Uncovering the Truth: Modeling a DNA Replication Error

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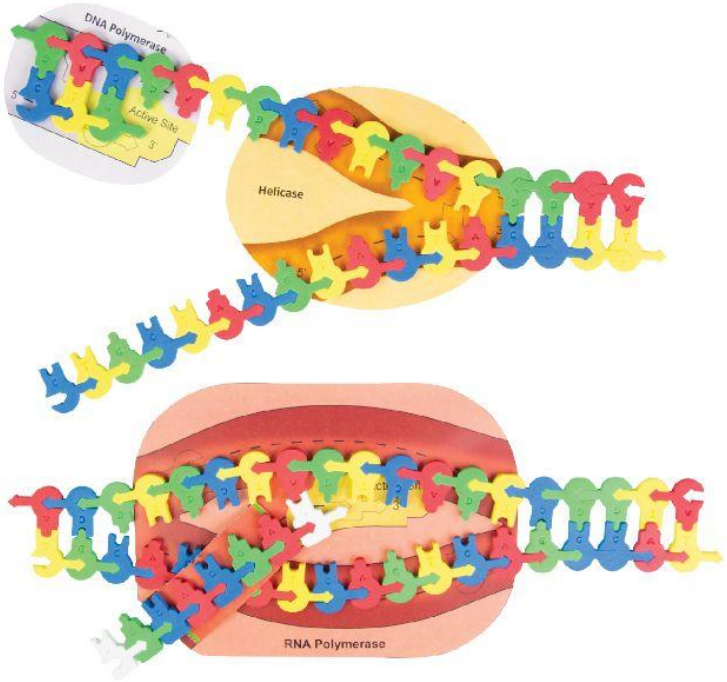
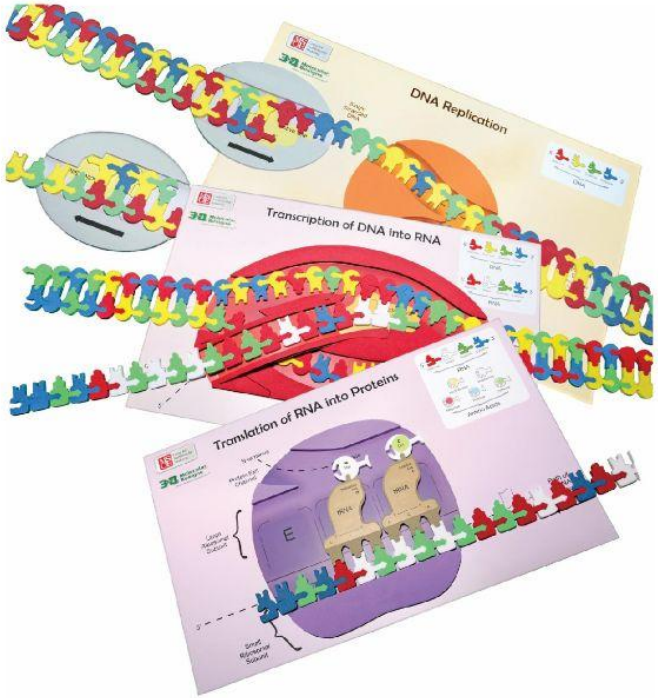
NABT 2023
Baltimore

Nucleotide Student Modeling Pack



Flow of Genetic Information Kit©



Workshop Goals

- Use the foam nucleotides to model DNA replication
- Use the model to learn about one type of DNA replication error
- Apply the DNA replication error in the context of a story

The Crime

A 26-year-old woman drove into a parking lot to park her car and return to work. She approached a man in the lot, thinking he was the parking attendant. The man told her that she had to move her car immediately or it would be towed, and he followed her back to the car. As she got in the car to leave, the man told her she could stay.

As she got back out, the man attacked her with a screwdriver and told her to give him the keys and her purse. She struggled with him and then decided to flee; she threw her purse on the seat and jumped out of the car. As she was exiting the car, she noticed that the man was bleeding and that there was blood inside the driver's side door.

She ran into a nearby parking garage and called the police.

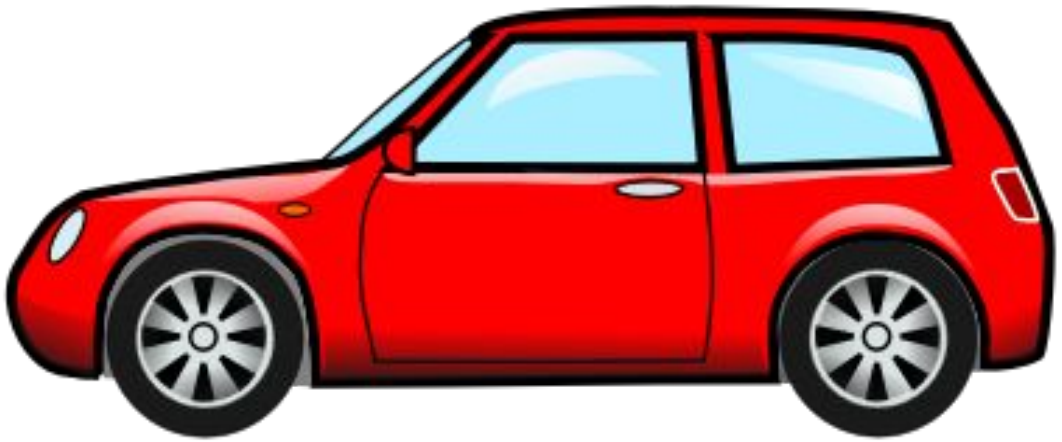
The Arrest

The victim described her attacker to police as a clean-shaven man wearing a baseball cap. She said the man was 5’10” tall and had a gap between his teeth. The next day, the victim helped police draw a composite sketch of the attacker.

Meanwhile, police recovered the victim’s car in another part of town. Latent fingerprints and swabs of the blood stain inside the driver’s side door were collected.

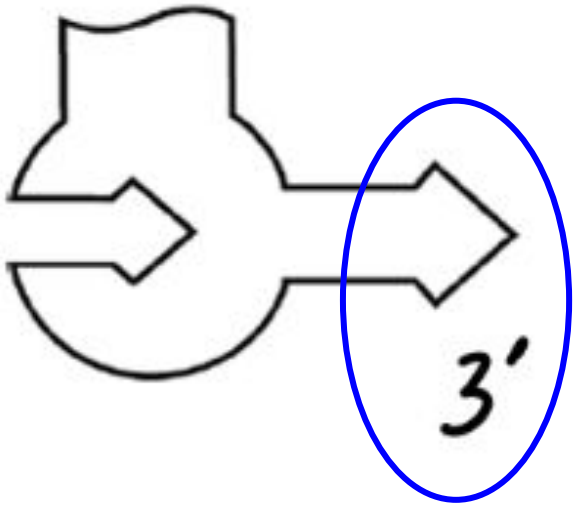
Six days later, a detective arrested Antonio B. because he thought Antonio resembled the composite sketch in this case. The same detective prepared a live lineup including Antonio and three other men – two of them police officers. The victim identified Antonio and he was charged with first-degree robbery.

Is Antonio guilty of the carjacking?



DNA replication

Important Reference:



What is the purpose of each of the following?

Helicase -

Primers -

Nucleotides -

DNA Polymerase -

DNA replication

DNA replication

What is the purpose of each of the following?

Helicase - breaks H bonds

Primers - short nucleotide sequence required for polymerase to begin replication, or extension, of the new strand of DNA

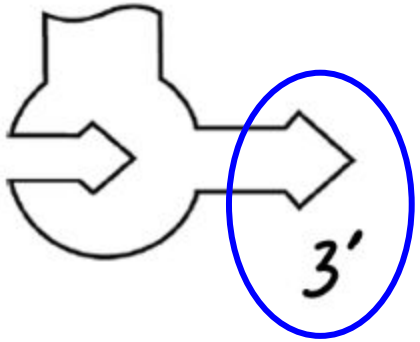
Nucleotides - added by polymerase to extend the new strand of DNA

DNA Polymerase - enzyme that adds nucleotides to to the growing strand of DNA in order to replicate DNA

Let’s Make a DNA Model!

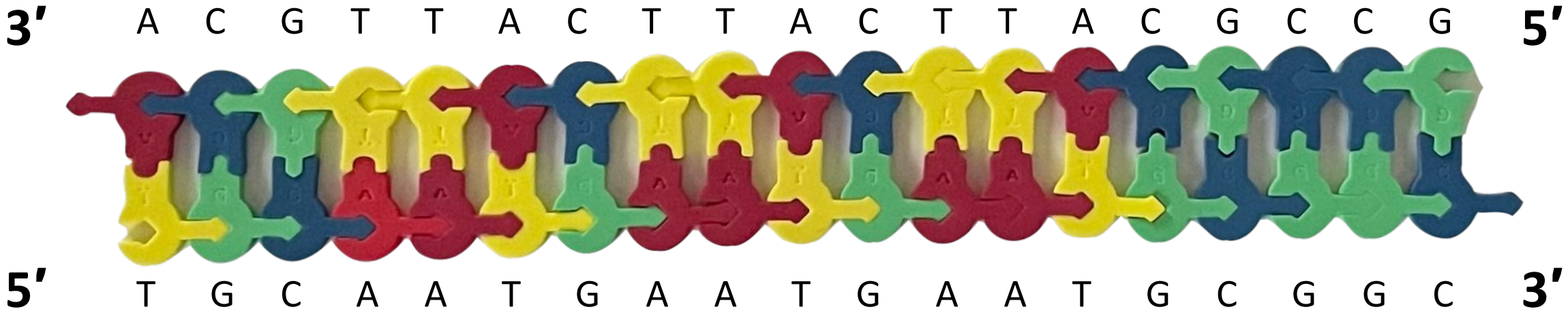
3′ A C G T T A C T T A C T T A C G C C G 5′

5′ T G C A A T G A A T G A A T G C G G C 3′

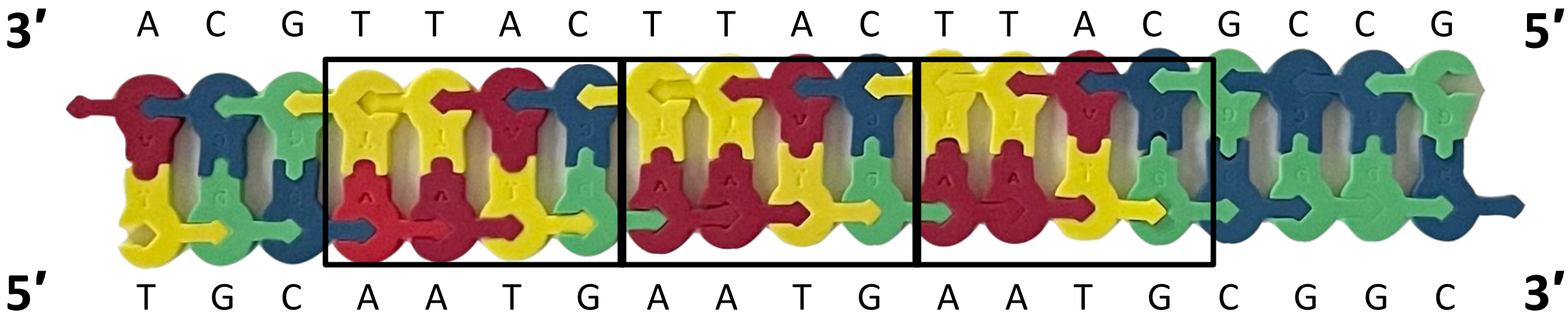


A = Red
T = Yellow
C = Blue
G = Green

Observe your DNA model. What do you notice?

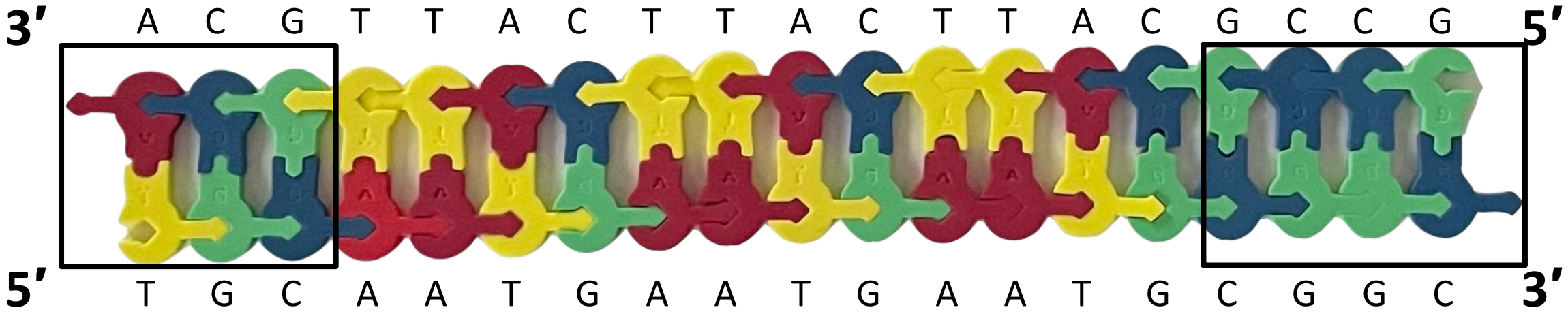


Observe your DNA model. What do you notice?



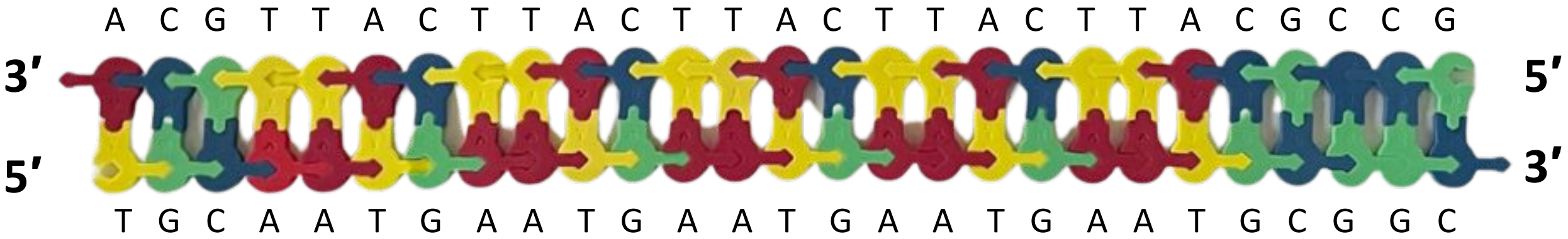
The unit of bases, AATG, is repeated three times along the bottom strand.
Genetic shorthand: $[AATG]_3$

Flanking Regions

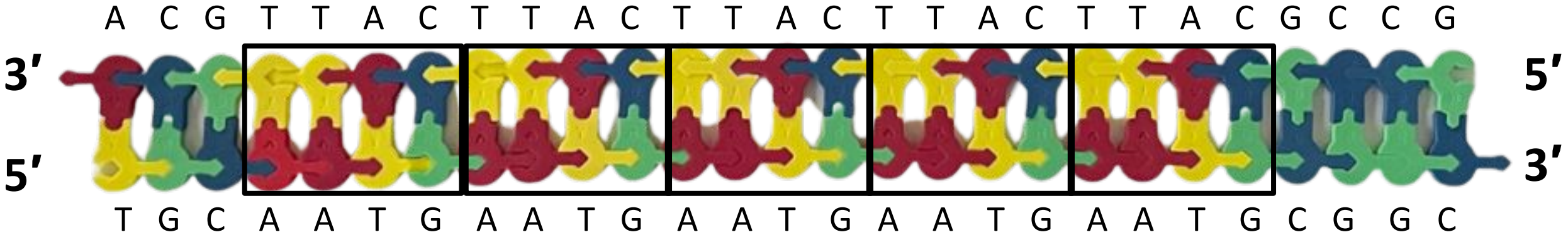


The unit of bases, AATG, is repeated three times along the bottom strand.
Genetic shorthand: $[AATG]_3$

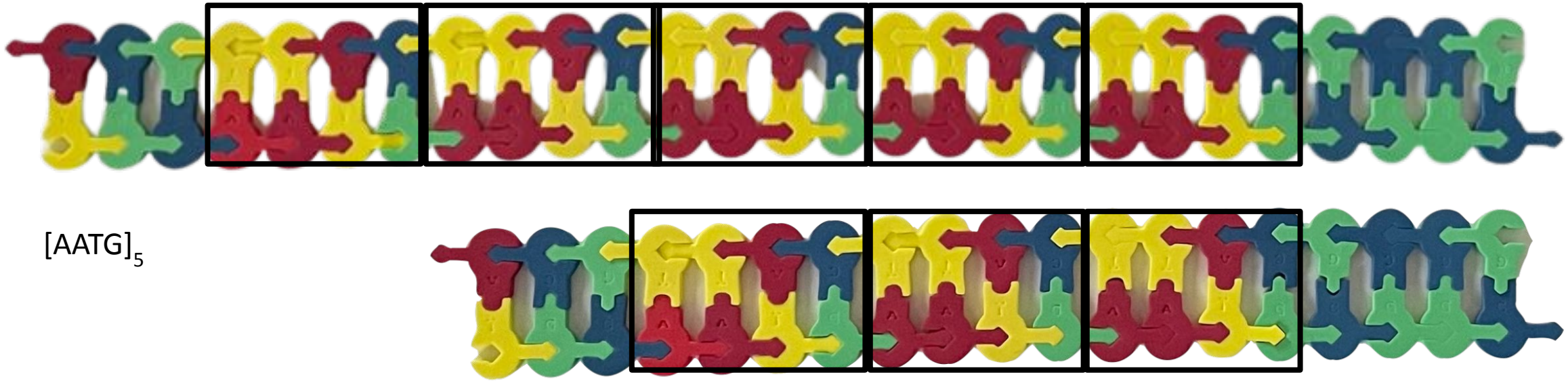
Observe this DNA model. What do you notice?



Five repeated AATG units: $[AATG]_5$



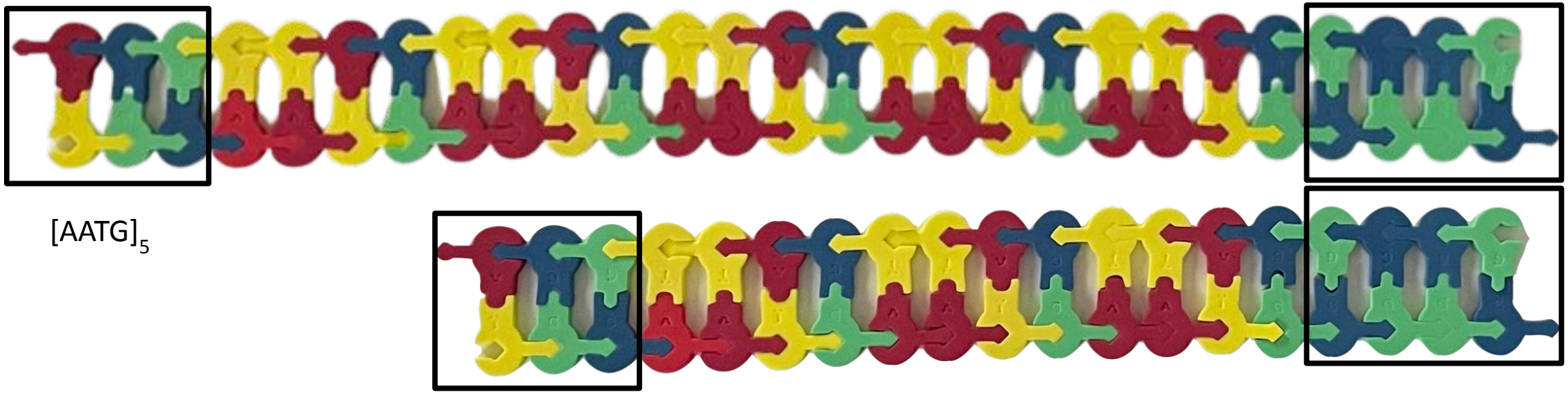
Short Tandem Repeats (STRs) = short repetitive units of nucleotides. The number of "repeats" can vary at a particular locus in the genome. For example, TPOX on Chromosome 2:



$[AATG]_5$

$[AATG]_3$

Compare both DNA molecules. What do you notice?



$[AATG]_5$

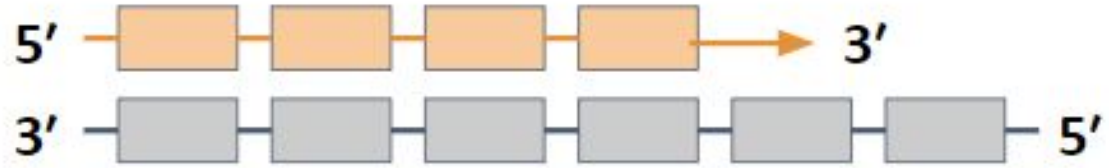
$[AATG]_3$

The flanking regions are identical

Pub Med Central:
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5054066/

What Causes a Different Number of Repeated Nucleotide Units?

Typical Replication

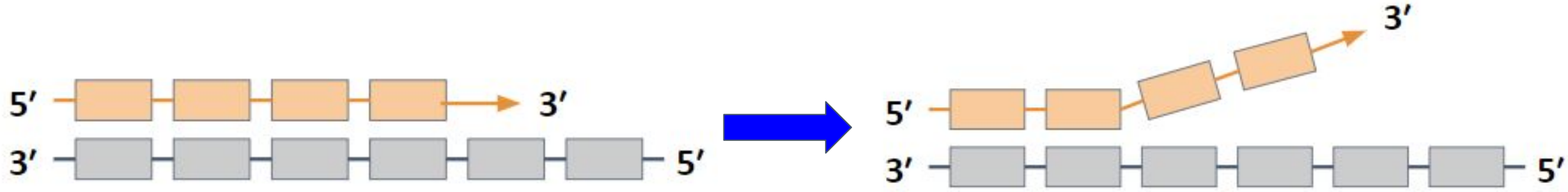


6 Repeat Units on
Template Strand (Gray)

1 **Replication**

Newly synthesized
strand is orange

Typical Replication…



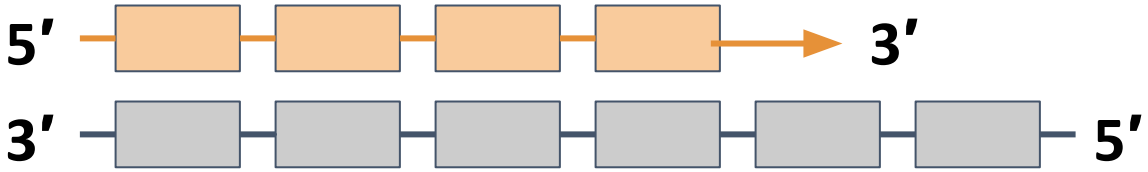
1 **Replication**

Newly synthesized strand is orange

2 **Strand Dissociation**

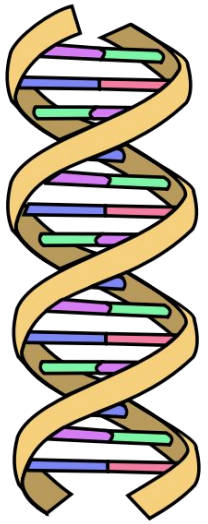
Polymerase pauses and can be temporarily released. The end of the newly synthesized strand can dissociate from the template

Original blocks and lines
that can be coped



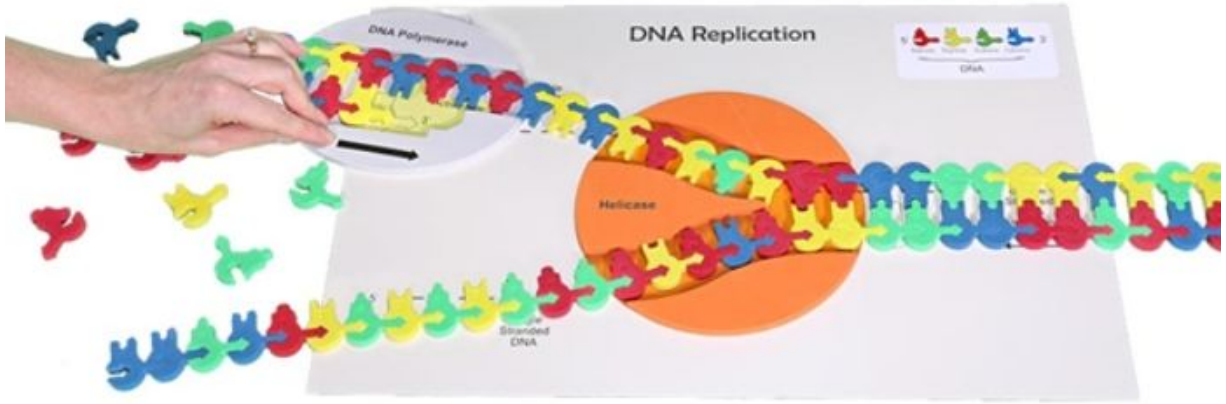
Hmmm…

How might this result in an inserted repeat (ex: AATG) in the newly synthesized strand?



Let’s Replicate!

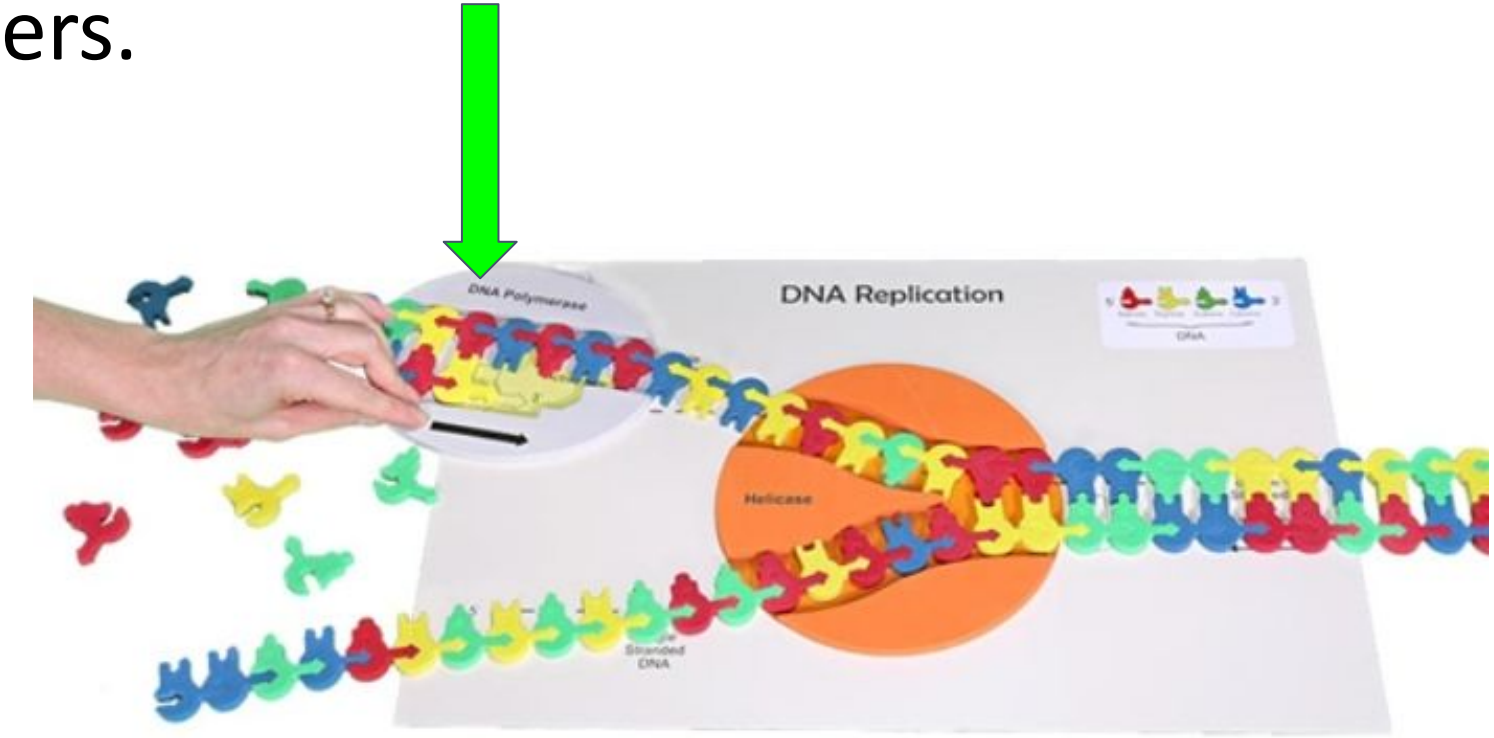
You will need your DNA model, DNA replication mat, and one DNA polymerase.



Let’s Replicate!

We will focus on replicating ONLY the top strand and we will <u>not</u> incorporate primers.

Continue replicating the top strand of DNA in the 5’ to 3’ direction, until you have added 15 nucleotides to the new strand of DNA.



New Strand of DNA

Polymerase pauses and is temporarily released…and the newly synthesized strand dissociates…

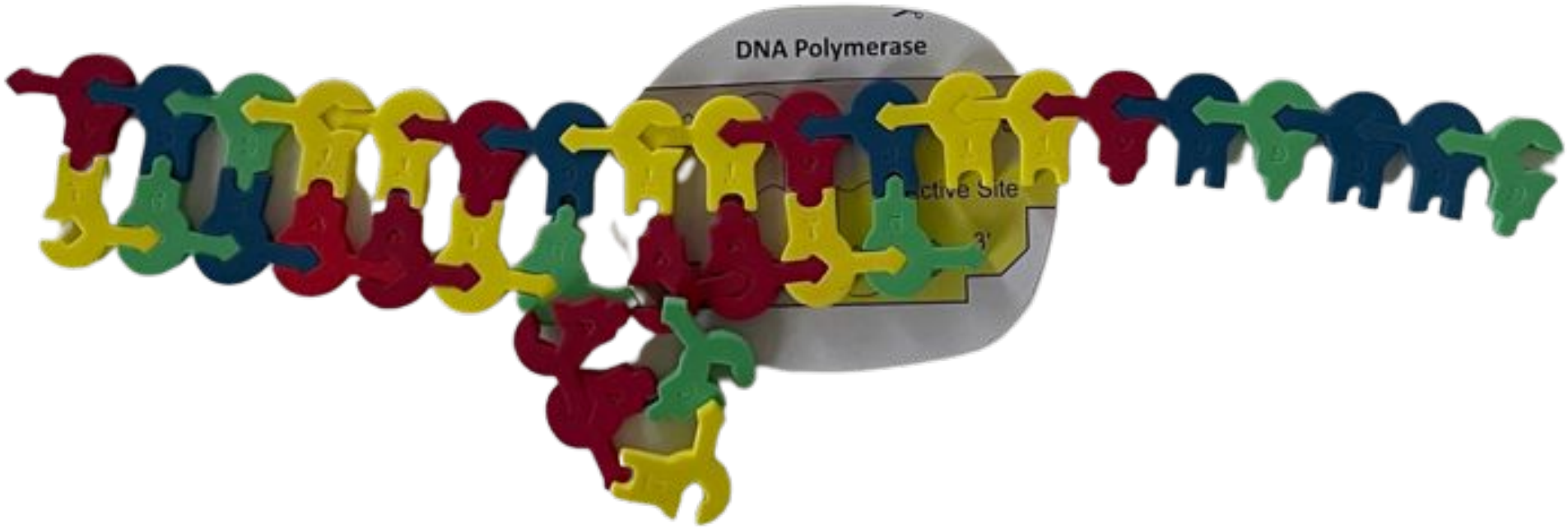
We will pause here to give you a few minutes to think about this “mistake”

Replication Mistake: Slipped-Strand Mispairing*

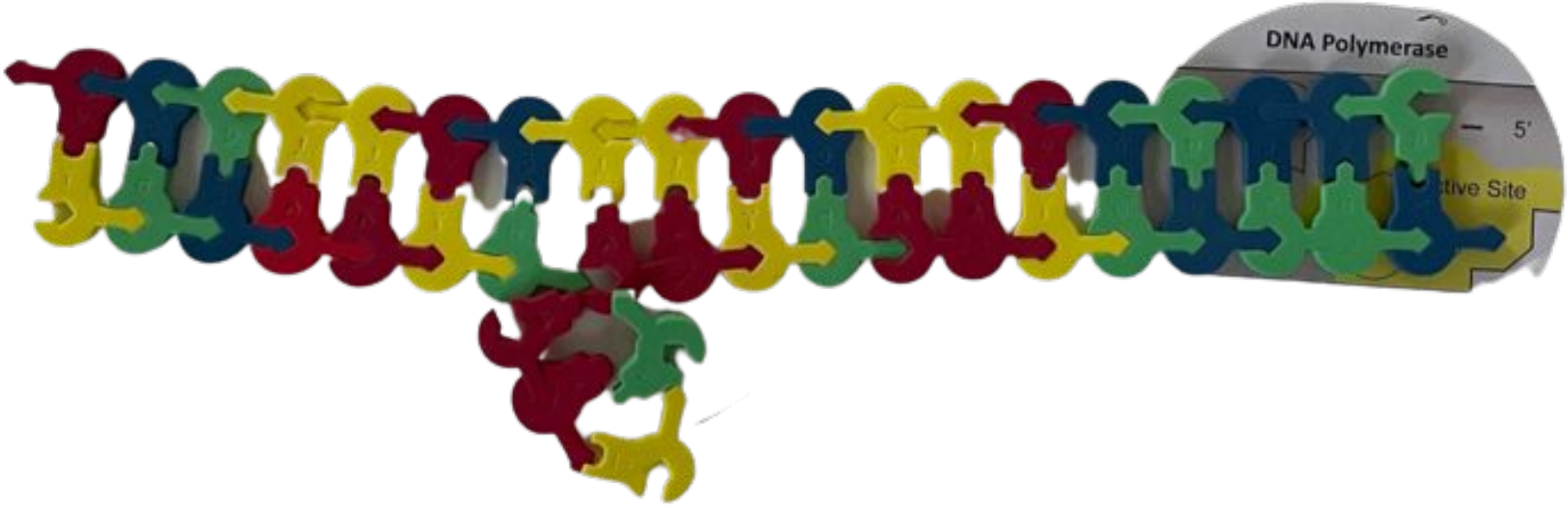
Use your model of DNA and polymerase to demonstrate slipped-strand mispairing

*Slipped-Strand Mispairing (a.k.a DNA Slippage, Polymerase Slippage, Replication Slippage, or Strand Slippage)

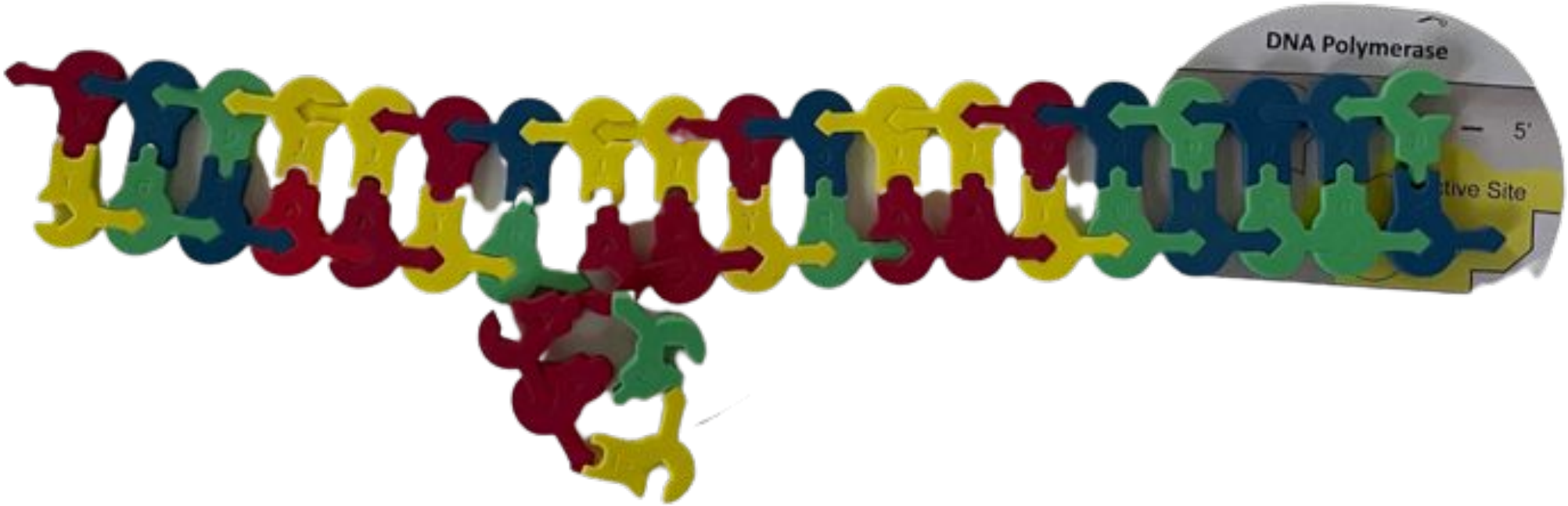
Replication Mistake: Mispairing Due to Repeated Unit



Replication Mistake: Polymerase Continues to Extend the New Strand



What will happen if the replication error isn’t repaired?



A mutation has occurred and will continue to be copied in subsequent DNA molecules

$[AATG]_3$

$[AATG]_4$

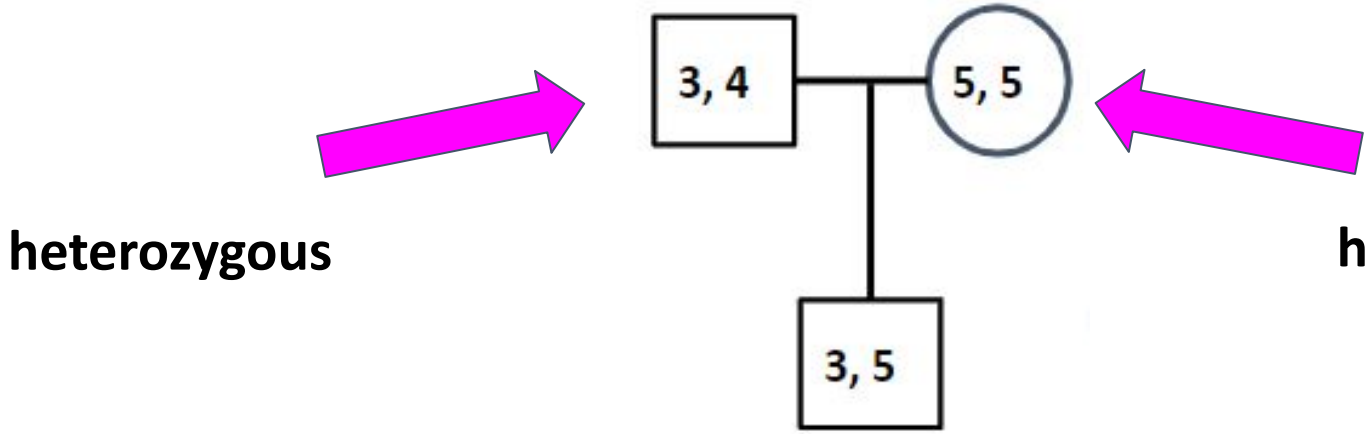
Replication Mistake: Short Tandem Repeats (STRs)

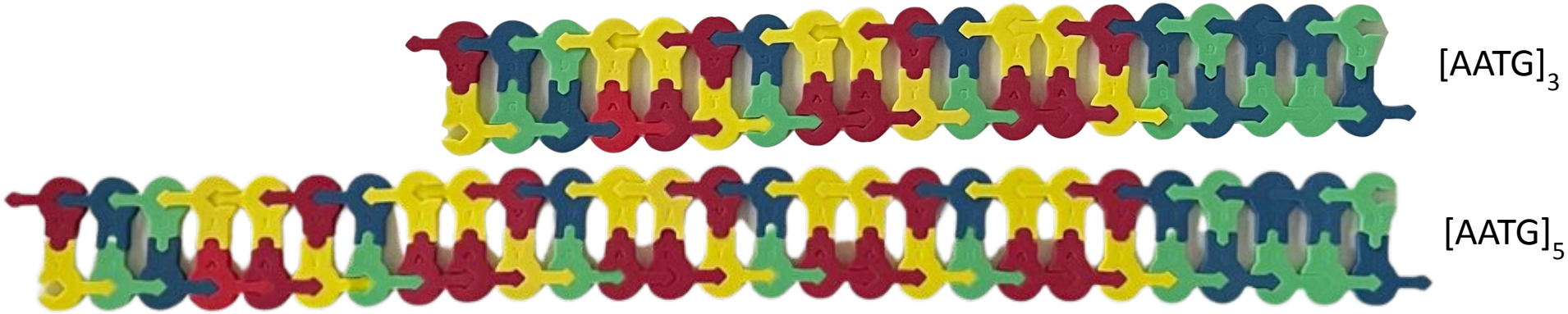
- Subject to high mutation rates
- Mutation rate can be up to 100,000x greater than a point mutation
- Account for approximately 3% of the genome
- Occurs in introns and exons
- DNA polymerase more likely to pause in an STR region (unsure why)
- Prone to slipped-strand mispairing
- Tandem repeats contain many copies of the same short sequence over and over, so it is easy for the two strands of DNA to become misaligned in this local region
- Results in multiple alleles for each STR

Citation: Myers, P. (2007) Tandem repeats and morphological variation. *Nature Education* 1(1):1

Genetic Inheritance of STRs

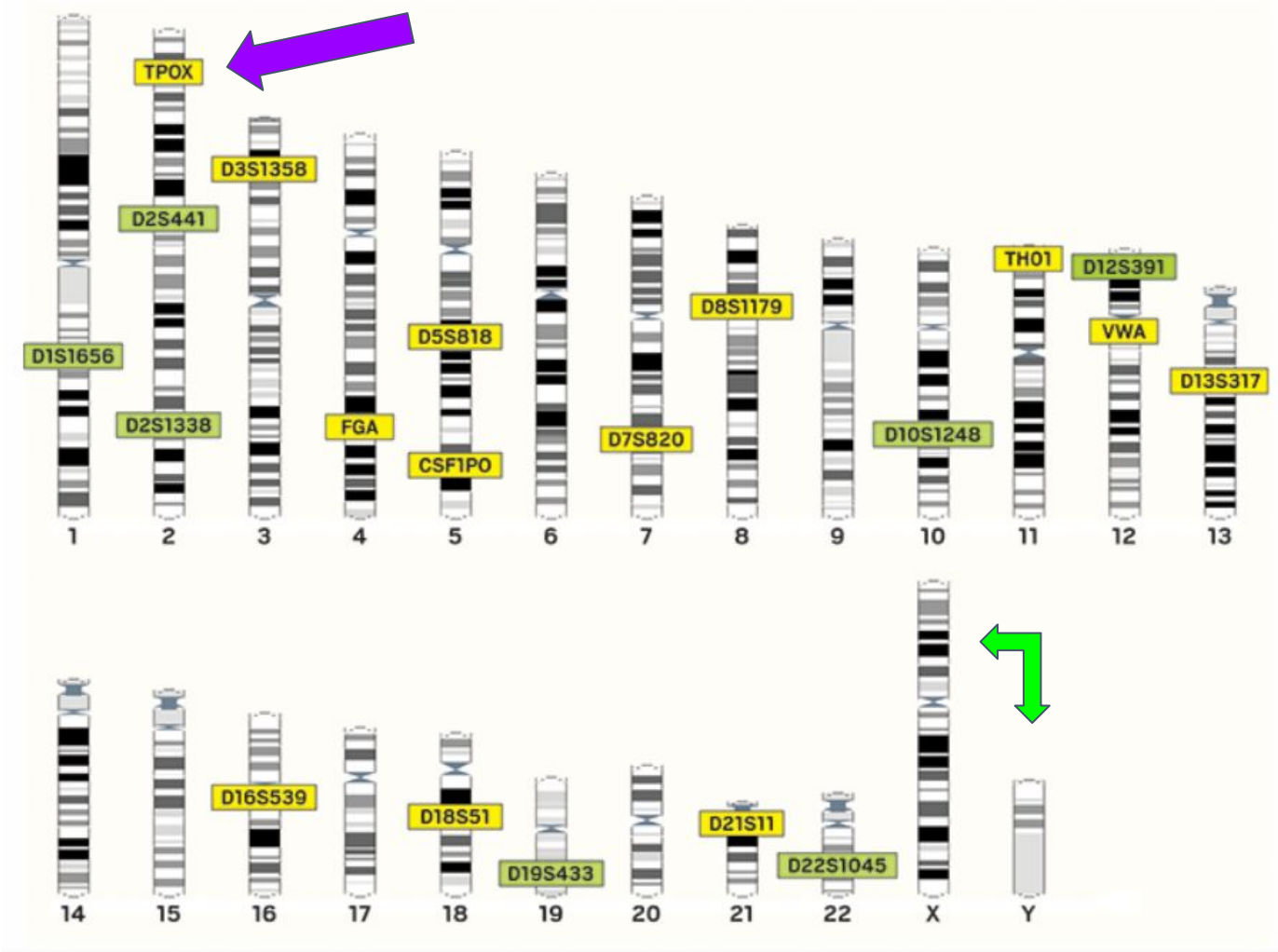
Example: TPOX on Chromosome 2 (AATG)





DNA Profiling

20 Core Loci



https://cen.acs.org/analytical-chemistry/Thirty-years-DNA-forensics-DNA/95/i37

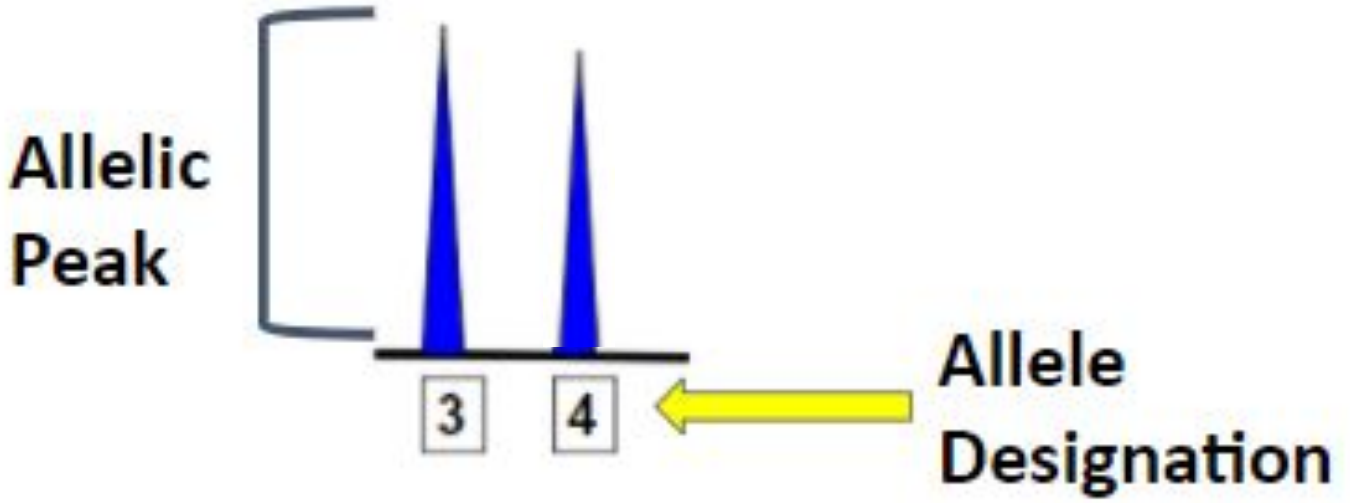
flanking regions serve as primer attachment

We learned how replication occurs in the cell, what will we need to make copies of DNA in the lab?

Kit Connection

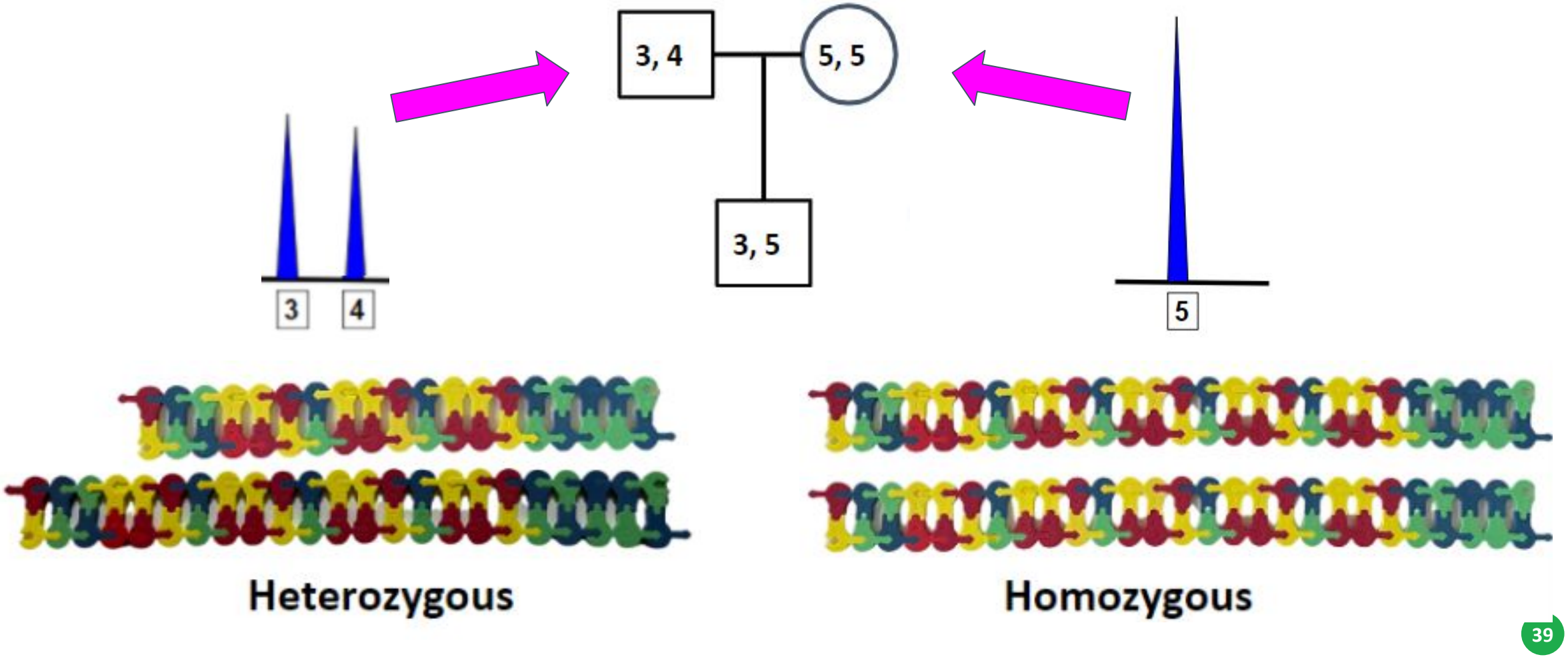
DNA Replication	PCR
Helicase breaks hydrogen bonds	High temperature breaks hydrogen bonds
Primers synthesized in the cell and attach to template strand	Primers made in a lab, added to the reaction tube, and temperature cooled so they can attach to template
DNA polymerase uses nucleotides to make a new strand of DNA	DNA polymerase uses nucleotides to make a new strand of DNA

Electropherogram



TPOX (AATG repeat)

Let’s Revisit our Pedigree



Allelic Ladder: Each allele represents a different number of repeat units at that locus

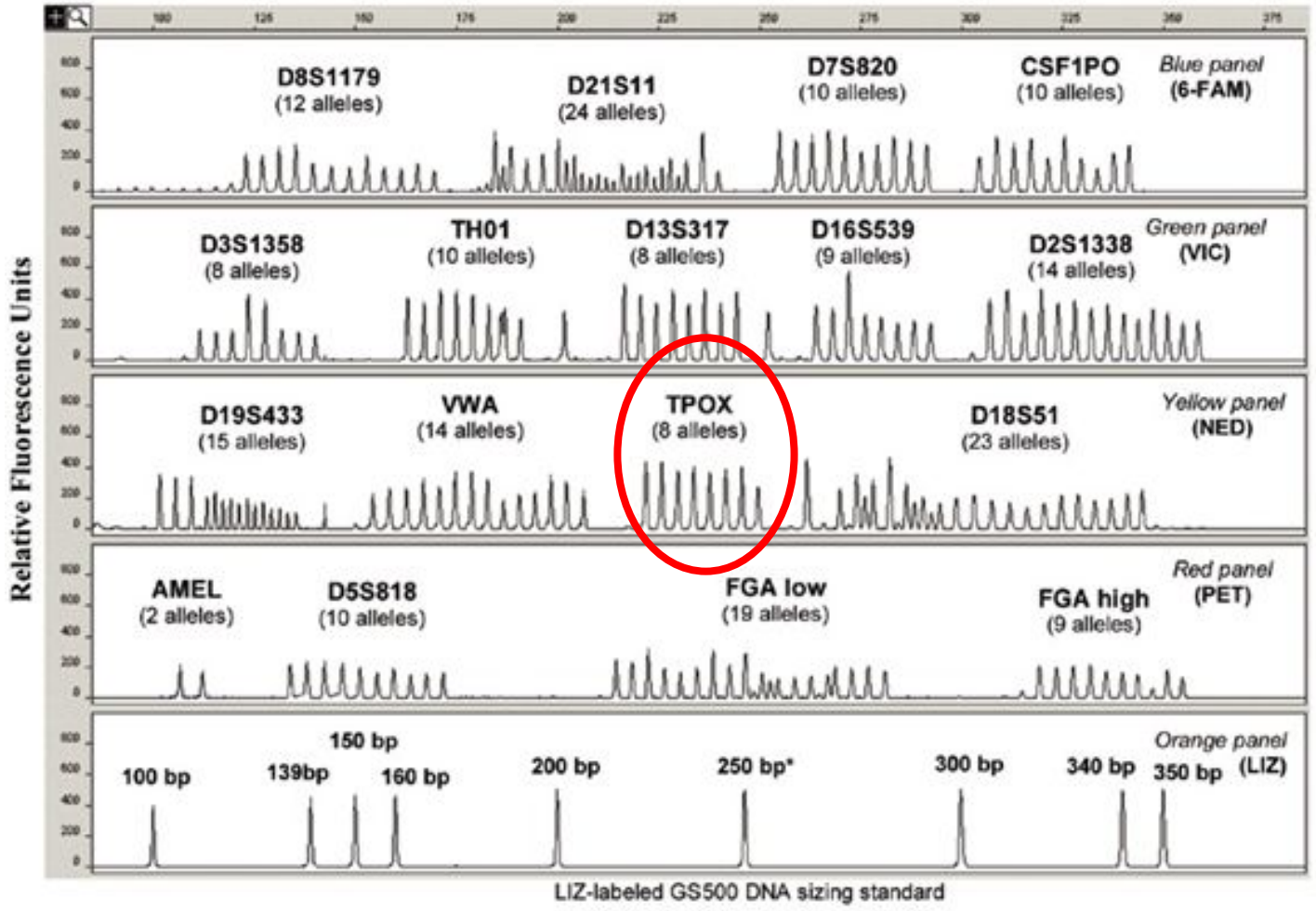


Diagram citation: Supplement to Vol. 43 ı No. 4 | 2007 www.biotechniques.com

Knowing that STRs are a powerful forensic tool, let's return to the scene of the crime…

Imagine DNA has been isolated and amplified from the blood found in the car…

The electropherogram report is ready to be analyzed!

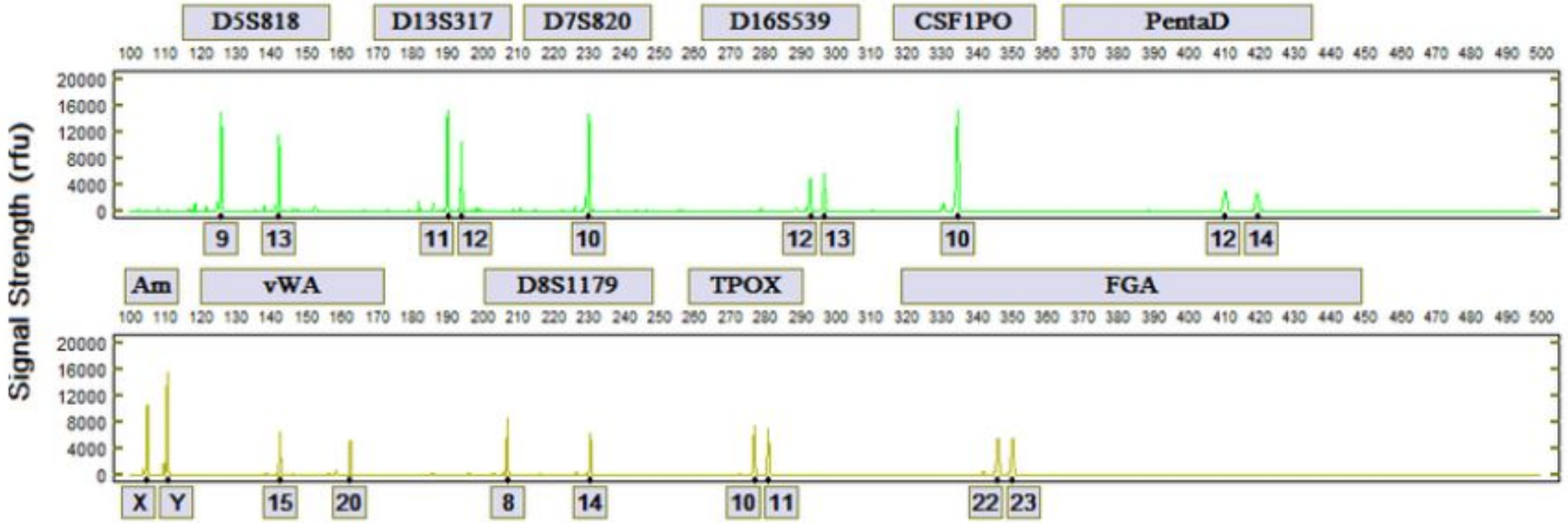
Electropherogram of the Evidence:

citation: https://www.researchgate.net/figure/Representative-electropherogram-developed-by-the-DNAscan-Expert-System_fig2_307556395

Is Antonio guilty of the carjacking? Cite one piece of evidence to support your answer.

Antonio
D5S818: 9, 13
D7S820: 10
D16S539: 11, 14
TPOX: 9, 12

Electropherogram of the Evidence:



citation: https://www.researchgate.net/figure/Representative-electropherogram-developed-by-the-DNAscan-Expert-System_fig2_307556395

The Trial

Antonio was charged with first-degree robbery and tried in April 1997. The victim testified that Antonio was the man who attacked her, the prosecution argued that the victim's clear memory of the crime meant that she was better able to identify the perpetrator.

The defense presented evidence showing that fingerprints collected from the victim's car – including prints from the driver's side and the rearview mirror – did not match the victim or Antonio. They argued that prints left on the rearview mirror indicated that the person who left them must have driven the car.

The Verdict

After several hours of deliberations over two days, the jury convicted Antonio of first-degree robbery.

He was sentenced to 18 years in prison.

The DNA Evidence

In 2001, Antonio filed a motion on his own behalf requesting DNA testing. The state opposed the motion, but the court granted a hearing on the issue in 2005.

The Innocence Project accepted Antonio's case and filed another brief on his behalf in 2006. The state agreed to testing on the blood swab from inside the car door in October 2006 and the results proved that Antonio could not have committed this crime. The DNA testing not only excluded Antonio as the source of the blood, but led to the identification of another man already incarcerated for other crimes.

Antonio was exonerated on March 29, 2007. He was 41 years old at the time of his exoneration and had served more than a decade in prison.

INNOCENCE PROJECT

After serving more than 10 years in Missouri prisons for a carjacking he didn't commit, DNA tests on blood stains at the crime scene proved Antonio Beaver's innocence and he was exonerated in 2007.

State: Missouri

Charge: First Degree Robbery

Conviction: First Degree Robbery

Sentence: 18 years

Incident Date: 08/15/96

Conviction Date: 04/25/97

Exoneration Date: 03/29/07

Served: 10 years

Workshop NGSS Connections

SEPs	CCCs	DCIs
Asking questions	**Patterns**	**LS3.A: Inheritance and Variation**
Developing and using models	**Cause and effect**	**LS3.B: Inheritance and Variation**
Constructing explanations supported by evidence		

Modeling the Molecular World

Session 1: July 29 - August 2

OR

Session 2: August 5 - August 9

Join us — the first and only team to put protein models in the hands of students — for a week of modeling! You will:

- Hear powerful molecular stories, told by master storyteller, Tim Herman, PhD
- Learn tips and techniques from experienced teachers who have fostered student modeling, discovery, and inquiry in their classrooms
- Become part of a community of outstanding educators utilizing 3D Molecular Design's kits and models in a variety of courses
- Learn how to incorporate modeling with NGSS standards
- Leave with an introductory set of innovative materials, including a Water Kit©, Amino Acid Starter Kit©, Dynamic DNA Kit©, a cellular landscape by David Goodsell, and more

https://3dmd.3dmoleculardesigns.com/summer-courses

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Cost of course: $900 (includes a collection of 13 collaborative kits and interactive models)
Early Bird Pricing: $750 - Pay by 2/1/24
Additional Staff Members: $450
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COVID-19 Policy: Masking is encouraged, free daily COVID testing is required

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Thank you for attending today's workshop!

Please use this QR code to complete a survey:

Use discount code FC23 for a 20% off most kits!

Join us for the next session from 3:30 - 4:00 pm: "Hydrophobic Marvels: Using Water Models to Unravel the Self-Cleaning Secrets of Lotus Leaves"