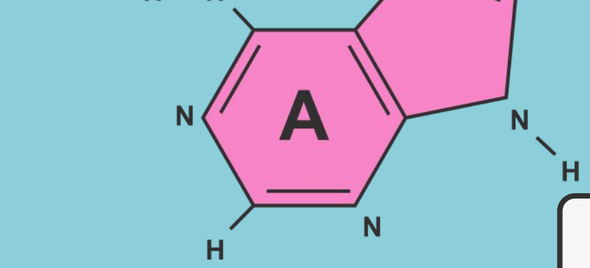
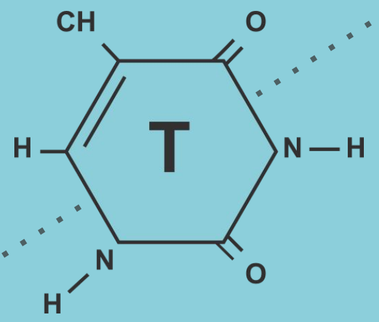
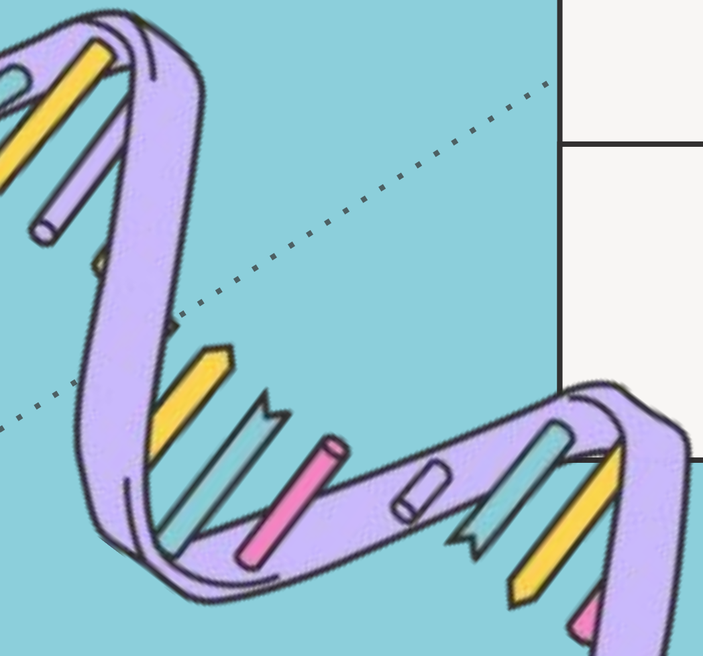
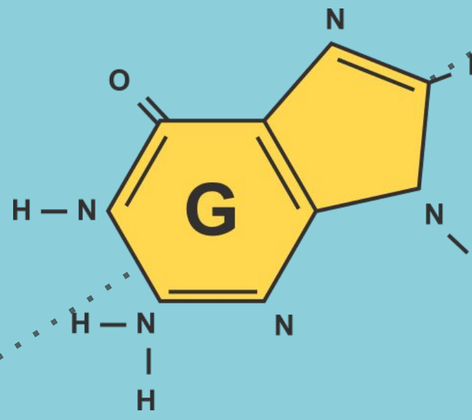




Correcting the Code:
CRISPR-Cas and PCR in Sickle Cell Anemia

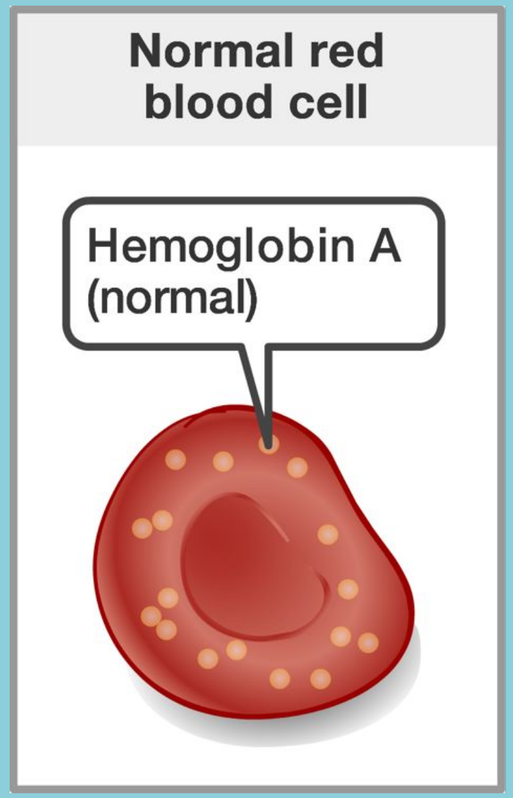
By: Kaitlyn Coley and Darcy Jones

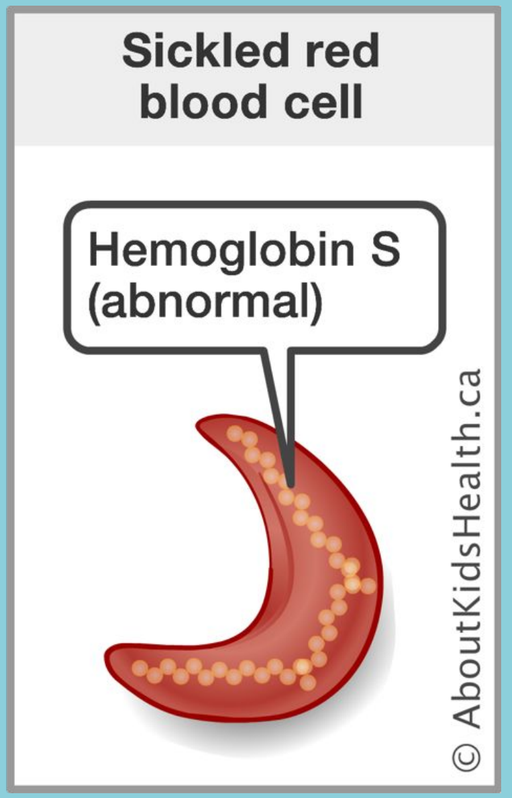




What is Sickle Cell Anemia?

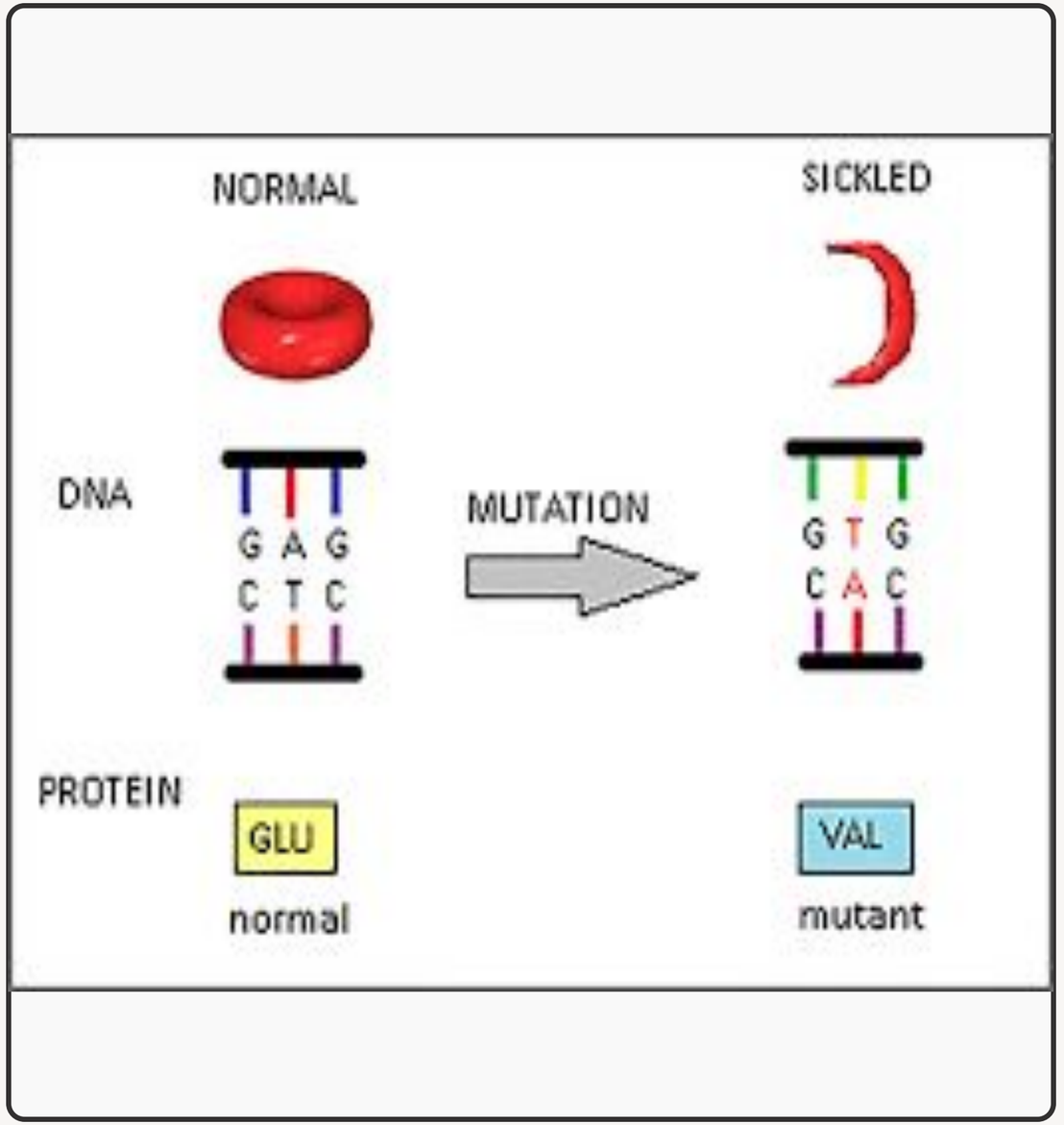
- Also known as Sickle Cell Disease (SCD)
- An **autosomal recessive** hemoglobinopathy
  - Produces abnormal hemoglobin called **hemoglobin S** (HbS)
- Low oxygen conditions = sickle-shaped RBC's

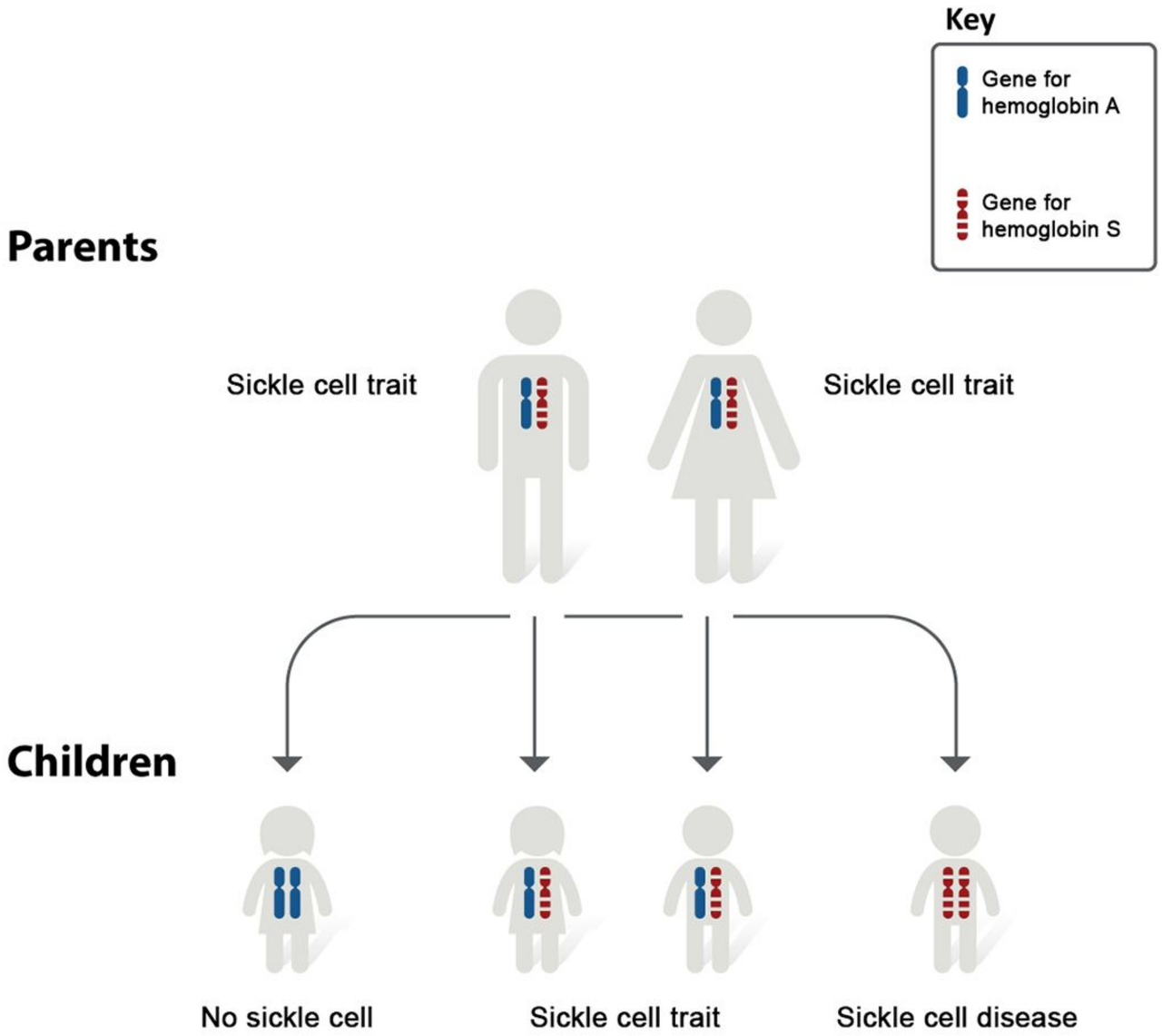




The Cause of the Crescent

- **Point Mutation** in **β-globin gene** (HBB)
  - Chromosome 11
  - **Single nucleotide substitution** at position 6 of HBB
    - **GAG —›GTG**
  - HbS produced
    - Low oxygen: HbS polymerizes





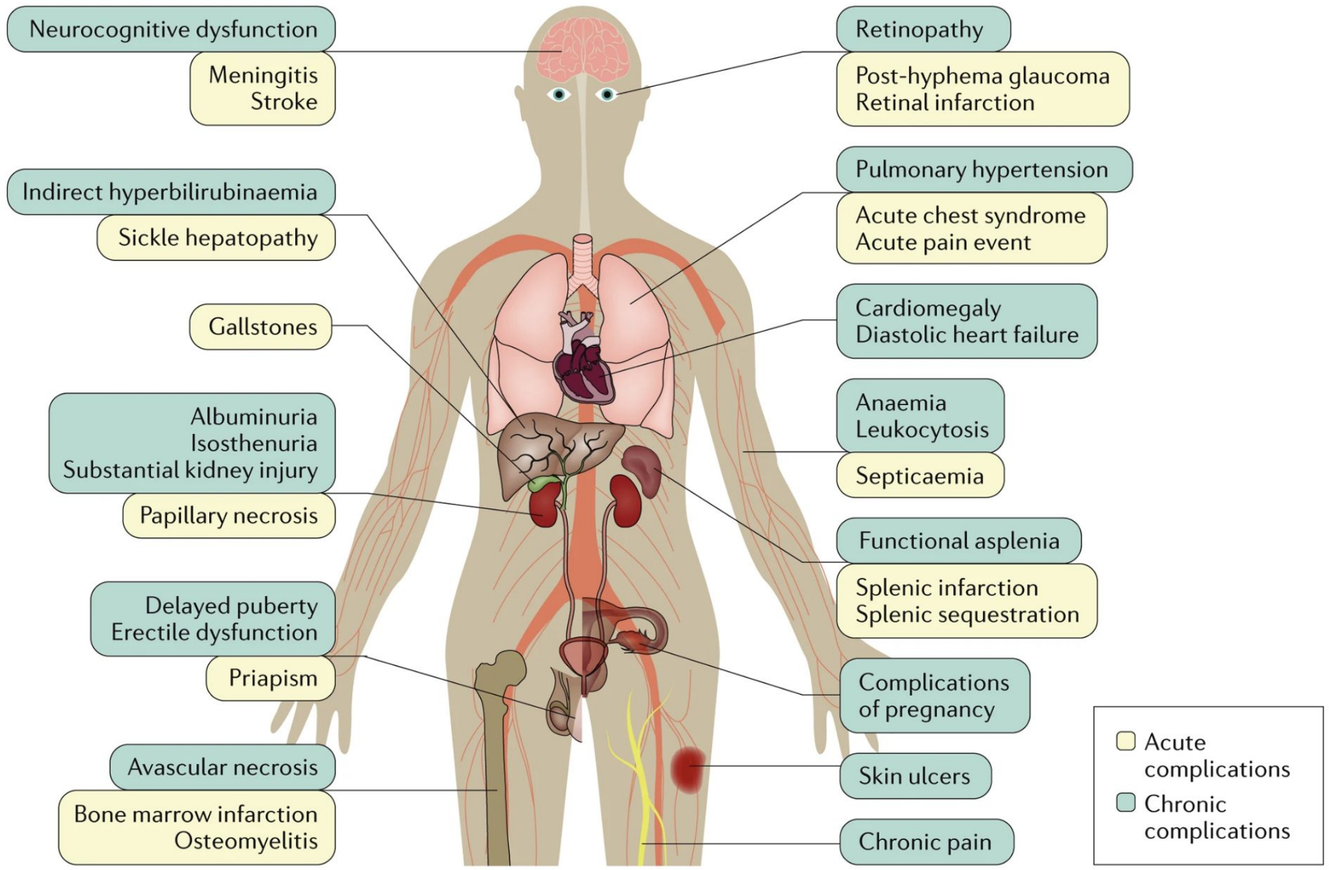
- **HbAA:** Normal
  - No disease, no carrier
- **HbAS:** Sickle cell trait
  - No disease, carrier
  - Malaria resistance
    - **Heterozygote advantage**
- **HbSS:** Sickle cell anemia
  - Disease, carrier
  - Symptomatic

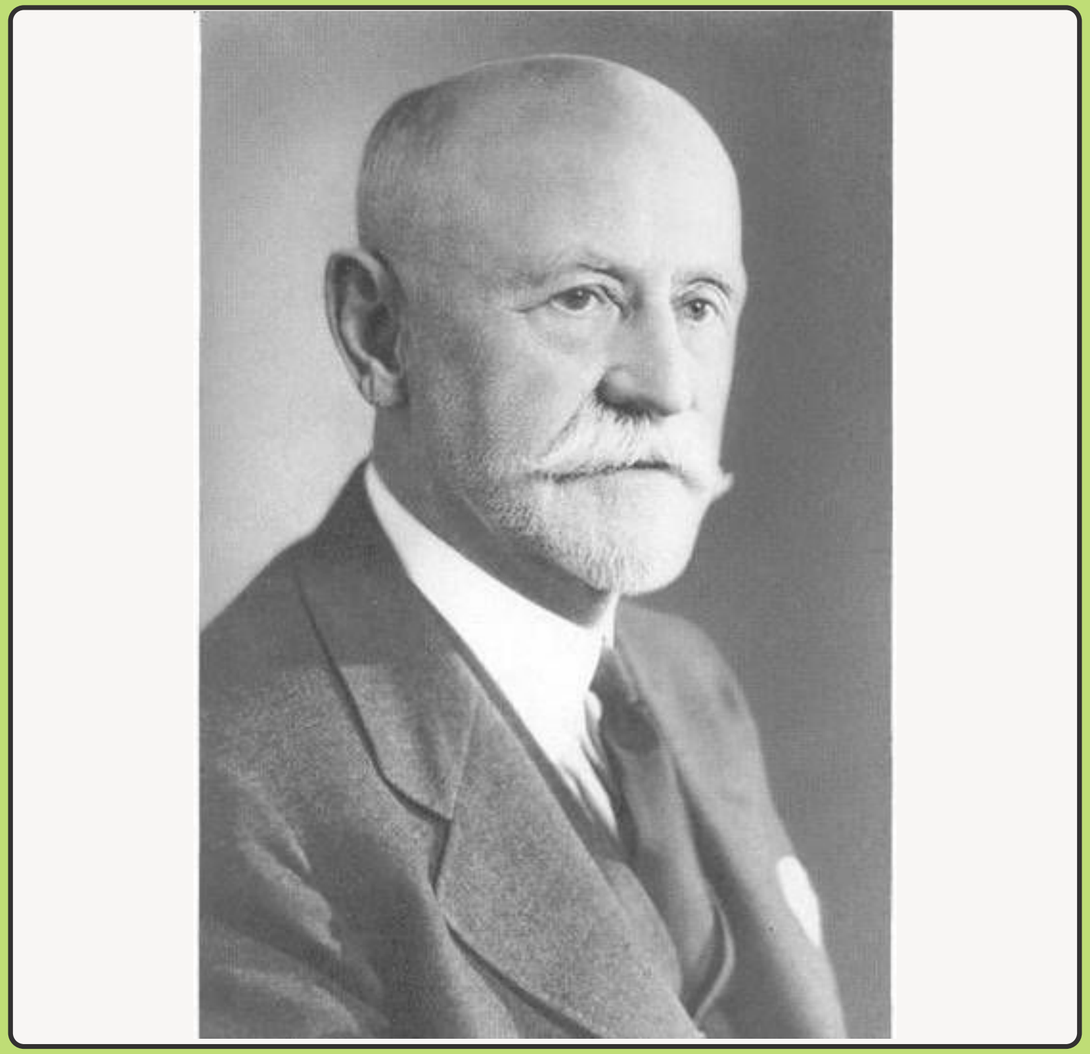
Complications

- **Hematologic**
  - Anemia, splenic dysfunction, gallstones
- **Vascular**
  - Stroke, severe pain, pulmonary hypertension, priapism
- **Organ Damage**
- **Growth and Development**
  - Delayed growth and puberty
- **Infections**

Figure 5: Sickle cell disease clinical complications.

From: Sickle cell disease

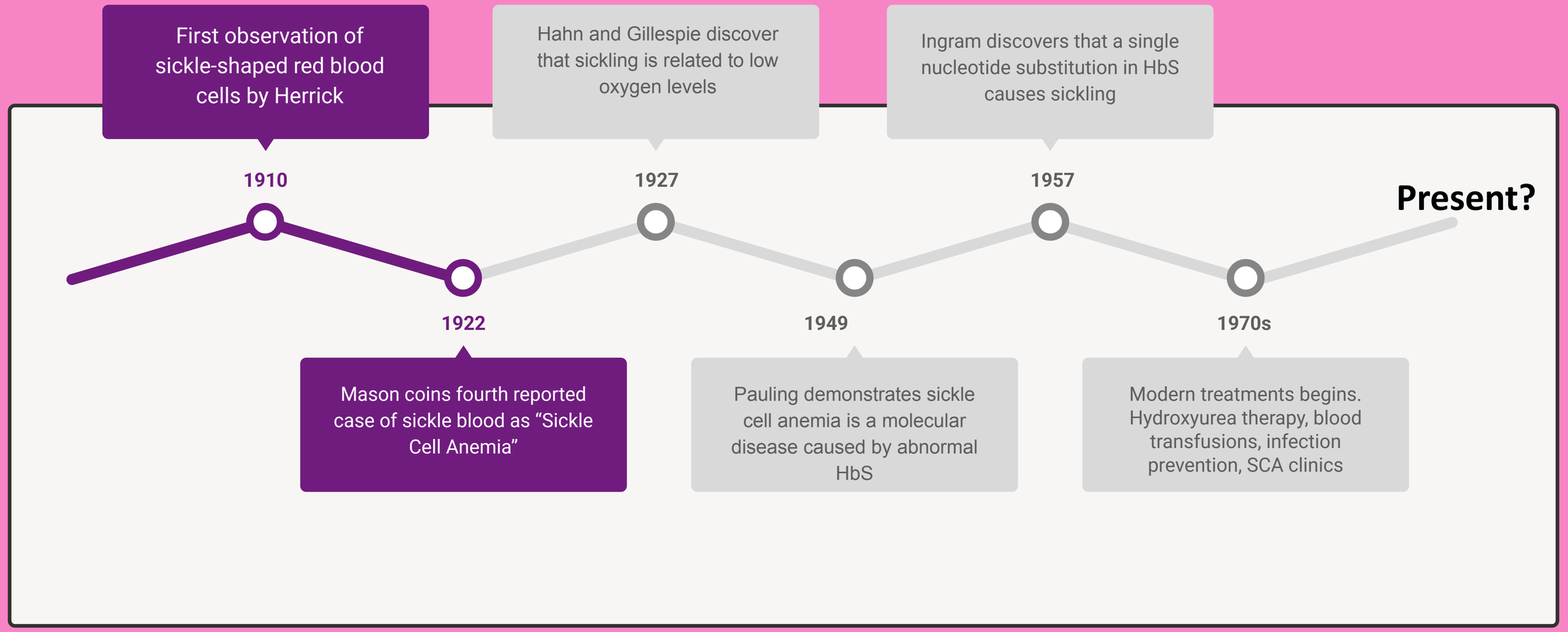




Seeing Sickle

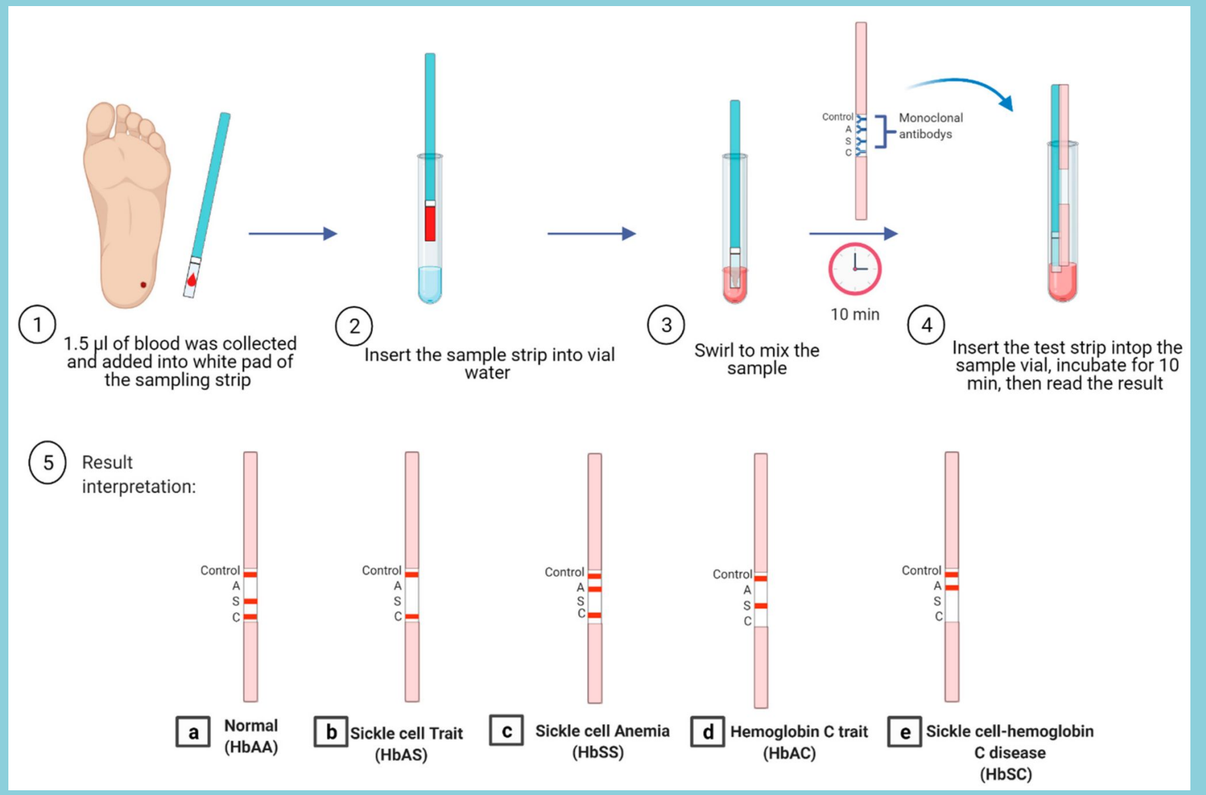
- **Dr. James B. Herrick**
  - 1861-1954
- American Physician + Professor of Medicine
  - Harvard Medical School
  - **1910**: Published **first** detailed case report of Sickle Cell Anemia

The History of Sickle Cell



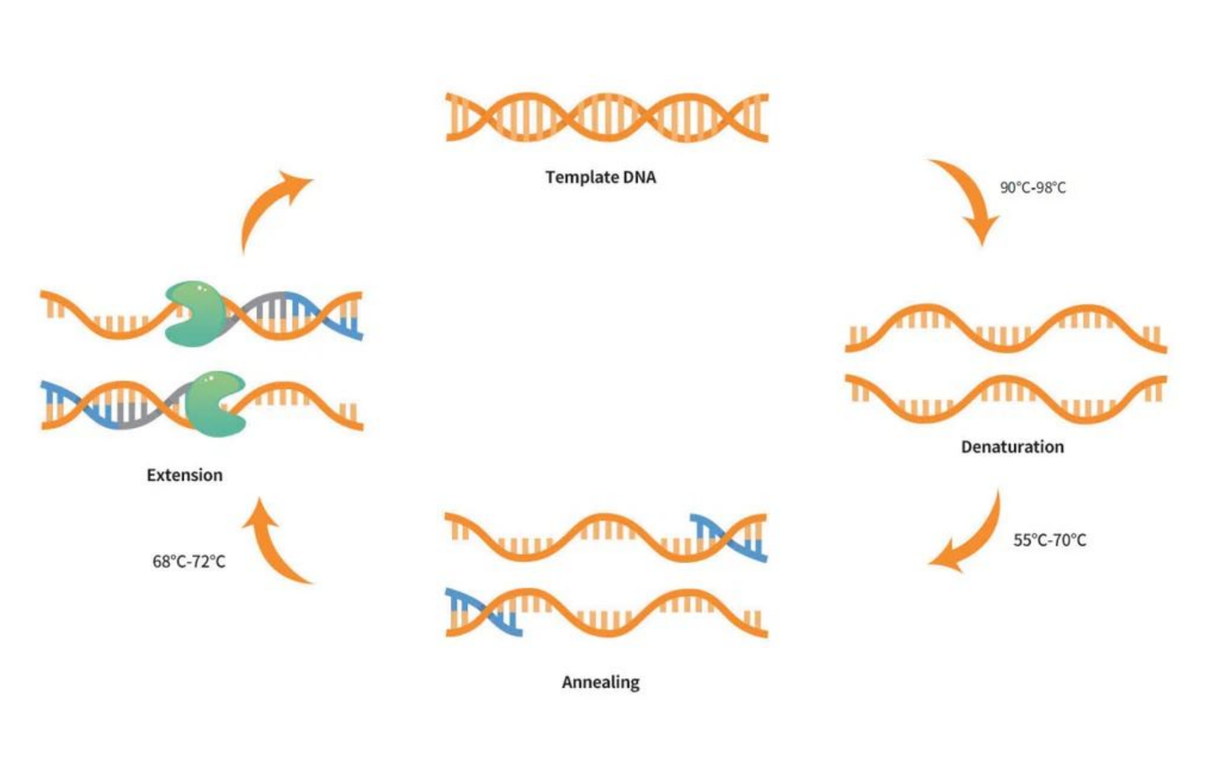
Modern Medicine

- Stem cell + bone marrow transplants
- New drugs + trials
  - L-glutamine, voxelotor, crizanlizumab
- Global screening + newborn programs
- **PCR + CRISPR-based therapies**



Rapid Recall: PCR

PCR: Laboratory **nucleic acid** amplification technique



Step 1: Denaturation

DNA heated to 95°C

Hydrogen Bonds disrupted between base pairs

Step 2: Annealing

Denatured DNA cooled to 55-72°C

Primers bind complementary strands

Step 3: Elongation

DNA heated to 75-80°C

Taq adds nucleotides

New DNA strand synthesized

PCR in Sickle Cell Anemia

Amplification Refractory Mutation System PCR (ARMS-PCR)

- **Allele-specific primers**
- Targets exact mutation (GAG→GTG)
- One primer matches HbA (normal allele)
- One matches HbS (mutant)
- Amplification = **allele present**
- HbAA = only normal band, HbSS = only mutant band, HbAS = both

Restriction Fragment Length Polymorphism (PCR-RFLP)

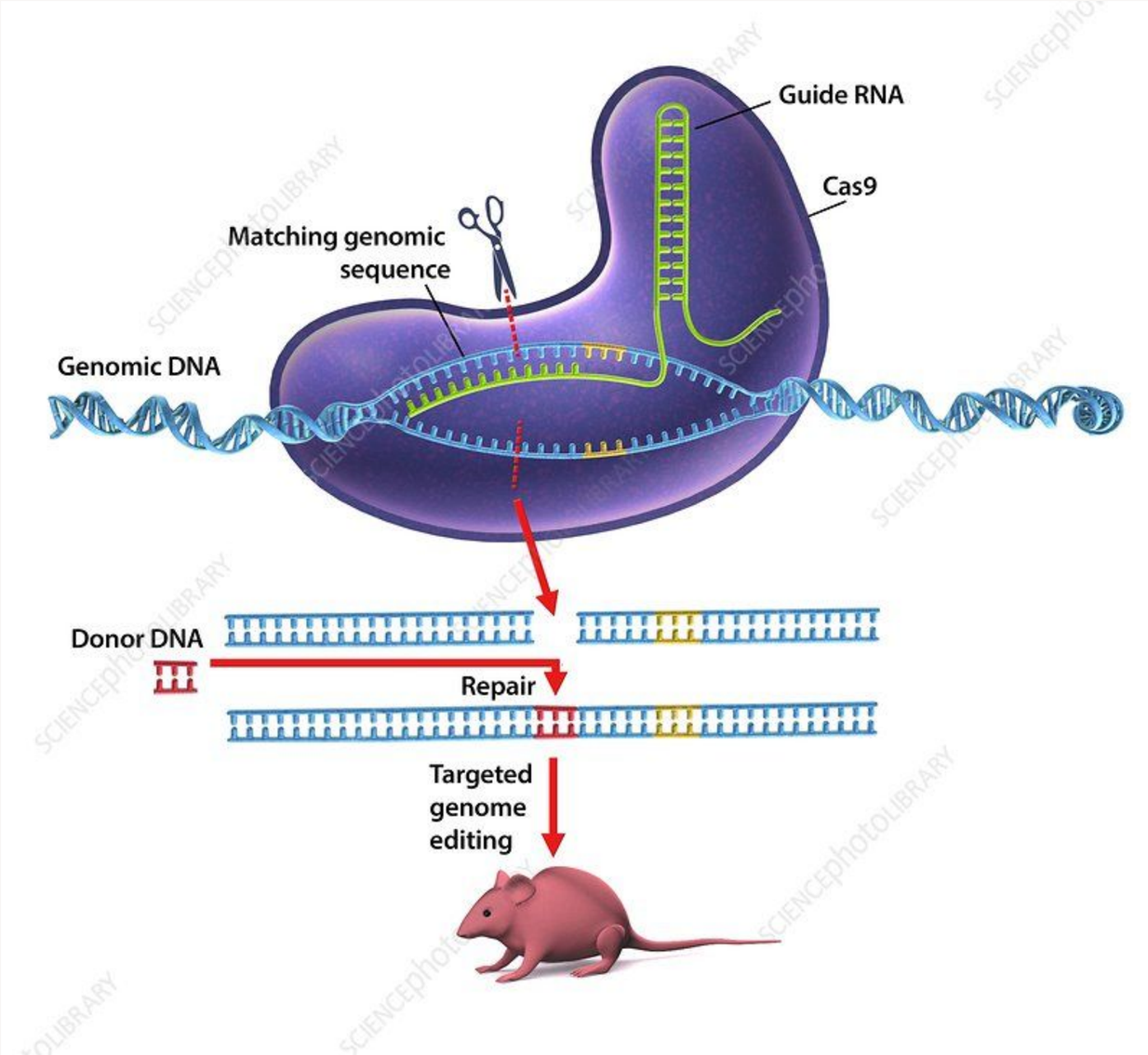
- Amplification of region around **codon 6 of HBB gene**
- Uses restriction enzymes
  - **MstII**
- Normal sequence (HbA): restriction site present → **DNA cut**
- Mutant sequence (HbS): mutation abolishes restriction site → **DNA remains uncut**
- Gel electrophoresis: HbAA = 2 smaller fragments (cut), HbSS 1 large fragment (uncut), HbAS = 3 bands (cut + uncut)

Transition: From **PCR** to **Gene Editing**

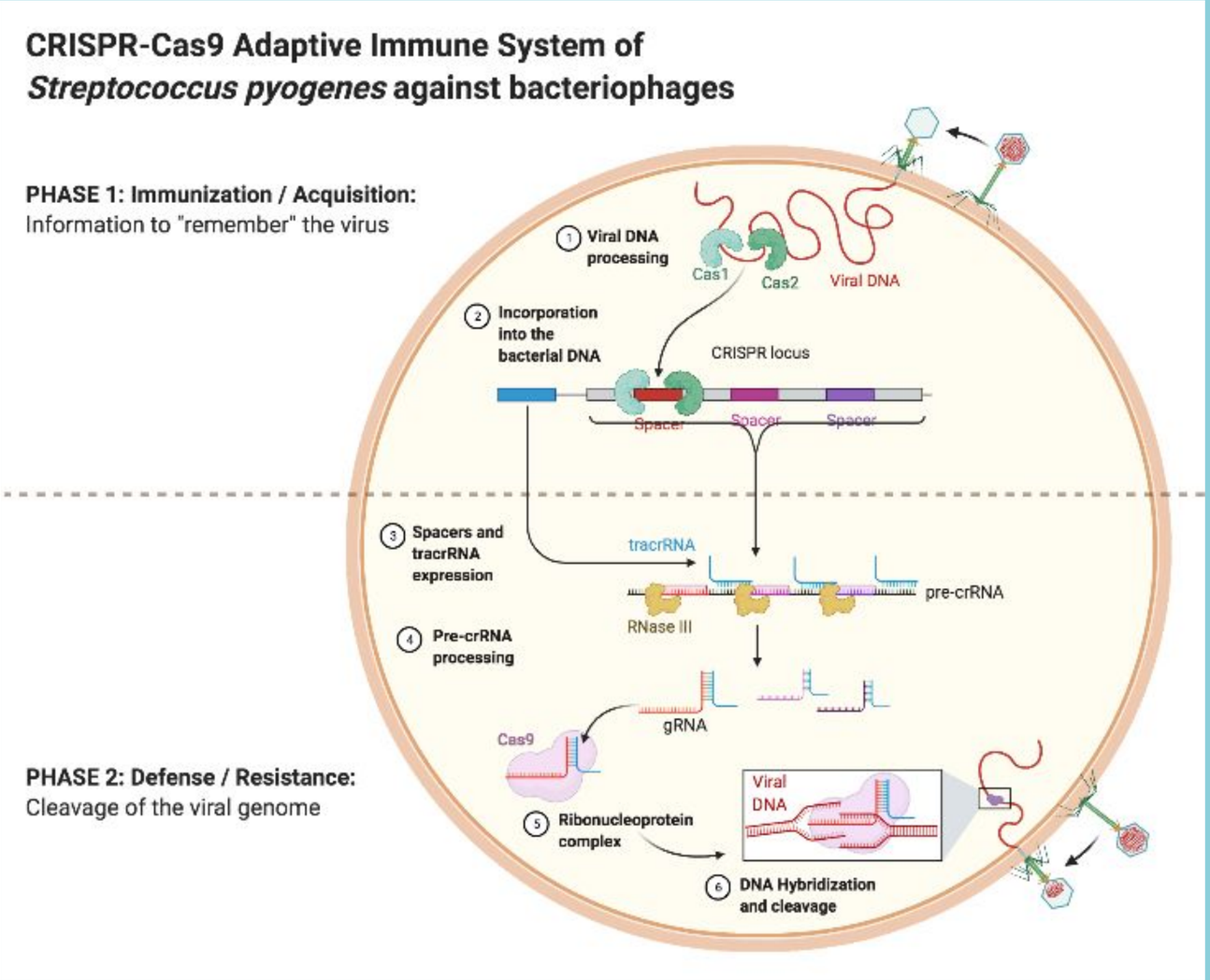
- **PCR** = amplification (diagnosis/research)
- **CRISPR** = **direct genome modification**
- Key question: *Can we fix the sickle mutation at its source?*

What is **CRISPR-Cas**?

- **Clustered Regularly Interspaced Short Palindromic Repeats**
- Derived from **bacterial adaptive immunity**
- **Cas9** = endonuclease that cuts DNA
- Guide RNA (**gRNA**) directs specificity



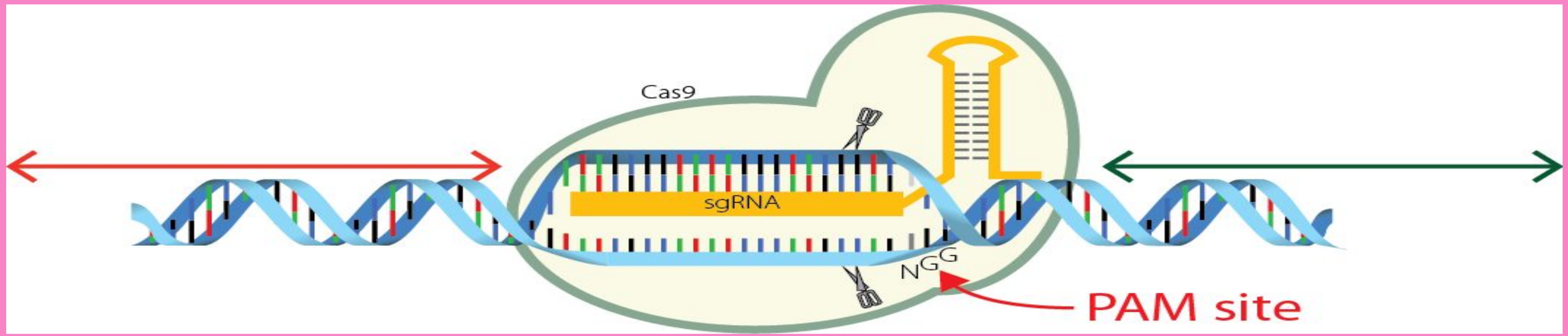
Natural Function (**Bacterial Immunity**)



- Bacteria store **viral DNA fragments** in CRISPR loci
- Upon reinfection → **Cas** proteins target matching sequences
- Acts as a **sequence-specific defense system**

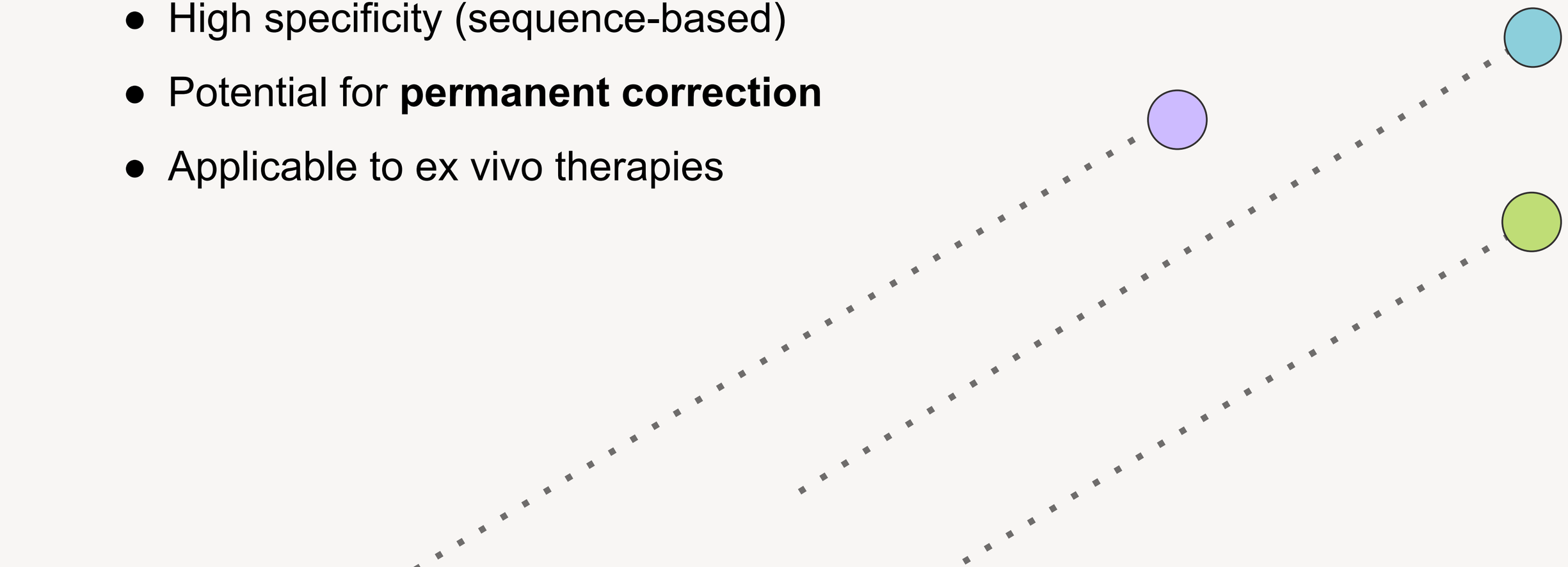
CRISPR-Cas9 Mechanism

- **gRNA** binds target DNA (complementary sequence)
- Cas9 introduces **double-strand break**
- Repair pathways:
  - **NHEJ** → insertions/deletions
  - **HDR** → precise editing



Why **CRISPR** Works for **Genetic Disease**

- Targets **single-gene mutations**
- High specificity (sequence-based)
- Potential for **permanent correction**
- Applicable to ex vivo therapies

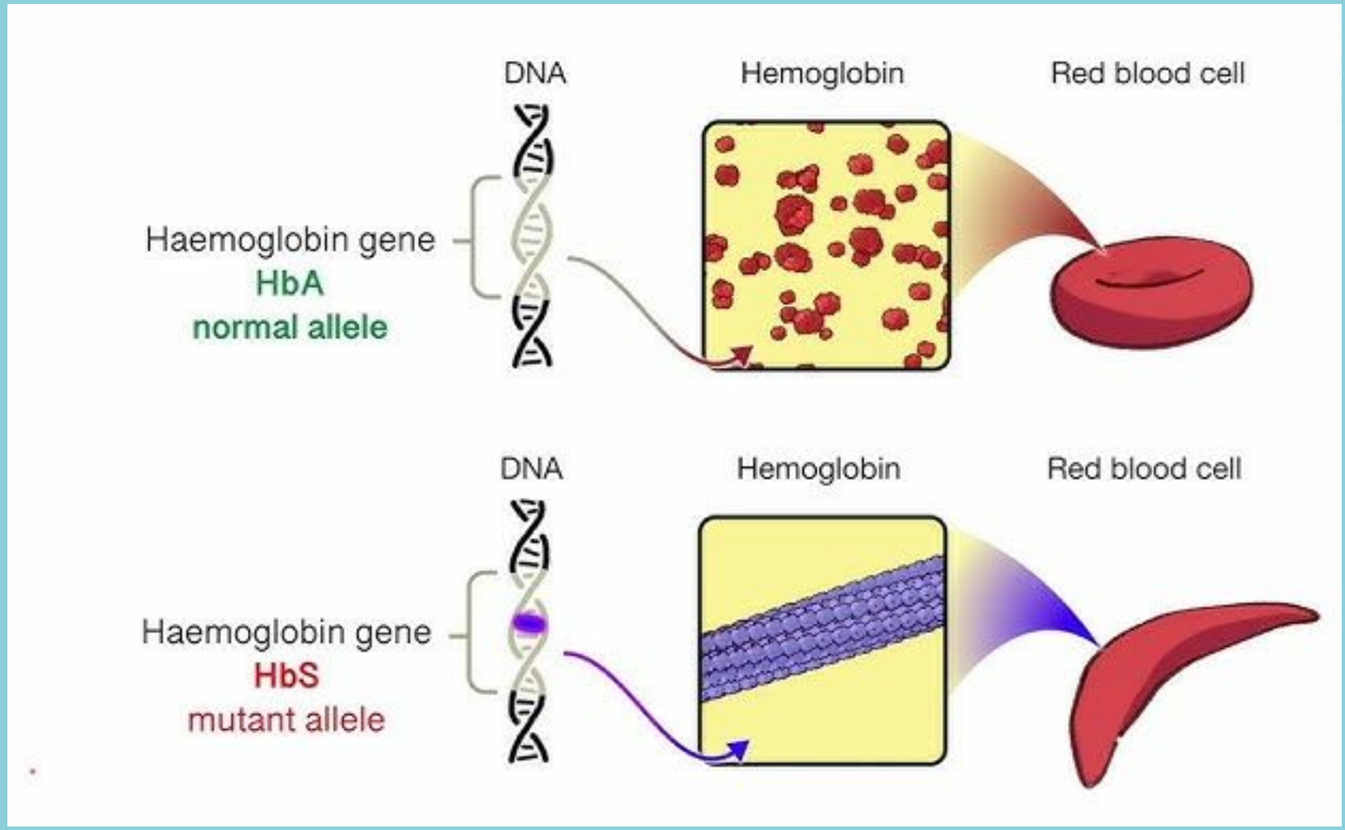


Recap: Genetic Basis of **Sickle Cell**

- Mutation in **β-globin gene (HBB)**
- Glutamic acid → valine substitution (E6V)
- Causes HbS formation and RBC sickling

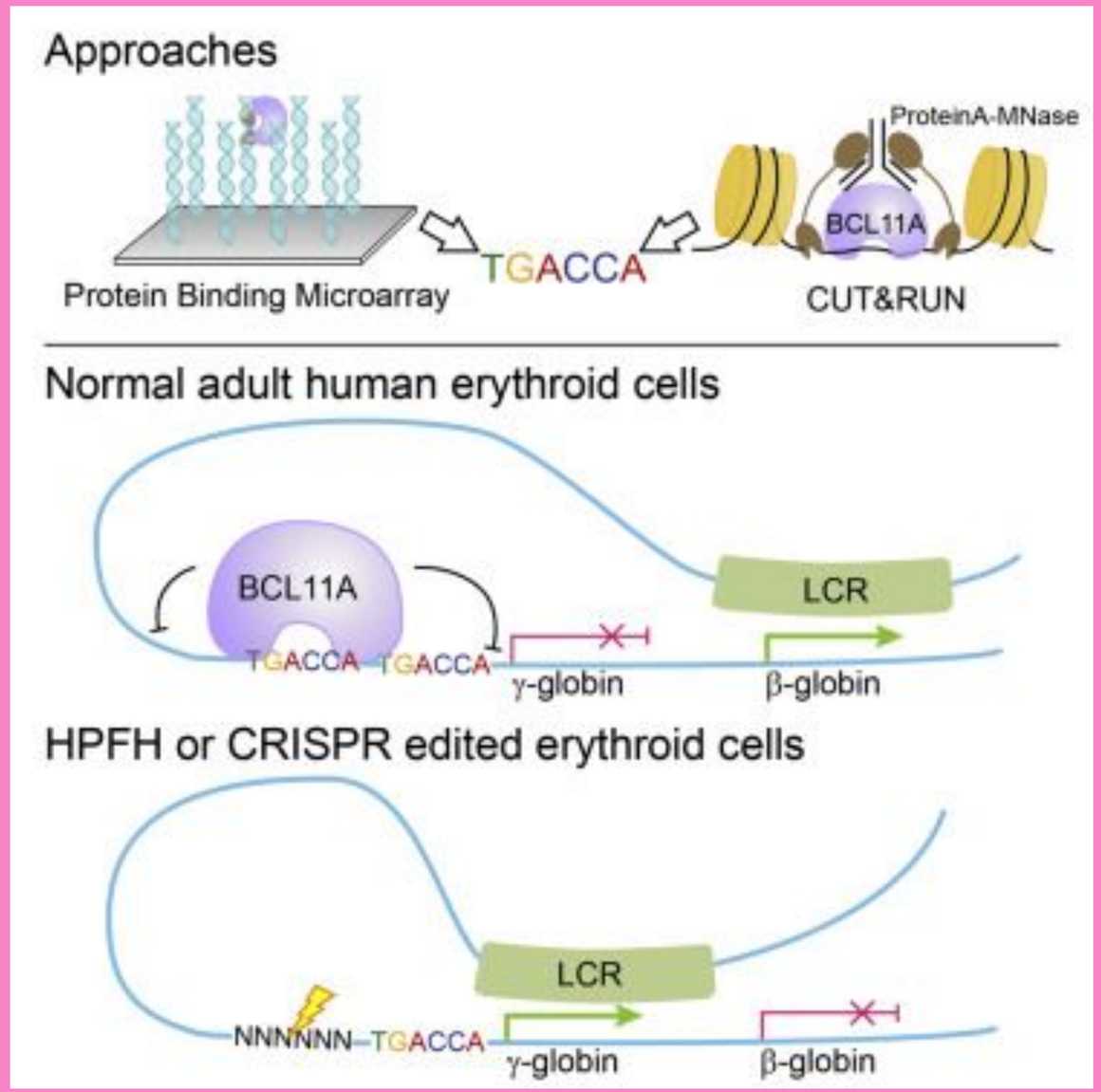
Strategy 1: **Direct Mutation Correction**

- Use CRISPR + **HDR** to repair **HBB mutation**
- Replace HbS allele with normal HbA
- Challenge: HDR efficiency is low in many cells



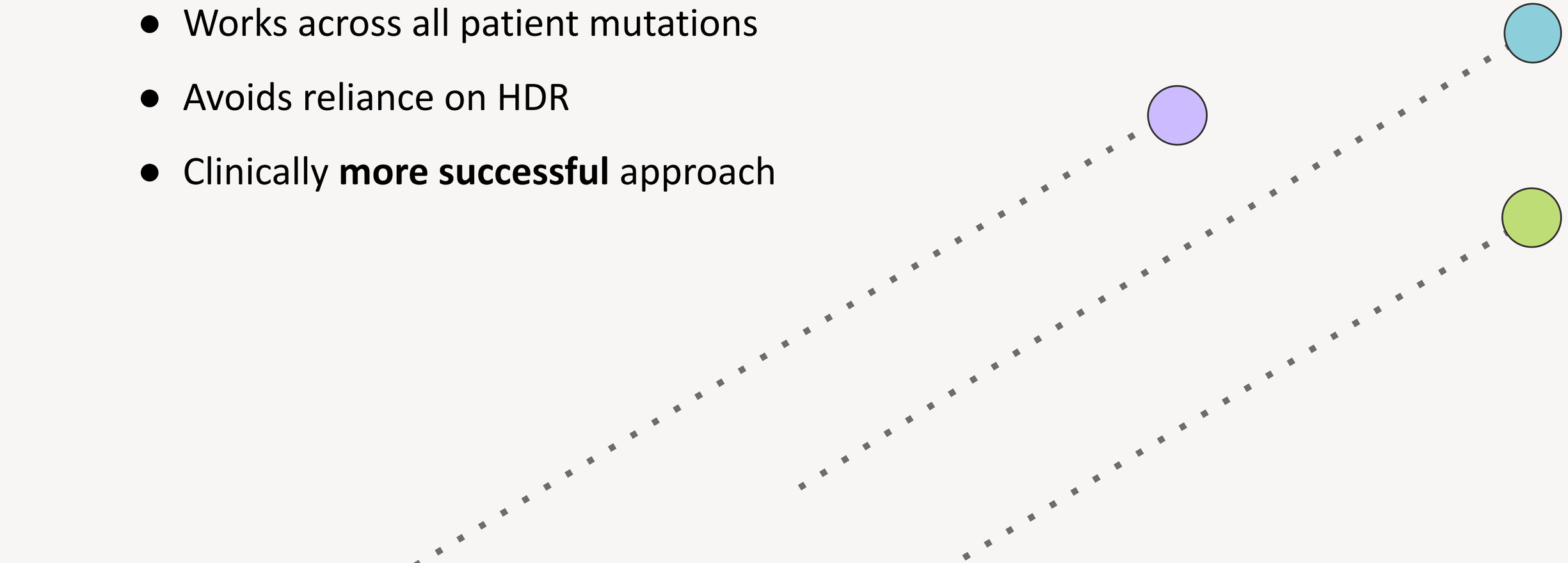
Strategy 2: **Reactivating Fetal Hemoglobin** (HbF)

- HbF does **not sickle**
- Normally silenced after birth
- CRISPR targets **BCL11A** (HbF repressor)
- Result → increased HbF → reduced symptoms



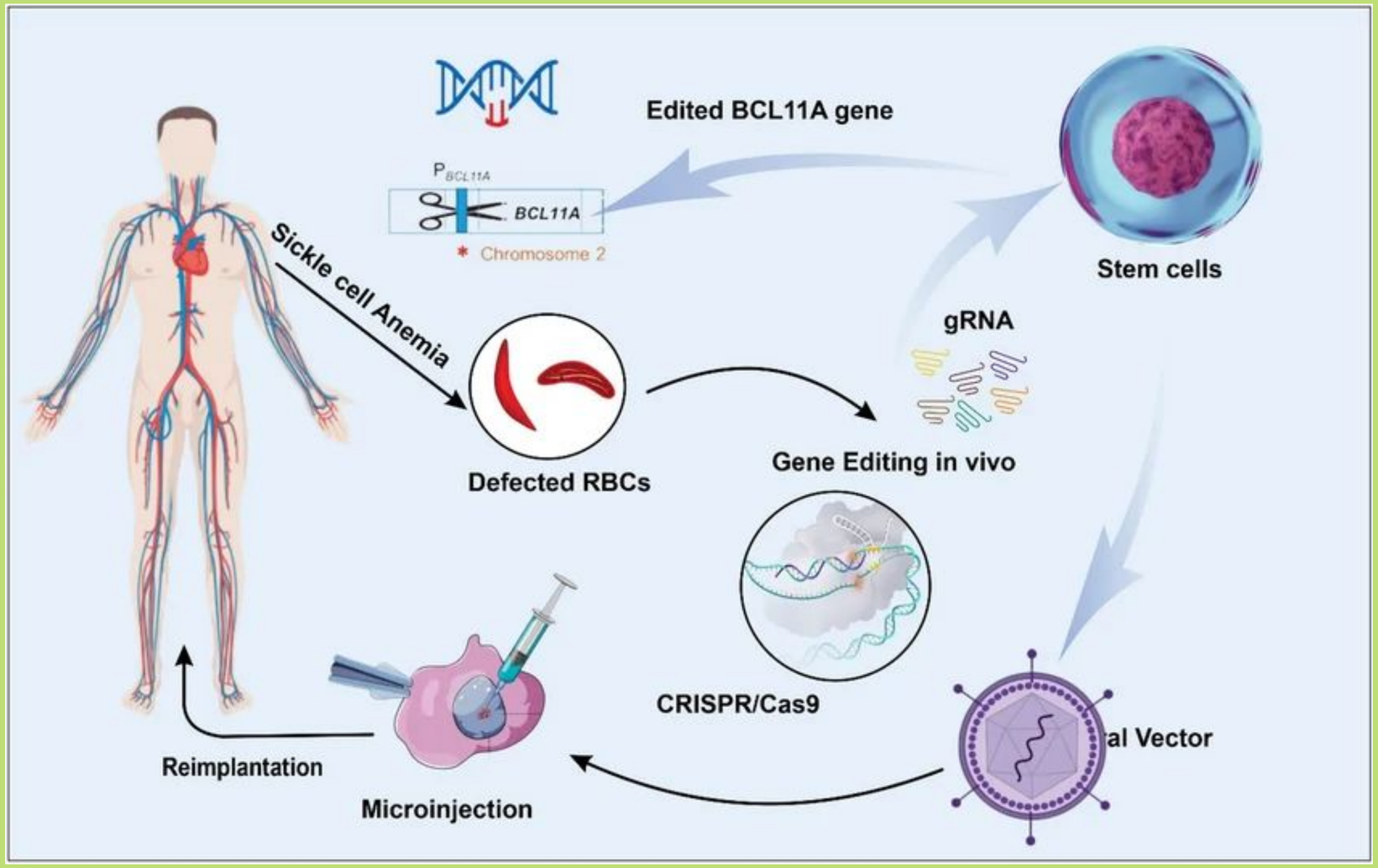
Why Target **BCL11A** Instead of **HBB**

- More **efficient** than direct repair
- Works across all patient mutations
- Avoids reliance on HDR
- Clinically **more successful** approach



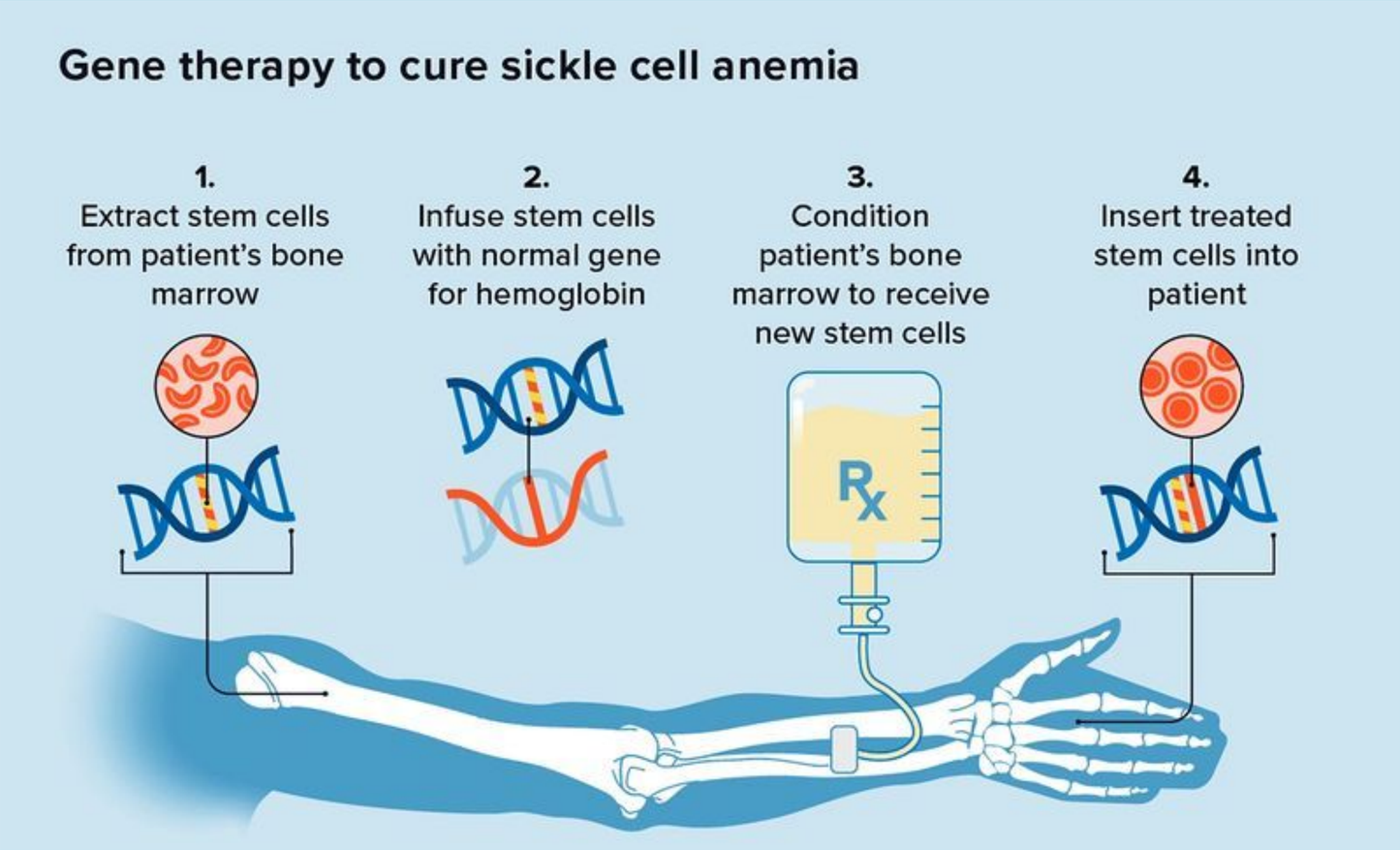
Ex Vivo Editing Workflow

- Harvest hematopoietic stem cells (HSCs)
- Edit cells using CRISPR
- Expand edited cells
- Reinfuse into patient
- Cells repopulate bone marrow



Clinical Example

- **Casgevy (exa-cel)**
- **First** CRISPR-based therapy approved (UK/US)
- Targets **BCL11A enhancer**
- Patients show:
  - Reduced pain crises
  - Increased HbF levels

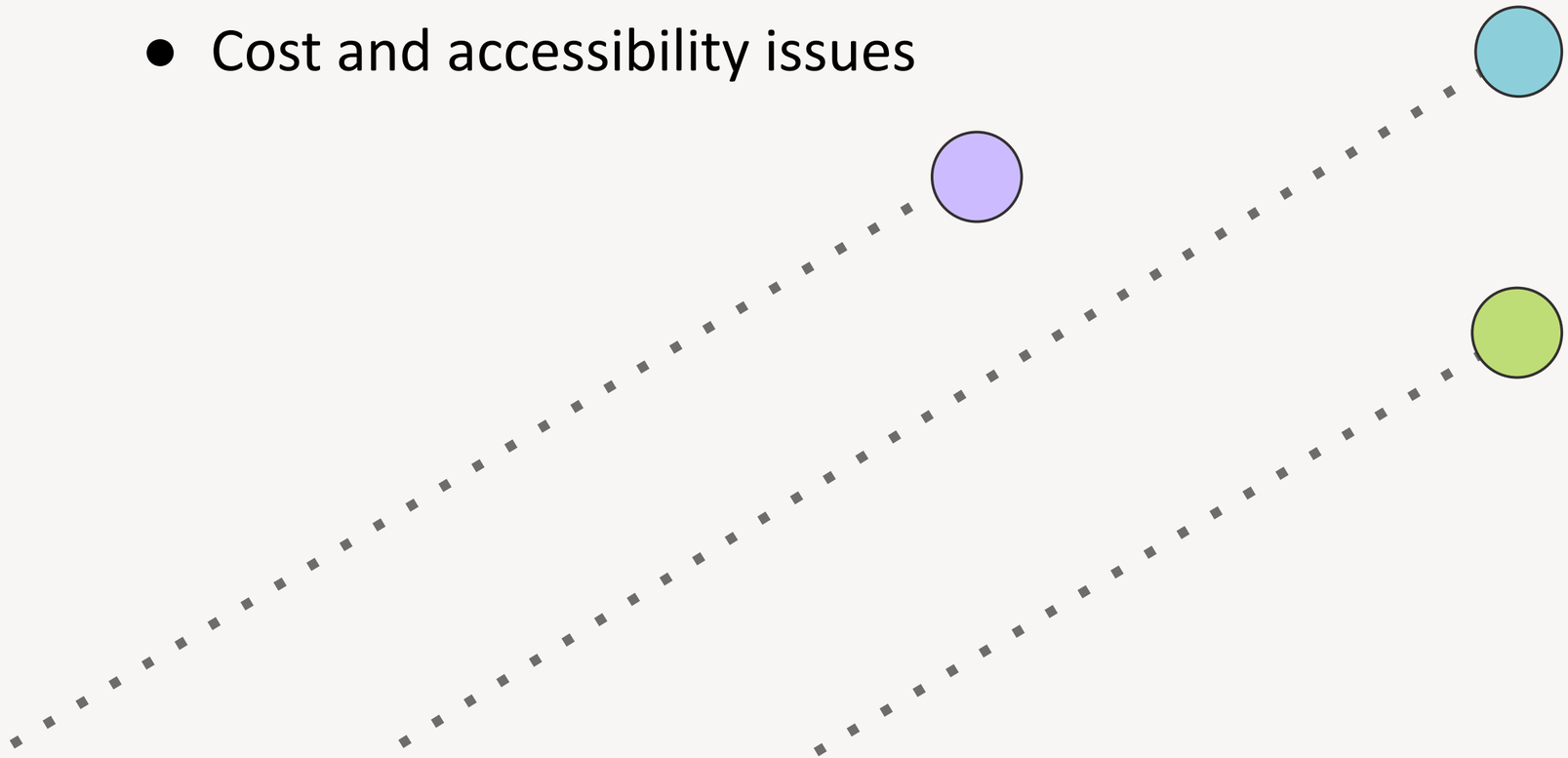


Effectiveness of **CRISPR Therapy**

- Many patients become **transfusion-independent**
- **Sustained HbF expression**
- Long-term data still emerging
- Functional **“cure-like”** outcomes

Limitations & Risks

- Off-target **mutations**
- Delivery challenges
- Conditioning (chemotherapy) required
- Cost and accessibility issues

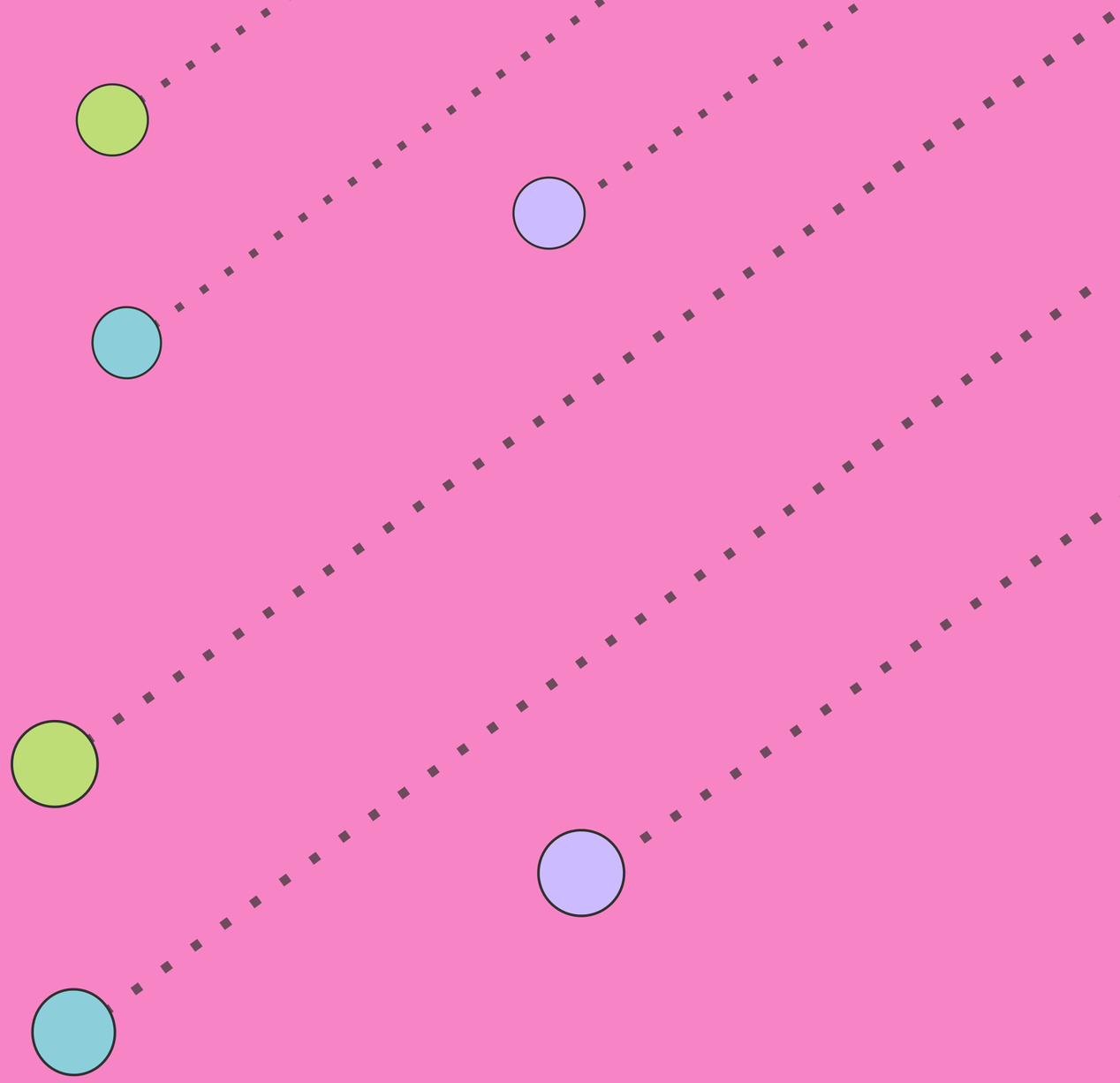


Ethical Considerations

- **Somatic** vs **germline** editing
- Equity of access
- Long-term **unknown** effects
- Regulatory frameworks evolving

Future Directions

- Improved **specificity** (base editing, prime editing)
- **In vivo** delivery systems
- Expansion to **other diseases**:
  - β-thalassemia
  - muscular dystrophy
- Toward **precision medicine**



REVIEW AND SUMMARY

Sickle Cell Anemia

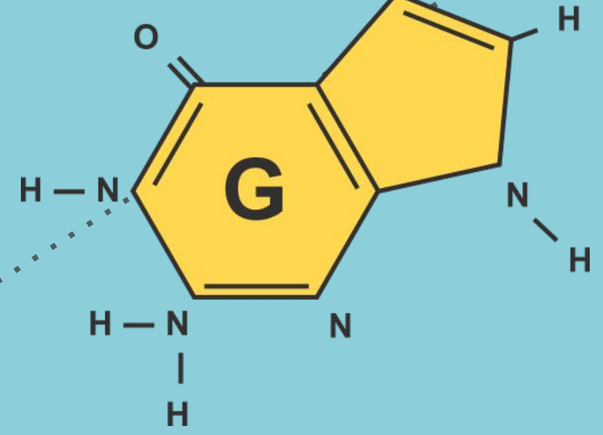
Inherited autosomal recessive hemoglobinopathy disease that comes with a list of complications for the affected individual

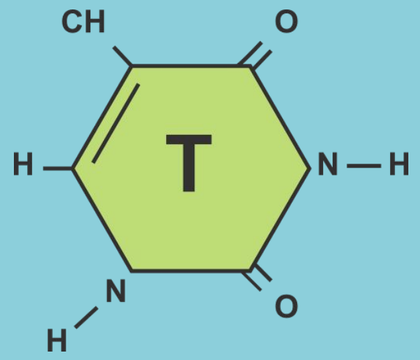
Polymerase Chain Reaction (PCR)

A laboratory technique used to identify the presence or absence of a genetic disorder.

CRISPR-CAS

CRISPR transforms sickle cell anemia from a lifelong genetic disorder into a potentially curable condition through precise genomic intervention





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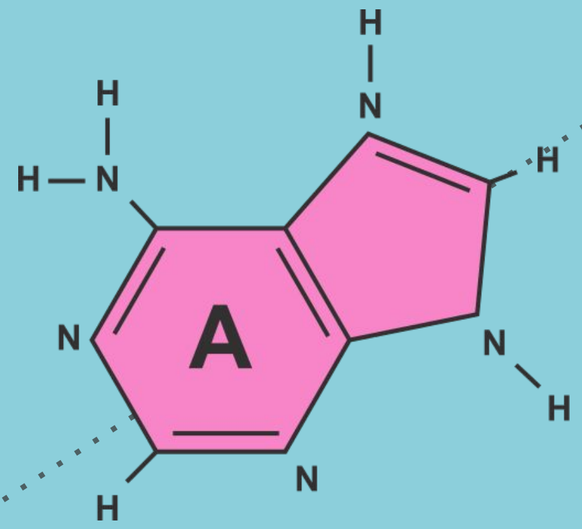

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