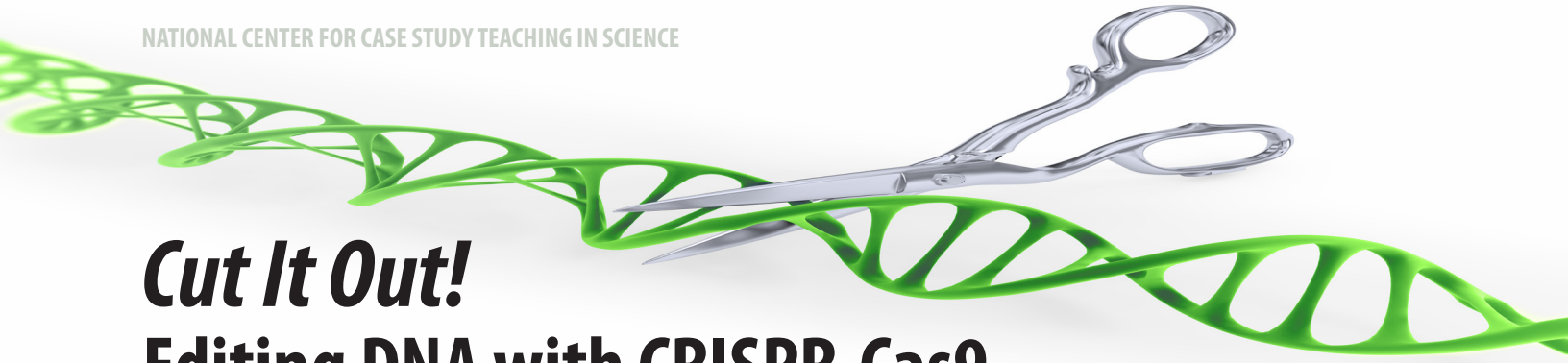

Cut It Out!
Editing DNA with CRISPR-Cas9

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Part I – Nadia's Grandfather

David paused as he walked towards his favorite reading chair, absorbing the early morning sunlight peaking through the window. Even though he had retired from his civil engineering career, he still woke early and liked to get started on his day. Rather than sitting in an office chair working on draft plans for a new community center, he now began his day here in his living room with his iPad. He had adjusted to this change in accessing information and appreciated that one didn't have to wait for the daily paper to see what was going on in the world. After a quick scan of the headlines—which revealed nothing too interesting—David navigated to the TED website. He had recently become hooked on TED Talks, and relied on these much more than the Discovery or History Channel to help him keep learning. In another life, he would have been a college professor.

He had just finished watching the 2015 best science talks playlist yesterday (a 16-year-old developing a test for pancreatic cancer? Wow!), so what next? What about genetics? What was new with all that human genome research? And wasn't his granddaughter Nadia taking a genetics class this term at UCLA? She'd be home for the holidays soon; maybe he could impress her with his up-to-date knowledge.

Scrolling through his search results, a name caught his eye: Jennifer, his late wife's name. What was this about—editing our DNA? Well, it must be fate—Jenny had had a long career as a writer and editor, so this should be a good place to start his morning

Fifteen minutes later, as the closing applause signified the end of the video (<http://www.ted.com/talks/jennifer_doudna_we_can_now_edit_our_dna_but_let_s_do_it_wisely>), David shook his head. Using bacteria's defense against viruses to cure human disease? This is why he had been a civil engineer. Living things were so much more complicated... .

Questions

1. Where did the CRISPR system first originate in nature and what is its purpose in that context?
2. How is Cas9 able to bind to specific sequences of DNA? What does Cas9 do to the sequences it interacts with?
3. What advantage does the CRISPR-Cas9 system offer compared to previous genome editing technologies?
4. David remembers one of his friends discussing how the government should focus funding more on clinical research rather than basic research. His friend felt that basic research in model organisms was not very important anymore since we know so much about genetics. Why are model systems an important part of research? What might David tell his friend about basic research, now that he's seen this TED video?

Part II – Nadia's Aunt

Audrey and her partner Andrew sat in the corner of the diner, trying to process the information they had received during their meeting with the team from the research hospital. Many couples planning for a pregnancy have anxieties and questions, but this pair felt they had more than most. During her teenage years, shortness of breath had led to genetic tests that revealed Audrey was heterozygous for a mutation in the dystrophin gene. The dystrophin gene is X-linked; since Audrey had a functional allele on her other X chromosome, the impacts on her health had been minimal. But now she and Andrew wanted to have children and they were concerned about the possibility of having a son with Duchenne Muscular Dystrophy (DMD), a disorder that results when no functional dystrophin is made.

At their first meeting with a genetic counselor, they had talked about *in vitro* fertilization (IVF) and pre-implantation diagnosis (PGD), which would allow them to select only female embryos or embryos that did not carry the altered dystrophin allele. They understood that this would allow for genetic screening of embryos and then a decision would have to be made about which embryo(s) to transfer for Audrey's uterus hopefully for successful implantation. But then one of Audrey's friends on Facebook highlighted a report about a new technology that helped mice with muscular dystrophy (<http://www.sciencemag.org/news/2015/12/crispr-helps-heal-mice-muscular-dystrophy>) and the couple realized they had more questions about their possible options. Audrey wished she had been able to schedule this meeting when her niece Nadia was visiting for Thanksgiving. Nadia was studying biology in college and had discussed the genetics underlying this situation with her aunt before. The medical team had shared with the couple the possibility of using new genome editing technology to modify the dystrophin gene in fertilized eggs or even to treat an individual diagnosed with DMD after birth. What was the name of that method? Andrew dug through his backpack and pulled out his notes—CRISPR-Cas9 it was called—and Audrey texted Nadia to ask about it.

Questions

1. Why is the couple concerned about having a son with DMD? Are their daughters not at risk?
2. Nadia's mother Rachel is Audrey's biological sister. Should Nadia herself be concerned about being a carrier of the DMD allele or having the disorder?
3. *(Version A)* Conventional gene therapy, described as introducing a functional copy of an allele into an individual that lacks this allele, has been explored as a clinical approach for decades. However, conventional gene therapy is not useful in treating certain genetic conditions.
   a. Given what you have learned about the dystrophin gene, why is conventional gene therapy difficult for treating DMD?
   b. When the gene product of a dominant genetic disorder is harmful, why is conventional gene therapy not easily used to treat such disorders?

3. *(Version B)* Conventional gene therapy, described as introducing a functional copy of a gene into an individual that lacks this allele, has been explored as a clinical approach for decades. Why is the CRISPR-Cas9 system more favorable compared to this conventional type of therapy?
4. *(Version A)* There are potential technical challenges with genetic modification in general and with CRISPR-Cas9 specifically.
   a. What challenges might there be in using genome editing to treat an individual diagnosed with DMD after birth?
   b. The article also refers to "off-target effects." Explain what you think is meant by this phrase, and why it might be a risk when using CRISPR-Cas9 clinically.

4. *(Version B)* What challenges might there be in using genome editing to treat an individual diagnosed with DMD after birth?
5. How might the way we think about using these gene editing technologies change if we imagine them being employed for purposes other than clinical disease prevention/treatment?

Part III – Nadia's Class

Nadia sat in her biology class with a box of tissues in front of her. She felt miserable and her doctor had told her that it was a viral infection. Since antibiotics wouldn't affect a viral infection, she was just going to have to let her immune system fight it off. She reached for another tissue when her instructor began talking about prokaryotes and how they have a simple adaptive immune system. Nadia perked up and wondered, *How could a bacteria or archaea have an immune system? Immune systems are really complex!* She thought about her own illness and figured that her body was recognizing the foreign virus and launching immune cells to target and destroy it. She always imagined an immune attack like a Star Wars space battle. Since prokaryotes are single cells, Nadia began to listen to her professor explain how a single cell can build up defenses against a viral attack. She copied down the reference her professor listed on the slide [Sampson, T.R. and Weiss, D.S. 2014. Exploiting CRISPR-Cas9 systems for biotechnology. *Bioessays* 36: 34–38] and planned to take a look at the first three sections that her prof had highlighted later (Introduction, Cas9 as a... platform, and Engineering Cas9…).

When Nadia got out of class, she took a quick glance at her phone as she was headed to lunch. A text from her aunt Audrey read, "Do you know anything about CRISPR-Cas9 technology? We met with the medical team today and they said it was a possible treatment option for us."

Whoa! thought Nadia. *That was the method that had been brought up in class today!* She settled into a corner table in the cafeteria and flipped open her laptop to look up the reference.

Questions

The Basics

1. What does CRISPR stand for?
2. Based on your understanding of nucleic acids, what type of bonds form between the CRISPR/guide RNA molecule and the target DNA? What type of bonds would an enzyme such as Cas9 affect?

DNA Repair

Figure 1 in the Sampson and Weiss review shows two types of DNA repair, non-homologous end joining (NHEJ) and homology directed repair (HDR).

3. When Cas9 induces cleavage resulting in NHEJ, it causes mutations in the cell. One common effect is a frameshift mutation: how does this affect gene expression?
4. How is HDR different and why would this be desirable?

Transcriptional Effects (Optional)

5. How is dCas9 different than wild-type Cas9? Explain how it is able to repress transcription.
6. How can dCas9 be further modified to *promote* transcription?

Part IV – Nadia's Research

Nadia found herself so intrigued by this technology—and all its possible applications—that she almost missed her afternoon class! After a nap and dinner, she was feeling slightly better and decided to look for more information specifically about CRISPR-Cas9 and DMD, so she could be helpful to her aunt. Her aunt had shared with her the news article about how the CRISPR-Cas9 technology had helped mice with muscular dystrophy and Nadia knew that looking at the primary literature the news article was based on would help her understand the research. Nadia discovered that the news article was actually based on three different primary papers published back-to-back in an issue of the journal *Science* (January 22, 2016). She began examining the work reported by Nelson et al. and read through the Introduction and the results depicted in Figure 1.

Questions

1. *(Optional)* The link Nadia got from her aunt is a secondary article. The three groups whose research was discussed in the secondary article published their work directly in primary literature articles. Access each of the three articles and compare them to each other and the secondary news article. What are some components you consistently see in scientific primary literature papers that distinguish them from secondary articles?
2. *(Optional)* We will focus here on the study published by Nelson et al. What is the name of that primary article? Why do you think these three articles were published "back-to-back"?
3. From the Nelson et al. article, state what type of mutation the *mdx* mouse carries and describe the result of such a mutation in terms of the final protein product.
4. In your own words, use Figure 1A to describe how this group used the CRISPR-Cas9 system to "correct" this mutation.
5. Nadia thought back to the article she read for class: *They could have done that a different way though!* What could the researchers have done instead to create a functional dystrophin protein product?
6. *(Version A)* What kind of assay was being used to show changes in the dystrophin transcript in Figure 1D of Nelson et al? Why are there multiple bands in the treated lanes?

6. *(Version B)* In Figure 1D, they do an assay called RT-PCR. Look up this method and then in your own words:
   a. Describe how this assay can be used to determine if their genome editing worked.
   b. Explain why there are multiple bands in the treated lanes and how that links to Figure 1E.
7. *(Optional)* What are the implications of this result for restoring muscle function?
8. Given this data and other considerations raised in this case study, what might Nadia share with her aunt about the current strengths and/or limitations of using a CRISPR-Cas9 approach to treat DMD postnatally?