



Hurry up and wait...

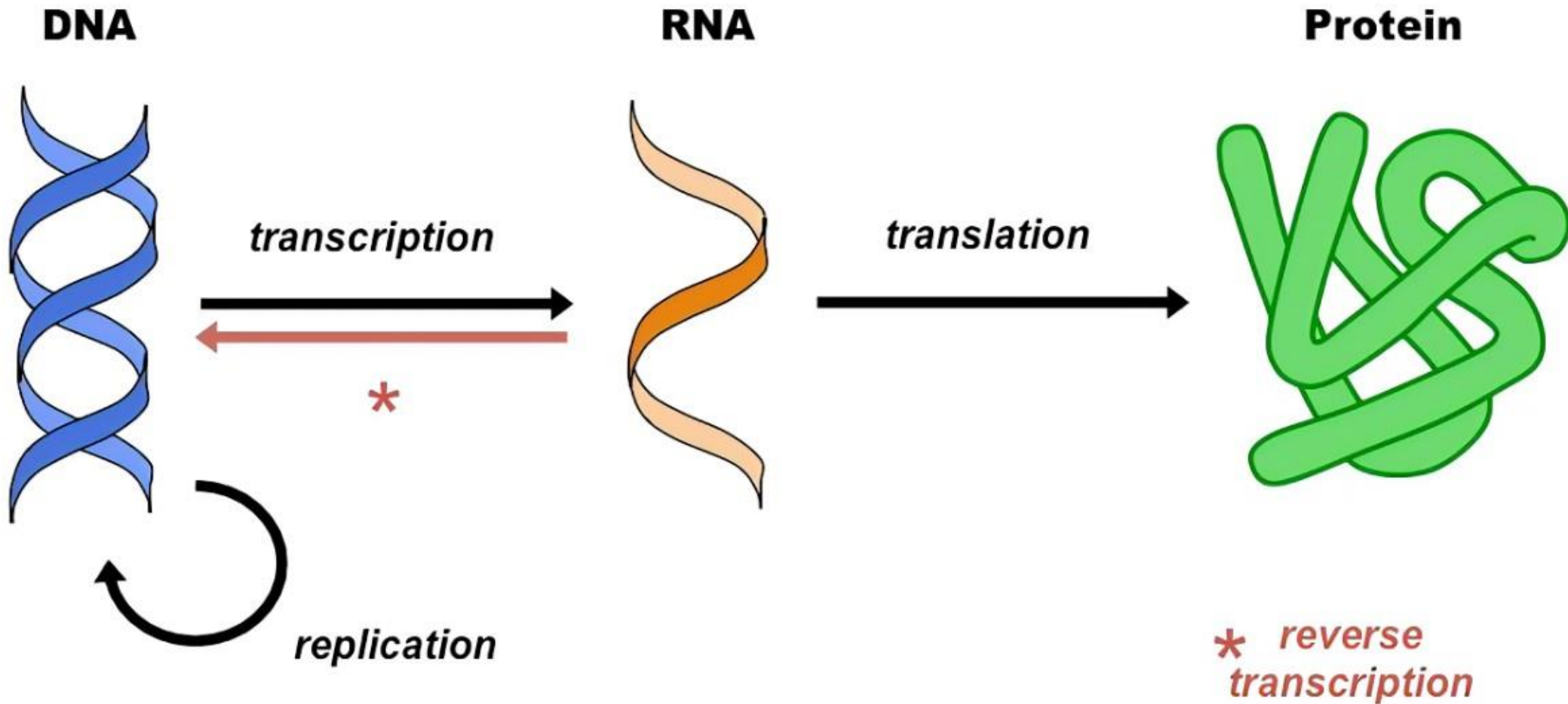
Plasmid Ligations



Mark "Arnie" Arnholt
3D Molecular Designs



The Central Dogma of Biology



Our simplified plasmid map:

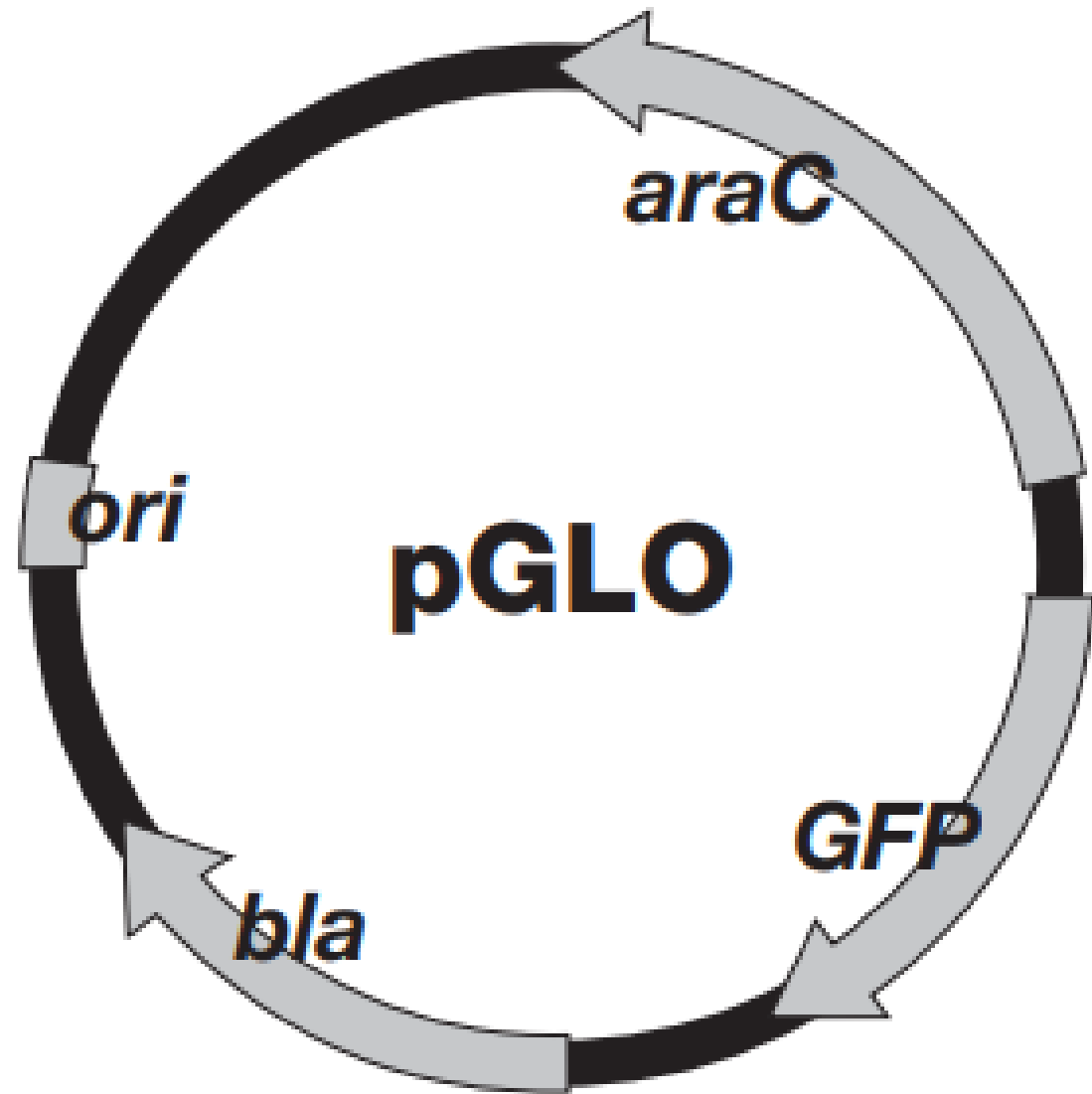
ori – origin of replication

bla- Beta-lactam resistance (amp R)

GFP – Green fluorescent protein

araC – Gene inducer

Where do you think we would find
 P_{BAD} - GFP Promoter?

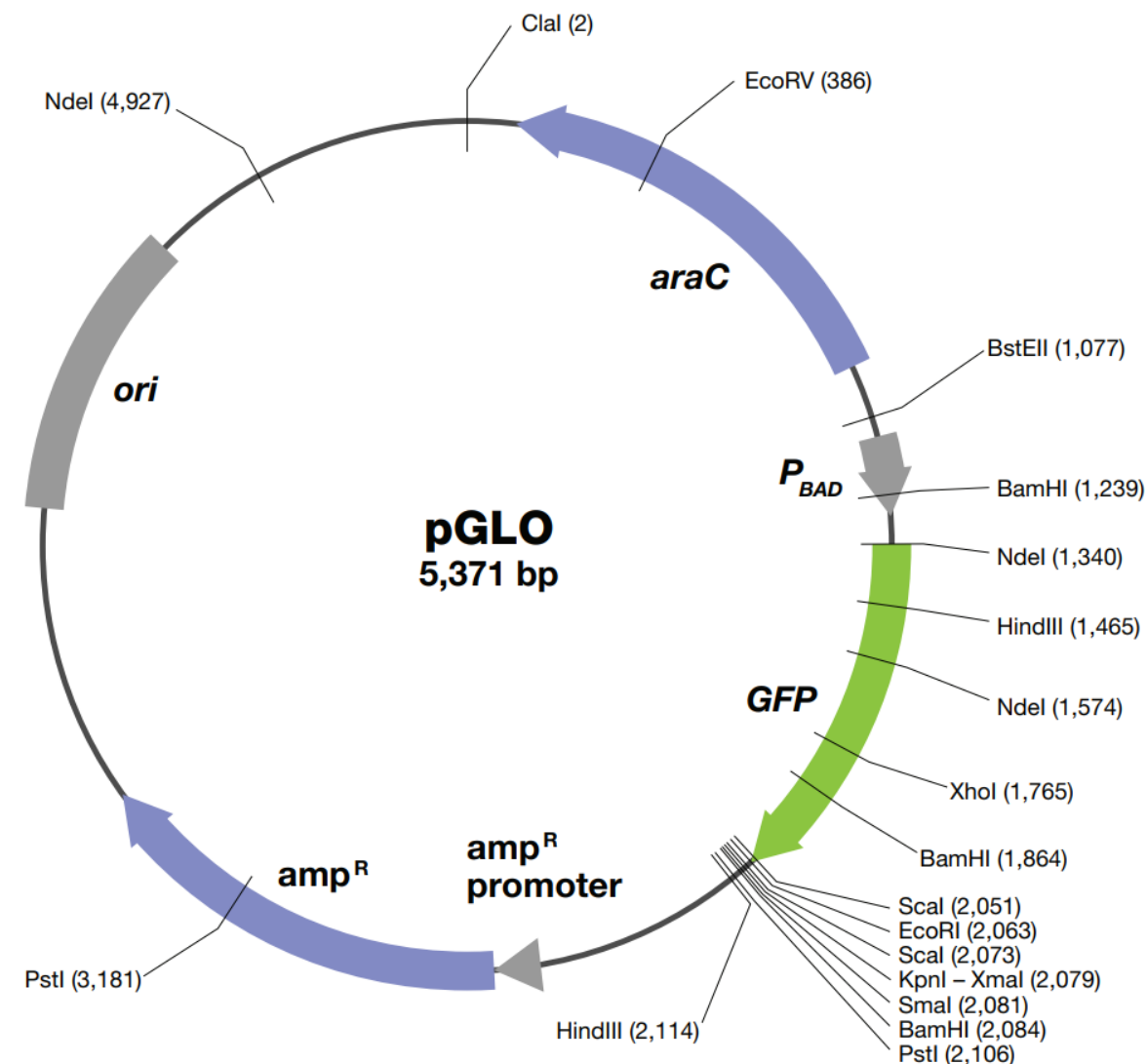


A little more detailed

pGLO is a 5,371 bp plasmid

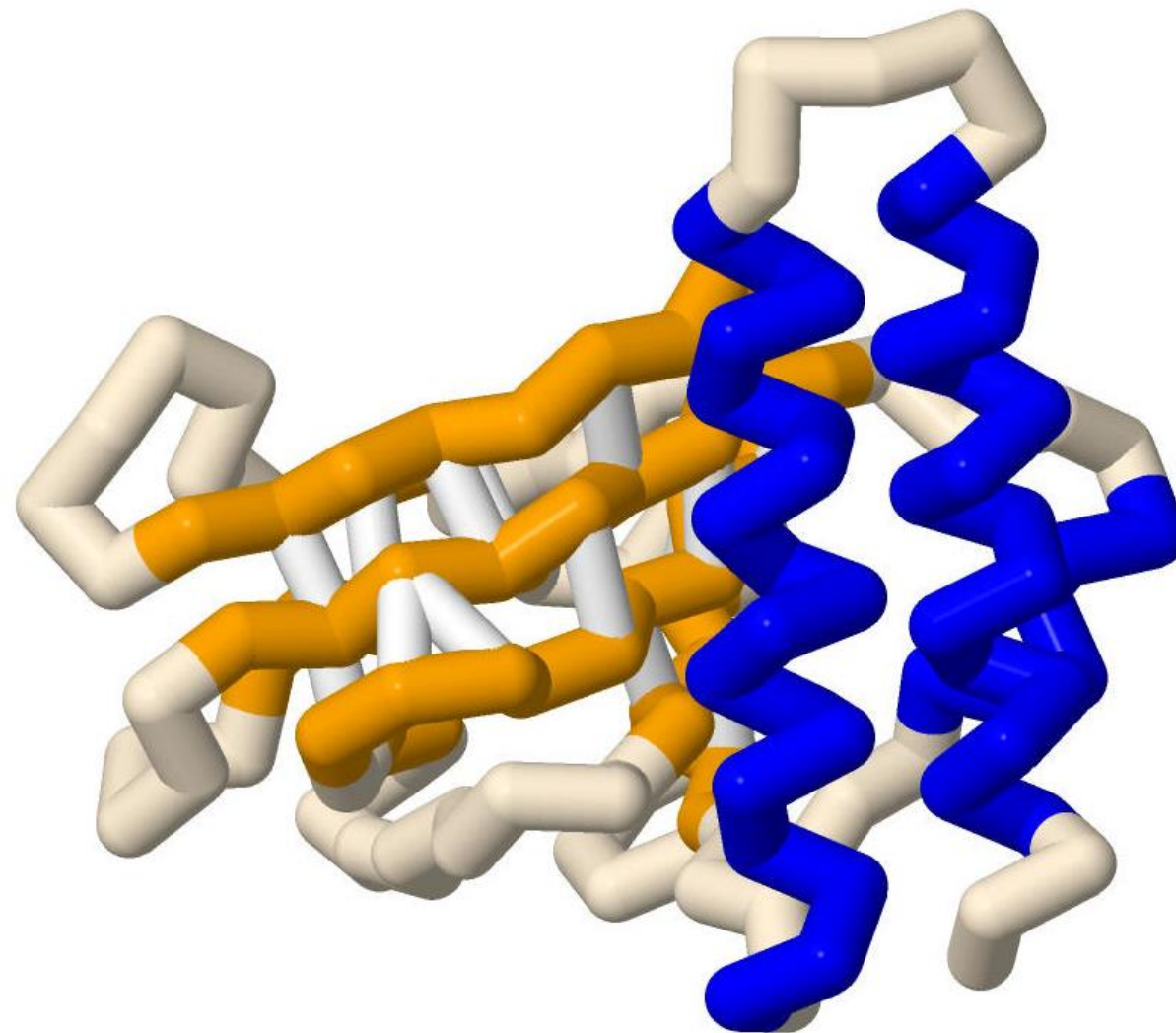
Promoters and restriction sites are identified

See handout-



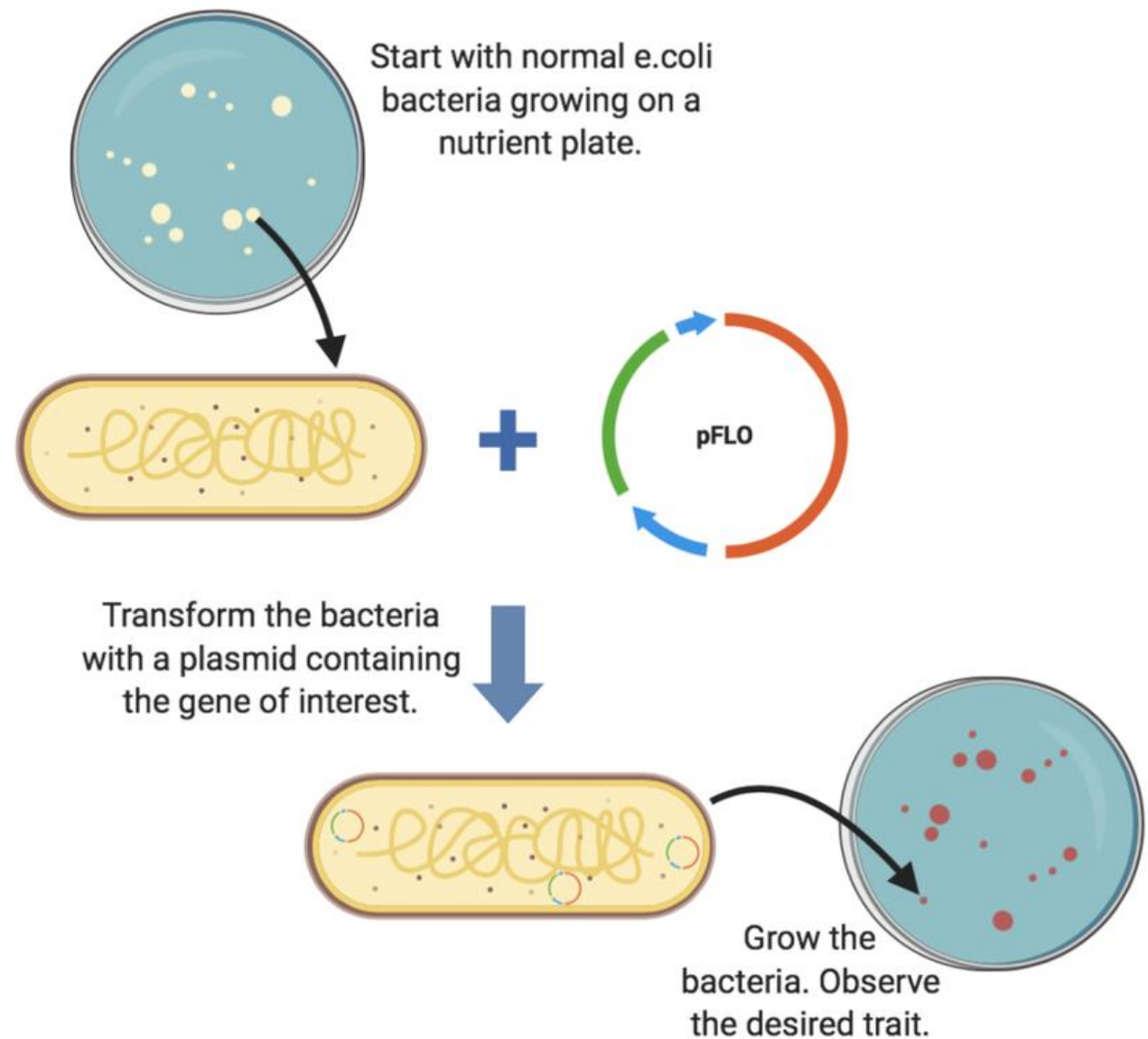
araC gene regulation

How does araC regulate the GFP transcription?



Harnessing the power of microbes

How do I transform a bacterial cell?



Bacterial Transformation

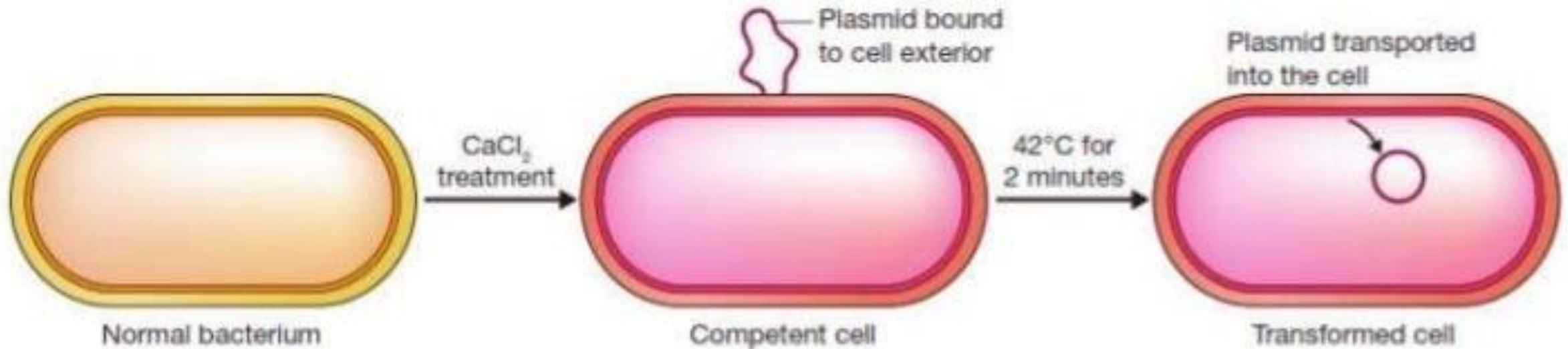


Fig 5.3. Brown, T.A. 1990. Gene Cloning and DNA Analysis

Screening our products:

Predict our outcomes on these plates.

-pGLO have not been transformed
+ pGLO have our plasmid

Plate I



Plate II

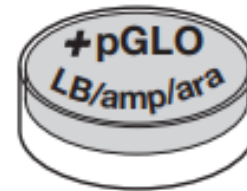


Plate III

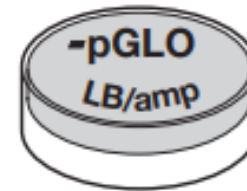


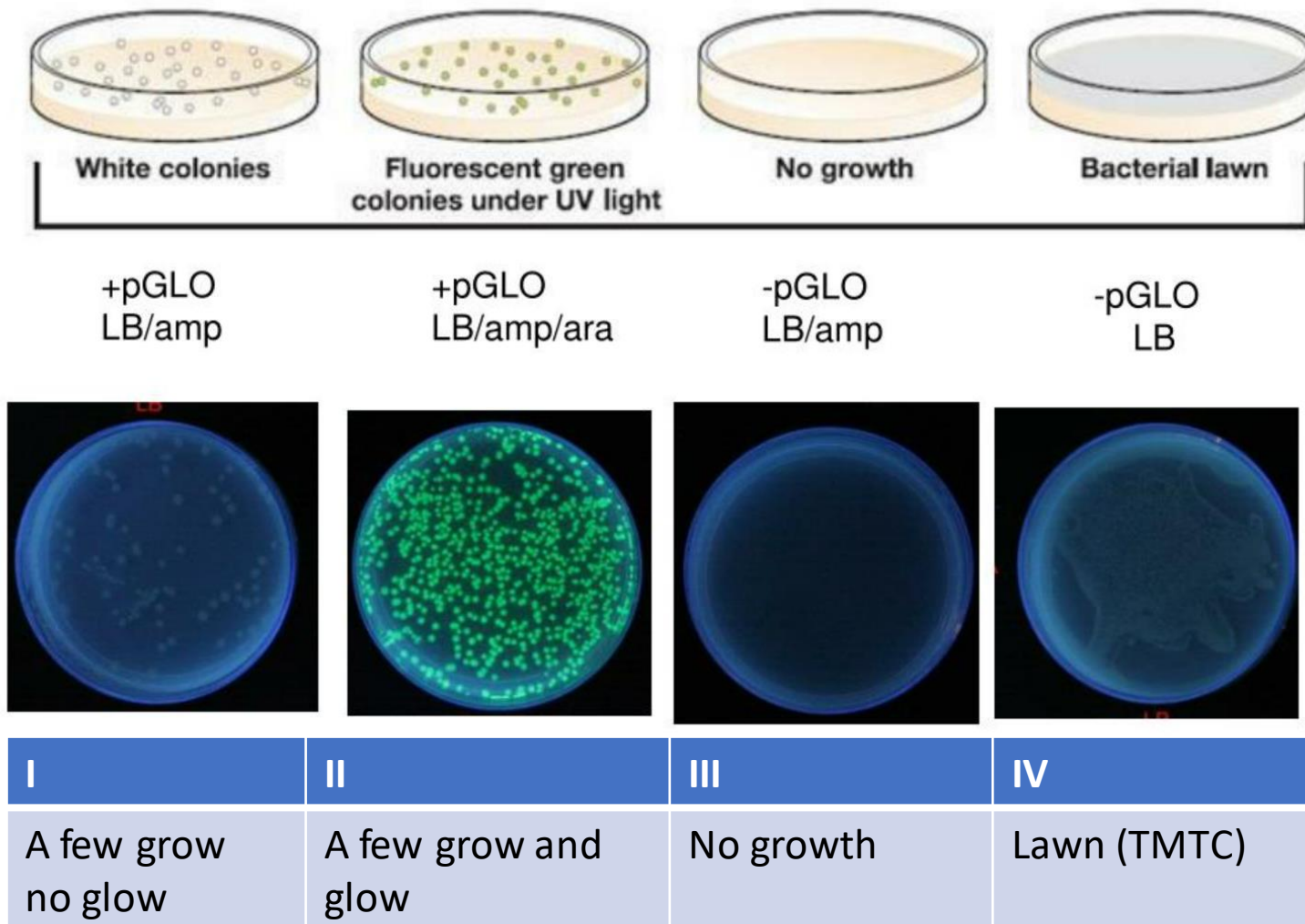
Plate IV



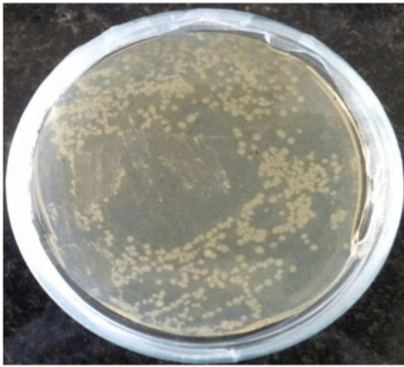
Predicted growth / glow

I	II	III	IV

Predicted results

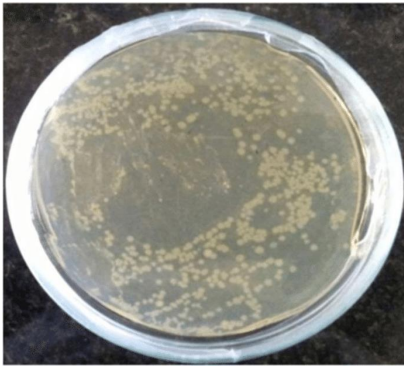


Our Actual plates:



I	II	III	IV
Lawn	Lawn	Lawn	Lawn

What happened?



I	II	III	IV
Lawn	Lawn	Lawn	Lawn

How can we troubleshoot this?

How do I get a gene of interest into a plasmid?

- 1) Identify the target gene
- 2) PCR
- 3) Restriction enzyme digest
- 4) Ligate plasmid
- 5) Bacterial transformation and screening



Let's look at our antibiotics...

- Ampicillin – inhibitor of transpeptidase – prevents cell wall formation
- Kanamycin – targets 30s subunit of a ribosome -> leads to incorrect amino acids being added to a peptide during translation
- We will try and insert a kanamycin resistance gene into our plasmid and screen our bacteria. We can induce the gene with arabinose.

Let's look at our target gene...



Our target gene has been truncated as the actual gene would be hundreds of BP's long.

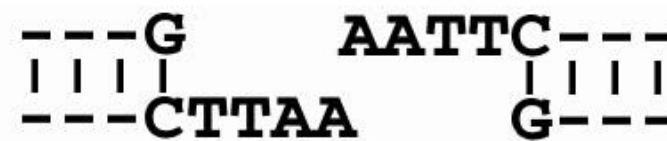
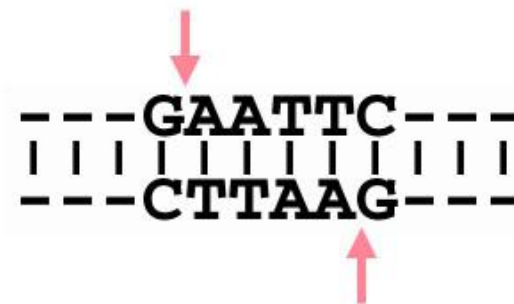
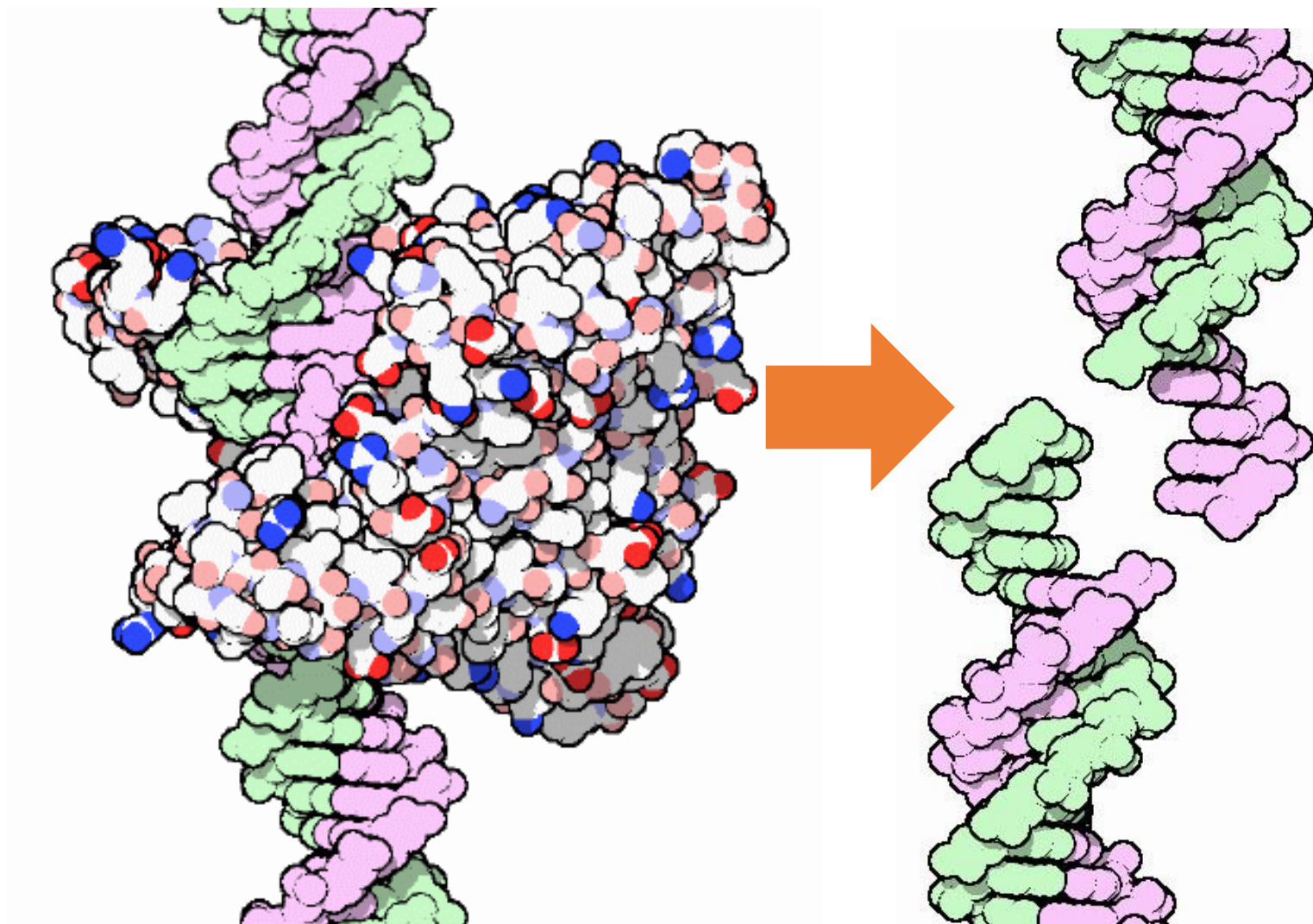
It also has lots of **restriction sites** in it-



Restriction enzymes – Molecular scissors

- Endonucleases that cleave DNA into fragments at specific recognition sites.
- Defense mechanism for bacteria and archaea to defend themselves against viruses
- Make incisions through the sugar-phosphate backbone of DNA

EcoRI



We will need to digest our target DNA

Restriction enzymes at hand include:

Eco RI

Bam HI

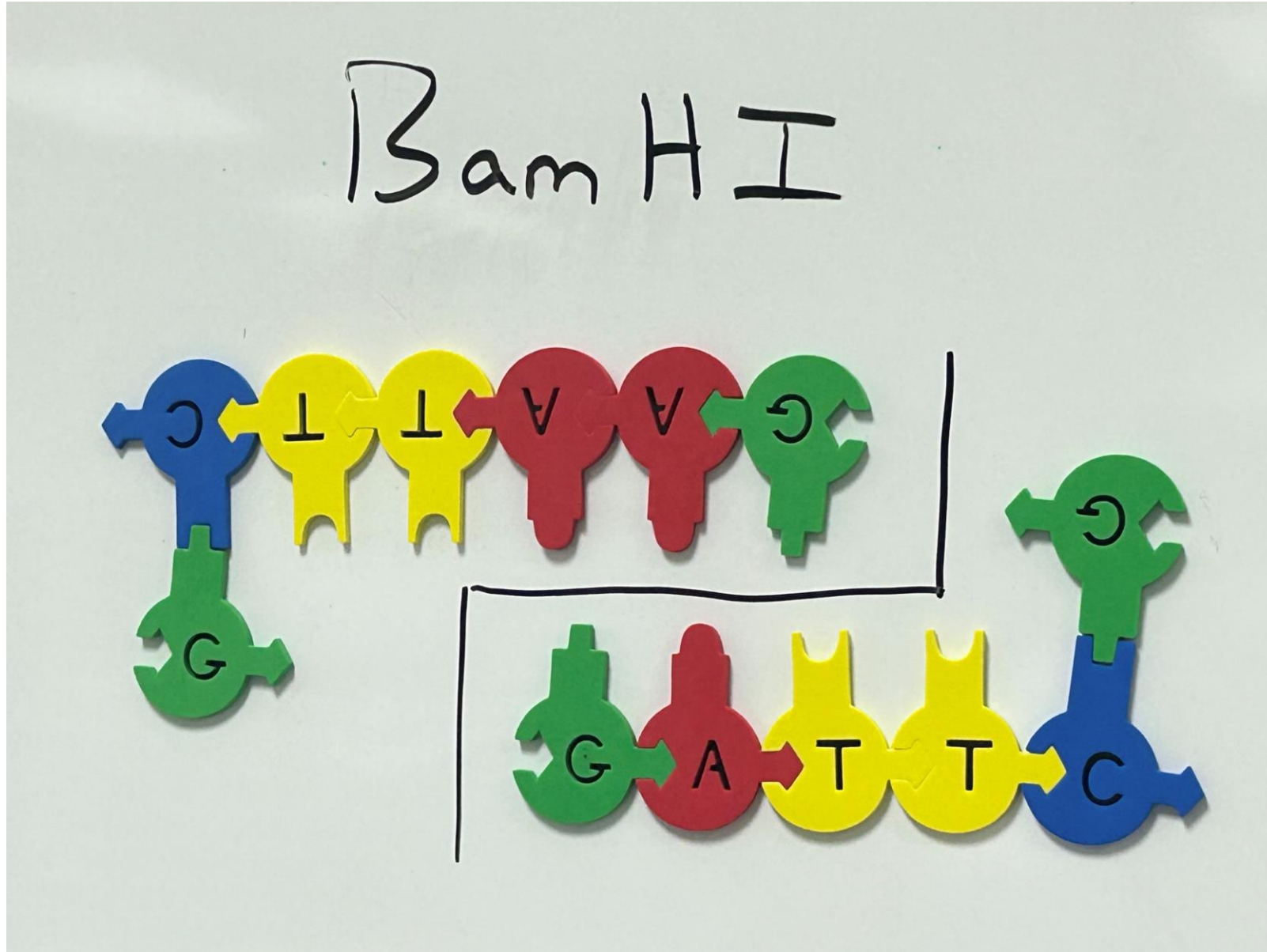
PstI

HIND III

Hae III

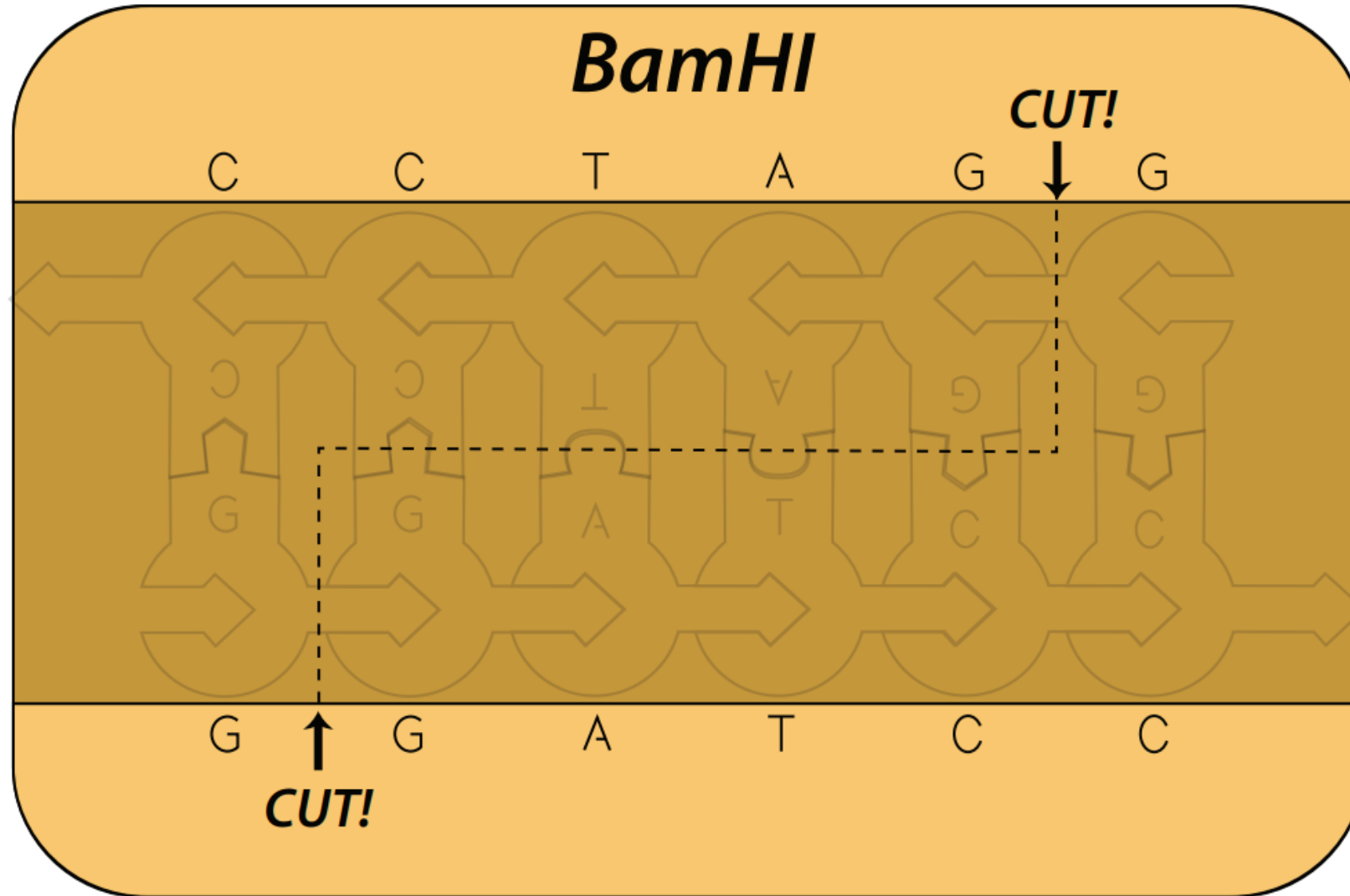


BamHI

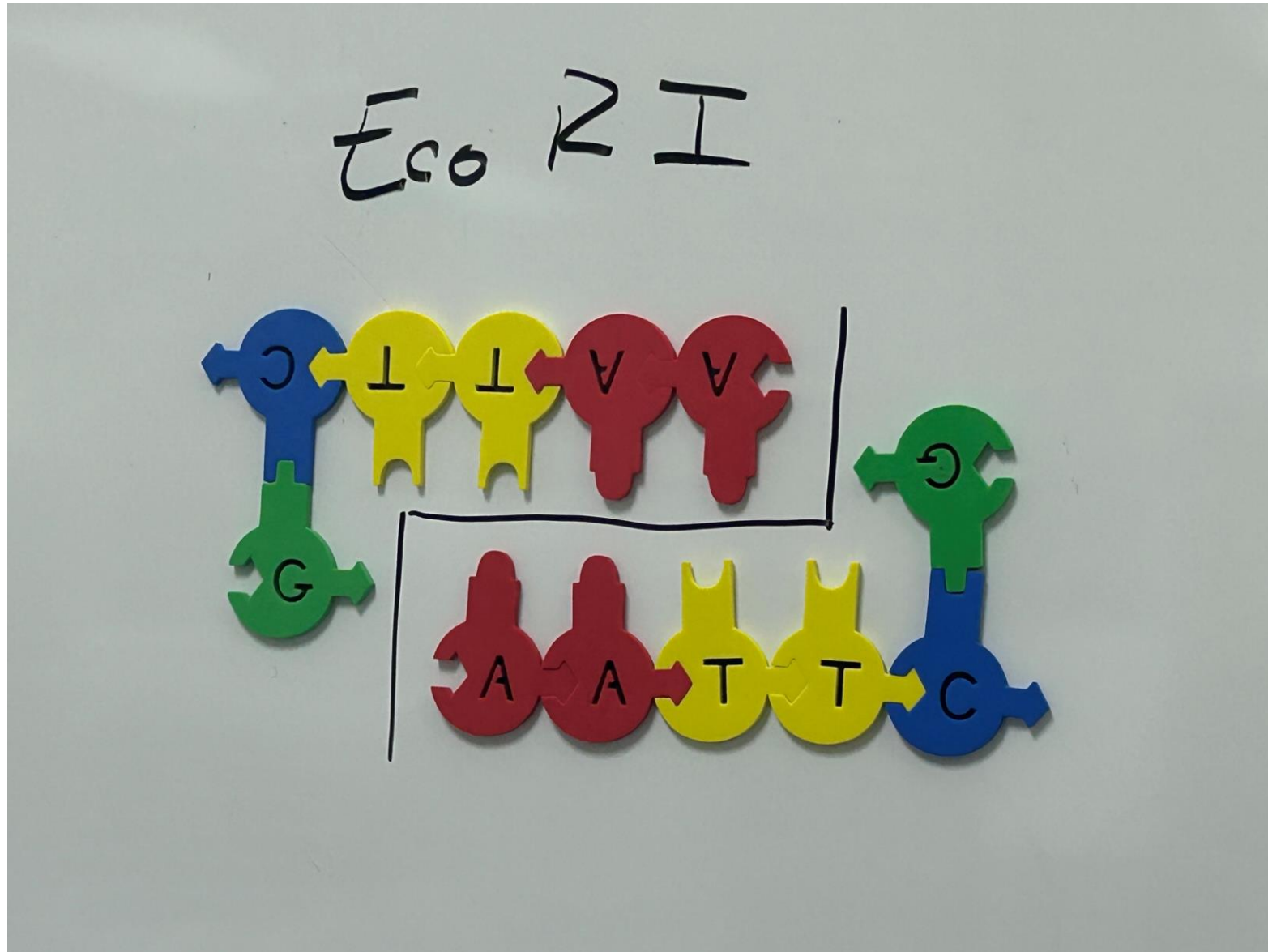




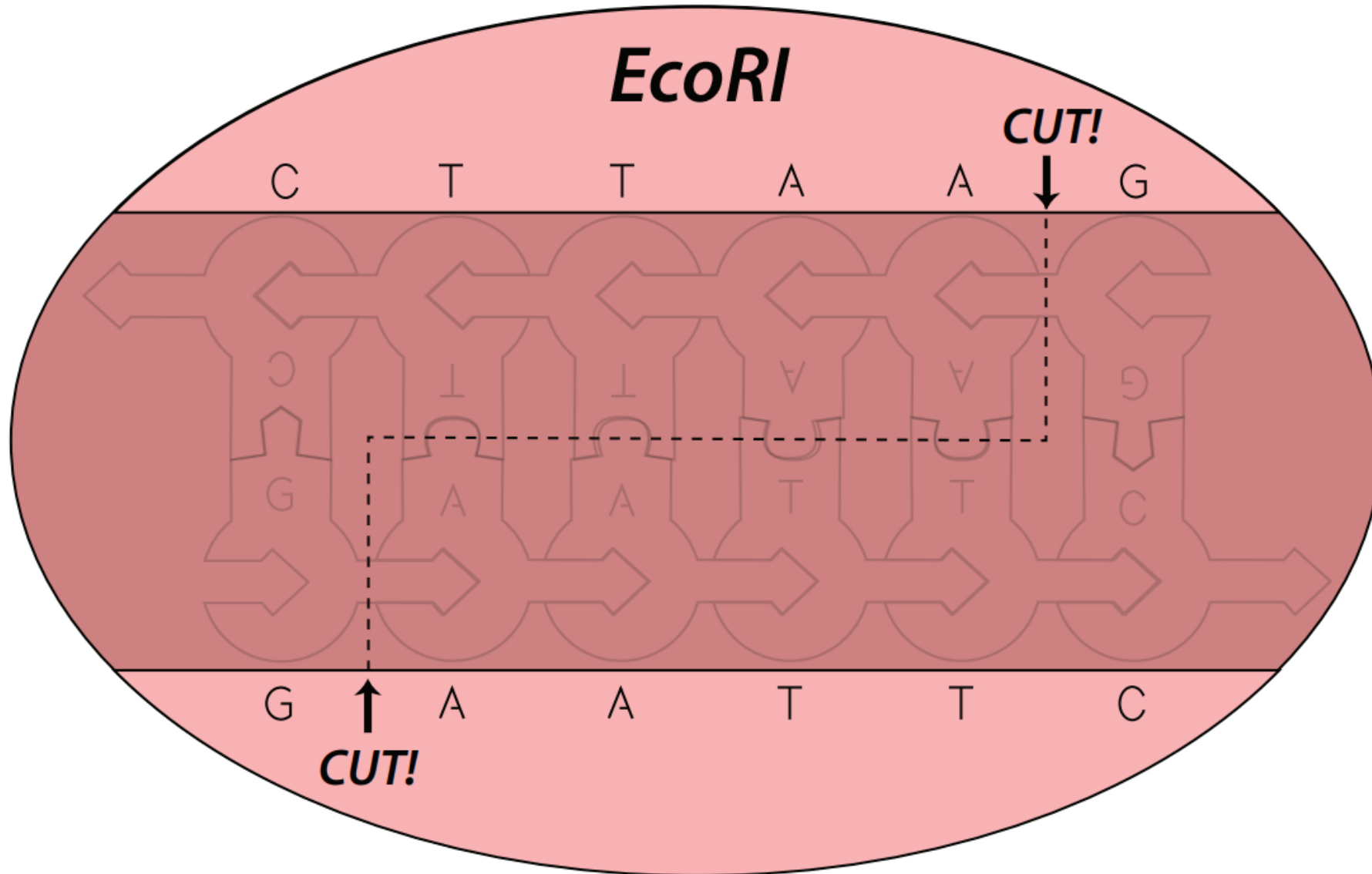
*Bam*HI



EcoRI

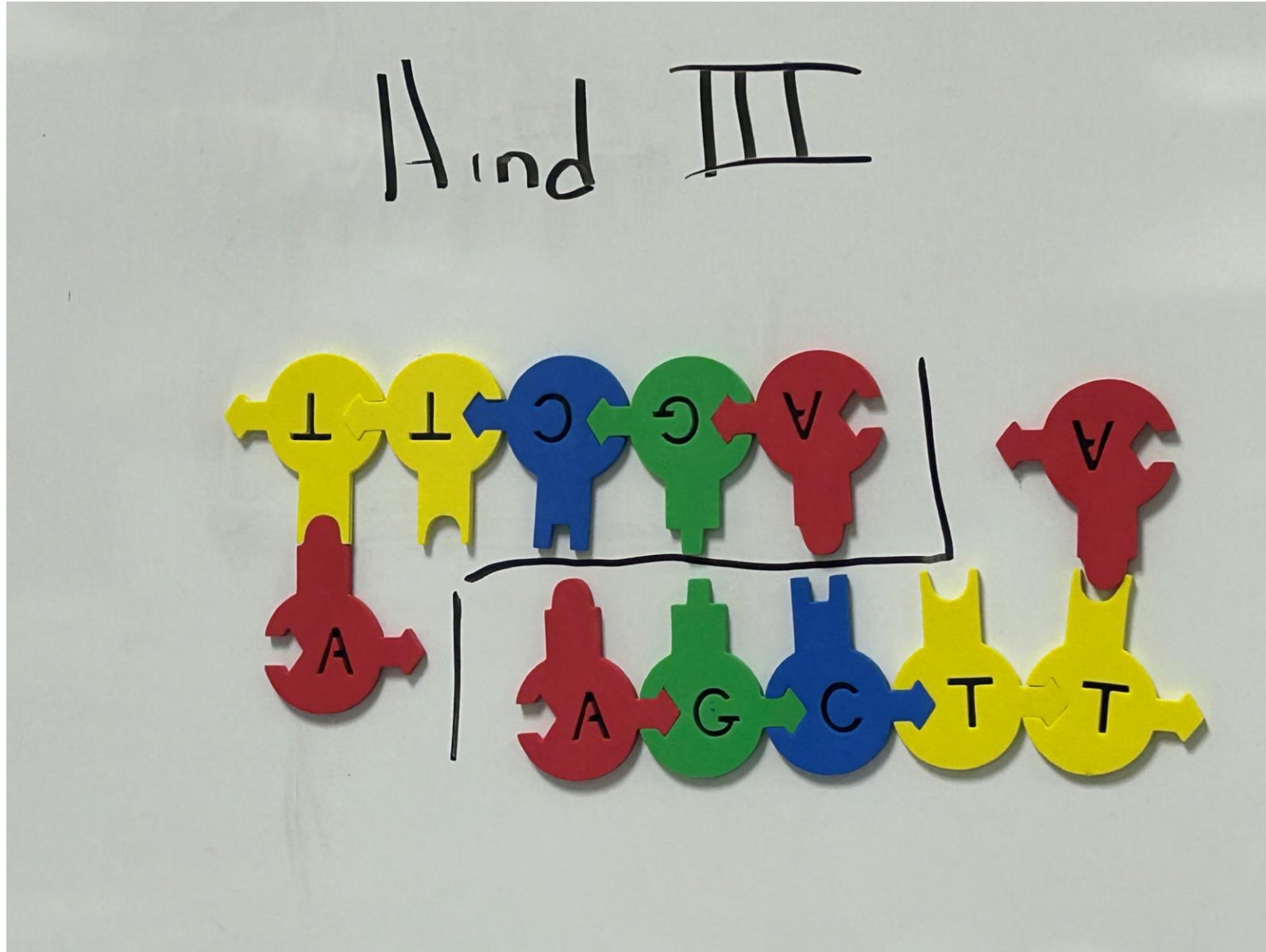


EcoRI



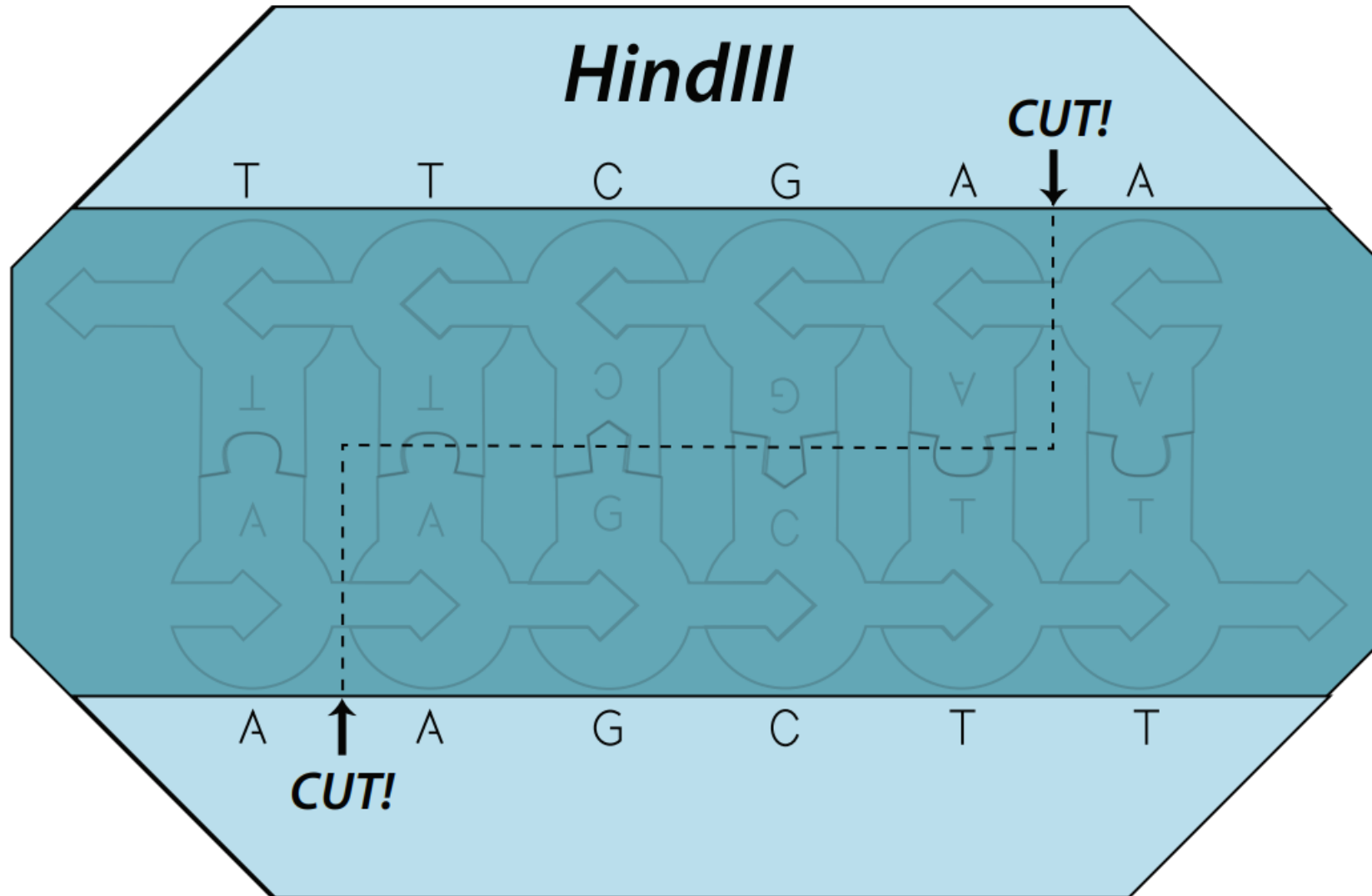


Hind III



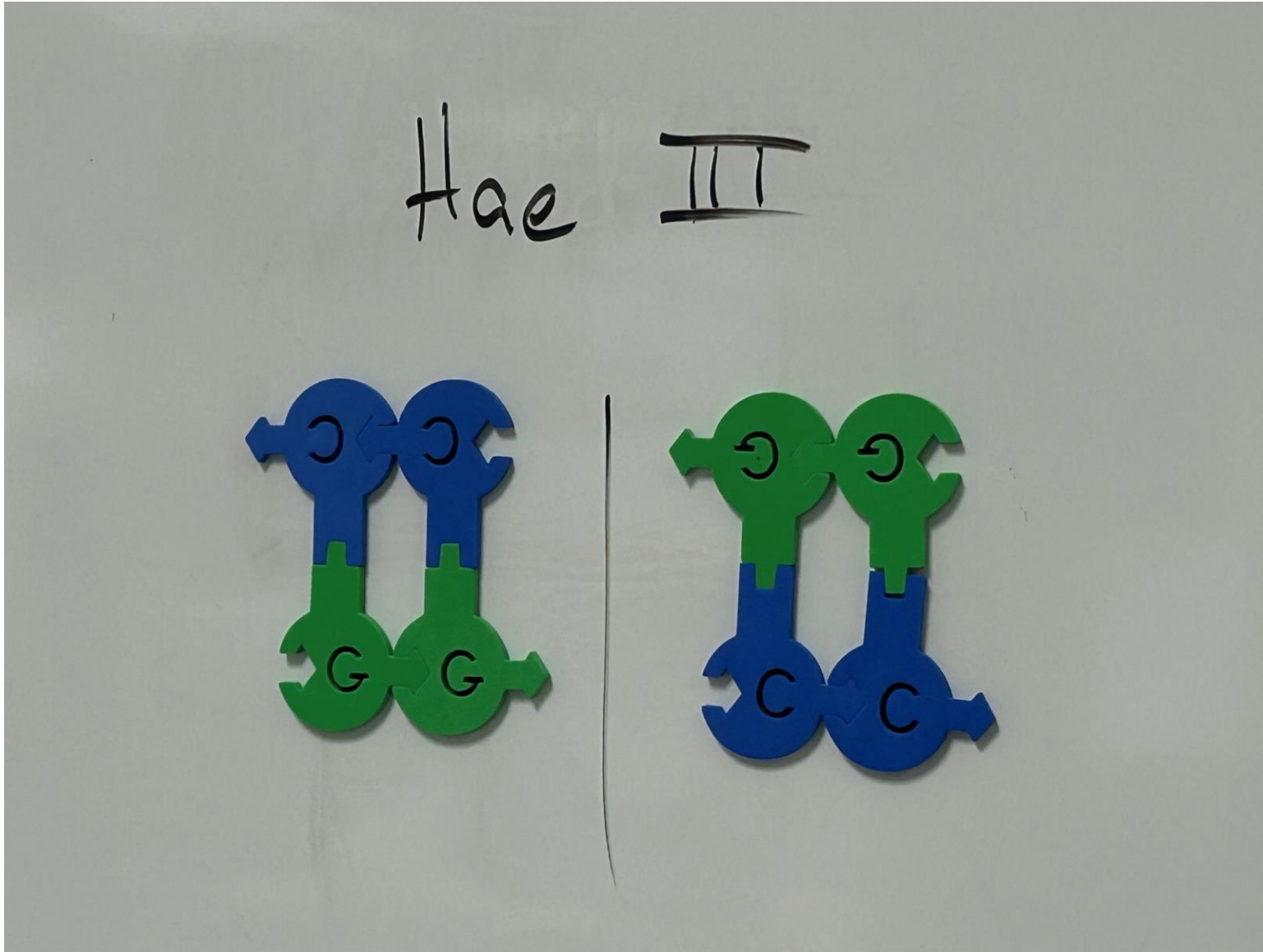


Hind III



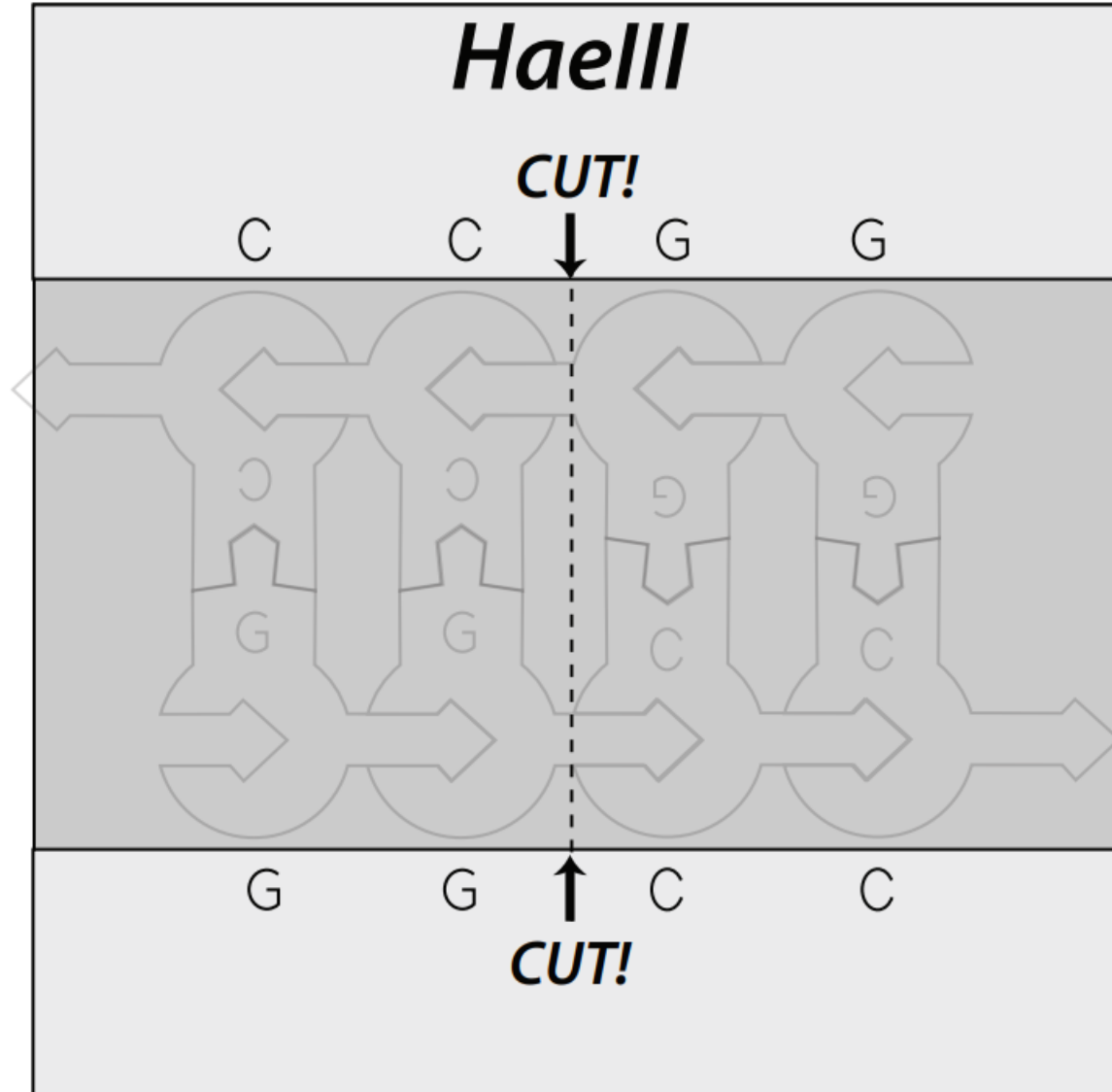


Hae III



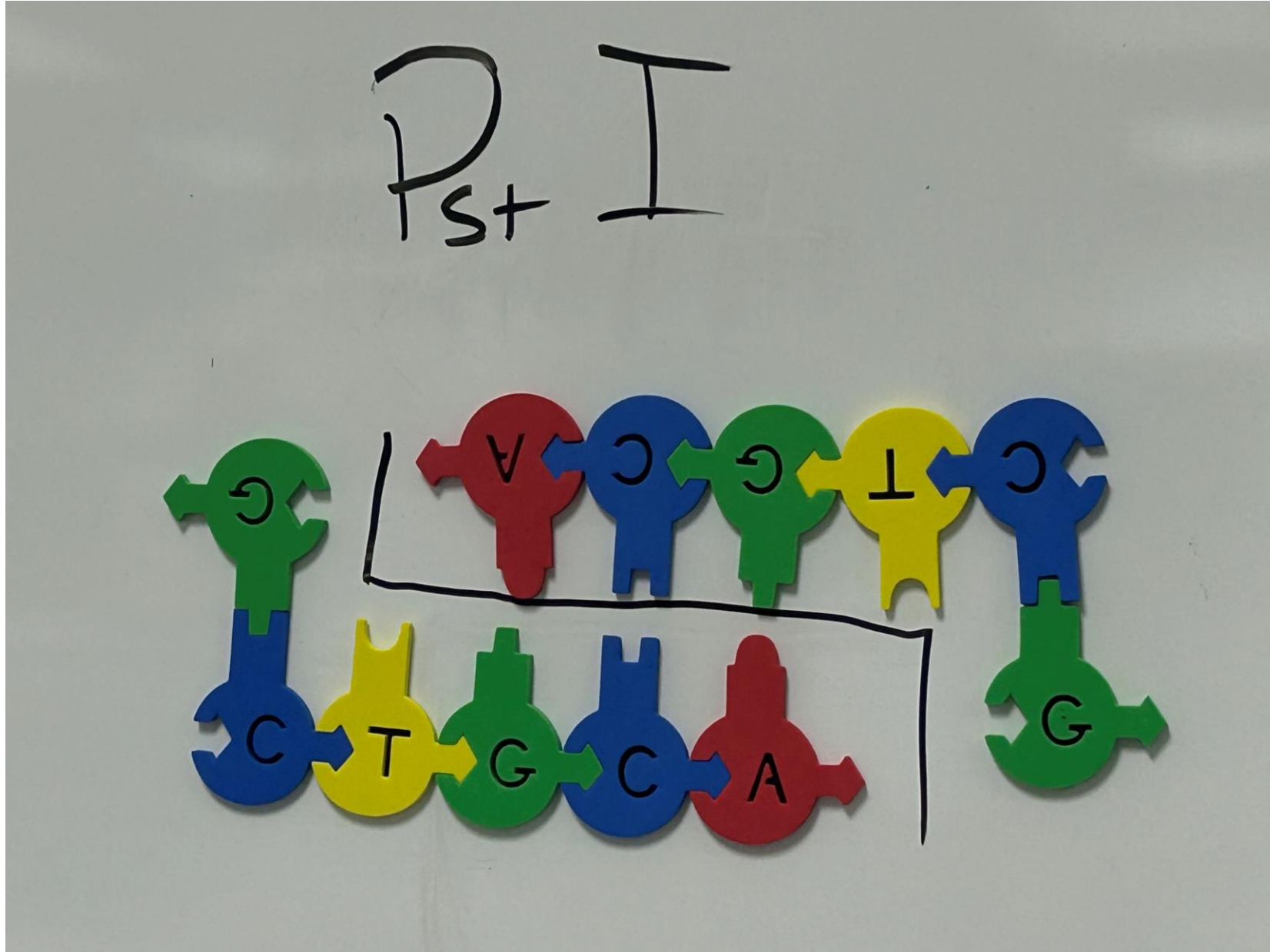


Hae III



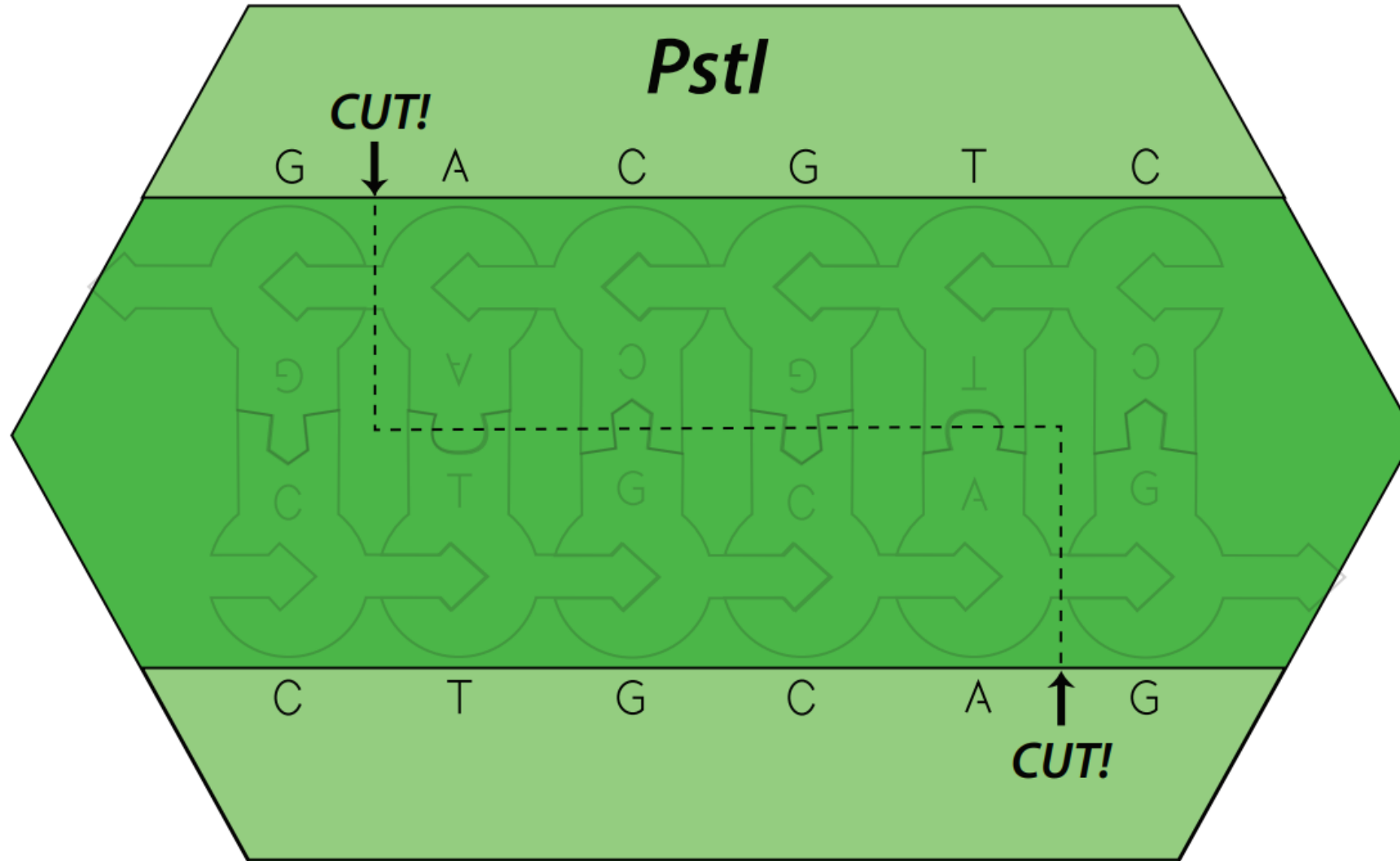


*Pst*I



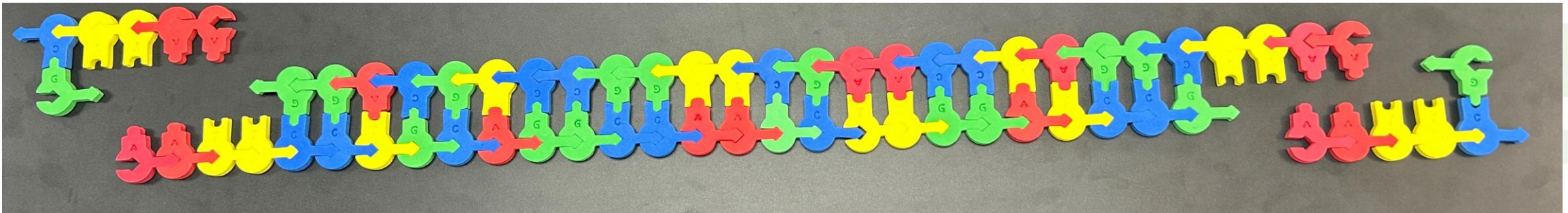


PstI





Our digests...



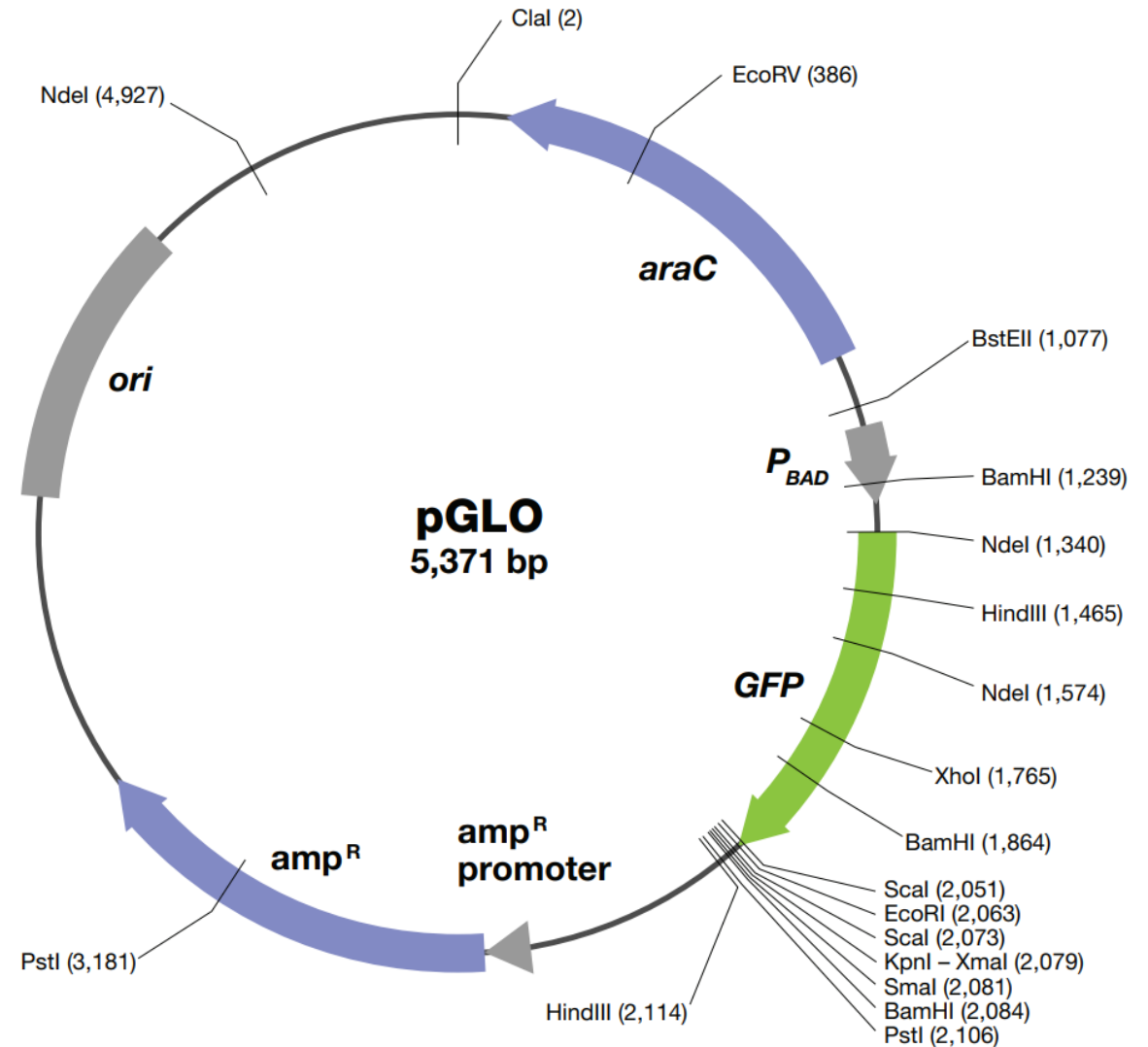


Our digests...



Our actual map:

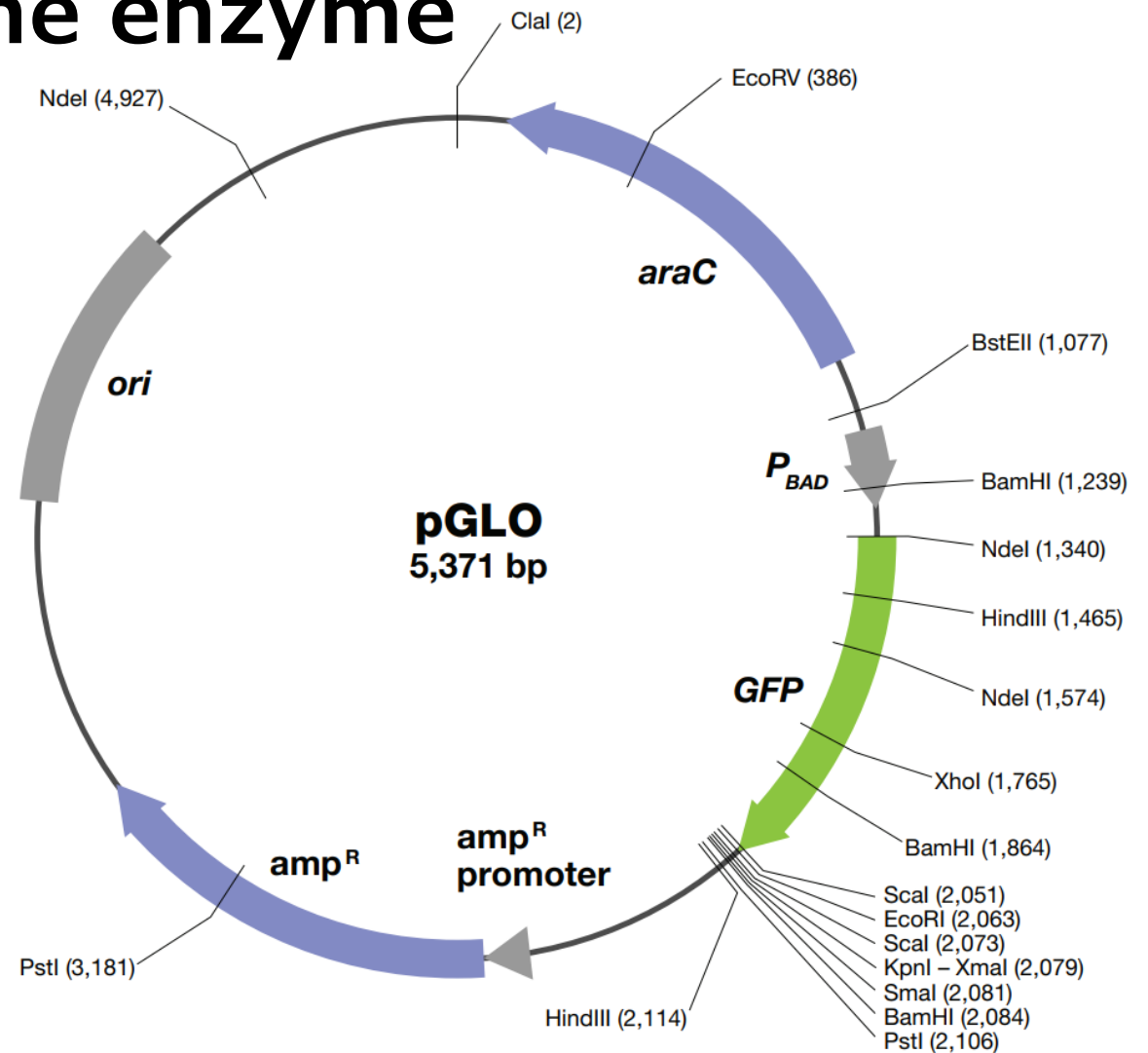
All of our potential restriction sites have been identified:
See handout-



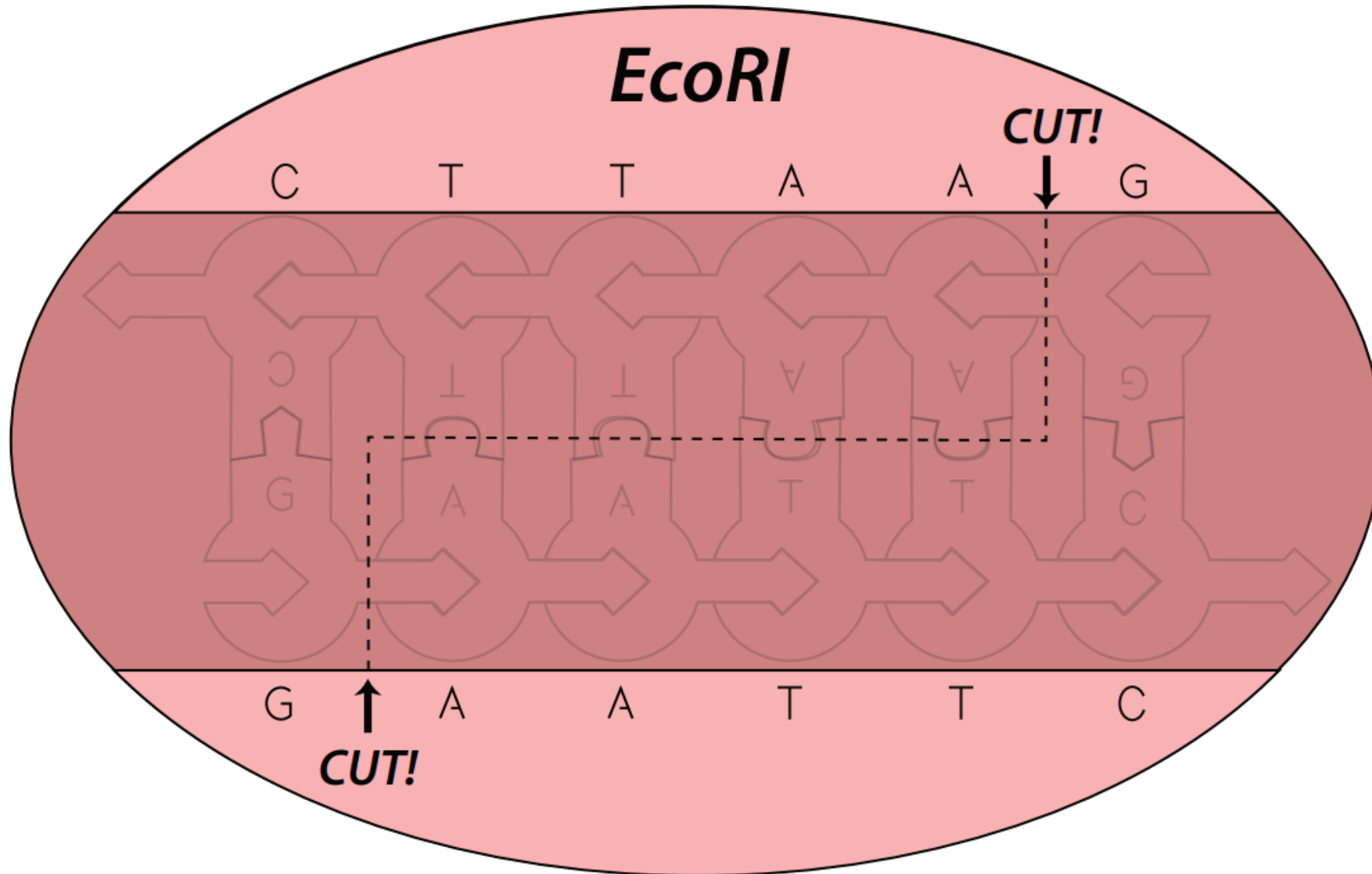
We need to use the same enzyme

The target DNA and the plasmid need to be digested with the same restriction enzyme.

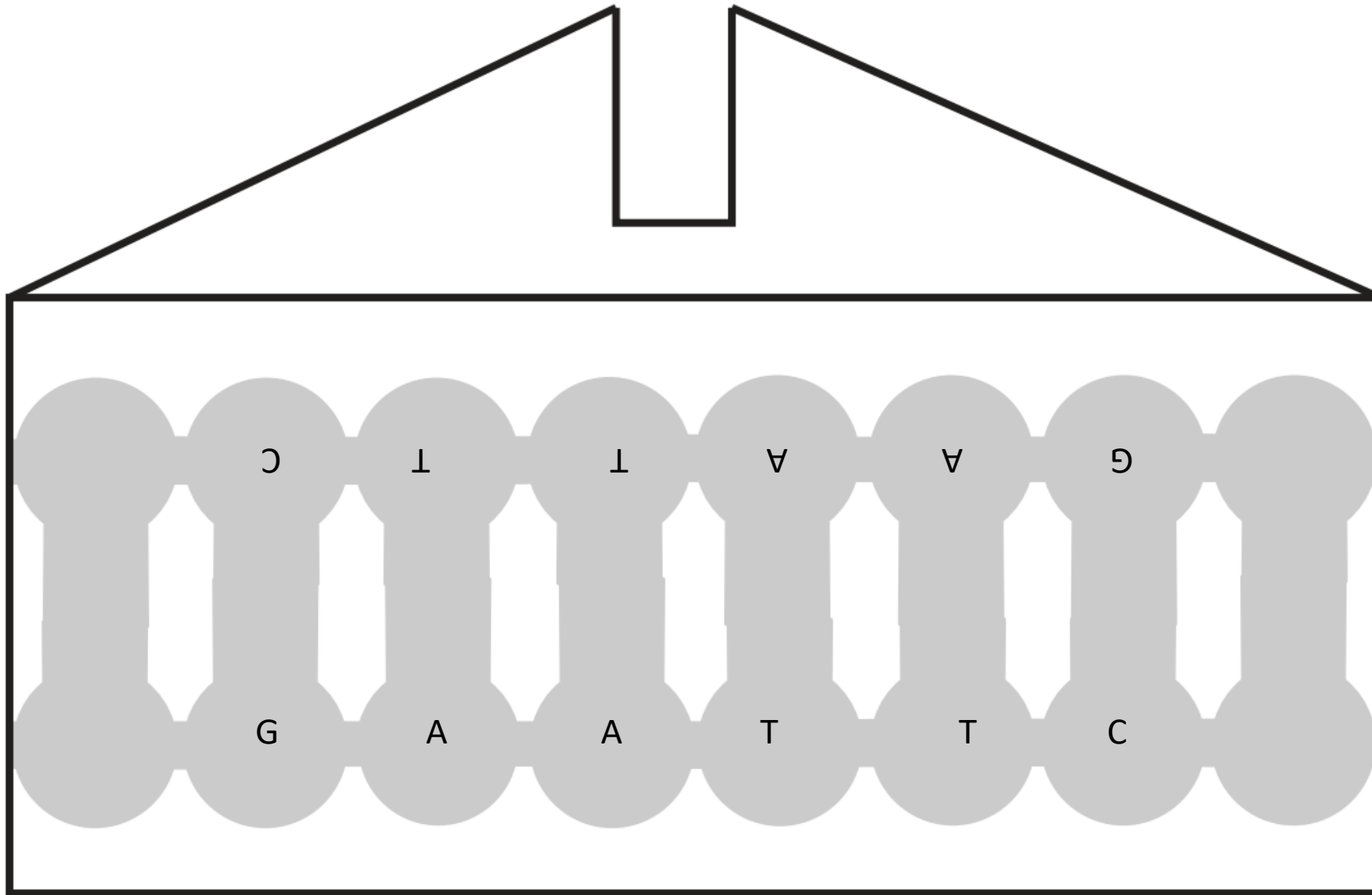
Which one should we use?



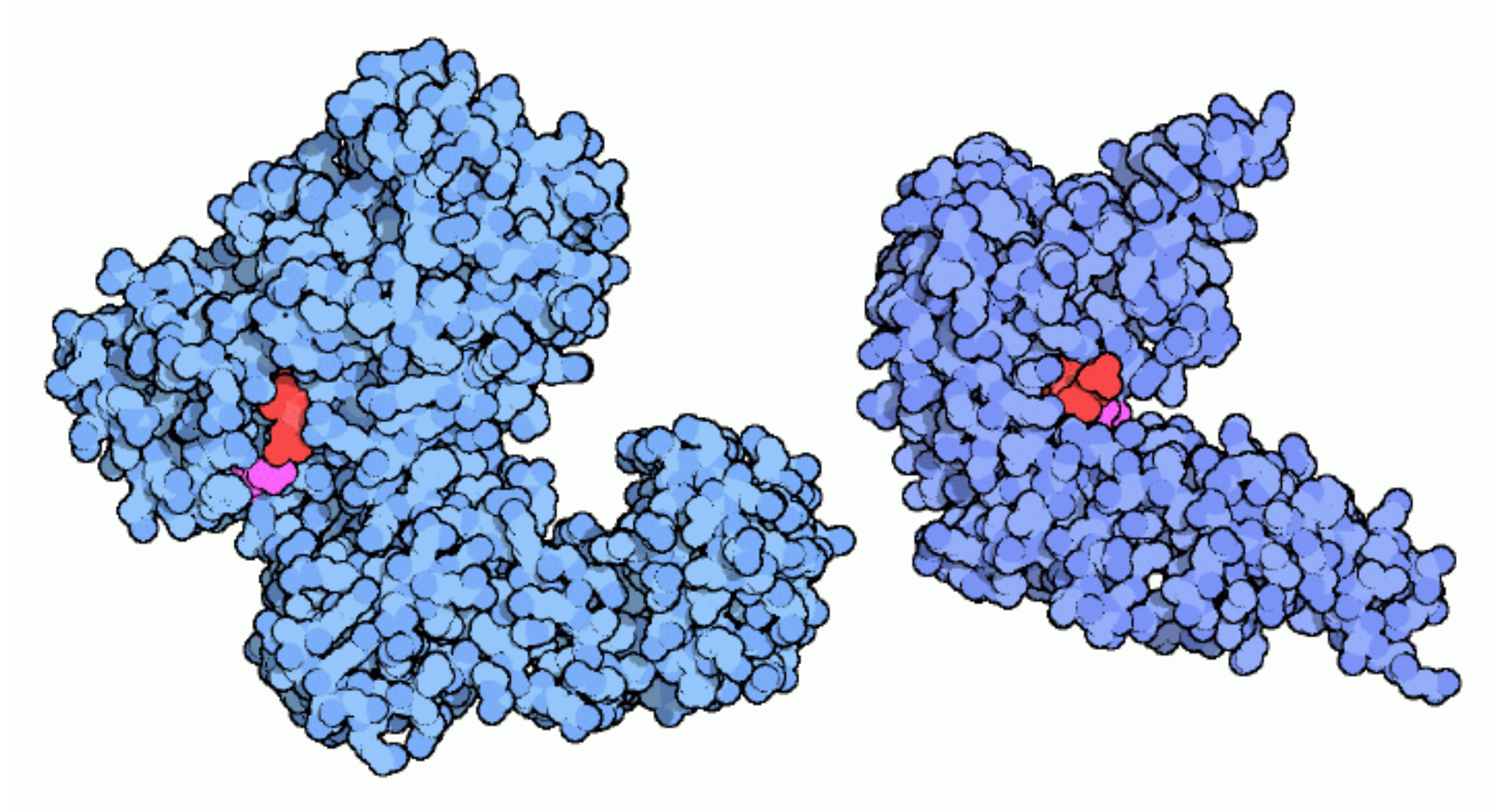
EcoRI !



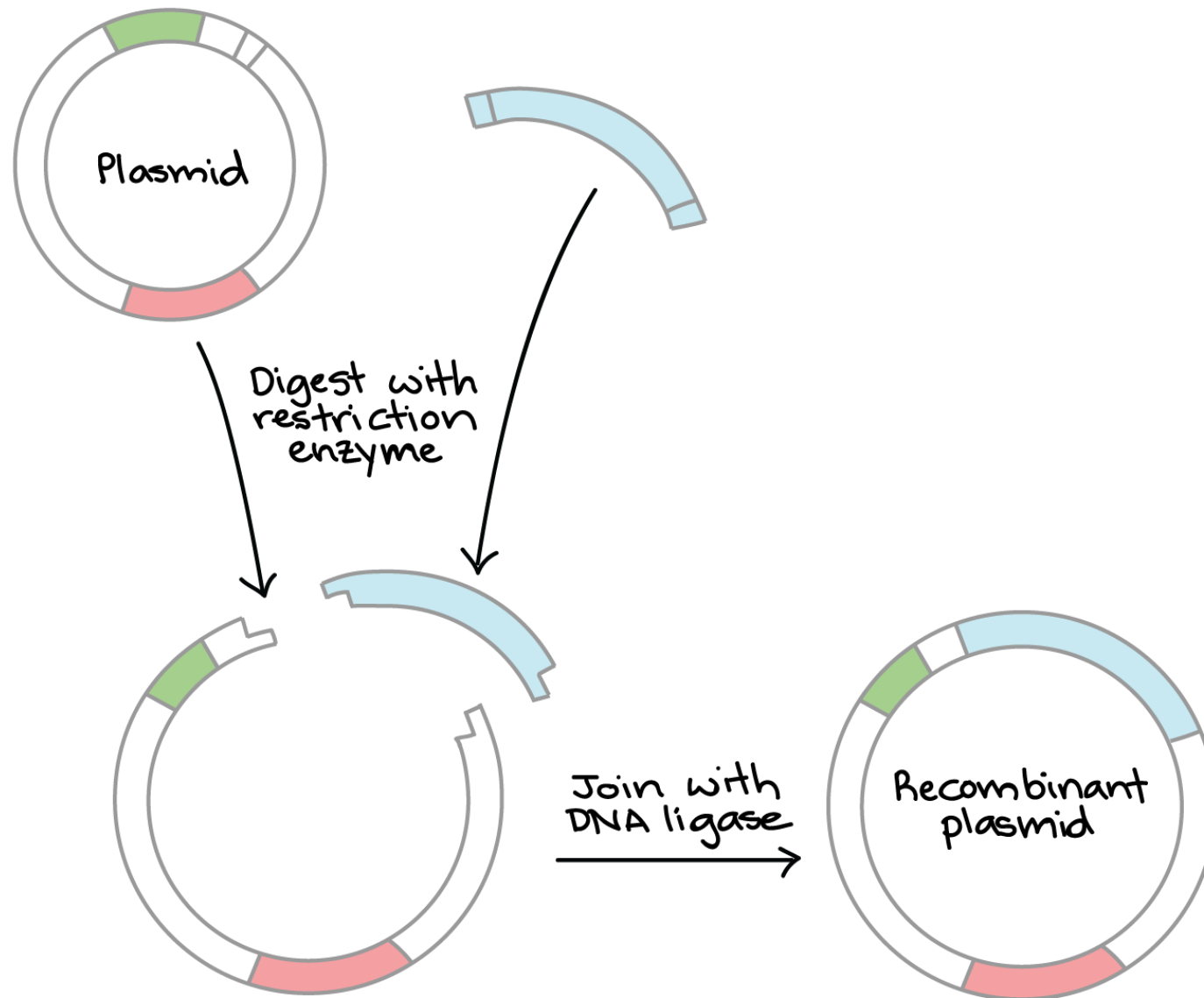
Build the EcoRI Sequence in gray -



DNA Ligase – Molecular tape



Digest and Ligate



Scan this QR code and fill out our survey!

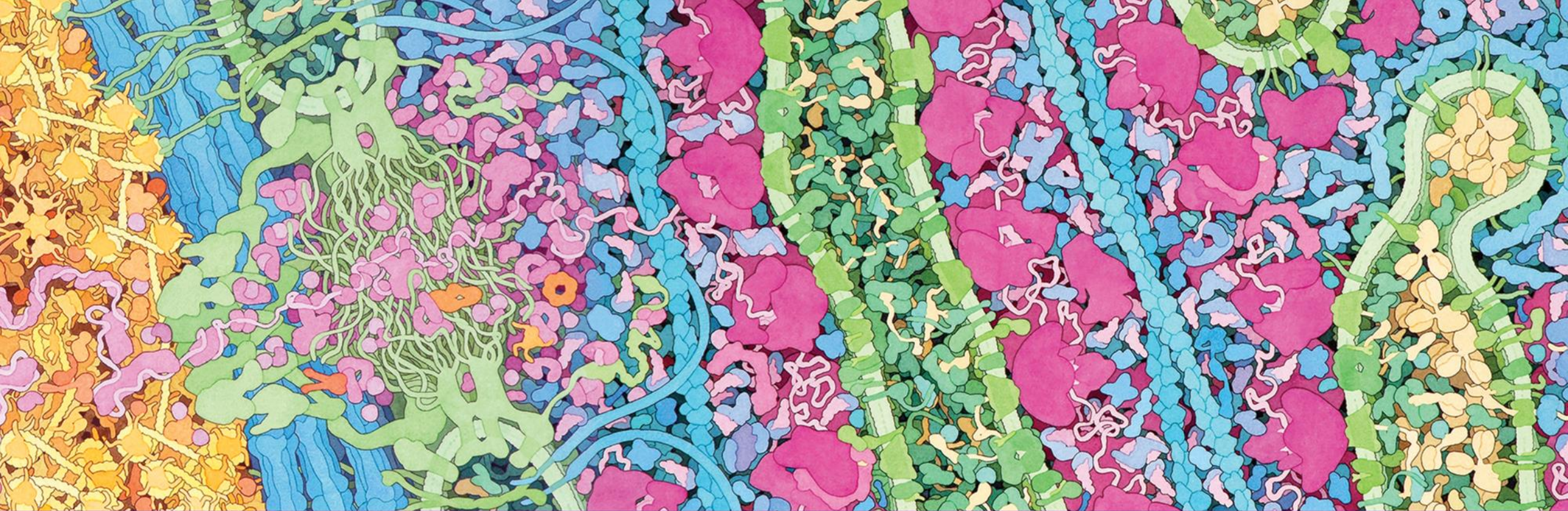


Show me your DNA molecule image
and I'll give you a model with AR
enhancement as a thank-you gift!



(6) The student explores the emerging field of biotechnology. The student is expected to:

- (A) define biotechnology and provide examples of biotechnology products such as recombinant proteins, fermented foods, biopharmaceuticals, and genetically modified foods;
- (i) Define biotechnology
- (ii) Provide examples of biotechnology products



3d molecular
designs

Transforming Science Learning