1. The talk in this video describes the CRISPR technology for gene editing. Note this talk is from 2015, which is prior to the events in subsequent parts of this module and before the speaker won the Nobel Prize for developing this technology. Please watch this video, and answer the question below.

A. To edit the DNA the DNA is first broken and then repaired. The CRISPR technology controls just one of these steps; how does the other step happen?

The CRISPR technology controls just the breaking of the DNA. Cells have the ability to detect broken DNA and repair it. When a plant or animal cell detects a 'double-stranded' break in its DNA, it can fix it by either pasting the two cleaved ends together, or by integrating a new piece of DNA at the site of the cut.

B. What type of molecule is Cas9? What type of molecule binds with Cas9 to form a "sentinel" that finds desired sites in the cell's DNA and then cleaves the DNA at these sites? How does the "sentinel" identify the desired sites? Does Cas9 help identify the site or carry out the cleaving?

Cas9 is a protein. Little bits of RNA from the CRISPR locus bind to the protein to act as a sentinel, finding the desired DNA. To find this, it searches through all the DNA in the cell and finds the sites that match the sequences in the bound RNA. The RNA allows the locating of the site, while the Cas9 cleaves the DNA.

C. The key advantage of CRISPR over previous genome editing technologies is explained in terms of an analogy to computers—what is this analogy?

Engineers can program these complexes to recognize specific DNA sequences, and make a break in the DNA at the site. Can allow cells to make a very precise change, just as we use word processing with computers to find spelling errors and find exactly where they occur. It is easily programmable, without having to completely rewrite anything entirely.

D. To use CRISPR to edit a desired gene, what type of molecule is "programmed" so that the DNA is broken at the location of the desired gene, and how does an engineer "program" this molecule?

The RNA is the programmed molecule. An engineer would determine the DNA that they would like to target, and would craft/find a strand of RNA that is the complement (or is compatible with) of this DNA to package with the Cas9 protein. This RNA will then be able to find the DNA through the sentinel, and then the Cas9 can cleave/break the specific location. Once the DNA is cleaved, the cell will heal it. Engineers will primarily target mutations and 'bad DNA' in order to make use of the technology and cells' inherent healing factor.

E. One of the slides refers to "perfect pitch". What is the speaker referring to, and what ethical question is the speaker addressing here?

She shows perfect pitch when showing a baby with 'ideal features'. Perfect pitch refers to the ability to hear a note or chord and immediately recognize it, just as regular people do with color

recognition. The ethical question involves both safety and the idea of creating ‘designer humans’. She is worried about the various implications with creating the ideal human, especially regarding social norms. She urges us to think of the unintended consequences of doing such a thing so early in the technology's development with respect to human testing.

2. This article describes research at CWRU that uses the CRISPR technology as part of a novel biosensor. Please read the article and answer the following questions.

A. This research at CWRU addresses a new "universal biosensing" point-of-care medical device. What does this biosensor detect?

This device would detect 'troublesome' viruses like HPV or parvo. It would detect them rapidly and accurately, eliminating the need for the existing tests which are expensive and take a long time to get an accurate result (3 to 5 days for the old vs under an hour for the new).

B. How does this biosensor make use of the CRISPR technology?

They modified the CRISPR technology into E-CRISPR, which uses an 'electrochemical platform' to identify and quantify viruses present in blood by making use of the accuracy of the CRISPR technique. They will program the CRISPR complex so that it looks for a specific target (virus) and to probe any activity electrochemically. If the Cas9 protein starts to cut, the probe will pick up on it indicating that a virus is present.

3. This news article reports the use of CRISPR to edit a gene in an embryo. Please read the article and answer the questions below.

A. What gene was edited and why was this gene chosen to be studied?

The researchers targeted a mutation in a gene called MYBPC3, which causes the heart muscles to thicken (a condition called hypertrophic cardiomyopathy) resulting in sudden death in young athletes. It is a dominant gene, so just one copy is required for the mutation to be present. Because of its dominance and that it's the leading cause of sudden death in these athletes, they chose this gene to study.

B. What are the two key safety hurdles to applying CRISPR technology? How successful was this study in these regards?

They had to tackle 1) the risk of making additional, unwanted, genetic changes called off-target mutations and 2) the risk of generating mosaics, where different cells in the embryo contain different genetic sequences. They have been largely successful, finding no evidence of off-target genetic changes, and having generated only a single mosaic in an experiment involving 58 total embryos.

C. What did Keith Joung say about the safety claims of this work?

Keith Houng said that just because the team didn’t find any off-target changes to the genome doesn’t mean that they aren’t present. In other words, don’t be fooled by our apparent success in eliminating the risk of our first key safety hurdle.

4. This science news article describes the response of the scientific community to the announcement of the first genome-edited baby. Please read the article and answer the following questions.

A. When and where were the world's first genome-edited babies born?

The twin girls were born in China during the month when the article was published, so sometime in November 2018.

B. What were the steps in the process that starts with a prospective mother and father and ends with a baby born with its genome having been edited?

He first found a couple that was affected by HIV so that they could have a child protected from a similar fate. Then, he impregnated the woman with her fertilized egg (embryos) that have been edited to disable the genetic pathway HIV uses to infect cells. The babies are then born healthy, having only the target gene changed/disabled.

C. What was the goal of editing this gene? Was it to correct a mutation that would lead to health problems?

The gene, called CCR5, encodes a protein that allows HIV to enter a cell. Editing the gene was done to disable it, hence attempting to prevent HIV from entering the person's body. It wasn't to correct a mutation, but to disable the gene entirely to prevent infection.

D. What did Paula Cannon say about the decision to edit this gene?

She noted that some strains of HIV don't even use that gene's passageway to get into cells, and just because CCR5 is disabled doesn't mean that the person is immune entirely from HIV. She also says it makes no sense that He found a family HIV-positive *father* because there is no real risk of transmission to the children. Her final remark is that the experiment exposes otherwise healthy and normal children to the risks of gene editing without any realized benefit.

5. This video shows the statement of the engineer who led the genome-edited baby project. The Ethical Decision Making Primer is a brief description of the key theories of ethics. Please watch the video and read the article, and answer the following questions.

A. What was Dr. He's explanation for why he believes he did the right thing?

Dr. He thinks he did the right thing because he was able to give the family another chance to have kids, and was able to allow the husband to be a part of society again, as HIV is widely shunned. He emphasized that his actions provided a chance for families to have healthy children and that his work is a continuation of advancements in reproductive technologies that have already benefited millions of families. Without the treatment, the children would have to live with a fatal disease all their lives.

B. Do you agree or disagree with Dr. He's analysis regarding the right thing to do, and why?

Yes, I think his analysis is correct. There is little to no downside to allowing families to have healthy children when they usually wouldn't be able to through conventional methods. Some families need this technology in order to lead happy lives, and I don't think there is anything wrong with this. This is exactly what Dr. He argues in his analysis as well. While the risks may be detrimental, I think the current short term gain is worth it. With more information about the negative side effects, I am almost positive that my feelings towards his analysis would change from positive to negative.

C. Would you consider Dr. He's explanation to follow a virtue ethics approach (and why)?

Yes, but I think there's a lot of consequentialism in his explanation. His virtue ethics comes through when talking about how wholesome it is that he can give this family a new beginning. He seems to be acting truly out of what he believes is virtuous, bringing in his personal life and mentioning his children that he holds dear. I think the consequentialism comes through when he acts out of the best interest for the family without really considering the long term consequences of the treatment. His lack of discussion about the negative side effects leads me to believe he follows a more virtuous approach, as a true consequentialist would be skeptical due to any unintended consequences.

6. This news article describes the legal consequences for the engineer who led the genome-edited baby project. Please read the article and answer the following questions.

A. What crimes was Dr. He convicted of?

A court in Shenzhen found that He and two colleagues forged ethical review documents and misled doctors into unknowingly implanting gene-edited embryos into two women. The court ruled that the defendants had deliberately violated national regulations on BME research and medical ethics. He was also fined, as were his two collaborators.

B. What did Guandong province's investigation conclude regarding the ethics of Dr. He's work? Do you agree with their conclusions (and why)?

Their investigation concluded that "He had defied government bans and conducted the research in the pursuit of personal fame and gain". I agree with their conclusions. He clearly acted out of self interest, and knew that there were consequences he was not considering due to the secrecy of his research. While he was trying to better the lives of his subjects, he would've found out about the lack of full coverage of the treatment if he was interacting with other scientists. He also forged ethical review documents, which means he knew that what he was doing wasn't going to be considered ethical. Making the decisions he made seems purely out of fame and gain, as if the procedure was 100% successful he would be leading the cutting-edge research into gene modifications. His denial of government regulations has little to do with ethics, as sometimes laws are not ethical and should not be used as an ethical compass. Ultimately, He acted unethically, and I agree with the conclusion of both Guandong province's investigation and the court's ruling.

7. Read the section "What about the other scientists implicated?" of this article.
In 100 words, describe your view on the ethical responsibilities of scientists, such as Mello and Porteus, who were informed by Dr. He about his plans to edit the genomes of babies?
I think it's particularly challenging to criticize the lack of action exhibited by the scientists who were in the know. From what I can gather, Mello and Porteus both tried to dissuade He, and tried to advise him against proceeding. If the research was in the US, reporting the malpractice would've been easier due to regulations, but I don't think there's much the scientists could've done as the research was in China, which makes it harder to find avenues for reporting things like this. Without sufficient precedent, criticising these onlookers is hard for me to do in good faith.