**Lesson 3:** Genetic Engineering and Optogenetics

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**LESSON OVERVIEW**

**Activity Time:**

One 50 minute class period. Additional time for homework.

**Lesson Plan Summary:**

In this lesson, students will first review the brain function mapping technologies discussed last class and highlight the fact that they do not reveal the brain function in real time with fine spatiotemporal resolution needed to understand this complex organ.

Students watch a video about optogenetics and how this technology is revolutionizing our understanding of brain function in unprecedented detail. Students will review essential vector components, learn about opsin protein and then design their optogenetics vector. They evaluate their design by comparing to existing optogenetics vector maps and highlight any improvements which can be made on their vectors. Students will contemplate other resources needed to conduct an optogenetics experiment in the real world. Students read a short article on innovation of flexible optical fibers to drive home the point that conducting experiments and solving complex problems need innovations transcending many different streams of science.

**STUDENT UNDERSTANDINGS**

**Investigative Phenomenon/Problem:**

* **Description of Phenomenon/Problem:** Optogenetics can be used to selectively manipulate neural circuits, helping us to study and map the brain function at an unprecedented spatiotemporal resolution.
* **Driving Questions:** 
  + How do different branches of scientific research -- such as prokaryotic biology, material science, genetic engineering, gene therapy and neuroscience -- come together to solve a complex real world problem like mapping the brain function?

**Learning Objectives:**

*Students will know…*

* The key elements/components of a vector.
* How opsins can be used to selectively excite or inhibit genetically engineered neurons.

*Students will be able to…*

* Step through a guided engineering design process (EDP) to design their own optogenetics vector.
* Evaluate their optogenetics vector designs with vectors available to use in research labs.
* Understand that complex experiments such as optogenetics need innovations in other areas of science such as materials science and physics (flexible optical fibers for optogenetics).

**Vocabulary**:

* **Optogenetics (optical stimulation):** A technique used by neuroscientists that uses both light and genetic manipulation to control neurons. Neurons are genetically manipulated to be light-sensitive, thus enabling light to cause neurons to fire.
* **Opsin:** Photo-receptive proteins that are an important part of optogenetics. Opsins are a group of proteins that, when expressed by a neuron, make the nerve cell’s plasma membrane sensitive to light.
* **Vector:** In biology, a vector is a carrier of disease (e.g., a mosquito) or a carrier of medication (e.g., a plasmid).

**Next Generation Science Standards:**

This lesson builds toward the following Performance Expectation (PE) and its integrated three dimensions of learning. Additional dimensions are denoted with an asterisk (\*).

| **High School Performance Expectations** | | |
| --- | --- | --- |
| [**HS-LS1-1**](https://www.nextgenscience.org/pe/hs-ls1-1-molecules-organisms-structures-and-processes) **From Molecules to Organisms: Structures and Processes**  Construct an explanation based on evidence for how the structure of DNA determines the structure of proteins which carry out the essential functions of life through systems of specialized cells.  [**HS-LS1-2**](https://www.nextgenscience.org/pe/hs-ls1-2-molecules-organisms-structures-and-processes) **From Molecules to Organisms: Structures and Processes**  Develop and use a model to illustrate the hierarchical organization of interacting systems that provide specific functions within multicellular organisms. | | |
| **Science and Engineering Practices (SEPs)** | **Disciplinary Core Idea(s)** | **Crosscutting Concepts (CCCs)** |
| Constructing Explanations and Designing Solutions    Developing and Using Models  \*Asking Questions and Defining Problems | LS1.A: Structure and Function  \*ETS1.A: Defining and Delimiting an Engineering Problem | Structure and Function  Systems and System Models  ***Connections to Engineering, Technology, and Applications of Science***  \*Science is a Human Endeavor |

**Common Core State Standards:**

* **CCSS.ELA-Literacy.RST.11-12.1:** Cite evidence to support analysis.
* **CCSS.ELA-LITERACY.RST.11-12.7:** Integrate and evaluate multiple sources of information presented in diverse formats and media (e.g., quantitative data, video, multimedia) in order to address a question or solve a problem.
* **CCSS.ELA-LITERACY.RST.11-12.8:** Evaluate the hypotheses, data, analysis, and conclusions in a science or technical text, verifying the data when possible and corroborating or challenging conclusions with other sources of information.
* **CCSS.ELA-LITERACY.RST.11-12.9:** Synthesize information from a range of sources (e.g., texts, experiments, simulations) into a coherent understanding of a process, phenomenon, or concept, resolving conflicting information when possible.
* **CCSS.ELA-LITERACY.RST.11-12.10:** By the end of grade 12, read and comprehend science/technical texts in the grades 11-CCR text complexity band independently and proficiently.

**TEACHER PREPARATION**

**Materials:**

| **Material** | **Description** | **Quantity** |
| --- | --- | --- |
| Chromebooks with access to internet | Student chromebooks | At least 1 per group or individual |
| Classroom Computer | Teacher computer with projector, speakers, and internet connection to project slideshow and videos | 1 |
| Supplies | Large whiteboards and whiteboard markers.  Colored pencils (class set). | 1 set per lab group |
| Documents | * Lesson 3 [Student Handout](https://docs.google.com/document/d/1OrLvO58b9hx25nG8FoDSHsqPNfXfaGqX/edit?usp=sharing&ouid=109206844755224090285&rtpof=true&sd=true): Introduction to Optogenetics * [Teacher Resource for student handout](https://docs.google.com/document/d/1fM9rT47IPUraqw5cBO-Ff3ha-CLUIRGN/edit?usp=sharing&ouid=109206844755224090285&rtpof=true&sd=true) and other background resources mentioned in this document * [Lesson 3 Slidedeck](https://docs.google.com/presentation/d/1DWd3YywvQq_39L6cWxg7WGalolvla_Xw/edit?usp=sharing&ouid=109206844755224090285&rtpof=true&sd=true) | 1 student handout per student |

**Preparation:**

1. Make copies of Lesson 3 [*Student Handout*](https://docs.google.com/document/d/1OrLvO58b9hx25nG8FoDSHsqPNfXfaGqX/edit?usp=sharing&ouid=109206844755224090285&rtpof=true&sd=true)*: Introduction to Optogenetics,* one per student.

2. Review the slide deck and modify it if needed.

**PROCEDURE**

*Use the Lesson 3 slide deck as you progress through the lesson procedure.*

**Activity 1: Review/Bridge & Introduce Optogenetics (5-10 minutes)**

1. Review the technologies used to discern brain function in the last class and the scale at which the brain is studied for each. Remind students that action potential is triggered due to flow of Na+ and K+ into the cells which depolarizes the neuron.
2. Introduce the concept of optogenetics and how it allows us to study brain function in real time. Pick any of the following videos which break down this technique into relevant details. Note that the last video option is more advanced in content and longer. Advise the students that they will be learning more about opsins and use their prior knowledge of genetic engineering to design their own vector for optogenetics.

**Select one video (Closed Captioning available through YouTube:**

**Optogenetics: Using Light to Control Your Brain** | SciShow 2018 (4:54 min)

<https://www.youtube.com/watch?v=D_9rdj4SJrc>

**Method of the Year 2010: Optogenetics** | Nature Video December 2021(4:26 min)

<https://www.youtube.com/watch?v=I64X7vHSHOE>

**What are Optogenetics?** | Neuro Transmissions August 2017 (4:41 min)

<https://www.youtube.com/watch?v=-8bMMuvpbkg>

**Advanced Option: Optogenetics - Light Gets On Your Nerves**

Max Planck Society March 2017 (10:12 min)

<https://www.youtube.com/watch?v=dJ5MQluKcZY>

1. Recap how introduction of artificial membrane channels and pumps - opsins by genetic engineering can trigger action potential (excite neurons) or inhibit neurons through flow of negative ions just by shining light on a specific area of the brain.
2. Note that the goal of this lesson is to build their own optogenetics vector.

**Activity 2: Introduction to Optogenetics - Part 1 of Student Handout**

*Review essential components of a vector used in genetic engineering.*

1. Split students into groups of 2-4 according to your class size. Give each student a Lesson 3 handout and inform them to stop at the stop signs and wait for further instructions. In this activity, students will review key components of a vector by comparing various plasmid maps.
2. **NOTE:** Teachers can substitute any other plasmid maps freely found on the internet. These are representative images which are licensed by Creative Commons.
3. Walk around to guide student groups to help them select ori, selectable marker, MCS (multiple cloning site) and promoter at the very least. Give students time to fill in the table by using any of the resources found on the internet. Remind students that the cDNA or gene to be expressed needs to be downstream of the promoter and also maintain the reading frame.
4. Proceed with the slide deck to cover the opsin proteins used in optogenetics. Various examples of opsins are provided in the slide deck. Teachers can point out how channelrhodopsin is tagged with a fluorescent protein to help locate the engineered neurons *in vivo*.

**Activity 3: Introduction to Optogenetics - Part 2 and 3 of Student Handout**

*Design and evaluate your own optogenetics vector.*

1. Ask students to design their own vector to introduce the opsin gene in their designer plasmid and briefly write about its essential function. **NOTE:** It is recommended that students use colored pencils or mini whiteboards if present so that they can improve on their designs.
2. Studentsgo to:<http://www.addgene.org/collections/optogenetics/>
3. Tell students: With your partner/group, design your optogenetic vector by selecting the options shown on the website. Draw a simplified version of your vector map and try to highlight the vector elements below. **Compare** the Addgene repository vector map to the one drawn by your group previously. Briefly highlight how you can **improve** your optogenetics designer vector by adding or removing vector components.
4. If time permits student groups can present their vector designs to the class.
5. Facilitate a teacher led class discussion (see slide deck). Ask students if they perceive any problems not addressed? Answers will vary and few examples are given below:

* Plasmids are bacterial vectors. We need other vectors to infect mammalian cells such as AAV (adenoviruses).
* How do you know which nerve cells are expressing the genetically engineered construct? We need to add a fluorescent tag to the opsin gene.
* How can we shine light through the skull and other layers of the brain? Design flexible and optically transparent chambers which can maintain aseptic conditions during the experiment while exposing specific areas of the brain to light/laser.

**Activity 4: Wrap up (5 min or finish as homework if needed)**

1. Students read about how researchers at the Center for Neurotechnology use flexible fiber probes to record and stimulate neural activity or a lengthier article from *The New Yorker*. The longer article could be assigned as additional homework.

**Researchers Use Flexible Fiber Probes to Record, Stimulate Neural Activity**

Mary Guiden, September 5, 2014 | Center for Neurotechnology

<https://centerforneurotech.uw.edu/engage-enable/post/researchers-use-flexible-fiber-probes-record-stimulate-neural-activity>

**Lighting the Brain: Karl Deisseroth and the Optogenetics Breakthrough**

John Colapinto, May 11, 2015 | *The New Yorker*

<https://www.newyorker.com/magazine/2015/05/18/lighting-the-brain>

**STUDENT ASSESSMENT**

**Assessment Opportunities:**

* Please see the [Teacher Resource](https://docs.google.com/document/d/1fM9rT47IPUraqw5cBO-Ff3ha-CLUIRGN/edit?usp=sharing&ouid=109206844755224090285&rtpof=true&sd=true) document and procedural details.

**DIFFERENTIATION FOR INCLUSIVE INSTRUCTION**

**Adaptations for Remote Learning Environments:**

| **Name of Activity** | **Remote Adaptations** |
| --- | --- |
| **Activity 1: Intro** | Video (please see the Procedure section to select one) can be played on Zoom/Google Meet to the whole class. |
| **Activity 2** | Review of vectors used in genetic engineering. Make Google Docs and use breakout rooms for student groups. |
| **Activity 3** | Refresher [video](https://www.youtube.com/watch?v=55meVwea5Ss) can be played on Zoom/Google Meet. Students can share their screens to show the Addgene optogenetics vector they selected. |
| **Activity 4** | Article URLs can be given to students to read independently . |

**Adaptations for Learners’ Needs:**

* Give students the [vector map which is annotated in detail](https://thebumblingbiochemist.com/wp-content/uploads/2019/10/plasmid-100-1024x1024.jpg) instead of having them explore different plasmid maps for common vector components. Also, add in this information to the student handout.

**Extension Activities to Build on Student Interest and Expertise:**

* Students can research the length of actual genes and the fluorescent tag using public databases such as ncbi.nlm.nih.gov to calculate the total length of their insert.
* Students can also research different types of promoters used in gene therapy vectors and further enhance their designed vector constructs.
* Students can watch the optional videos not shown in class.
* Technical reading resource:

Zhang, F., Vierock, J., Yizhar, O., Fenno, L. E., Tsunoda, S., Kianianmomeni, A., Prigge, M., Berndt, A., Cushman, J., Polle, J., Magnuson, J., Hegemann, P., & Deisseroth, K. (2011). The microbial opsin family of optogenetic tools. *Cell*, *147*(7), 1446–1457. <https://doi.org/10.1016/j.cell.2011.12.004>

**TEACHER BACKGROUND & RESOURCES**

**Background Information:**

* Optogenetics guide: <https://www.addgene.org/guides/optogenetics/>
* Molecular Biology Reference: <https://www.addgene.org/mol-bio-reference/>
* Viral Plasmids and resources: <https://www.addgene.org/viral-vectors/>
* Download eBook from Addgene: (Comprehensive background material on page 193) <https://info.addgene.org/download-addgenes-ebook-plasmids-101-3rd-edition?_ga=2.14864880.733119364.1627351532-1957784241.1624381283>

**Additional Resources:**

* The Beginner’s Guide to Reading Plasmid Maps (can be used as teacher resource or can be given to students who need additional support) <https://bitesizebio.com/43119/the-beginners-guide-to-reading-plasmid-maps/>