Isothermal Recombinase Polymerase Amplification

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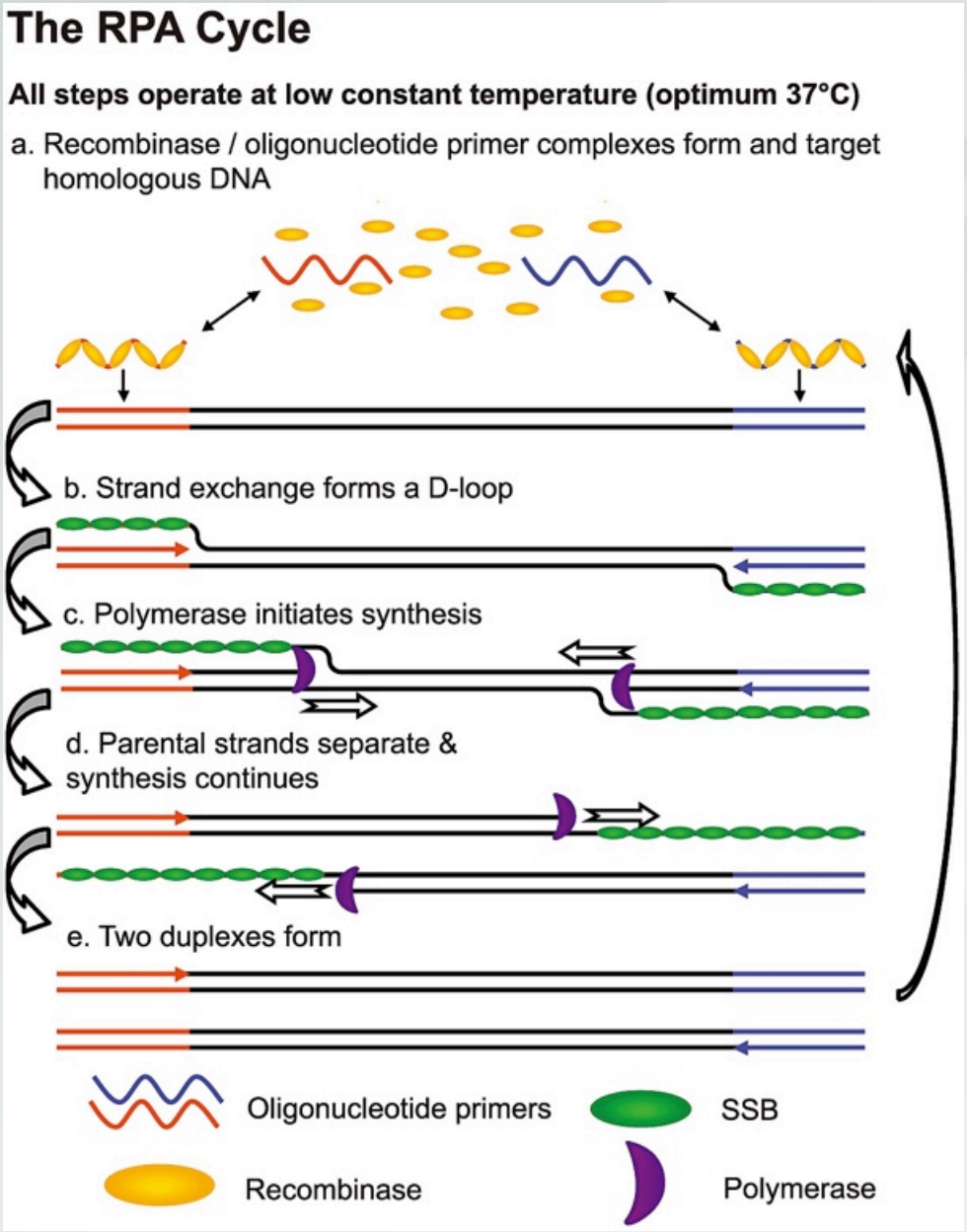
The RPA Cycle



Kit components:

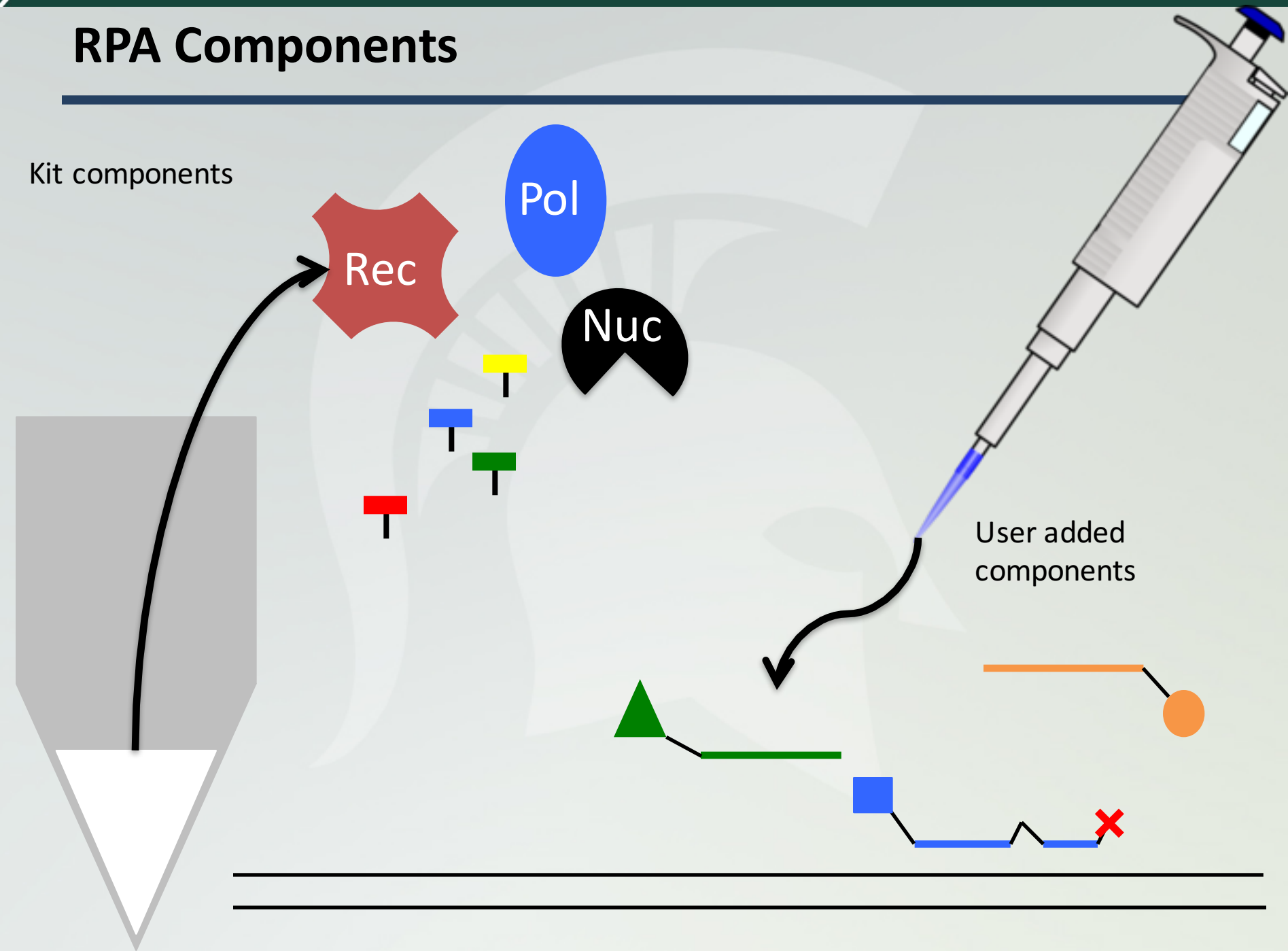
- Recombinase
- Polymerase
- Special nuclease
- dNTPs
- Single strand binding proteins

User added components:

- DNA template / target
- Forward primer
- Reverse primer
- Unique probe
- Buffer
- Water

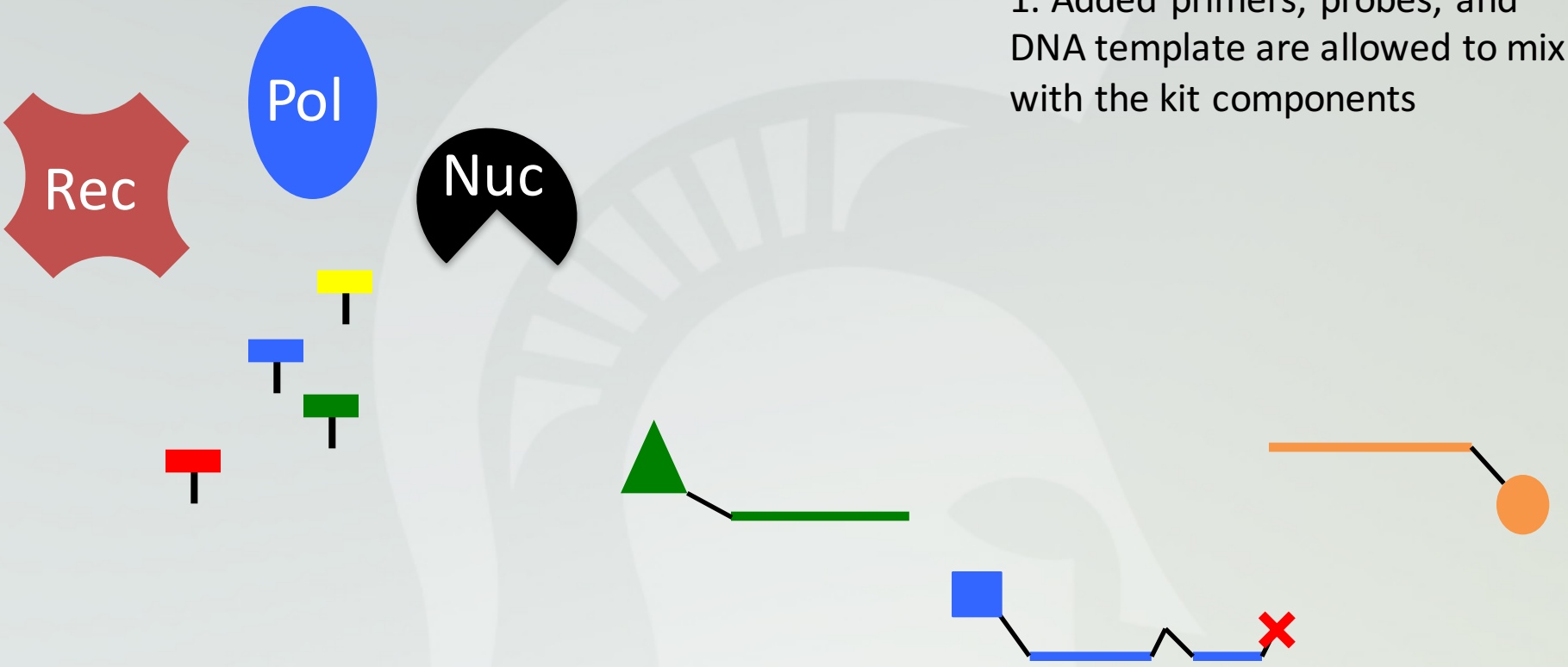
http://www.twistdx.co.uk/our_technology/

RPA Components



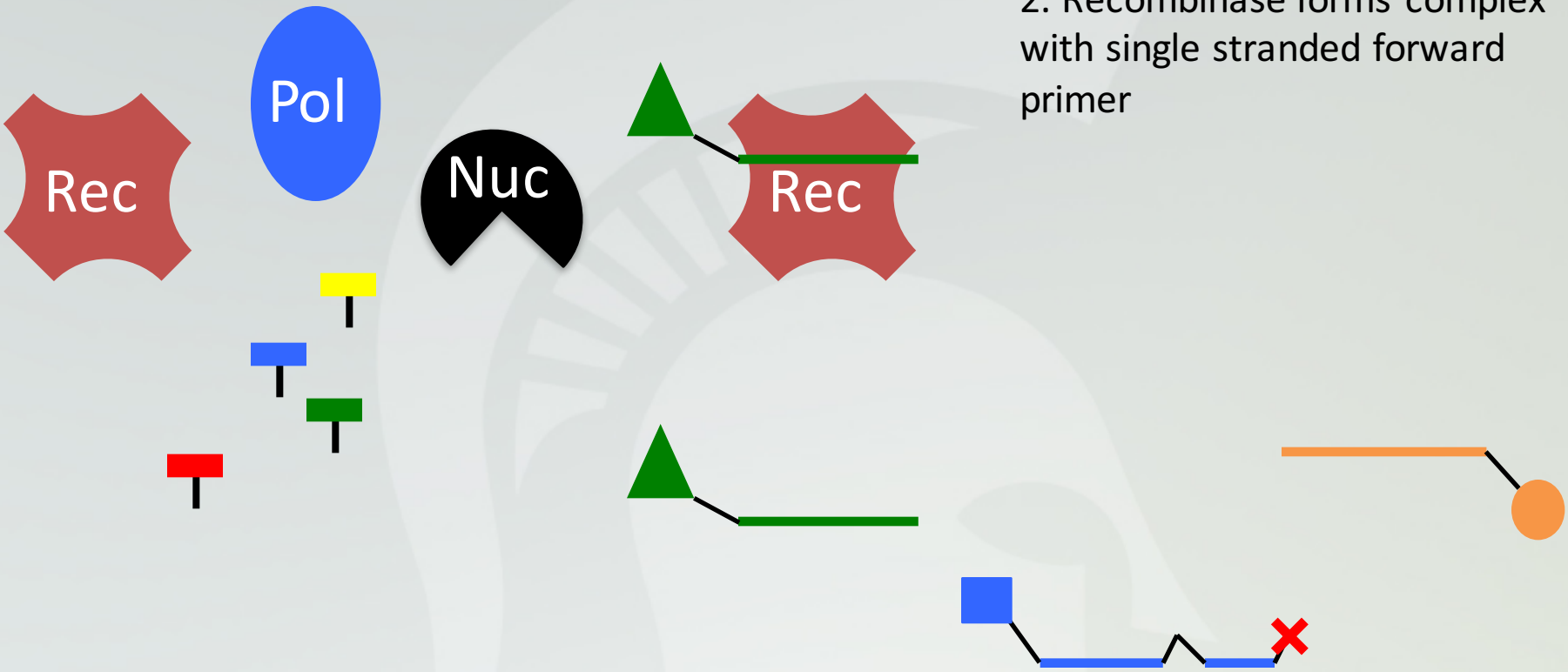
RPA Reaction Steps

1. Added primers, probes, and DNA template are allowed to mix with the kit components



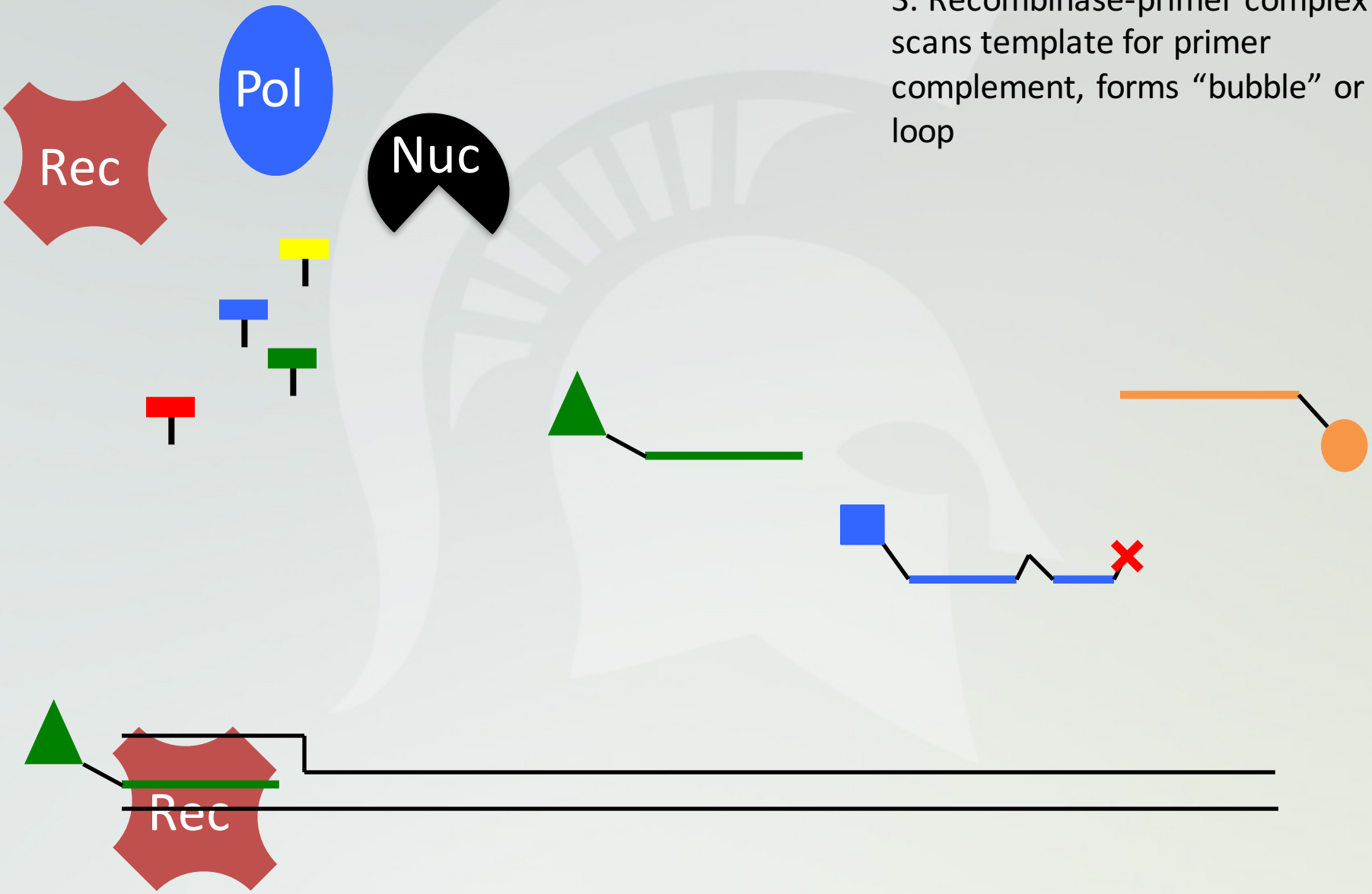
RPA Reaction Steps

2. Recombinase forms complex with single stranded forward primer



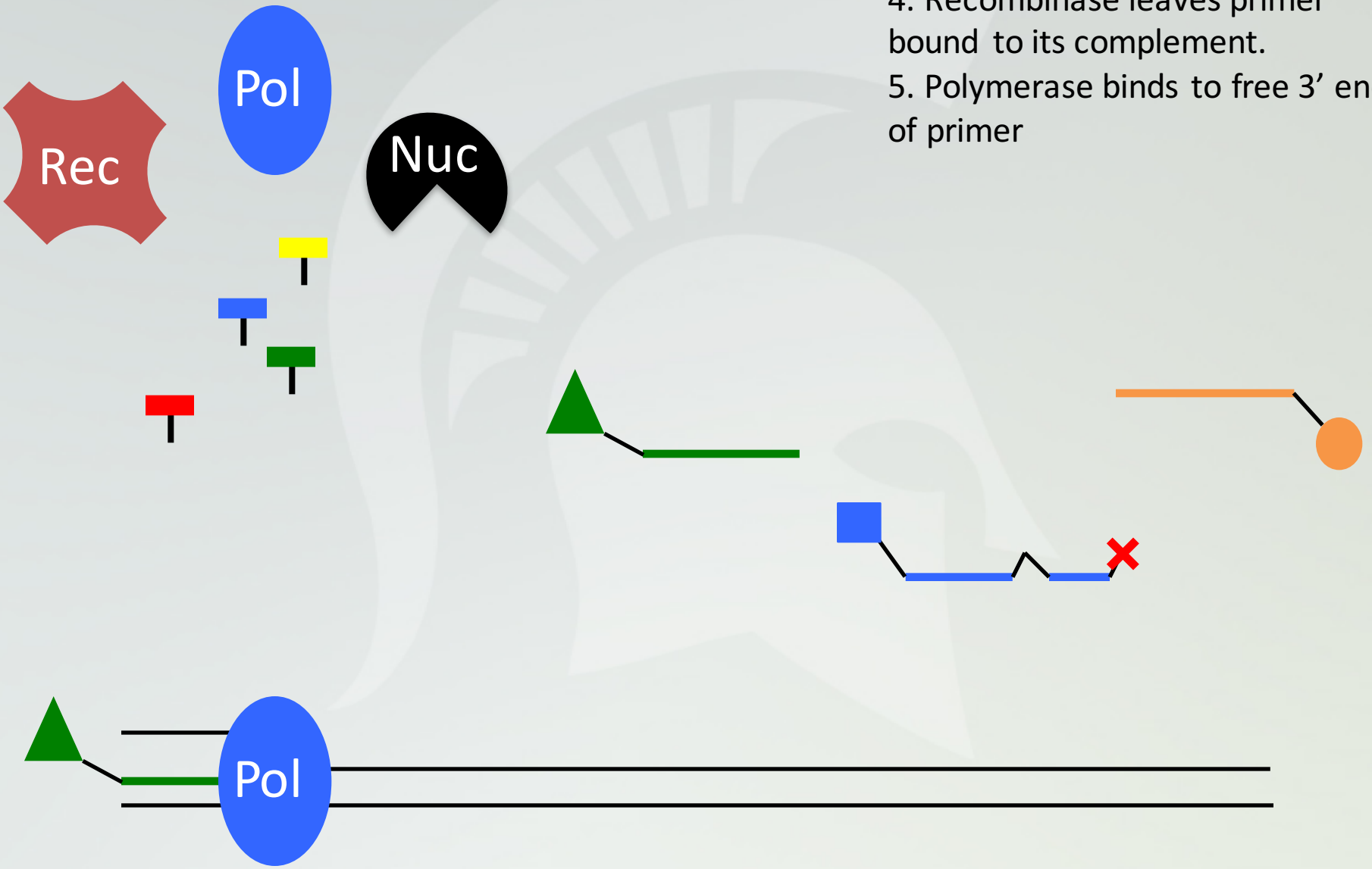
RPA Reaction Steps

3. Recombinase-primer complex scans template for primer complement, forms “bubble” or D-loop



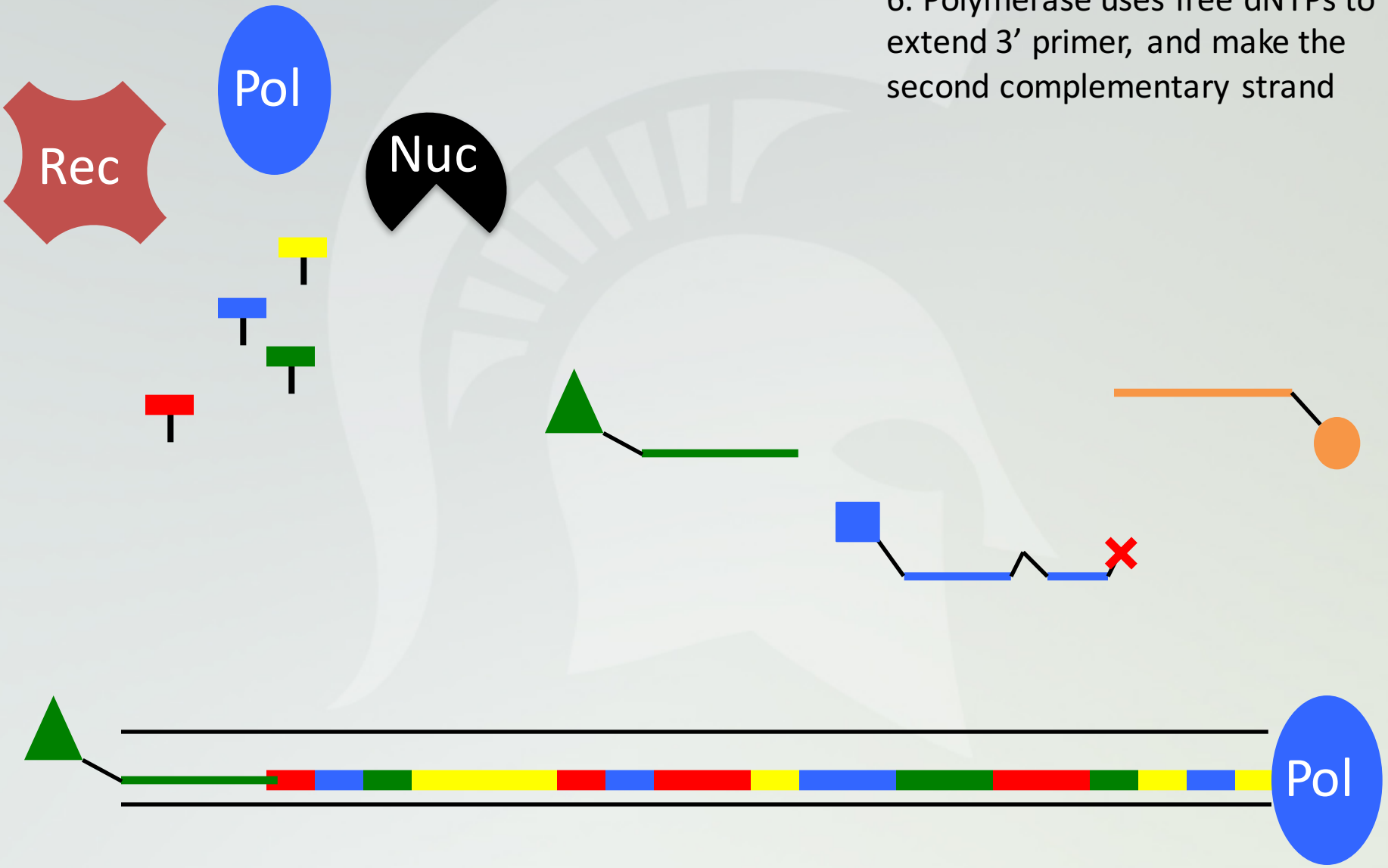
RPA Reaction Steps

4. Recombinase leaves primer bound to its complement.
5. Polymerase binds to free 3’ end of primer

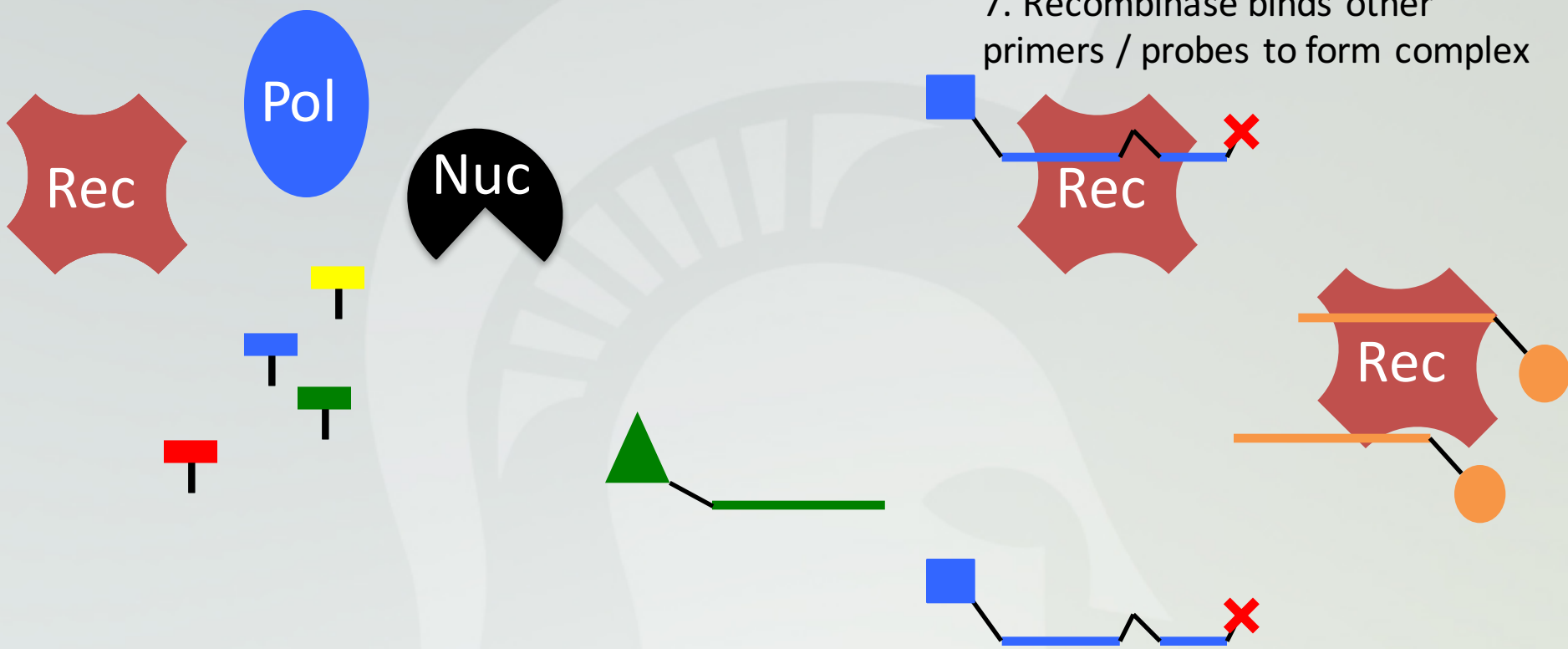


RPA Reaction Steps

6. Polymerase uses free dNTPs to extend 3’ primer, and make the second complementary strand

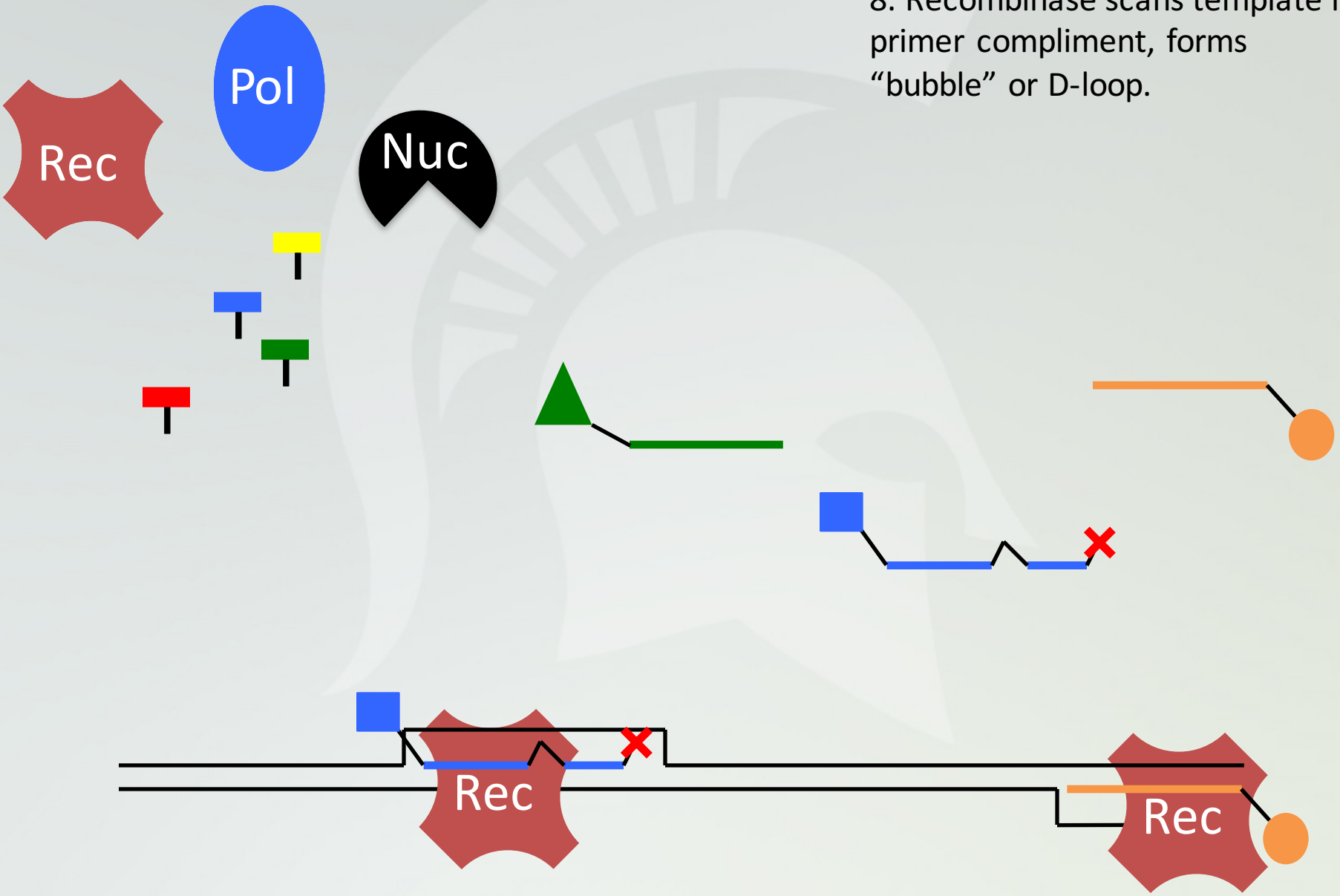


RPA Reaction Steps

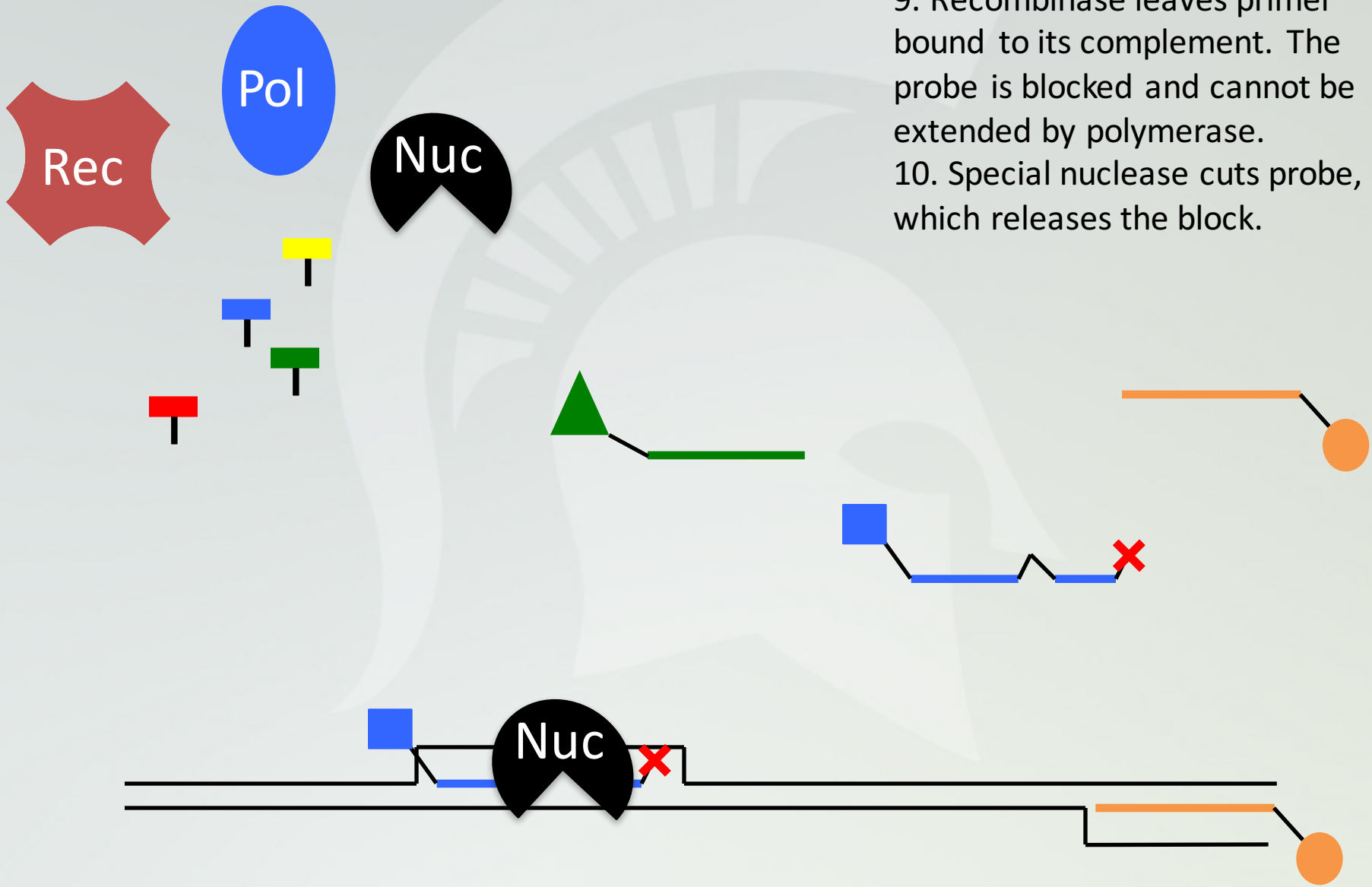


RPA Reaction Steps

8. Recombinase scans template for primer compliment, forms "bubble" or D-loop.



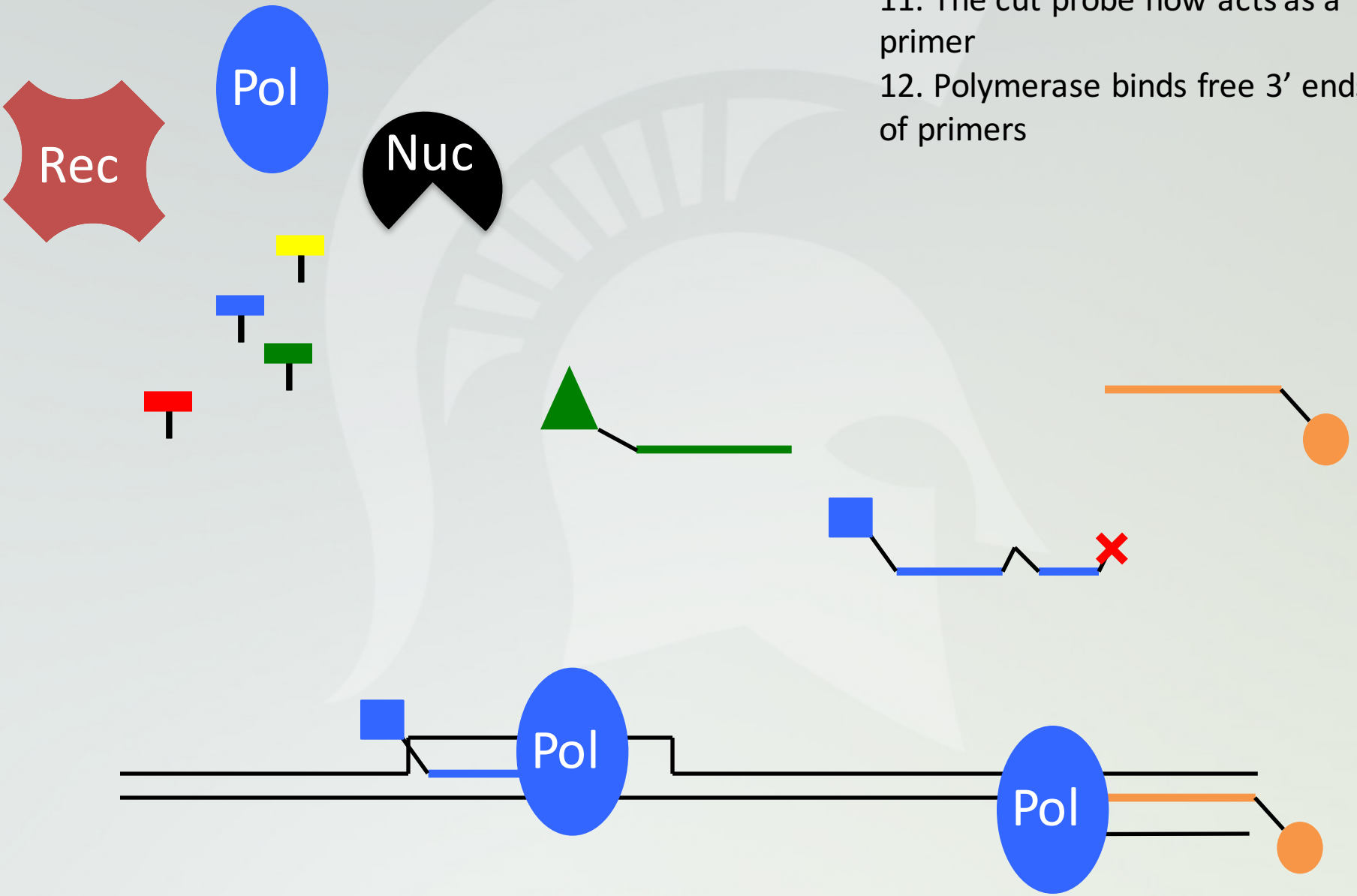
RPA Reaction Steps



9. Recombinase leaves primer bound to its complement. The probe is blocked and cannot be extended by polymerase.
10. Special nuclease cuts probe, which releases the block.

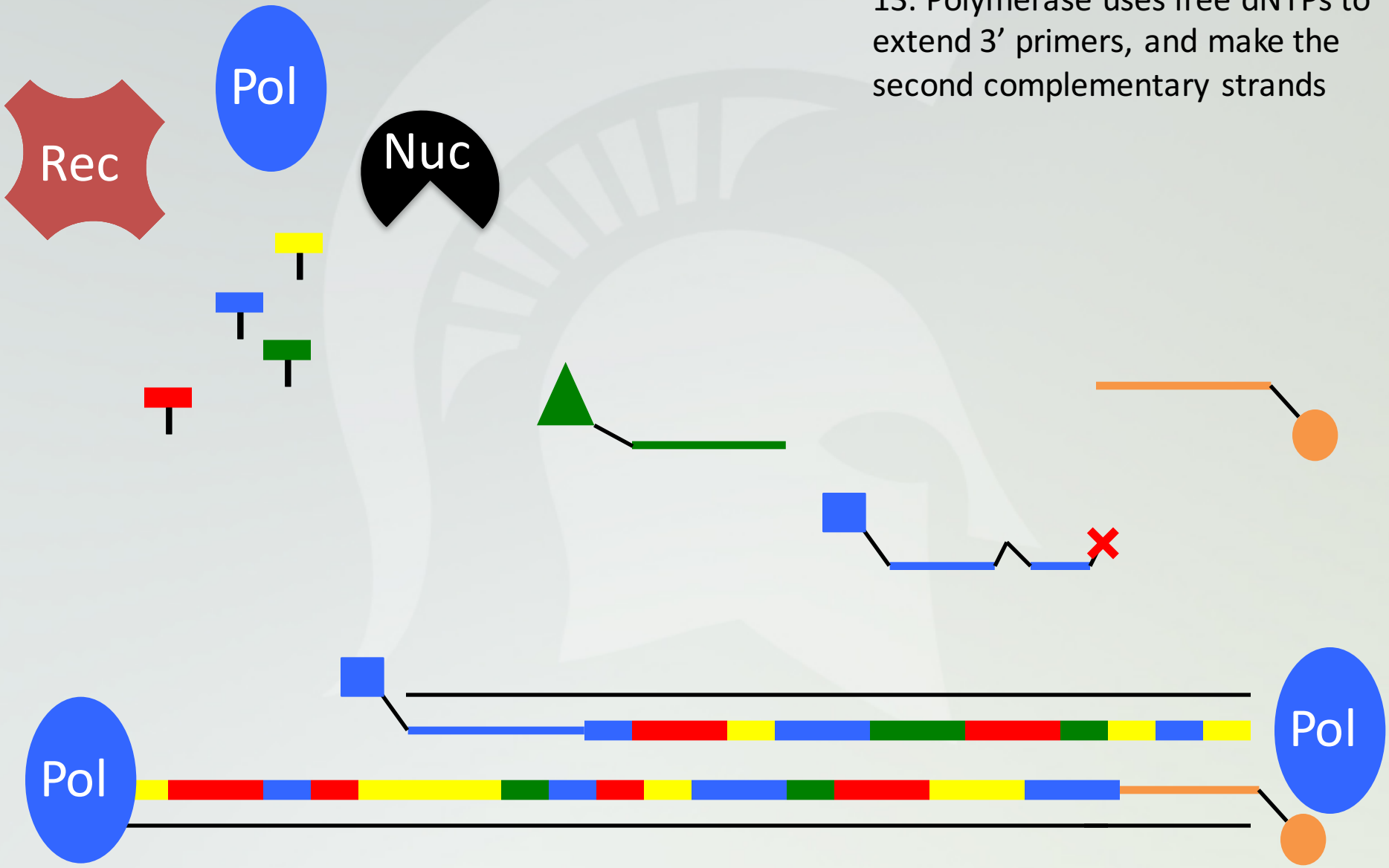
RPA Reaction Steps

11. The cut probe now acts as a primer
12. Polymerase binds free 3’ ends of primers

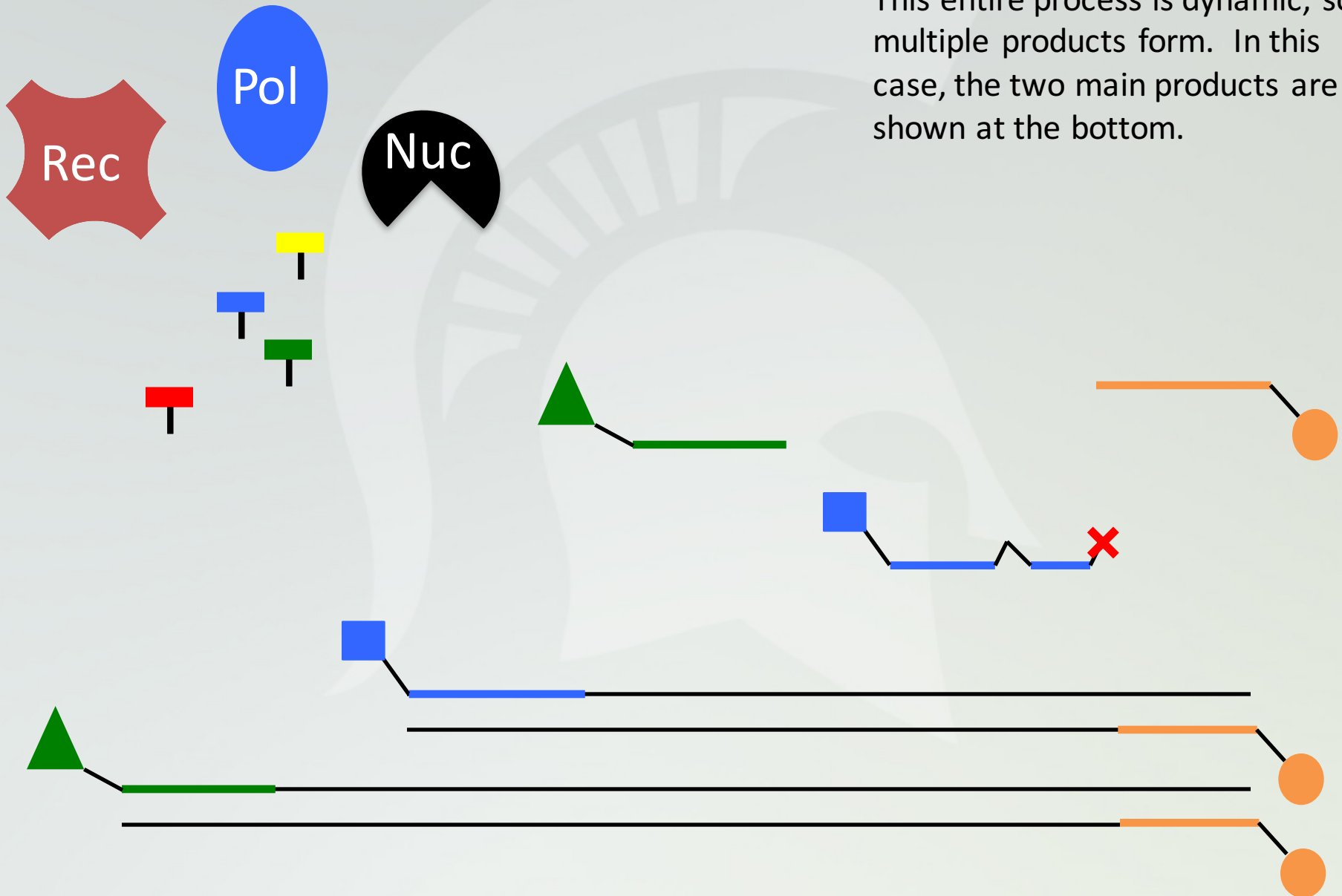


RPA Reaction Steps

13. Polymerase uses free dNTPs to extend 3’ primers, and make the second complementary strands

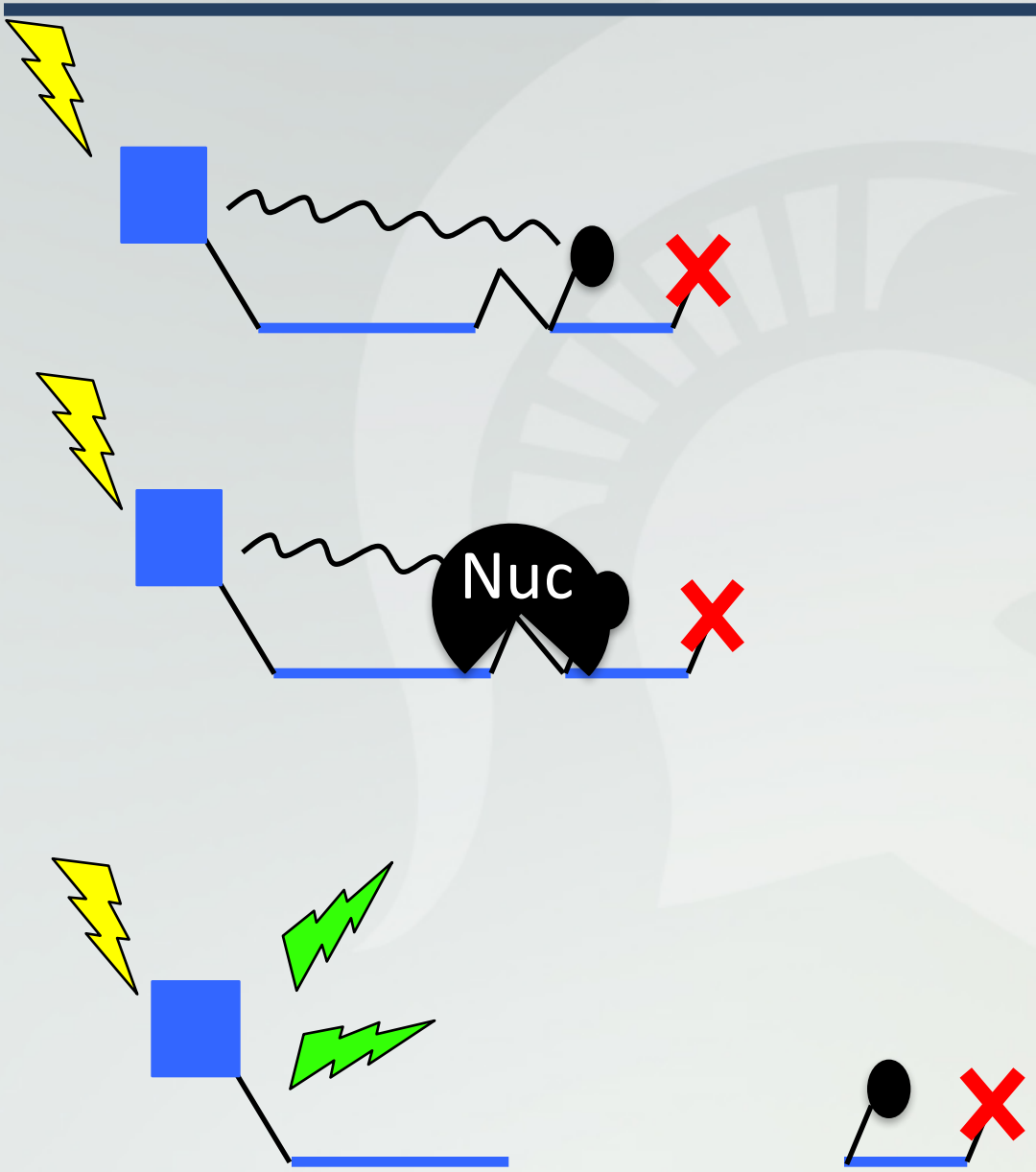


RPA Reaction Steps



This entire process is dynamic, so multiple products form. In this case, the two main products are shown at the bottom.

Interpreting Results – real time



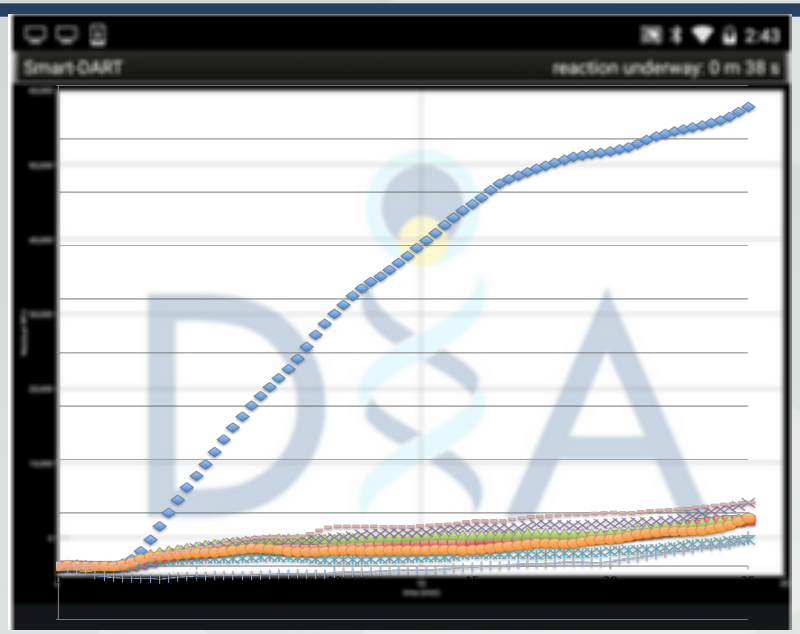
Exposing the probe to light causes it to emit a different wavelength of light. This emitted light is absorbed by an internal quencher. The 3' (right) end of the probe is also blocked so polymerase cannot extend from this position.

The special nuclease present in RPA kits will cleave the probe at a specific site called a THF site. This cleavage separates the fluorophore from the quencher and block.

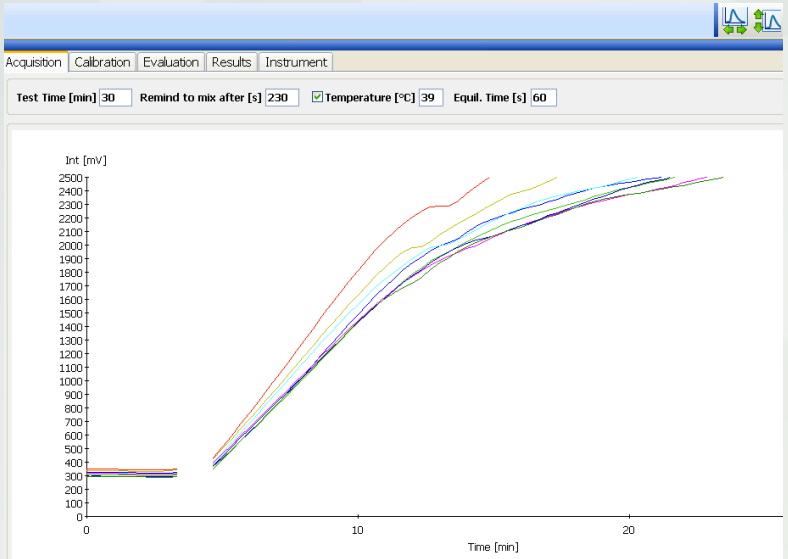
With the quencher absent, the emitted light from the fluorophore is detectable in a real-time fashion. Also, the new 3' end is unblocked, and polymerase can extend from this free end.

Interpreting Results – real time

Smart-DART
Example output



Twista
Example output



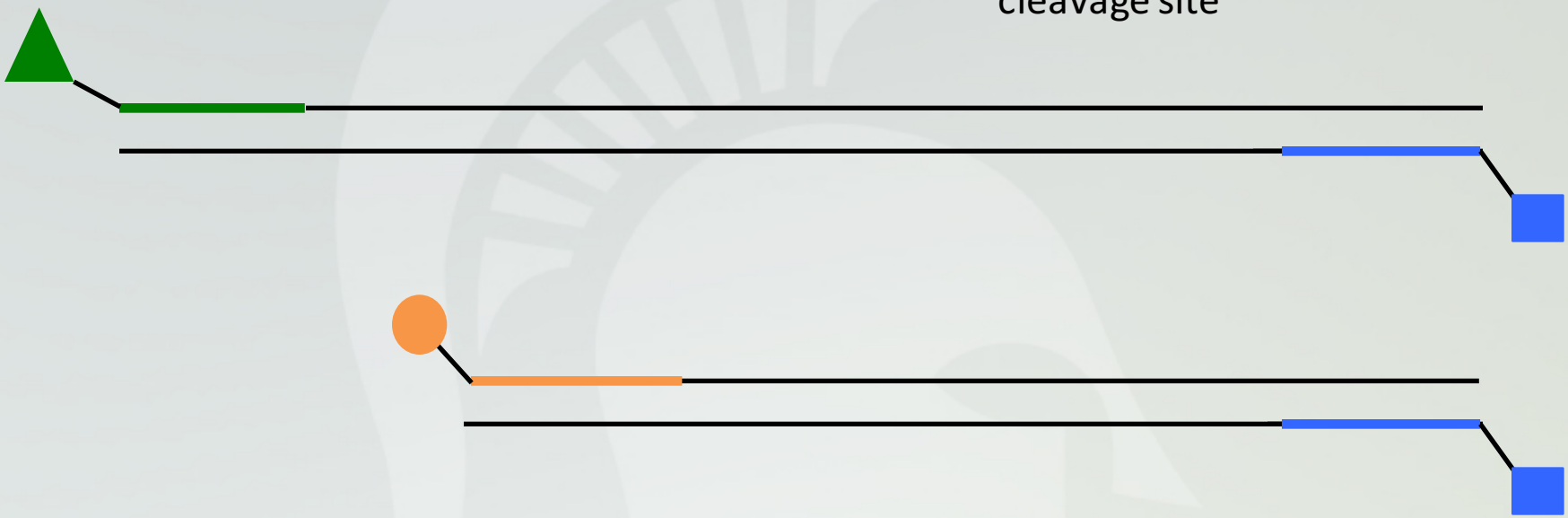
The y axis is fluorescence values, while the x axis is time.

If fluorescence is increasing over time, this indicates that the probe is finding its compliment sequence and being cut. This is a positive result meaning your target pathogen is present.

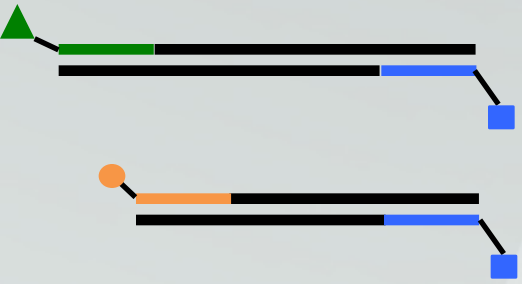
If fluorescence is not increasing over time, or increasing very minimally, then the probe is not finding its compliment nor being cut. This is a negative result, meaning your target pathogen is absent.

Interpreting Results – end point

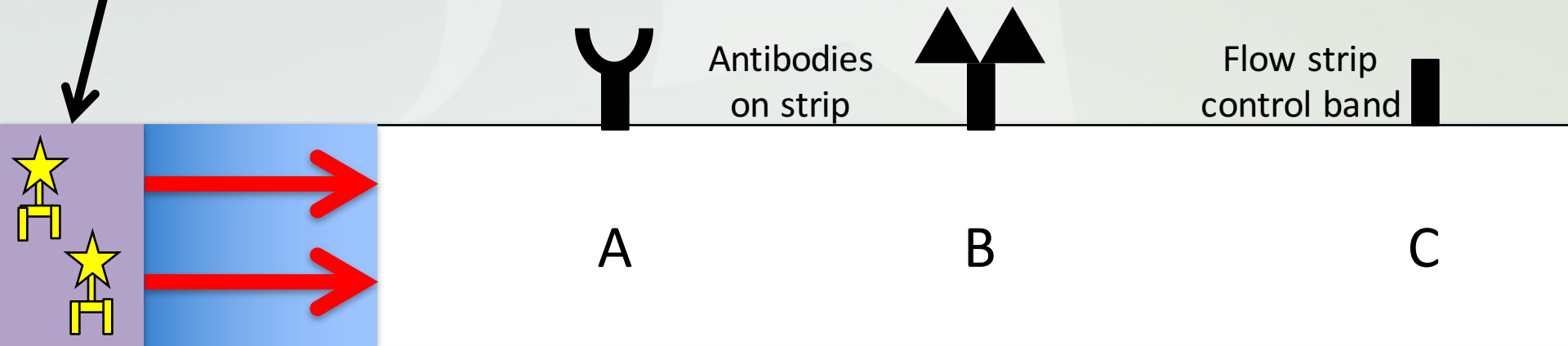
In this end-point detection, probe and primer have switched places, and the probe does not have a cleavage site



Interpreting Results – end point

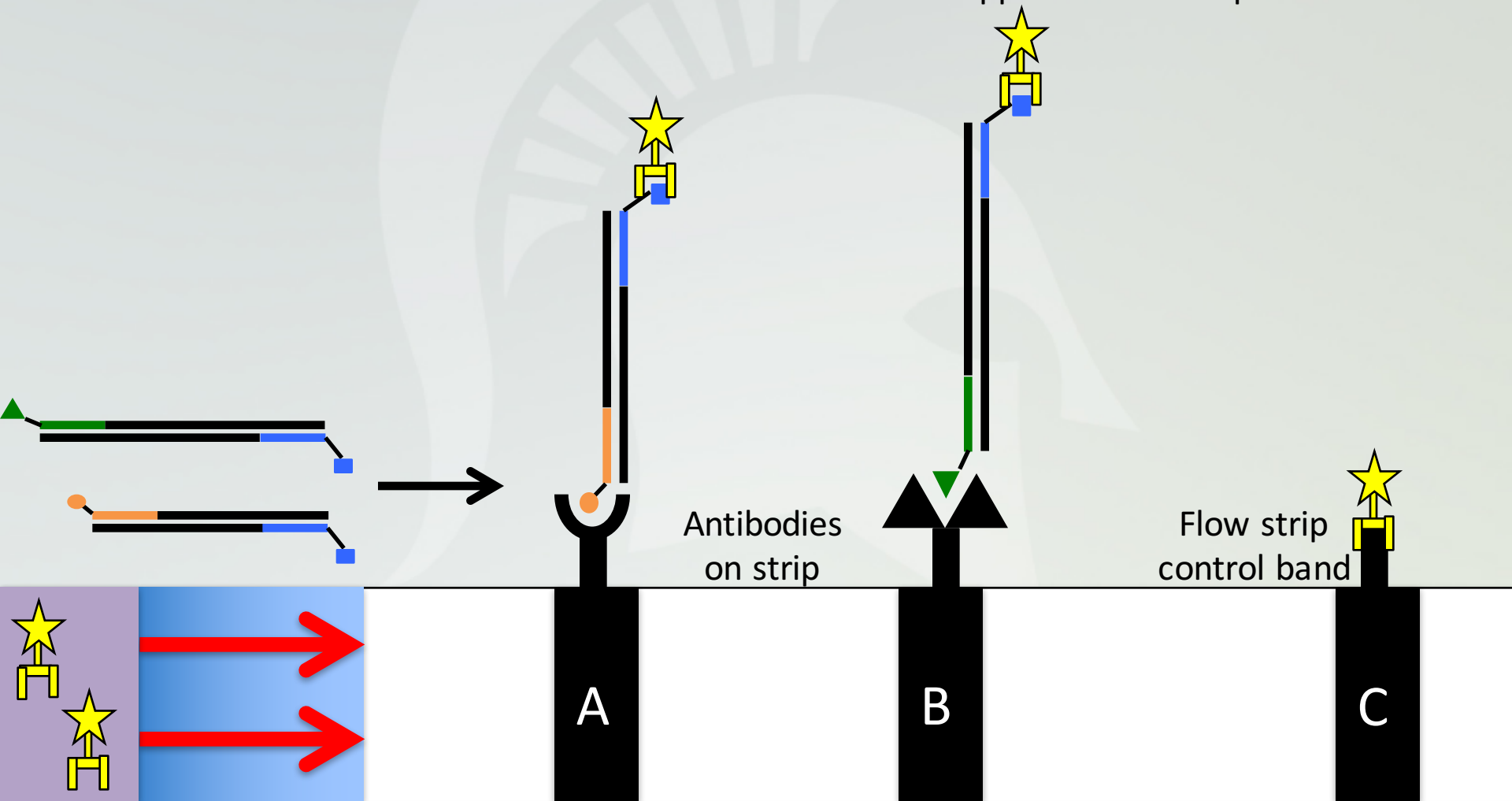


The reaction sample is added to a flow strip, where it mixes with gold particles and migrates towards antibodies.



Interpreting Results – end point

If the reaction successfully made both targets, they will bind to the antibodies and cause a band to appear on the strip.



Example Results