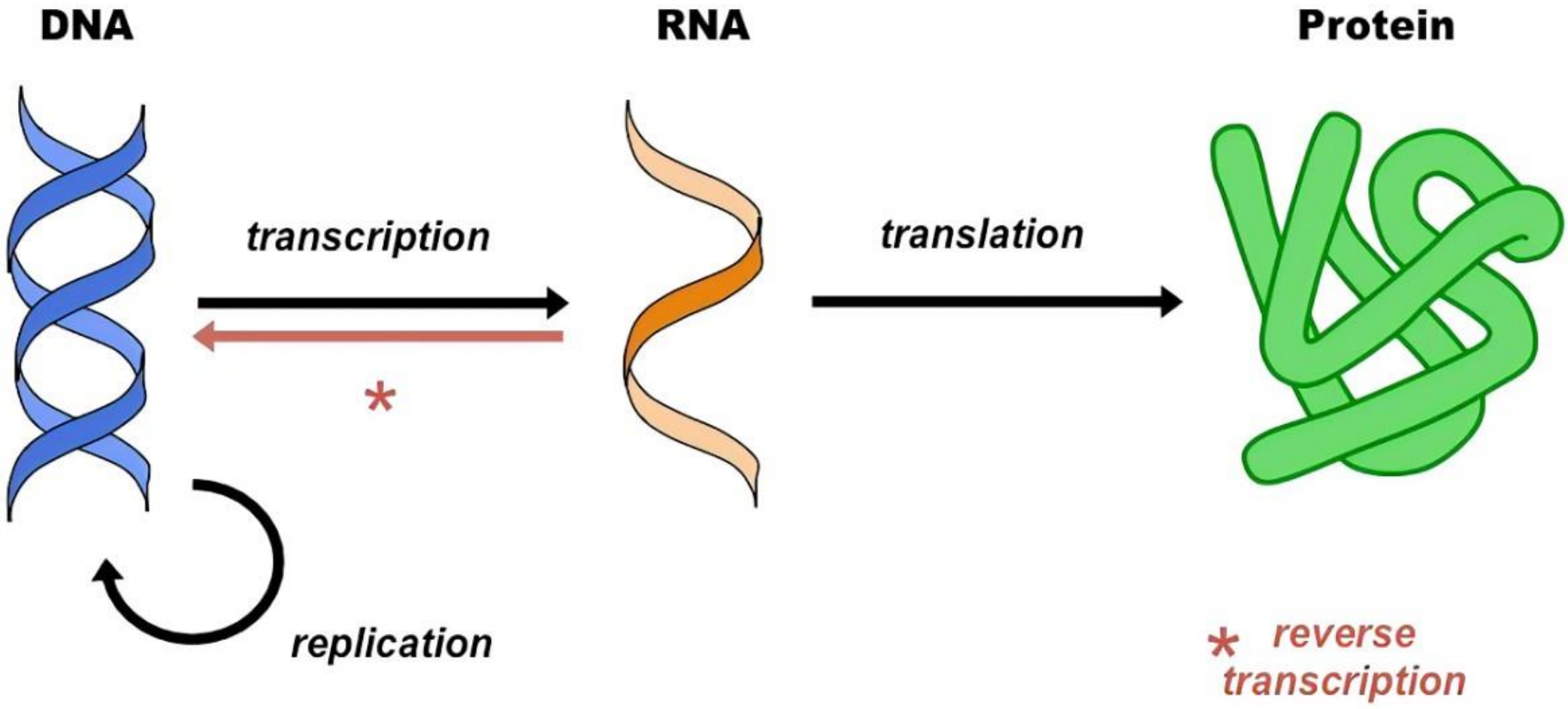
3d molecular designs
Transforming Science Learning

Hurry up and wait… Plasmid Ligations

Mark “Arnie” Arnholt
3D Molecular Designs

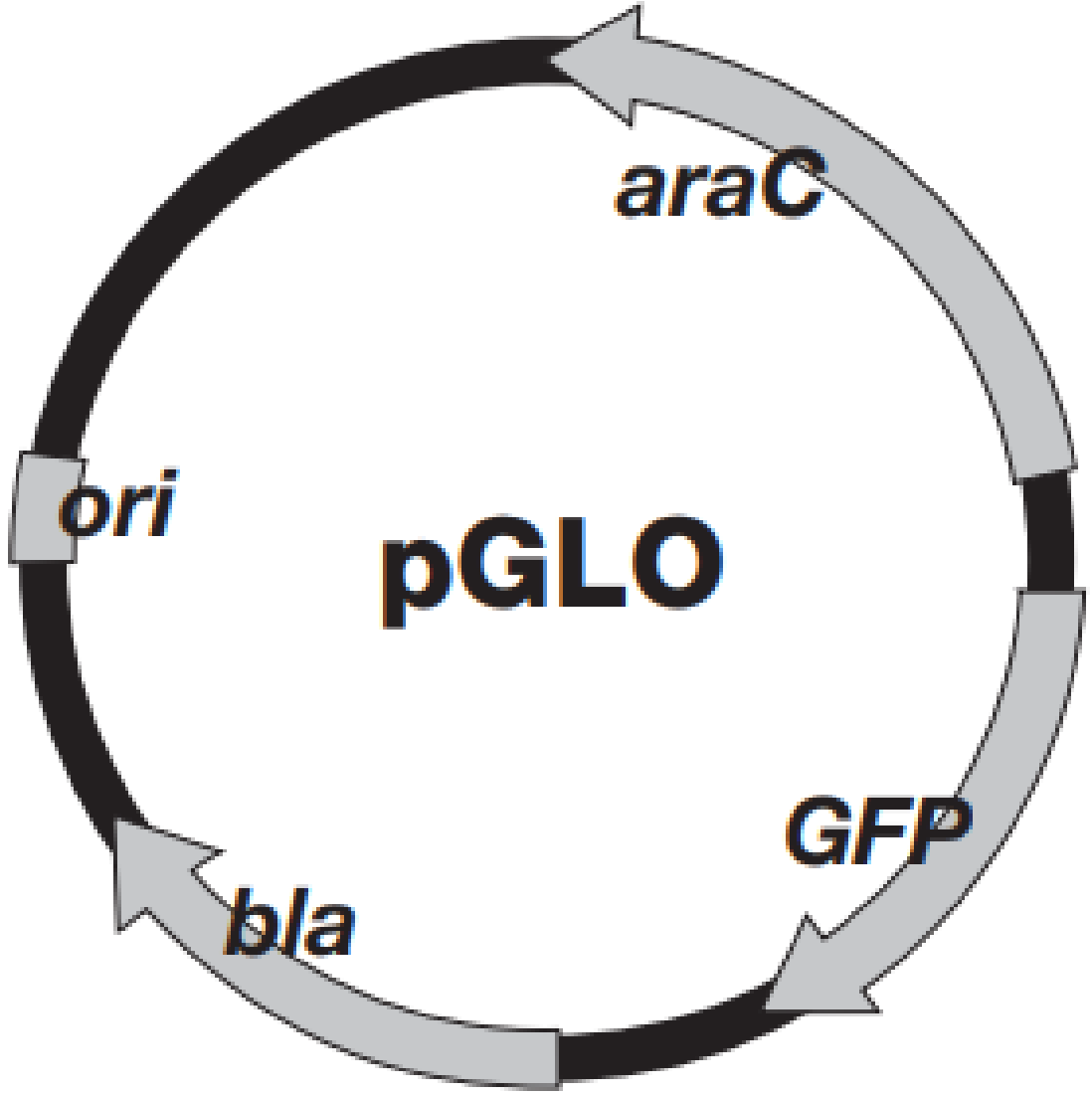
The Central Dogma of Biology



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?

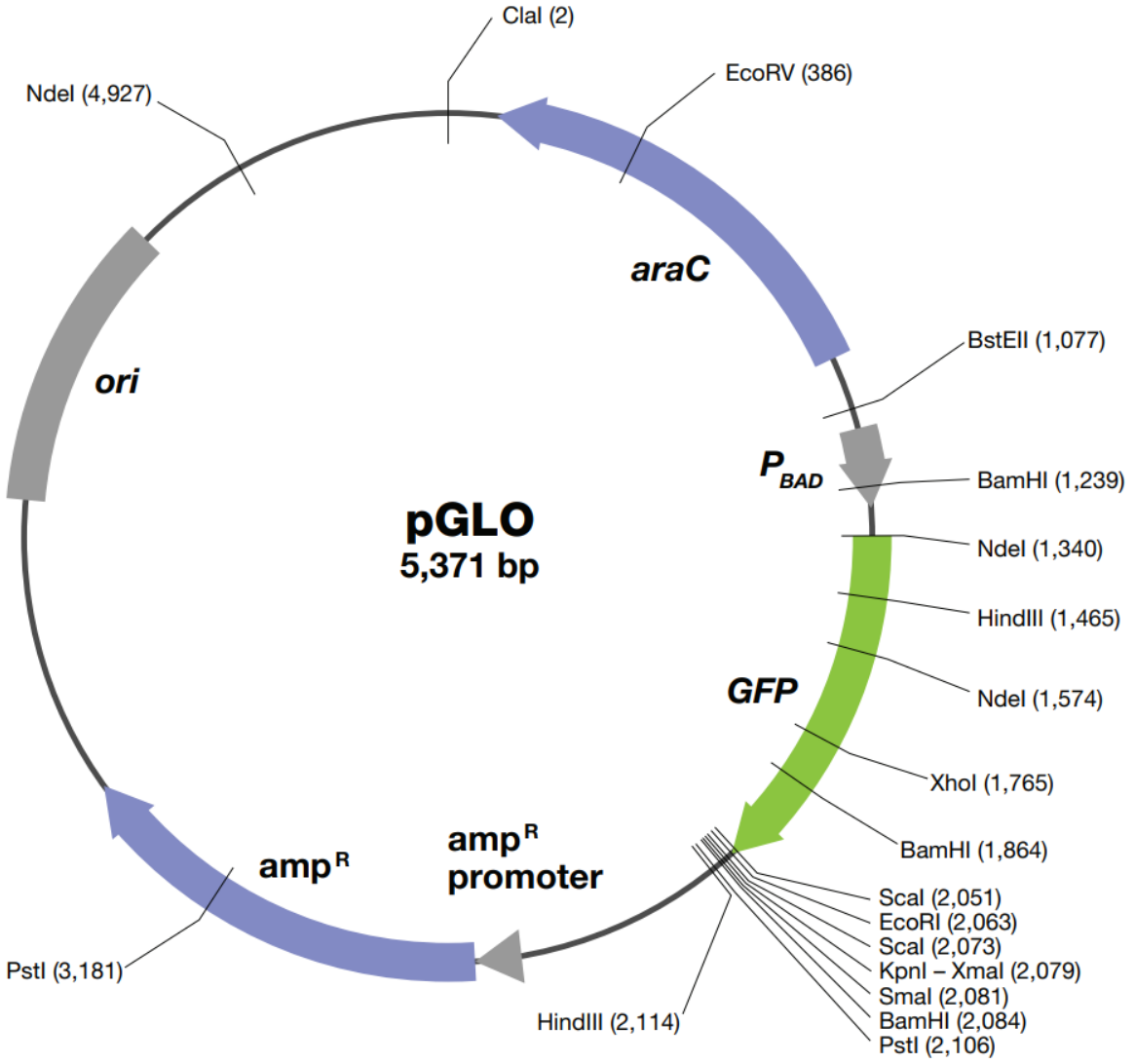


A little more detailed

pGLO is a 5,371 bp plasmid

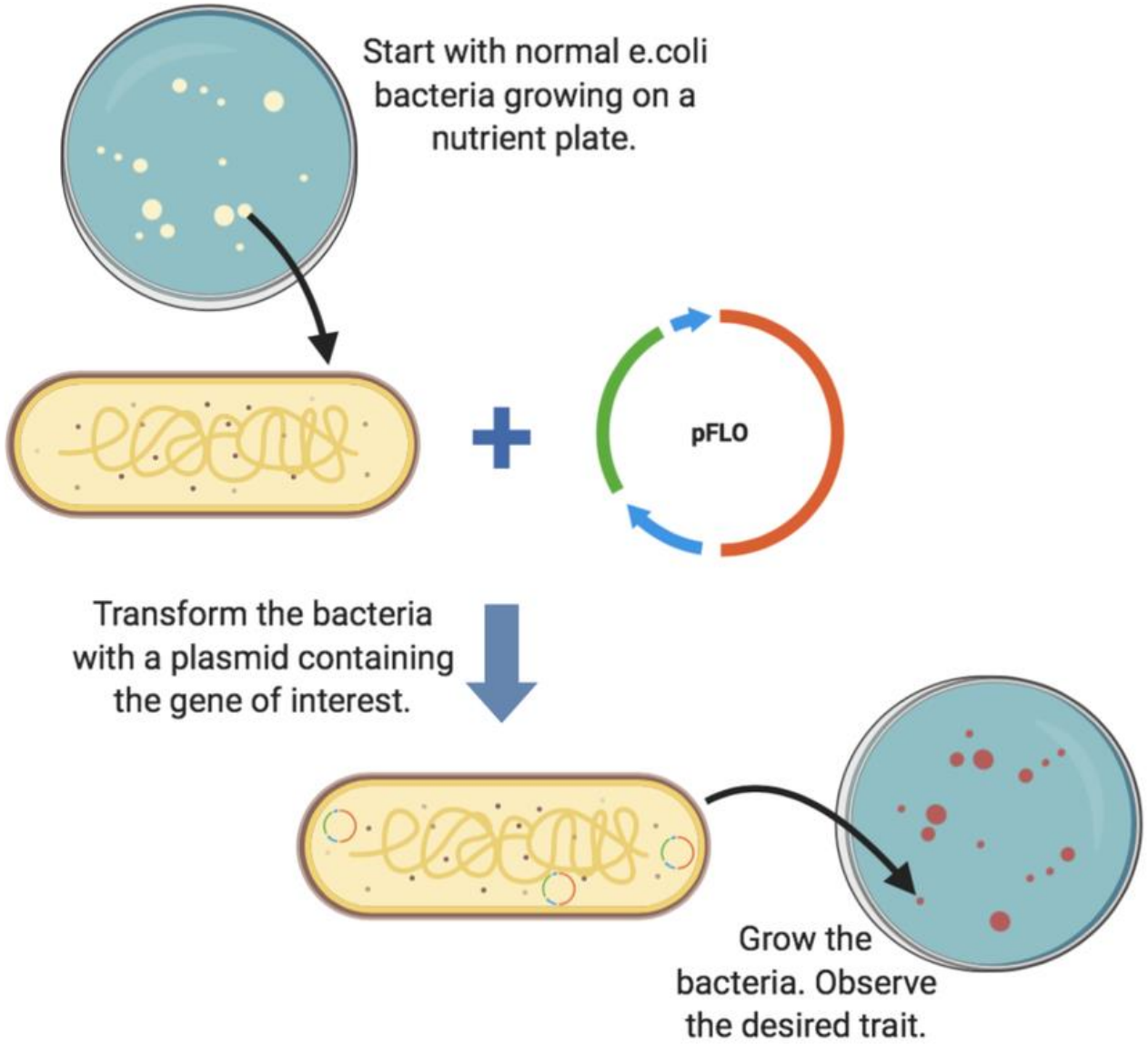
Promoters and restriction sites are identified

See handout-



Harnessing the power of microbes

How do I transform a bacterial cell?



Bacterial Transformation

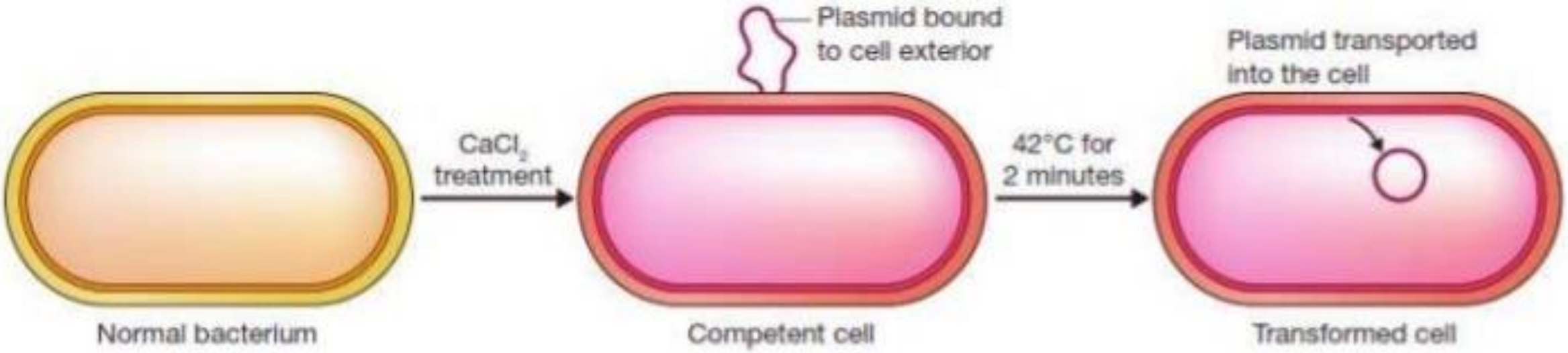
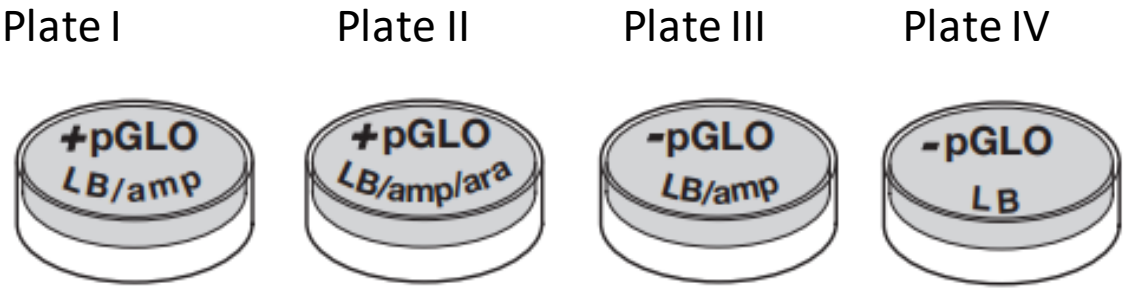


Fig 5.3. Brown, T.A. 1990. Gene Cloning and DNA Analysis

Screening our products:

Predict our outcomes on these plates.

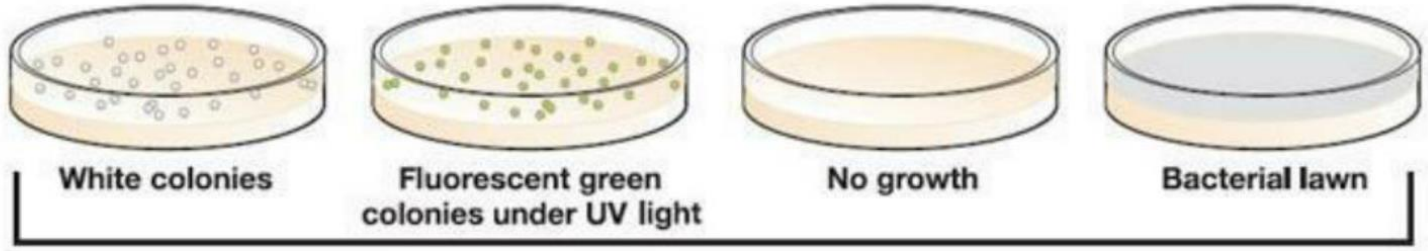
-pGLO have not been transformed
+ pGLO have our plasmid



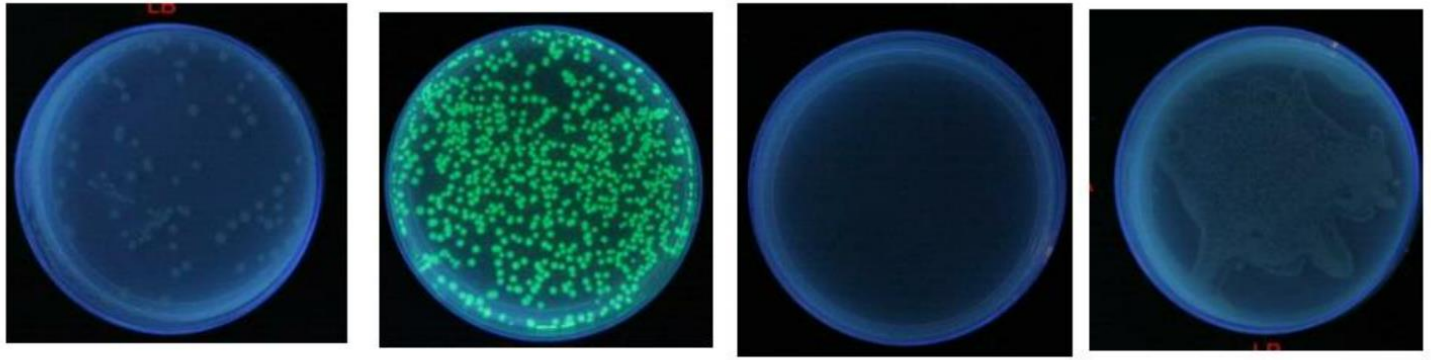
Predicted growth / glow

I	II	III	IV

Predicted results

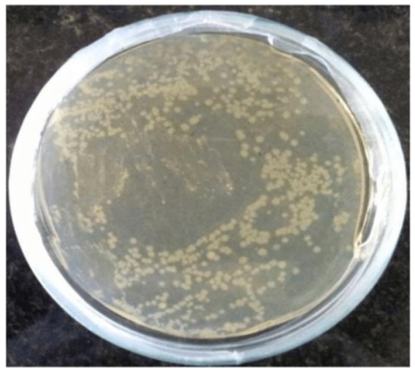
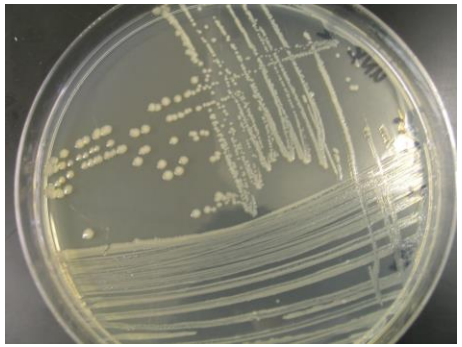


+pGLO LB/amp	+pGLO LB/amp/ara	-pGLO LB/amp	-pGLO LB



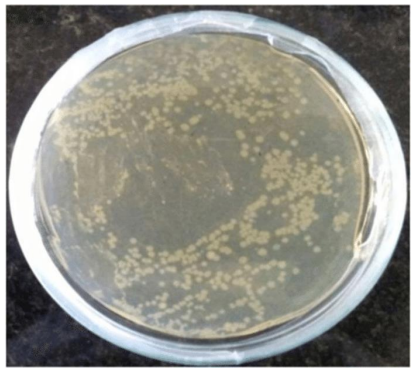
I	II	III	IV
A few grow no glow	A few grow and glow	No growth	Lawn (TMTC)

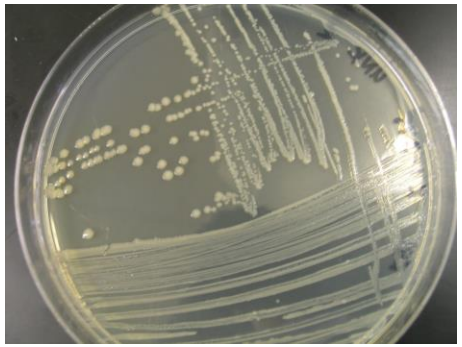
Our Actual plates:

I	II	III	IV
Lawn	Lawn	Lawn	Lawn

What happened?





I	II	III	IV
Lawn	Lawn	Lawn	Lawn

How can we troubleshoot this?

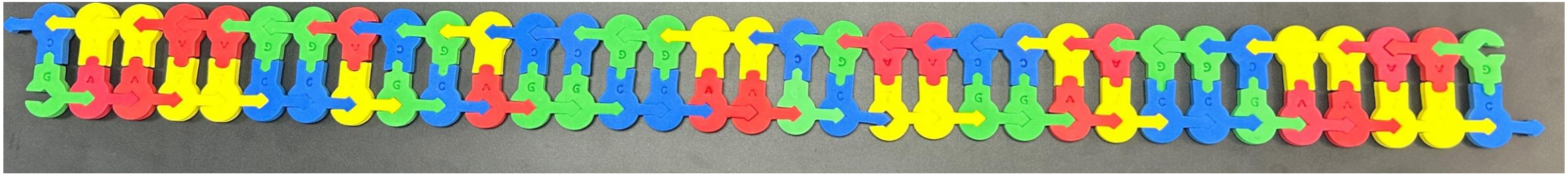
How do I get a gene of interest into a plasmid?

- 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 out target gene…



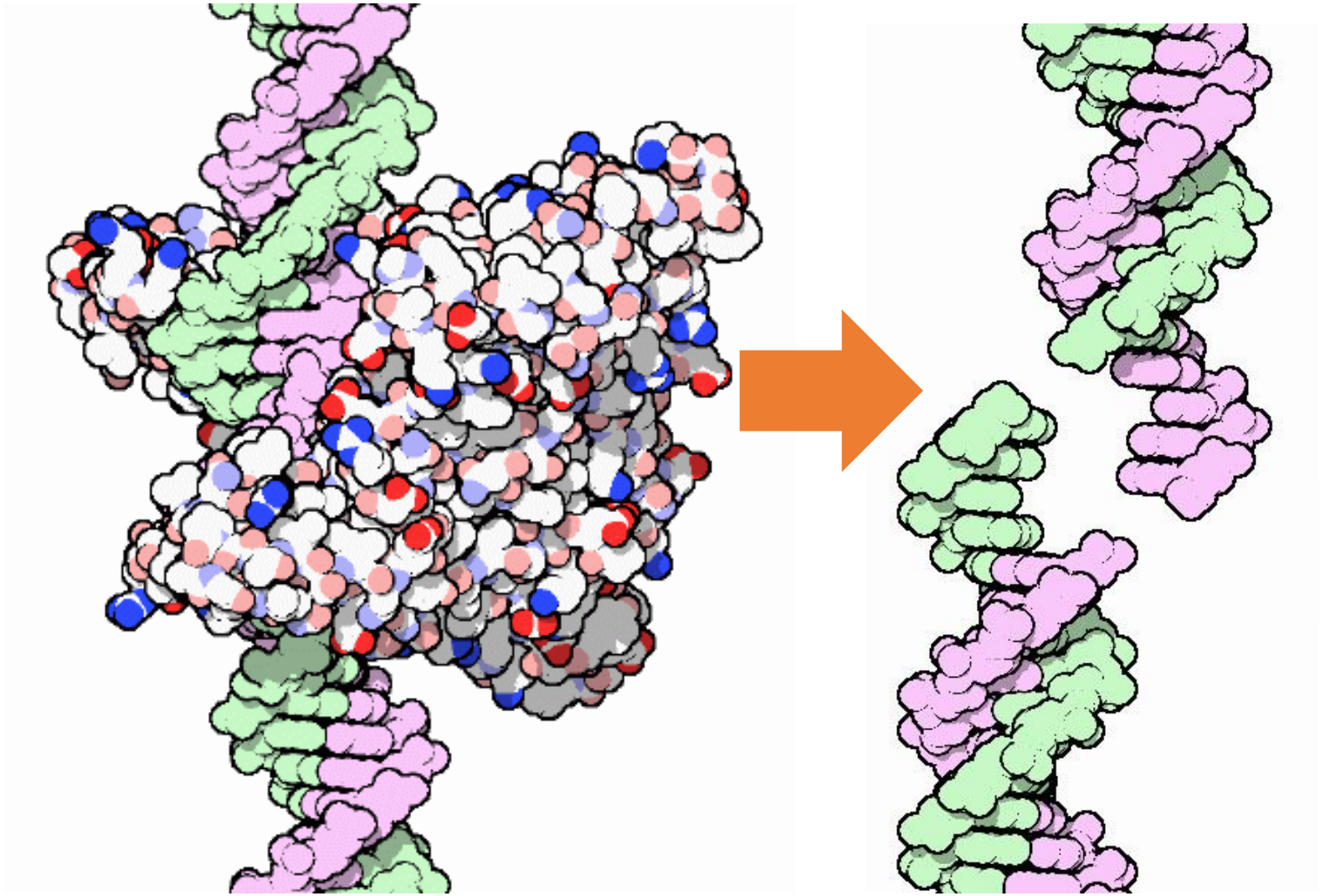
Our target gene has been truncated as the actual gene would be hundreds of BP’s long.

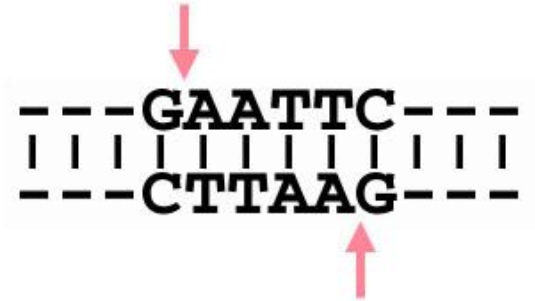
It also has lots of restriction sites in it-

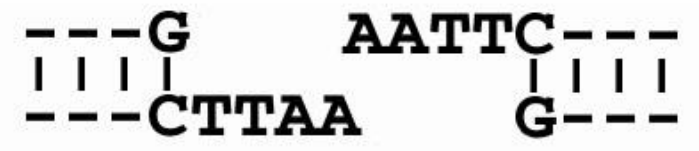
Restriction enzymes – Molecular scissors

- 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





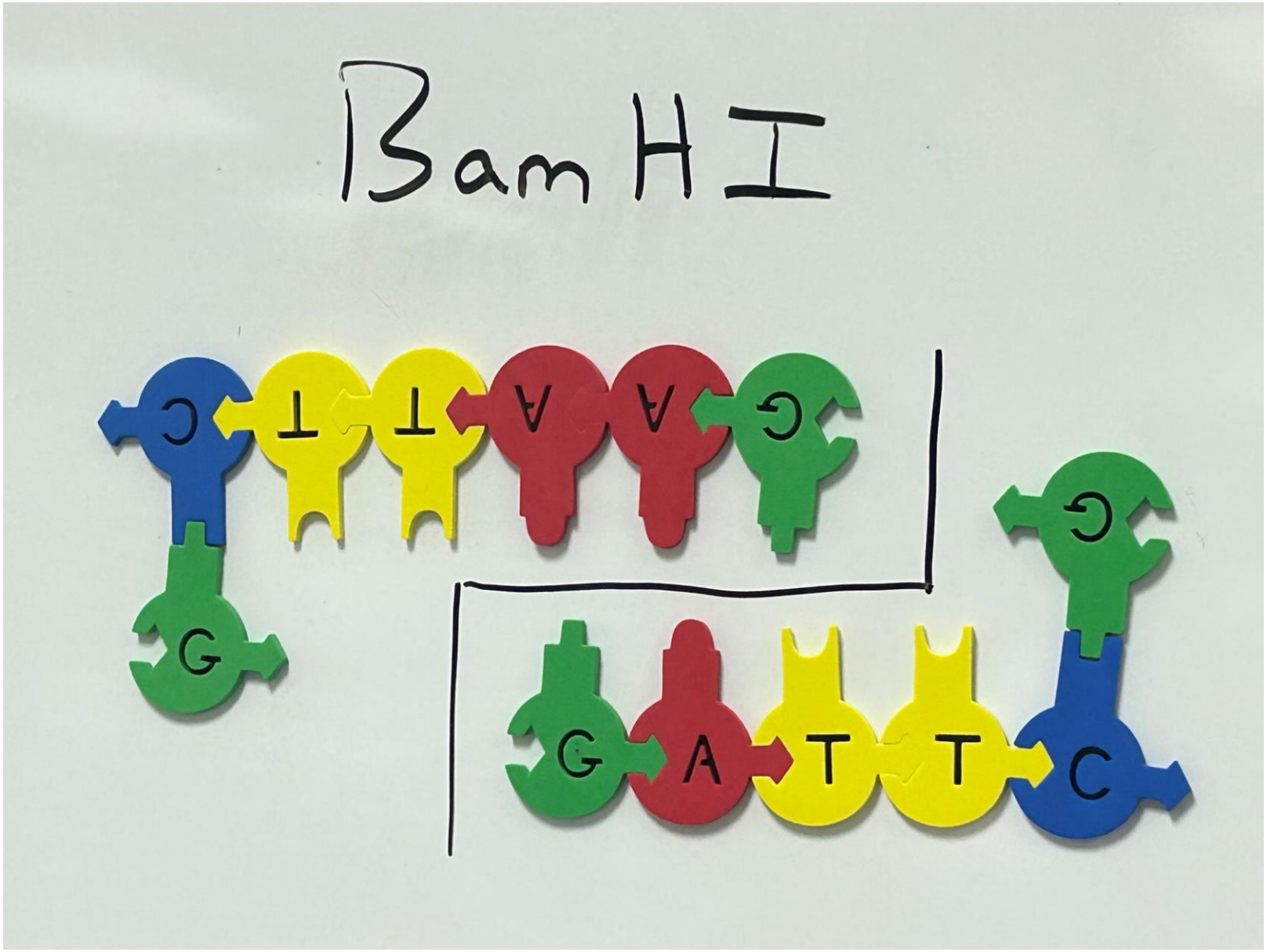


We will need to digest our target DNA

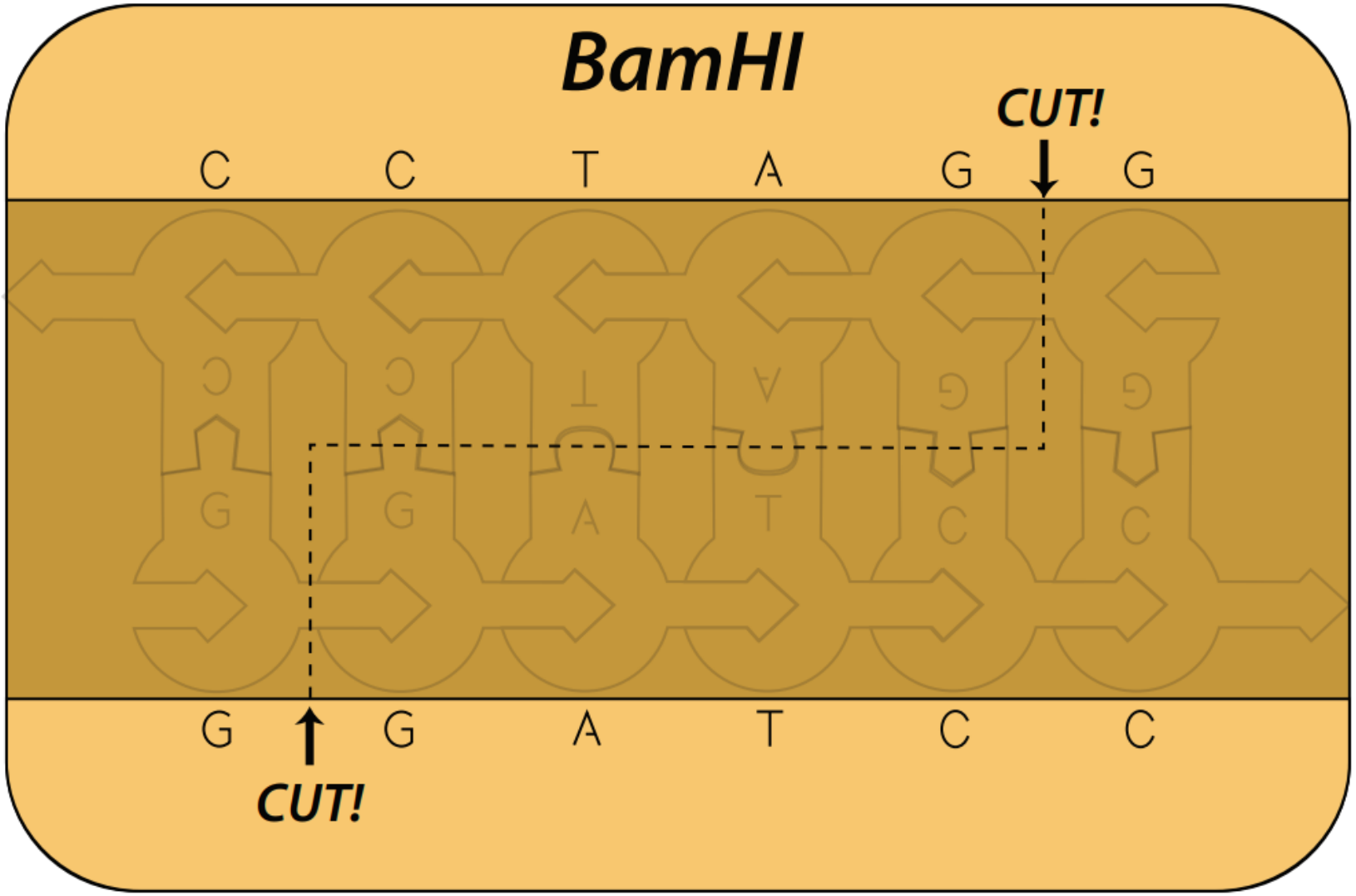
Restriction enzymes at hand include:

Eco RI
Bam HI
PstI
HIND III
Hae III

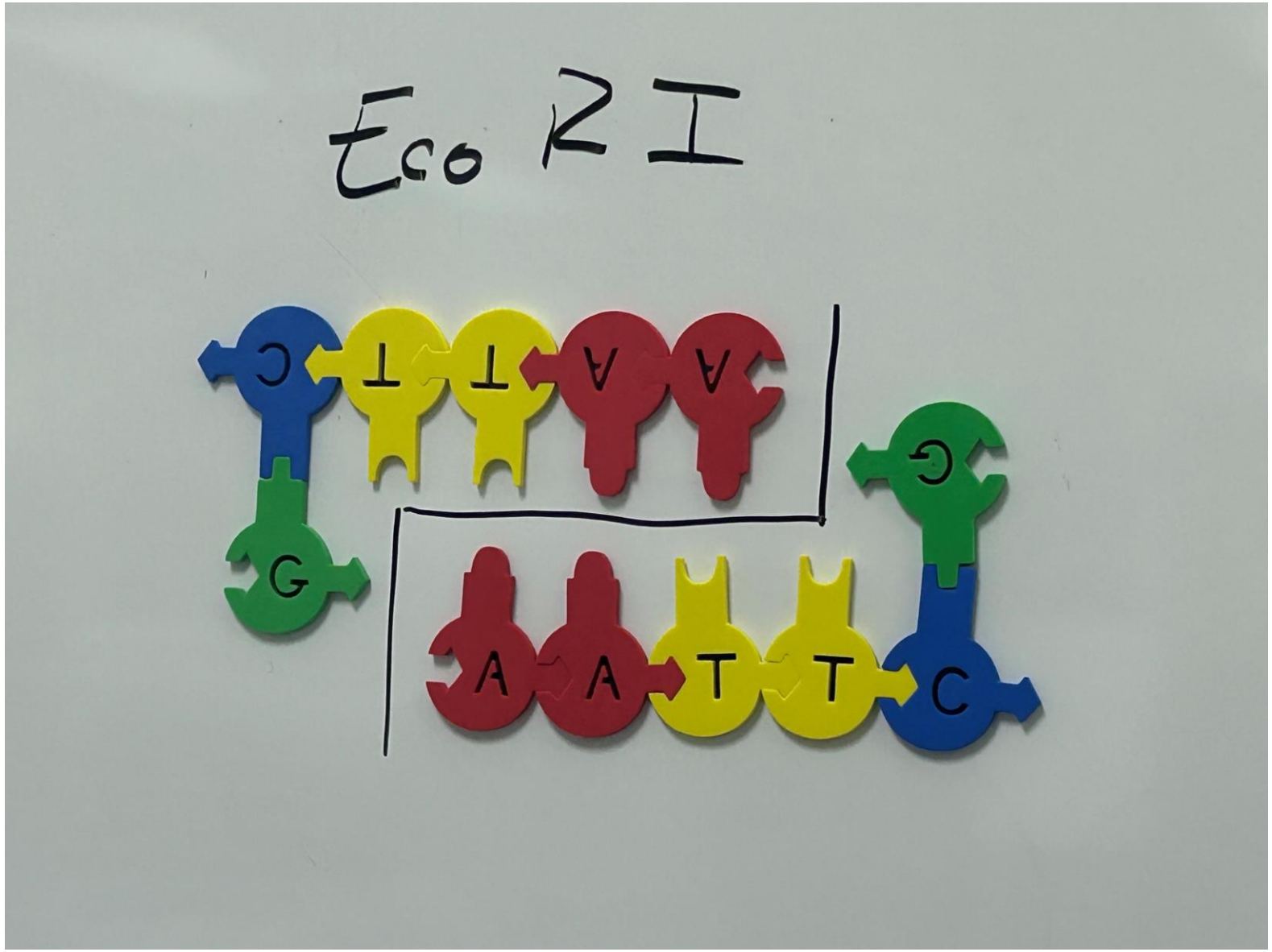
BamHI



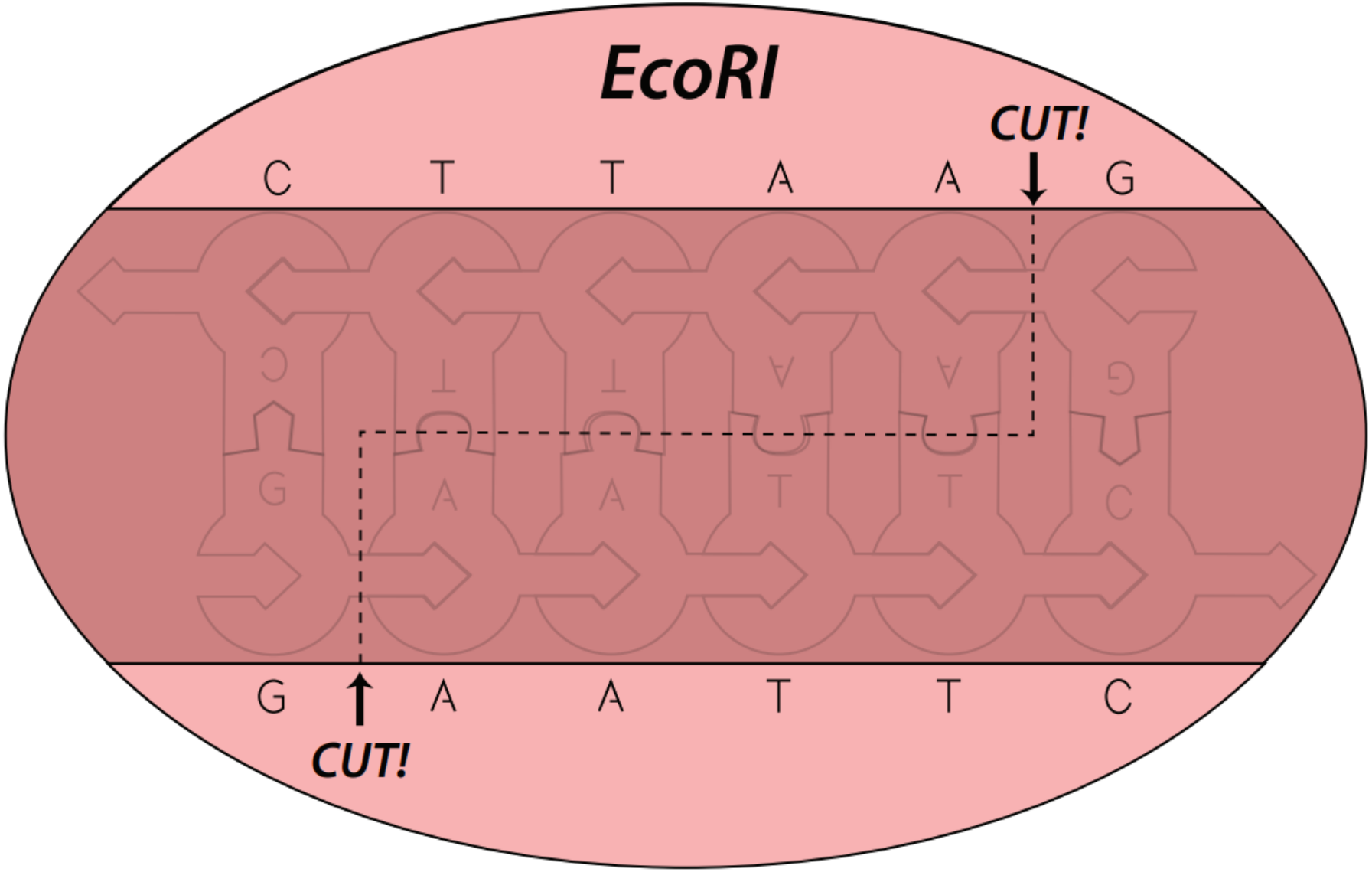
BamHI



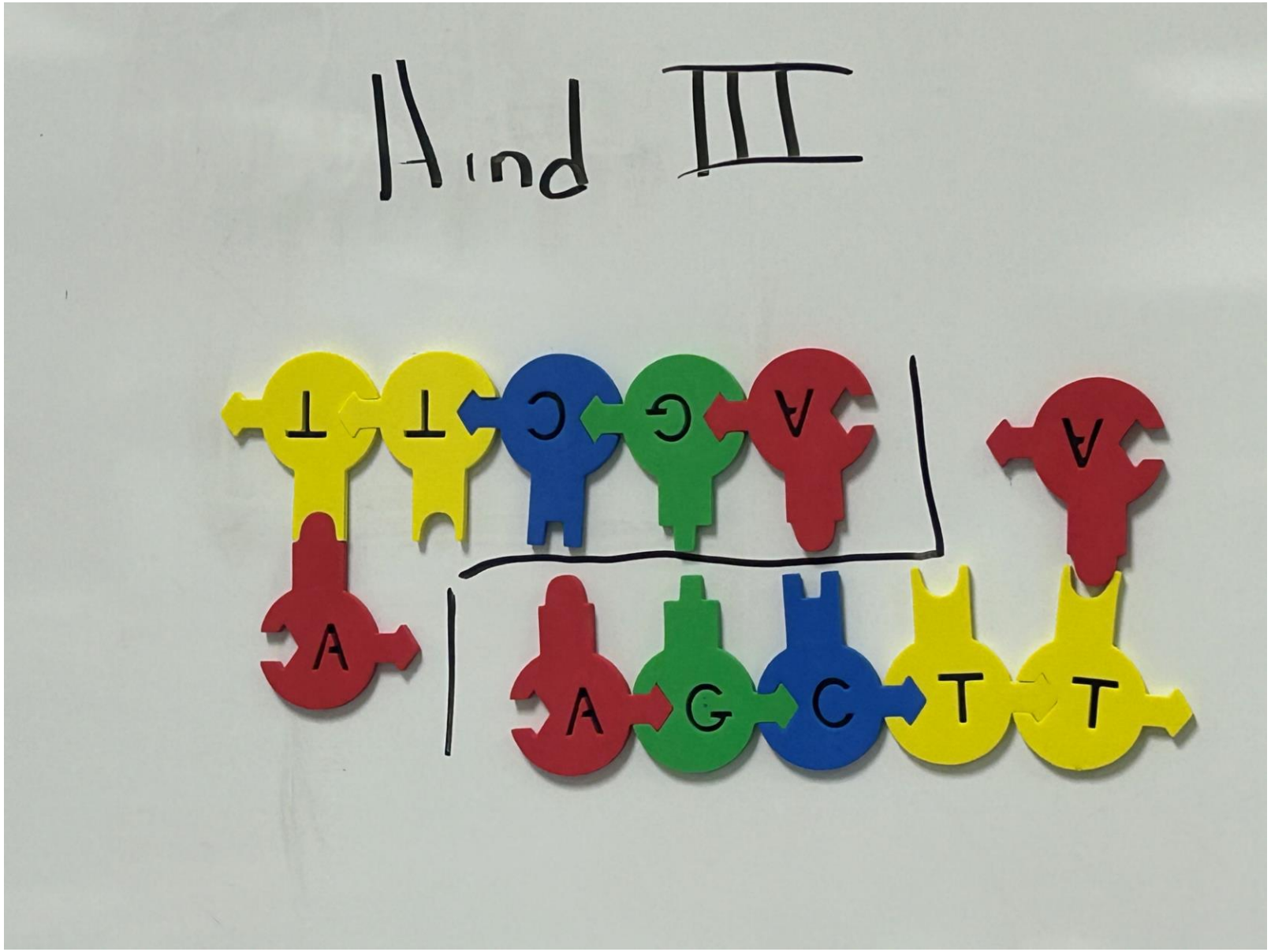
EcoRI



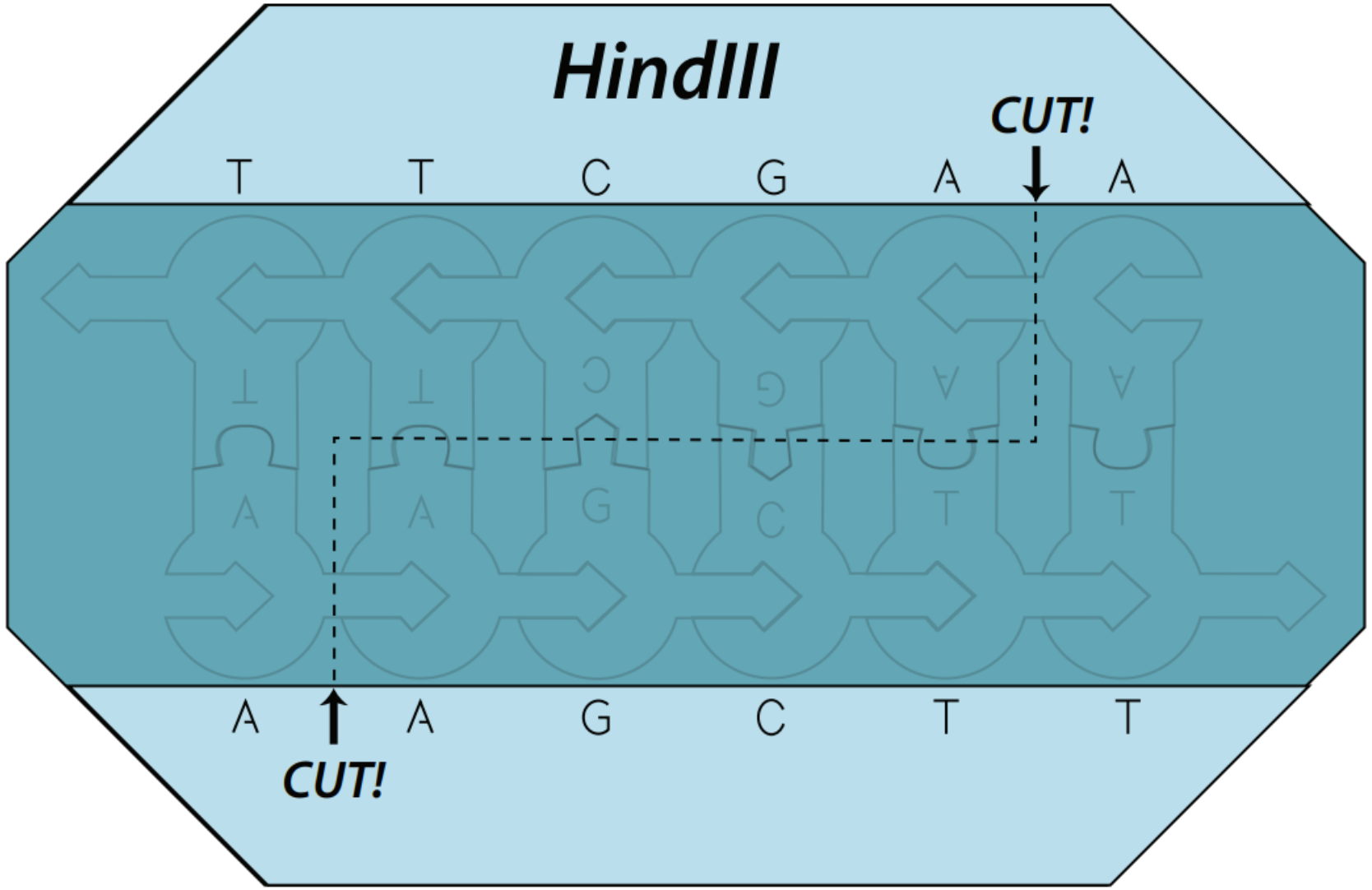
EcoRI



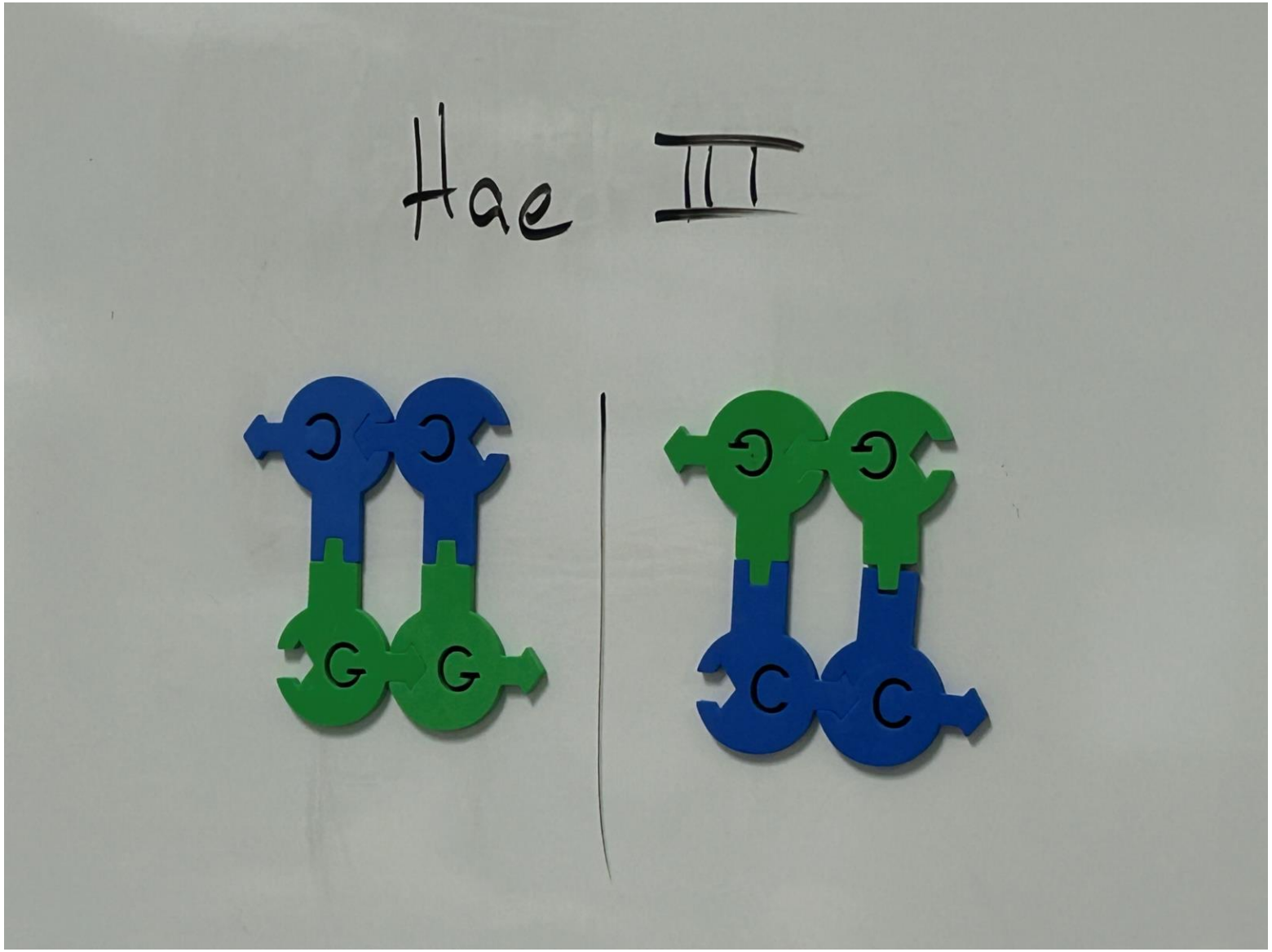
Hind III



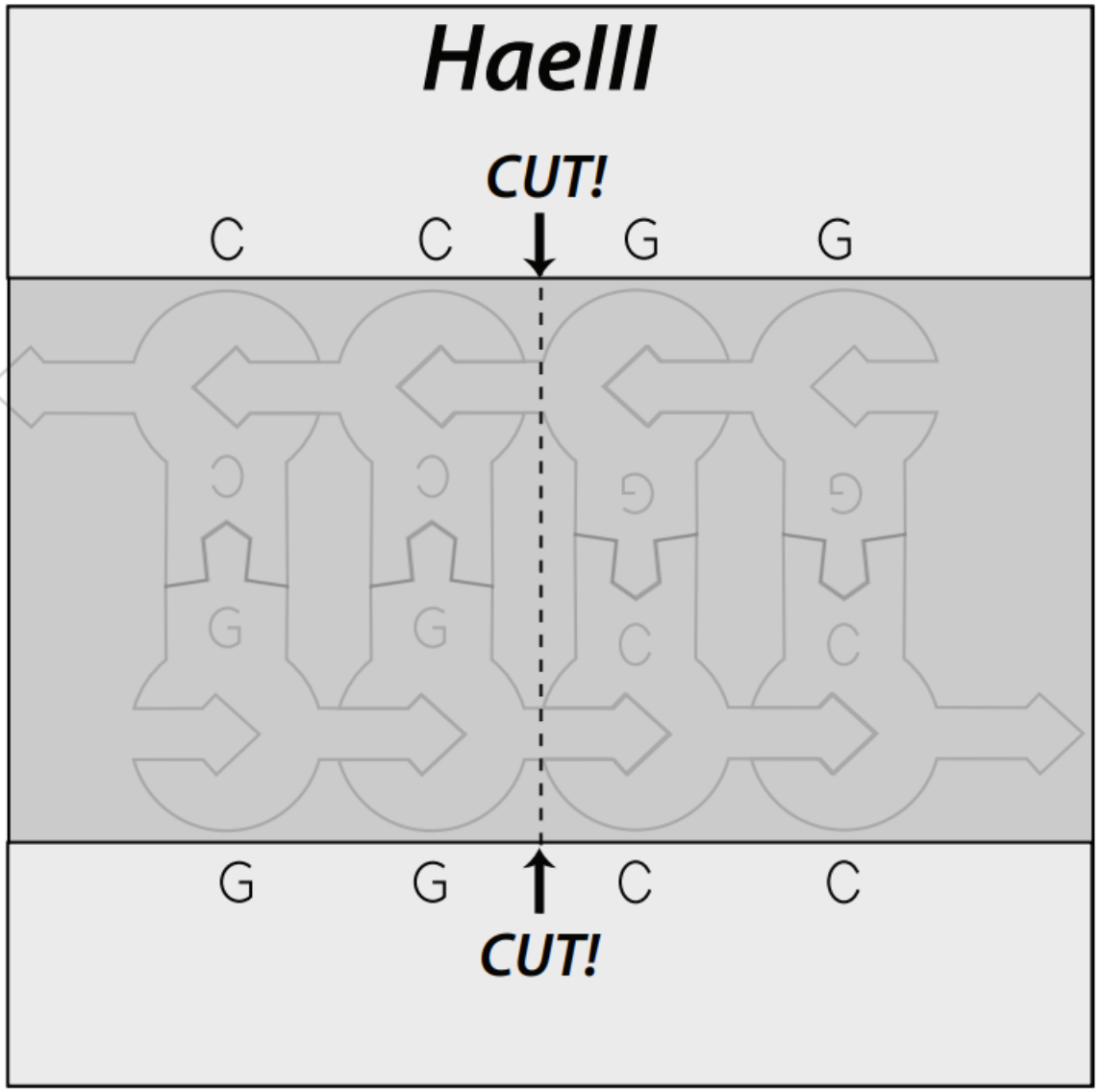
Hind III



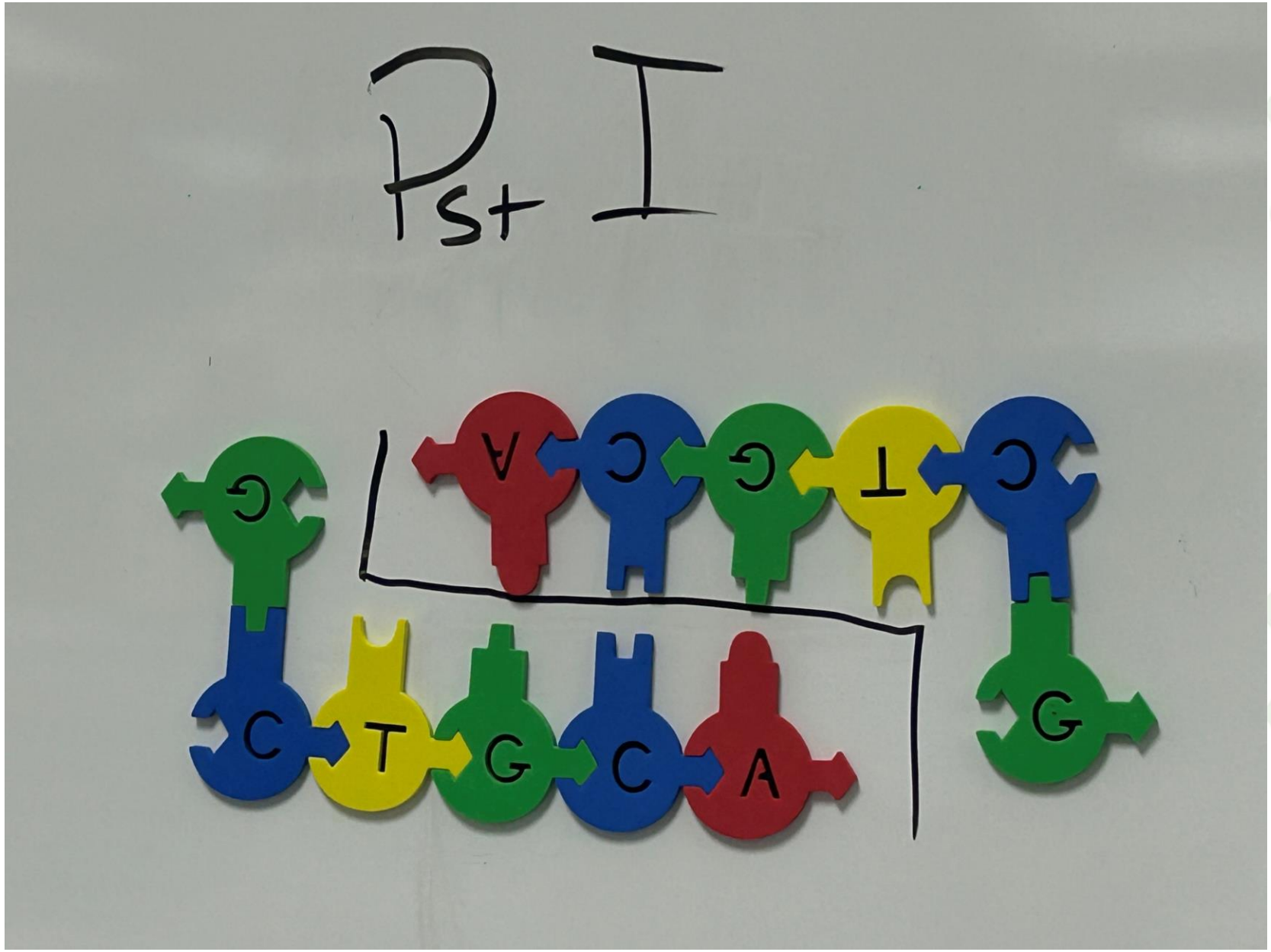
Hae III



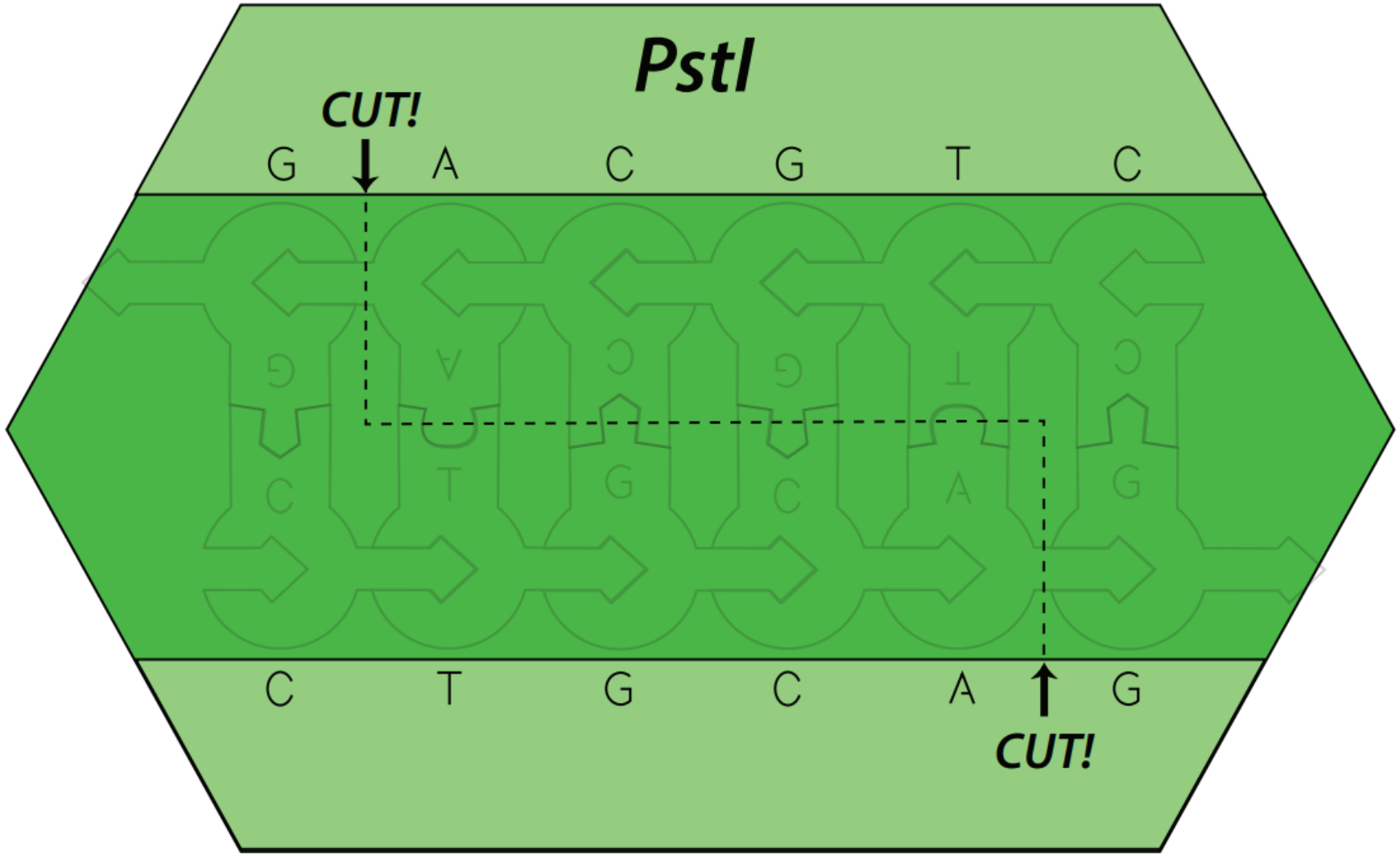
Hae III



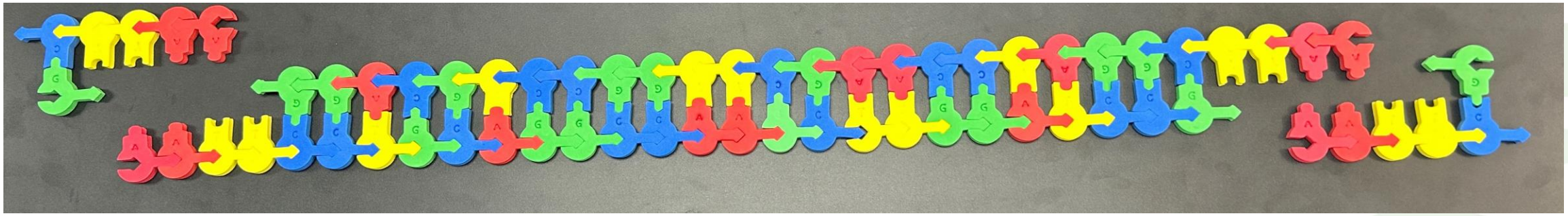
PstI



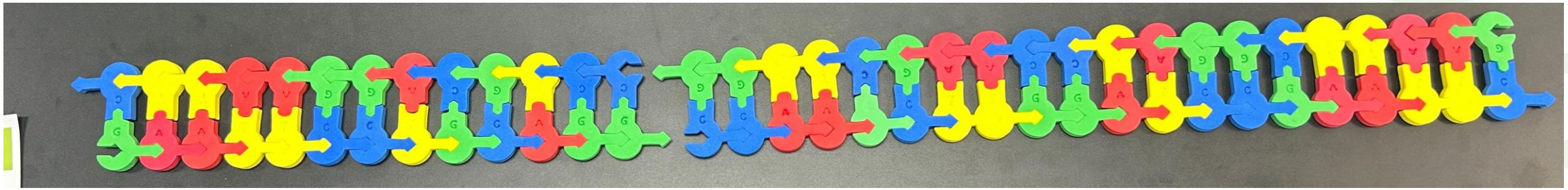
PstI

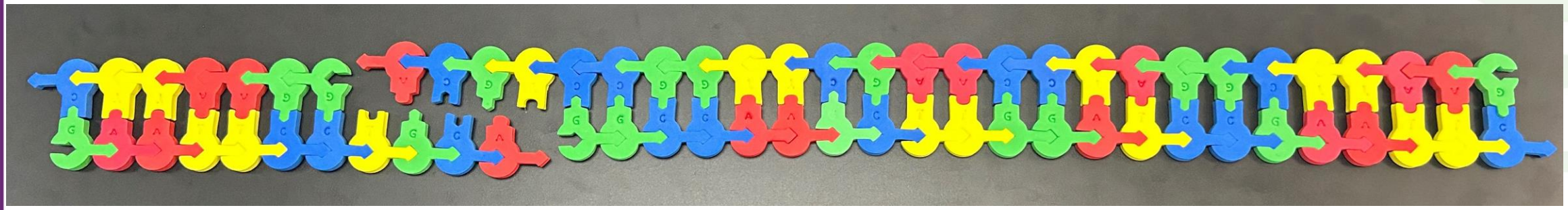


Our digests…



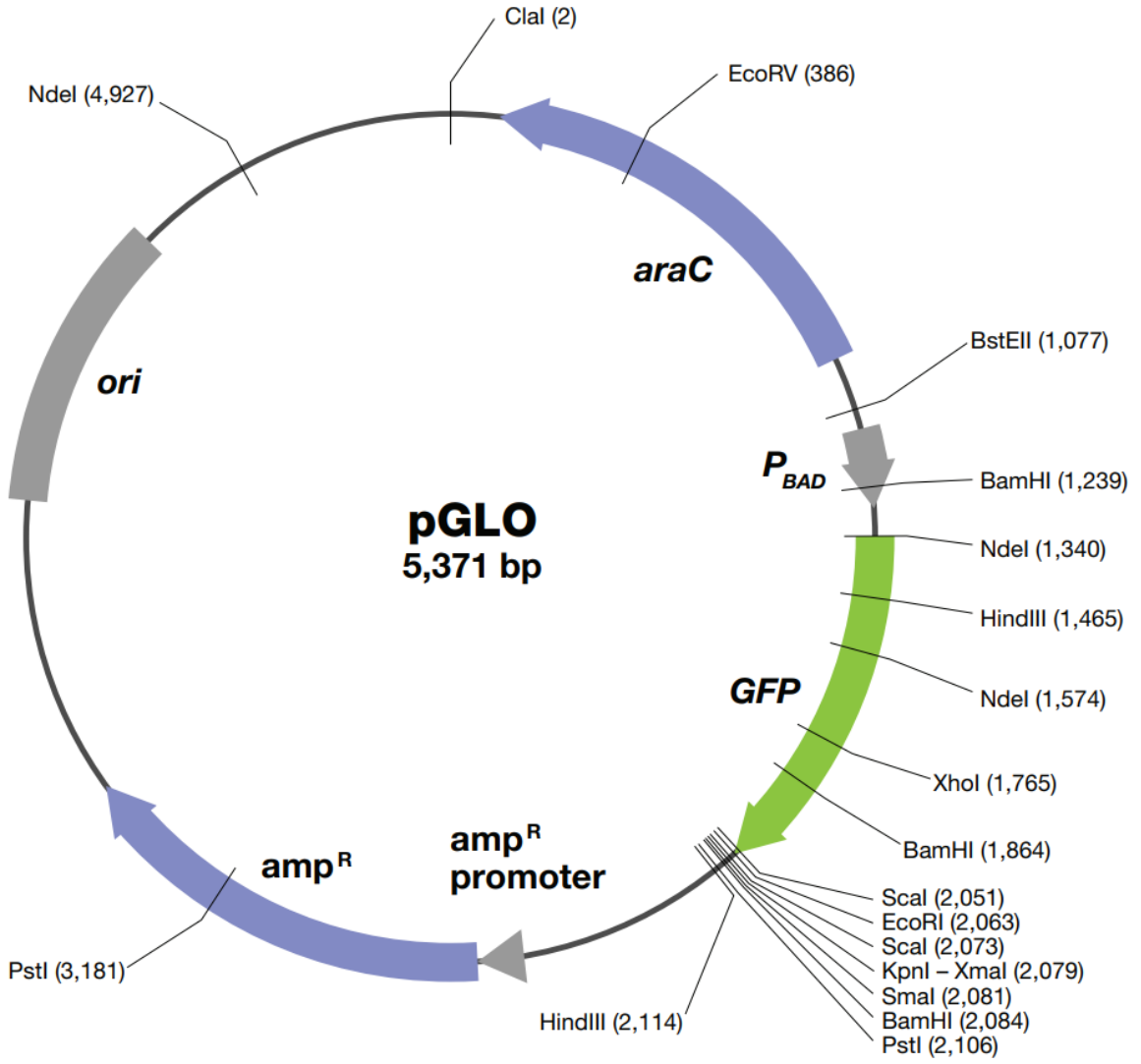
Our digests…





Our actual map:

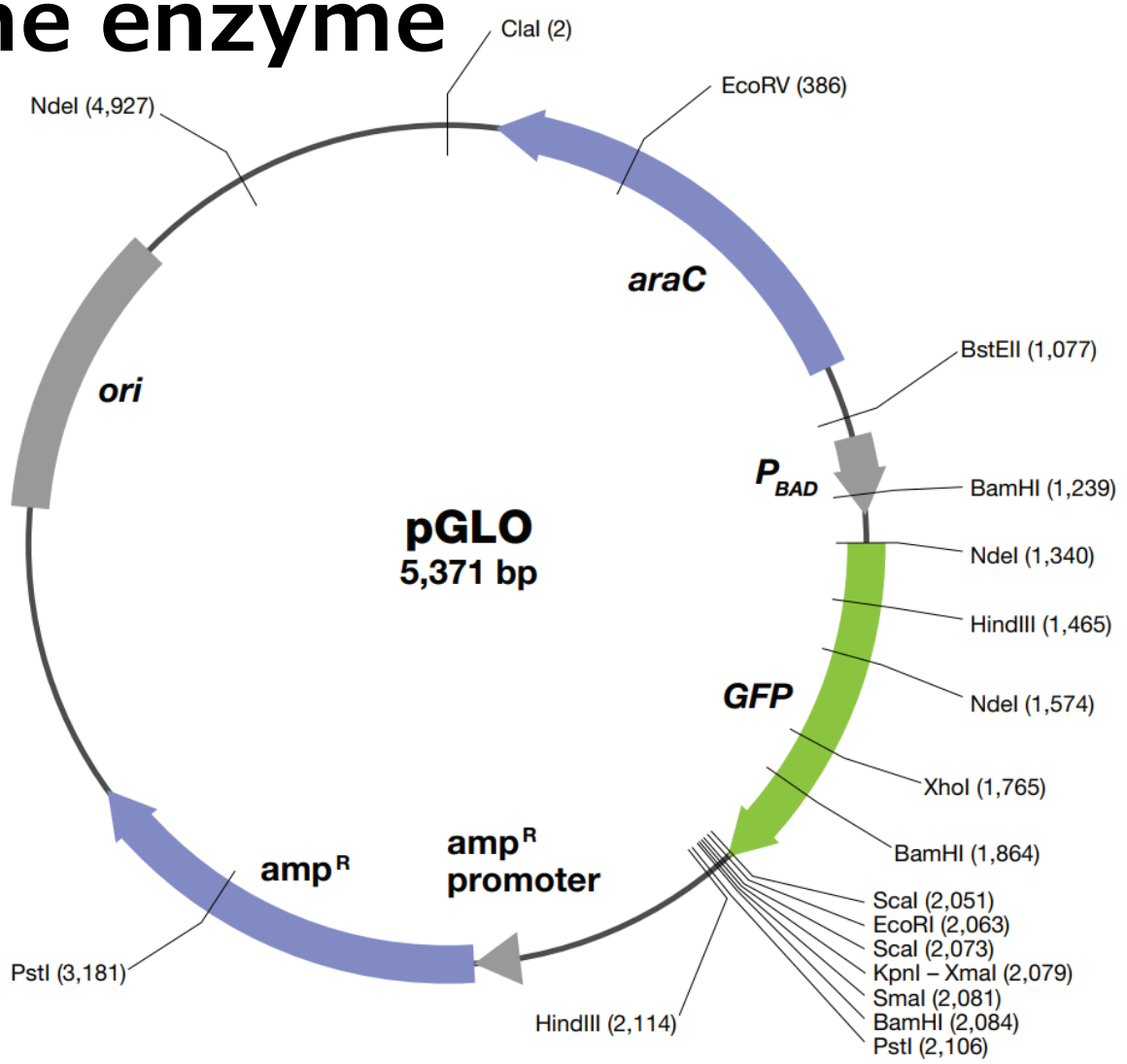
All of our potential restriction sites have been identified:
See handout-



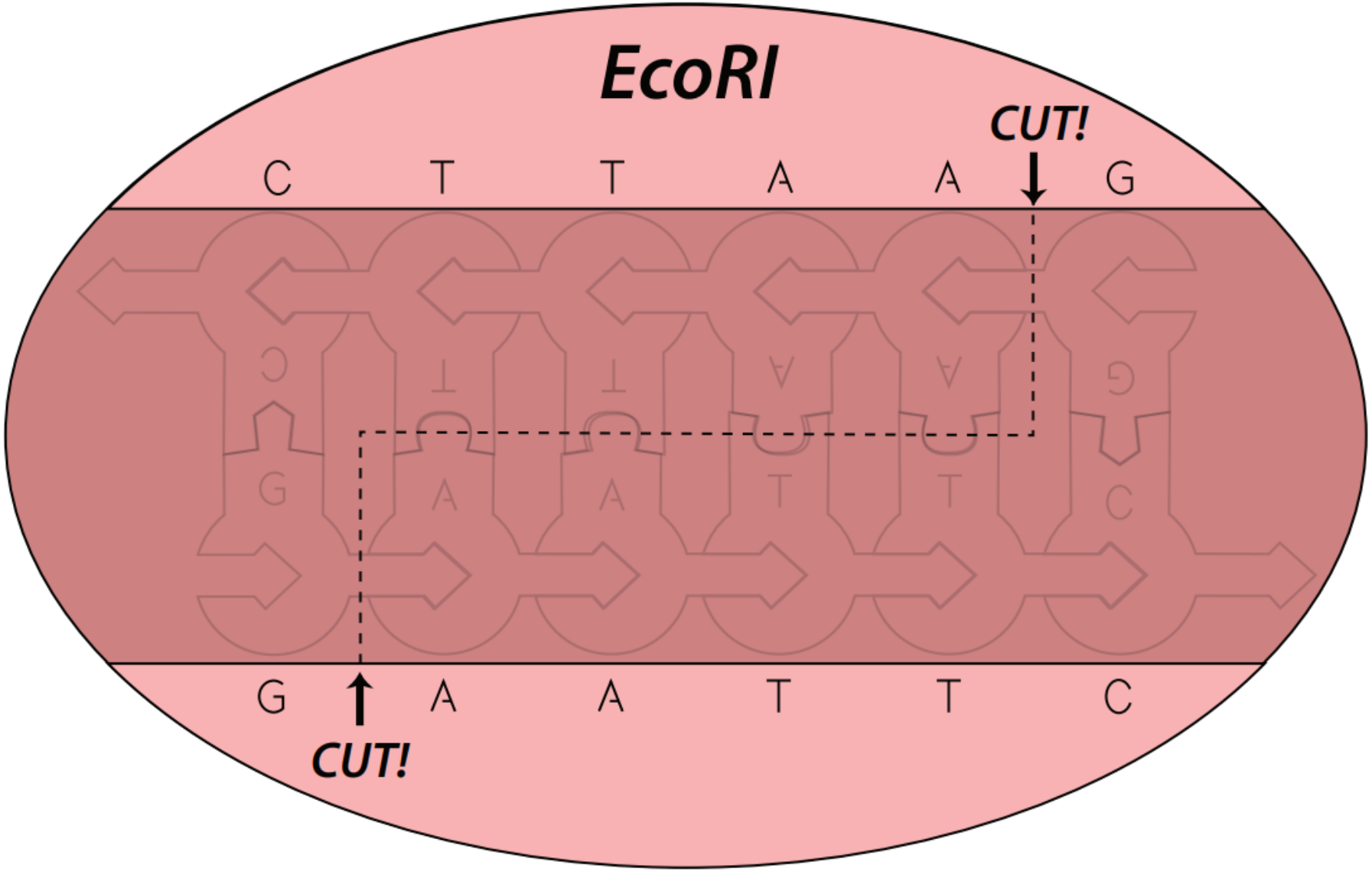
We need to use the same enzyme

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

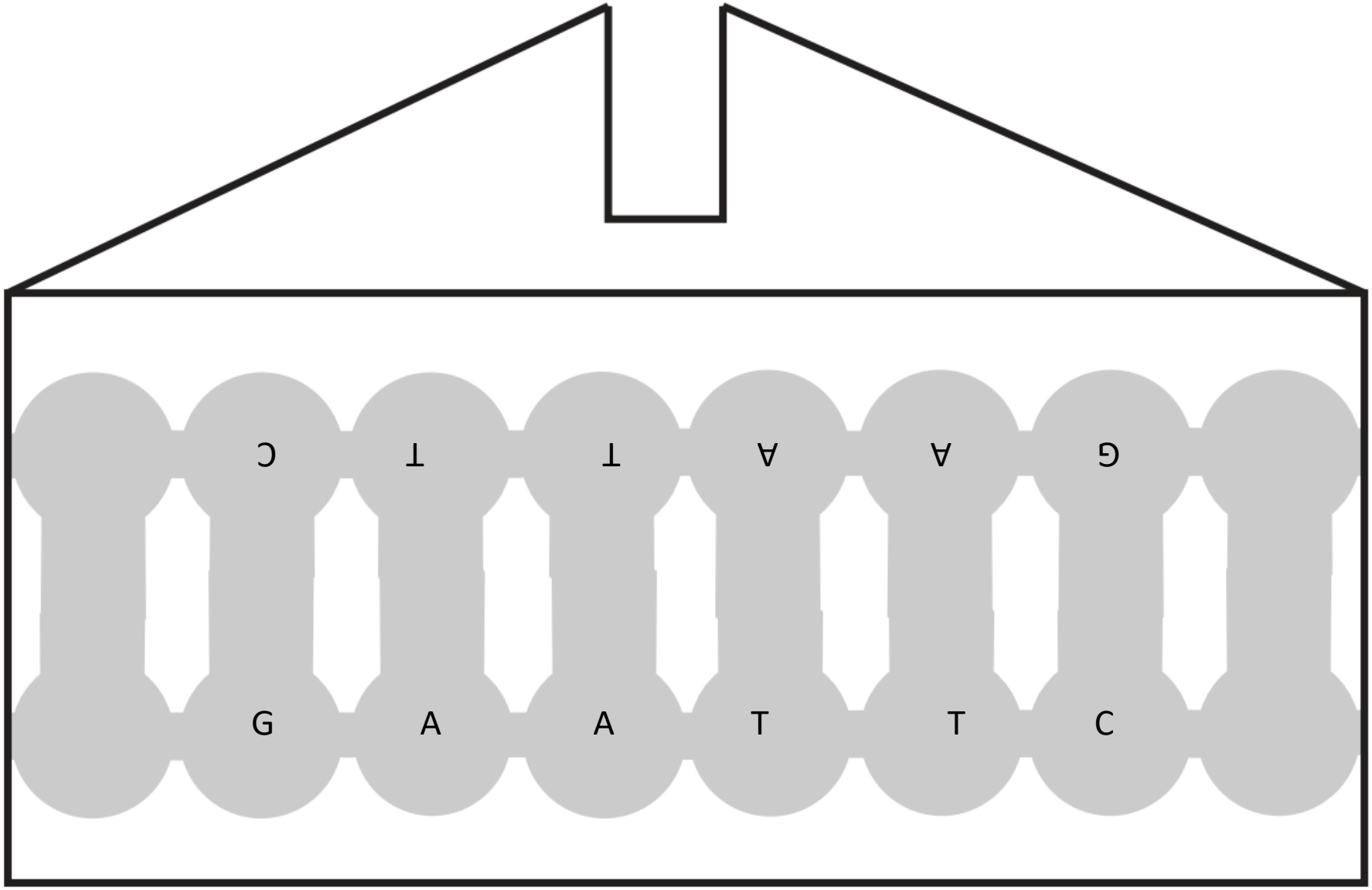
Which one should we use?



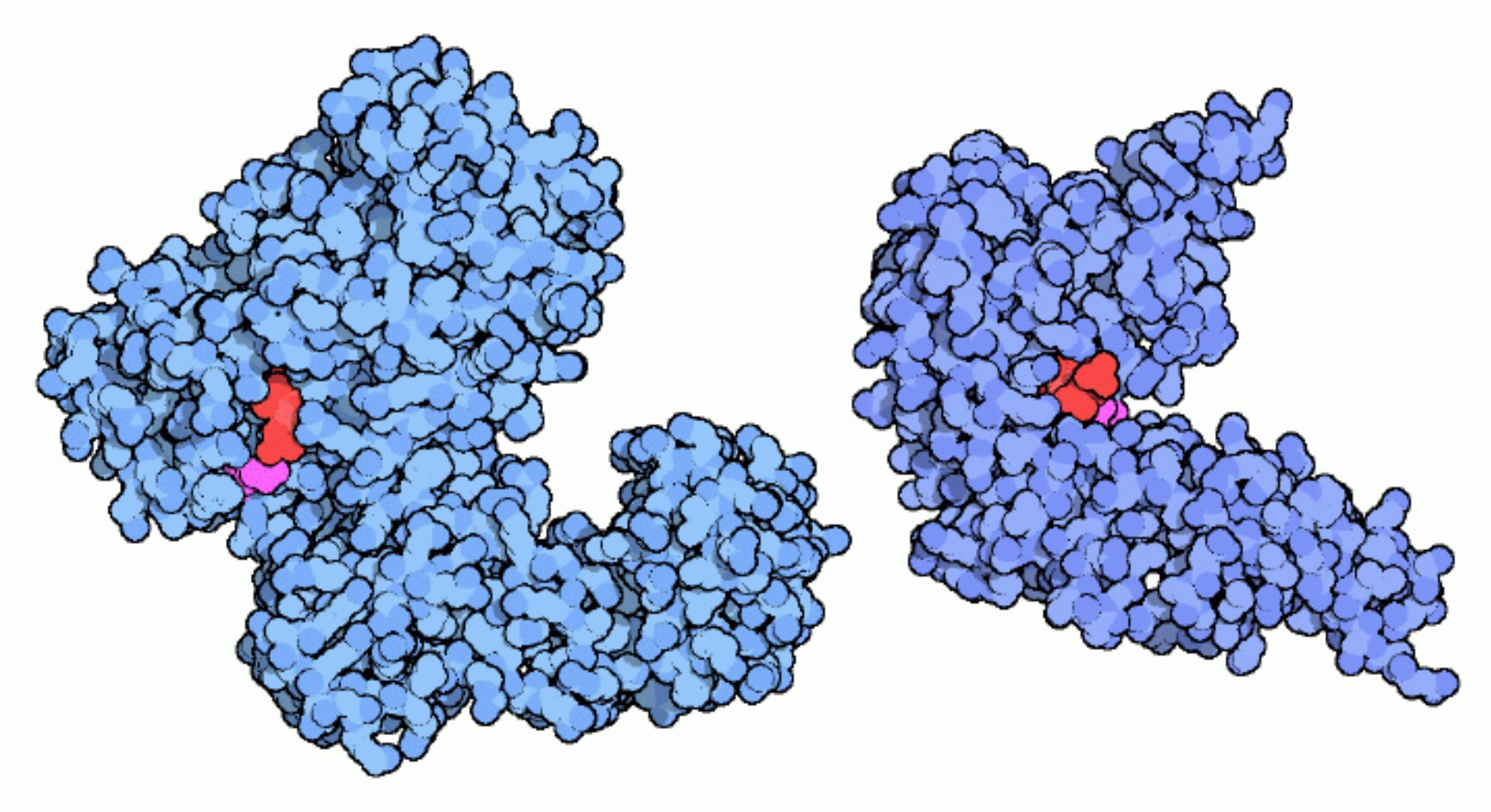
EcoRI !



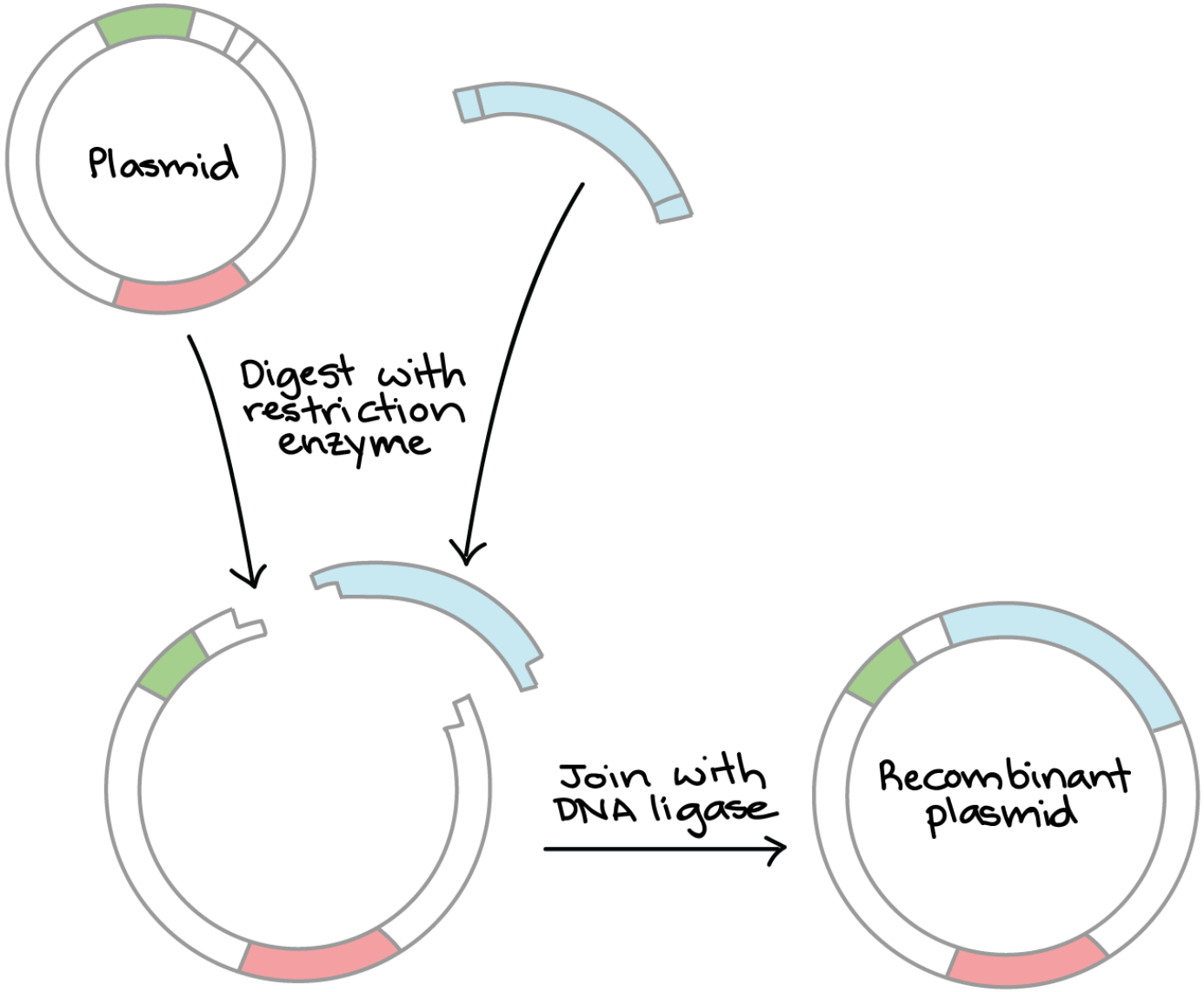
Build the EcoRI Sequence in gray -



DNA Ligase – Molecular tape



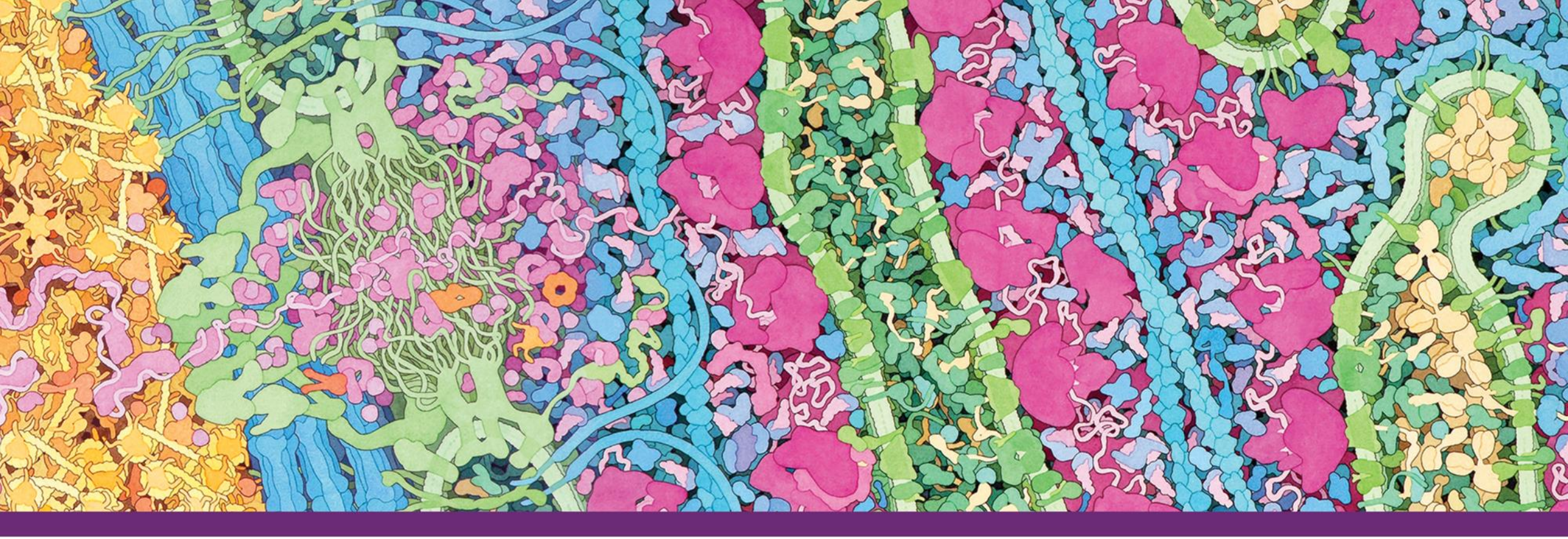
Digest and Ligate



Scan this QR code and fill out our survey!

3d molecular designs

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



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