Assessing Student Conceptions of Protein Synthesis with a Case Study in CRISPR and De-extinction

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Abstract
Scientific modeling is a practice that we use frequently in our undergraduate biomedical applications course for nonscience majors. We use case studies in which students apply course concepts to create cause-and-effect models. In this article, we describe a case study assessment on protein synthesis that examines the use of CRISPR to bring back the mammoth (i.e., de-extinction). Students learn about protein synthesis throughout the course and work on various case study scenarios to apply those concepts. Their final assessment is a team project to illustrate how protein synthesis is influenced by gene editing, including gene expression and its regulation, transcription, translation, protein structure and function, and the ultimate impact on an organism’s phenotype. Although we use this case study as an assessment, it is also appropriate as a class activity in which students practice modeling the CRISPR gene-editing system.

Key Words: CRISPR; de-extinction; DNA; gene editing; mammoth; protein synthesis.

Introduction
Calls for undergraduate education reform recommend focusing courses on scientific practices, crosscutting concepts, and core ideas (Cooper et al., 2015). To meet these calls, our undergraduate students develop scientific models (a scientific practice) to explain how molecular structures and processes influence an organism’s physiology and appearance (core ideas from HS-LS1 in NGSS, 2013). Prior to each modeling activity, students prepare by viewing informative videos with an associated open-note quiz that covers basic concepts. During class, students apply those concepts to novel cases, such as how gene expression regulation causes stickleback fish to develop—or not develop—spines (activity modified from HHMI Biointeractive, 2011). For each unit of the course, students repeat this process on related concepts for a few class periods and then apply all concepts from the unit in a modeling assessment regarding a single case study.

This course focuses on protein synthesis and gene regulation, such as how enhancers influence gene expression, and during the last unit of the course, students apply those concepts to illustrate how various biotechnologies work and influence gene expression. The assessment described in this article focuses on an emerging gene-editing biotechnology: CRISPR. First, we describe CRISPR and the associated assessment case study on mammoth de-extinction. We explain the team-based modeling assessment activity and present students’ performance results.

Instructional Context
The course is an undergraduate introductory biology course offered to nonscience majors and enrolls 100–150 students. We implemented this assessment to determine students’ knowledge of protein synthesis. We used a flipped-style approach in which students complete short readings and preparatory quizzes prior to each class period. During class, students work in teams consisting of three to four members. We created these teams using a team-maker software called CATME (Purdue University, www.catme.org), which creates a student survey and places students in teams based on the results of that survey. The survey includes questions about demographics, team preferences, and expected effort in the class. We have teams composed of individuals with similar team preferences but we also ensure that no one is by themselves within a team (e.g., not having one person that identifies as female with teammates that all identify as male).

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Assessment Objectives

Our assessment uses CRISPR as a grounding element to measure students’ understanding of protein synthesis. The following are the assessed objectives, which incorporate concepts from the previous units, including amino acid properties, protein structure and function, transcription, translation, gene expression and regulation (or regulation of gene expression), and mutations.

- Develop and use a scientific model based on amino acid properties to test and illustrate protein structure and function.
- Make appropriate inferences of protein structure and function models.
- Predict the extent of similarity of protein structures based on the extent of similarity in protein functions and evolutionary relatedness.
- Identify the switches, promoter, and transcription starting regions on a DNA molecule.
- Describe how activators, transcription factors, and polymerase proteins are used in transcription.
- Predict how mutations in various parts of a DNA sequence influence protein expression.
- Explain how different cell types perform different functions.
- Describe the roles of mRNA, tRNAs, and ribosomes in protein translation.
- Describe how translation starts and ends, including the functions of start and stop codons.
- Describe the anatomy of a tRNA.
- Interpret a codon–amino acid chart.
- Describe examples of protein synthesis regulation.
- Create a model to explain how cells regulate gene expression using switches or exons and introns.

In doing this assessment, students learn about CRISPR and meet the following objectives. These objectives, though, were not part of the assessment, so students were welcome to ask questions during class to help in their understanding of these objectives.

- Identify the molecular steps and their variations in CRISPR.
- Develop a scientific model that illustrates how CRISPR works in the context of a novel case study.
- Apply concepts of genetic mutations, protein synthesis, gene expression, regulation, and protein structure and function to CRISPR.

○ Case Study Background

Scientists modify genes in a variety of ways, including cloning (inserting DNA from an individual into a host egg cell of another), reconstructing the DNA chemically (if the full sequence is available), and using CRISPR (editing specific sequences). The basis of the technology is that short RNA sequences guide the protein Cas9 to its matching DNA sequences where Cas9 binds and cuts the target gene (Broad Institute, 2018). Once the DNA is cut, researchers allow the cell’s natural repair mechanisms to repair the genetic material; this creates a random mutation in the gene, which most often makes it nonfunctional (National Institutes of Health, 2020). Alternatively, scientists can insert a new piece of DNA.

One possible application of CRISPR is de-extinction, which has recently come to light as a highly debated scientific technology due
to ethical, social, and ecological concerns in the media as well as among scientists at public events and even at special symposia at academic conferences (Novak, 2018). The International Union for the Conservation of Nature guidelines define de-extinction as generating a new species that is altered to serve a similar ecological function as the original species (Novak, 2018). Because an extinct species lineage can never be fully recovered, its ecological function cannot be identical to the original, extinct species (Novak, 2018).

Within the mass media, one of the most commonly discussed candidate species for de-extinction is the woolly mammoth. Mammoths likely had a huge impact on the ecosystem by maintaining grasslands in areas that later transformed to woodlands after their extinction (McCauley et al., 2016). Given that woolly mammoths went extinct thousands of years ago, the samples of DNA sequences that scientists are able to extract are contaminated with DNA from other organisms such as bacteria (e.g., CCGB, 2013), so they need to use a “template” (or surrogate species) for developing the entire genome. Therefore, it is likely that CRISPR is the most applicable tool in comparison to DNA reconstruction or cloning, should scientists ever decide to bring back the woolly mammoth. CRISPR is used to edit the genes of the surrogate’s embryo so that it closely resembles a woolly mammoth, and then the surrogate parent carries the embryo to term. Therefore, the surrogate species is the link to generate the new species of mammoths. The closest genetic living relative of the mammoth is the Asian elephant (Rohland et al., 2007), and therefore, it is the most likely surrogate species. Again, the extinct mammoth species will likely never be recovered, but CRISPR makes it possible to use the partial DNA available from the extinct mammoth and DNA of its closest living relative, the Asian elephant, to create a new species capable of serving a similar ecological function as the woolly mammoth. Because Asian elephants live in a very different environment from the woolly mammoth, there are a few key traits that would likely need to change, including the blood’s ability to release oxygen at low temperatures, subcutaneous fat for insulation and storage, thick hair, and smaller ears and tail (see Lynch et al., 2015, for a review).

Student Activities

Overview

This activity is an assessment that applies protein synthesis concepts from throughout the semester to a novel case study; that is, how an edit to a gene via CRISPR may change how that gene is synthesized and/or how that edit affects the organism. In this case, an Asian elephant’s genome is modified and inserted into an embryo so that the embryo results in mammoth-like Asian elephant. To prepare for this assessment, students create two in-class models that apply course concepts to other biotechnologies (i.e., genetically modified organisms and gene therapy) and complete a homework assignment that introduces students to how CRISPR works. They were introduced to the general idea of CRISPR and its controversial issues earlier in the semester by reflecting on the following TED Talks:

- “Changing the Human Story with CRISPR-Cas9”
- “The Ethical Dilemma of Designer Babies”
- “What You Need to Know About CRISPR”

The first video gives a very positive position on CRISPR by an undergraduate researcher, including how easy it is to use. Having a video by an undergraduate was purposely selected to give students a more relatable speaker. The second video, by a biologist, focused on a potential ethical dilemma of CRISPR, and thereby providing a negative view on CRISPR. The third video, by an established CRISPR researcher, describes both positive and negative concerns of CRISPR.

Preparation Case Studies

In preparation for the CRISPR case study, the two class periods before the assessment involve practicing modeling how a biotechnology works and how the concepts from the course help explain how that biotechnology influences an organism’s phenotype: one case is on genetically modified foods, and the other is on gene therapy. The GMO case study on AquAdvantage salmon prepares students by having them model the genetic process of the normal Atlantic salmon and the AquAdvantage salmon. Then students use this information to explain how the cell types are different. The gene therapy case study on cystic fibrosis has students model protein synthesis and challenges students to apply concepts of protein synthesis, gene expression regulation, and the effects of gene therapy on protein structure and function. Students practice using these activities to piece together the process in which an organism can be edited using biotechnology techniques.

Preassessment Homework

To prepare for the CRISPR modeling assessment, students view the following series of videos and participate in an interactive that shows how CRISPR works:

- Rachel Haurwitz (2016), “CRISPR: Editing Out Genetic Instructions,” https://www.youtube.com/watch?v=wktwXGAbF_Q
- McGovern Institute (2014), “Genome Editing with CRISPR-Cas9,” https://www.youtube.com/watch?v=2pp17E4E-O8
- Seeker (2016), “What Is CRISPR and How Could It Edit Your DNA?,” https://www.youtube.com/watch?v=5yAo5I1YgUw
- Yourgenome (2016), “What Is CRISPR-Cas9?,” https://www.yourgenome.org/facts/what-is-crispr-cas9
- HHMI Biointeractive (2019), “CRISPR-Cas9 Mechanism & Applications,” https://www.biointeractive.org/classroom-resources/crispr-cas-9-mechanism-applications

Then students complete a multiple-choice quiz that addresses the main components and steps of CRISPR as a homework assignment that is graded based on correctness.

In-Class Modeling Assessment

For the modeling assessment, students answer the question “How can CRISPR be used to modify DNA in the Asian elephant to produce protein found in a mammoth?” In the worksheet, students first identify natural ways in which two species can create similar but different proteins. In our class, students often explored this question: e.g., a mutation causing a deletion of a switch that had turned on the gene in a specific cell type) and had to address it in writing as they learned about additional mechanisms. Without this background, though, the question may result in confusion, as students may think it is just referring to CRISPR rather than which changes in a gene result in different proteins.

Students use the concepts that they learned throughout the semester to create a model explaining how an edit in a gene of an Asian elephant embryo affects protein synthesis and results in a genetically modified elephant that is similar to a woolly mammoth...
(see the worksheet in Figure 1 for the specific questions that the model must address).

While students work on the model, the instructor walks around, providing guidance to students who request assistance in understanding how the model should explain certain concepts about gene editing and tying all the processes together. The students’ most common questions are about the mechanism of CRISPR and how it works. The instructor answers any questions regarding CRISPR and de-extinction but not questions on how protein synthesis works, because the students are being assessed on that knowledge. If a student simply asks if something is correct, the instructor refers them to the grading rubric.

**Model Evaluations**

We graded students’ models using an analytic rubric (Table 1). Overall, students’ models met the exemplary level in the rubric and demonstrated an understanding of protein synthesis (Figure 2). Most students correctly transcribed a DNA sequence to an mRNA sequence and translated it into an amino acid sequence (see Figures 3 and 4 for examples and Supplement Material, available with the online version of this article, for an exemplar model developed by the authors). Several models lacked a protein shape—that is, how the charges of the amino acids influence the shape of the protein (Figure 3). It is unclear, though, if this trend was due to a lack of understanding of the concept or a lack of explicit instructions. The rubric (Table 1) used for this analysis was revised from the original grading rubric to explicitly state that exemplary work demonstrates an understanding how the model should explain certain concepts about protein synthesis, including enhancers that turn genes on and off and alternative splicing of exons after transcription. We encouraged students to consider any possible mutation, such as a mutation in a switch preventing protein synthesis. They were asked to

![Figure 1](image36x319to153x397)

**Figure 1.** Student worksheet. Part 1 prepares students to create the model, and Part 2 is the modeling activity.

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**Table 1.** Grading rubric for evaluating student models.

| CRISPR | DNA Sequence | DNA Editing | mRNA Sequence | Amino Acid Sequence | Protein Structure | Protein Function |
|--------|--------------|-------------|----------------|---------------------|-------------------|-----------------|
| Exemplary | Light gray | Light gray | Light gray | Light gray | Light gray | Light gray |
| Proficient | Gray | Gray | Gray | Gray | Gray | Gray |
| Needs Improvement | Light gray | Light gray | Light gray | Light gray | Light gray | Light gray |
| Not Evident | Light gray | Light gray | Light gray | Light gray | Light gray | Light gray |

![Figure 2](image36x319to153x397)

**Figure 2.** Heat map illustrating evaluation results of all student models. Each row represents one model, and each column is a criterion from the rubric (Figure 2). Shading aligns with the levels in the rubric: light gray is exemplary, and dark gray is not evident (see key).
Table 1. Rubric used to assess students’ models. Bold and italicized text are phrases used in this assessment but not the original grading of the models.

| Critical Elements | Exemplary (100%) | Proficient (75%) | Needs Improvement (55%) | Not Evident |
|-------------------|------------------|------------------|--------------------------|-------------|
| CRISPR (30% of total grade) | Model explains how Cas-9 enzyme and guide RNA are used to edit DNA. | Model explains how CRISPR works but does not include specific molecules (Cas-9 or RNA). | Model explains how CRISPR works but does not show it. Or, some aspects of the model pertaining to CRISPR are incorrect. | Model does not include the mechanism of CRISPR. |
| DNA Sequence (10%) | Model provides original and edited DNA sequences, including switch, promoter, gene, termination sequence, and actual base sequence. | Model provides original and edited DNA sequences but has some inaccurately labeled parts or does not include base sequence. | Model provides original and edited DNA sequences but does not label switch, promoter, gene, and termination sequence. Or, model includes only an original or edited DNA sequence. | Model provides a DNA sequence that is missing a base sequence, and parts of DNA sequence are not labeled or are inaccurately labeled. |
| DNA Edit (20%) | Model illustrates which part of the DNA sequence is edited to make a mammoth protein (select any trait from the case study). Also includes a description of why that particular edit was done (i.e., which part of the sequence was edited and how much was edited). | Model illustrates which part of the DNA sequence is edited to make a mammoth protein. Also includes a description of why that particular edit was done, but it explains only which part of the sequence was edited or how much was edited. | Model incorporates either an illustration of what was edited in the DNA sequence or a description of why that particular edit was done. | Model does not include an illustration or description of which part of the DNA sequence was edited. |
| mRNA Sequence (10%) | DNA is transcribed correctly with no errors. | DNA is transcribed with few errors. | DNA is transcribed with some errors. | Specific mRNA sequence is not provided or does not align with DNA sequence. |
| Amino Acid Sequence (10%) | mRNA is translated correctly with no errors. | mRNA is translated with few errors. | mRNA is translated with some errors. | Specific amino acid sequence is not provided or does not align with mRNA sequence. |
| Protein Structure (10%) | Tertiary shape of protein contains specific amino acids and reflects amino acid properties. | Tertiary shape of protein reflects amino acid properties with few errors. | Tertiary shape of protein is provided but does not align with amino acid properties. Or, object is a squiggly line. | Tertiary shape of protein is not provided. |
| Protein Function (10%) | Model explains (can include written explanation) resulting protein function, which includes its relation to a mammoth trait and is compared to the original Asian elephant trait. | Model explains resulting protein function, but it only includes its relation to a mammoth trait or is only compared to the original Asian elephant trait. | Model explains that the protein’s function is determined by its shape but is not specific to a certain trait. | Model does not relate back to protein function. |
develop a biologically plausible prediction rather than to research mammoth de-extinction. Most students modeled a mutation in the protein-coding region, resulting in a new, functioning protein. Therefore, students may have felt most comfortable with modeling this kind of mutation, but some created mutations in other parts of the gene. One model illustrated a mutation in an exon and labeled it as alternative splicing but did not model that a different exon was being spliced—just that the last exon was different. Another model showed an insertion of a mammoth enhancer. The latter included a detailed description that illustrated connections across the concepts:

Since the Asian elephant lives in a warmer climate, it has evolved to not use the protein that releases blood oxygen at low temperatures. The amino acid sequence for the protein can still be in the DNA, but there is no switch [enhancer] that activates it. By using CRISPR-CAS9 to insert the switch, the Asian elephant can produce the protein again.

**Student Survey**

Toward the end of the first semester of using this assessment, students anonymously completed an internal evaluation survey. This instrument is used in courses within our department to assess students’ perceptions of each course’s activities and pedagogical strategies. It contains a core set of questions used in every class and a set of questions specific to the course, such as certain class activities. This was the first semester that the survey was used in the department, and so the primary goal was to test the instrument. We took advantage of this opportunity by adding a question about the CRISPR modeling assessment. Students completed it during class for extra credit and were made aware that the instructor could not access the results until after submitting final grades. Of the 138 students enrolled in the course, 132 students completed at least the multiple-choice portion of the survey (96%), and 120 students also answered most of the open-response questions (87%).

One question asked to rate the helpfulness of the CRISPR modeling assessment. On a five-point Likert scale in which 5 meant that the assessment greatly helped and 1 meant that it did not help, the average score was 3.81 (n = 132), with 29% of students selecting 5 and 3% choosing 1 (Figure 5).

When asked to comment on how the class activities throughout the semester helped in their learning, students often offered positive comments regarding the general setup of the course, and two mentioned this specific assessment, such as “this class was taught with working with other peers to solve complex issues such as modeling how CRISPR works.” Another student commented:

*The class is taught so that you work in groups and work on problems of a specific topic with the members of your group to help solve it. For example . . . we had to make a CRISPR model working*
with the members of our team, using knowledge that we each had to help complete it.

Out of the 120 students that provided comments for this question, nine provided negative feedback. This feedback was mostly generic comments, but except for the following, which is a constructive comment that provided an idea on how to improve the activities:

If they were well explained and the online activity was actually good prep for it, then they were very helpful. Other times, there was way too big of a jump from the content of the online module and the class activity, and since there is no instructional time in the course, I very often felt lost during class.

This concern alludes to the importance of having pre-activity materials that prepare students for upcoming class periods. For the second semester, we added the interactive tutorial from HHMI Biointeractive, “CRISPR-Cas 9 Mechanism & Applications,” to the homework, and the instructor noticed nearly all teams had at least one student opening and going through the interactive while working on their CRISPR model.

**Conclusion**

This modeling assessment gave students a chance to not only learn about CRISPR, an emerging biomedical application, but also apply concepts of protein synthesis, gene regulation, and mutations to a novel case. Most recently, we used this assessment as a regular in-class activity, and then the assessment had students create a similar CRISPR model for another topic that we randomly assigned to each student, such as HIV treatment or crop longevity. They completed these activities on their own time, and the models are similar to the assessment models analyzed in this article.

The dissemination of these results was approved by the institution’s review board (Study ID: STUDY00003803).

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