CRISPRkit Instructions for Teachers

This document aims to help educators and teachers understand how CRISPRkit aligns with high school biology standards, as well as provides recommendations on how CRISPRkit can be incorporated into high school curriculum and integrated into classrooms for optimal student learning and success. This document contains clickable links to the content on our website.

Outline:

- How does CRISPRkit help students develop skills to meet the learning goals and standards in AP Biology?
- What is the ideal student audience for CRISPRkit?
- How can the experiment be integrated into the curriculum? Recommendations and Teacher Guide.

How does CRISPRkit help students develop skills to meet the learning goals and standards in AP Biology?

Learning biology for high school students is not just about the conceptual understanding of biological mechanisms and the regurgitation of facts. Rather, it is a holistic endeavor that involves the honing of an array of skills that will help develop students into astute critical thinkers and problem solvers.

We believe the AP Biology learning framework is an excellent model for cultivating good scientific practices in high school students. In this section, we will demonstrate how CRISPRkit closely aligns with these practices. We hope to help educators and teachers understand the relevance of CRISPRkit and how it can be integrated into their teaching strategies.

Skill	**Description**	**CRISPRkit Relevance**
Concept Explanation	Explain biological concepts, processes, and models presented in written format	Students are encouraged to go through the Key Concepts page and visit the Overview page to understand the fundamental biological concepts underpinning CRISPR. Students should be able to describe the mechanisms of CRISPR and differentiate between CRISPR and CRISPRi before experimenting with CRISPRkit.
Visual Representations	Analyze visual representations of biological concepts and processes	The melanin metabolism pathway allows for easy visualization of gene expression. Through observing a binary result in reaction tubes, students can explore and understand how CRISPR represses and modulates gene expression.

Question and Method	Determine scientific question and method	Alongside the melanin experiment, CRISPRkit additionally offers a guess-and-test experiment where students are agnostic about the melanin's final expression. Through conducting the experiment and observing how color changes, students will hypothesize how different reactants will affect the experiment results.
Representing and Describing Data	Represent and describe data	CRISPRkit is not just about conducting the experiment on Day 1. It also involves collecting and describing the data on Day 2. With CRISPectra, students can upload their tube images and analyze how much pigment output is in their reactions to approximate gene expression quantitatively.
Statistical Tests and Data Analysis	Perform statistical tests and mathematical calculations to analyze and interpret data	In addition to interpreting observed trends in graphical data generated using CRISPectra, students can quantitatively evaluate the performance of CRISPRkit through mathematical analysis. This involves calculating two crucial metrics: efficiency and specificity, which serve as concrete measures to assess how effectively and precisely the CRISPRkit represses gene expression.
Argumentation	Develop and justify scientific arguments using evidence	Leveraging graphical data and computed metrics, students can rely on the gathered evidence and develop scientific arguments to evaluate and justify the success of their CRISPRkit experiment.

How do the concepts covered in CRISPR connect to AP Biology course contents?

Big Idea 2: Biological systems use energy and molecular building blocks to grow, reproduce, and maintain dynamic homeostasis. (ENE)

- ENE 1: 3.1 Enzyme Structure
- ENE 3: 3.2 Enzyme Catalysis

Big Idea 3: Living systems store, retrieve, transmit, and respond to information essential to life processes. (IST)

- IST 6: 6.5 Regulation of Gene Expression
- IST 6: 6.6 Gene Expression and Cell Specialization
- IST 6: 6.8 Biotechnology

CRISPRkit is ideally suited for the classroom environment, offering a practical approach to its incorporation into educational settings. This section aims to provide educators and teachers with practical advice on how to effectively integrate the kits into their curriculum and classroom instruction.

What is the ideal student audience for CRISPRkit?

The CRISPRkit is designed for AP Biology or specific biotechnology/bioengineering classes catering to 10^{th} to 12^{th} graders. Our experience indicates that students in these grades are optimally positioned to not only perform the experiments but also to understand the underlying principles of CRISPR and gene regulation. While younger students may be able to carry out the experiments, they might struggle with grasping these complex concepts behind genetic engineering. We find that a class size of 20-30 students is ideal, allowing teachers to adequately address questions and closely monitor the experiment groups. It is advised to organize students into groups of 3, where each group shares a single kit to conduct their experiment. With multiple students sharing the same kit, it helps these students build confidence, and understand the concepts via discussion, and teamwork.

How can the experiment be integrated into the curriculum?

For AP Biology classes, we suggest introducing this lab during Unit 6: Gene Expression and Regulation. Topics such as *Genetic Expression and Biotechnology* and *Cellular Energetics* are directly relevant to our kit, making our CRISPRkit experiment a fitting addition to the subjects taught. For classes focusing on general biotechnology or bioengineering, it is best to conduct this lab when discussing CRISPR and gene editing technologies. Our kits also closely align with supporting the teaching of enzyme-substrate reactions, this experiment would be an amazing way to merge and reinforce concepts about CRISPR and enzymatic reactions. This timing enhances students' engagement by directly connecting the experiment with the concepts being learned. We recommend breaking down the lab or the process of conducting the CRISPRkit experiment into three parts.

Part 1: Pre-Lab Orientation Duration: 30~45 minutes (Recommended to be conducted in a separate class session, ideally 1-2 days prior to the experiment for optimal content absorption)

Before commencing the experiment, students must understand the foundational concepts associated with CRISPR, CRISPRi, and CRISPRkit. Allocate sufficient time to this segment based on the students' grasp of the subject matter, aiming to familiarize them with the CRISPR mechanisms. Teachers should also hand out Day 1 protocols to students so they can know what comprises each reaction tube / experimental condition.

Resources:

Key Concepts
CRISPRkit Overview
Day 1 Melanin Kit Protocol

Part 2: Doing the Experiment Duration: ~30 minutes (Day 1 of the experiment)

Organize students into small groups of 2-3 individuals, and assign a CRISPRkit to each group. Then the groups will follow the Day 1 protocol to complete the experiment.

Resources:

Day 1 Melanin Kit Protocol
Experiment Worksheet (for students to check off components along the way) Day 1

Experiment Video Demo

Part 3: Analysis and Discussion Duration: ~30 minutes (Day 2 of the experiment - 24-48 hours after

Day 1 Experiment for the dual-color experiment. Different experiments may need different gap times to obtain optimal results. For example, a 4-hour gap is recommended for the melanin experiment. This can be done by moving the reactions to a fridge).
The Day 2 protocol should be handed out to students, and the kits will be returned to students to analyze experimental outcomes using CRISPectra. After analyzing the data (instructions on Day 2 protocol), a class debrief should be conducted by the teacher to discuss the results collectively, focusing on the interpretation of quantification metrics such as efficiency and specificity, thereby concluding the CRISPRkit experience with a review of the findings.

Resources:
Day 2 Dual Color Kit Protocol
Day 2 Results Analysis Video
CRISPectra GUI
CRISPectra Documentation

How to store the kits until use?
We recommend the teachers to store all reagents in a -20C freezer for long-term storage, or a 4C fridge for 1-2 day short-term storage. If the reagents are stored in a -20C freezer, the teacher should thaw the reagents before classroom use.

How to dispose of the tubes, loops, and liquids and relevant NIH guidelines. The tubes and loops can be placed directly into regular trash, and the liquids can be poured down the drain without being autoclaved or treated with a chemical disinfectant such as bleach.

The CRISPRkit is exempt from NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (Version April 2024). On Page 24 (https://osp.od.nih.gov/wp content/uploads/NIH_Guidelines.pdf) Section III-F-2, it states 'those that are not in organisms, cells, or viruses and that have not been modified or manipulated (e.g., encapsulated into synthetic or natural vehicles) to render them capable of penetrating cellular membranes'. Because the CRISPRkit is not used in organisms or cells and thus can be disposed of as regular trash.

How to ensure consistent quality of phone image?
We recommend all images be taken with the phone (or camera) placed 6 inches away from the imaging tubes to ensure optimal pixels for downstream image analysis.