3d molecular designs
Transforming Science Learning

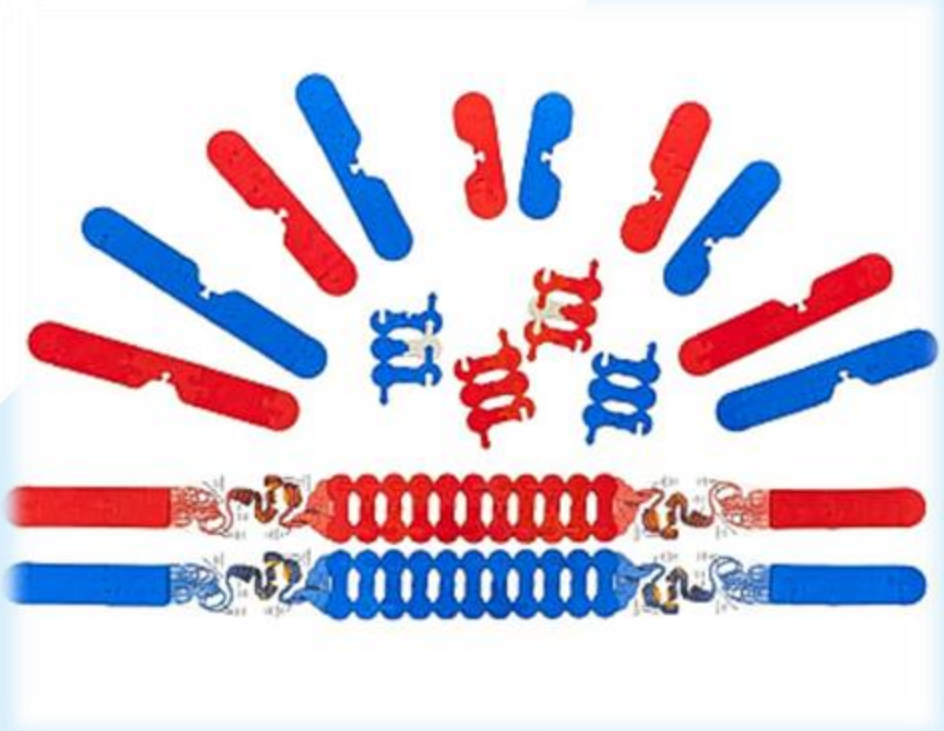
Making the Cut with CRISPR that Changes Lives

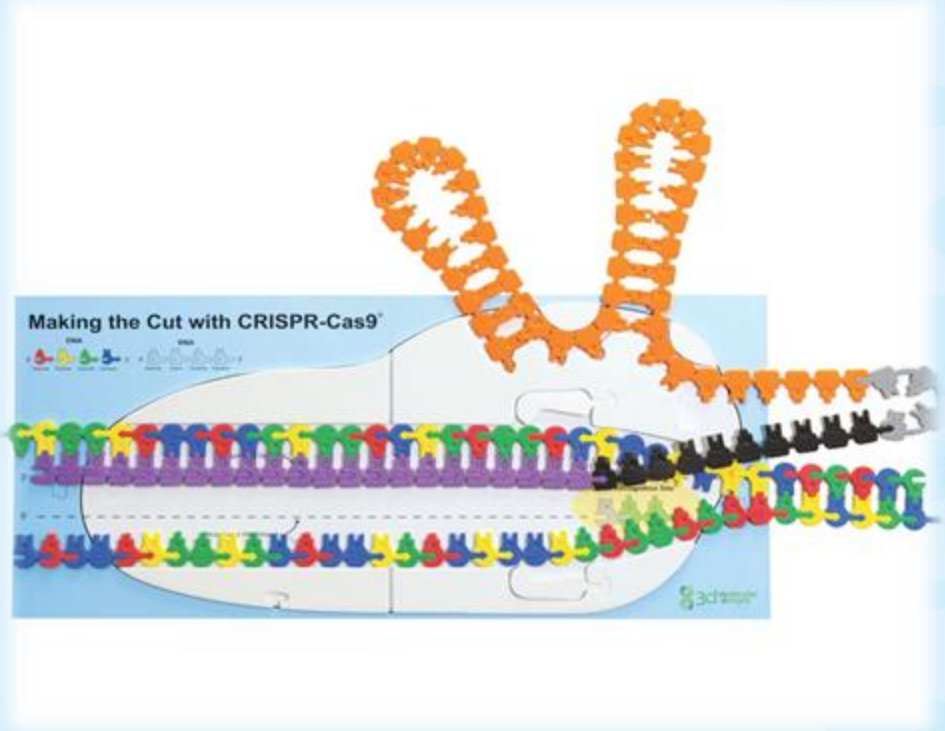
CAST24
Everything's Connected
Presented by STAT SCIENCE TEACHERS ASSOCIATION of TEXAS

Ruth Hutson, MSSE

3d molecular designs

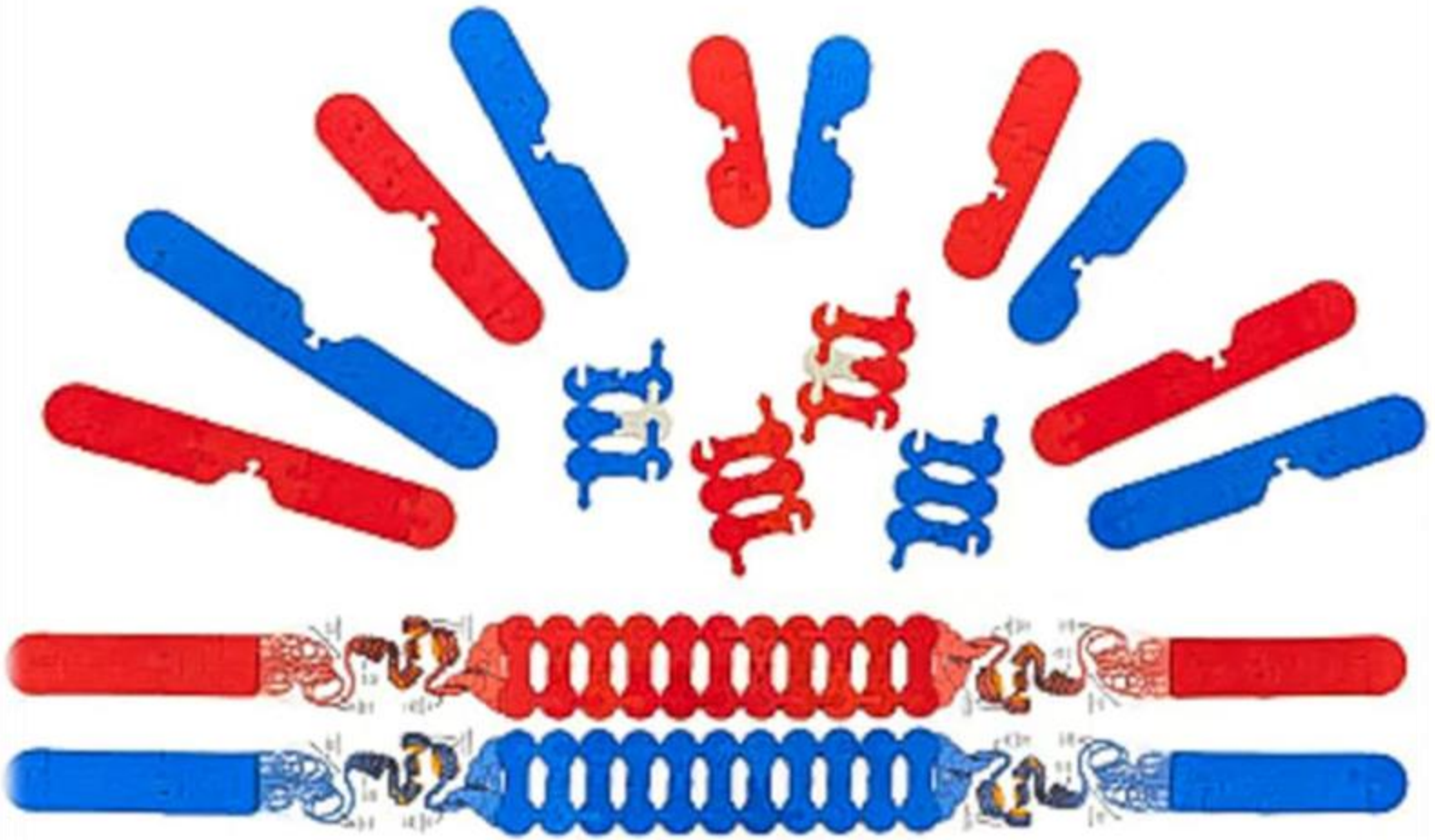
Transforming Science Learning





Workshop Outcomes

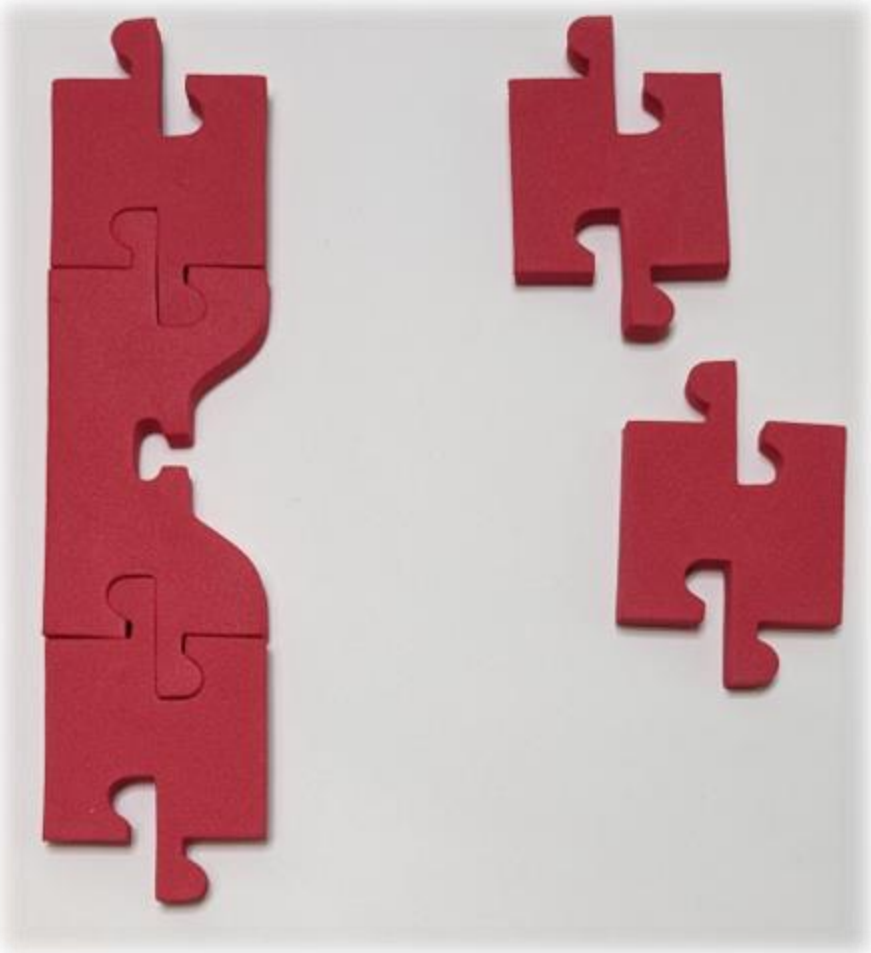
- Make and defend a claim based on evidence that genetic variations result from a variety of factors.
- Understand how the Cas9 protein binds to a specific sequence of double-stranded DNA at a PAM site and cuts it.
- Explain how CRISPR technologies are applied to edit genomes.
- Have fun!



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



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- **Add a cap piece to each end of the chromatid.**

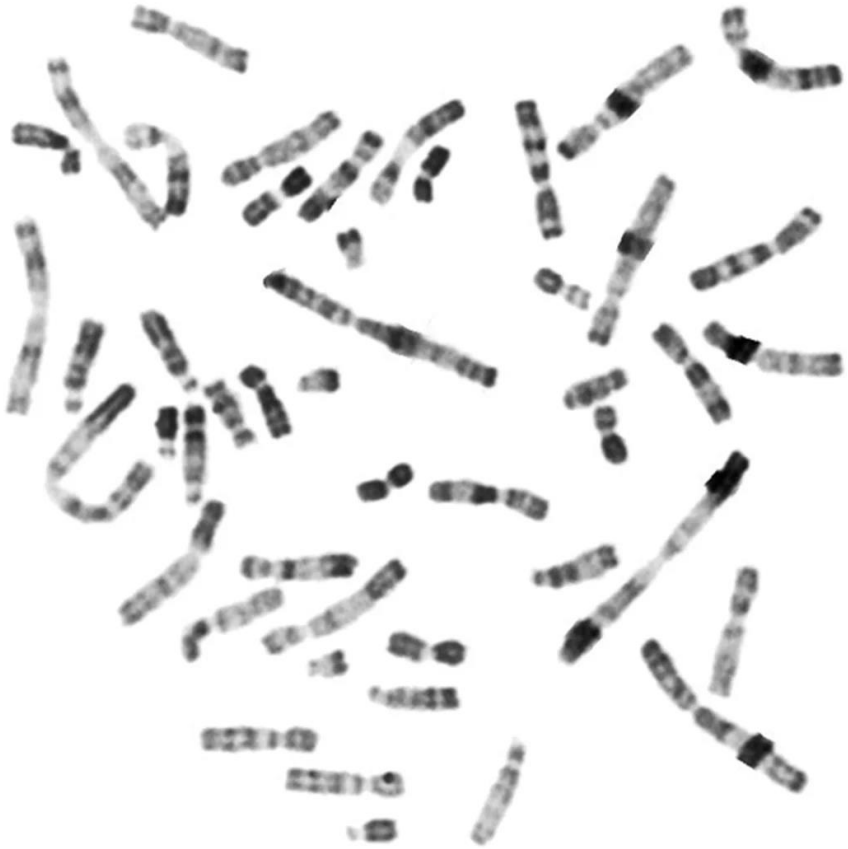
Create a sister chromatid

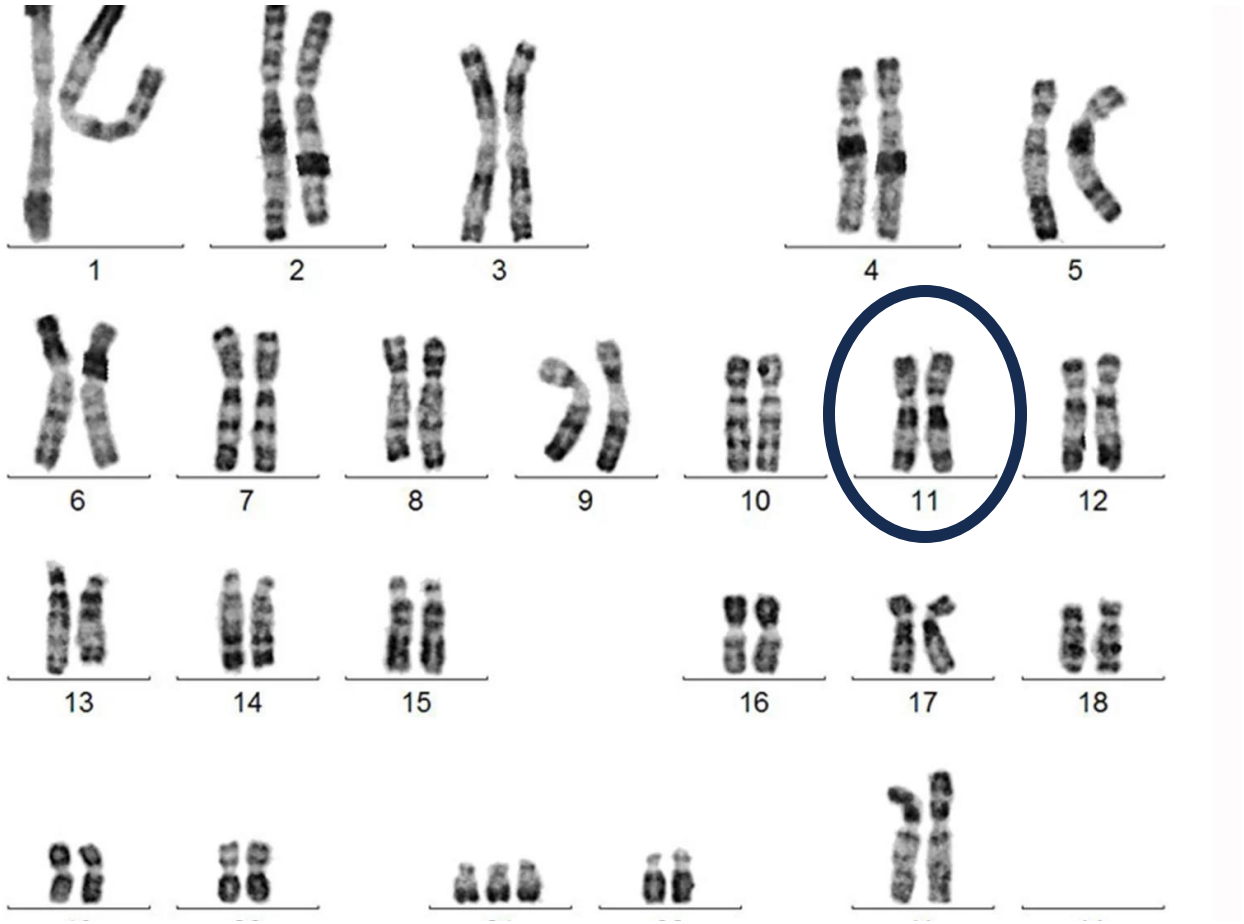
- Create a duplicate copy of the chromosome to make a sister chromatid.

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.

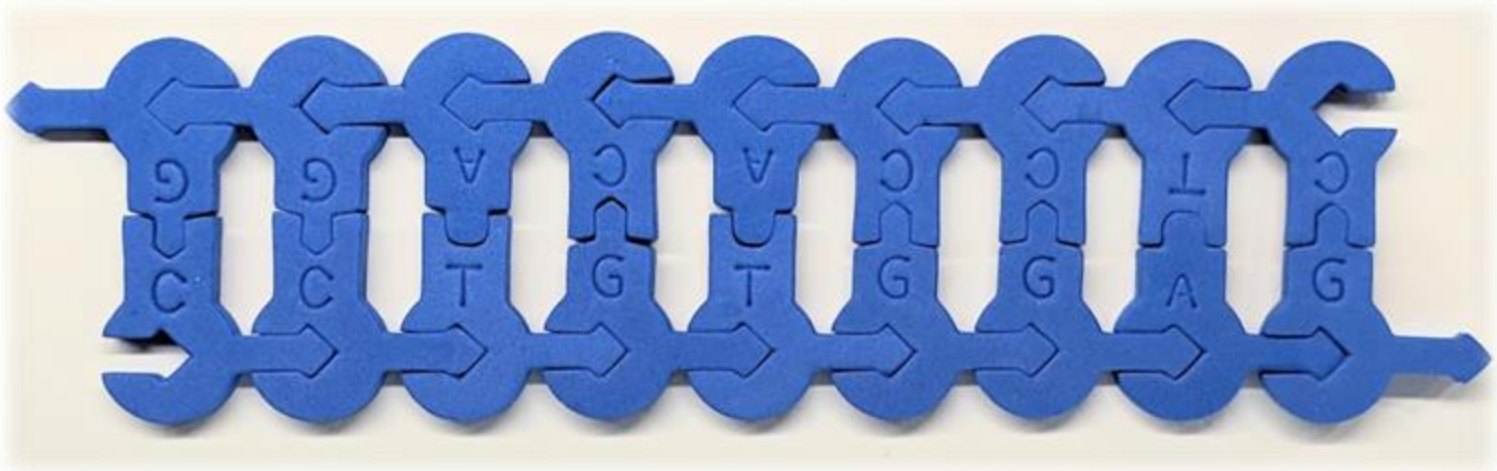
The human B-globin locus is a 60-kb segment on the short arm and contains five functional genes and two pseudogenes. We will look at a small part of this locus.

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

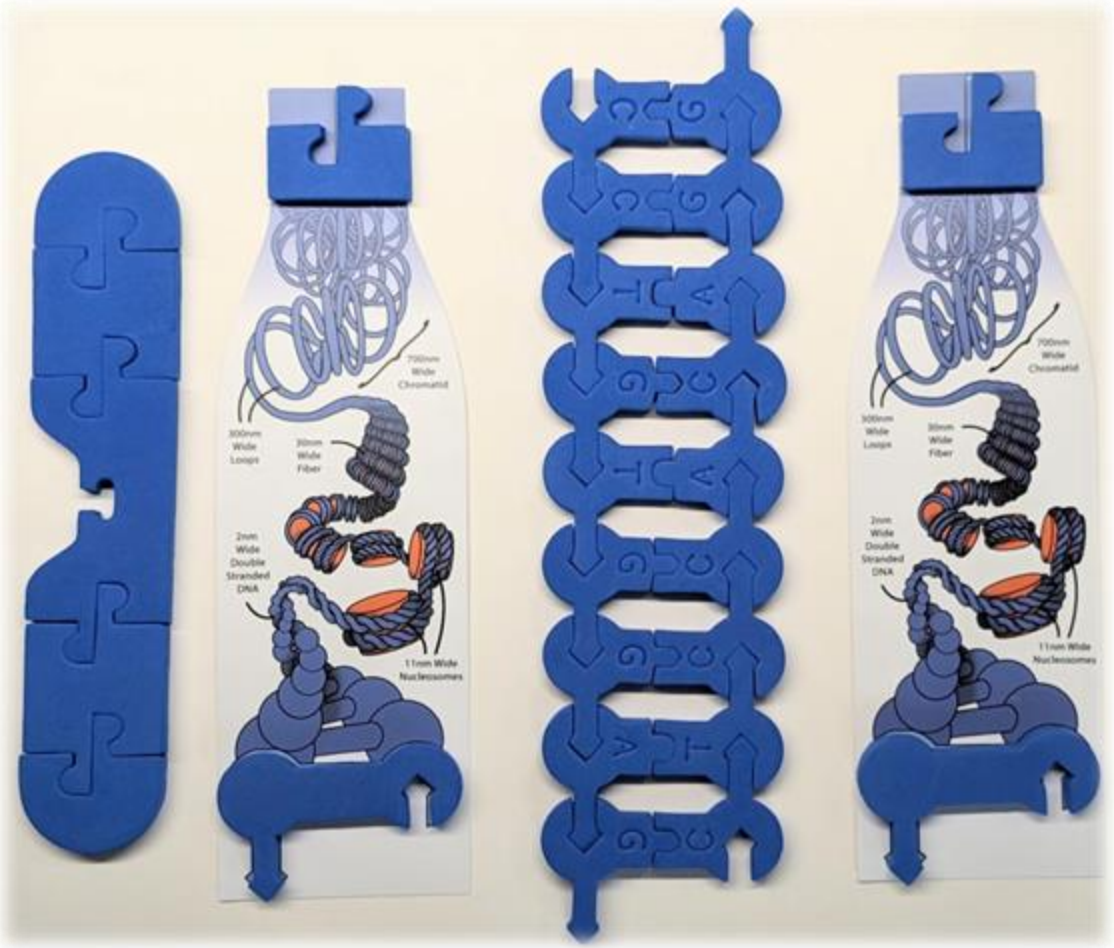
Examine this beta-globin DNA gene sequence.

3′ G G A C A C C T C 5′

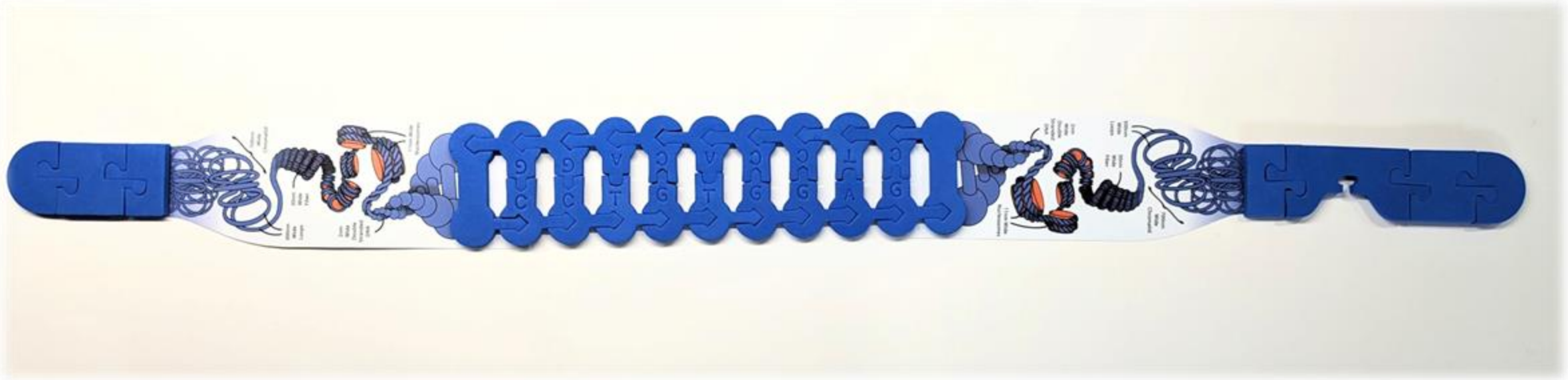
5′ C C T G T G G A G 3′



Use insert pieces to assemble the paternal chromosome

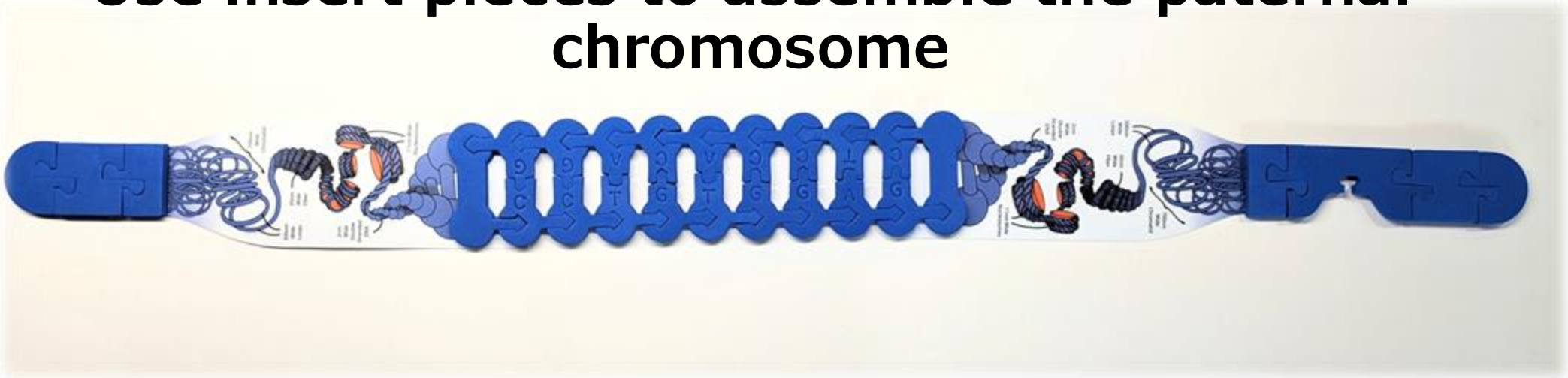


Use insert pieces to assemble the paternal chromosome



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	G – C
A – T	T – A
G – C	G – C

What is the difference between the two sequences?

The Genetic Codon Chart©

	U	C	A	G	
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Amino Acid Properties

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

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How might that affect the resulting protein?

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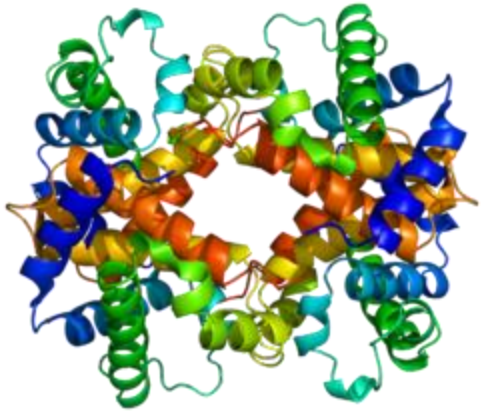
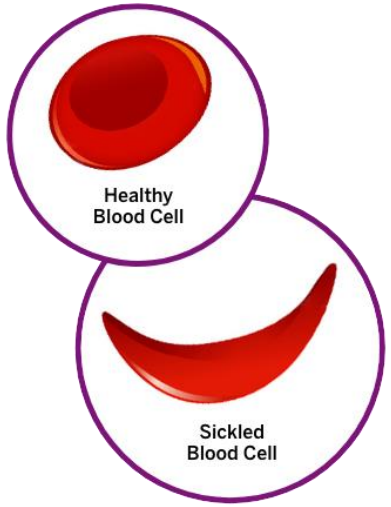


Beta-globin



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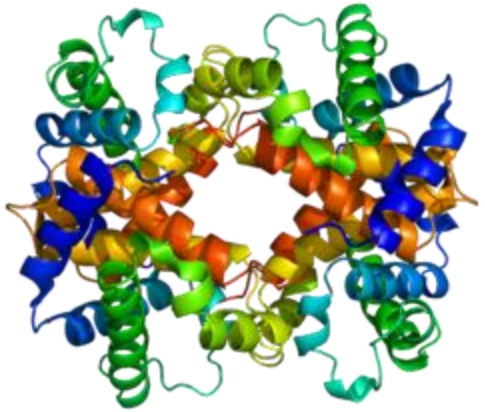

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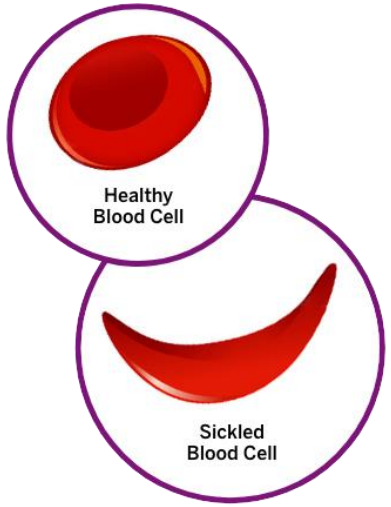
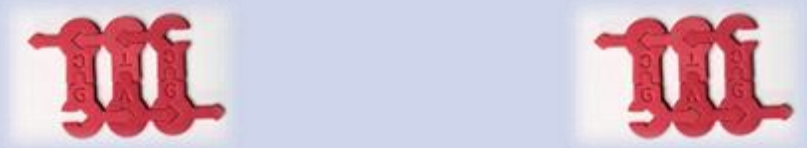
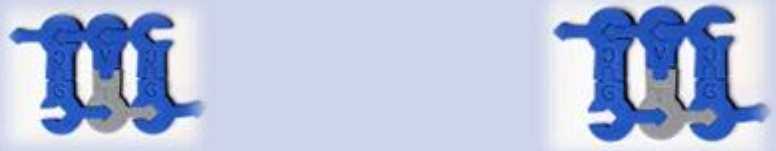


Understanding Codominance

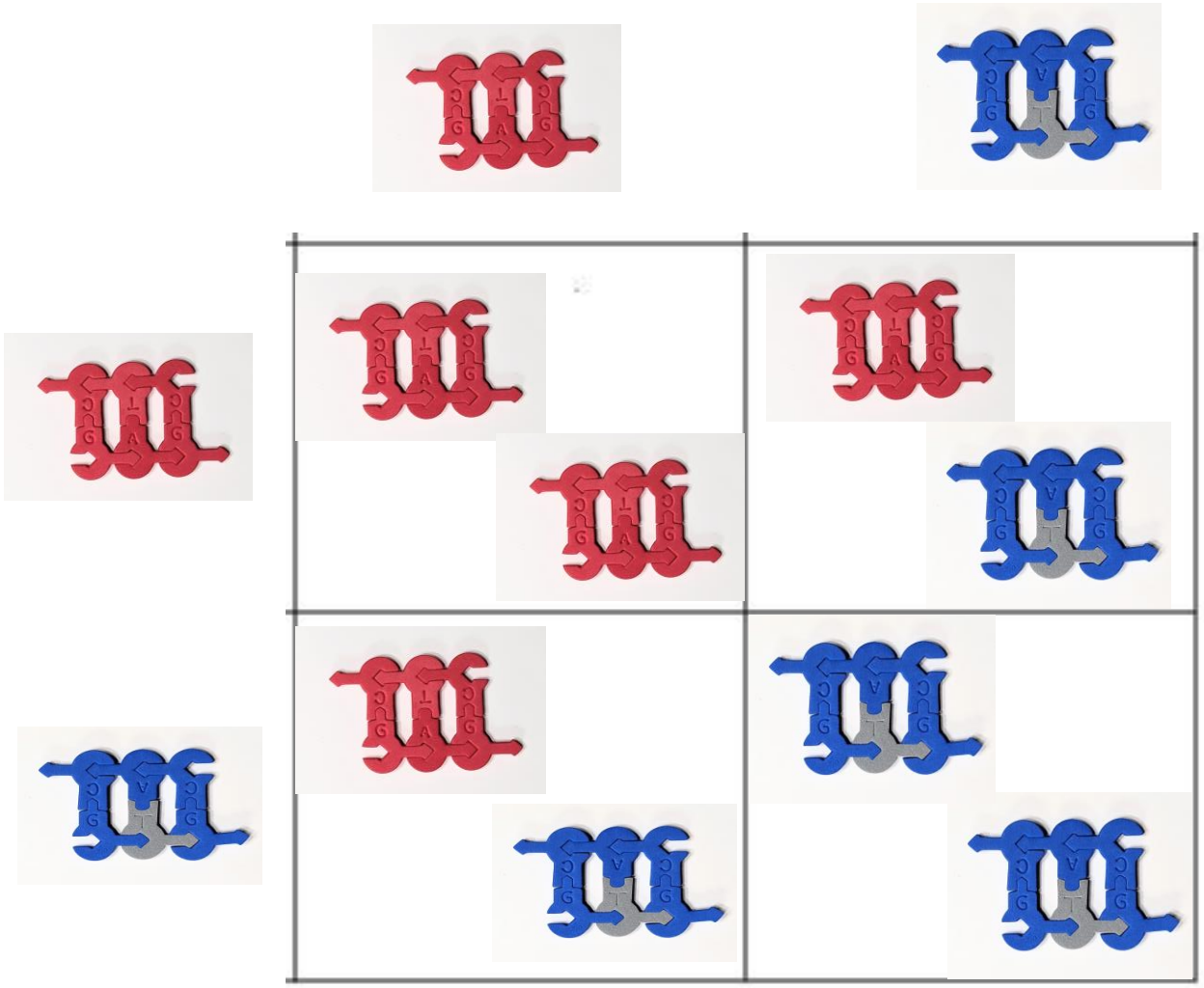
Sickle Cell Anemia Possible Genotypes and Phenotypes	DNA sequence
SS Homozygous Typical beta-globin	
Ss Heterozygous Carrier Typical beta-globin and Atypical beta-globin	
ss Homozygous Atypical beta-globin	

A visit to the genetic counselor

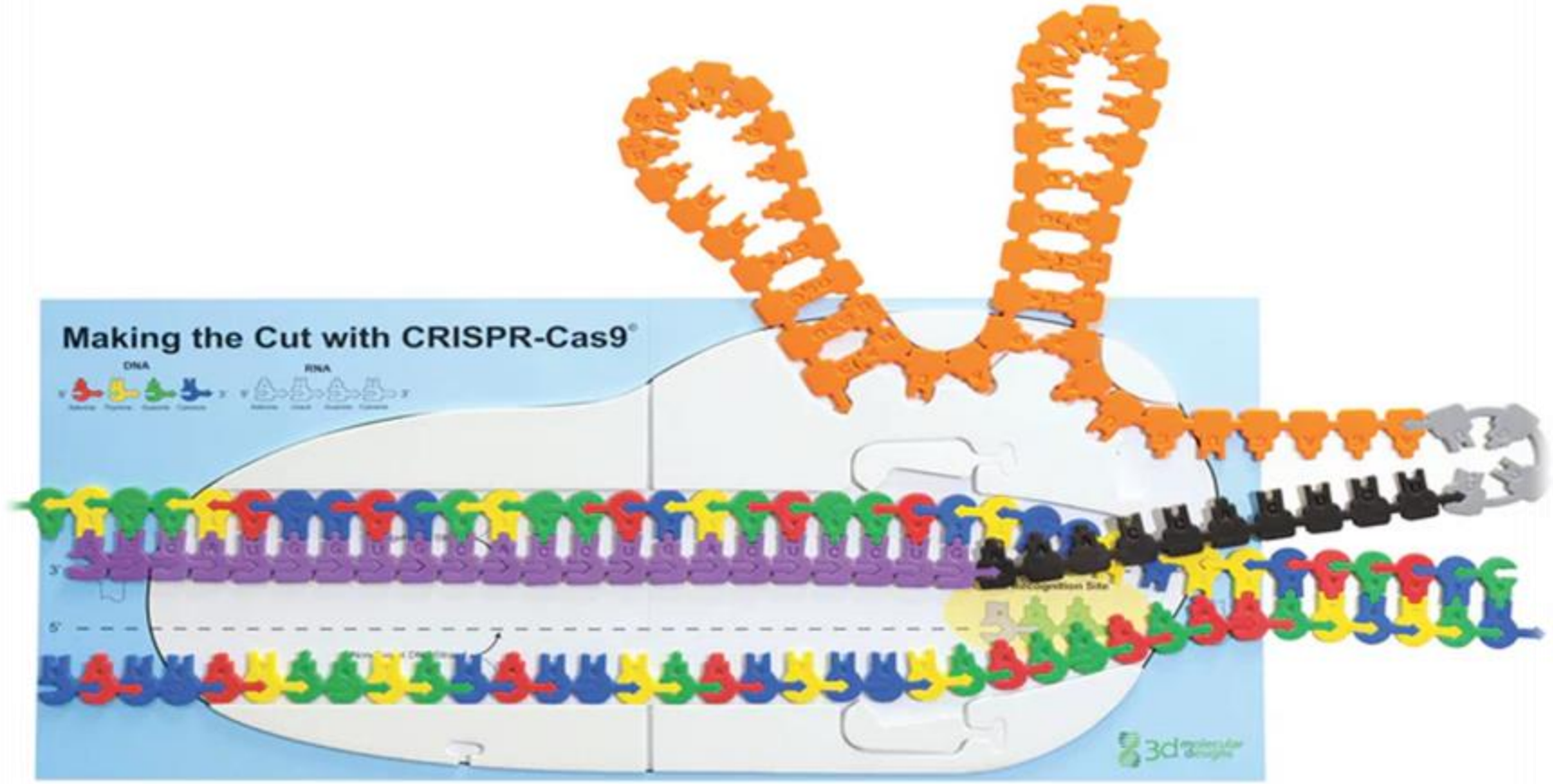
Two individuals with sickle cell trait want to know their chances of having a child with sick cell anemia. Complete the Punnett square using DNA sequences to fill in each of the squares.

How would you advise them?

A visit to the genetic counselor



What are the implications?


Making the Cut with CRISPR-Cas9®
DNA
RNA
3'
5'

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?***

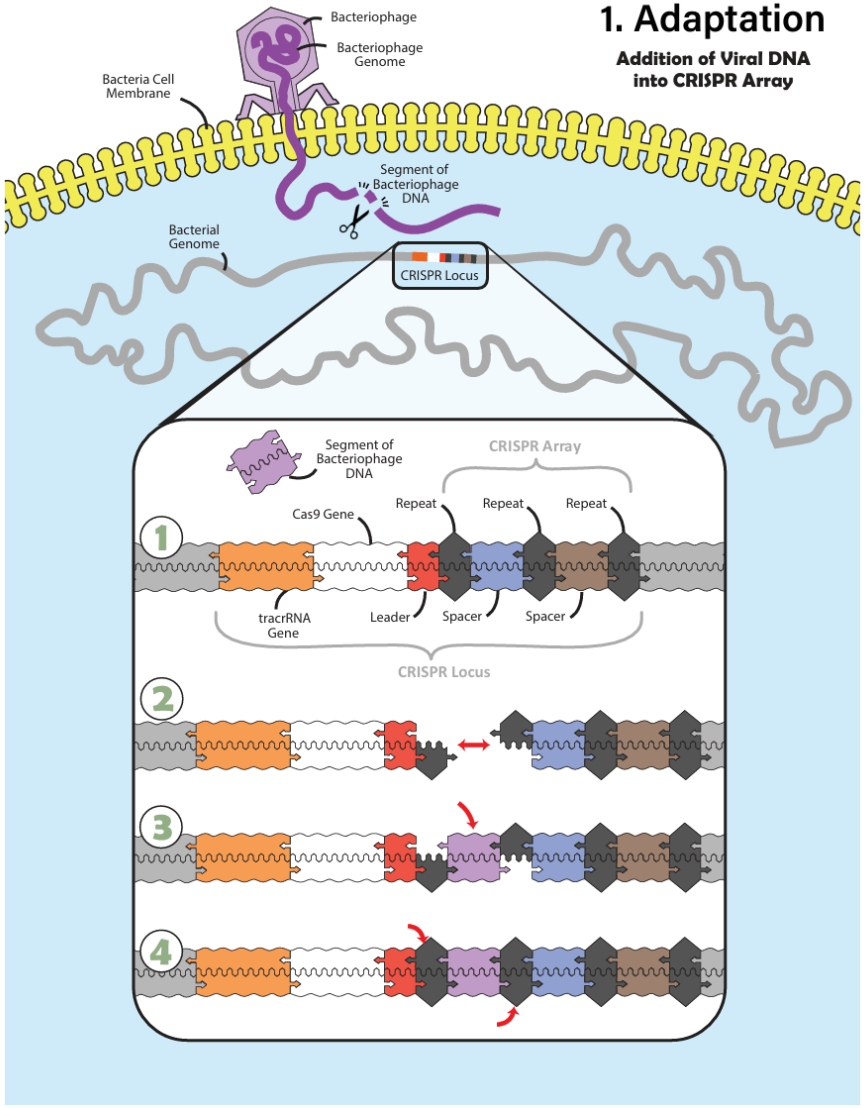
The first report of what was later to become known as the CRISPR system appeared in a paper from a Japanese research group) in 1987. These researchers noticed an unusual pattern in the sequence of nucleotides that occurred just downstream from an E. coli gene they were studying. They had no idea what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

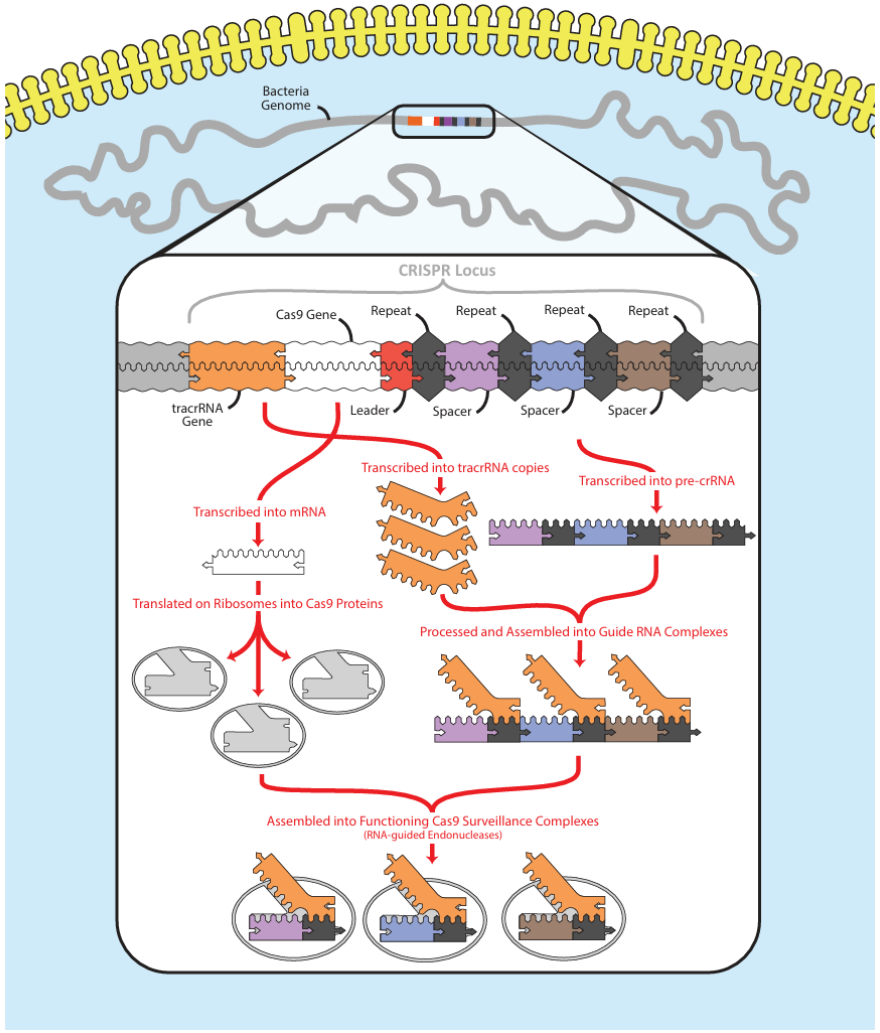
Over the next ten years, other nucleotide sequences with this pattern were reported in the genomes of other bacteria. Eventually, this pattern was named CRISPR – Clustered Regularly Interspaced Short Palindromic Repeats.

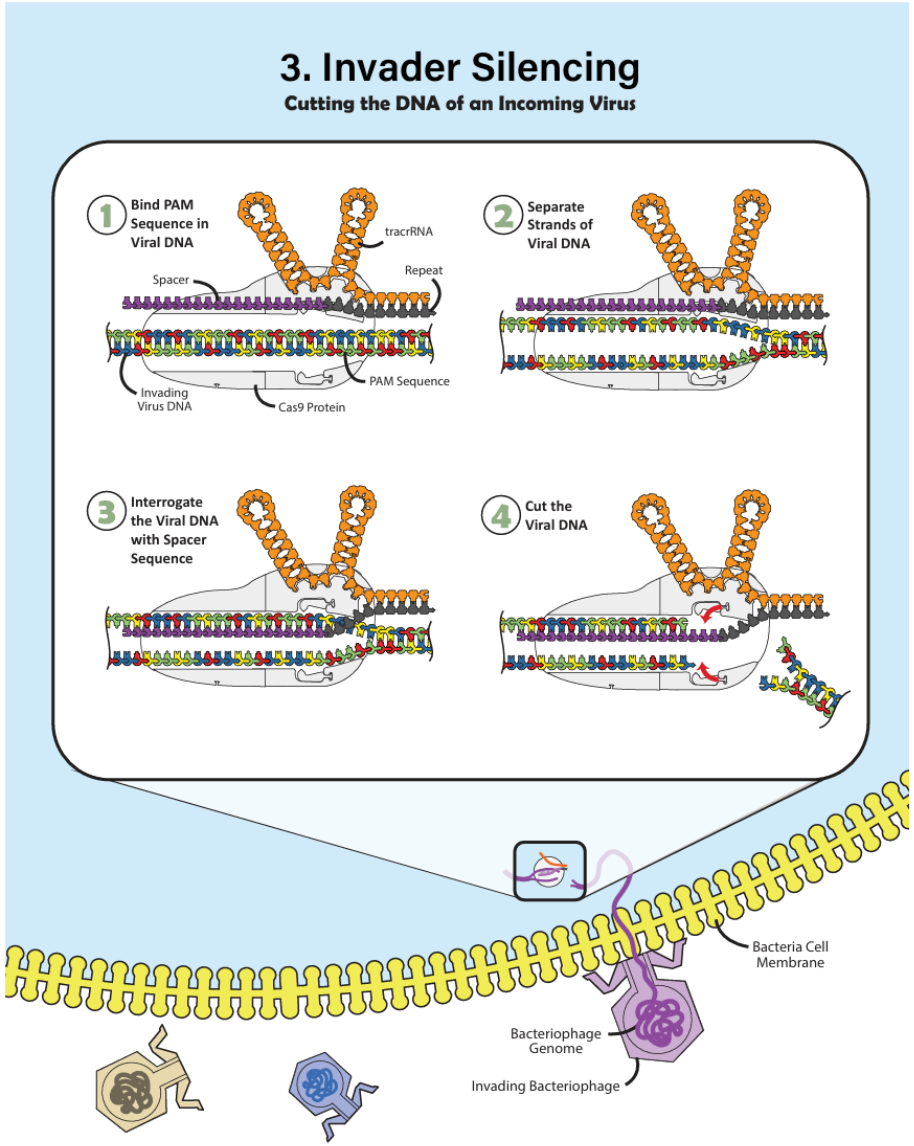
So, here is the challenge: ***Give your students the following sequence of nucleotides. . .***

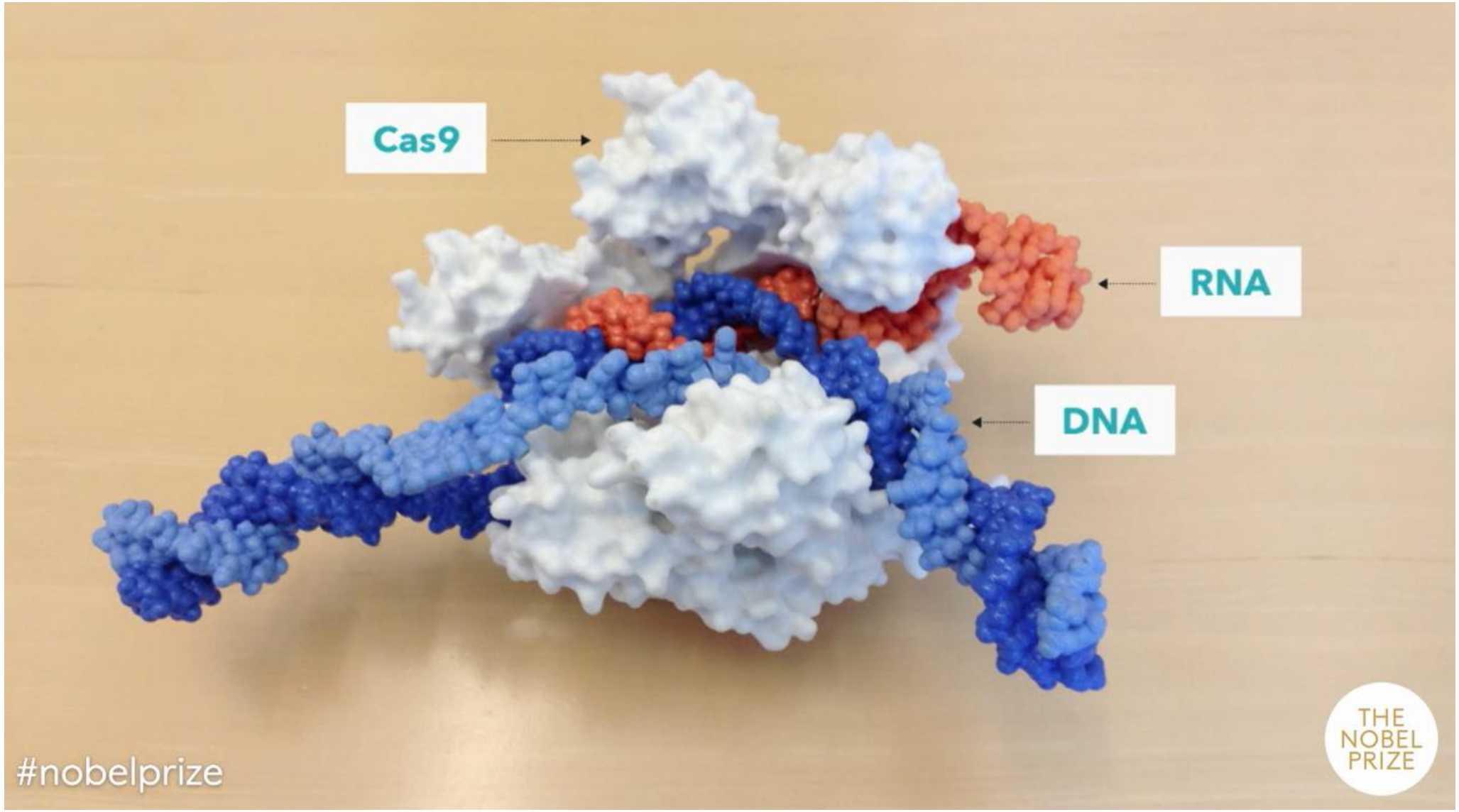
GGAGTTCTACCGCAGAGGCGGGGGAACTCCAAGTGATATCCATCATCGCATCCAG
TGCGCCCGGTTTATCCCCGCTGATGCGGGGAACACCAGCGTCAGGCGTGAAATCT
CACCGTCGTTGCCGGTTTATCCCTGCTGGCGCGGGGAACTCTCGGTTCAGGCGTT
GCAAACCTGGCTACCGGGCGGTTTATCCCCGCTAACGCGGGGAACTCGTAGTCCA
TCATTCCACCTATGTCTGAACTCCCGGTTTATCCCCGCTGGCGCGGGGAACTCG

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


1. Adaptation
Addition of Viral DNA into CRISPR Array
Bacteriophage
Bacteriophage Genome
Bacteria Cell Membrane
Segment of Bacteriophage DNA
Bacterial Genome
CRISPR Locus
Segment of Bacteriophage DNA
CRISPR Array
Cas9 Gene
Repeat
Repeat
Repeat
tracrRNA Gene
Leader
Spacer
Spacer
CRISPR Locus
1
2
3
4


2. Expression of CRISPR Locus Genes
Building Cas9 Surveillance Complexes
Bacteria Genome
CRISPR Locus
Cas9 Gene
Repeat
Repeat
Repeat
Repeat
tracrRNA Gene
Leader
Spacer
Spacer
Spacer
Transcribed into tracrRNA copies
Transcribed into pre-crRNA
Transcribed into mRNA
Translated on Ribosomes into Cas9 Proteins
Processed and Assembled into Guide RNA Complexes
Assembled into Functioning Cas9 Surveillance Complexes
(RNA-guided Endonucleases)


3. Invader Silencing
Cutting the DNA of an Incoming Virus
1 Bind PAM Sequence in Viral DNA
tracrRNA
Repeat
Spacer
PAM Sequence
Invading Virus DNA
Cas9 Protein
2 Separate Strands of Viral DNA
3 Interrogate the Viral DNA with Spacer Sequence
4 Cut the Viral DNA
Bacteria Cell Membrane
Bacteriophage Genome
Invading Bacteriophage


Cas9
RNA
DNA
#nobelprize
THE NOBEL PRIZE

The NEW ENGLAND JOURNAL of MEDICINE

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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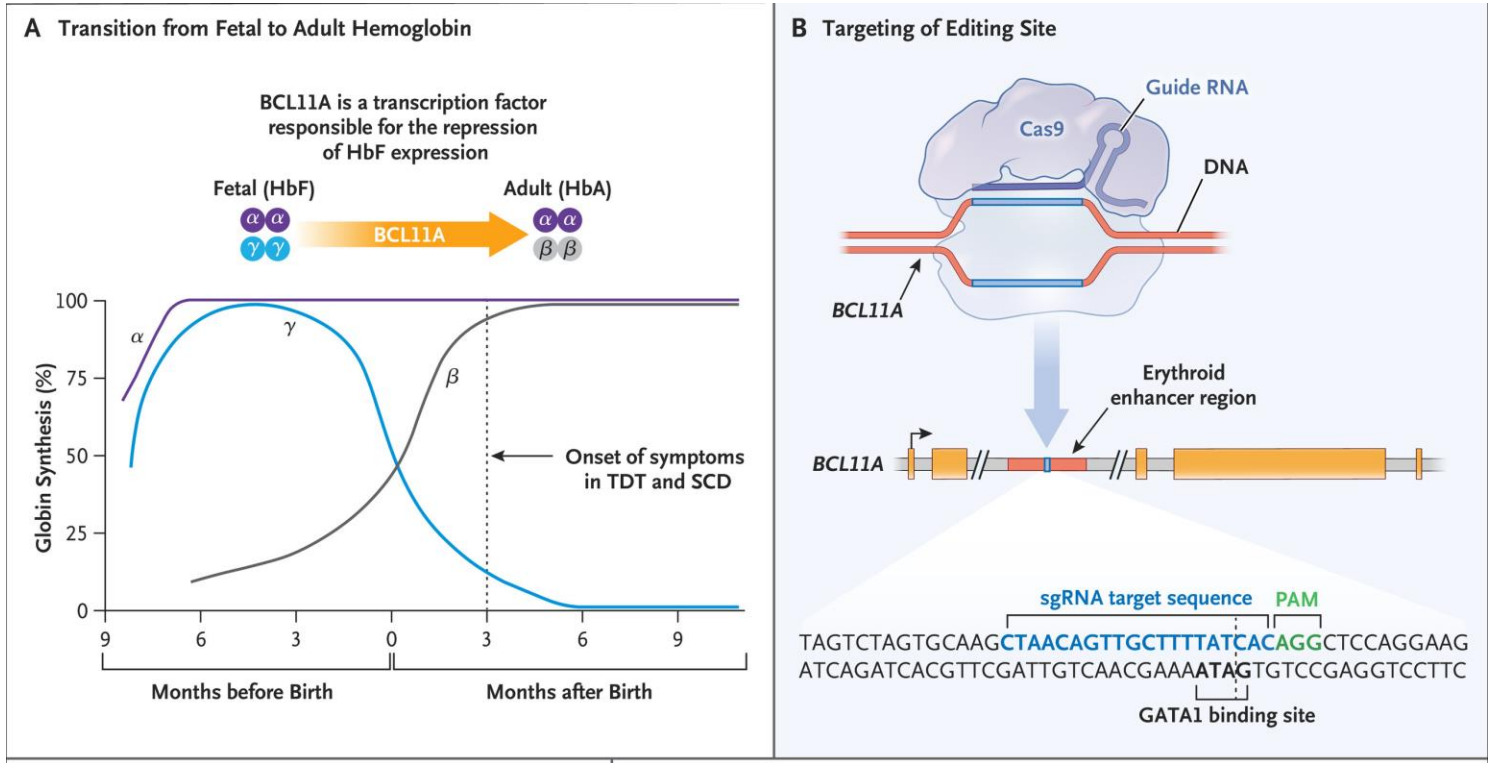
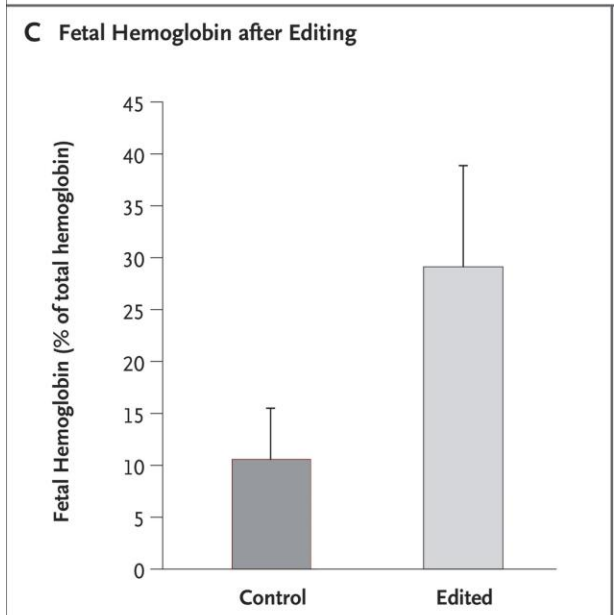
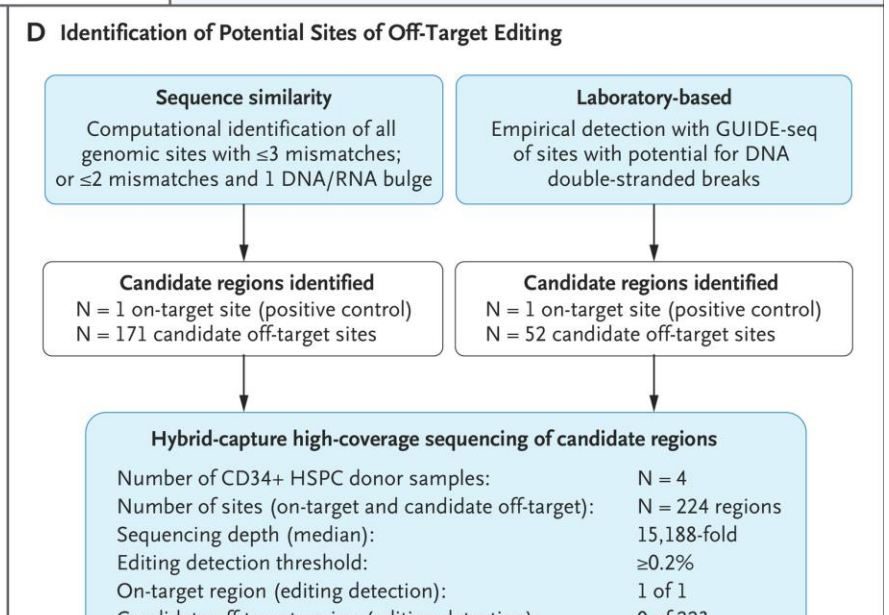
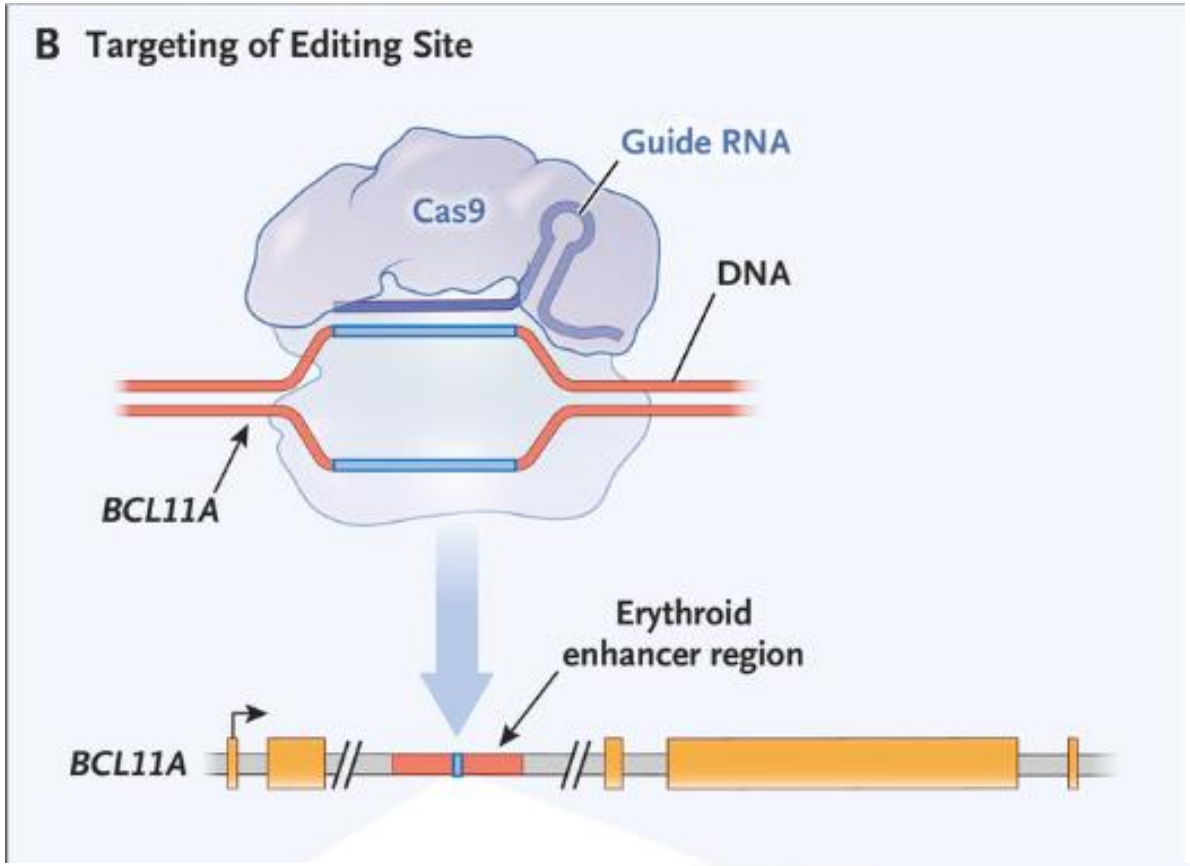
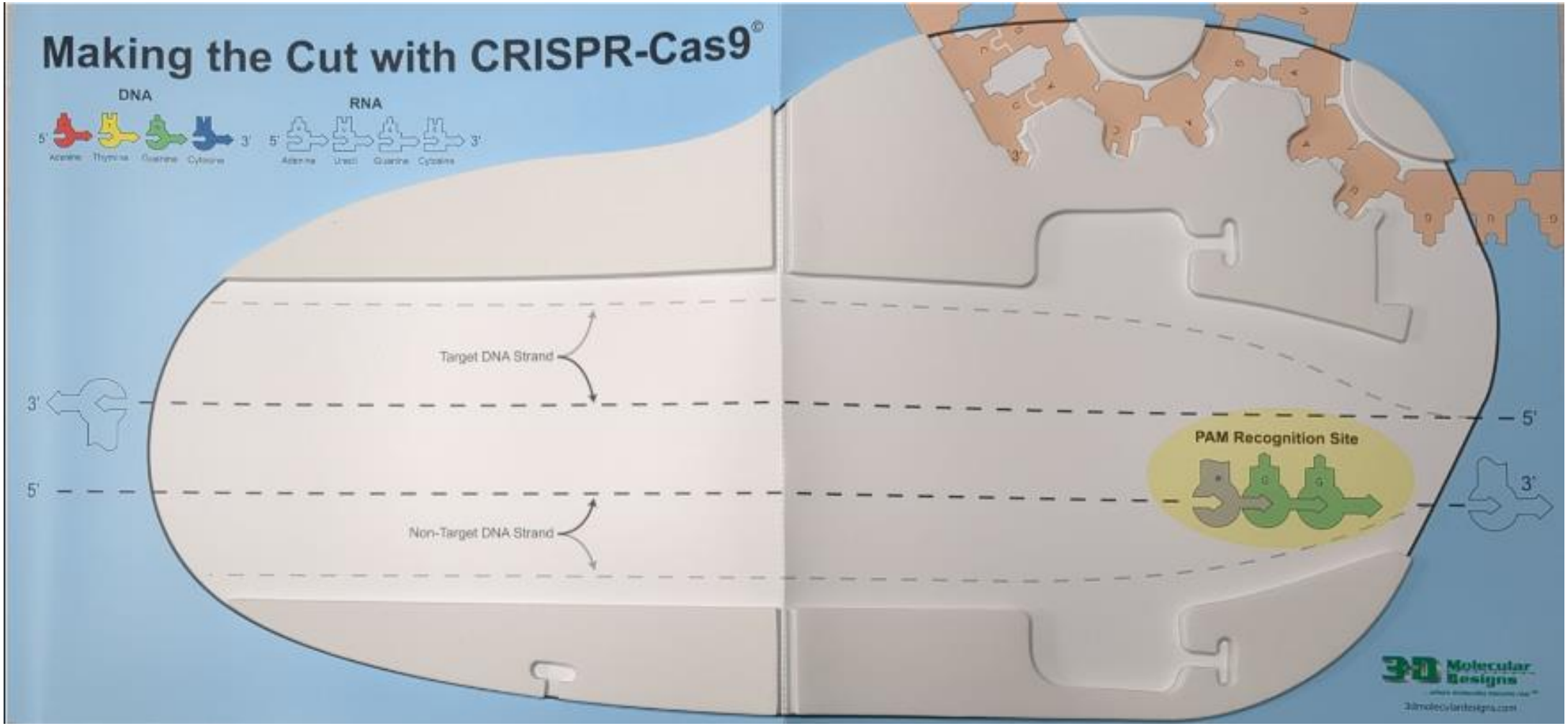

A Transition from Fetal to Adult Hemoglobin
BCL11A is a transcription factor responsible for the repression of HbF expression
Fetal (HbF)
Adult (HbA)
BCL11A
Globin Synthesis (%)
100
75
50
25
0
α
γ
β
Onset of symptoms in TDT and SCD
9
6
3
0
3
6
9
Months before Birth
Months after Birth
B Targeting of Editing Site
Guide RNA
Cas9
DNA
BCL11A
Erythroid enhancer region
BCL11A
sgRNA target sequence
PAM
TAGTCTAGTGCAAGCTAACAGTTGCTTTTATCACAGGCTCCAGGAAG
ATCAGATCACGTTCGATTGTCAACGAAAATATAGTGTCCGAGGTCCTTC
GATA1 binding site


C Fetal Hemoglobin after Editing
Fetal Hemoglobin (% of total hemoglobin)
45
40
35
30
25
20
15
10
5
0
Control
Edited

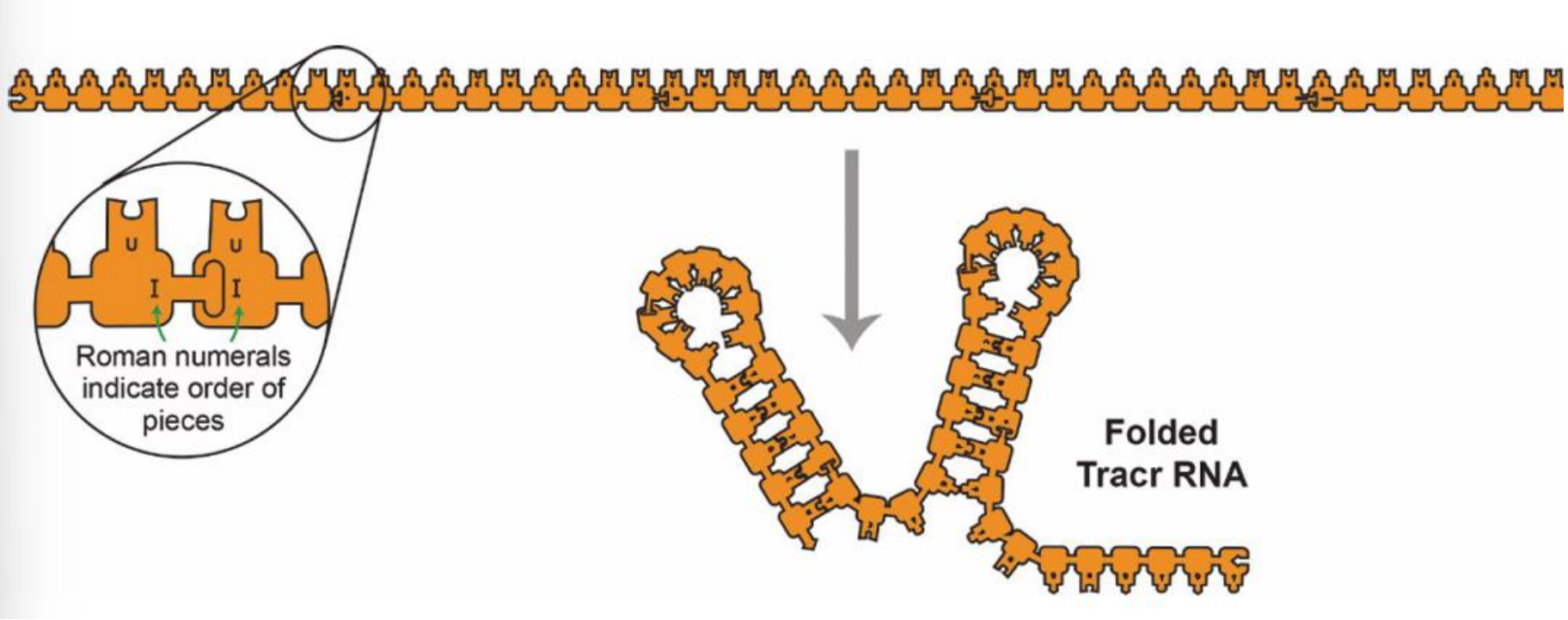

D Identification of Potential Sites of Off-Target Editing
Sequence similarity
Computational identification of all genomic sites with ≤3 mismatches; or ≤2 mismatches and 1 DNA/RNA bulge
Laboratory-based
Empirical detection with GUIDE-seq of sites with potential for DNA double-stranded breaks
Candidate regions identified
N = 1 on-target site (positive control)
N = 171 candidate off-target sites
Candidate regions identified
N = 1 on-target site (positive control)
N = 52 candidate off-target sites
Hybrid-capture high-coverage sequencing of candidate regions
Number of CD34+ HSPC donor samples: N = 4
Number of sites (on-target and candidate off-target): N = 224 regions
Sequencing depth (median): 15,188-fold
Editing detection threshold: ≥0.2%
On-target region (editing detection): 1 of 1
Candidate off-target region (editing detection): 0 of 223

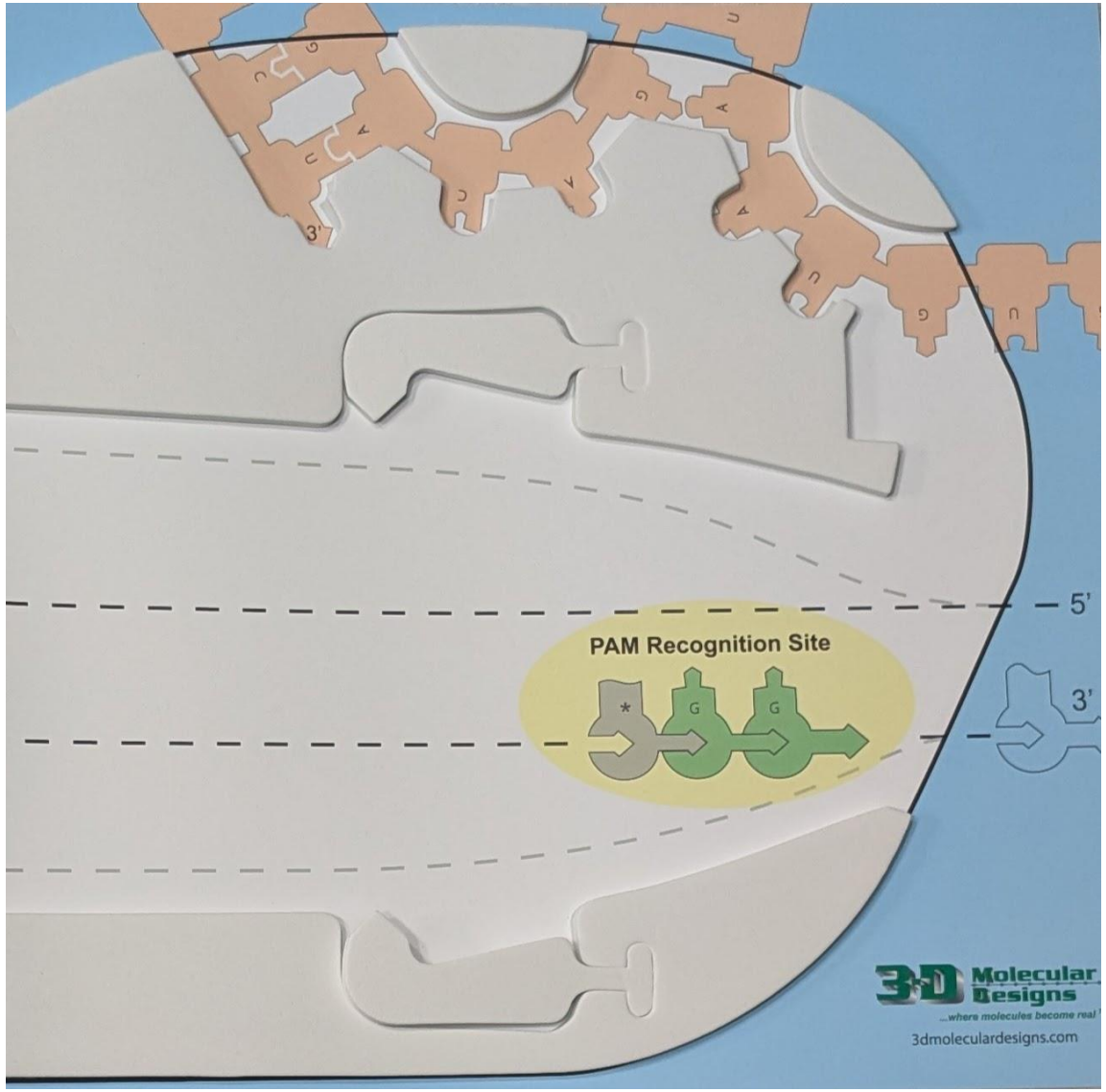

B Targeting of Editing Site
Guide RNA
Cas9
DNA
BCL11A
Erythroid
enhancer region
BCL11A


sgRNA target sequence
PAM
TAGTCTAGTGCAAGCTAACAGTTGCTTTTATCACAGGCTCCAGGAAG
ATCAGATCACGTTCGATTGTCAACGAAAATAGTGTCCGAGGTCCTTC
GATA1 binding site


Making the Cut with CRISPR-Cas9®
DNA
RNA
5'
3'
Target DNA Strand
Non-Target DNA Strand
PAM Recognition Site
3'
5'
5'
3'

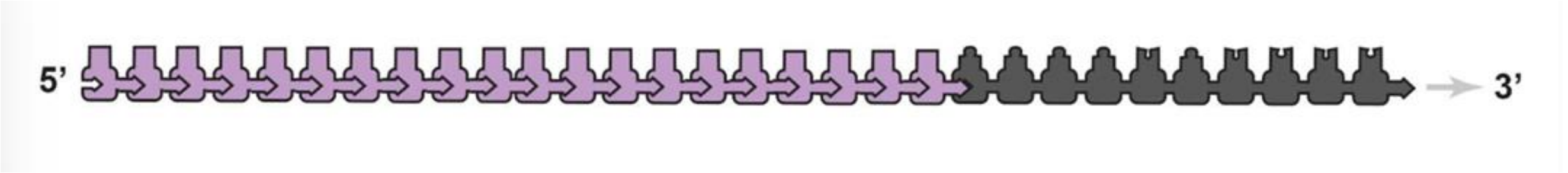
Construct a model of tracrRNA.




3'
5'
PAM Recognition Site
*
G
G
3'
3D Molecular Designs
...where molecules become real
3dmoleculardesigns.com

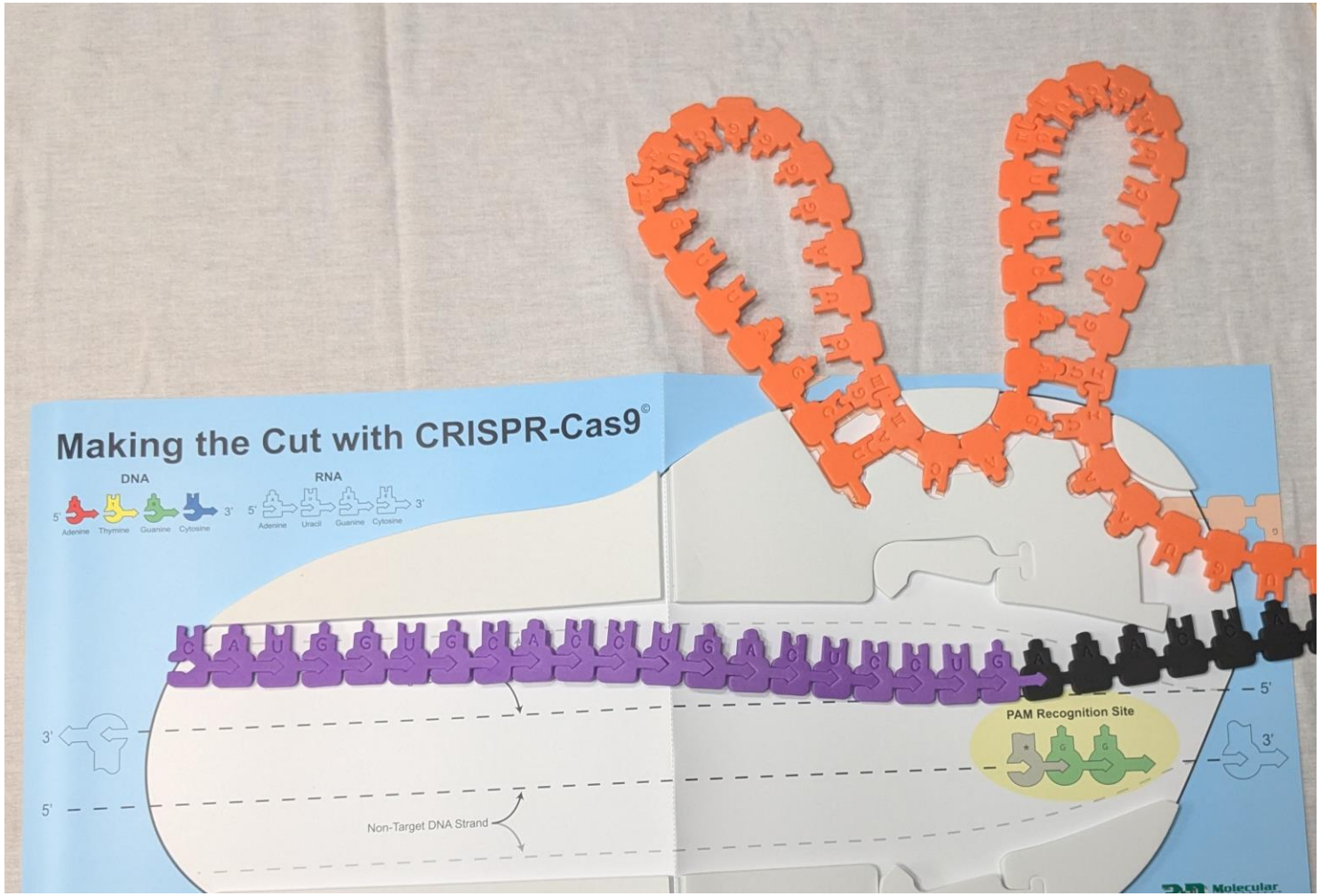
What do you notice about this sequence?

Construct your CRISPR RNA (crRNA).



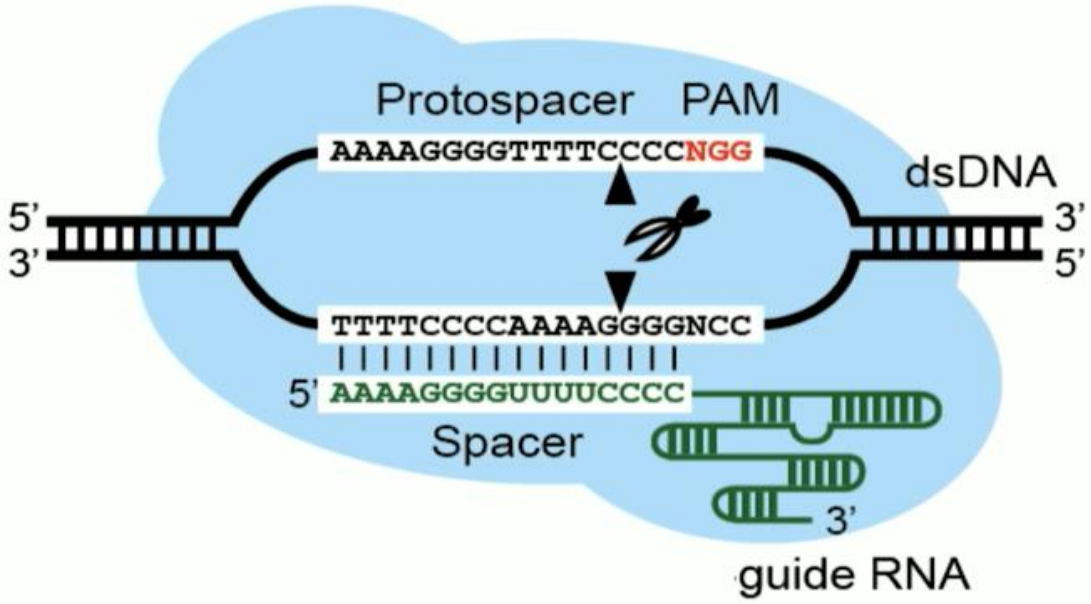
What do you notice? What questions do you have?

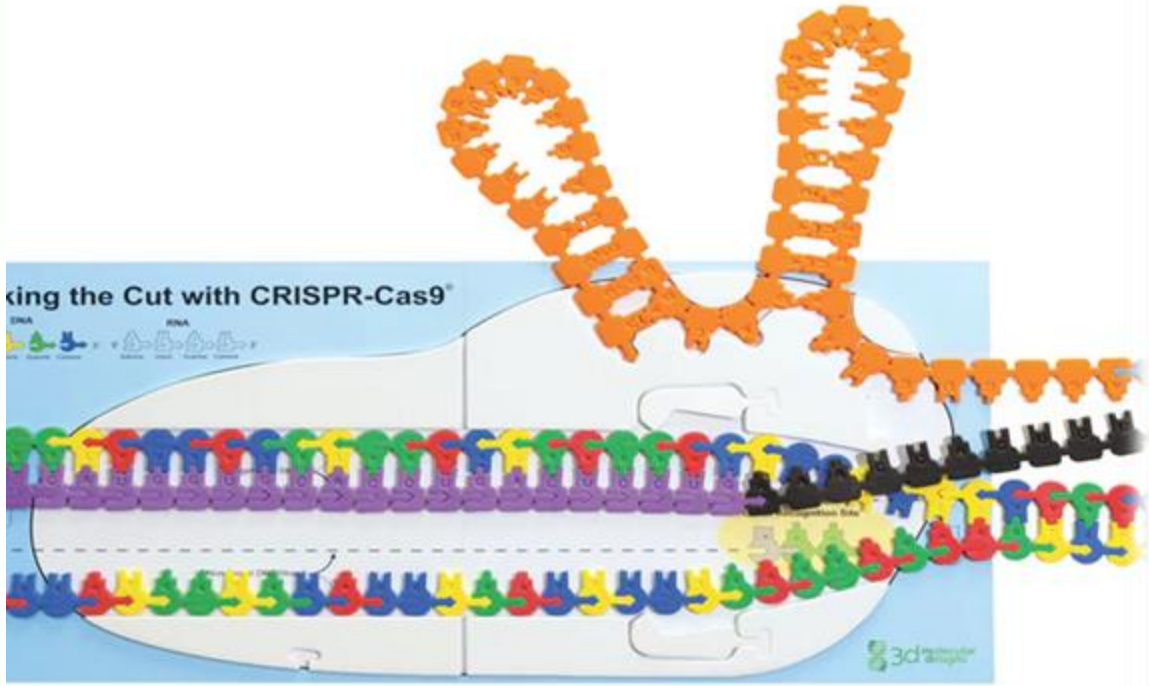
Construct the dual guide RNA. Add it to the Cas9 protein model.

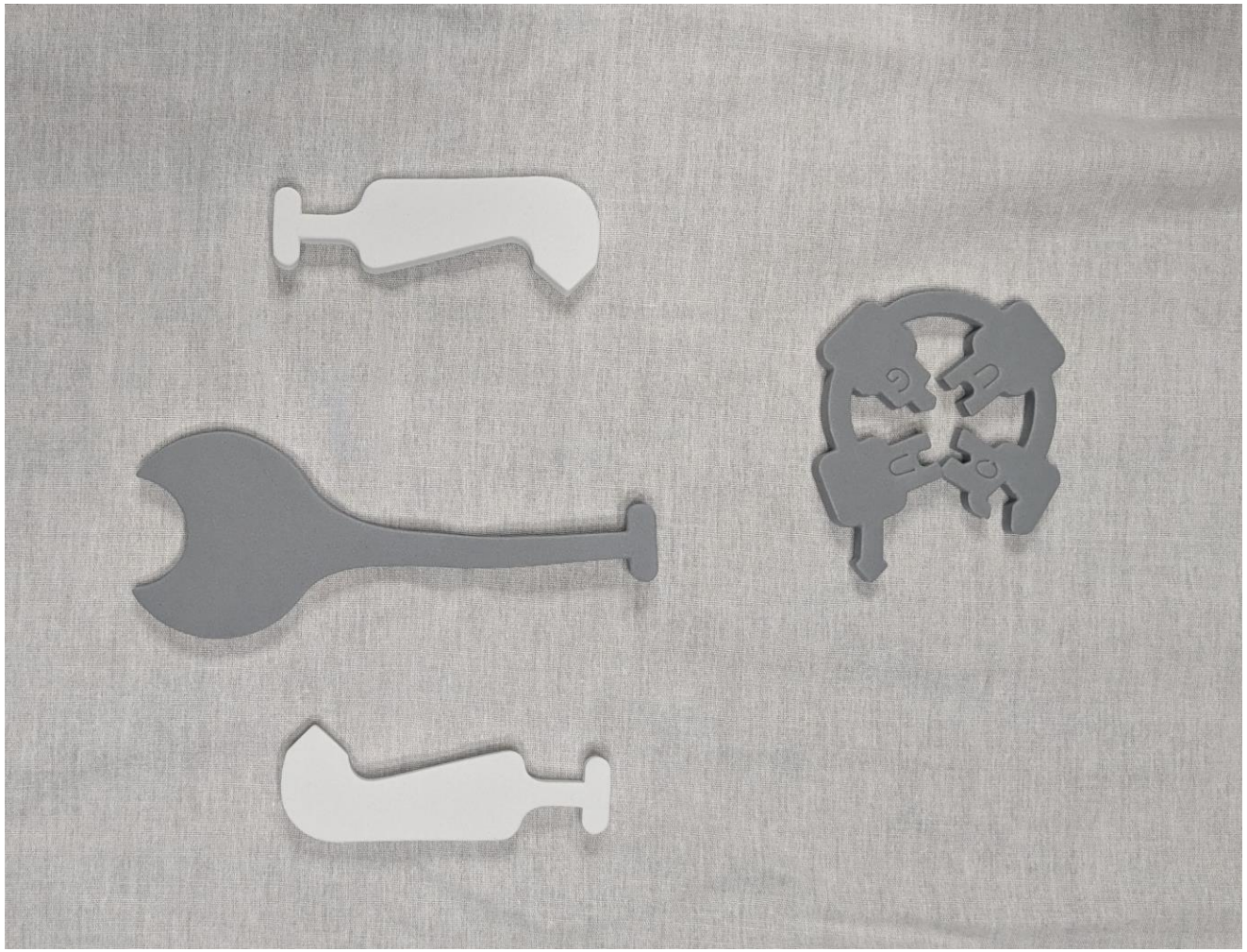


What do you notice about this sequence?

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]*

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

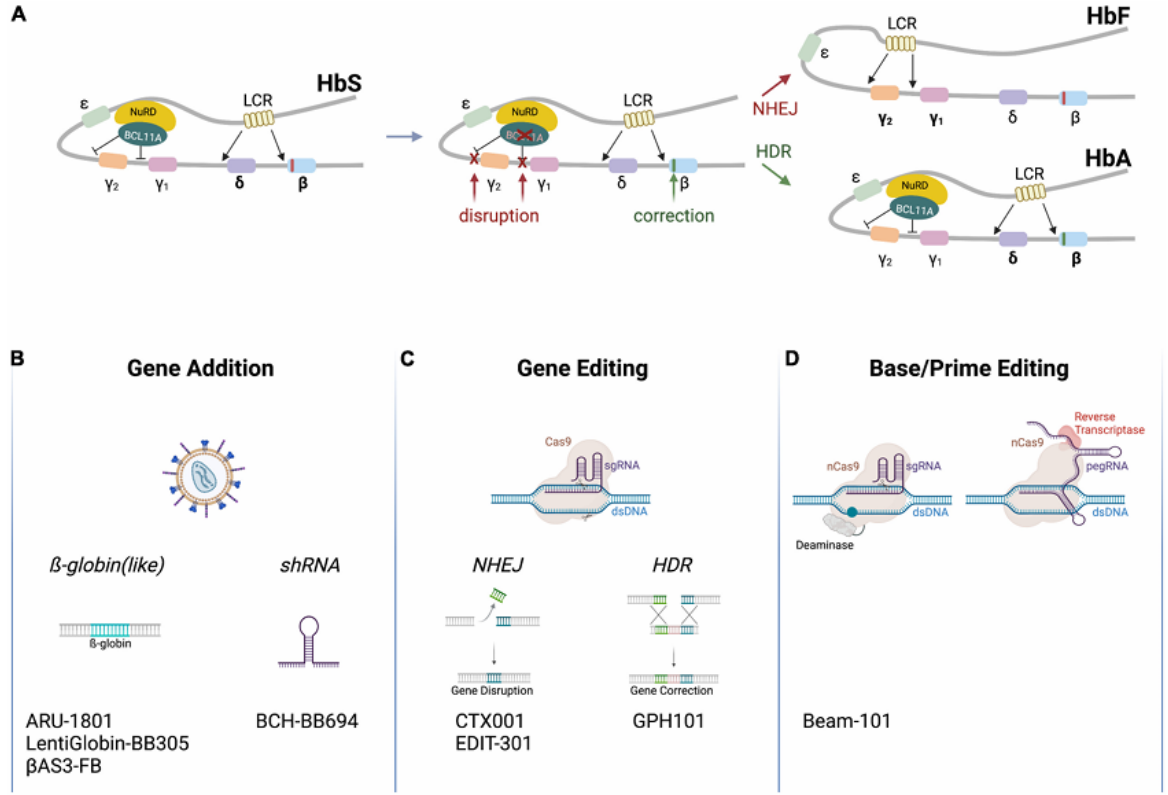
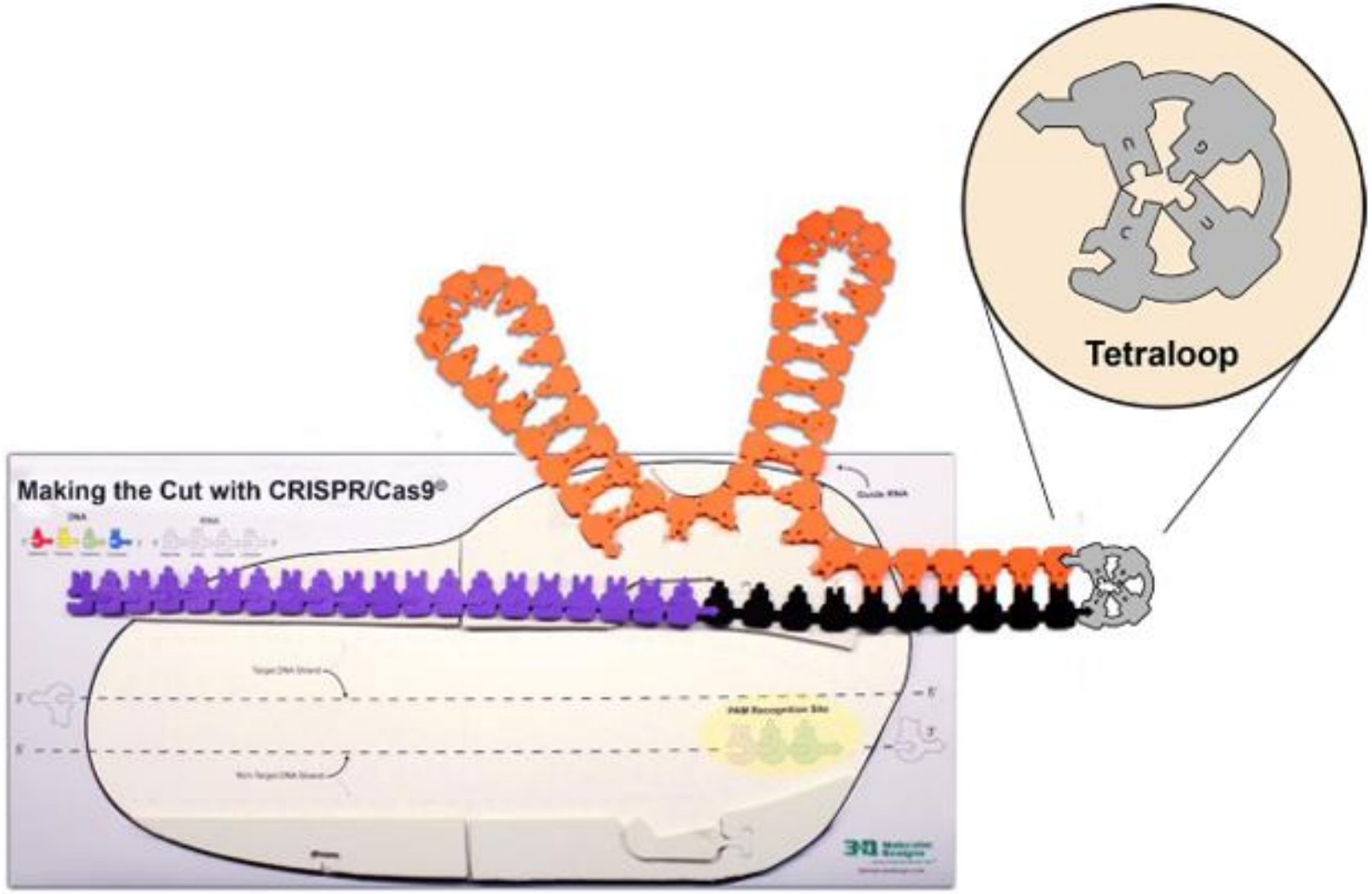


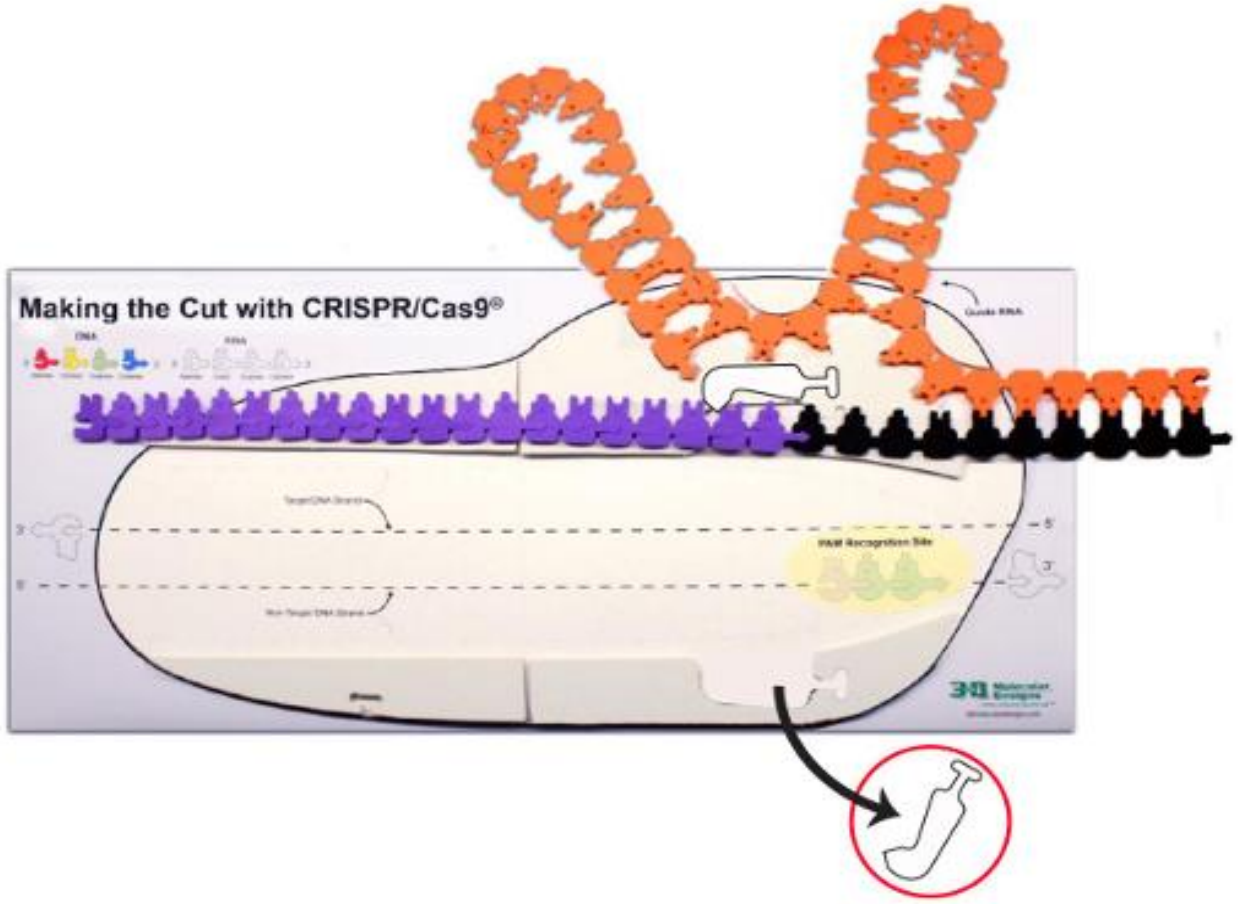
FIGURE 1

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).

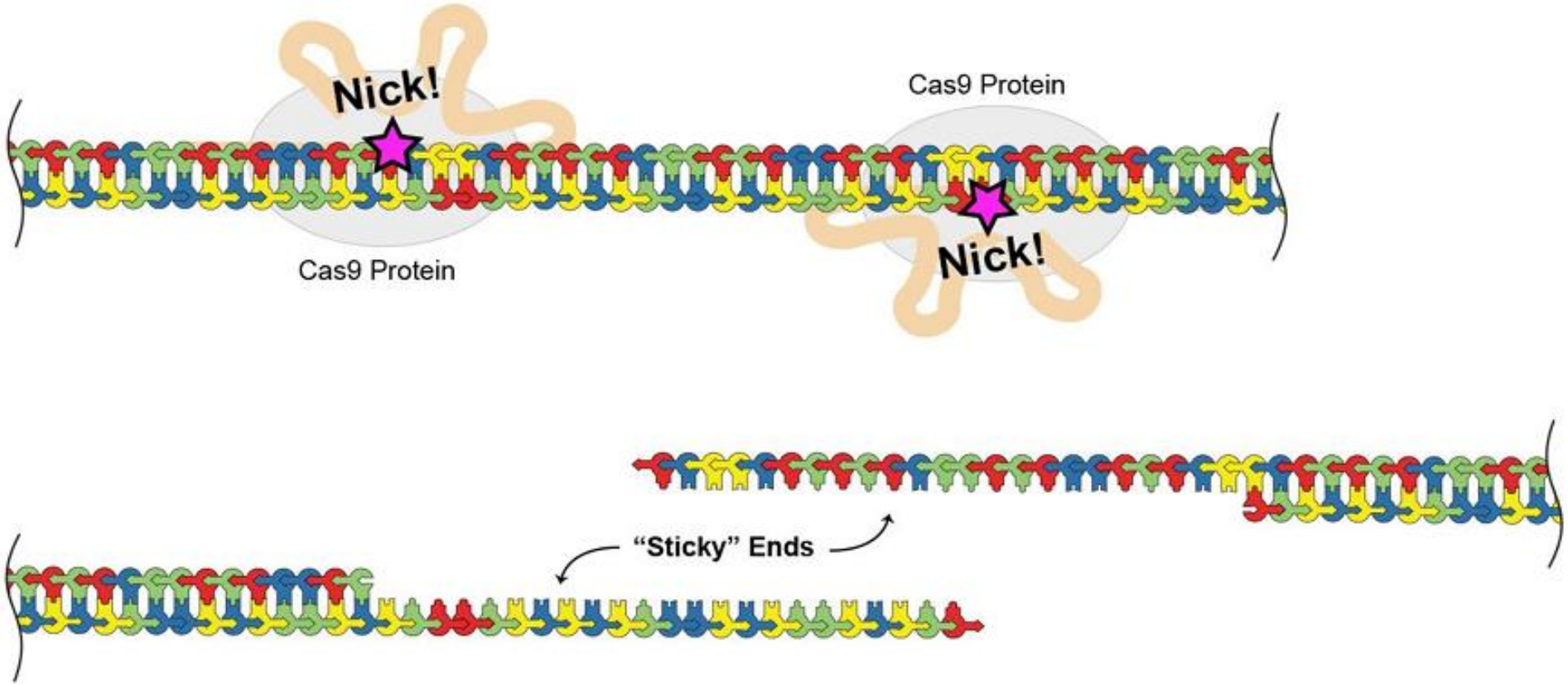
Knocking Out a Gene



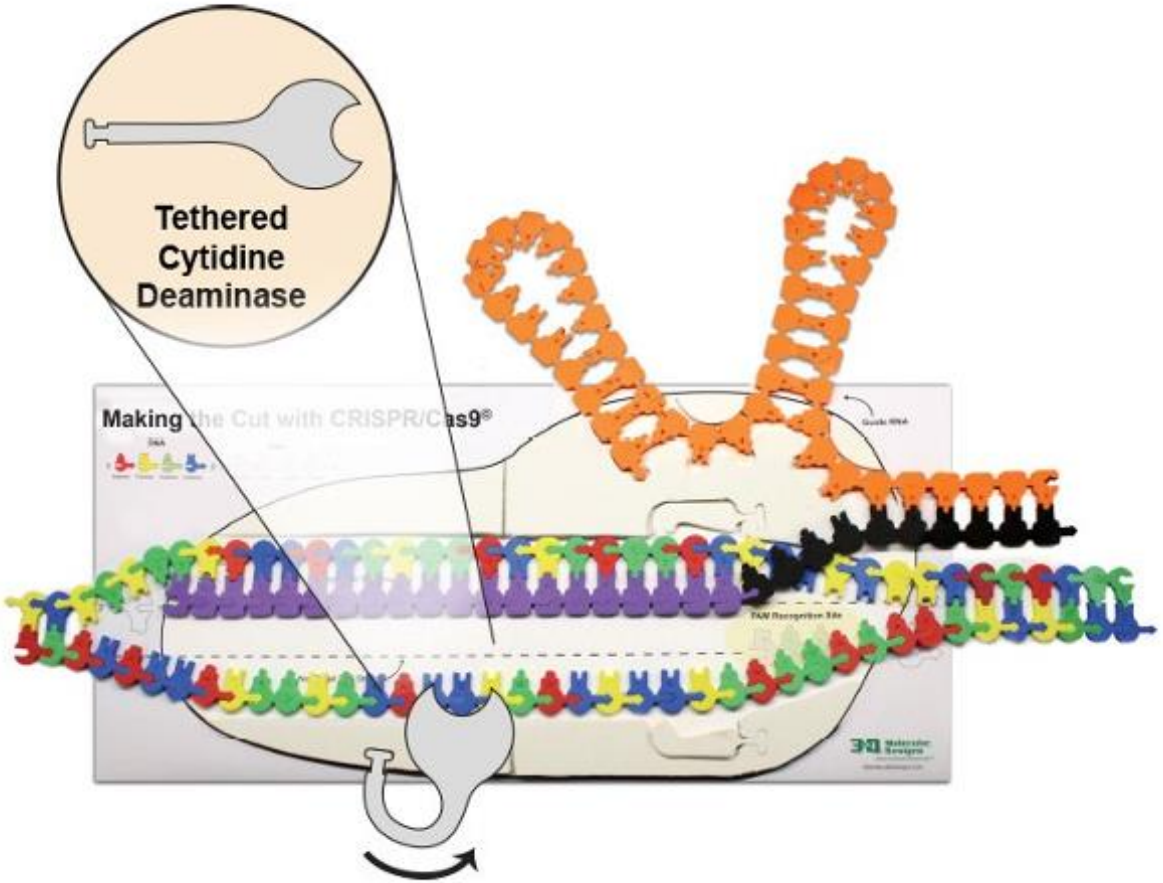
Nicking One Strand



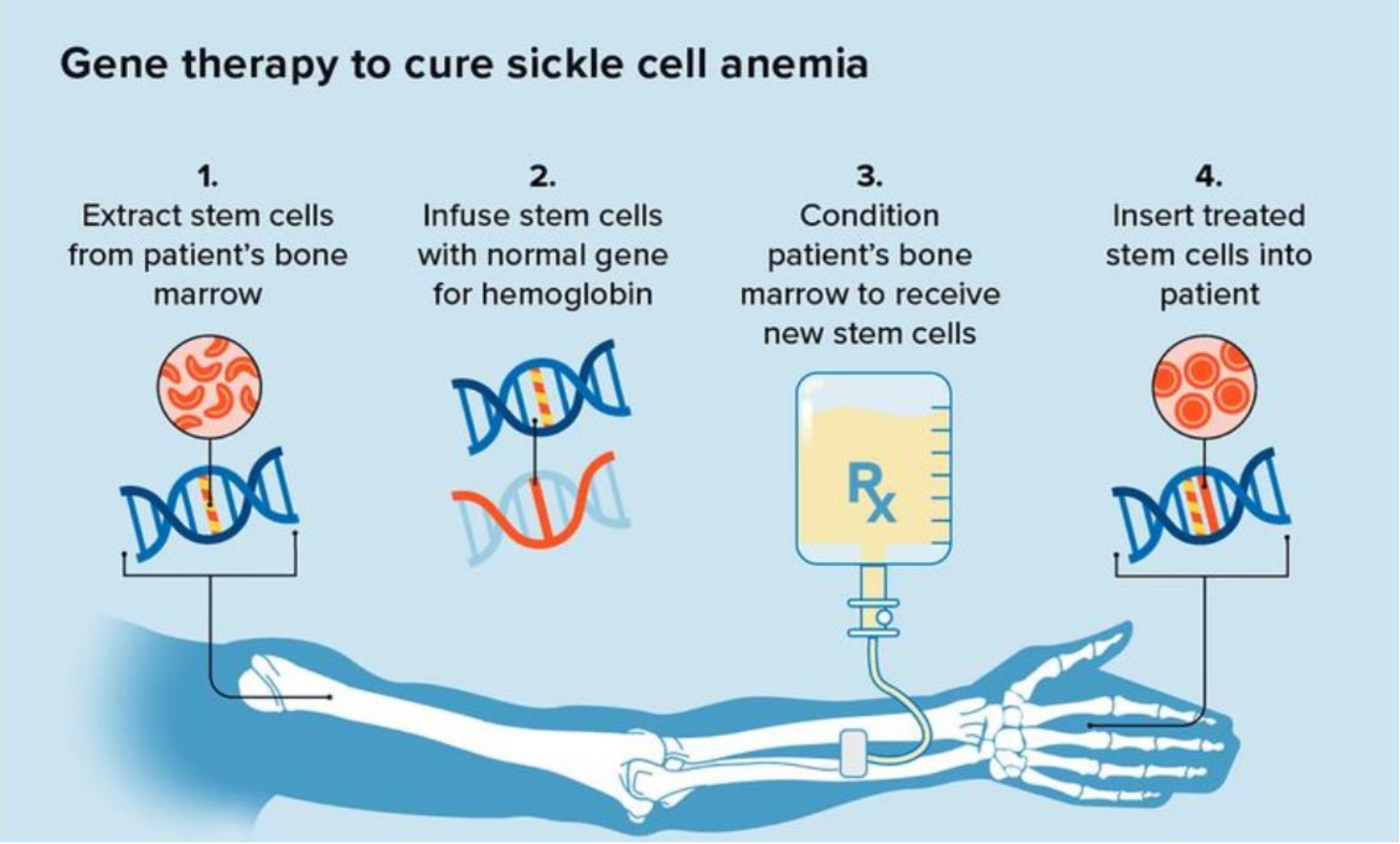
Nicking One Strand

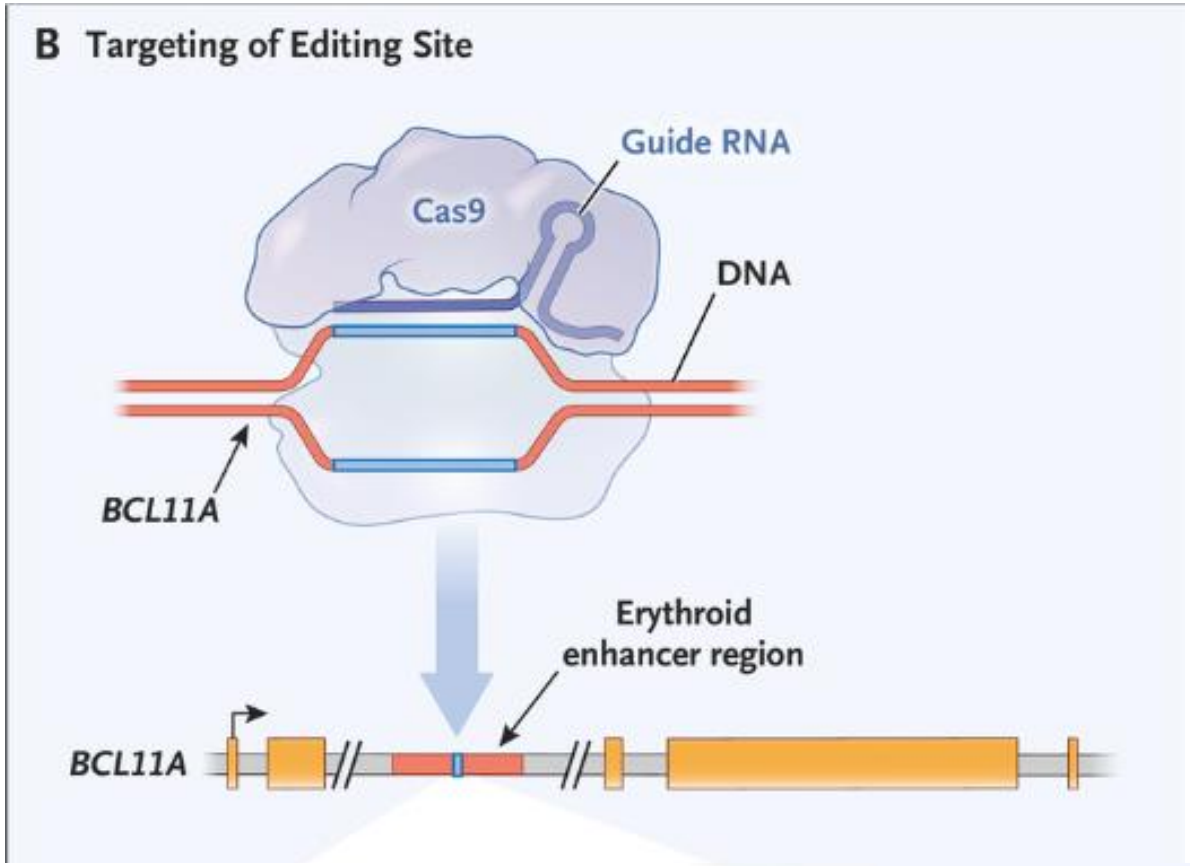


Base Pair Editing



What about Casgevy?

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enhancer region
BCL11A


sgRNA target sequence
PAM
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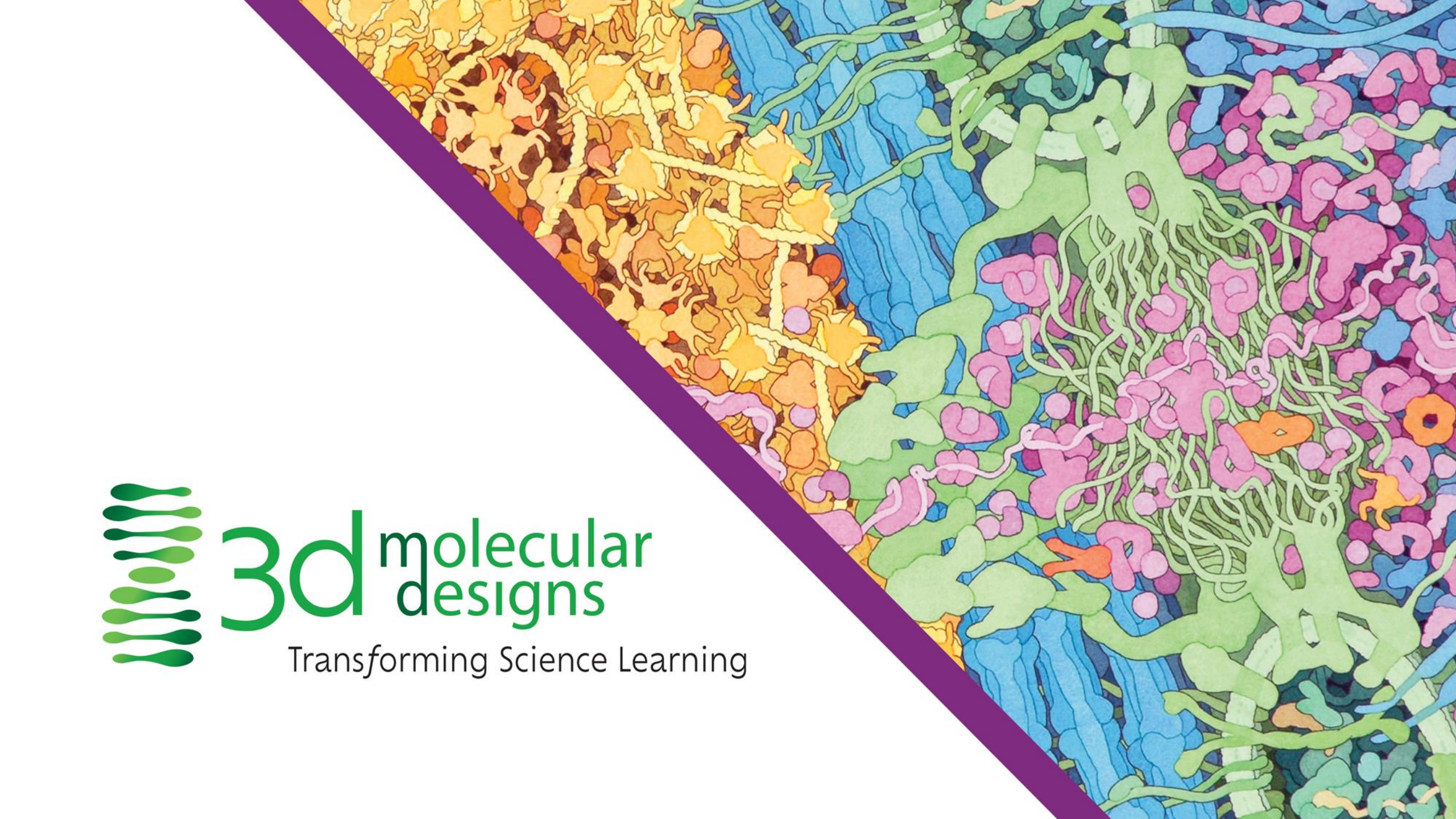
ATCAGATCACGTTCGATTGTCAACGAAAATAGTGTCCGAGGTCCTTC
GATA1 binding site

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 β-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



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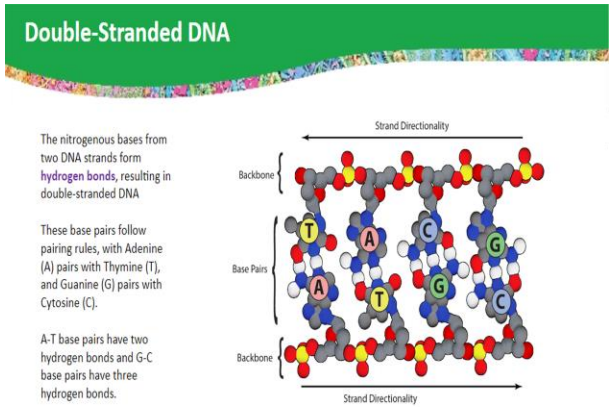

Look at our new
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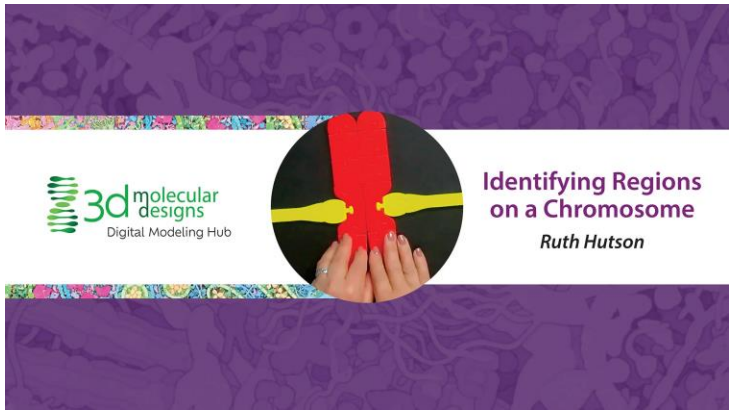
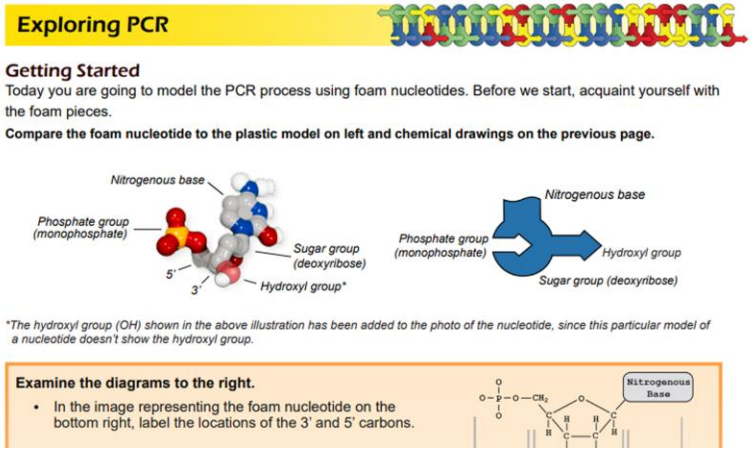
and CRISPR Making the Cut Kit.





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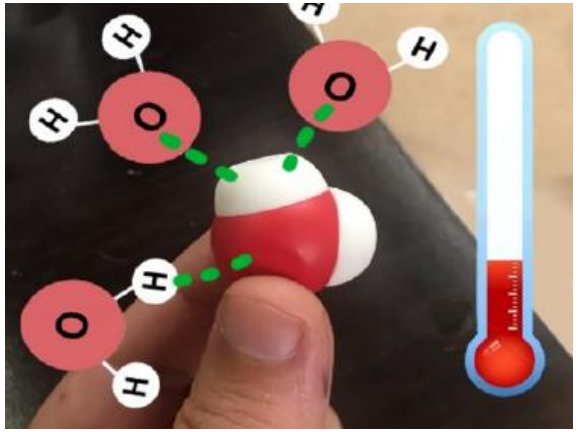

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