

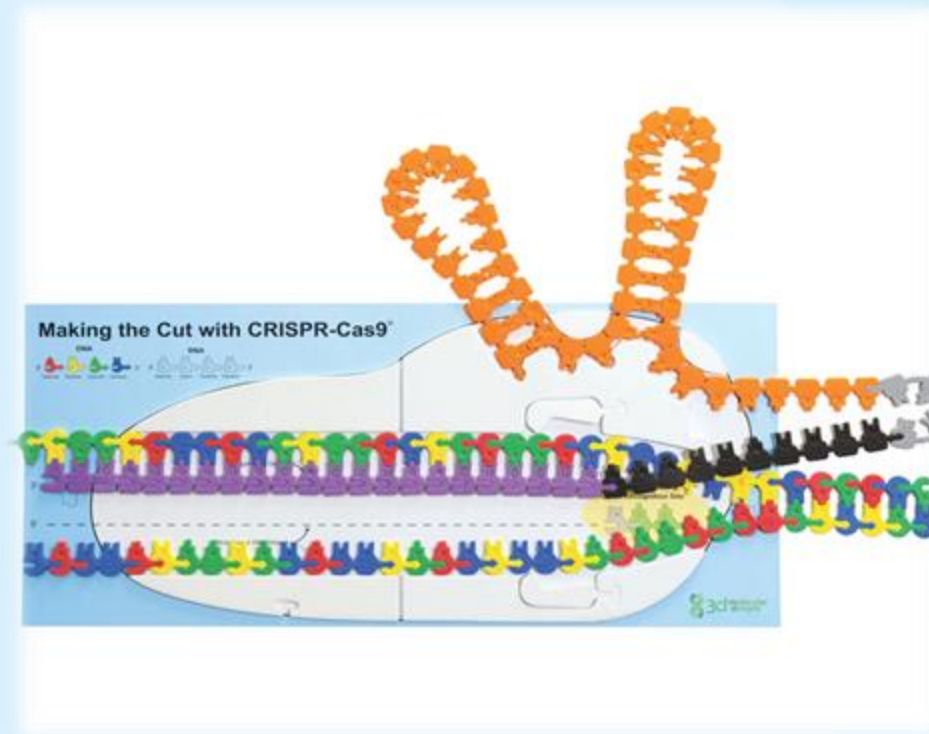
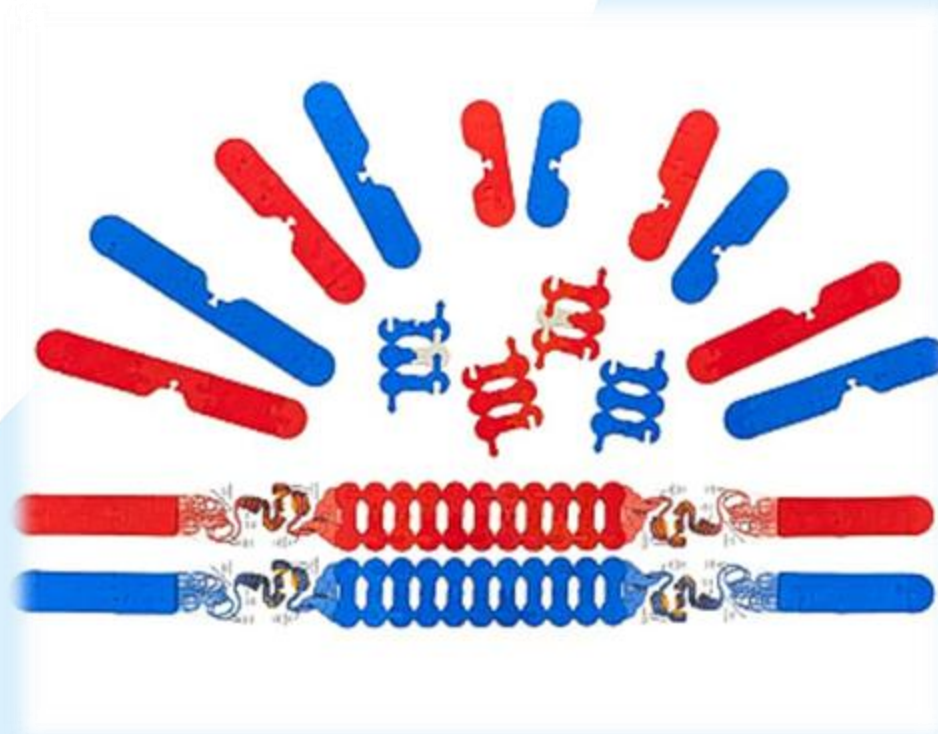
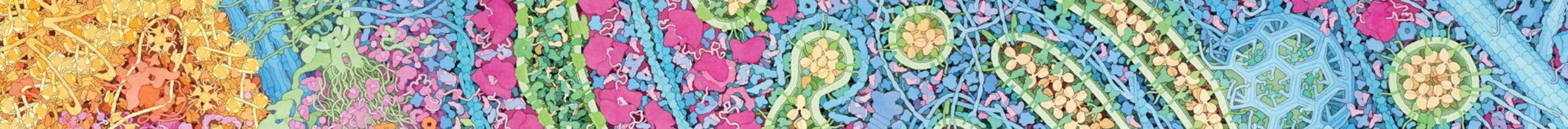


Making the Cut with CRISPR that Changes Lives

Ruth Hutson, MSSE

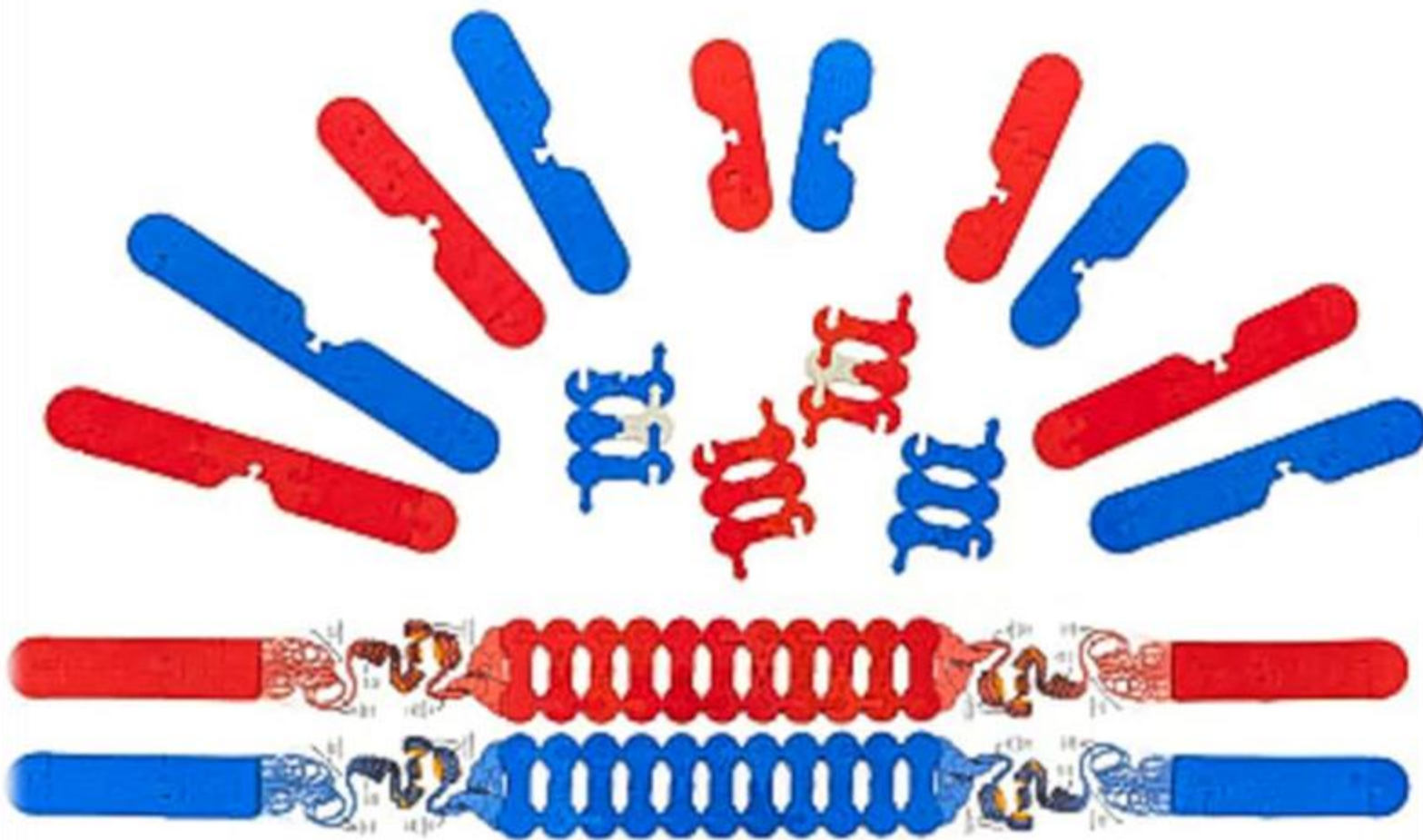


 **nsta**
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Workshop Outcomes

- Make and defend a claim based on evidence that genetic variations result from a variety of factors.
- Understand how the Cas9 protein binds to a specific sequence of double-stranded DNA at a PAM site and cuts it.
- Explain how CRISPR technologies are applied to edit genomes.
- Have fun!

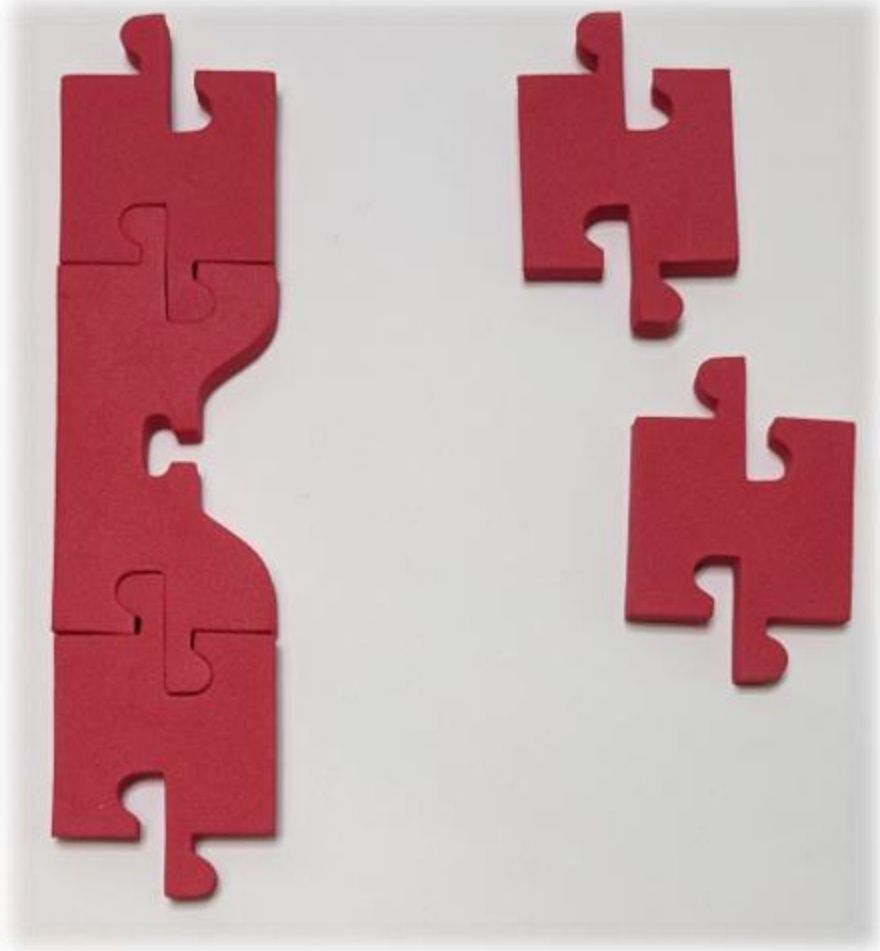


Construct a chromosome



- **Start with one centromere**
- Add 3 to 4 chromosome connectors to each side of the centromere
- Add a cap piece to each end of the chromatid.

Construct a chromosome



- Start with one centromere
- **Add 3 to 4 chromosome connectors to each side of the centromere**
- Add a cap piece to each end of the chromatid.

Construct a chromosome



- Start with one centromere
- Add 3 to 4 chromosome connectors to each side of the centromere
- **Add a cap piece to each end of the chromatid.**

Create a sister chromatid



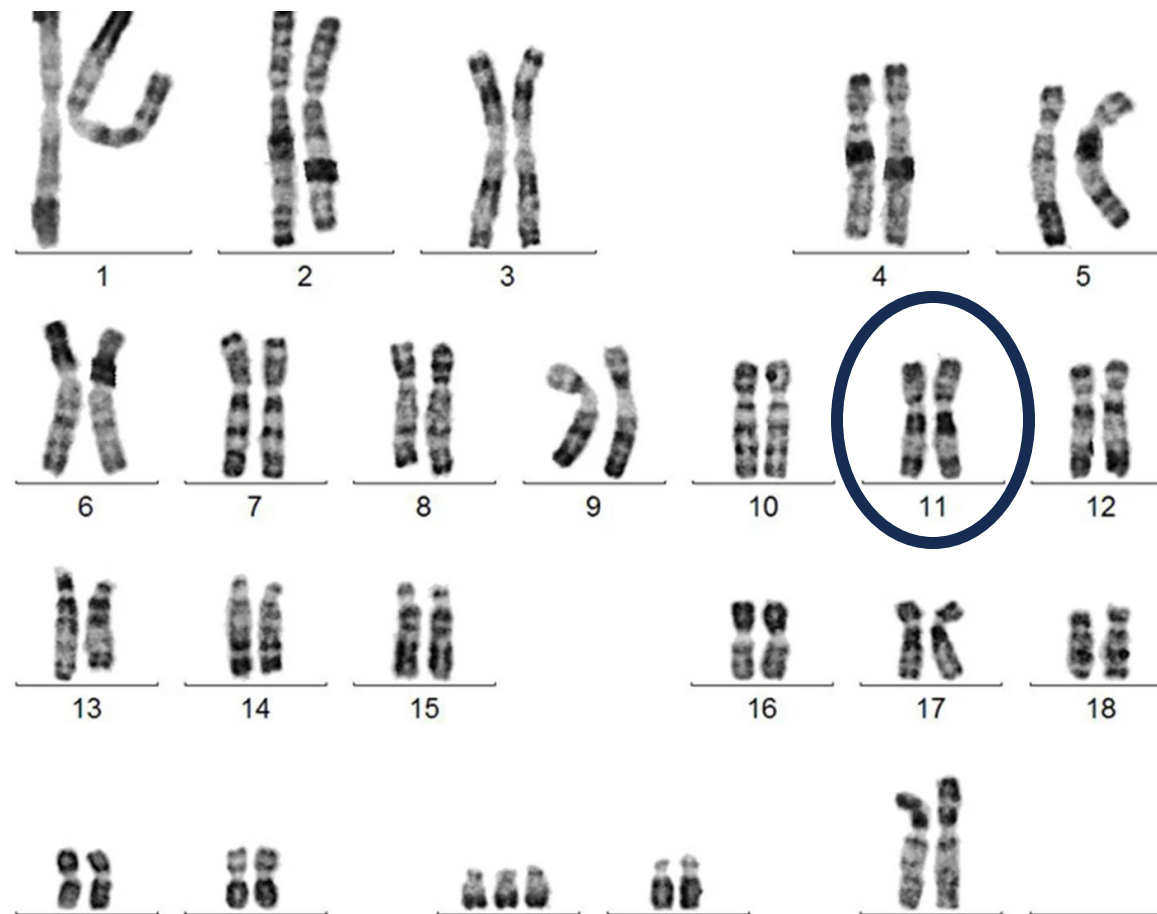
- Create a duplicate copy of the chromosome to make a sister chromatid.

Create a sister chromatid



- Create a duplicate copy of the chromosome to make a sister chromatid.

Let's look at chromosome 11



Examine this beta-globin DNA gene sequence.

3' G G A C T C C T C 5'
5' C C T G A G G A G 3'



Use insert pieces to assemble the maternal chromosome



Use insert pieces to assemble the maternal chromosome



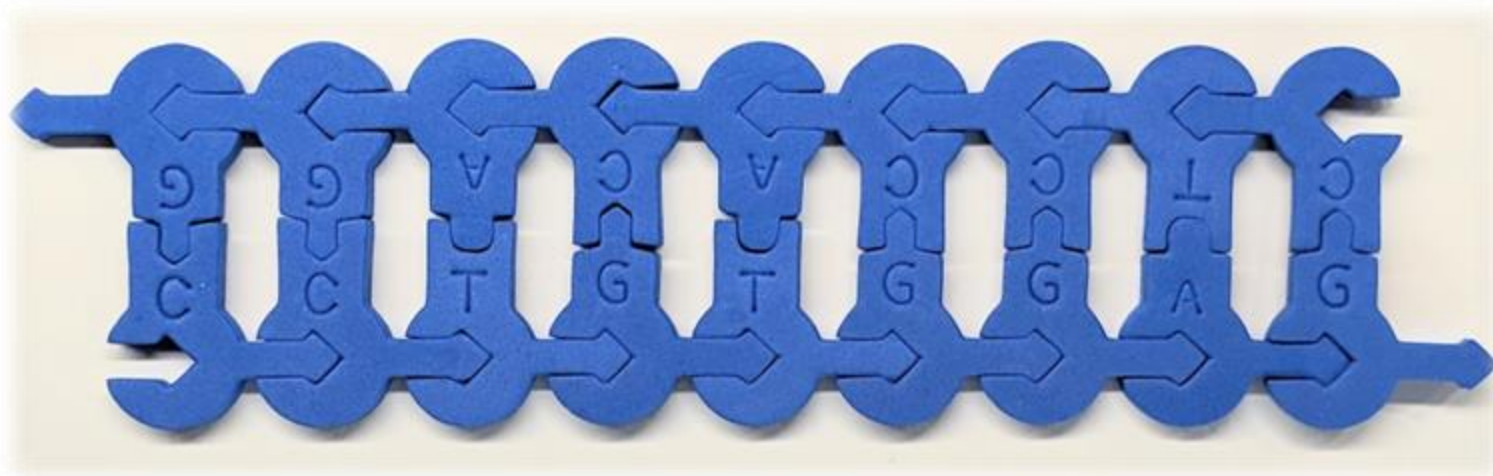
Human B-globin is located on the p-arm of chromosome 11.

The human B-globin locus is a 60-kb segment on the short arm and contains five functional genes and two pseudogenes. We will look at a small part of this locus.

It is responsible for the creation of the beta parts of the oxygen transport protein, hemoglobin.

Examine this beta-globin DNA gene sequence.

3' G G A C A C C T C 5'
5' C C T G T G G A G 3'



Use insert pieces to assemble the paternal chromosome



Use insert pieces to assemble the paternal chromosome



What is the difference between the two sequences?

Use insert pieces to assemble the paternal chromosome



What is the difference between the two sequences?

G – C

A – T

G – C

G – C

T – A

G – C

What is the difference between the two sequences?

The Genetic Codon Chart®

	U	C	A	G	
U	UUU → Phe F	UCU → Ser S	UAU → Tyr Y	UGU → Cys C	U
	UUC → Phe F	UCC → Ser S	UAC → Tyr Y	UGC → Cys C	C
	UUA → Leu L	UCA → Ser S	UAA → Stop	UGA → Stop	A
	UUG → Leu L	UCG → Ser S	UAG → Stop	UGG → Trp W	G
C	CUU → Leu L	CCU → Pro P	CAU → His H	CGU → Arg R	U
	CUC → Leu L	CCC → Pro P	CAC → His H	CGC → Arg R	C
	CUA → Leu L	CCA → Pro P	CAA → Gln Q	CGA → Arg R	A
	CUG → Leu L	CCG → Pro P	CAG → Gln Q	CGG → Arg R	G
A	AUU → Ile I	ACU → Thr T	AAU → Asn N	AGU → Ser S	U
	AUC → Ile I	ACC → Thr T	AAC → Asn N	AGC → Ser S	C
	AUA → Ile I	ACA → Thr T	AAA → Lys K	AGA → Arg R	A
	AUG → Met M	ACG → Thr T	AAG → Lys K	AGG → Arg R	G
G	GUU → Val V	GCU → Ala A	GAU → Asp D	GGU → Gly G	U
	GUC → Val V	GCC → Ala A	GAC → Asp D	GGC → Gly G	C
	GUA → Val V	GCA → Ala A	GAA → Glu E	GGA → Gly G	A
	GUG → Val V	GCG → Ala A	GAG → Glu E	GGG → Gly G	G

Amino Acid Properties

 Translation Start Codon	 Hydrophobic / Non-polar	 Negative Charge	 Cysteine
 Translation Stop Codon	 Hydrophilic / Polar	 Positive Charge	

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How might that affect the resulting protein?



What is the difference between the two sequences?

The Genetic Codon Chart®

	U	C	A	G	
U	UUU → Phe F	UCU → Ser S	UAU → Tyr Y	UGU → Cys C	U
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How might that affect the resulting protein?





What is the difference between the two sequences?

The Genetic Codon Chart®

How might that affect the resulting protein?

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What is the difference between the two sequences?

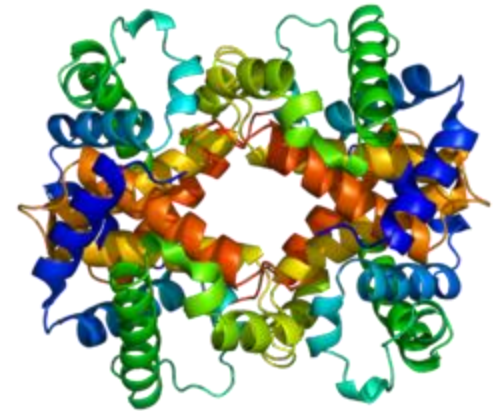
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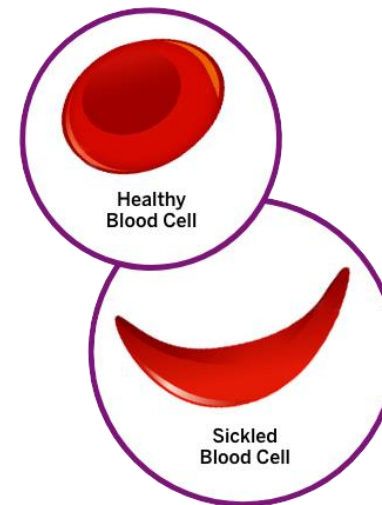
Amino Acid Properties

 Translation Start Codon	 Hydrophobic / Non-polar	 Negative Charge	 Cysteine
 Translation Stop Codon	 Hydrophilic / Polar	 Positive Charge	

How might that affect the resulting protein?



Beta-globin



What is the difference between the two sequences?

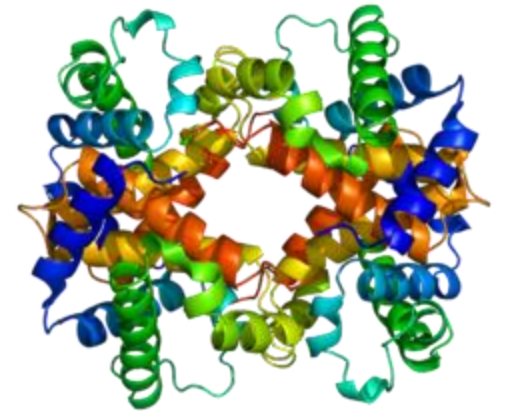
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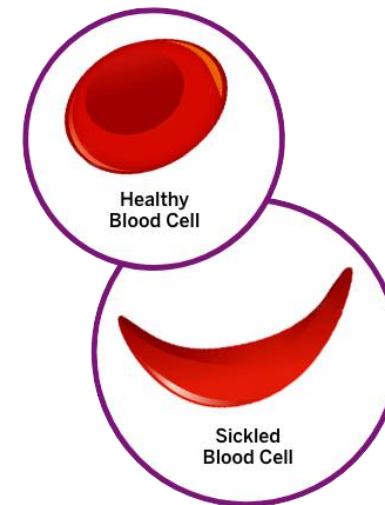
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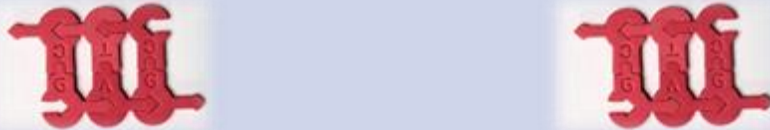
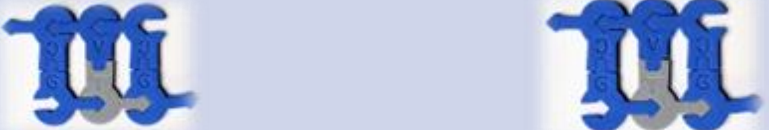
How might that affect the resulting protein?



Beta-globin



Understanding Codominance

Sickle Cell Anemia Possible Genotypes and Phenotypes	DNA sequence
SS Homozygous Typical beta-globin	
Ss Heterozygous Carrier Typical beta-globin and Atypical beta-globin	
ss Homozygous Atypical beta-globin	

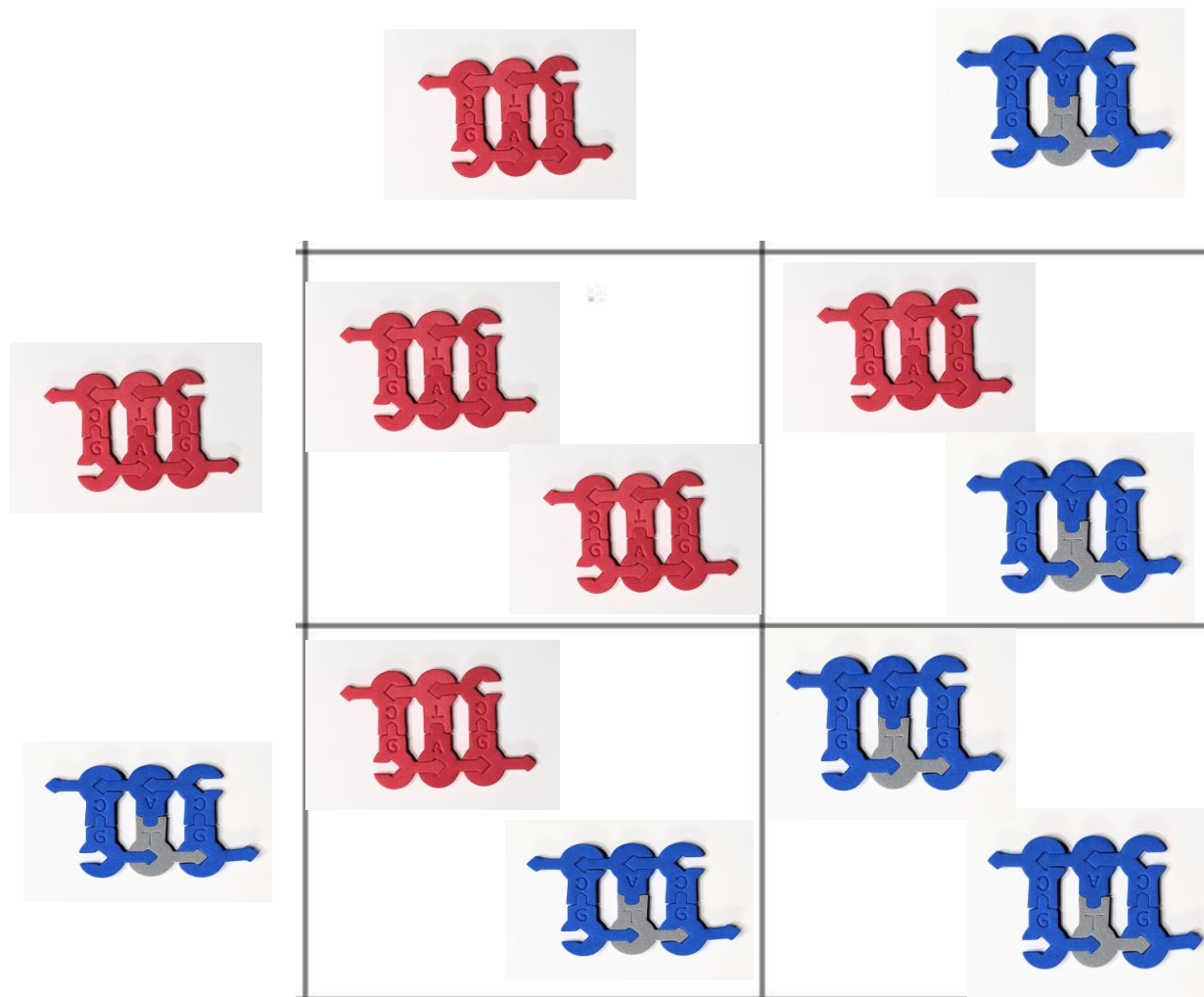
A visit to the genetic counselor

Two individuals with sickle cell trait want to know their chances of having a child with sick cell anemia. Complete the Punnett square using DNA sequences to fill in each of the squares.

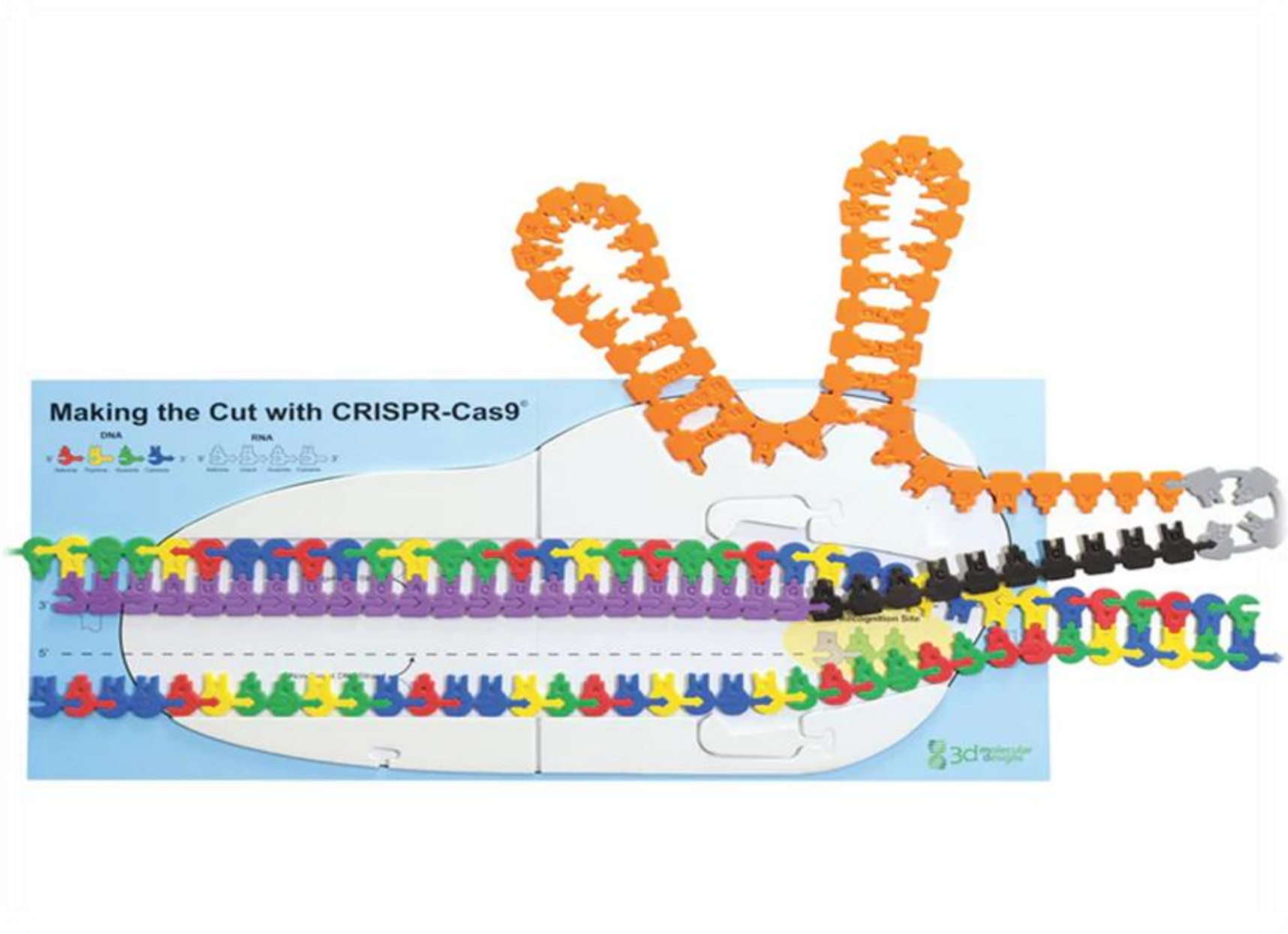


How would you advise them?

A visit to the genetic counselor



What are the implications?



Teacher Notes - How to Generate Interest in CRISPR

Below is a suggestion as to how you might get your kids interested in this topic. We suggest that you challenge your class to consider the question: *Could you have discovered CRISPR?*

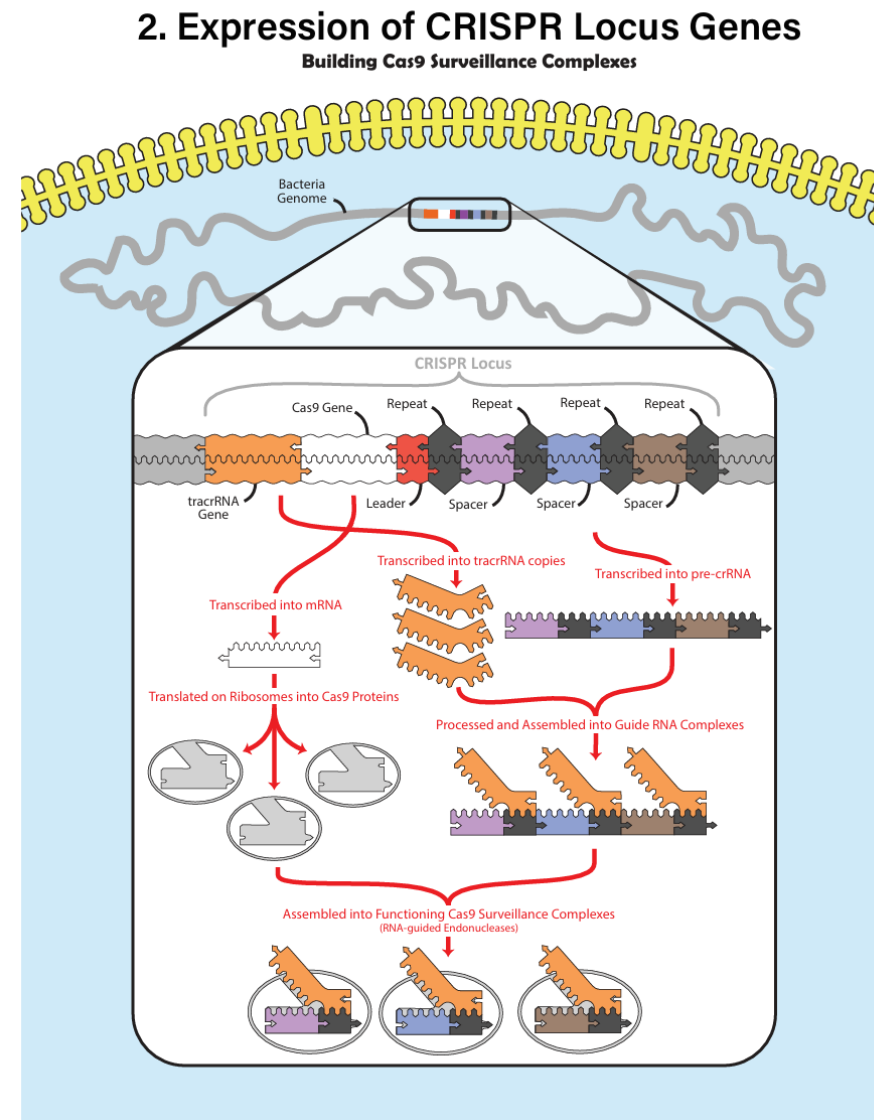
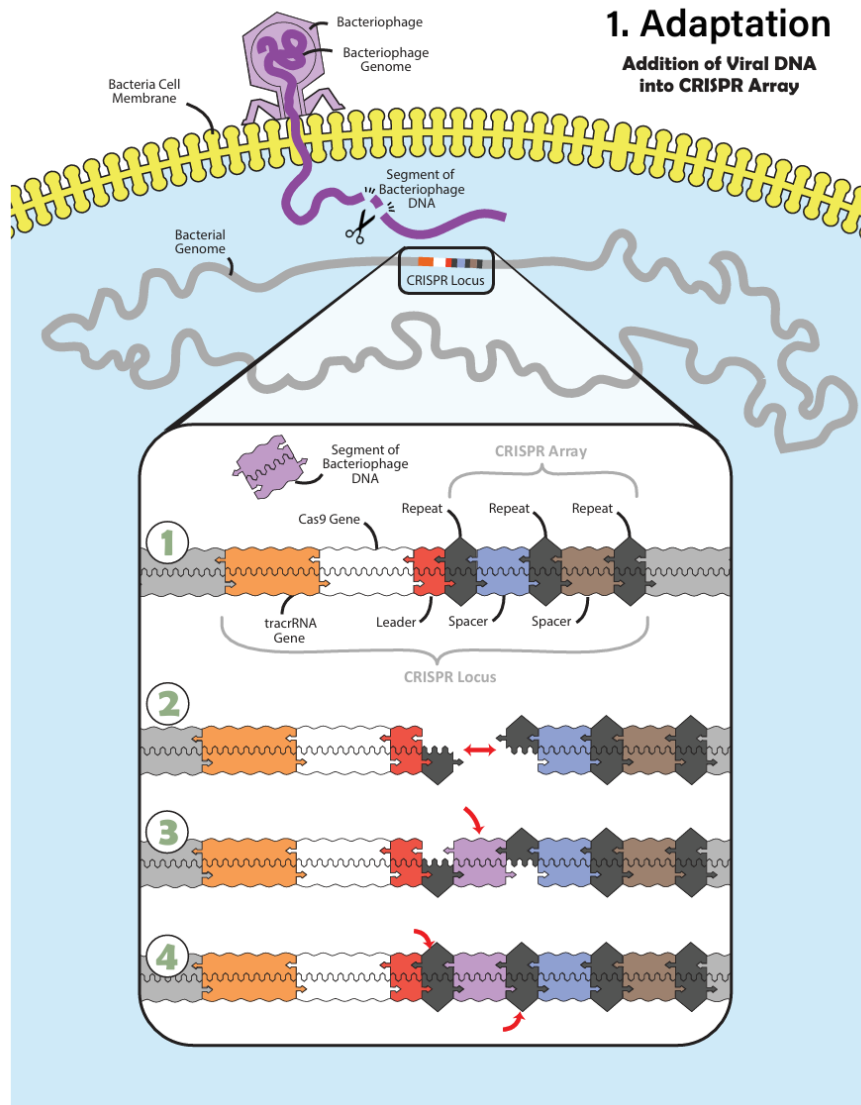
The first report of what was later to become known as the CRISPR system appeared in a paper from a Japanese research group) in 1987. These researchers noticed an unusual pattern in the sequence of nucleotides that occurred just downstream from an E. coli gene they were studying. They had no idea what this sequence meant, only that it was interesting, and worth reporting to the scientific community.

Over the next ten years, other nucleotide sequences with this pattern were reported in the genomes of other bacteria. Eventually, this pattern was named CRISPR – Clustered Regularly Interspaced Short Palindromic Repeats.

So, here is the challenge: *Give your students the following sequence of nucleotides. . .*

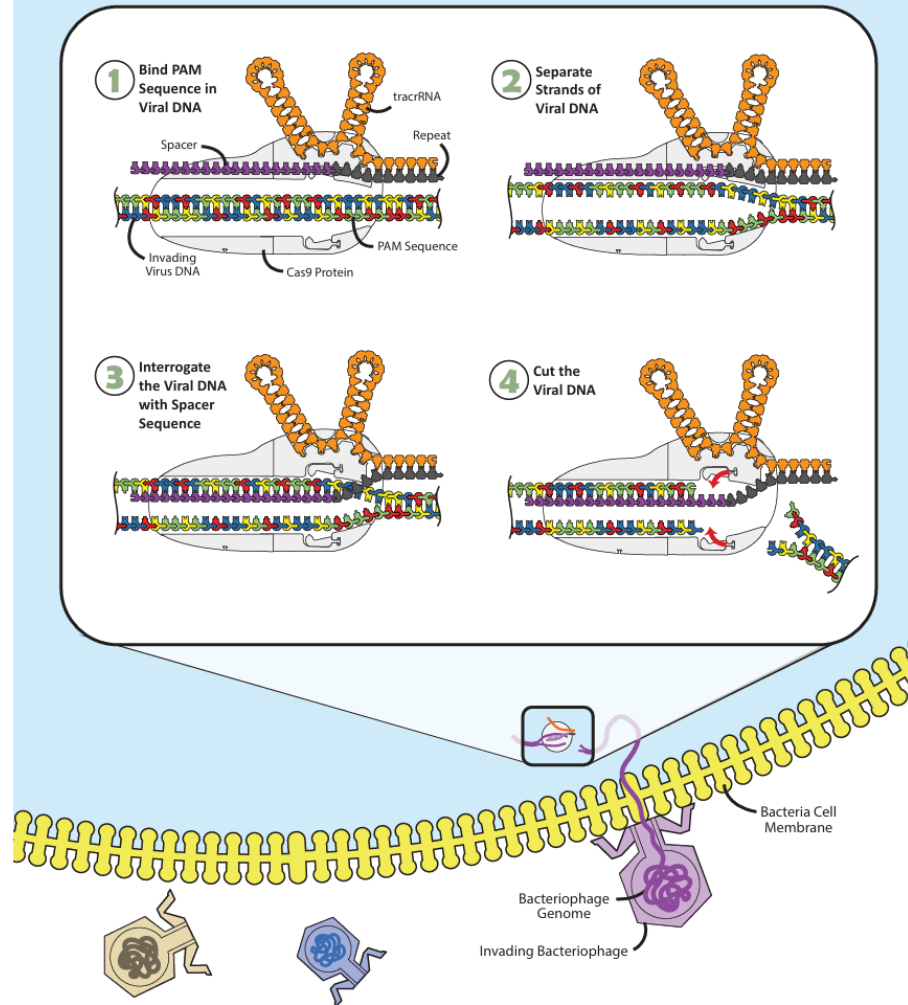
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GGAGTTCTACCGCAGAGGCGGGGGAAGTCCAAGTGATATCCATCATCGCATCCAG
TGCGCCCGGTTTATCCCCGCTGATGCGGGGAACACCAGCGTCAGGCGTGAAATCT
CACCGTCGTTGCCGGTTTATCCCTGCTGGCGCGGGGAAGTCTCGGTTTCAGGCGTT
GCAAACCTGGCTACCGGGCGGTTTATCCCCGCTAACGCGGGGAAGTCGTAGTCCA
TCATTCCACCTATGTCTGAAGTCCCGGTTTATCCCCGCTGGCGCGGGGAAGTCTG
```

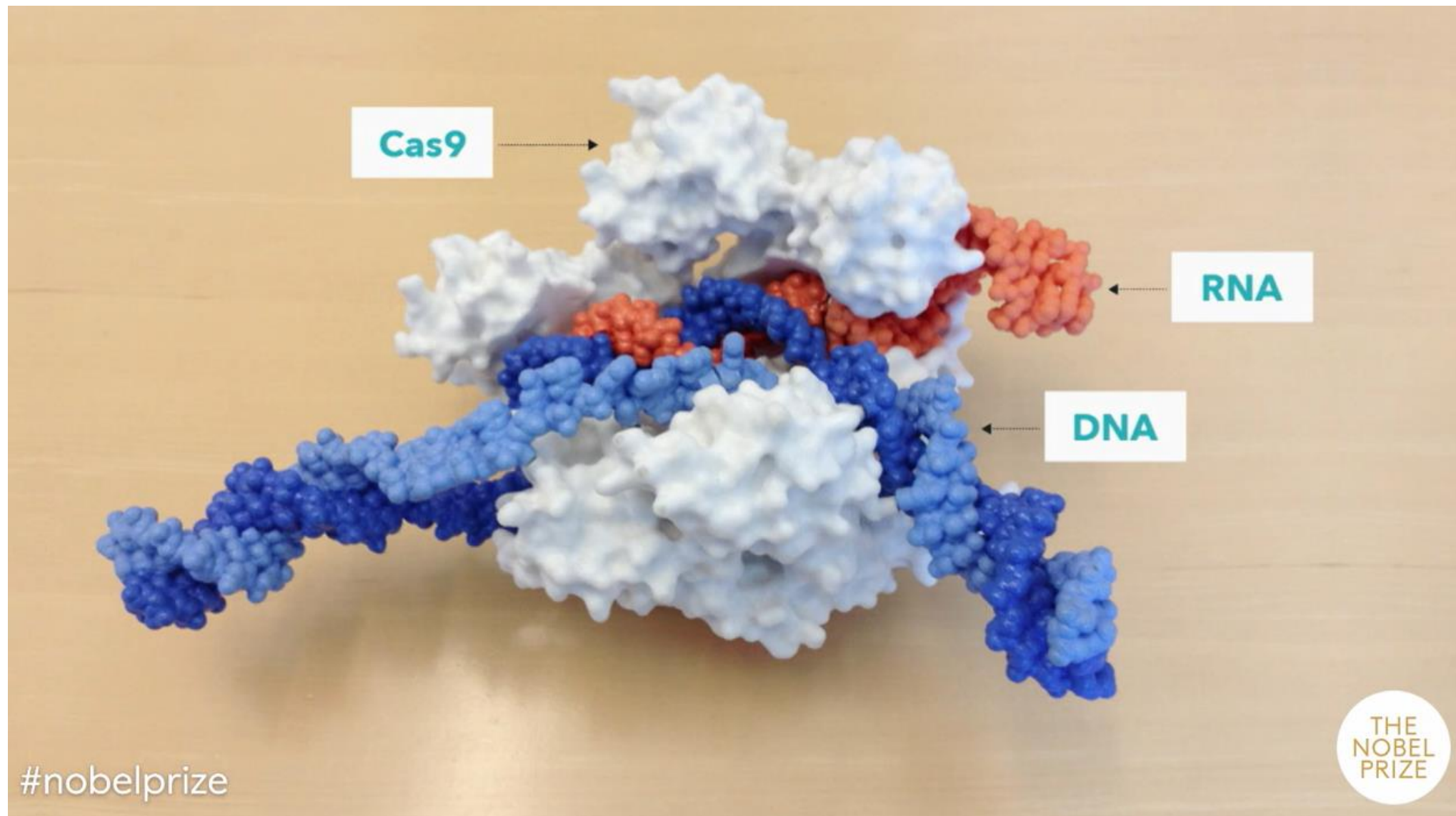
. . .and ask them if they can see the unusual pattern in this sequence of nucleotides.



3. Invader Silencing

Cutting the DNA of an Incoming Virus







The NEW ENGLAND
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ORIGINAL ARTICLE | BRIEF REPORT



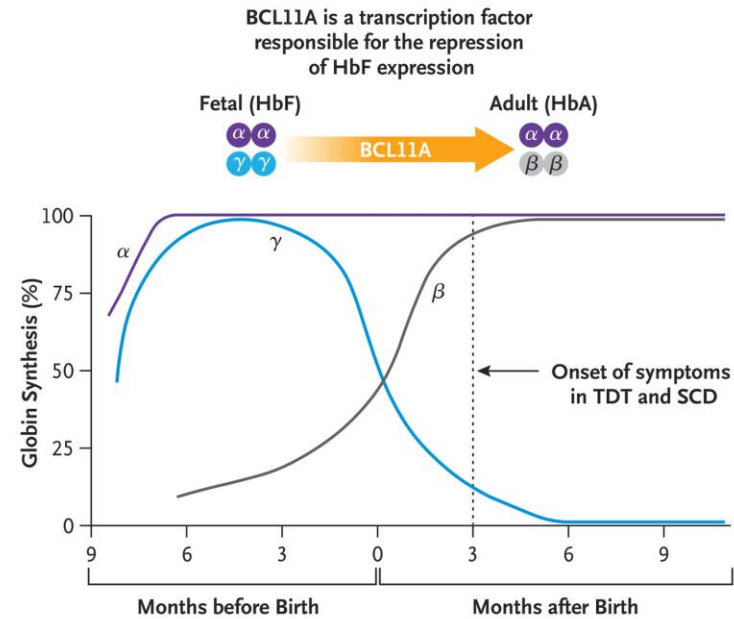
CRISPR-Cas9 Gene Editing for Sickle Cell Disease and β -Thalassemia

Authors: Haydar Frangoul, M.D., David Altshuler, M.D., Ph.D., M. Domenica Cappellini, M.D., Yi-Shan Chen, Ph.D., Jennifer Domm, M.D., Brenda K. Eustace, Ph.D., Juergen Foell, M.D.,  +18, and Selim Corbacioglu, M.D. [Author Info & Affiliations](#)

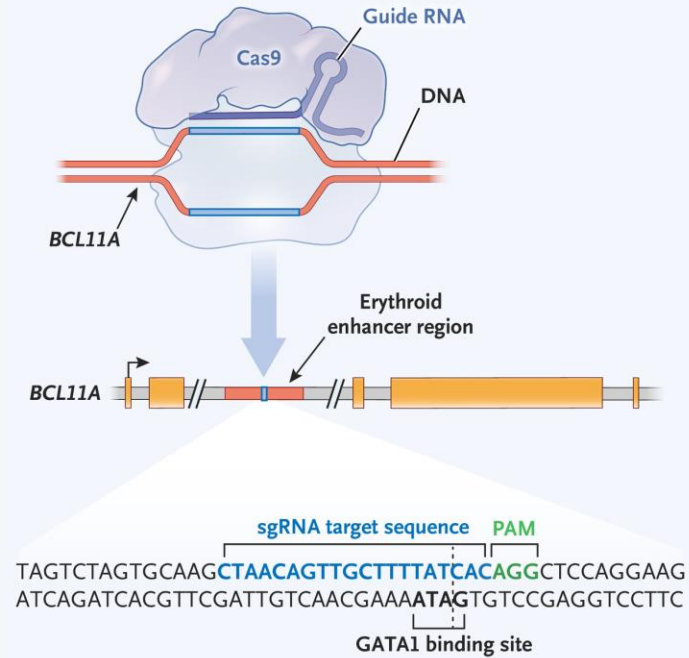
Published December 5, 2020 | N Engl J Med 2021;384:252-260 | DOI: 10.1056/NEJMoa2031054

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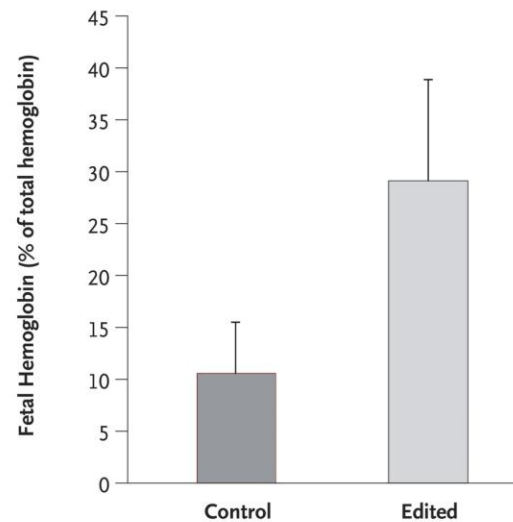
A Transition from Fetal to Adult Hemoglobin



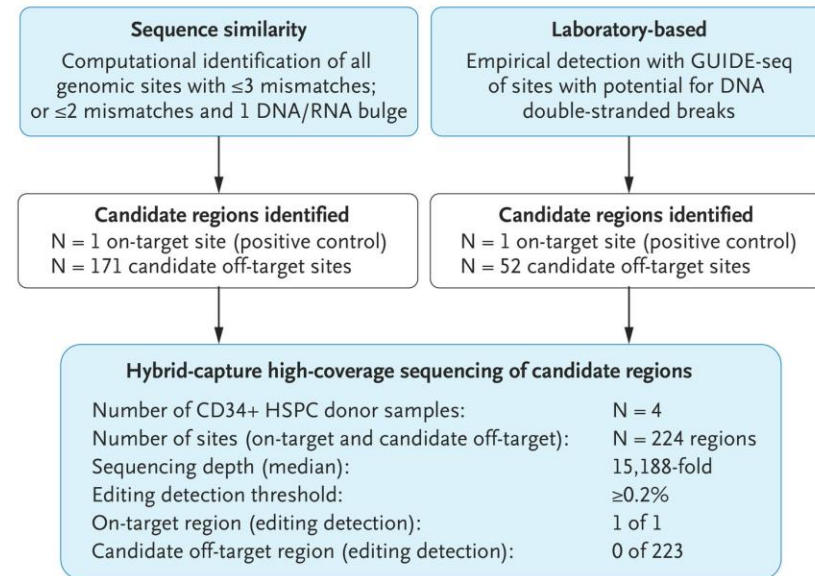
B Targeting of Editing Site



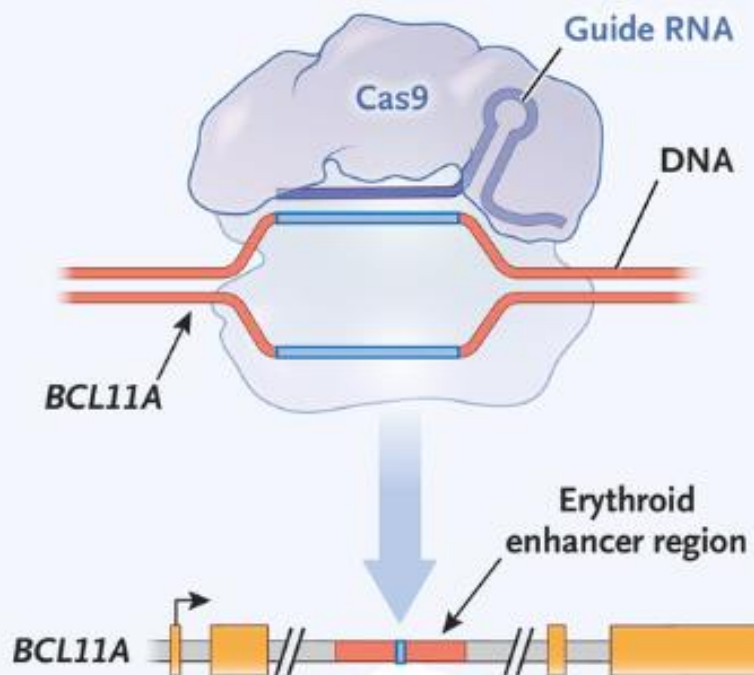
C Fetal Hemoglobin after Editing



D Identification of Potential Sites of Off-Target Editing



B Targeting of Editing Site



sgRNA target sequence PAM

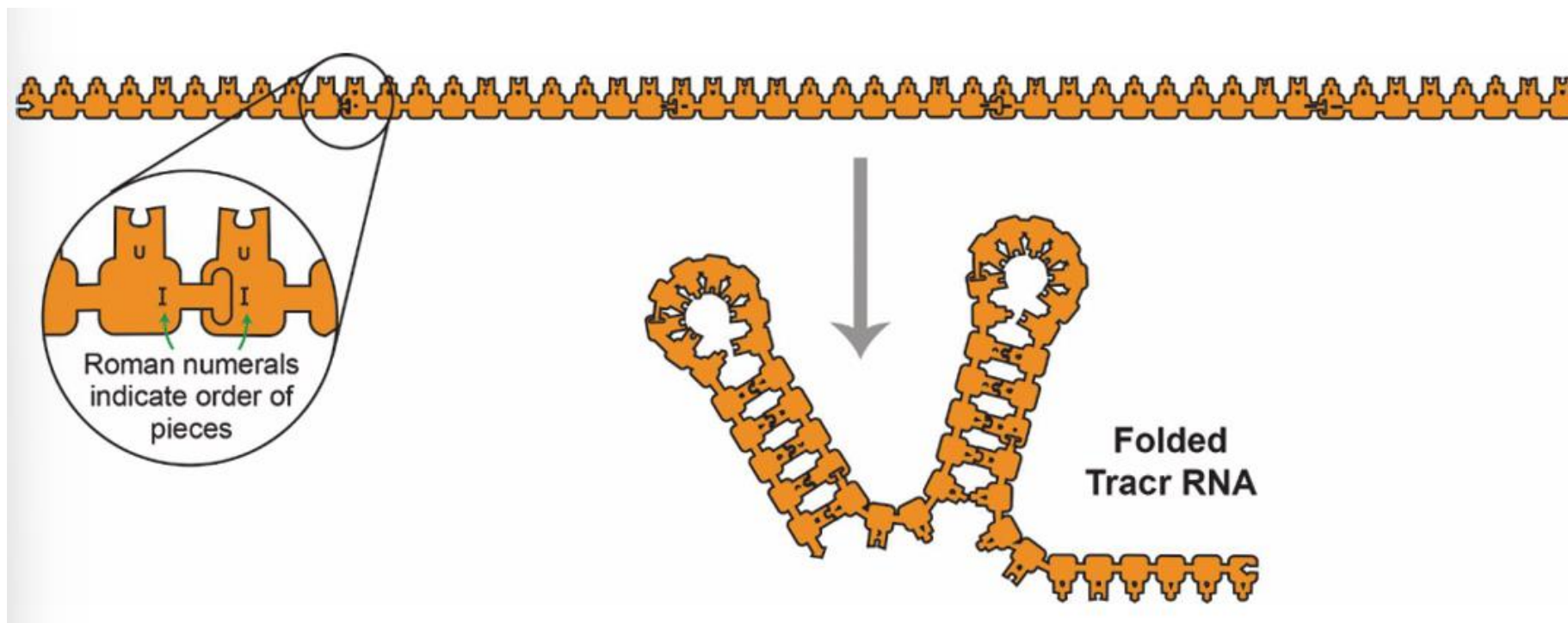
TAGTCTAGTGCAAGCTAACAGTTGCTTTTATCACAGGCTCCAGGAAG
 ATCAGATCACGTTTCGATTGTCAACGAAAAATAGTGTCCGAGGTCCTTC

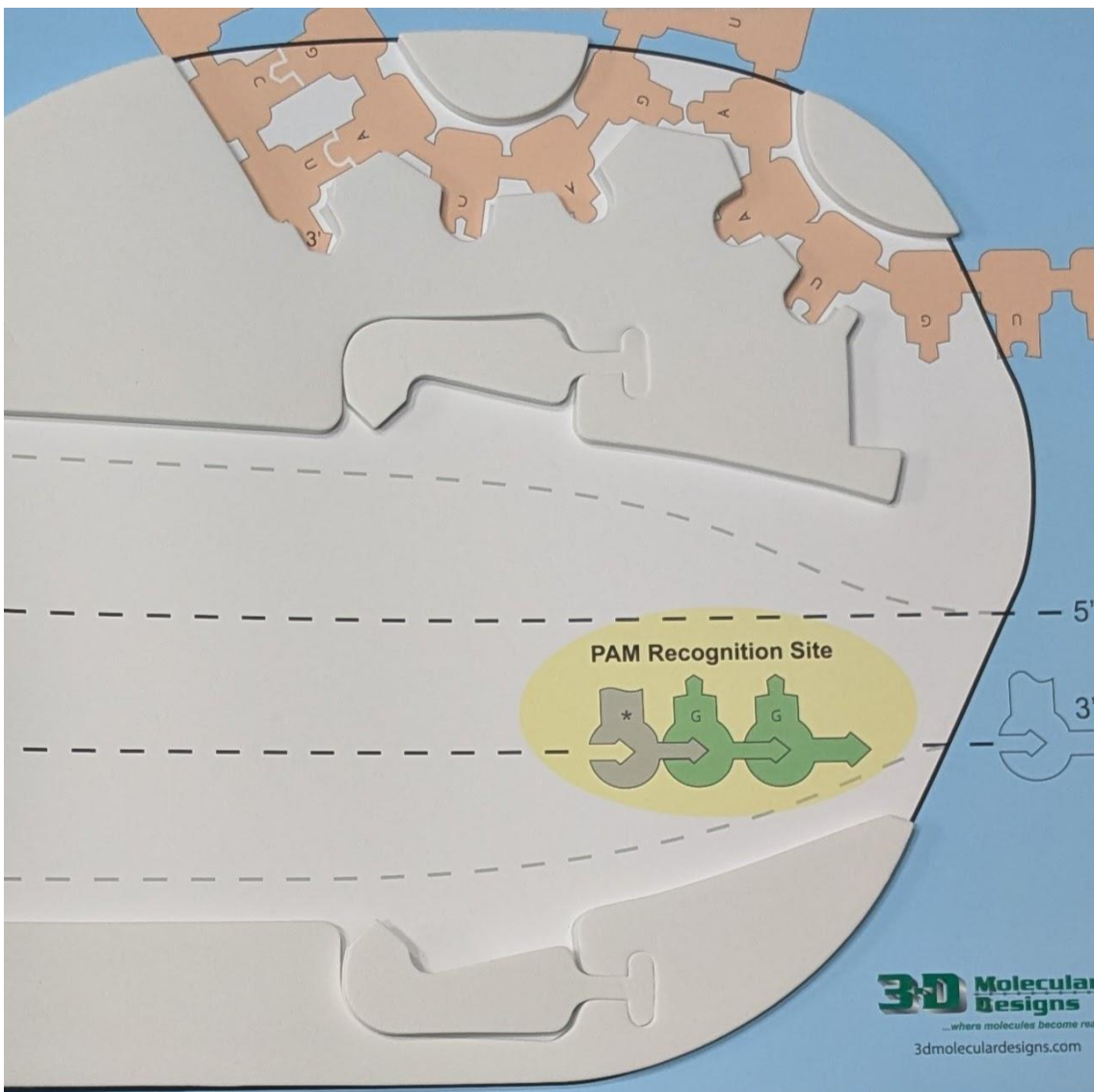
GATA1 binding site





Construct a model of tracrRNA.





What do you notice about this sequence?

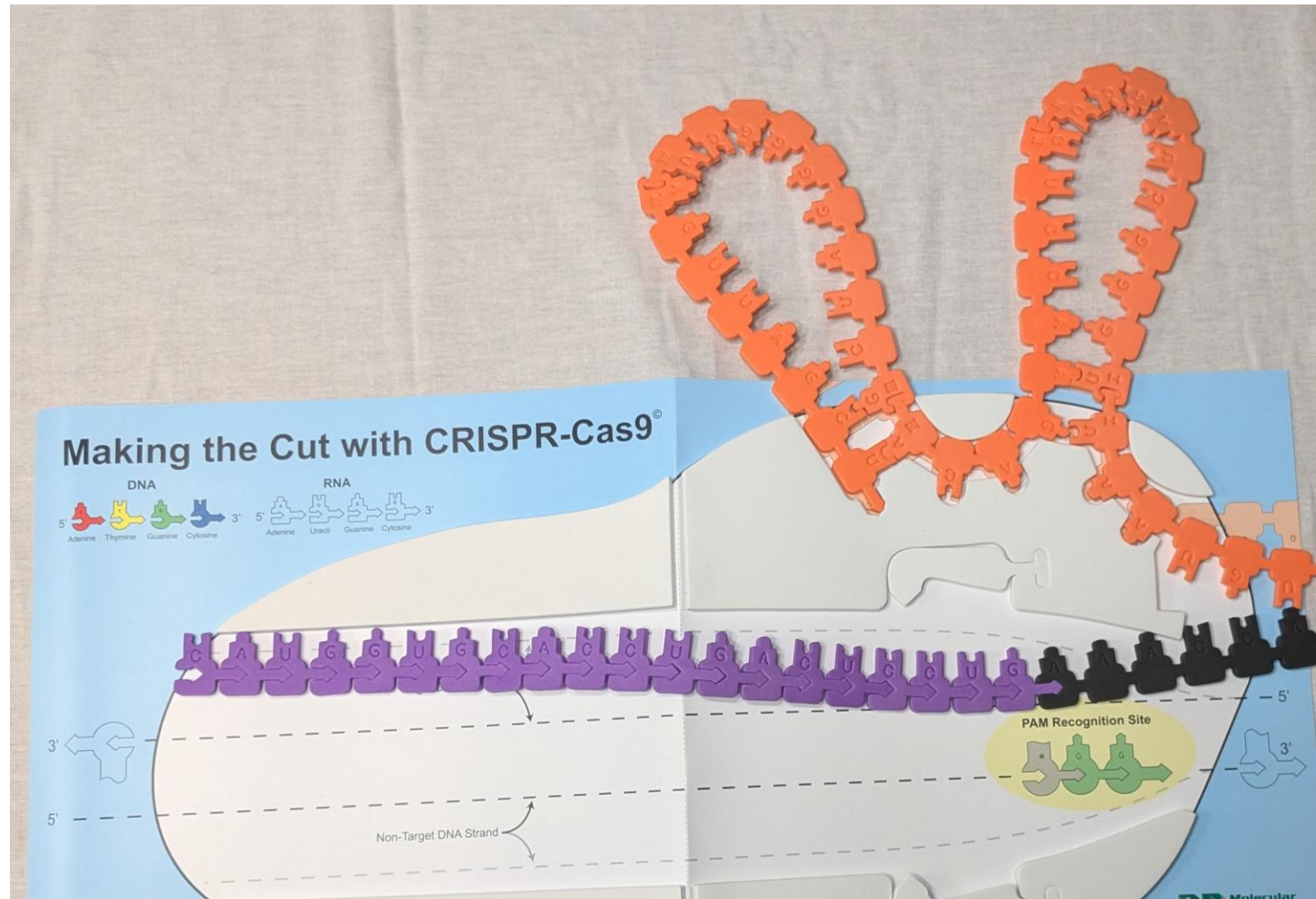


Construct your CRISPR RNA (crRNA).



What do you notice? What questions do you have?

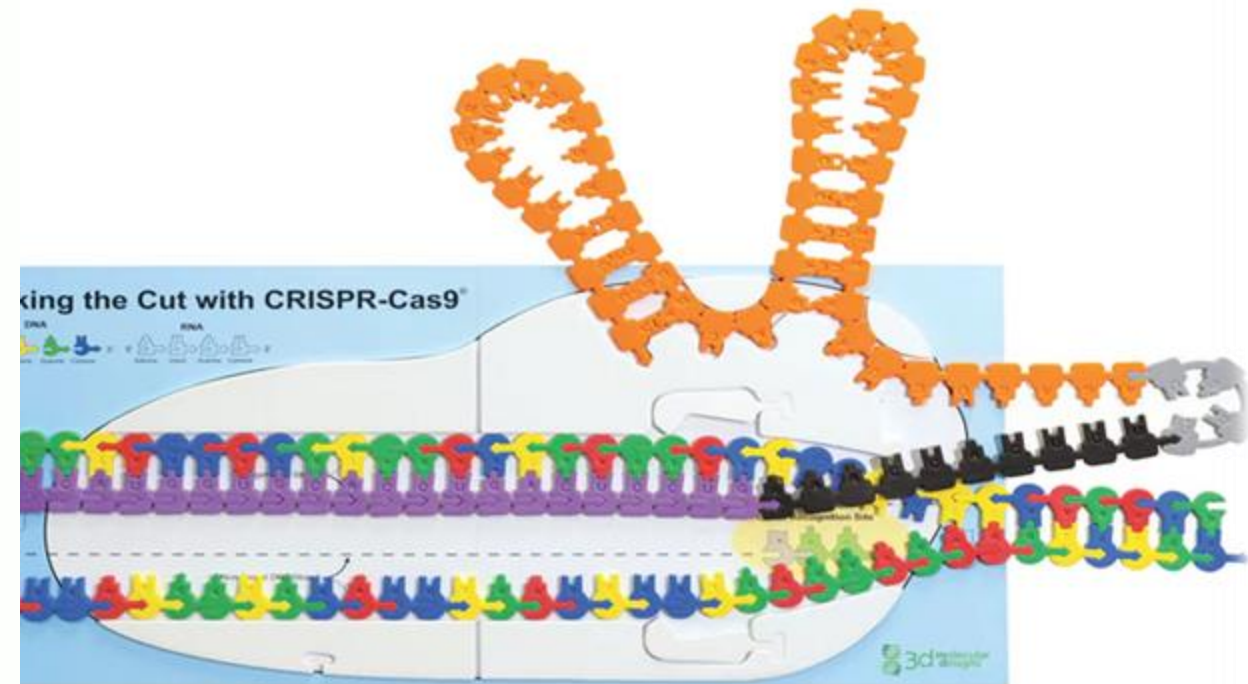
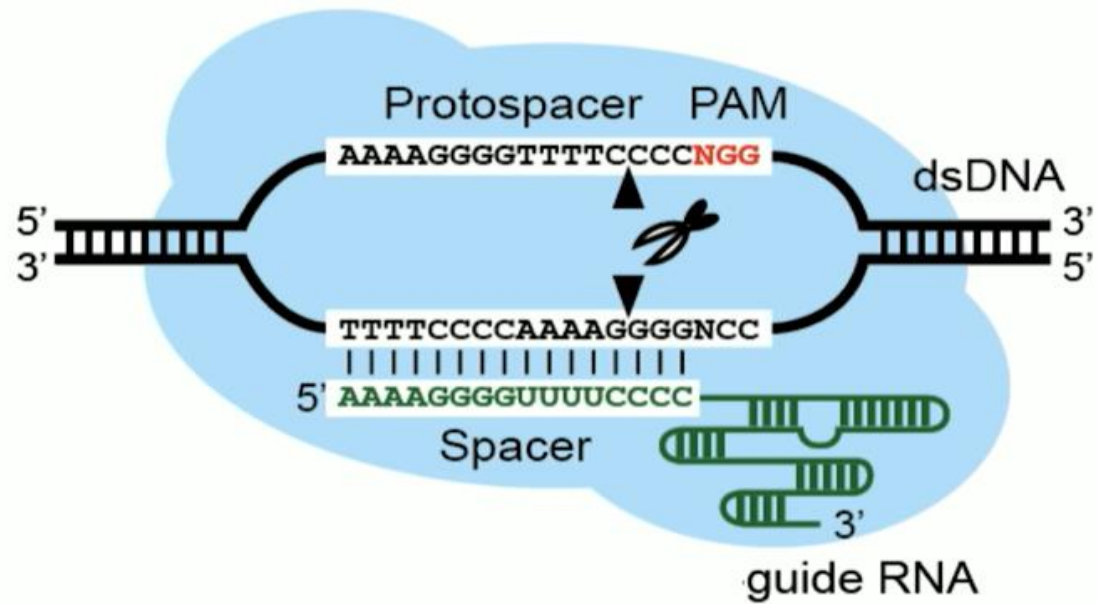
Construct the dual guide RNA. Add it to the Cas9 protein model.

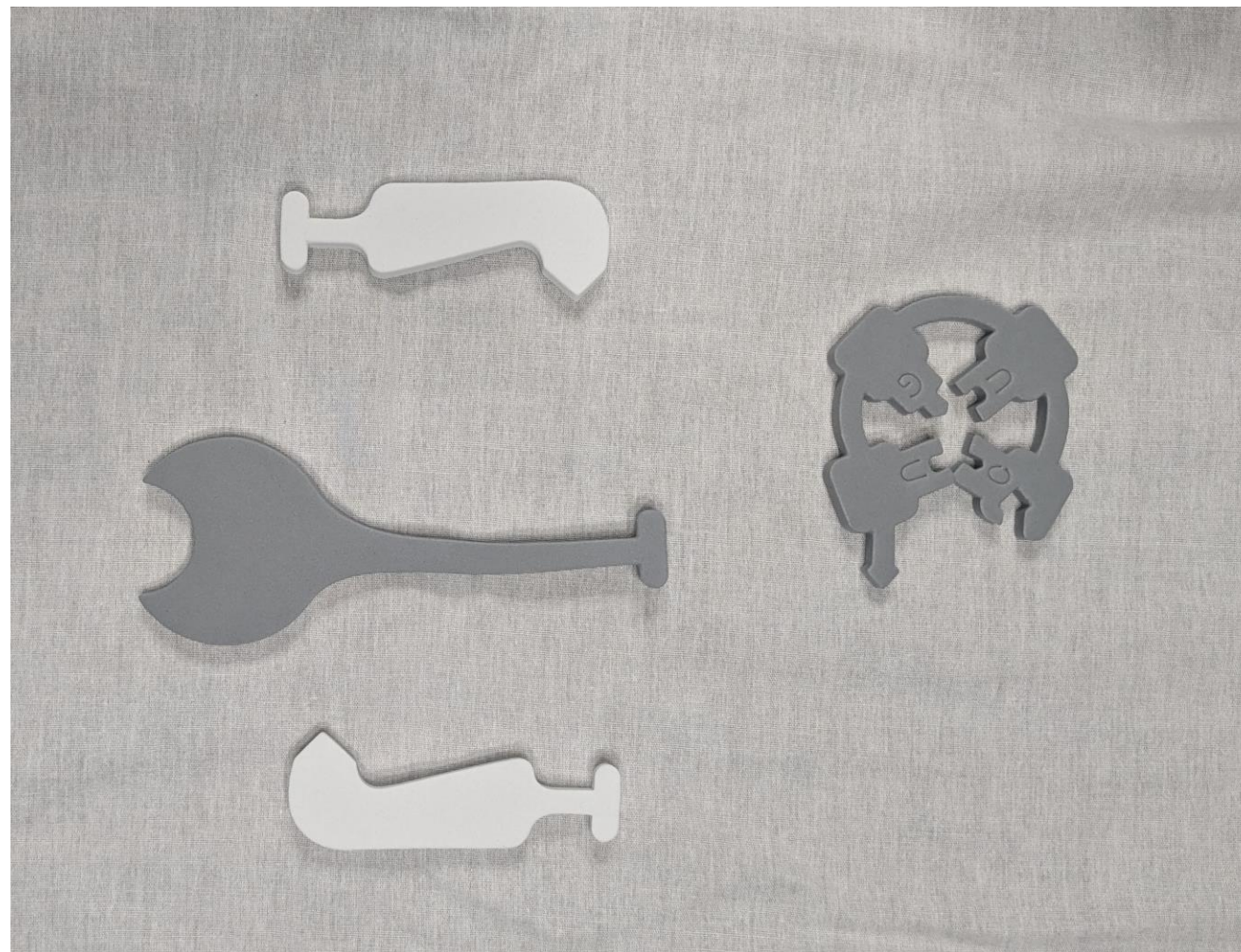


What do you notice about this sequence?



Cas9: RNA-guided DNA cutter





Clinical genome editing to treat sickle cell disease—A brief update

Parinaz Zarghamian^{1,2,3}, Julia Klermund ^{1,2} and Toni Cathomen^{1,2*}

¹Institute for Transfusion Medicine and Gene Therapy, Medical Center — University of Freiburg, Freiburg, Germany, ²Center for Chronic Immunodeficiency (CCI), Faculty of Medicine, University of Freiburg, Freiburg, Germany, ³Ph.D. Program, Faculty of Biology, University of Freiburg, Freiburg, Germany

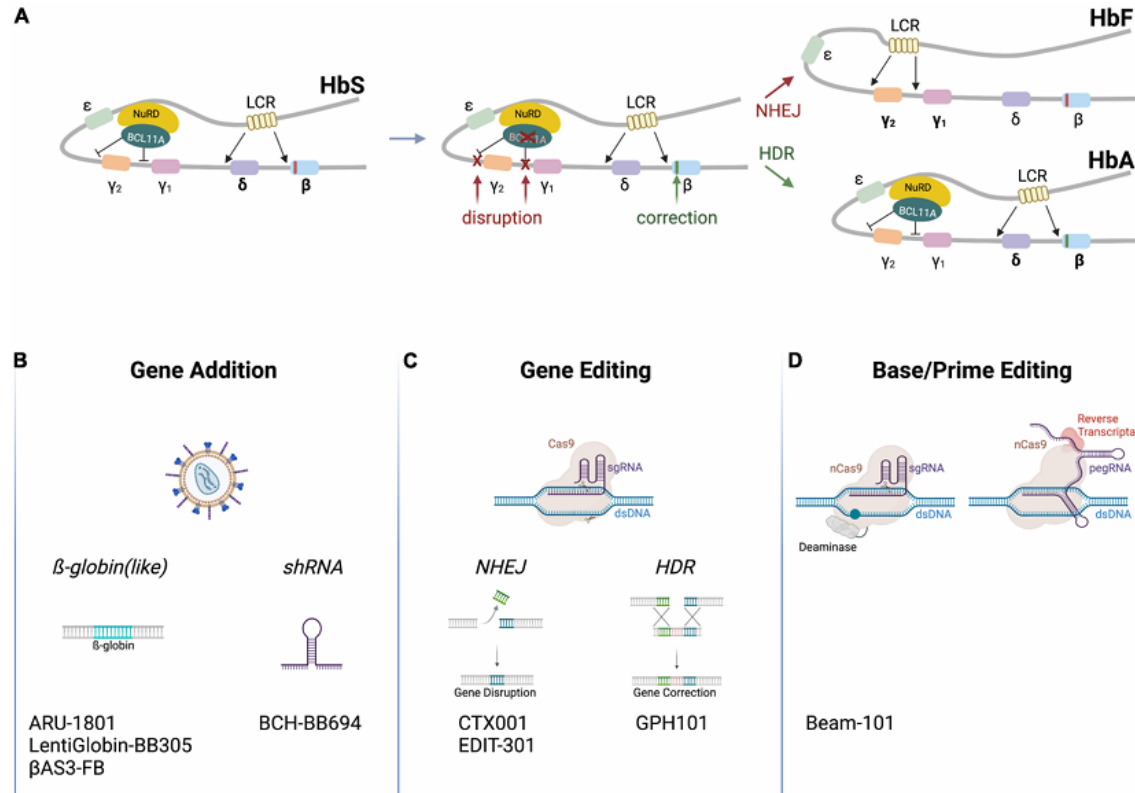
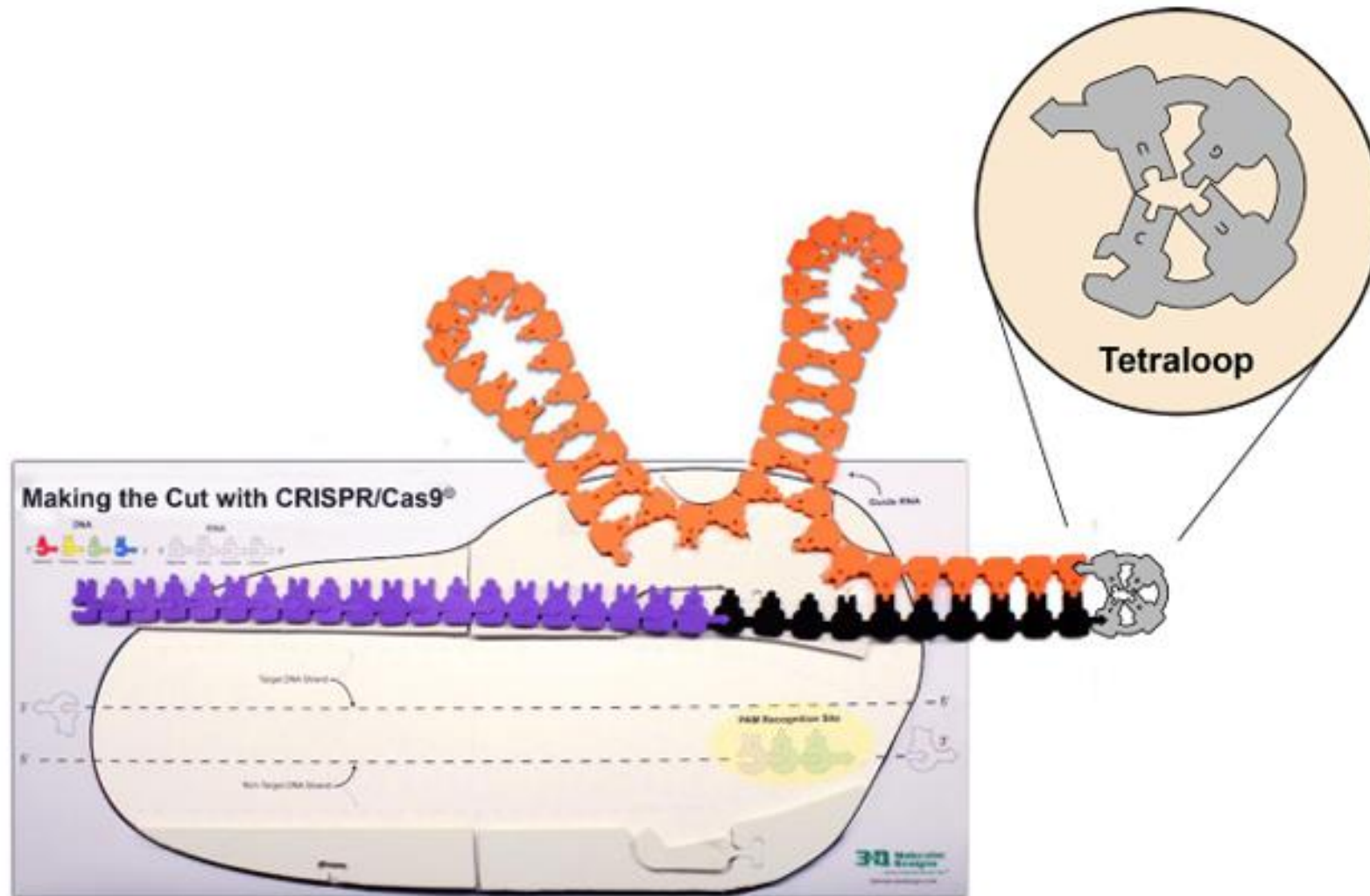


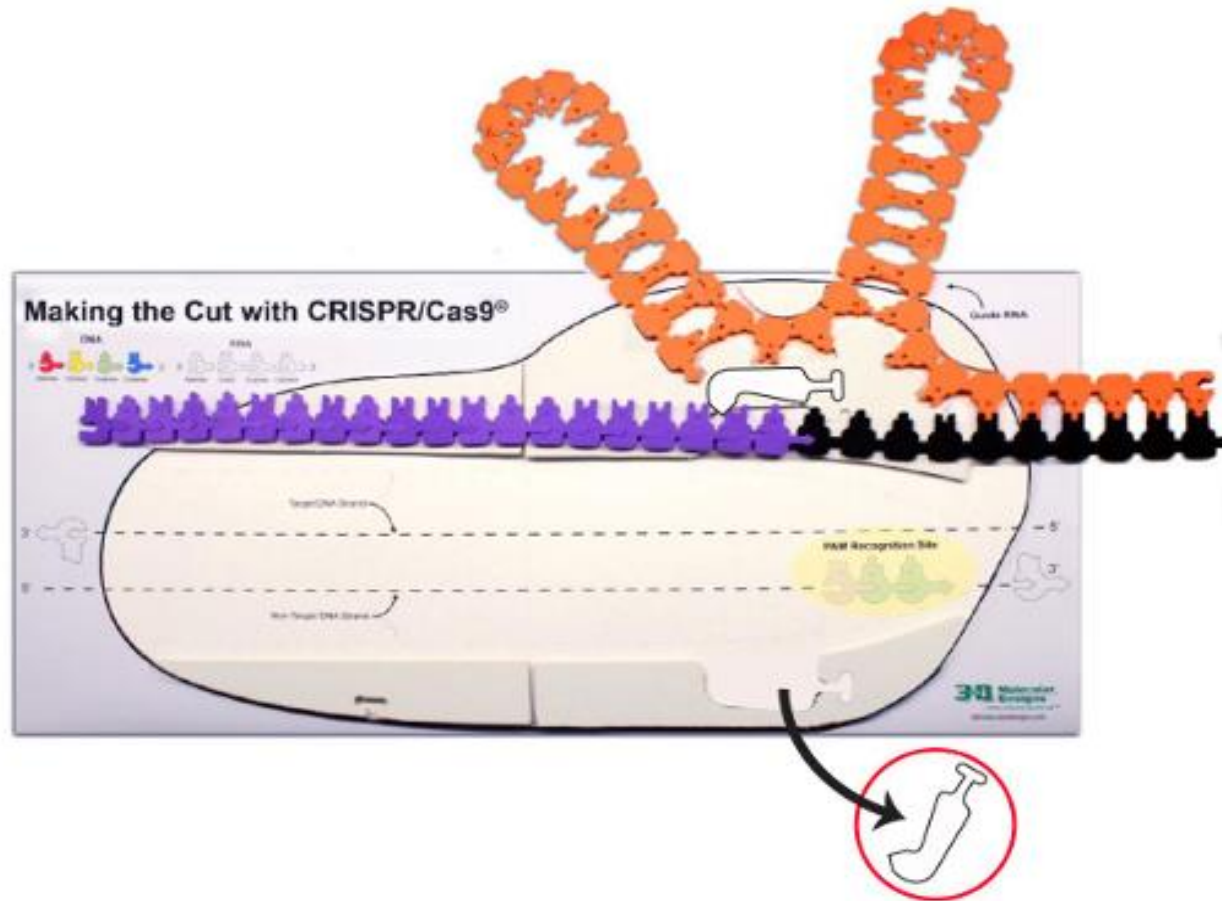
FIGURE 1

Schematic of clinical genome editing approaches for SCD. **(A)** The β -globin locus. The locus encompasses *HBE* (encoding ϵ -globin), *HBG2* and *HBG1* (γ -globin), *HBD* (δ -globin) and *HBB* (β -globin). A locus control region (LCR) and various factors (depicted are *BCL11A* and *NuRD*) regulate developmental stage-specific expression of the hemoglobin genes. A point mutation in *HBB* (red box) leads to expression of HbS. Therapeutic gene editing strategies aim at correcting *HBB* via HDR (green box) or at disrupting *cis*-regulatory elements in the *HBG2*/*HBG1* promoters or in *BCL11A* via NHEJ to re-activate γ -globin expression (red Xs), resulting in the expression of HbA ($\alpha_2\beta_2$) or HbF ($\alpha_2\gamma_2$), respectively. **(B–D)** Platform technologies used for the treatment of SCD. Clinically employed are **(A)** lentiviral vectors to transfer β -globin like genes or an shRNA targeting *BCL11A* mRNA, **(B)** genome editors to disrupt *cis*-regulatory elements by NHEJ or correct *HBB* by HDR, or **(C)** base and prime editors to disrupt *cis*-regulatory elements or off-set the mutation in *HBB*. The respective autologous, genome-engineered cell products are listed on the bottom (Created with BioRender.com).

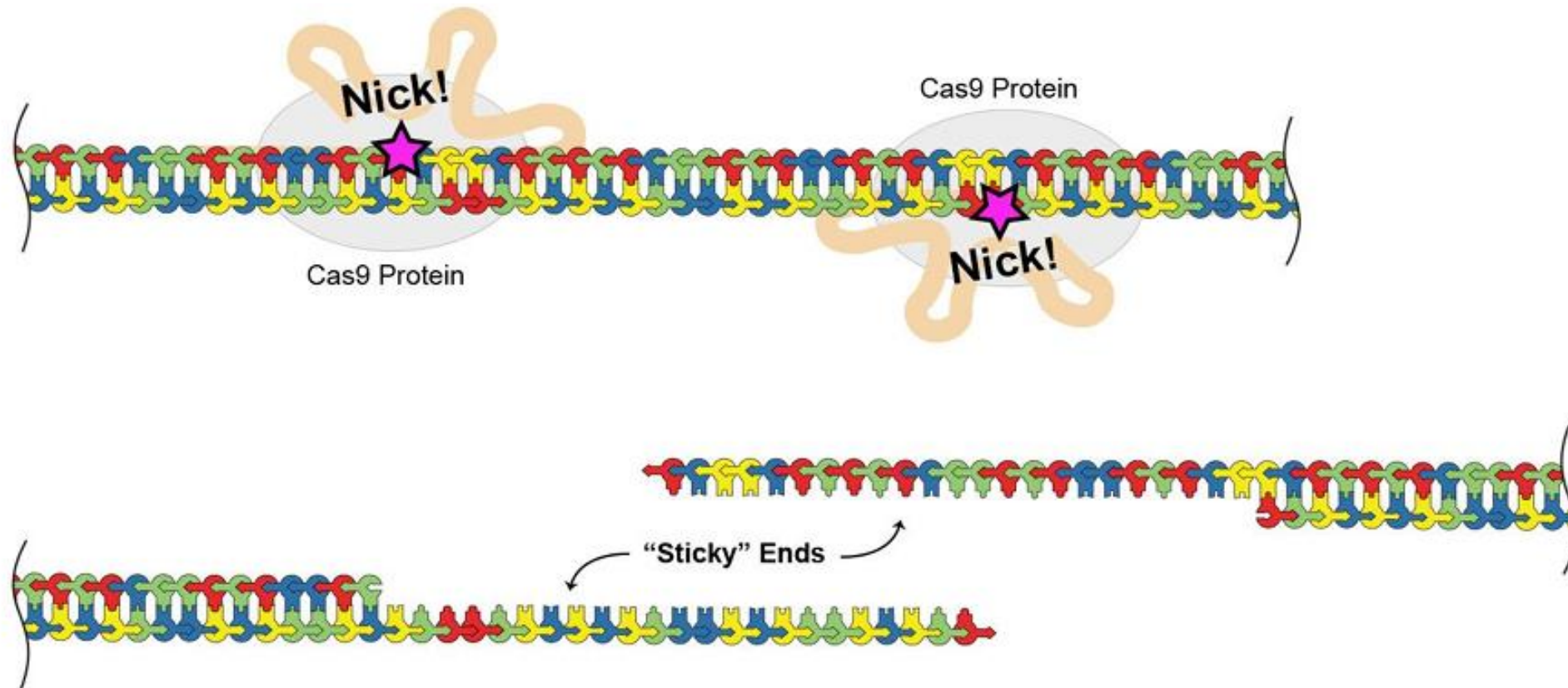
Knocking Out a Gene



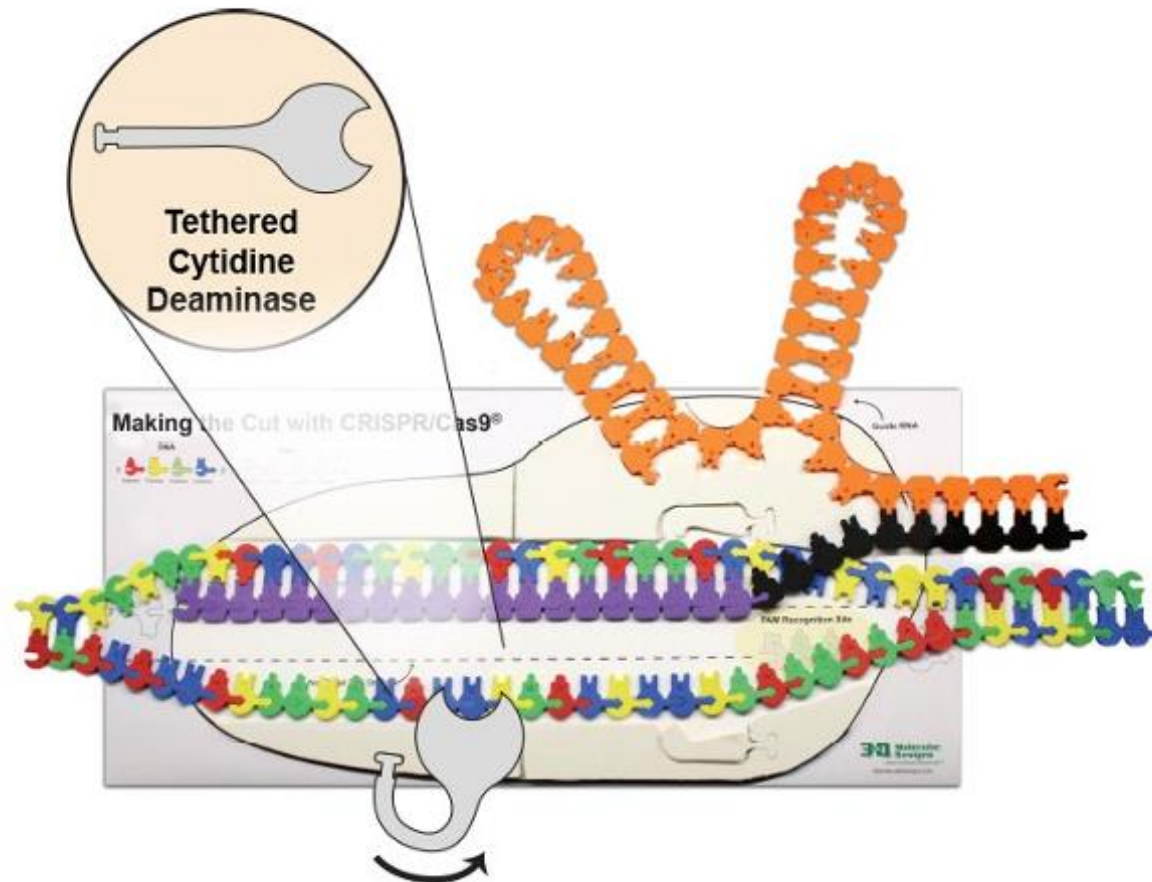
Nicking One Strand



Nicking One Strand

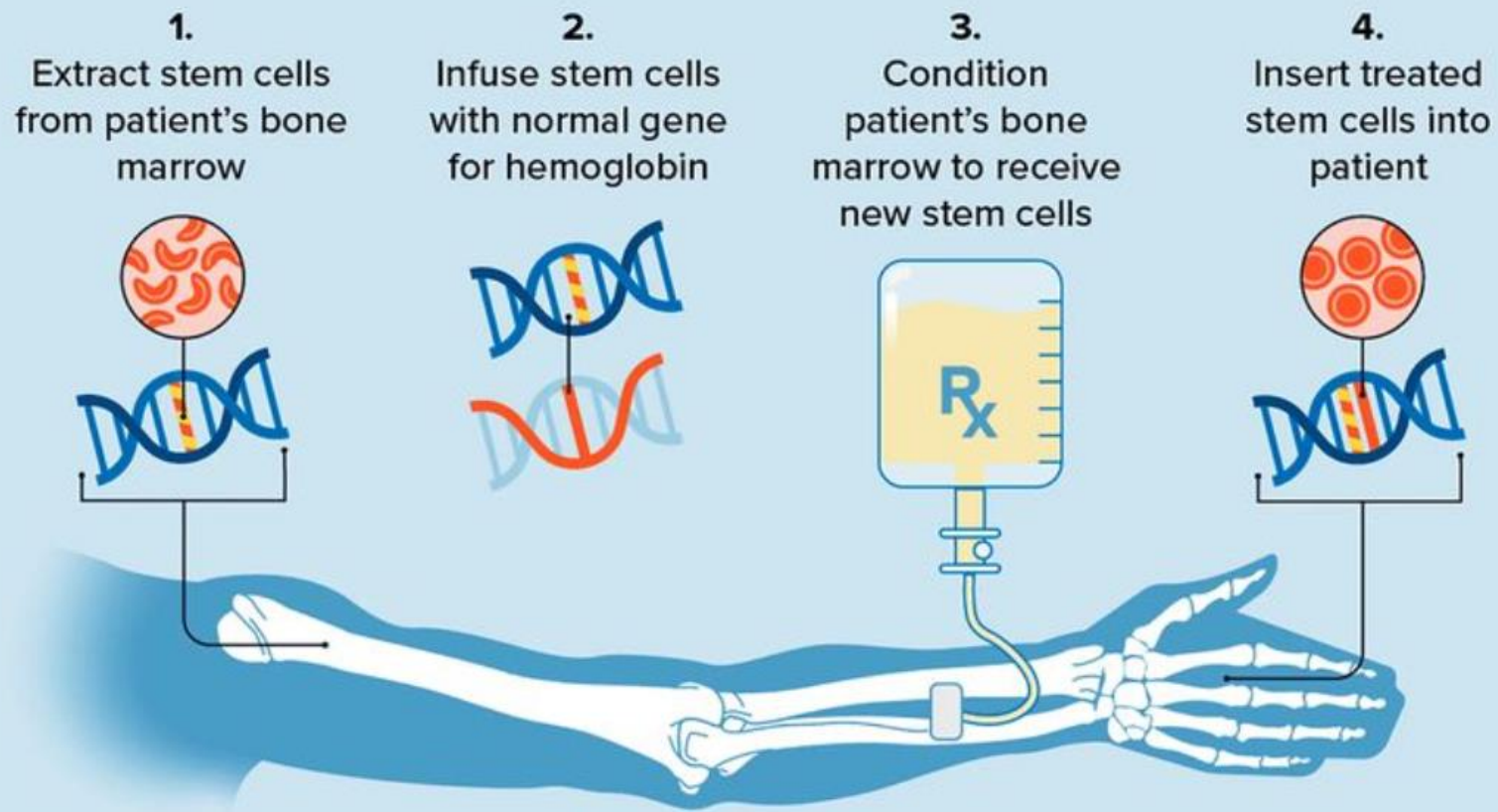


Base Pair Editing

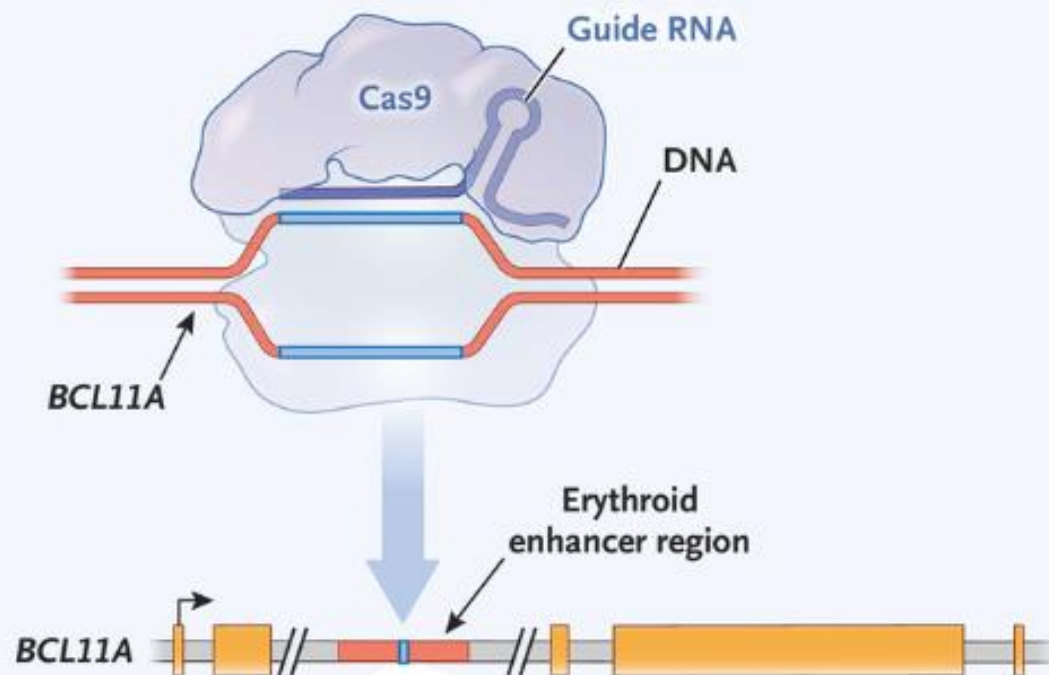


What about Casgevy?

Gene therapy to cure sickle cell anemia



B Targeting of Editing Site



sgRNA target sequence PAM

TAGTCTAGTGCAAGCTAACAGTTGCTTTTATCACAGGCTCCAGGAAG
 ATCAGATCACGTTTCGATTGTCAACGAAAAATAGTGTCCGAGGTCCTTC

GATA1 binding site

Additional Resources

- [Understanding Sickle Cell - Sickle Cell Speaks](#)
- [Sickle hemoglobin \(HbS\) allele and sickle cell disease: a HuGE review - PubMed \(nih.gov\)](#)
- [CRISPR: History of Discovery \(youtube.com\)](#)
- [The Heroes of CRISPR: Cell](#)
- [CRISPR-Cas9 Gene Editing for Sickle Cell Disease and \$\beta\$ -Thalassemia | New England Journal of Medicine \(nejm.org\)](#)
- [Treatment by CRISPR-Cas9 Gene Editing — A Proof of Principle | New England Journal of Medicine \(nejm.org\)](#)
- [Clinical genome editing to treat sickle cell disease—A brief update - PMC \(nih.gov\)](#)
- [FDA approves Casgevy, 1st gene-editing therapy to treat sickle cell \(sicklecellanemianews.com\)](#)



Additional Resources

- [The status of the human gene catalogue | Nature](#)
- [Human Genome Is Much More Than Just Genes | Science | AAAS](#)
- [An integrated encyclopedia of DNA elements in the human genome | Nature](#)



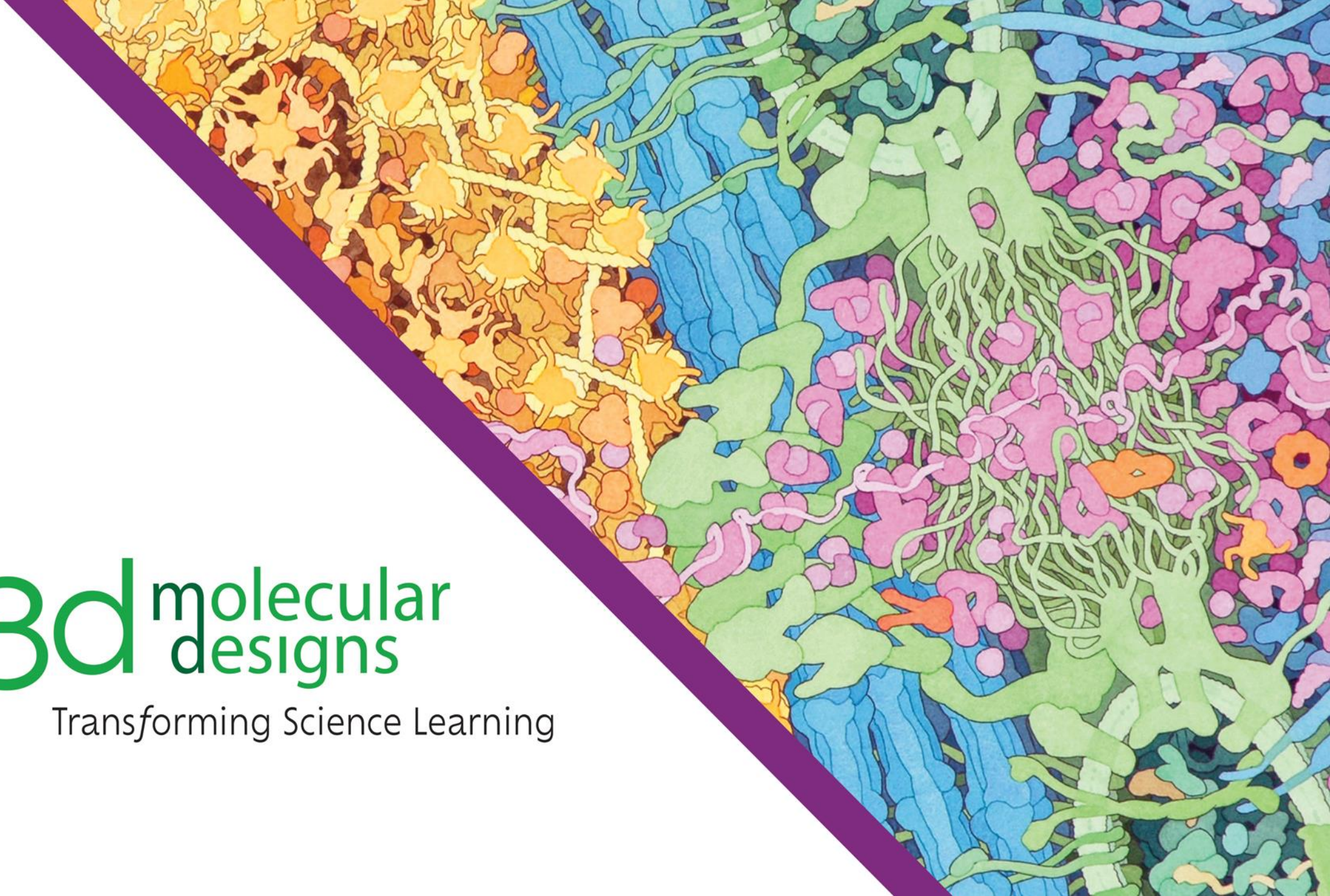
Workshop NGSS Connections

SEPs	CCCs	DCIs
Asking Questions and Defining Problems	Systems and System Models	HS-LS1-4
Developing and Using Models	Cause and Effect	HS-LS3-2
	Scale, Proportion, and Quantity	



3d molecular
designs

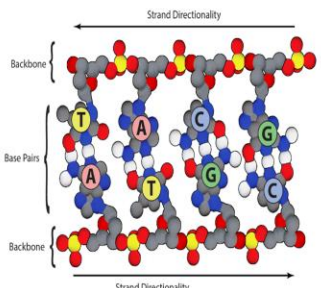
Transforming Science Learning



Digital Modeling Hub

We're enhancing our kits and models to inspire more hands-on learning with our classic content, Interactables, short content videos, and much more!! Check it out!

Double-Stranded DNA



The nitrogenous bases from two DNA strands form hydrogen bonds, resulting in double-stranded DNA.

These base pairs follow pairing rules, with Adenine (A) pairs with Thymine (T), and Guanine (G) pairs with Cytosine (C).

A-T base pairs have two hydrogen bonds and G-C base pairs have three hydrogen bonds.

Look at our new
Digital Modeling Hub for
additional resources to use with
the Chromosome Connection Kit
and CRISPR Making the Cut Kit.



Identifying Regions
on a Chromosome

Ruth Hutson

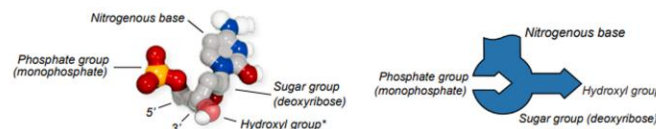
Exploring PCR



Getting Started

Today you are going to model the PCR process using foam nucleotides. Before we start, acquaint yourself with the foam pieces.

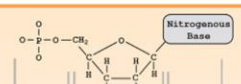
Compare the foam nucleotide to the plastic model on left and chemical drawings on the previous page.



*The hydroxyl group (OH) shown in the above illustration has been added to the photo of the nucleotide, since this particular model of a nucleotide doesn't show the hydroxyl group.

Examine the diagrams to the right.

- In the image representing the foam nucleotide on the bottom right, label the locations of the 3' and 5' carbons.



Get your access
code by
responding to
our survey!

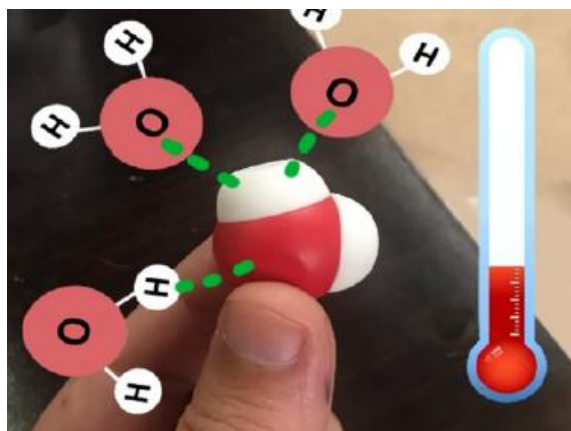
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You'll take home all these classroom resources!

We value your opinion!
Let us know what you
think.

Scan the QR
Code and
complete this
short survey.



A visit to the genetic counselor
