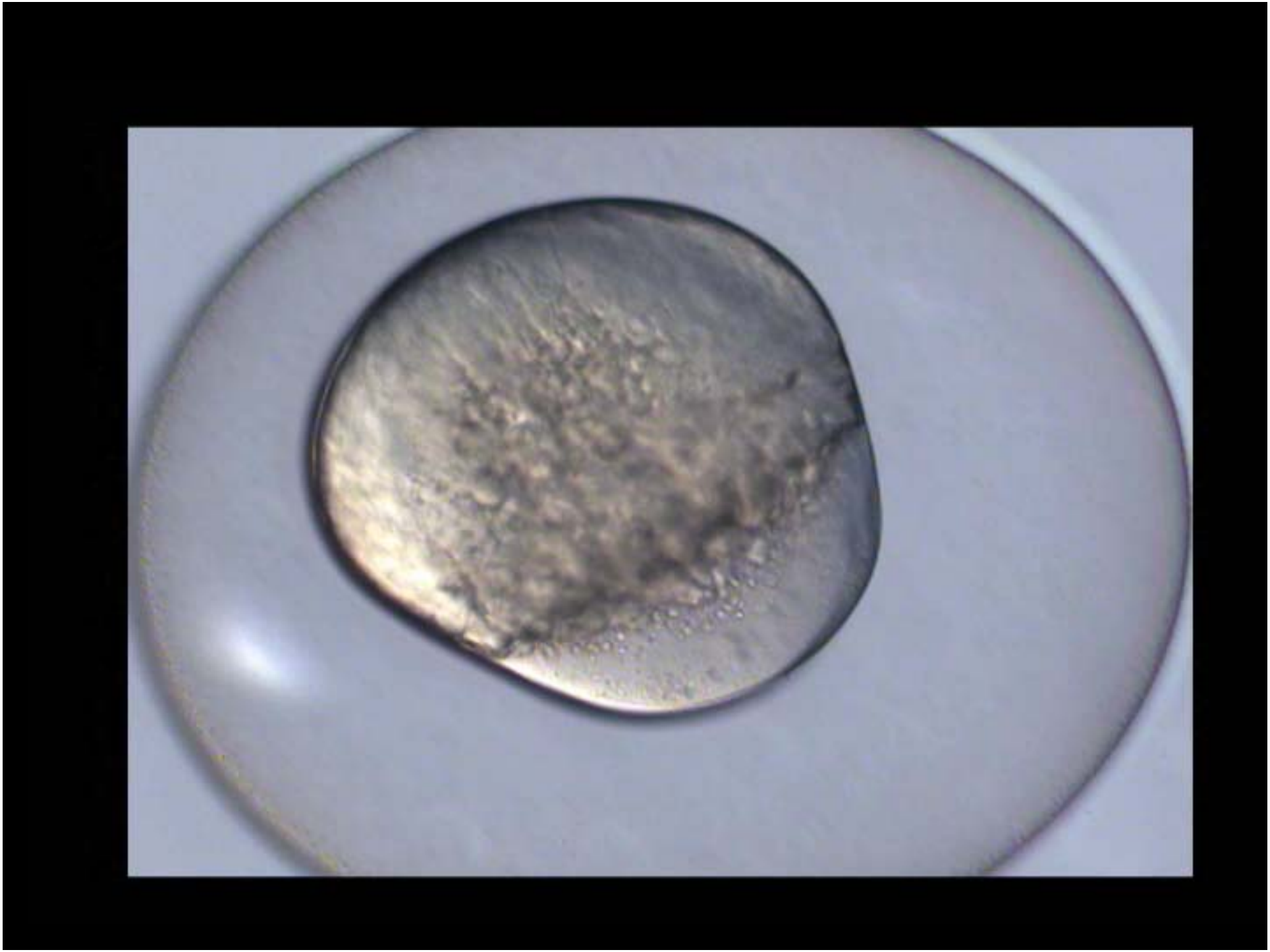
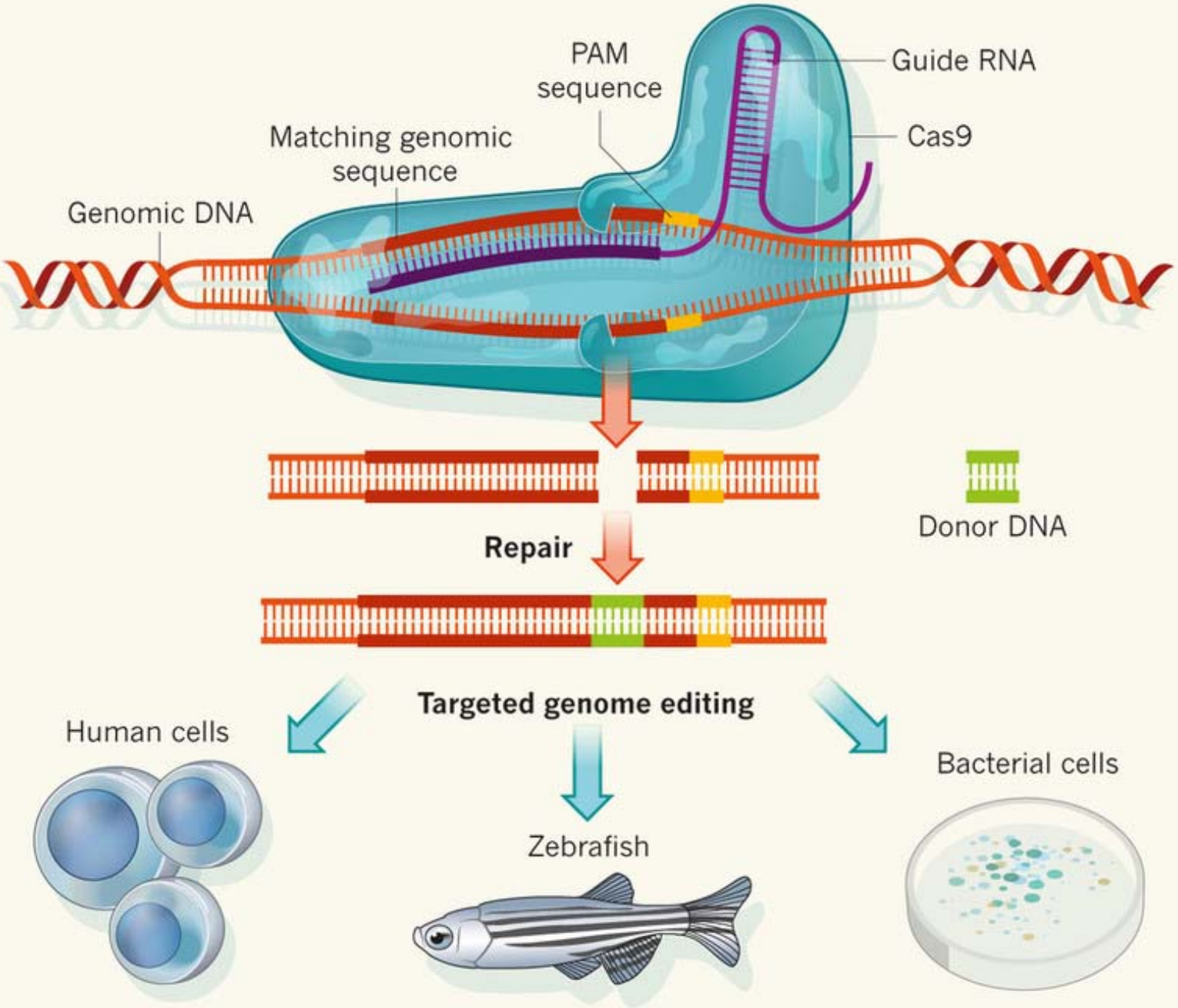
CRISPR/Cas9 Mediated Mutagenesis in *Danio rerio*

Cassie Bullard



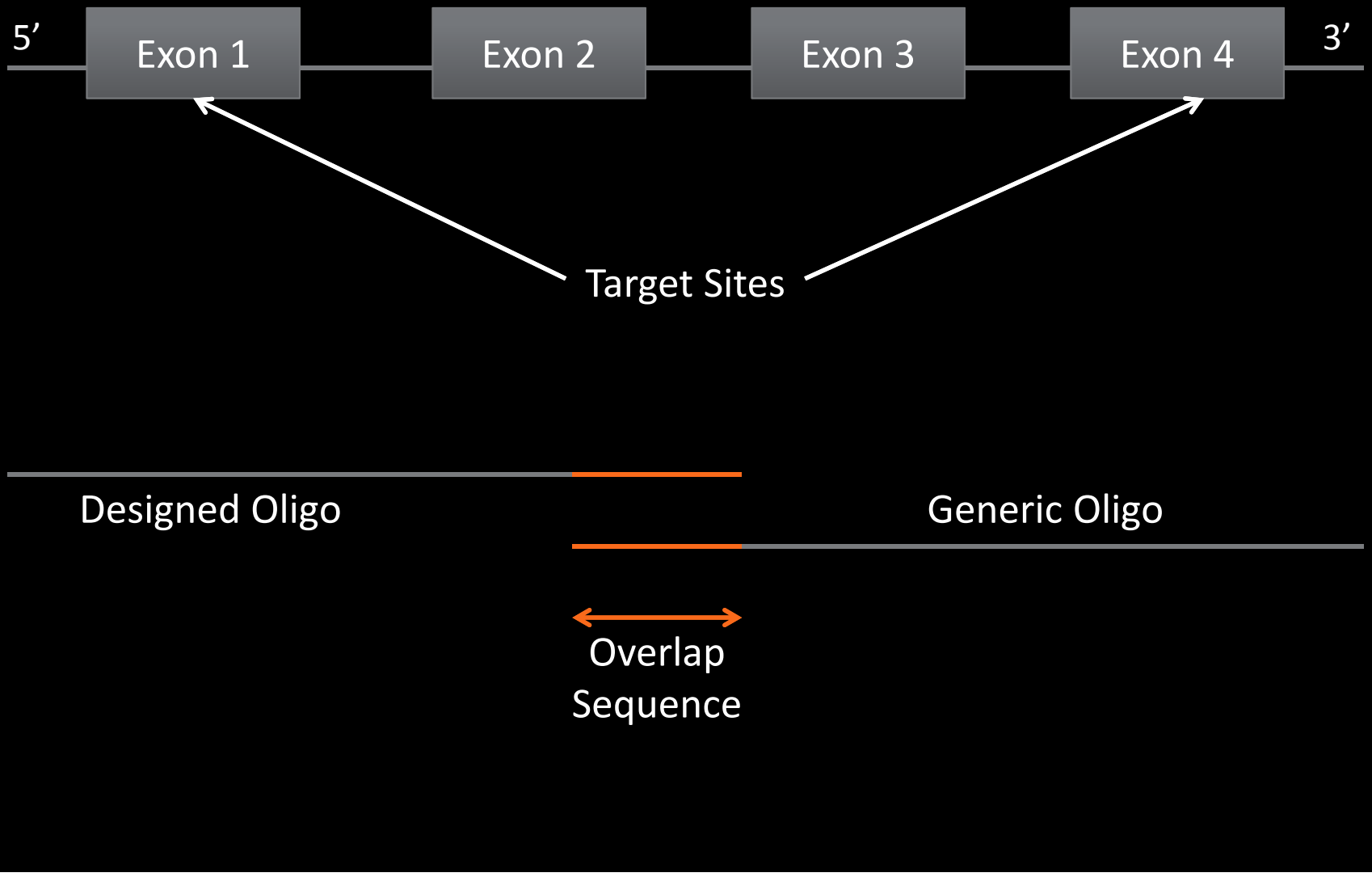
CRISPR/Cas9

- Acts as a rudimentary immune system in bacteria and archaea
- Creates double strand breaks
- Needs only single guide RNA
- Has recently been optimized for zebrafish

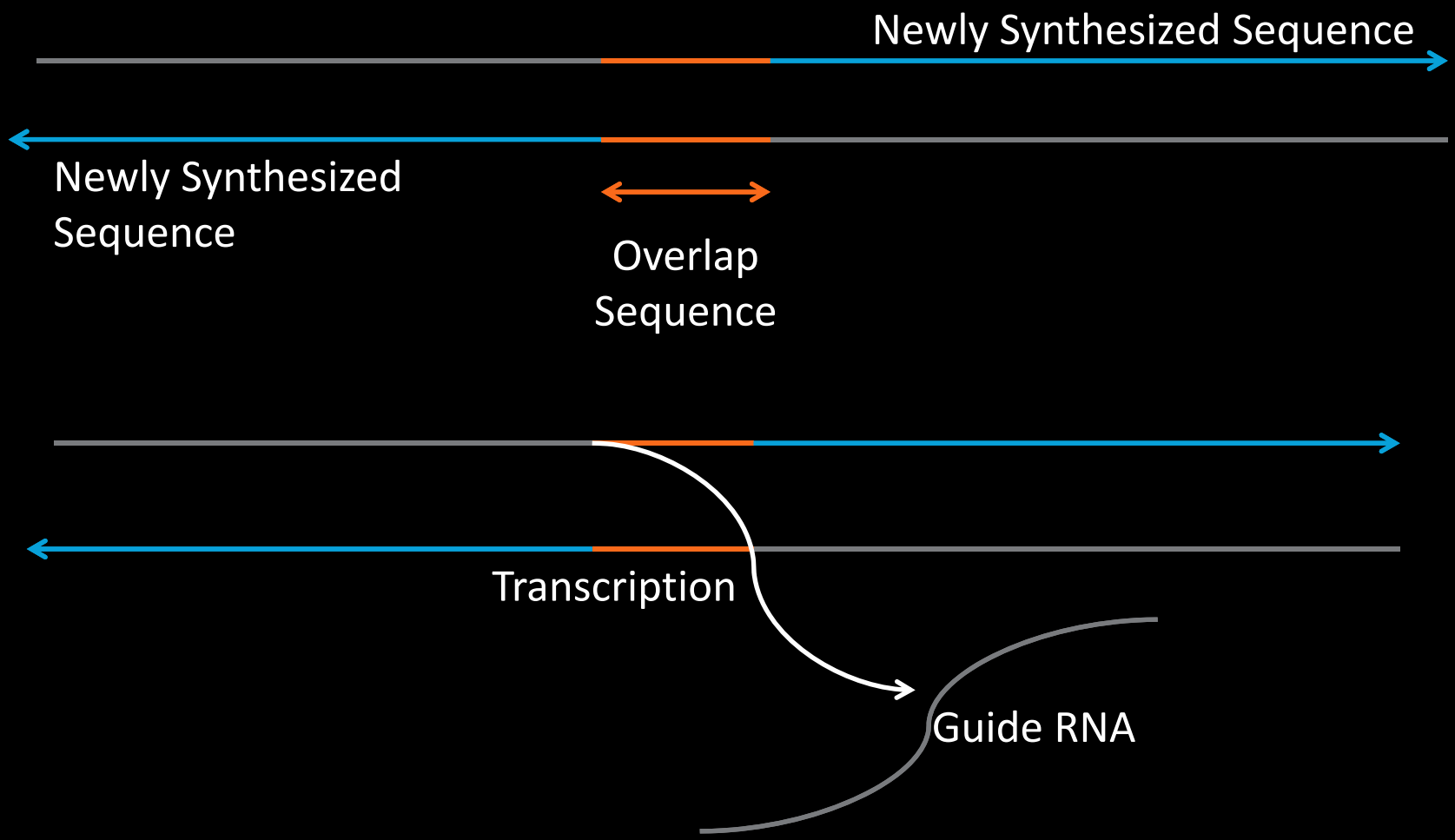


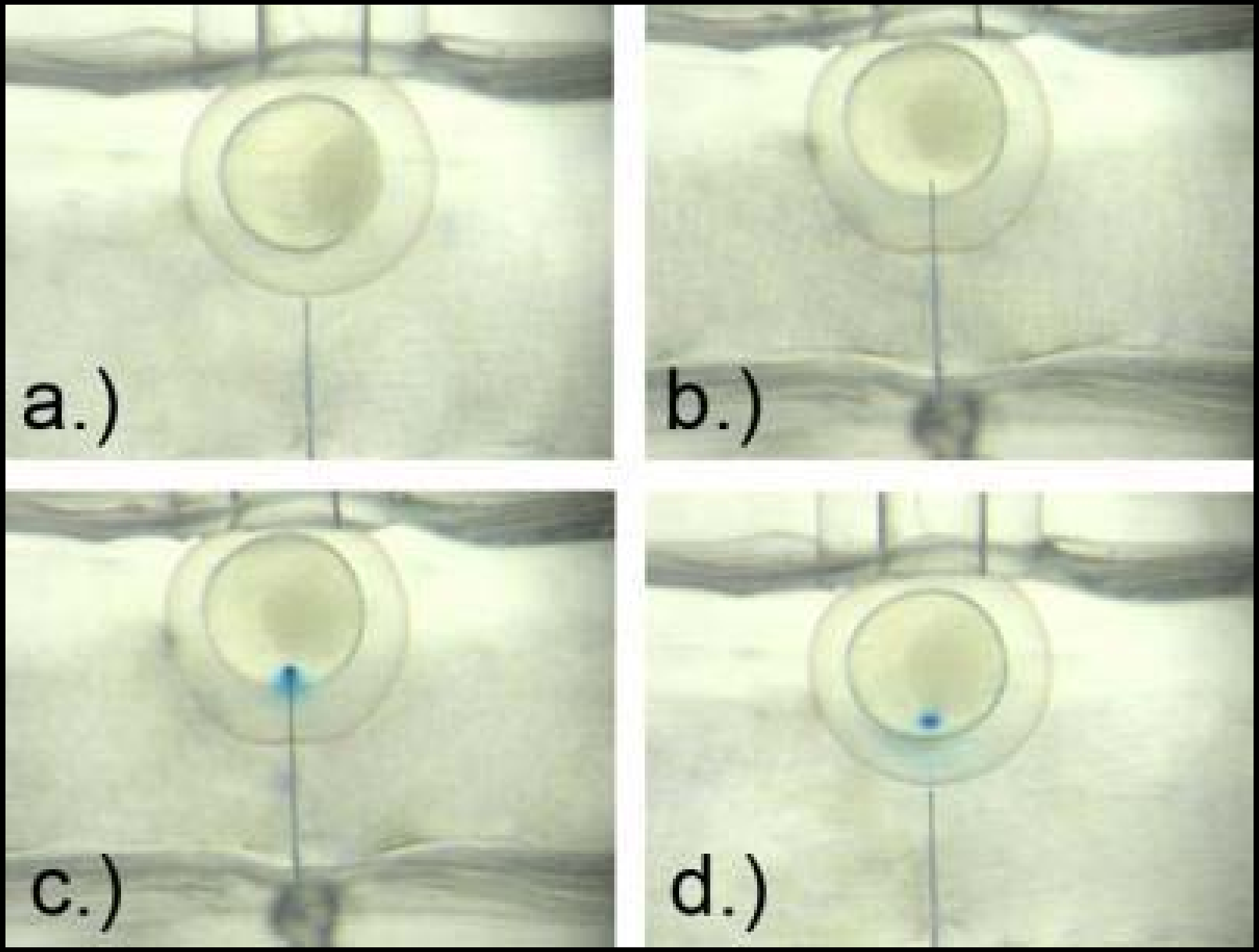
Emmanuelle Charpentier, and Jennifer A. Doudna. "Biotechnology: Rewriting a Genome." *Nature* 495.7439 (2013): 50. Web.

Design


5’
Exon 1
Exon 2
Exon 3
Exon 4
3’
Target Sites
Designed Oligo
Generic Oligo
Overlap
Sequence

Extension and Transcription





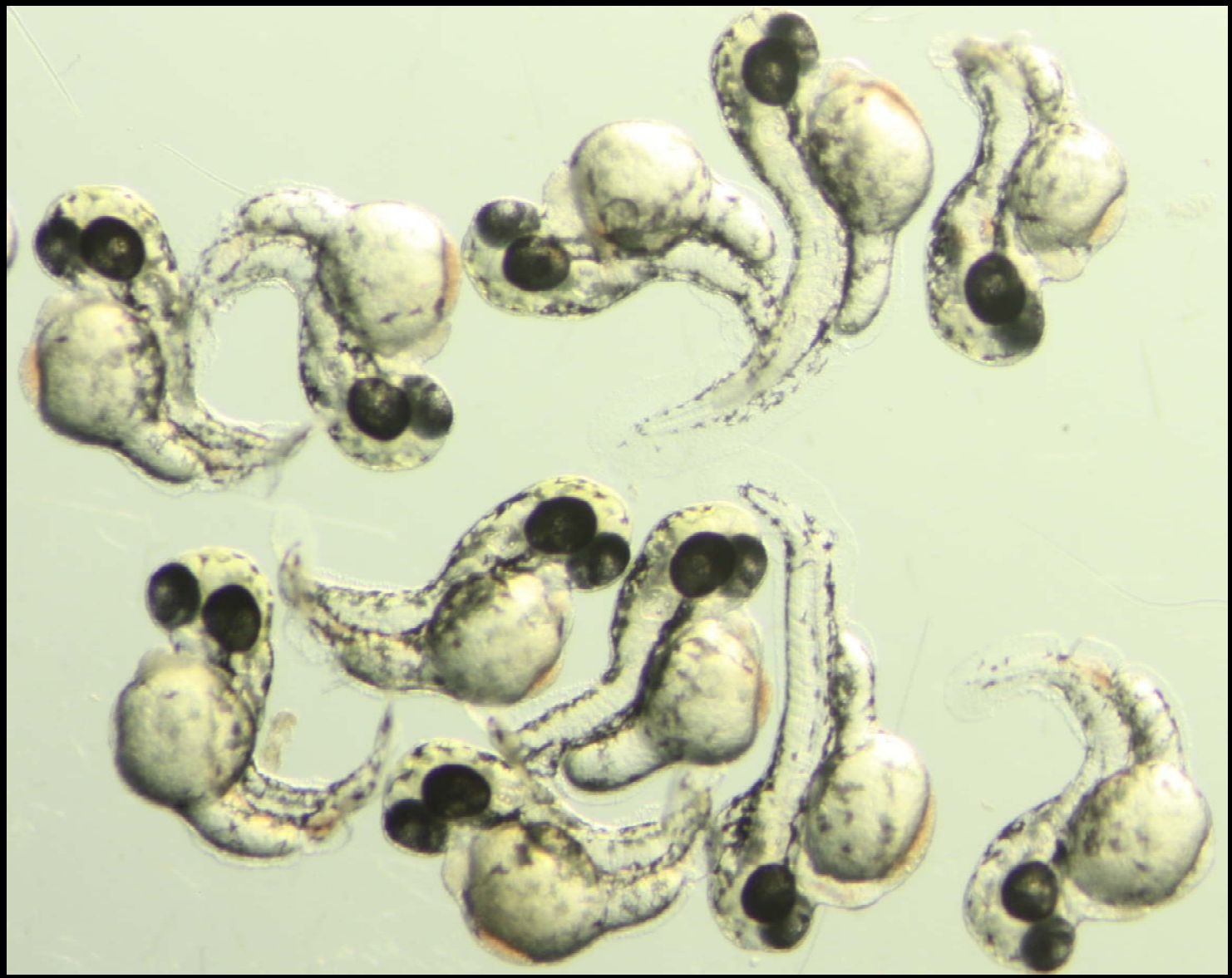
Nakonecheny, Gene. "Tiny injector to speed development of new, safer, cheaper drugs. *McMaster University EurekAlert!. 4 Nov. 2009. Web.*

Floating Head

- Involved in notochord development
- If disrupted, embryos have just a head
- Known and well established phenotype

Wildtype

Floating Head Mutants



Where It's Headed

- Ongoing project
- Can be used for individualized medicine
- Can possibly remove retroviruses (HIV)
- Gene therapy

Questions?