates containing ampicillin to select for cells that take up the plasmid. The plates are then incubated overnight at 37°C to allow colonies to grow (as shown in Figure 3), which the students then use to inoculate 5 mL cultures of LB media to grow up more cells that are harvested for plasmid extraction with a miniprep kit (Zymo). Samples of each purified plasmid are then shipped to Plasmidsaurus for Nanopore sequencing to determine the sequence of each plasmid. Finally, students use BLAST to align the sequencing reads to the sequence of their desired plasmid to determine if their experiments were successful (Figure 3). If any of these experiments fail, the students are required to repeat the preceding steps until they obtain their desired plasmid. Fortunately, most students obtain their plasmids on the first try, with only a few groups making mistakes that are usually easy to address.

Task 3: Compare transfection methods

Before the students evaluate their new promoters, they compare three different transfection methods to determine which method is the best choice for their target cells (HEK-293T). In these experiments, the students deliver two types of GFP expression plasmids – one with a weak core promoter (MinPro) and another with a strong EF1α promoter (Addgene #11154). These experiments also allow students to get some initial training in aseptic technique and analytical methods like fluorescent microscopy and flow cytometry.

In the first transfection experiment, students mix different amounts of the cationic polymer polyethyleneimine (PEI) with a constant mass of plasmid DNA (200 ng) and then use the resulting polyplexes to transfect HEK-293T cells in a 24-well plate. The amounts of PEI mixed with each plasmid are specifically selected to create samples in which the ratio of positively charged amines on the PEI (N) to negatively charged phosphates on the DNA (P) ranges from 0.75 to 75. Specifically, the polyplexes that are prepared at an N/P ratio of 0.75 should have a net negative charge (P>N), which means that they will be unable to bind the negatively charged cell membrane and fail to deliver the plasmid. In contrast, polyplexes with an N/P ratio of 7.5 and 75 have a positive charge and deliver the plasmids with varying transfection efficiencies (i.e., fraction of cells expressing GFP or %GFP+ cells) and mean fluorescence intensities per cell (MFI). The results of this experiment are shown in Figure 4, which indicate that the PEI polyplexes prepared at an N/P ratio of 75 are only able to successfully transfect about 3% of the target cells with a relatively low MFI. (Note: Due to the toxicity of PEI, the cell culture media is replaced with fresh media that lacks PEI 6 hours after transfection).

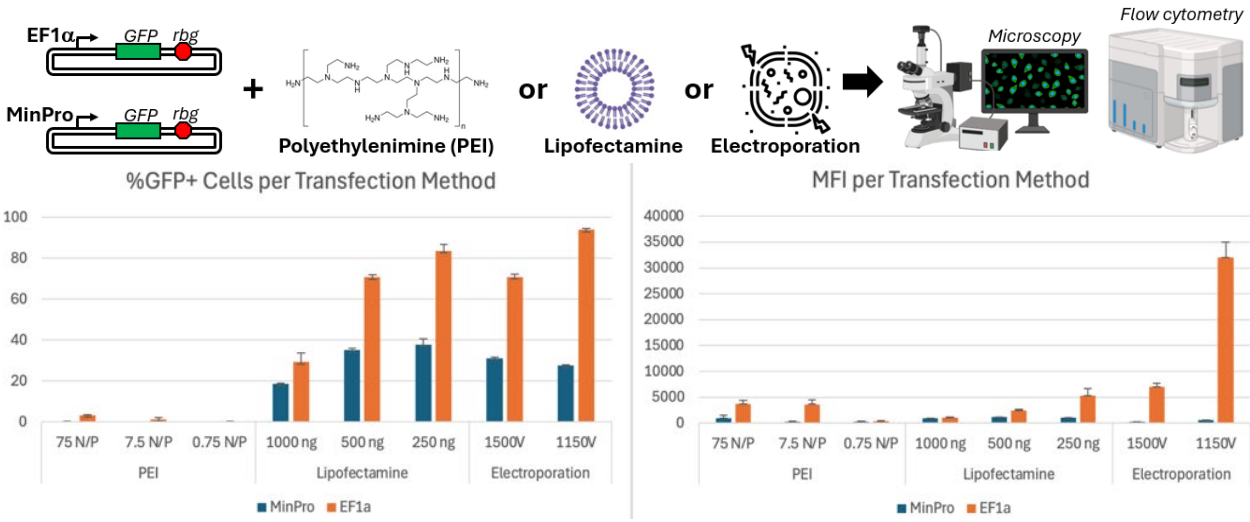


Figure 4: Task 3 – Compare non-viral transfection methods (PEI, Lipofectamine, & electroporation). The flow cytometry data/plots are an example of student work (the absence of labels on the y-axes led to a subsequent discussion of proper graph formatting!)

In contrast, the next experiment shows that transfection of the HEK-293T cells with the cationic lipid Lipofectamine LTX (ThermoFisher) achieves transfection efficiencies that are much higher. In these experiments, the amount of Lipofectamine used to prepare each sample of lipoplexes is held constant (0.5 uL/well), while the amount of DNA mixed with the Lipofectamine

is varied from 250-1,000 ng. These experiments show that Lipofectamine is a much more efficient delivery vehicle than PEI, as the students obtain much higher transfection efficiencies (>80% GFP+ cells) and MFI values (~2x higher than PEI). An interesting outcome of these experiments is that the students observe an inverse relationship between the amount of DNA used to form lipoplexes and transfection efficiency, which allows us to continue our discussion on the importance of forming lipoplexes with a net positive charge (i.e., adding too much DNA results in negatively charged lipoplexes that are unable to enter the cell). Furthermore, plasmid DNA can also induce a potent innate immune response in some cell types (e.g., PC-3) that restricts transgene expression.

In the third transfection experiment, students use a ThermoFisher Neon electroporation system to deliver plasmid DNA to the HEK-293T cells. Since the cells must be detached and transferred to a cuvette or pipette for electroporation, this experiment is also an excellent opportunity to train the students on trypsinization and cell passaging, while emphasizing the importance of aseptic technique. Indeed, if the cells in any of these transfections are contaminated, the students are required to repeat the experiment! After the students have aseptically detached their cells, they mix them with a constant amount of DNA (750 ng) and then use different voltages (1150 or 1500V) for 30 milliseconds to determine the optimal electroporation conditions that maximize transfection efficiency. While these experiments tend to yield the highest transfection efficiencies (>90% GFP+ cells) and MFI values that are 5-fold higher than those obtained with Lipofectamine, the students also use microscopy to reveal that electroporation kills more than half of the cells. Consequently, the students reach the conclusion that Lipofectamine is more effective than PEI and less toxic than electroporation, so they use lipofection to compare their promoters.

Task 4: Compare New Promoters to a Minimal Promoter and a Strong Promoter (EF1α)

The lab section of the course concludes with a series of transfections in which the students use Lipofectamine LTX to deliver their new plasmids to HEK-293T cells and compare them to a negative control (pMinPro) and a positive control (pEF-GFP). The transfected cells are subsequently analyzed via flow cytometry to measure transfection efficiency (%GFP+ cells) and transgene expression levels (MFI), along with plasmid copy number (via qPCR). Each transfection is repeated three times, which allows students to use simple statistical tests to determine if their promoters provide a transfection efficiency or MFI that is significantly higher than pMinPro or significantly lower the pEF-GFP. The results of these experiments are shown in Figure 5, alongside the structure and length of each synthetic promoter.

The results of these experiments are a source of disappointment for some groups and excitement for other groups. Unfortunately, some promoters either fail to express GFP at all (e.g., bGI in Figure 5) or provide expression levels that are similar to the minimal promoter that they replaced in Task 2. However, a few students each year are able to design promoters that provide significant improvements over the minimal promoter and nearly comparable to the strong EF1α promoter in some cases (e.g., promoter NFY in Figure 5). It is important to mention that even if these promoters do not exceed the activity of EF1α, they may still be useful because they are much smaller and some applications may require moderate expression levels. However, I am careful to explain in lecture that HEK-293T cells are generally permissive to transfection, so any promising promoters would have to be tested in other cell lines that are commonly used in cell therapies (e.g., primary T cells) before the potential usefulness of each promoter can be determined.

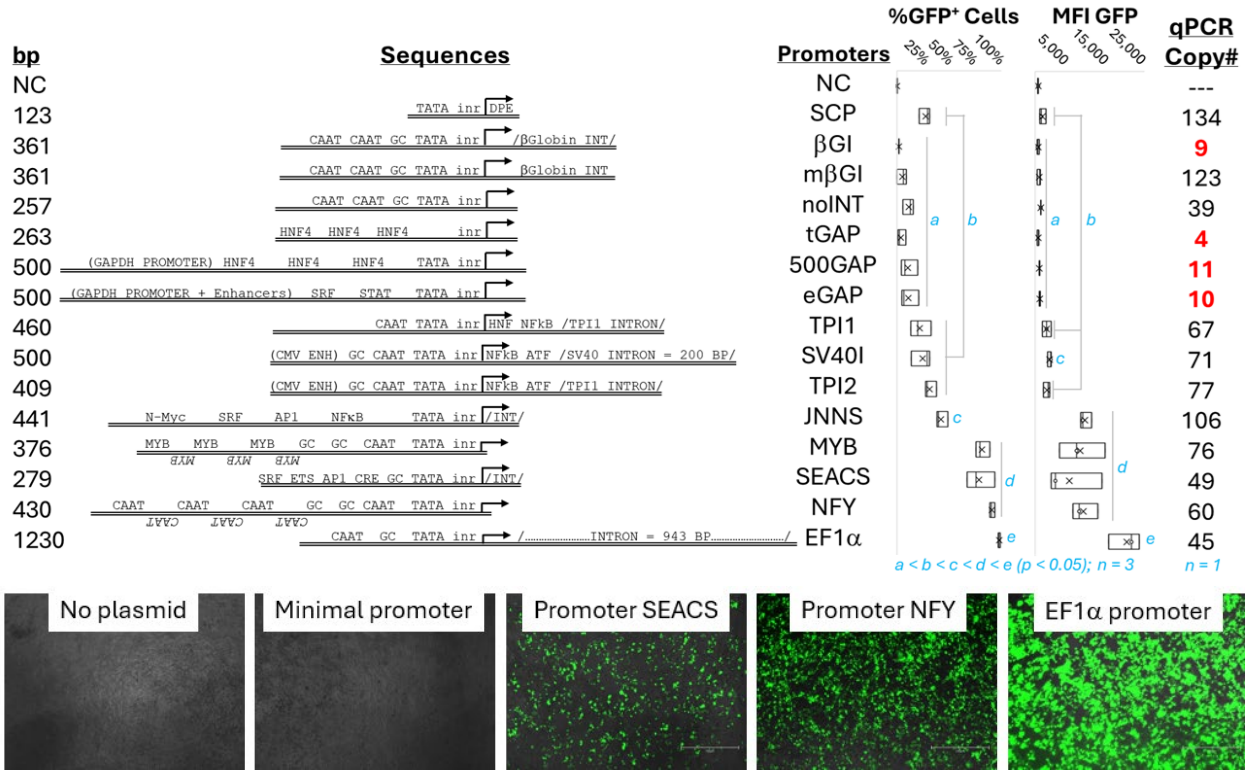


Figure 5: Task 4 – Comparison of new promoters to a super core promoter (SCP or MinPro) and EF1α. Statistically significant differences between groups determined by a Student's t-test ($p<0.05$) are represented by blue letters (a-e). The bottom panel shows fluorescent microscopy images from a representative transfection experiment in which HEK-293T cells were transfected with Lipofectamine LTX and no plasmid, the minimal promoter, two student-designed promoters (SEACS and NFY) or the strong EF1α promoter (from left to right).

In addition to measuring transfection efficiency and MFI, I also explain to the students that it is important to determine the mechanism by which their sequences enhance transgene expression. For example, a higher level of GFP expression could be obtained by either designing a promoter with a higher level of transcription or increasing nuclear uptake of the plasmid, such that the cells obtain a higher plasmid/gene copy number (as has been observed with the SV40 enhancer sequence). Consequently, the final experiment that the students run after their transfections requires them to extract the genomic (and plasmid) DNA from the transfected cells, which they then analyze via qPCR using primer pairs that either bind a genomic target (e.g., GAPDH) or the plasmid DNA. The qPCR results (C_T values) are then analyzed using the ΔΔCt method to determine the copy numbers obtained with each plasmid relative to the pMinPro plasmid. Some groups observe that their plasmids obtain a higher copy number than EF1α, but none of the plasmid copy numbers exceed the value observed for the plasmid with the minimal promoter, which is also the smallest plasmid.

Practical Considerations

The following sections include reflections by the instructor on the main tenets of the course.

Mastery

I have run this course with a requirement for mastery four different times now. It has required more work on my part, but it is worthwhile. Overall, only ~20% of the students make a mistake at some point during the cloning process and need to repeat one or more of the experiments in Task 2. I (thankfully) have not had any students contaminate the animal cell cultures used in Tasks 3 and 4. Speaking of which, it is worth mentioning that one of the more demanding aspects of this course is the maintenance and passaging of a HEK-293T line throughout the second half of the semester (e.g., passaging and plating twice a week), but this might be accomplished by a TA if the instructor is not available.

It is unfortunate that the mastery requirement requires both the students and the instructor to spend more time in lab than usual, but a significant benefit is that repeating failed experiments reinforces their learning experience, since the students have to think about what could have gone wrong in their experiments and then troubleshoot them. However, most students only need to repeat an experiment once, so this is only a minor inconvenience and I have never gotten a complaint from the students about the mastery requirement in this course, either via anonymous surveys or direct communication.

When students needed to repeat an experiment, they would schedule a time to meet with me in the lab during normal working hours (e.g., 9 a.m. to 5 p.m.), so I could provide some troubleshooting advice and supervise them as they worked in the lab. While this has certainly been an inconvenience for me as the instructor, it only happens with a small fraction of students and experiments, such that I have chosen to continue this practice for current and future offerings of the course. However, with that being said, others may not make the same choice and can certainly abandon this mastery requirement.

Technical Communication – Memos and Reports

I believe the communication of experimental results is just as important as collecting and analyzing the corresponding data, so I require the students to submit multiple writing assignments over the course of the semester. The current version of the course requires students to submit 8 memos and a final lab report that summarizes the memos.

Throughout the semester, each group is required to submit a memo the describes the motivation, methods, and results associated with each experiment before they can move on to the next experiment. I find that this requirement improves the quality of the final lab reports, since it forces students to organize and analyze their data immediately after they perform an experiment while everything is still fresh in their memory. More importantly, the memos also give me a chance to give the students feedback on both the quality of their writing (spelling, grammar, structure, and flow) and the technical content of the memos several times over the semester, so that their writing can improve by the time the final lab report is due. I also use at least one lecture to have a technical writing workshop, in which we discuss expectations for technical communication. Finally, I share the rubrics for the memos and lab report with the students on the first day of the semester, so they know exactly how their work will be graded.

Budget

This course requires some unique pieces of lab equipment (e.g., biological safety cabinet(s), thermal cyclers, centrifuges, and cell culture incubators) that are listed in Table 3. The most important common piece of equipment to consider would be the biological safety cabinet (BSC). On one hand, students absolutely need to learn how to aseptically culture animal cells in a BSC, since most of their future careers will require such expertise. On the other hand, BSCs take up at least 12 sq. ft. of space, cost more than $10,000 each, and can only accommodate 1-2 students at a time. We are fortunate to have a recently constructed bioengineering teaching space with 4 BSCs, so I can simultaneously supervise 4 groups of students doing aseptic cell culture or transfections. I also make sure that any experiments that require the BSCs will only take 60-90 minutes, such that I can have 8 groups of 2-3 students doing those types of experiments in a given 3 hour period. Therefore, I can have a maximum of 16-24 students in one section of this course. Any instructors that are considering adopting this course at a larger University should plan to offer multiple lab sections if they anticipate higher enrollments. The most limiting factors for instructors that are considering adoption of the course may be the requirement of an electroporation system, fluorescent microscope, and a flow cytometer. However, if these instruments are not available, the course may be modified as follows:

- **Electroporation system:** If an electroporator is not available or the consumables are too expensive, this transfection method may be replaced with a different method.
- **Fluorescent microscope:** If necessary, microscopy can simply be omitted from the lab requirements and replaced with detailed lectures on the subject.
- **Flow cytometer:** In lieu of GFP, the expression plasmids can be constructed with a luciferase gene, which allows the measurement of transgene expression using a luciferase assay that produces light, which can be measured with a plate reader/luminometer.

Table 3 – Equipment required for the course

Task	Experiment	Equipment
2	Digest+Gibson Assembly	Thermal Cycler(s)
	Digests	Gel Electrophoresis System(s)
		UV Transilluminator/GelDoc
	DNA Quantification	NanoDrop or Plate Reader
3	Sterilization	Autoclave
	Aseptic cell culture	Biological safety cabinet
		Bacterial shaker/incubator
		CO2 incubator (animal cells)
	Transfections	Neon Electroporator
		Fluorescent microscope
		Flow cytometer
4	Plasmid copy number	qPCR instrument

A detailed list of the consumables and equipment required for this course is shown in Table 4. Overall, the course typically requires a consumable budget of just over $5,000 per semester, assuming an enrollment of 10 students. These costs will increase if more students enroll (e.g., each synthetic promoter costs $75 to synthesize and $15 to sequence), but some of the costs can be defrayed by borrowing reagents or having students work together to design a single promoter sequence, if necessary (but that dampens the design aspect of the project).

Table 4 – Consumable costs for lab portion of course

Task	Experiment	Consumable/Equipment	Cost
1	Promoter design	Synthetic DNA	$75 x N
2	Restriction Enzyme (SalI) Digest	pMinPro plasmid	$0*
		SalI-HF enzyme (NEB R3138s)	$75
		Agarose (VWR 89406-784)	$145
		Ethidium bromide (Biorad 1610433)	$59
		DNA ladder (NEB N3232S)	$64
		Gel extraction kit (Zymo D4001)	$111
	Gibson Assembly	HiFi DNA Assembly Mix (NEB E2621S)	$186
	Transformation	NEB 5alpha *E. coli (NEB C2987I)*	$173
		LB agarose (VWR 76346-496)	$40
		Ampicillin (VWR 100219-890)	$112
		Petri dishes (VWR 89107-632)	$73
	Miniprep	LB media (VWR 76346-610)	$74
		50 mL tubes, sterile (VWR 21008-940)	$225
		Miniprep kit (Zymo, D4209)	$106
	DNA Sequencing	Nanopore sequencing (Plasmidsaurus)	$15 x N
3 & 4	Transfections	pEF-GFP plasmid (Addgene 11154)	$0*
		HEK-293T cells (ATCC CRL-3216)	$490
		Polyethylenimine 25 kDa (Sigma 408727)	$101
		Lipofectamine LTX (Thermo 15338030)	$292
		DMEM media (VWR 1677-200)	$10
		24 well plates (VWR 10062-896)	$130
		T-75 flasks (VWR 10062-860)	$113
		Trypsin/EDTA (VWR 82003-690)	$28
		PBS (Sigma P3813-1pak)	$10
		96 well plates (VWR 29442-070)	$169
	Plasmid Copy Number	gDNA extraction kit (Zymo D4068)	$163
		SYBR green master mix (Thermo a25741)	$113
		PCR primers (2xGAPDH + 2xpDNA)	$30
		OptiAmp qPCR well plates	$100
		Adhesive film for plates (VWR 75853-868)	$53
All	General plastics	P10 pipettes (Amazon, n = 5)	$100
		P10 tips (VWR 76323-388)	$67
		P100 pipettes (Amazon, n = 5)	$110
		P100 tips (VWR 76323-390)	$68
		P1000 pipettes (Amazon, n = 5)	$125
		P1000 tips (VWR 76323-456)	$104
		Eppendorf tubes (VWR 87003-294)	$112
		Nitrile gloves (n = 5, VWR 82026-)-426)	$340
		Safety glasses (VWR 89187-984)	$121
		Total (Assuming 10 students)	$5,292

**Available upon request; N = number of students, plasmids, or promoters*

Student Feedback

Feedback from the students is highly positive, according to student surveys that are administered by the University at the end of each semester. As shown in Table 5, the students generally find the course intellectually stimulating and indicate that they learned a great deal. When asked about the overall value of the course, they scored it highly (4.5-5 out of 5, with 5 being the highest possible value). To put these scores into context, Table 5 also includes the average scores for all courses offered in our college for each survey question, which shows that the scores for this elective course are consistently among the highest in the college.

Table 5 – Student Feedback (Survey Results)

Survey Prompt	2019	2021	2023	2025	College Average
I found the course intellectually stimulating.	4.9	5.0	4.6	4.8	4.4
I learned a great deal in this course.	4.9	5.0	4.8	4.9	4.5
Overall value of this course (5 = highest)	5.0	4.9	4.5	4.6	4.4

Scale = 1-5, with 5 being highest

Student comments:

Anonymous student comments collected in these surveys also tend to be positive. Some examples of constructive feedback from the students and my thoughts on them are listed below.

Re: Designing and ordering all promoters at the beginning of the course

"I like the idea of testing the promoters one at a time. It was difficult to create a promoter with only a few lectures of material to base it off of. When writing the report, I realized some things that I should have done back when creating the promoter that I did not do."

I have often thought about giving the students to iteratively improve their promoters, but thus far I have tended to err on the side of caution and suggest that all of the students order their promoter sequences early in the semester, just in case they need time to repeat experiments later on. The other difficulty associated with a trial and error approach would be that the instructor would have to keep the HEK-293T cell line going for a longer period of time and there would be more weeks in which flow cytometry would have to be performed. Overall, the logistics of letting a group iteratively improve their promoter are daunting, but I may allow it for the next offering.

Re: Lecture content

"Possibly including more homework assignments, like designing micro RNA fragments, etc."

"There could be more of a high level introduction to material rather than jumping into very complex terminology. Additional info could also be provided in the notes on blackboard to help define new terms."

"The course could be improved by starting off with the basics in lecture, prior to jumping into the specificities of various techniques being used in experiments."

"This class was way to complex for my understanding. I think [the instructor] assumed that most people had a background knowledge (since most of them somewhat did) but I found myself too far behind in understanding from the beginning to catch up. I think if he provided more of a

background and then covered less material slower and focused on understanding rather than covering all of it, I would have enjoyed the course a lot more."

This course is offered as a technical elective in a biochemical engineering program. It does not currently have any prerequisites like General Biology or Genetics. I do my best to offer links for review videos on basic concepts related to the topics we discuss each day, but I still tend to get comments like this each semester. I have addressed the first comment by adding more graded homework to the course. I have also started posting the recorded lessons for students to view and review at their convenience. I have always let the students know that they can come to my office hours or schedule a meeting to clarify anything they do not understand in lecture, but this approach does not work. In other words, the students do not share the sentiments above until the survey at the end of the course.

Re: Course structure (lecture + lab schedule)

"The only factor that I would change would be potentially adding a second day of lecture each week so that there is more time to learn the fundamentals and background. I think this course would be so much better if we did not try to cover so much material."

"This course requires way more time than just one class period and one lab period a week. I know that making the course require two lab periods a week may not be appealing to students who are registering for this course, however, it is significantly more realistic."

One potential solution to the previous problem and the comments above would be to offer the course as a 4-credit course (see below). This 3-credit course currently consists of a 1.5 hour weekly lecture with an expectation of 4.5 hours per week in the lab. The lab work occurs mostly during a scheduled 3 hour block in the student's schedule, whereas the remaining time is individually scheduled with the instructor to either complete or repeat experiments in the lab or work with group members to prepare memos and lab reports. After reading these comments, I am considering the possibility of changing the course to a 4-credit course, which would include increased lecture time (either 3x50 minute or 2x75 minute lectures per week) and reduce the lab work expectation to 3 hrs per week. This schedule should still allow the students to complete all the required experiments (~10 labs) within the semester if some of the experiments are combined.

Reflection on Learning Outcomes

The learning outcomes in Objectives 2, 3, and 4 primarily relate to the students' understanding of concepts relevant to eukaryotic translation, how lab equipment works (e.g., microscopy), and issues with gene therapies in the clinic. These outcomes are assessed via midterm and final exams.

Alternatively, Objectives 1 (understanding of transcription), 5 (construction of expression cassettes), and 6 (culture and transfection of animal cells) are evaluated through activities in the lab and the corresponding memos and lab reports. As shown in Figure 5, some students excel in these categories by designing and evaluating novel promoter sequences that provide high levels of GFP expression in HEK-293T cells. The promoters that do not perform well tend to be either truncated promoters from the human genome (e.g., sections of the GAPDH promoter) that are probably missing key upstream enhancers, since I restrict the students to only using 500 bases for their promoter sequences. Furthermore, a key pitfall that I have observed is the accidental introduction of start codons (ATG) between the transcription start site and the GFP gene. This

mistake causes premature translation initiation instead of the intended translation of the GFP open reading frame. These anecdotes are shared with students in the subsequent course offerings to avoid repeating those mistakes. Overall, however, the results from the lab experiments seem to indicate that all of the students successfully completed Objective 4 (i.e., no failed transfections or contaminated cell lines) and most satisfactorily achieved Objectives 2 and 3 (design and construction of a eukaryotic expression cassette). Finally, I will reiterate that many of the students whose promoters did not perform well all expressed the desire to redesign their promoters if they had more time in the semester (see student comments on previous page). It is interesting how these students are potentially embracing the mastery element of the course, in which they need to repeat an experiment if it fails. However, allowing students to iteratively design and test promoters would require much more work on the instructor's part.

Enrollment

This course has been offered in a Chemical Engineering department for a total of 4 times in 2019, 2021, 2023, and 2025. The enrollment data for those offerings are shown in Table 6. First of all, it is important to note that our College of Engineering did not offer a degree in Biomedical Engineering until the fall semester of 2026. Instead, previous classes would mostly enroll in a Chemical & Biological Engineering (CBE), Mechanical Engineering (ME), or Electrical & Computer Engineering (ECE) degree program and pursue a minor in Biomedical Engineering. That is why BME is not listed as a major in Table 6.

Year	Total	Sex		Year				Major			
		Male	Female	FR	SO	JR	SR	CBE	Neuro.	Biology	Biochem.
2019	14	4	10	0	0	1	13	12	1	1	1
2021	13	9	4	1	6	4	2	11	0	0	1
2023	21	6	15	0	0	12	9	20	0	1	0
2025	15	8	7	0	0	3	12	9	0	3	3
Totals	63	27	36	1	6	20	36	52	1	5	5

CBE = Chemical & Biological Engineering, Neuro = Neuroscience, Biochem = Biochemistry

Overall, the course enrolls an average of 15 students per semester from a department with a total of 240 students (i.e., ~60 students per class/year). The distribution of male and female students varies from semester to semester, but the enrollment from all four offerings of the course was 43% male and 57% female. The students are mostly junior and seniors, but there have been some underclassmen that have taken the course, including one freshman student. Freshmen typically do not register for this course, since it is offered in the fall semester and most of our incoming students have their schedules automatically generated for them (i.e., they are not advised by our faculty until halfway through their first fall semester). Finally, it is also worth noting that the course attracts a few students from our College of Liberal Arts and Sciences (CLAS) each year. The number of CLAS students enrolled in this course has increased recently due to the launch of our Cellular Engineering minor, which encourages students from CLAS to take courses from the College of Engineering directed towards cell culture and genetic engineering.

Potential Collaborations: A Consortium for Crowd-Sourcing Promoter Sequences

Perhaps the most exciting aspect of this course is that most of the students were able to independently design compact promoters that gave moderately high GFP expression levels! These

promoters are not quite as strong as the EF1α promoter we used as a positive control, but their smaller size could make them attractive in situations that require compact promoter sequences. In the future, I plan to share the sequences and results from past offerings of the course with new students, so that they can learn from the successes (and failures) of previous students. This may enable future students to design even stronger promoters. Indeed, other CUREs have already shown that dissemination of CURE materials to other institutions can lead to the creation of consortia (e.g., the Genomics Education Partnership at Washington University in St. Louis, [18]) involving dozens of different institutions and thousands of students that all work to address the same research problem (e.g., phage genomics, [19]).

If there are any other instructors that are interested in adopting this course, I am willing to share the sequences, flow cytometry data, microscopy images, and plasmid copy number measurements that we have collected thus far. Our odds of finding stronger or smaller promoters will increase drastically if more institutions get involved. In addition, it could create new collaborations between instructors that could enhance both the lab and lecture portions of this course. Finally, recruiting more participants could also enable us to explore exciting new variables, including:

- Evaluation and comparison of promoters in different cell types (including clinically relevant cell types like Jurkat or primary T cells)
- Optimization of the mRNA sequence (e.g., 5' UTR, coding sequence, and 3' UTR) to enhance transcript stability while also maintaining a high rate of translation
- Expression of a therapeutic gene (instead of GFP), such as the clotting factor 8 (FVIII) gene used to treat Hemophilia A. The activity of FVIII can also be measured with a clotting assay.

IRB Statement:

This work does not use data from humans and therefore does not meet the definition of human subjects research; IRB review is not required.

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