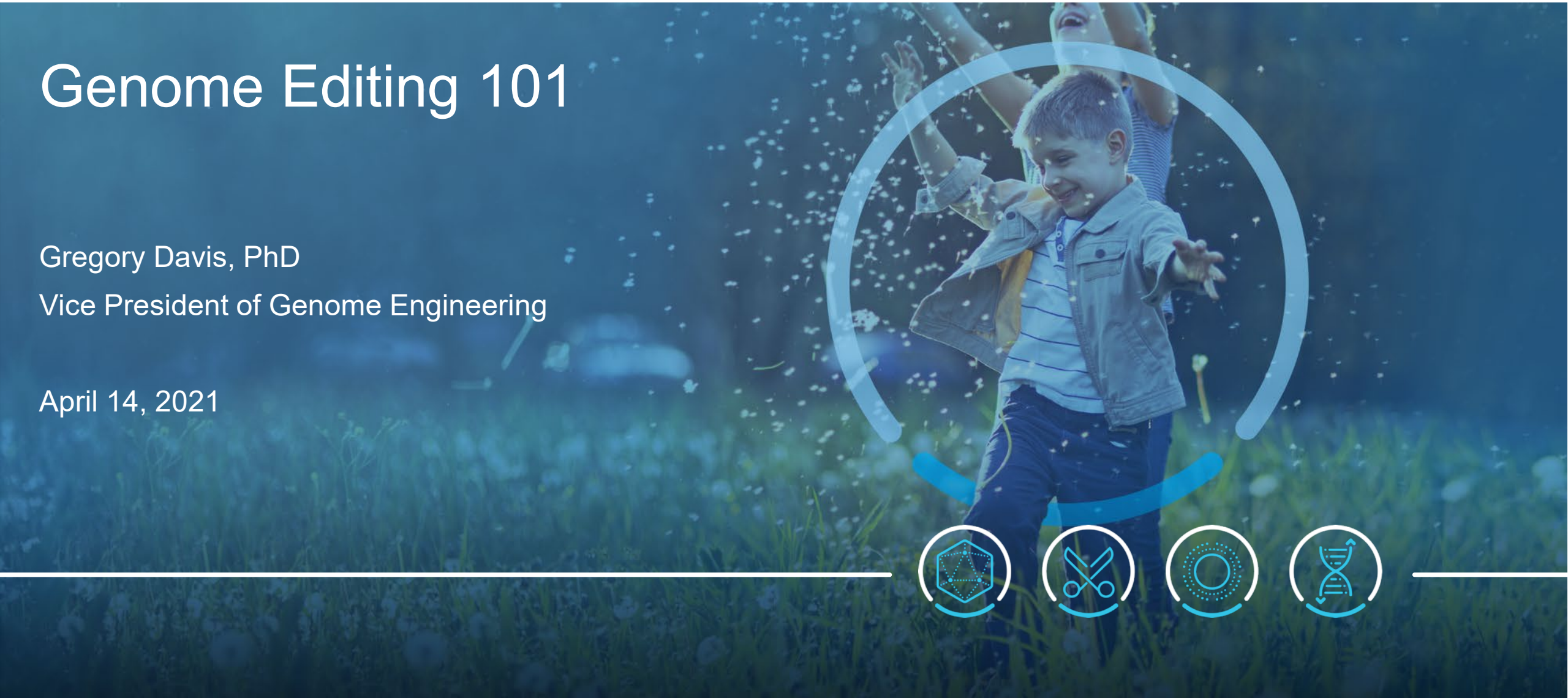
Sangamo THERAPEUTICS

Genome Editing 101



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Vice President of Genome Engineering

April 14, 2021

Pop Quiz

What are these?

Pop Quiz

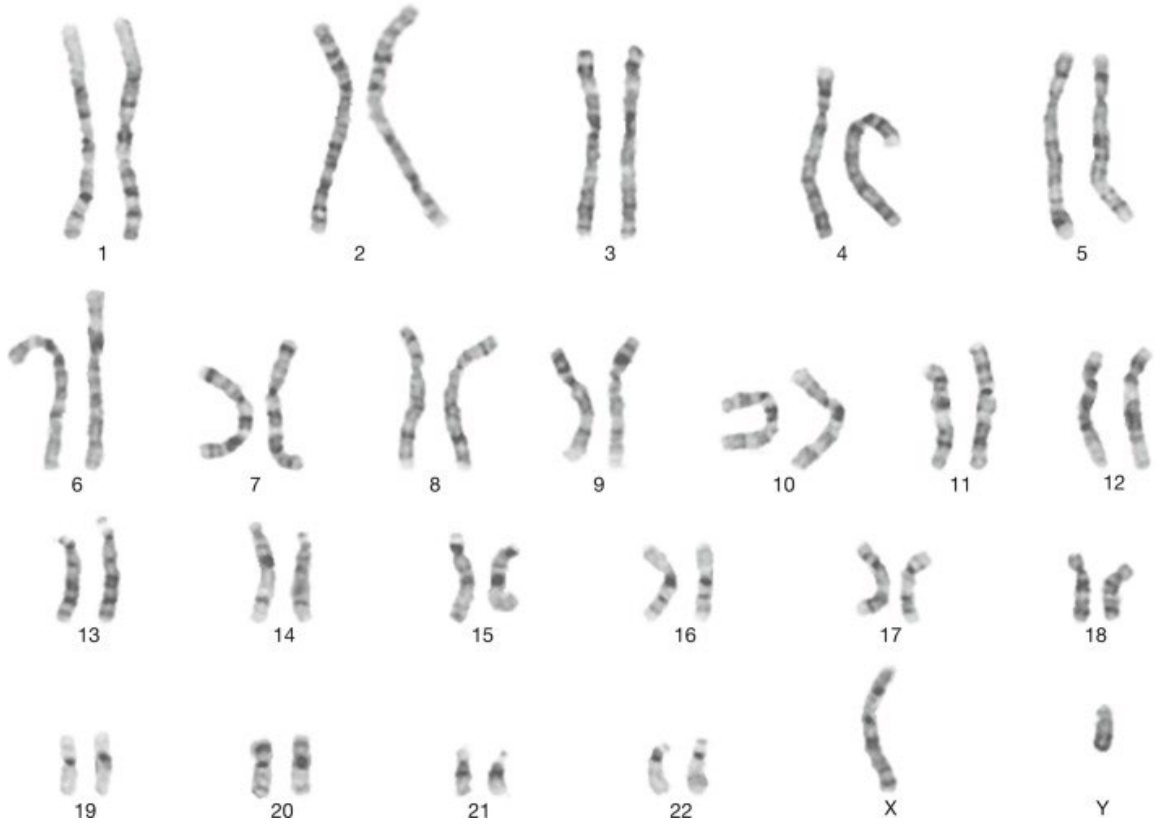
What are these?

Art by Gina Glover

Displayed in Guys Hospital, London

Look like chromosomes, but they are socks!!

What is the human genome?

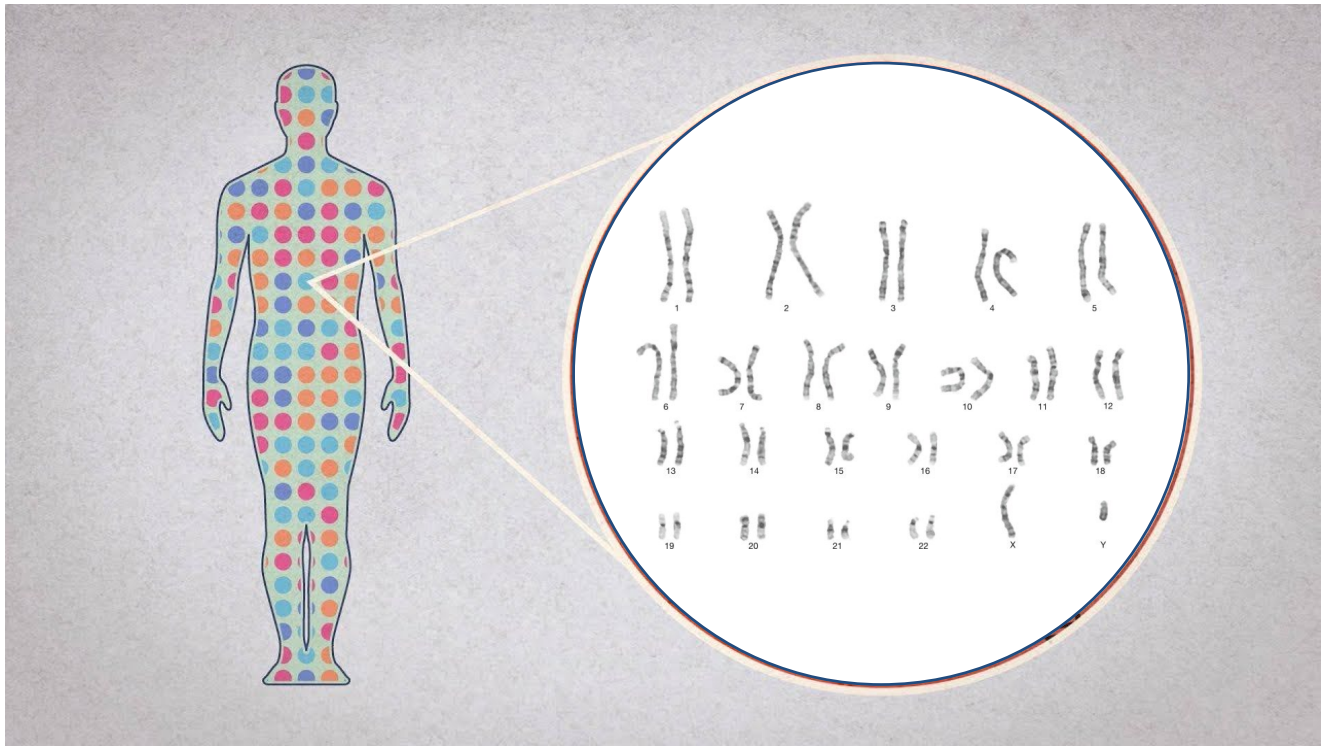


The human genome is a collection of long pieces of DNA called chromosomes (named 1,2,3, etc.)

- The human genome is spelled with about 3 billion DNA letters (A,C,G,T).
- Has about 20,000 genes.
- "Genes" are small parts of the genome that each have a unique function.

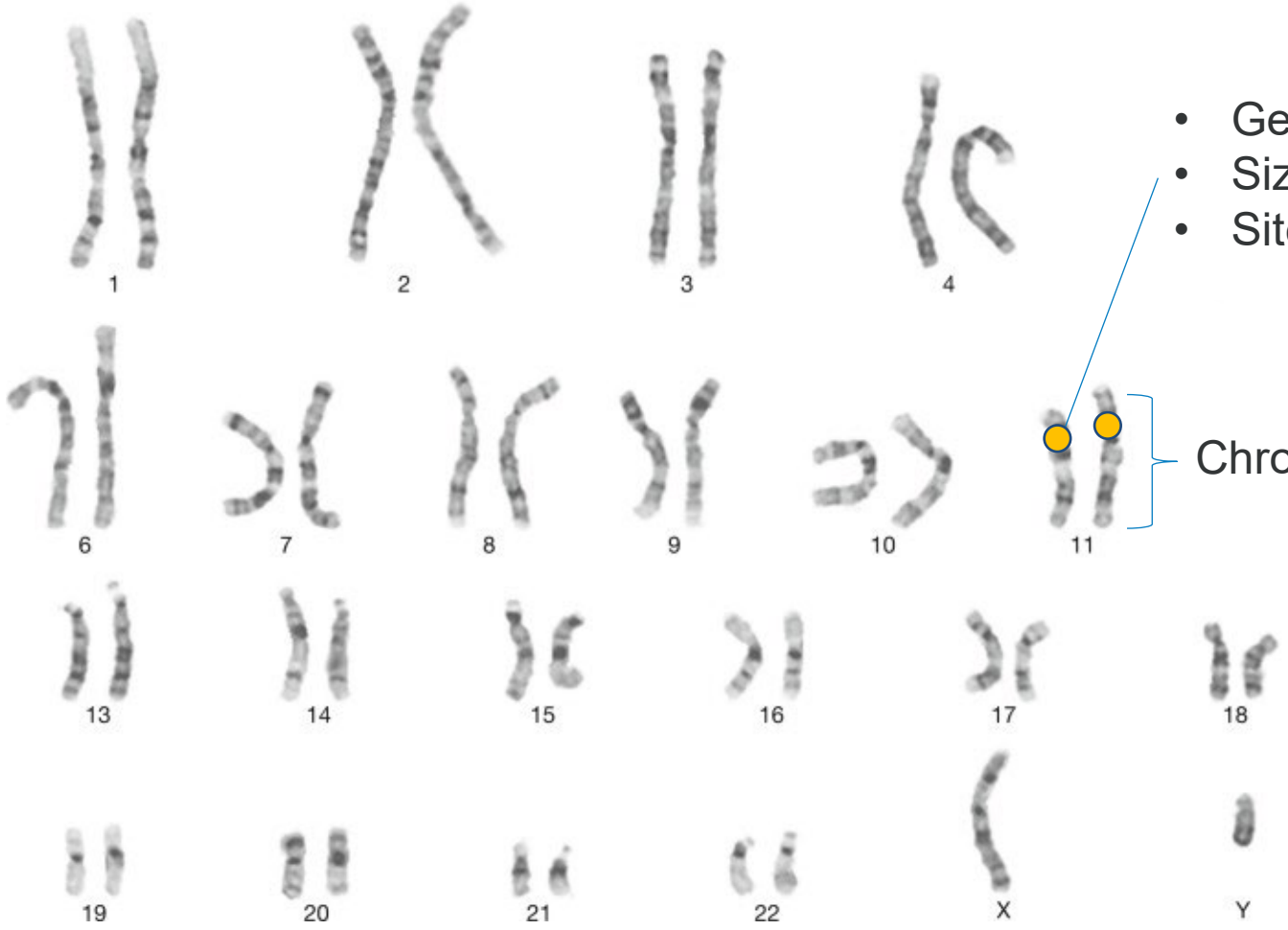
chromosome view, close up

Where is the genome?



- Most cells in the body have a copy
- Cells typically have two copies
- Each person has trillions of cells
- Each cell "expresses" different parts of genome called genes.

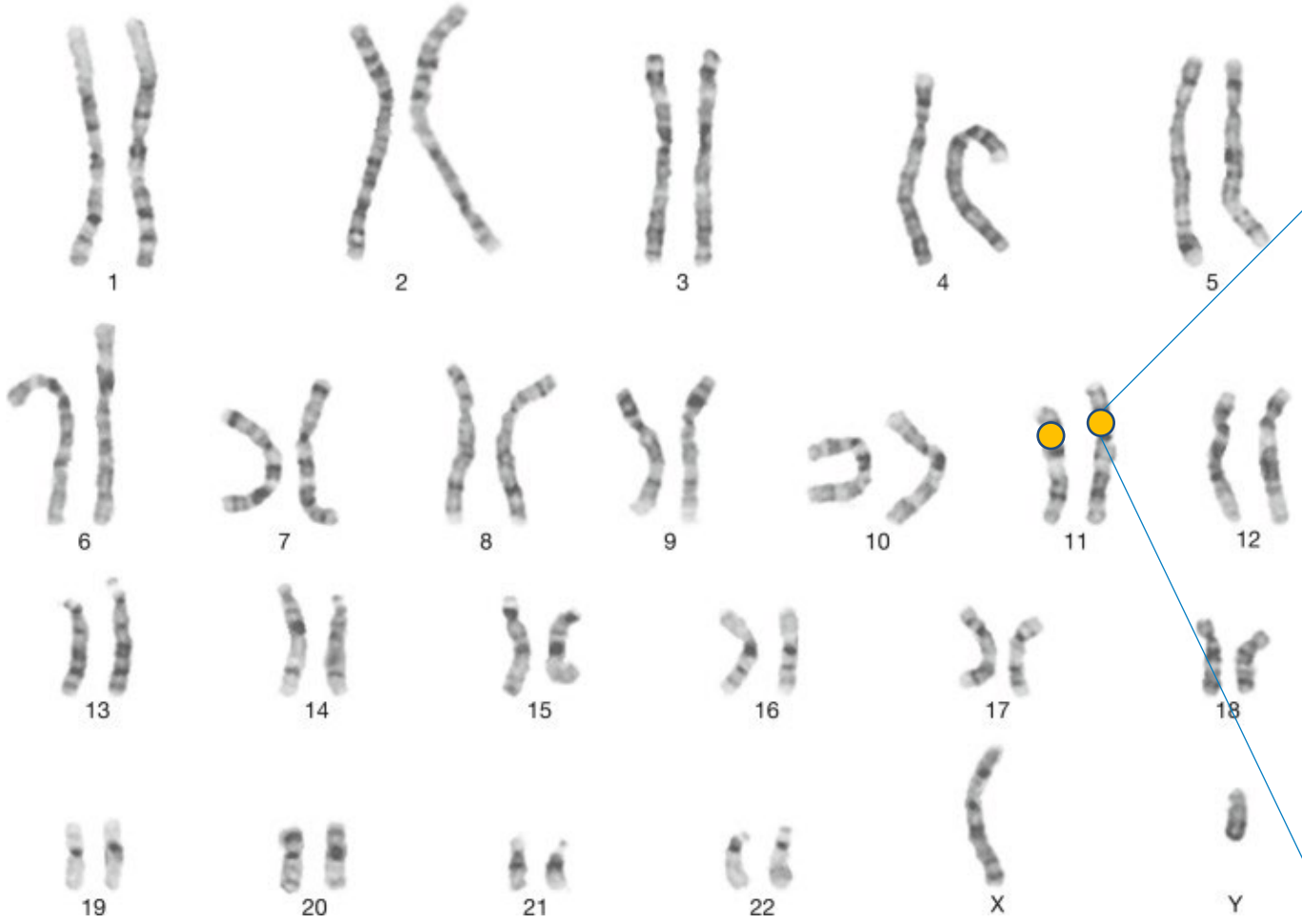
What is a gene and how does it cause disease?



- Gene for hemoglobin beta
- Size = about 1,600 bases
- Site of the sickle cell mutation

Chromosome 11 = 135 million bases

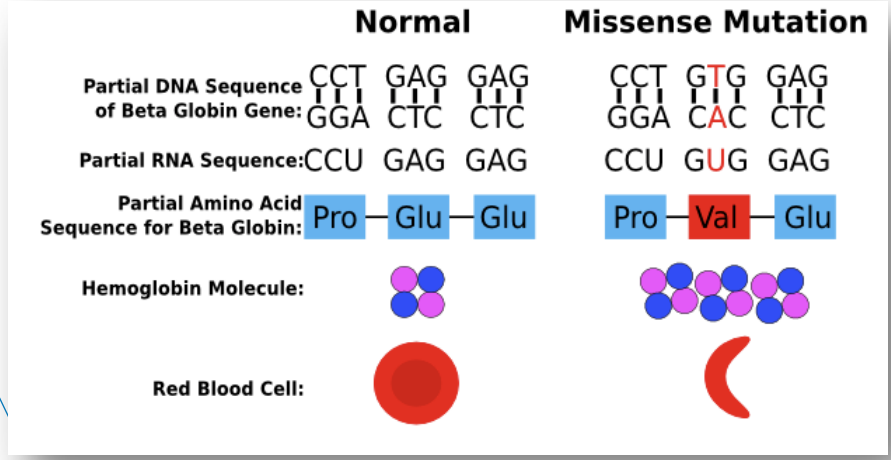
What does an incorrect gene look like? Sickle Cell Anemia



DNA sequence of the human hemoglobin beta (HBB) gene

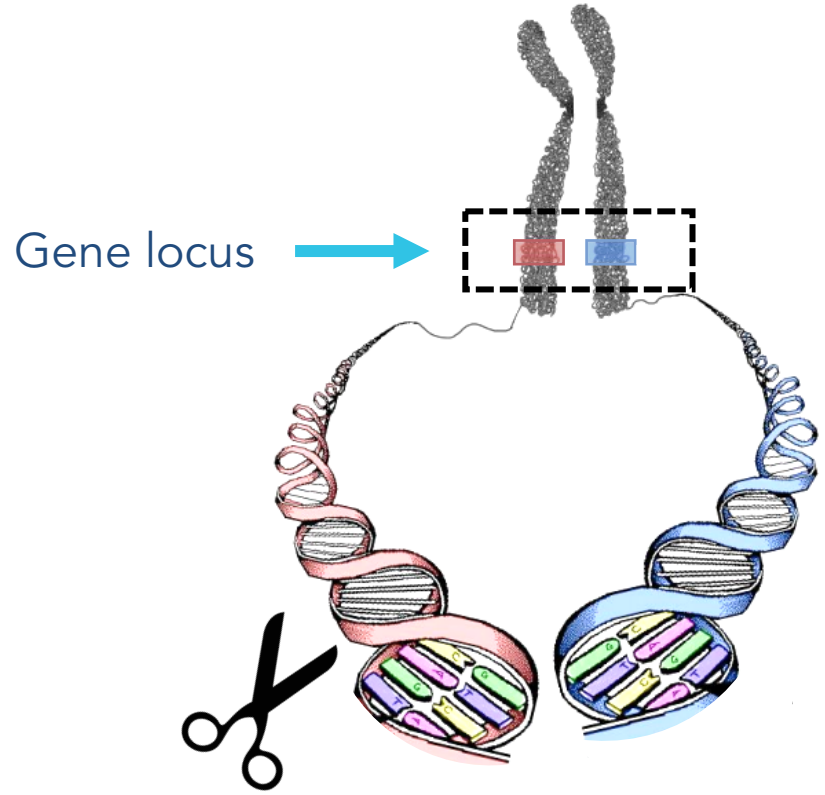
GCCAGGGCTGGGCATAAAAGTCAGGGCAGAGCCATCTATTGCTTACATTT
GCTTCTGACACAACTGTGTTCACTAGCAACCTCAAACAGACACCATGGTG
CATCTGACTCCTGTGGAGAAGTCTGCCGTTACTGCCCTGTGGGGCAAGGT
GAACGTGGATGAAGTTGGTGGTGAGGCCCTGGGCAGGTTGGTATCAAGGT
TACAAGACAGGTTTAAGGAGACCAATAGAAACTGGGCATGTGGAGACAGA
GA GTCT
AT GTTC
TT ACCC
TA GGCC
TG GCTG
CA TATG
GGACGCTTGATGTTTTCTTTCCCCTTCTTTTCTATGGTTAAGTTCATGTC
ATAGGAAGGGGATAAGTAACAGGGTACAGTTTAGAATGGGAAACAGACGA
ATGATTGCATCAGTGTGGAAGTCTCAGGATCGTTTTAGTTTCTTTTATTT
GCTGTTCATAACAATTGTTTTCTTTTGTTTAATTCTTGCTTTCTTTTTTT
TTCTTCTCCGCAATTTTTACTATTATACTTAATGCCTTAACATTGTGTAT
AACAAAAGGAAATATCTCTGAGATACATTAAGTAACTTAAAAAAAAACTT
TACACAGTCTGCCTAGTACATTACTATTTGGAATATATGTGTGCTTATTT

How do we correct this misspelled (mutated) gene?



What types of genome editing tools can we use?

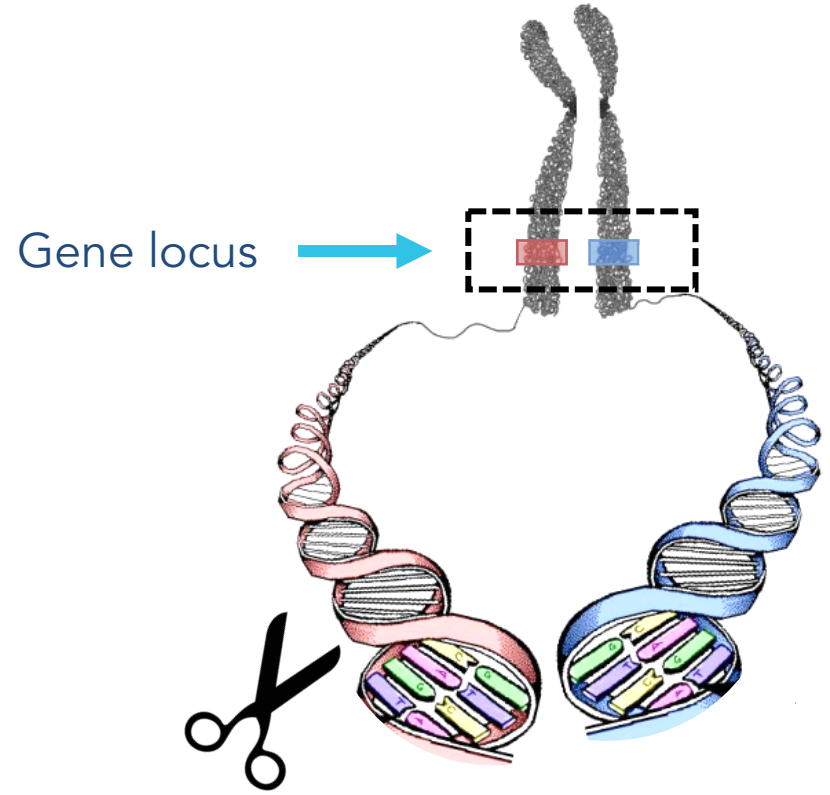
- Zinc Finger Nucleases (ZFNs)
- TALENs
- CRISPR



ZFN, TALEN, or CRISPR

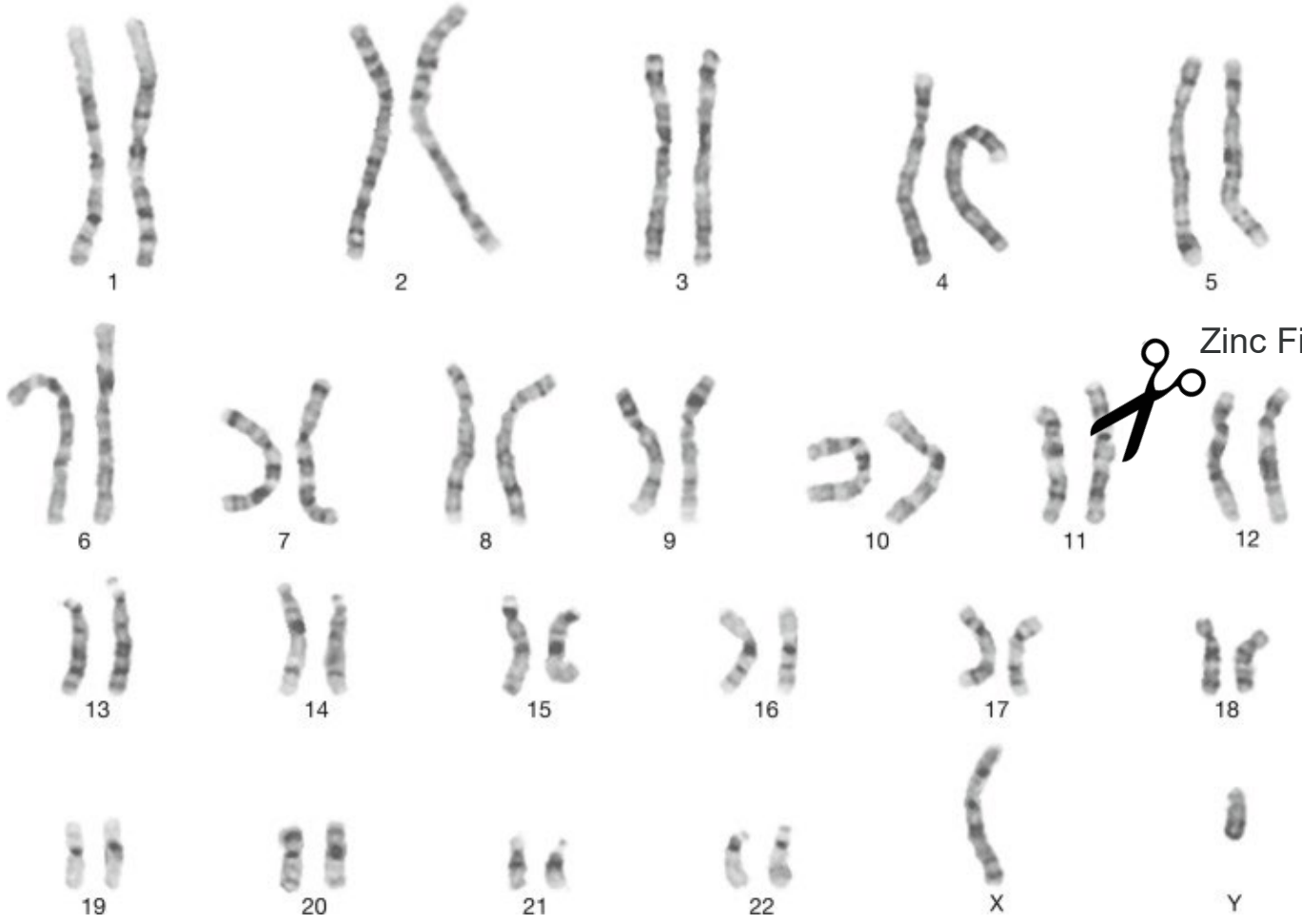
How are genome editing tools different?

- All bind to genomic DNA
- ZFs are native to mammals
- CRISPRs and TALs are native to bacteria
- Both can be engineered to edit genes
- Different genetic diseases require different molecular tools



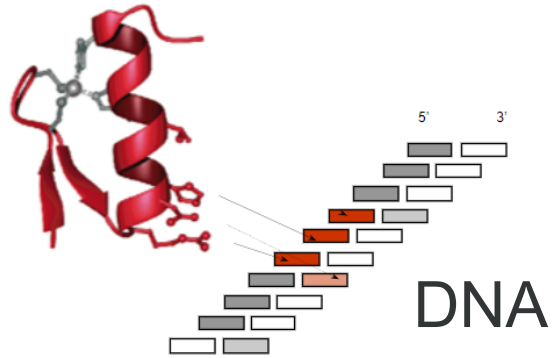
ZFN, TALEN, or CRISPR

Zinc fingers can read, grab, and edit the genome!

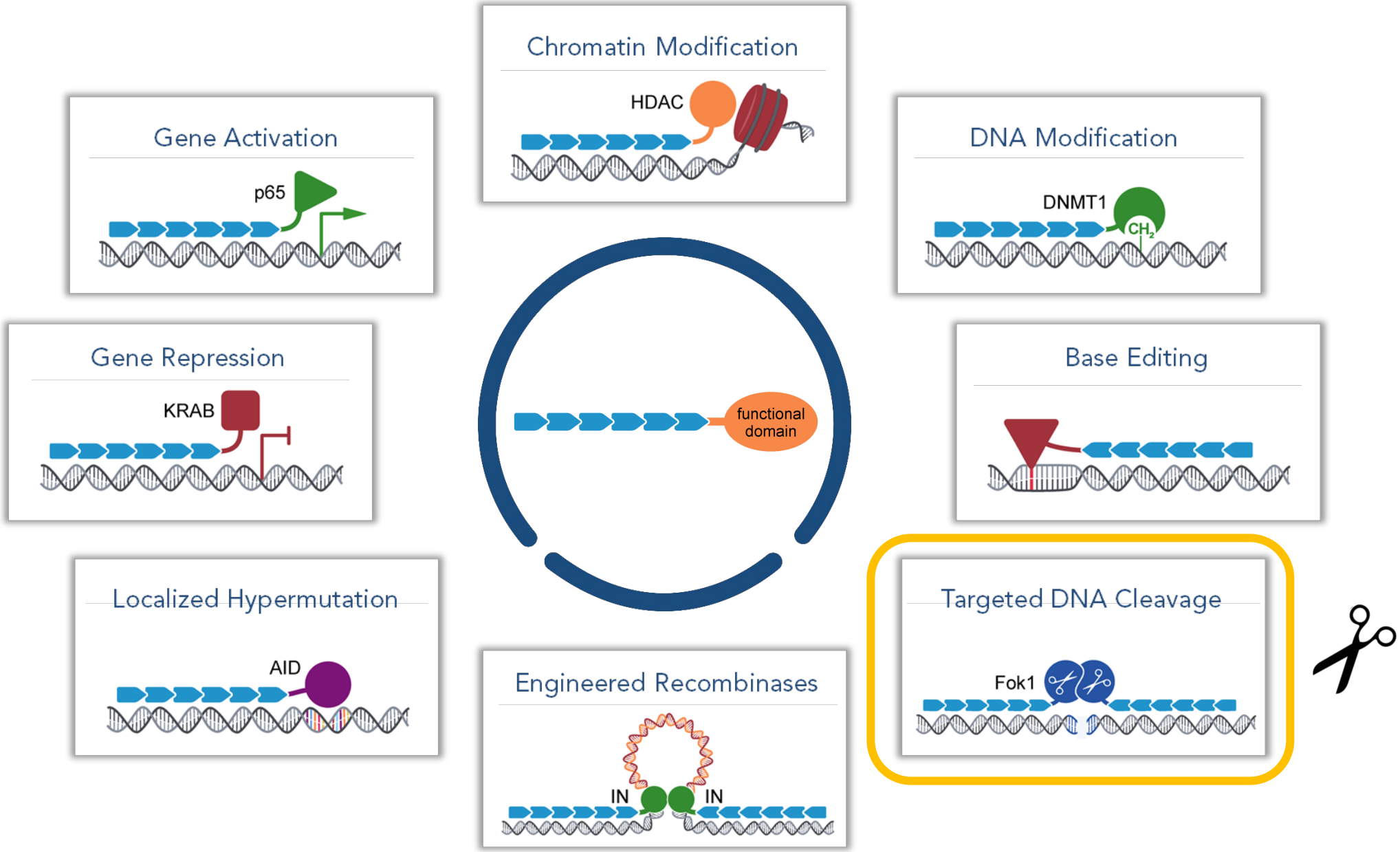


Zinc Finger Nuclease (ZFN)

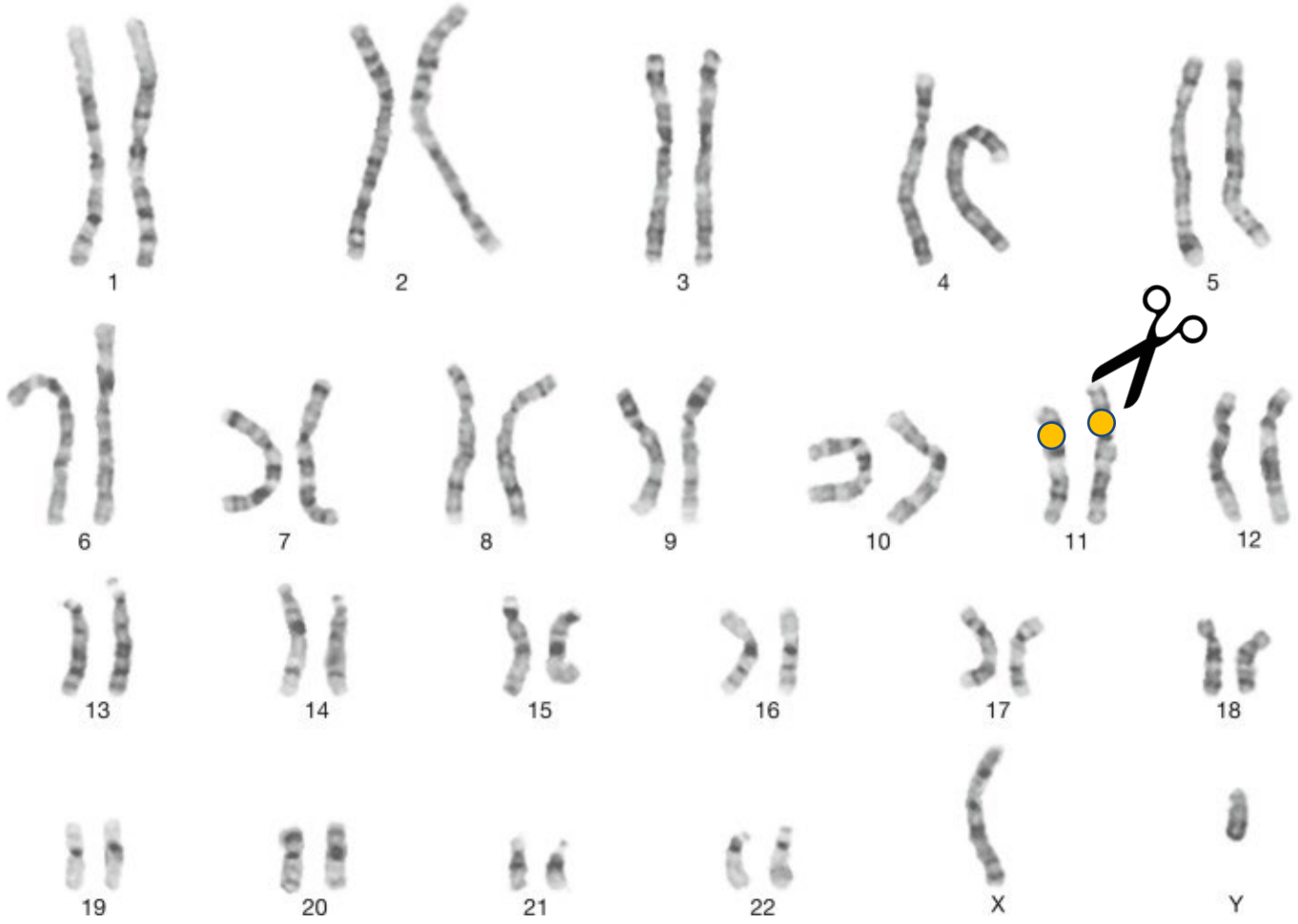
Zinc Finger



After grabbing DNA, zinc fingers can do many things...



Sickle Cell Example 1: correct a single letter (genome editing)



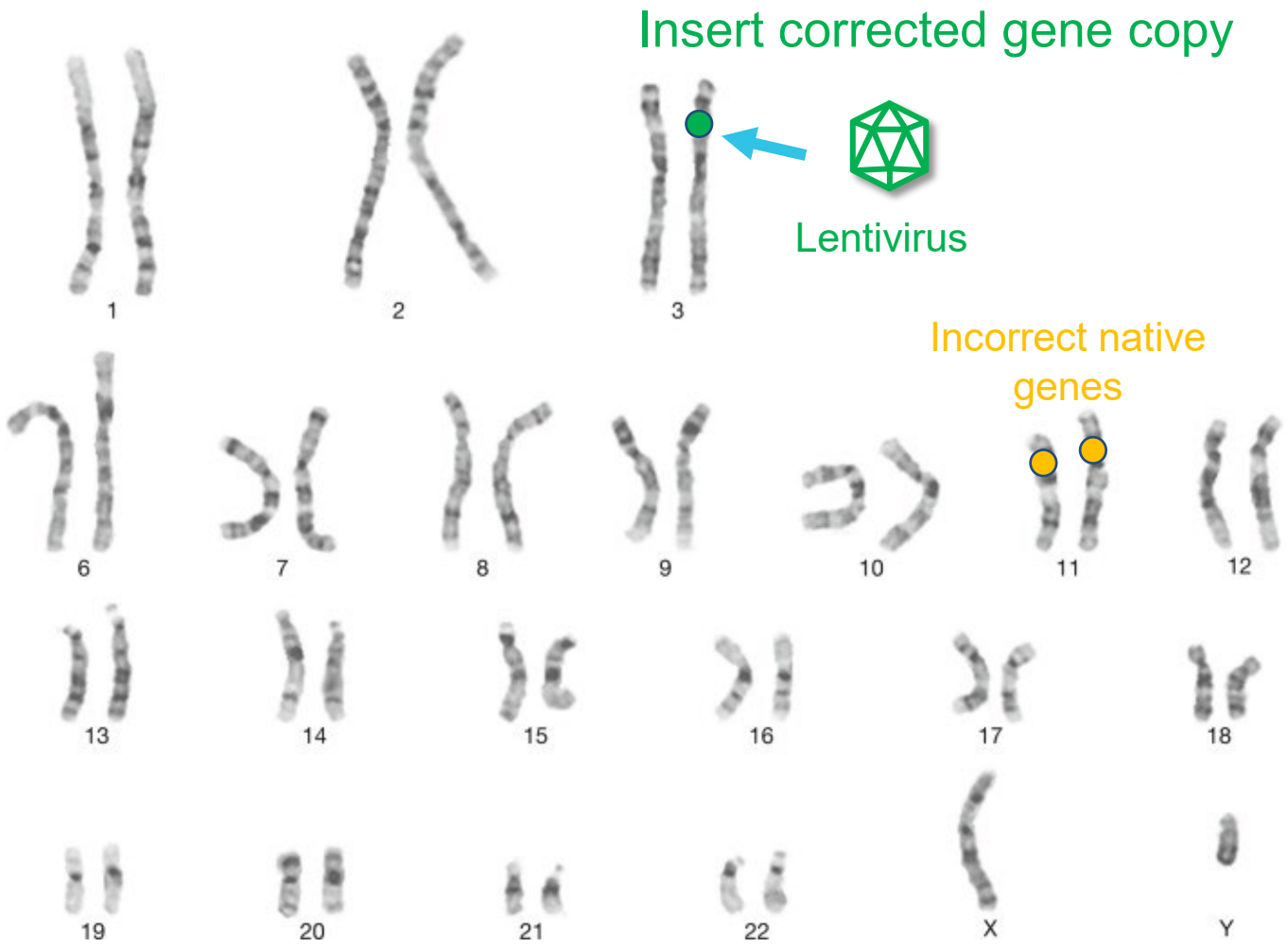
GTCAGGGCAGAGCCATCTATTGCTTACATTTGCTTCTG
ACACAACTGTGTTCACTAGCAACCTCAAACAGACACCA
TGGTGCATCTGACTCCTG**T**GGAGAAGTCTGCCGTTACT
GCCCTGTGGGGCAAGGTGAACGTGGATGAAGTTGGTGG
TGAGGCCCTGGGCAGGTTGGTATCAAGGTTACAAGACA

Cut and paste a new sequence

GTCAGGGCAGAGCCATCTATTGCTTACATTTGCTTCTG
ACACAACTGTGTTCACTAGCAACCTCAAACAGACACCA
TGGTGCATCTGACTCCTG**A**GGAGAAGTCTGCCGTTACT
GCCCTGTGGGGCAAGGTGAACGTGGATGAAGTTGGTGG
TGAGGCCCTGGGCAGGTTGGTATCAAGGTTACAAGACA

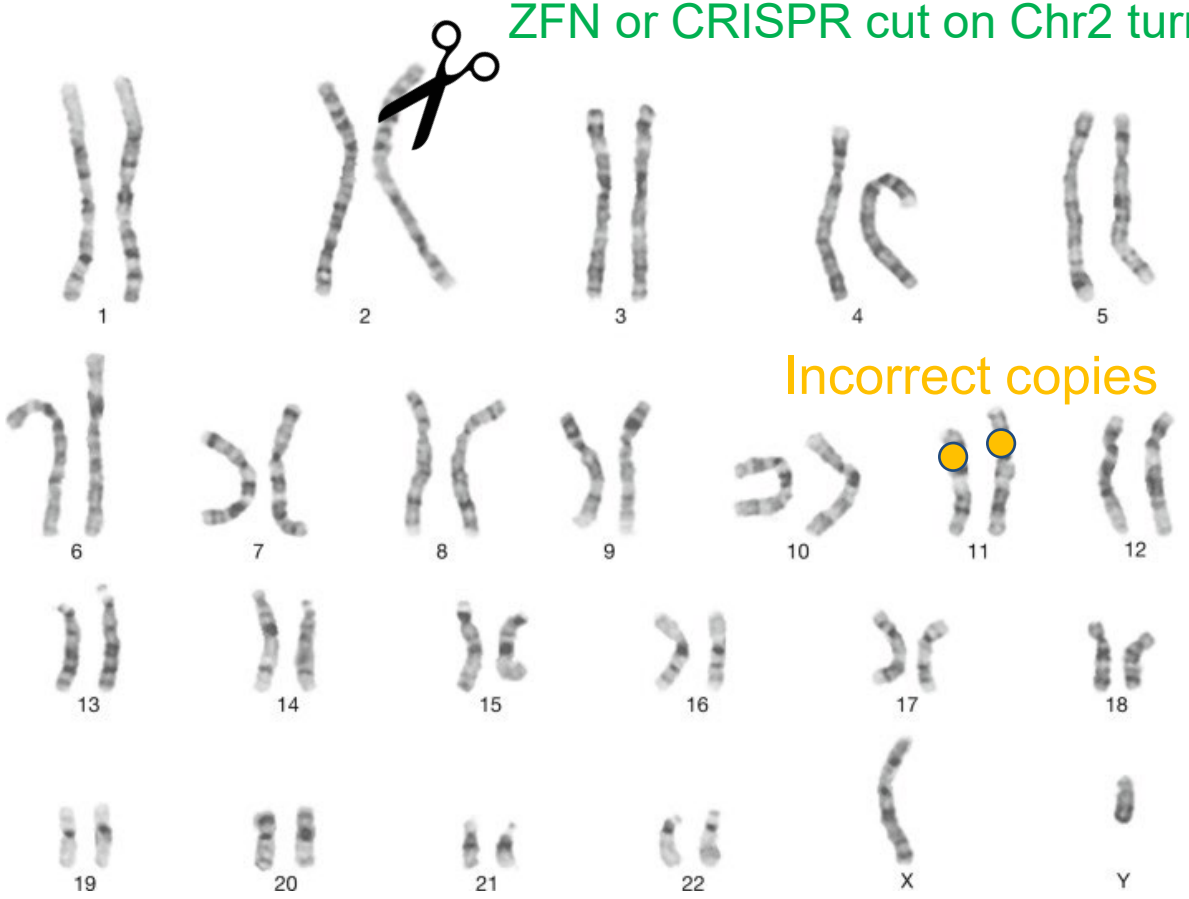
- Works well in the lab.
- Does not work well for sickle cell patients.

Sickle Cell Example 2: paste in a correct paragraph (gene therapy)



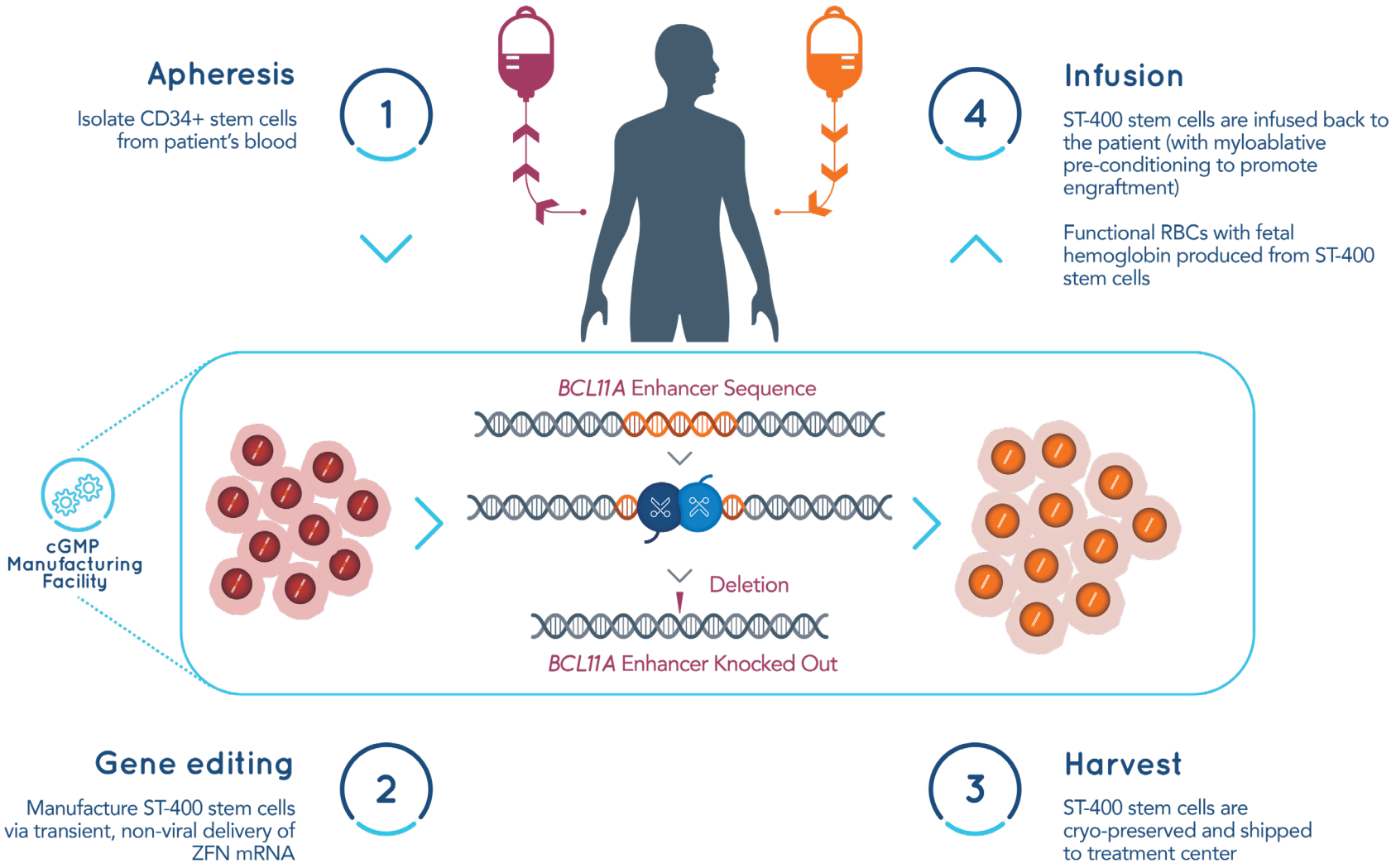
- Paste in an entire corrected gene (not just a single letter)
- This approach is known as a "gene therapy".
- A vector (called lentivirus) can place a correct copy in the genome
- Bluebird Bio has treated patients using this approach

Sickle Cell Example 3: turn on a similar gene (genome editing)

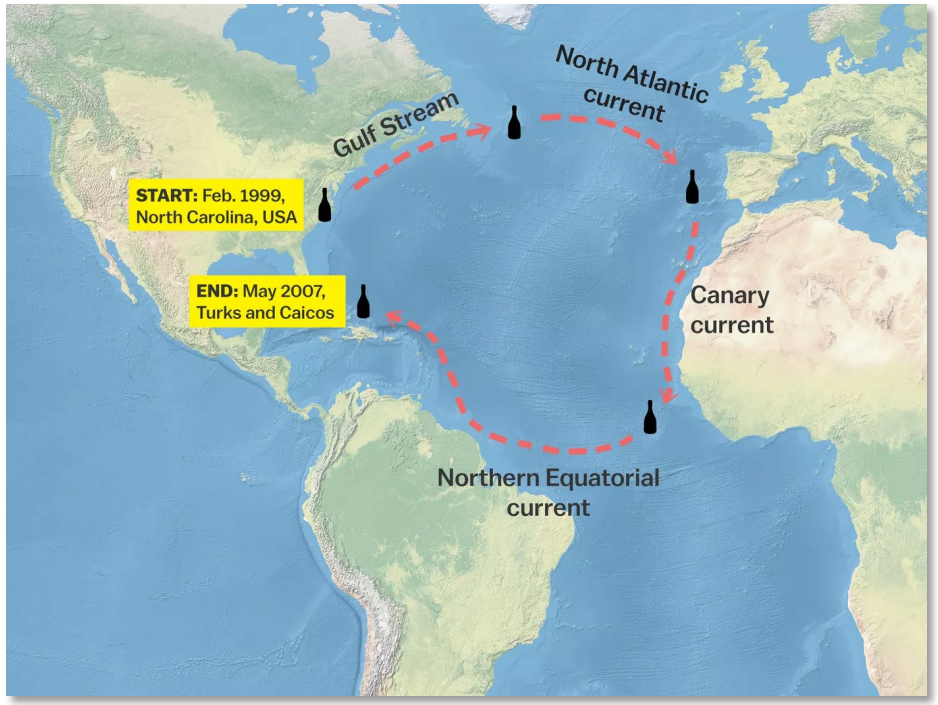


- Use ZFNs to cut the genome on chromosome 2 and turn on "fetal hemoglobin".
- Fetal hemoglobin compensates for loss of hemoglobin-beta.
- Sangamo in partnership with Sanofi (as well as others) are developing this treatment.

Sickle Cell Example 3: turn on a similar gene (genome editing)



What's next for genomic medicine? Delivery is key.



We need new technologies to navigate zinc finger proteins to the brain, liver, blood cells, lung, kidneys, etc.

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Adeno-associated-virus (AAV)

	AAV1	AAV2	AAV3B	AAV4	AAV5	AAV6	AAV8	AAV9	AAVrh.10
Primary Receptor	N-linked sialic acid	HSPG	HSPG	O-linked sialic acid	N-linked sialic acid	N-linked sialic acid and HSPG	Unknown	N-linked galactose	Sulfated LacNAc
Prominent tissue tropisms	Heart, skeletal muscle	Brain (direct injection), Eye	Liver	Eye	Liver Lung	Liver, heart, skeletal muscle	Liver	CNS, heart, skeletal muscle	CNS Liver

AAV can traffic corrected genes to various tissues in the body

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Gene Therapy using Adeno-associated Virus (AAV)

Adeno-associated viruses (AAVs) are naturally occurring viruses that infect humans but aren't known to cause disease. In gene therapy, we can deliver a corrected gene using AAV.

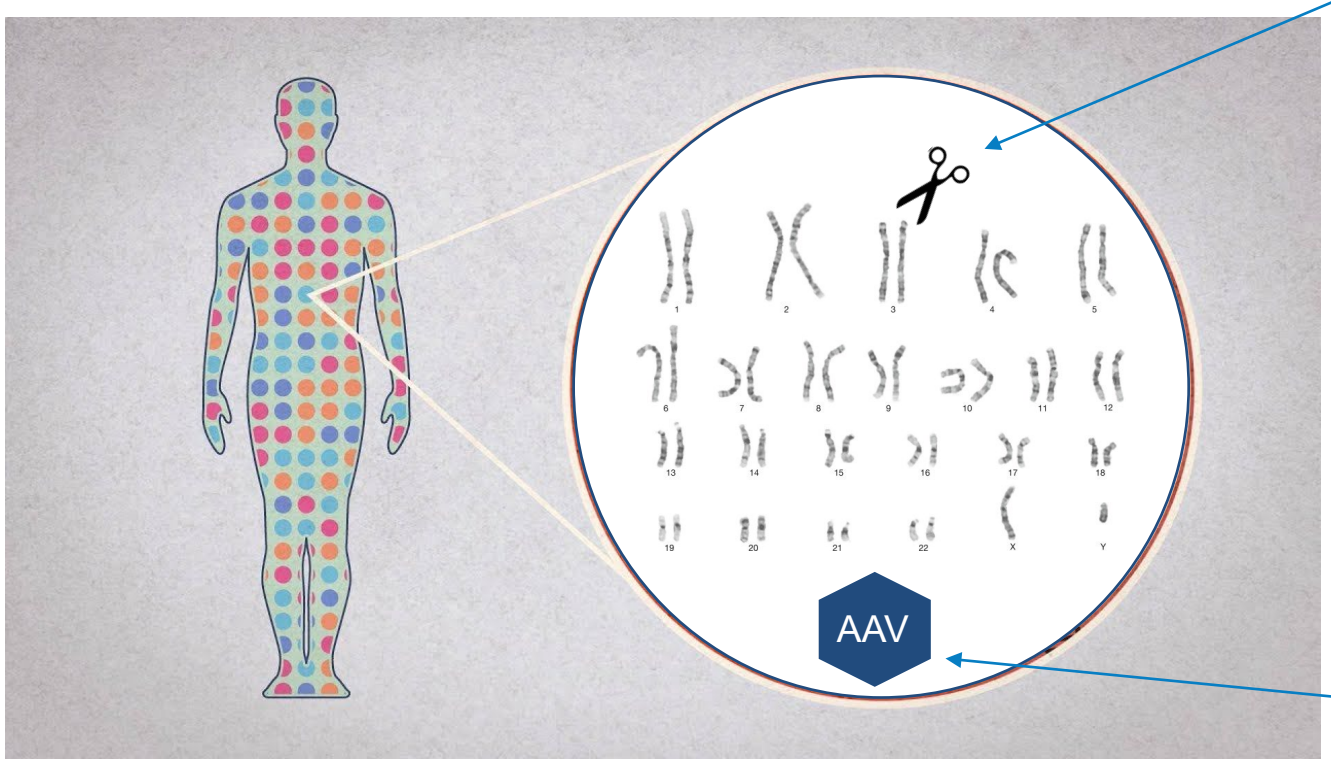
AAV medicines in development:

Haemophelia
Phenylketonuria (PKU)
Spinal muscular atrophy (SMA)
Fabry disease

How are AAV and lentivirus different?

- Both AAV and lentivirus deliver corrected copies of genes to cells.
- Lentivirus inserts the corrected gene into the genome.
- AAV maintains the corrected gene outside the genome, but still within the cell nucleus near the native chromosomal DNA.

Genome Editing vs. Gene Therapy



Genome Editing edits chromosomal DNA like text
(using Zinc Fingers or CRISPR)

Gene Therapy adds a new copy of a corrected gene to the cell
(using AAV or lentivirus)

Thank you!!!

Questions?

Pre-reads

CRISPR gene therapy shows promise against blood diseases. Nature 588, 383 (2020)
https://www.nature.com/articles/d41586-020-03476-x

CRISPR and another genetic strategy fix cell defects in two common blood disorders. Science. Dec 5, 2020.
https://www.sciencemag.org/news/2020/12/crispr-and-another-genetic-strategy-fix-cell-defects-two-common-blood-disorders