

THE MAGIC BOXES

AND THE MOLECULAR BIOLOGY BEHIND THE MARVELS

APRIL 28, 2016

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DISCLOSURE

Raymond P. Podzorski, Ph.D., D(ABMM)

April 28, 2016

No relevant financial relationships do disclose.

OBJECTIVES

- **Illustrate how the number of nucleic acid amplification methods has grown over the years**
- **Review the technology behind some of the popular nucleic acid amplification test systems**
- **Discuss some of the mechanisms used to detect amplified product in these systems**
- **Show the hardware associated with this technology**

NUCLEIC ACID AMPLIFICATION SYSTEMS

- **Polymerase Chain Reaction (PCR)**
- **Nucleic Acid Sequence Based Amplification (NASBA)**
- **Strand Displacement Amplification (SDA)**
- **Transcription Mediated Amplification (TMA)**
- **Ligase Chain Reaction (LCR)**
- **Q β Replicase**
- **Branched DNA (bDNA)**

NUCLEIC ACID AMPLIFICATION SYSTEMS

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- **Multiple Displacement Amplification (MDA)**
- **Transcription Mediated Amplification (TMA)**
- **Ligase Chain Reaction (LCR)**
- **Q β Replicase**
- **Cycling Probe Reaction (CPR)**
- **Hybrid Capture (HCII)**
- **Cleavase-based Amplification (INVADER)**
- **Branched DNA (bDNA)**
- **Nicking Enzyme Amplification Reaction (NEAR)**
- **Loop-Mediated Isothermal Amplification (LAMP)**
- **Helicase Dependent Amplification (HDA)**

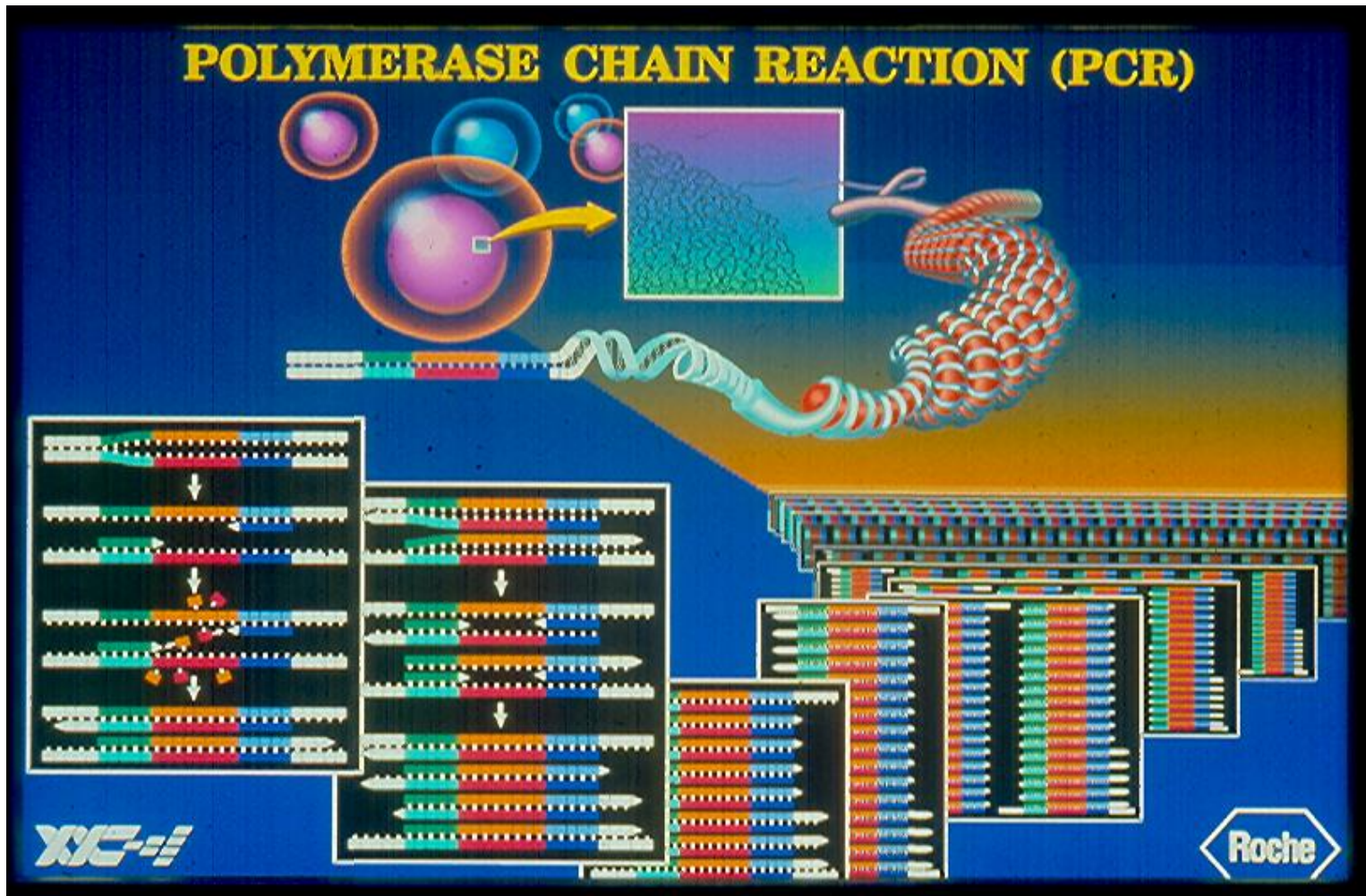
ISOTHERMAL AMPLIFICATION SYSTEMS

- **Helicase Dependent Amplification (HDA)**
- **Loop-Mediated Isothermal Amplification (LAMP)**
- **Multiple Displacement Amplification (MDA)**
- **Nucleic Acid Sequence Based Amplification (NASBA)**
- **Nicking Enzyme Amplification Reaction (NEAR)**
- **Ramification Amplification Method (RAM)**
- **Rolling Circle Amplification (RCA)**
- **Strand Displacement Amplification (SDA)**
- **Signal Mediated Amplification of RNA Technology (SMART)**
- **Single Primer Isothermal Amplification (SPIA)**
- **Transcription Mediated Amplification (TMA)**

ISOTHERMAL AMPLIFICATION SYSTEMS

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TARGET AMPLIFICATION SYSTEM - PCR



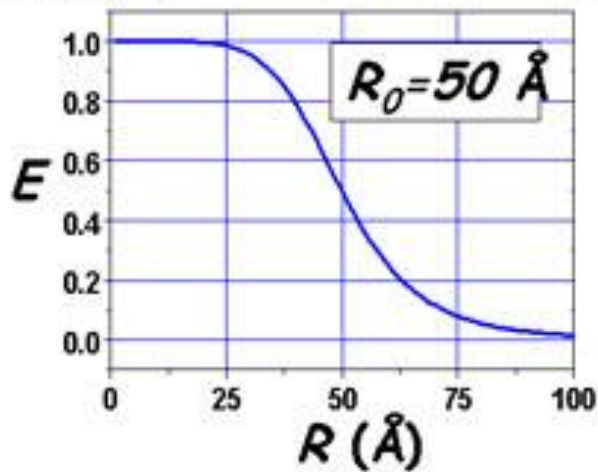
FRET CONCEPT



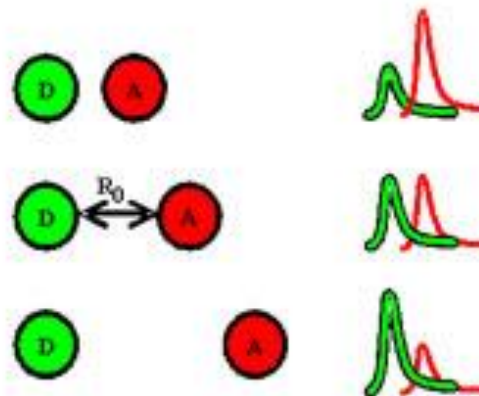
Energy Transfer Efficiency

$$E = \frac{1}{1 + (R/R_0)^6}$$

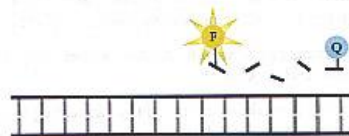
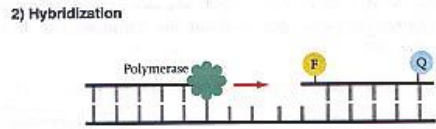
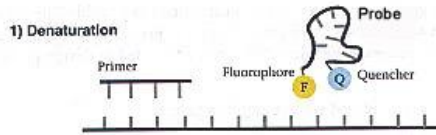
$R_0=50\%$ transfer efficiency distance
3nm~7nm



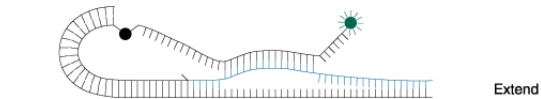
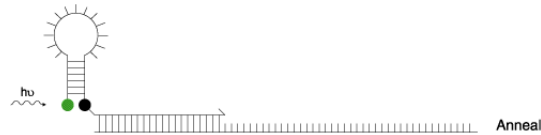
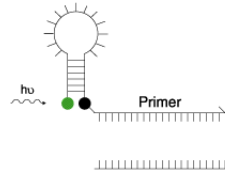
“Spectroscopic Ruler”



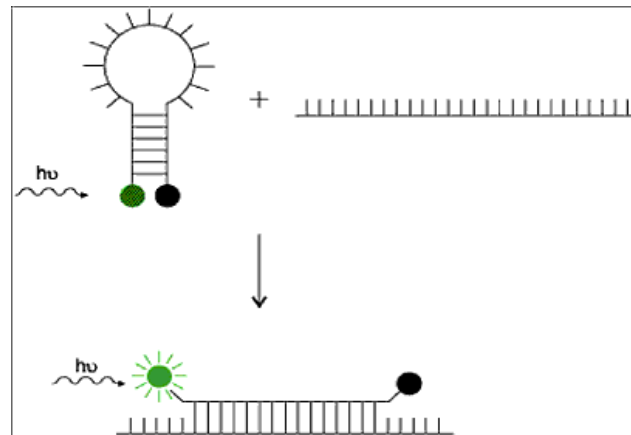
FRET PROBES



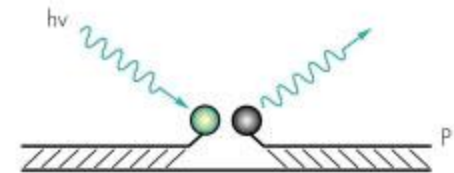
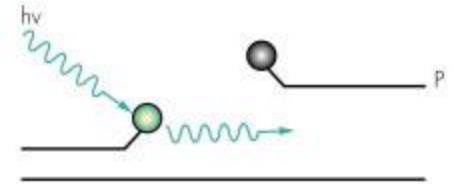
TaqMan
(hydrolysis)



Scorpion

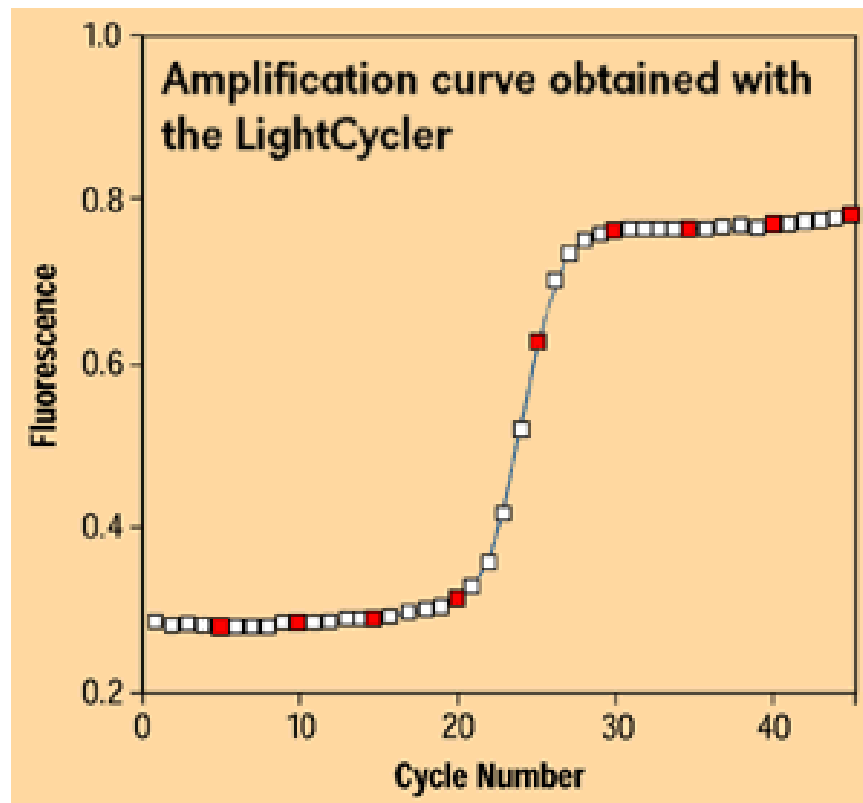
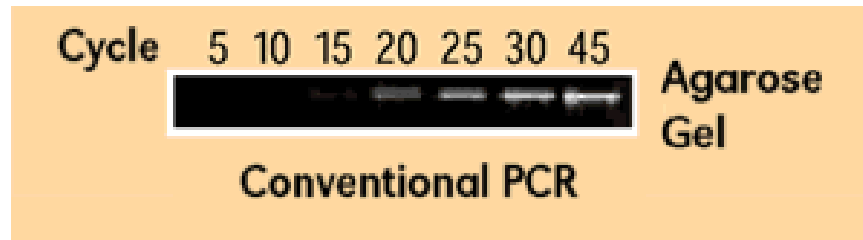


Molecular Beacon



Adjacent Hybridization
Probes

FRET PROBES



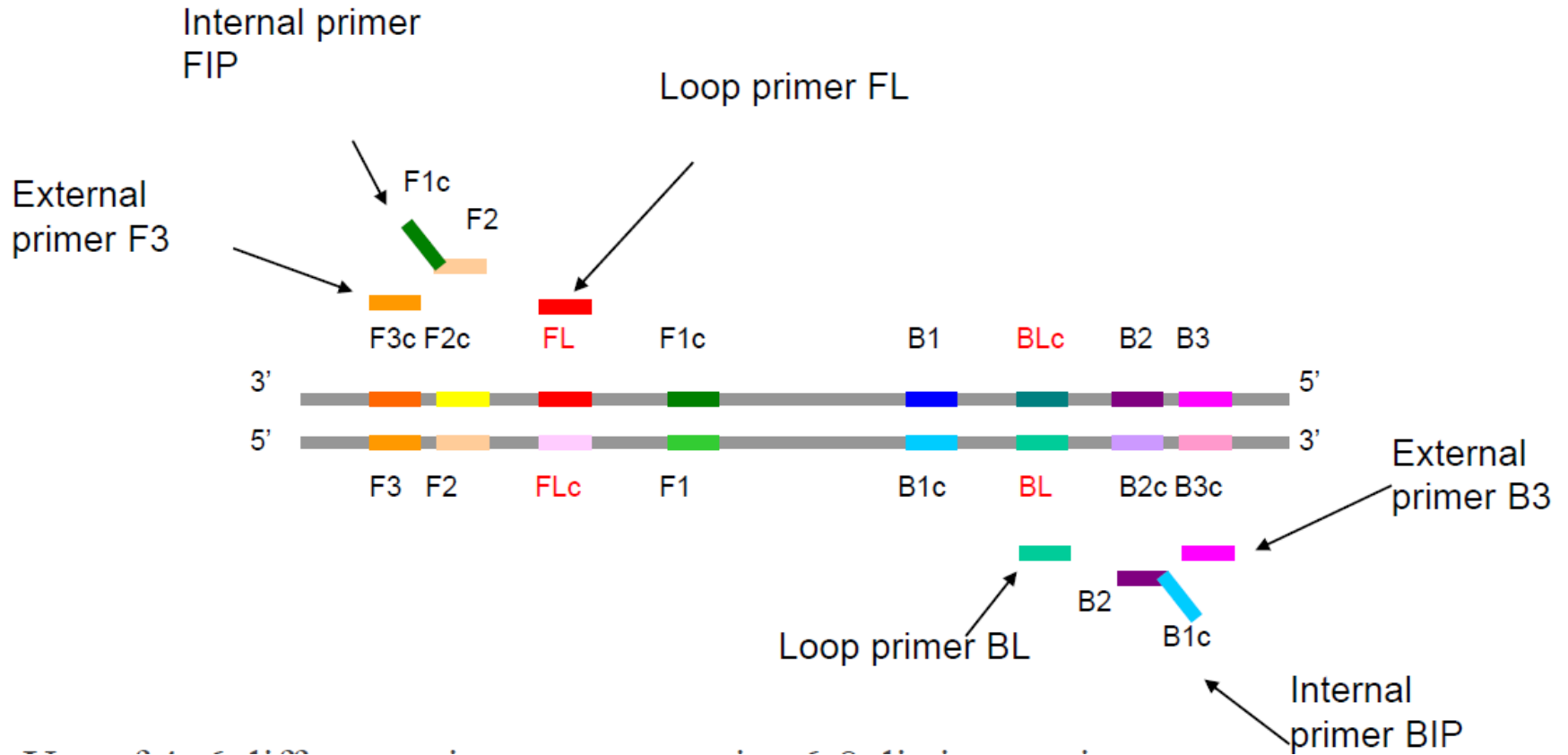
ILLUMIGENE



Loop Mediated Isothermal Amplification

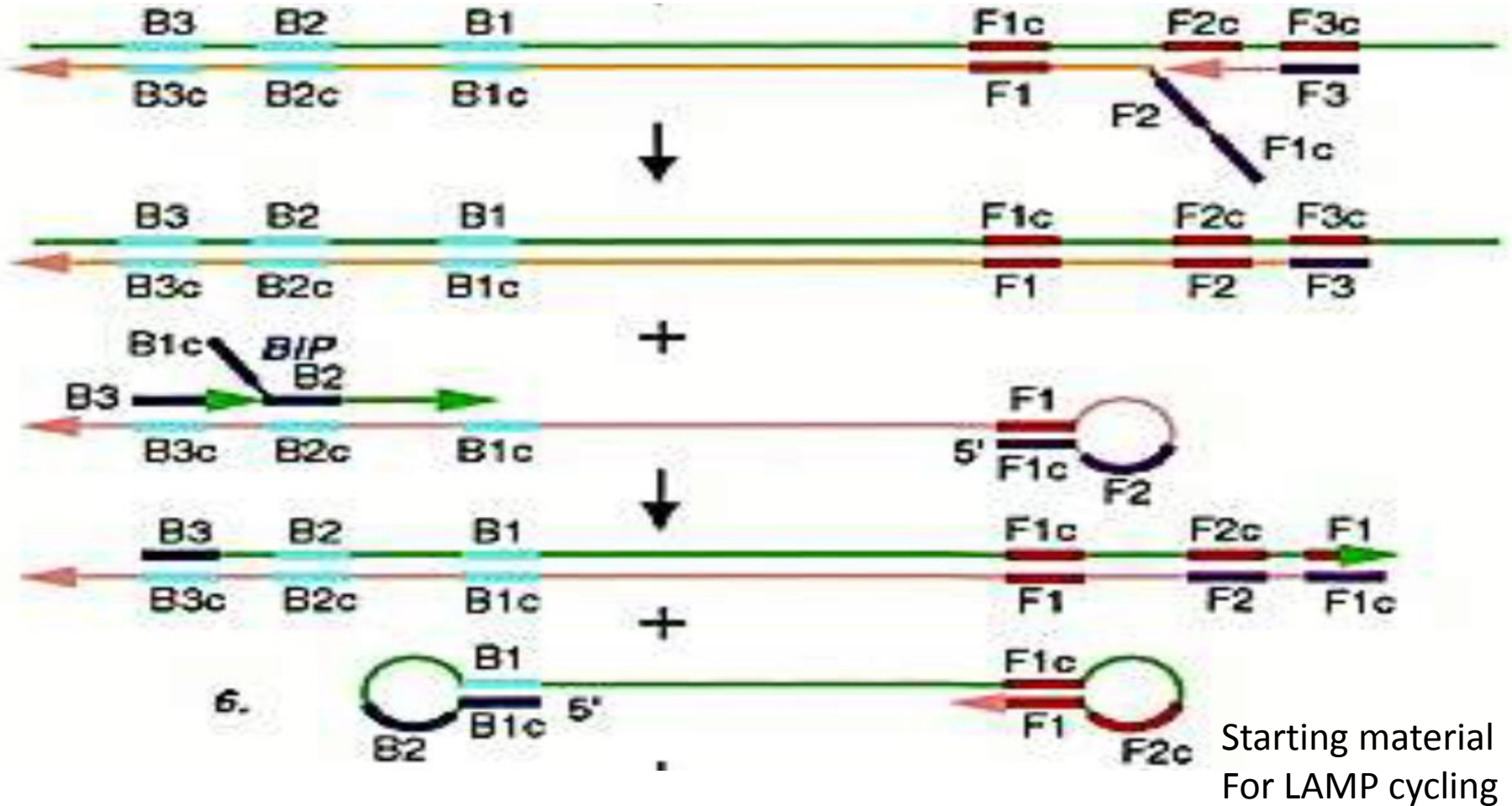
LAMP

I. Production of Starting Material - All 4 primers used at the start



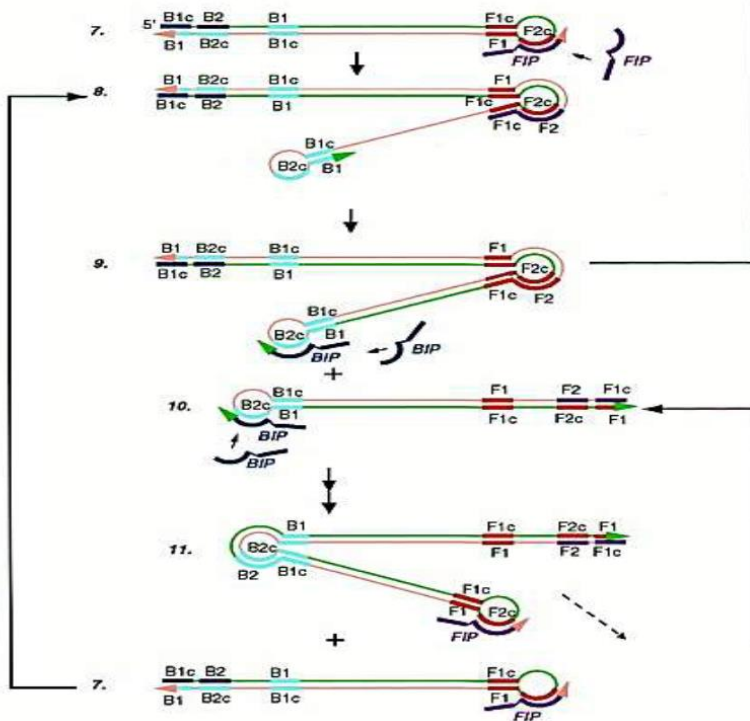
LAMP

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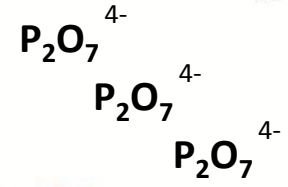
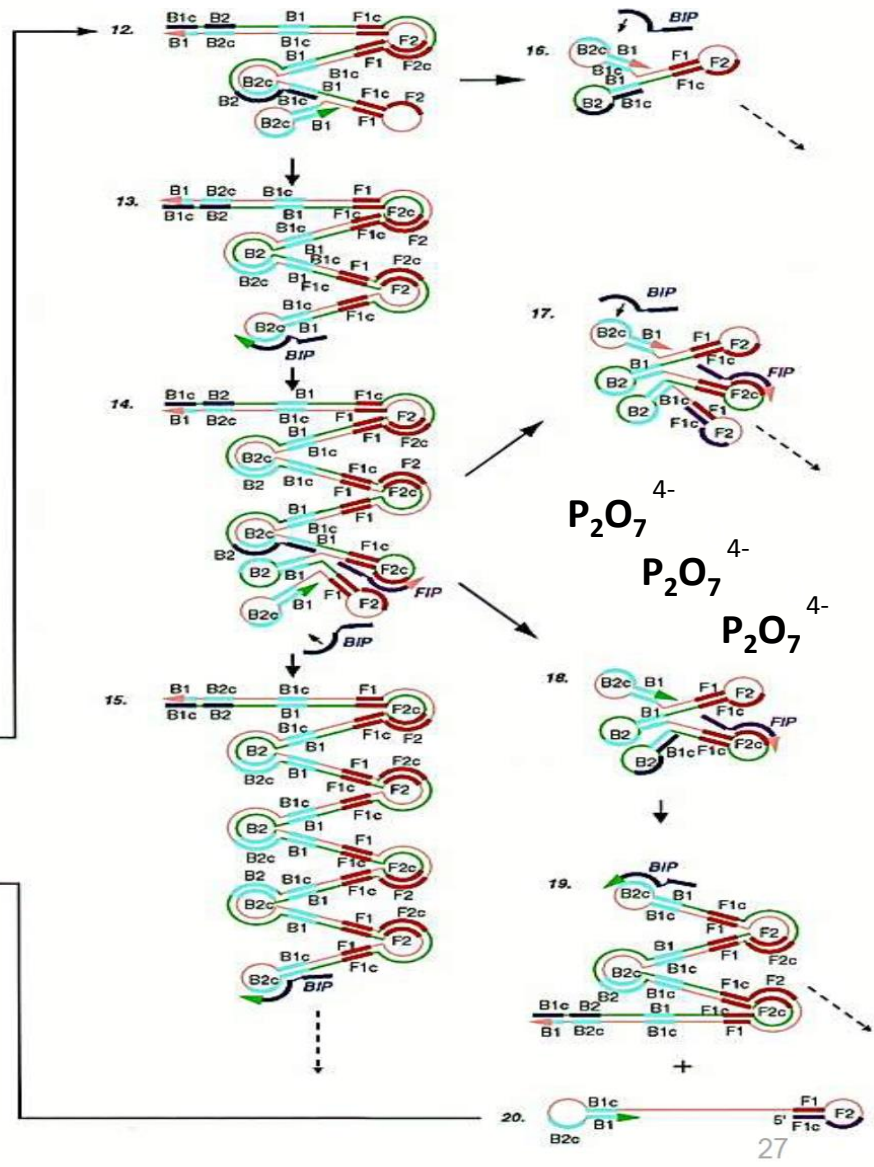


LAMP

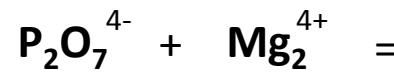
II. Cycling Amplification



III. Elongation and Recycling Step



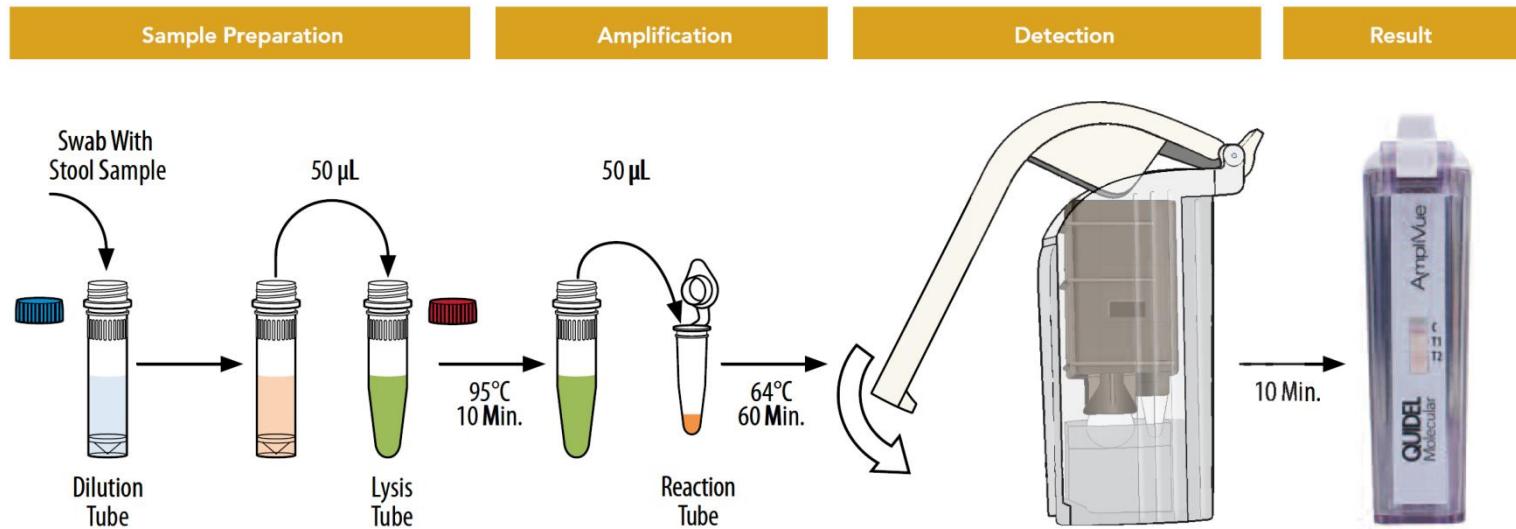
ILLUMIGENE



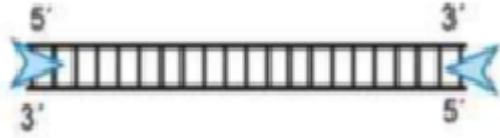
Loop Mediated Isothermal Amplification

AMPLIVUE

Ampli-fied DNA/RNA + Vue-able Results



Helicase Dependent Amplification

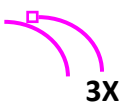


SSB ●

Helicase ➤

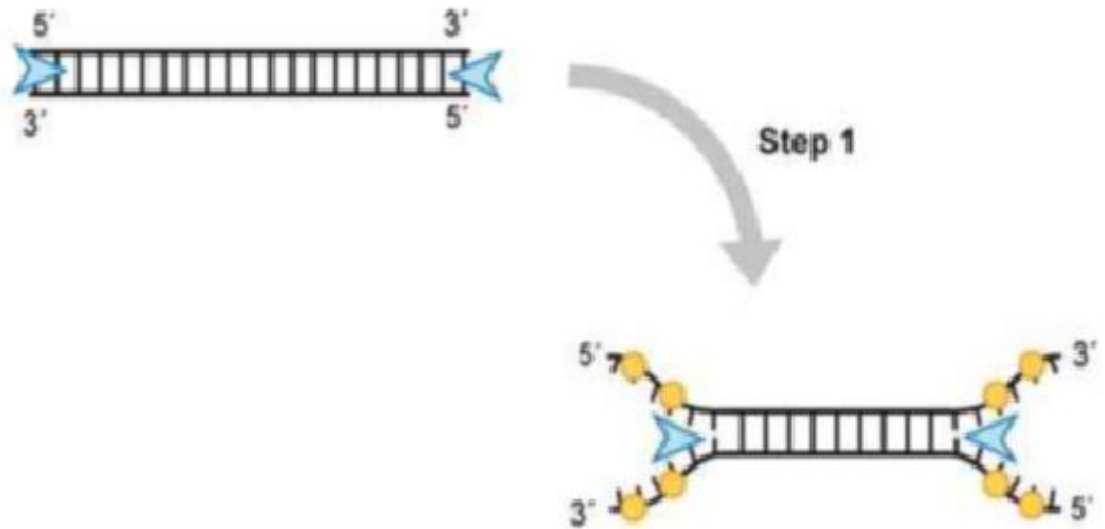
Polymerase ◀

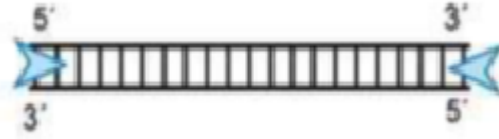
Primers



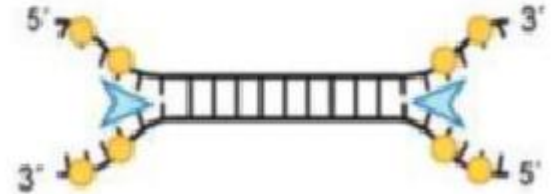
FITC-Probe



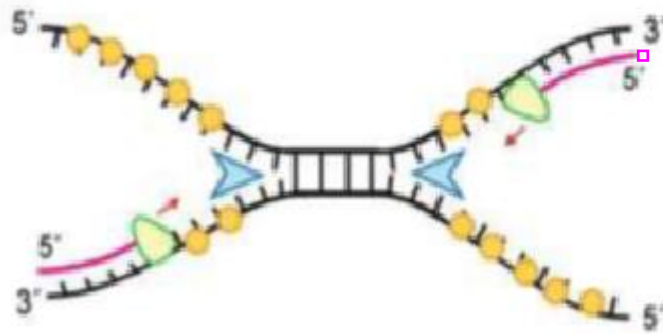




Step 1



Step 2



SSB



Helicase



Polymerase



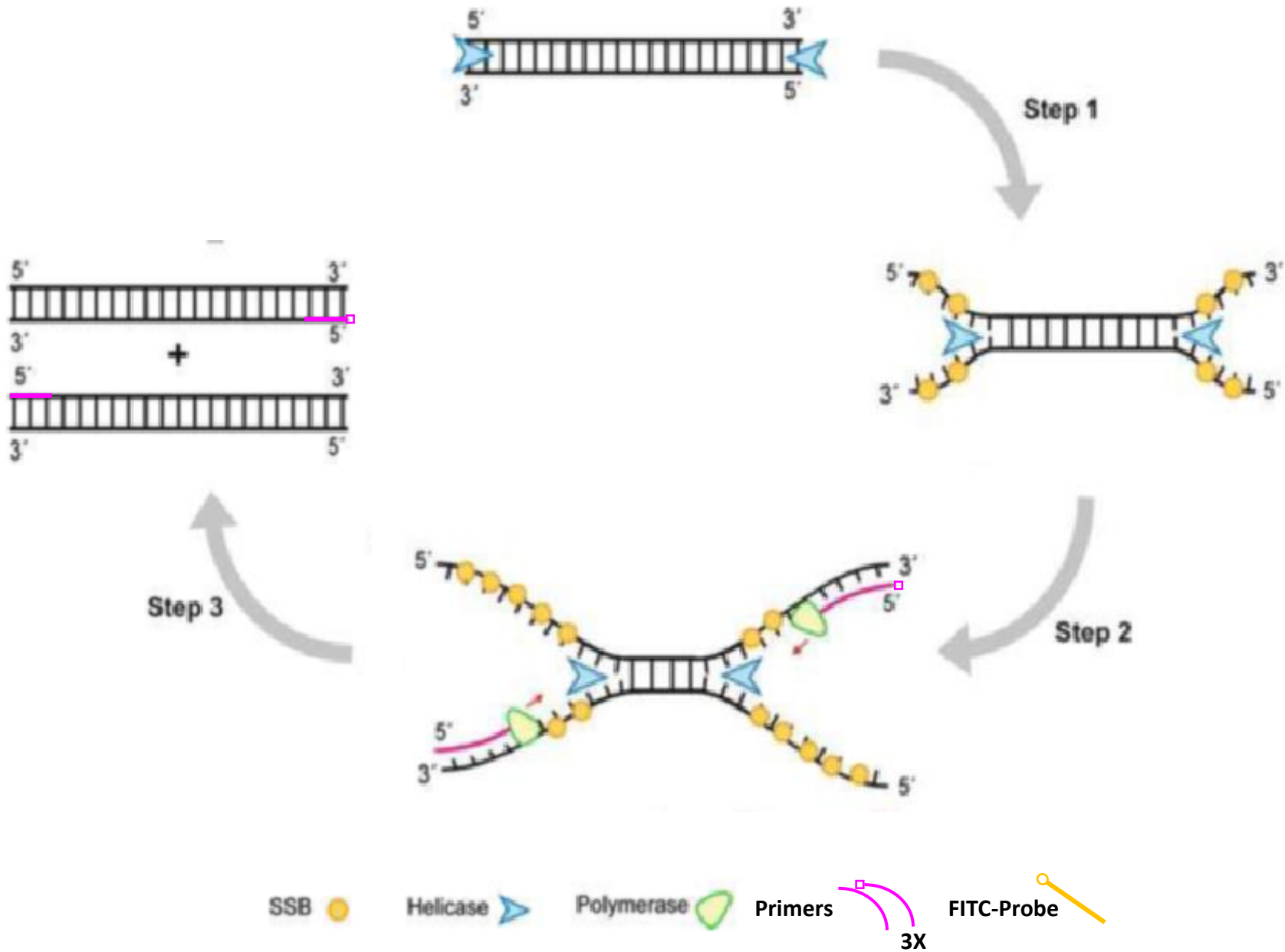
Primers

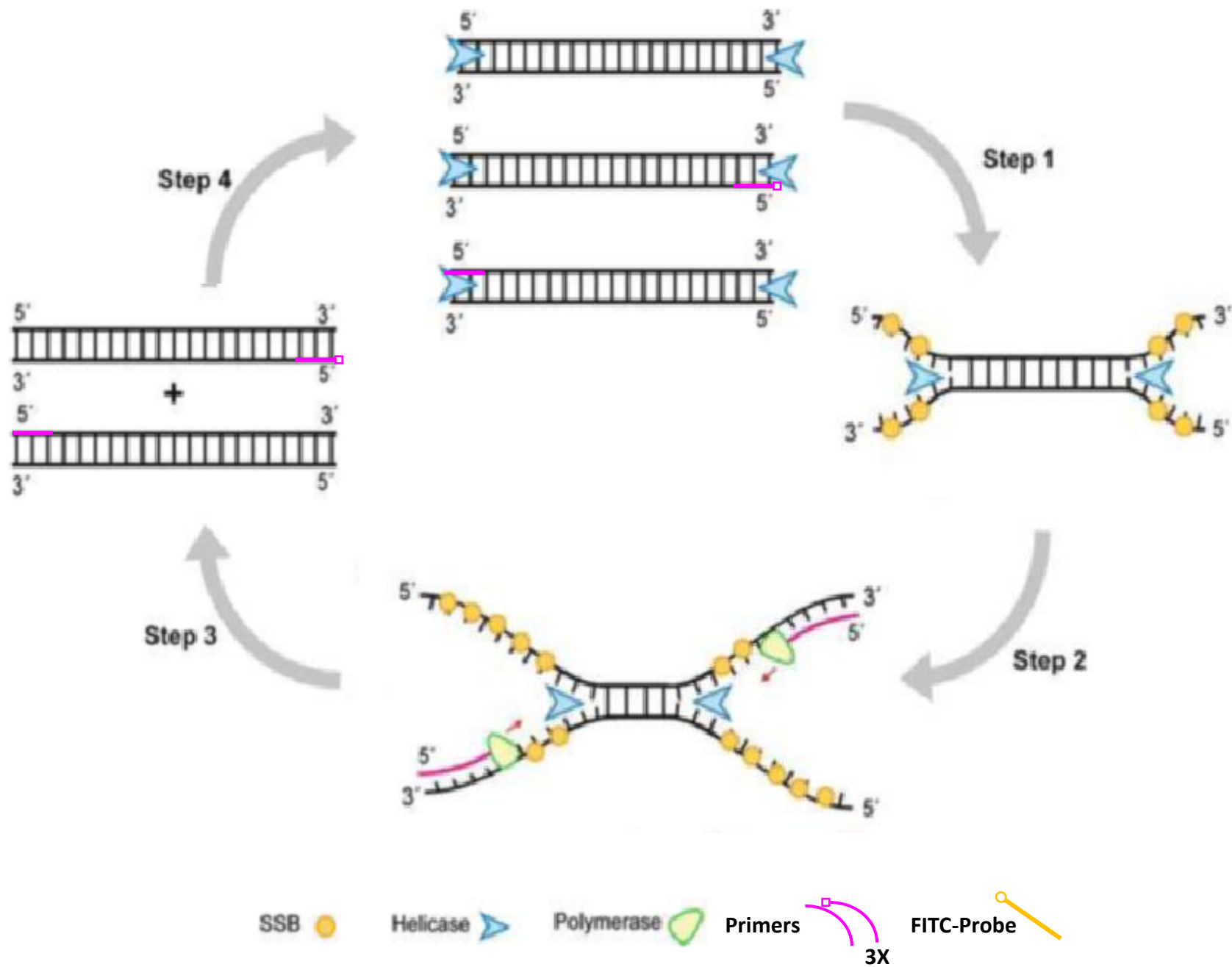


3X

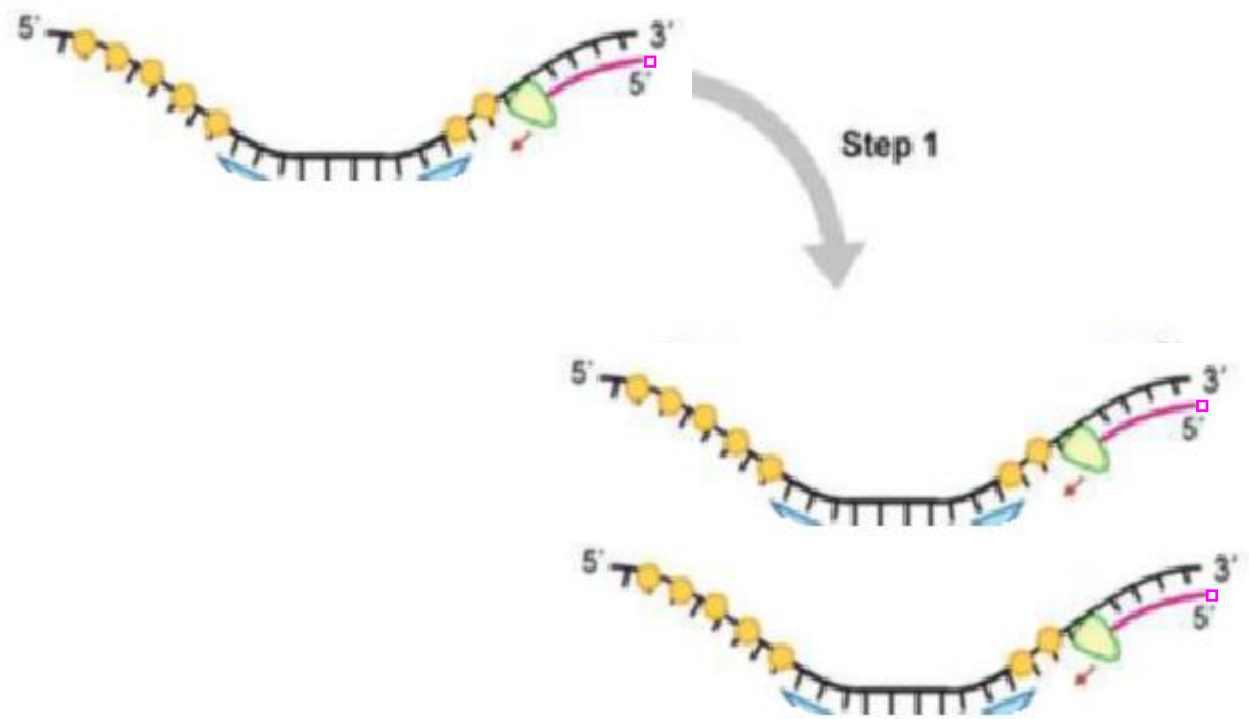
FITC-Probe

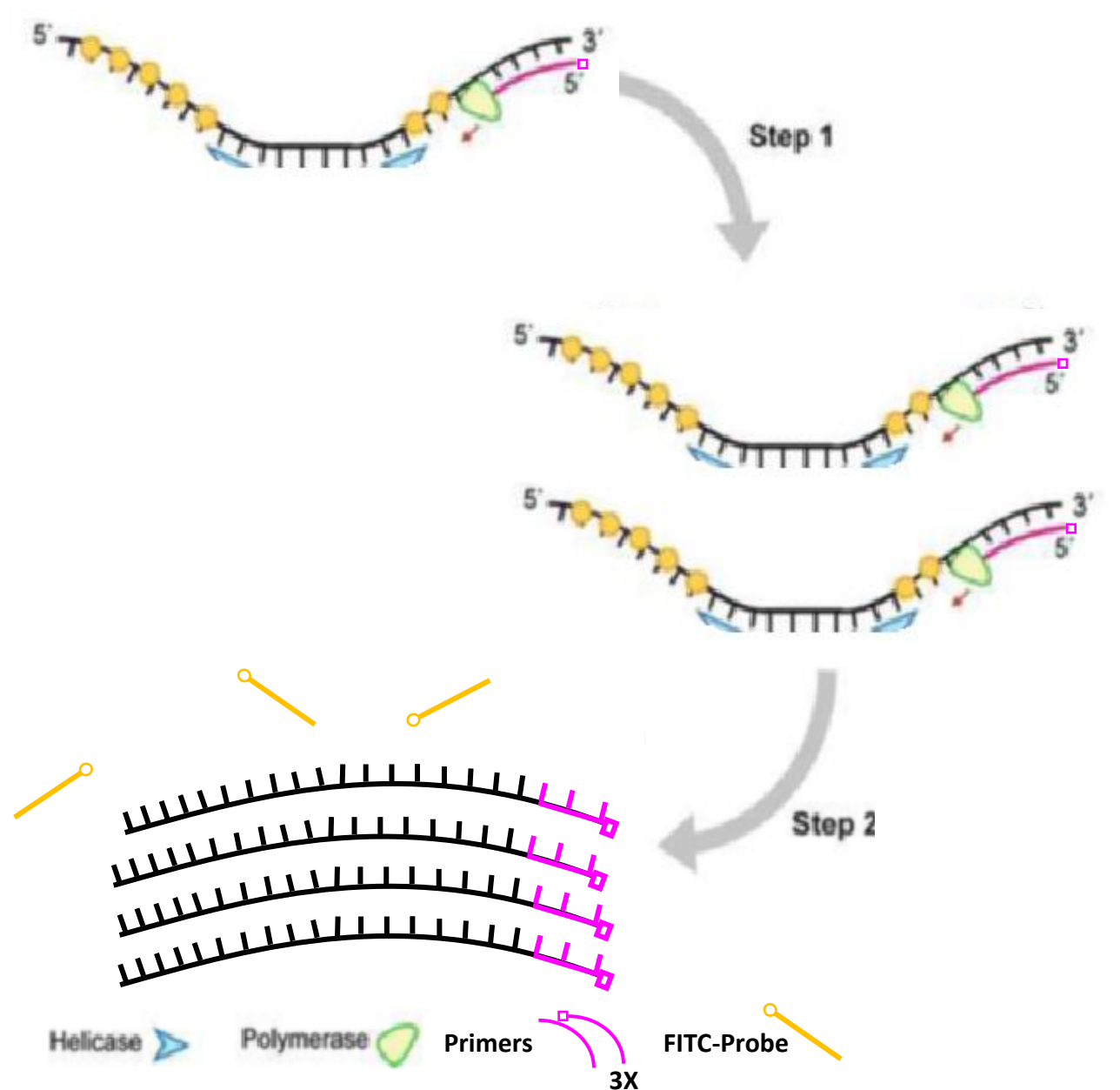


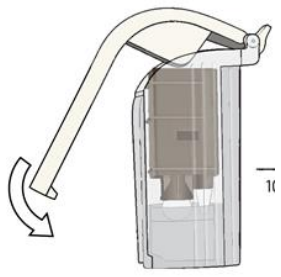








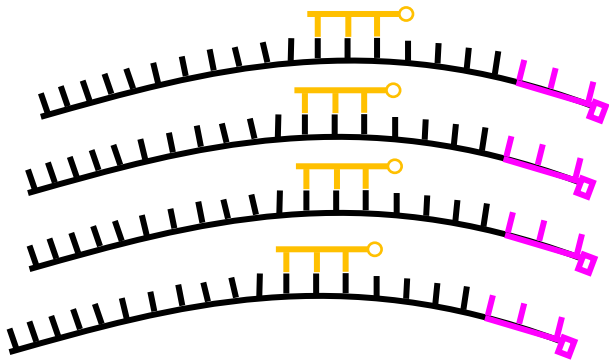
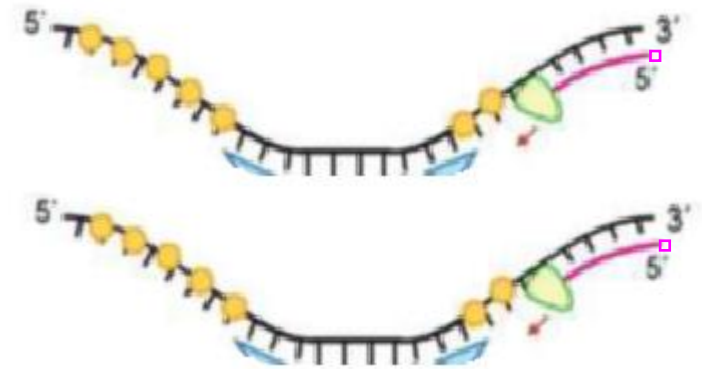




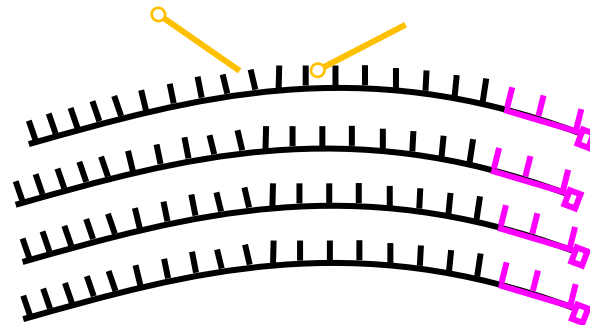
Step 4



Step 1



Step 3



Step 2

SSB



Helicase



Polymerase



Primers



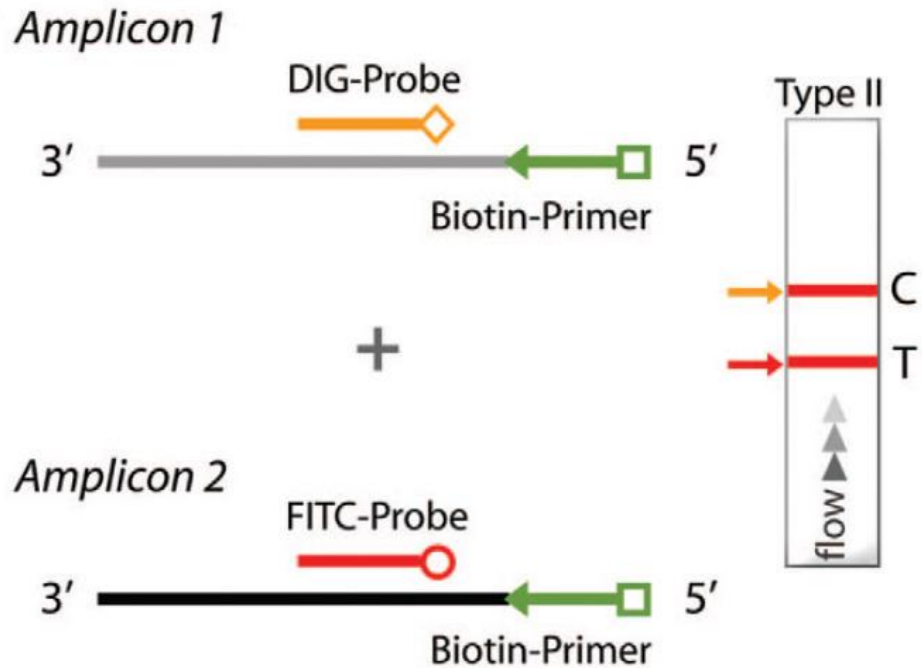
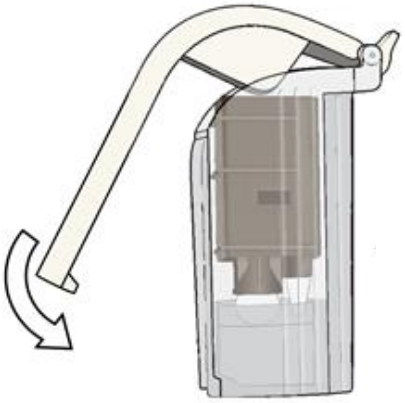
FITC-Probe



3X

AMPLIVUE

Ampli-fied DNA/RNA + Vue-able Results



Streptavidin coated latex beads imbedded in strip colored red

Anti-DIG Ab at C-line

Anti-FITC Ab at T-line

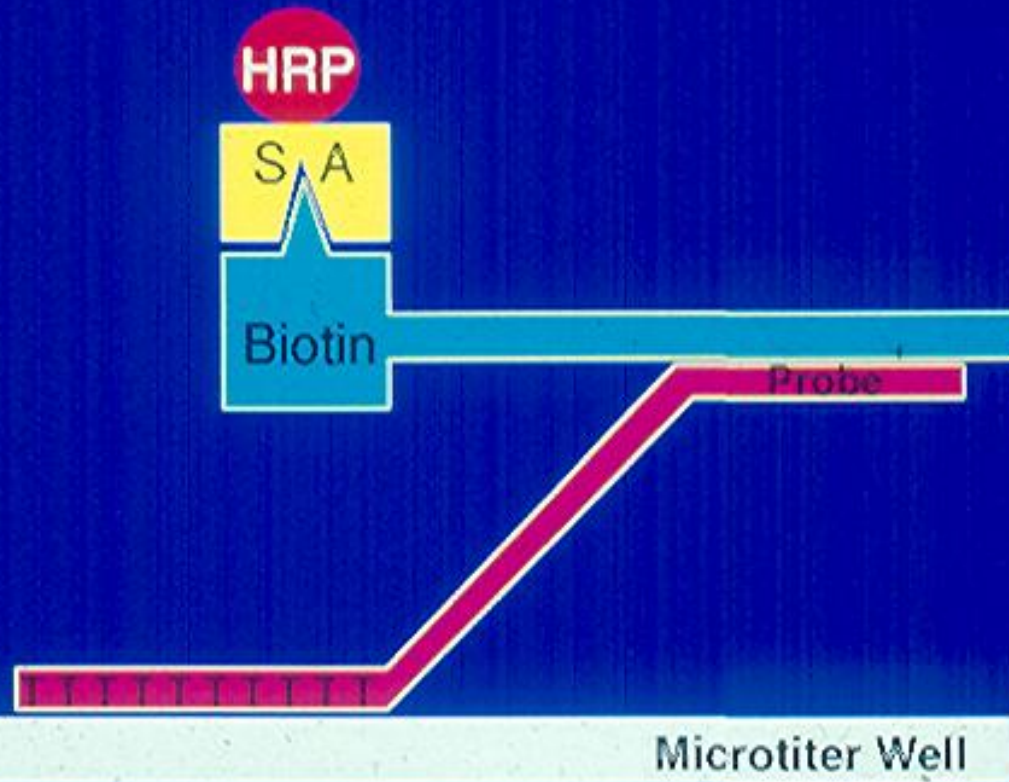
- B. pertussis*
- C. difficile* toxin A
- S. pyogenes*
- S. agalactiae*
- HSV 1+2
- T. vaginalis*

○ FITC
Probe target

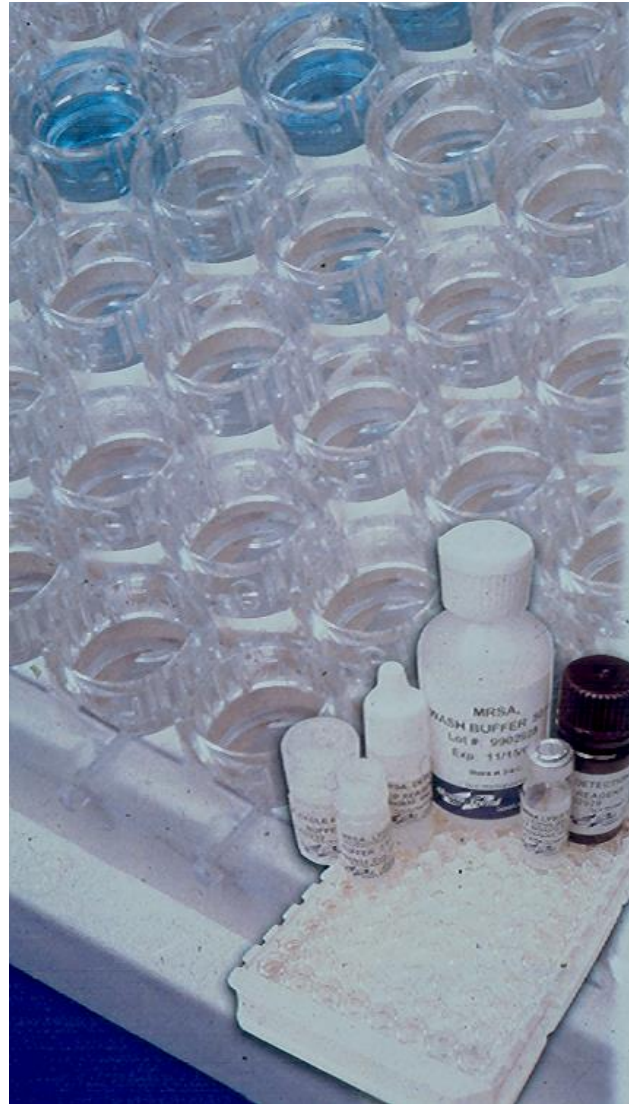
□ Biotin

◇ DIG
Probe control

Reverse Dot-Blot



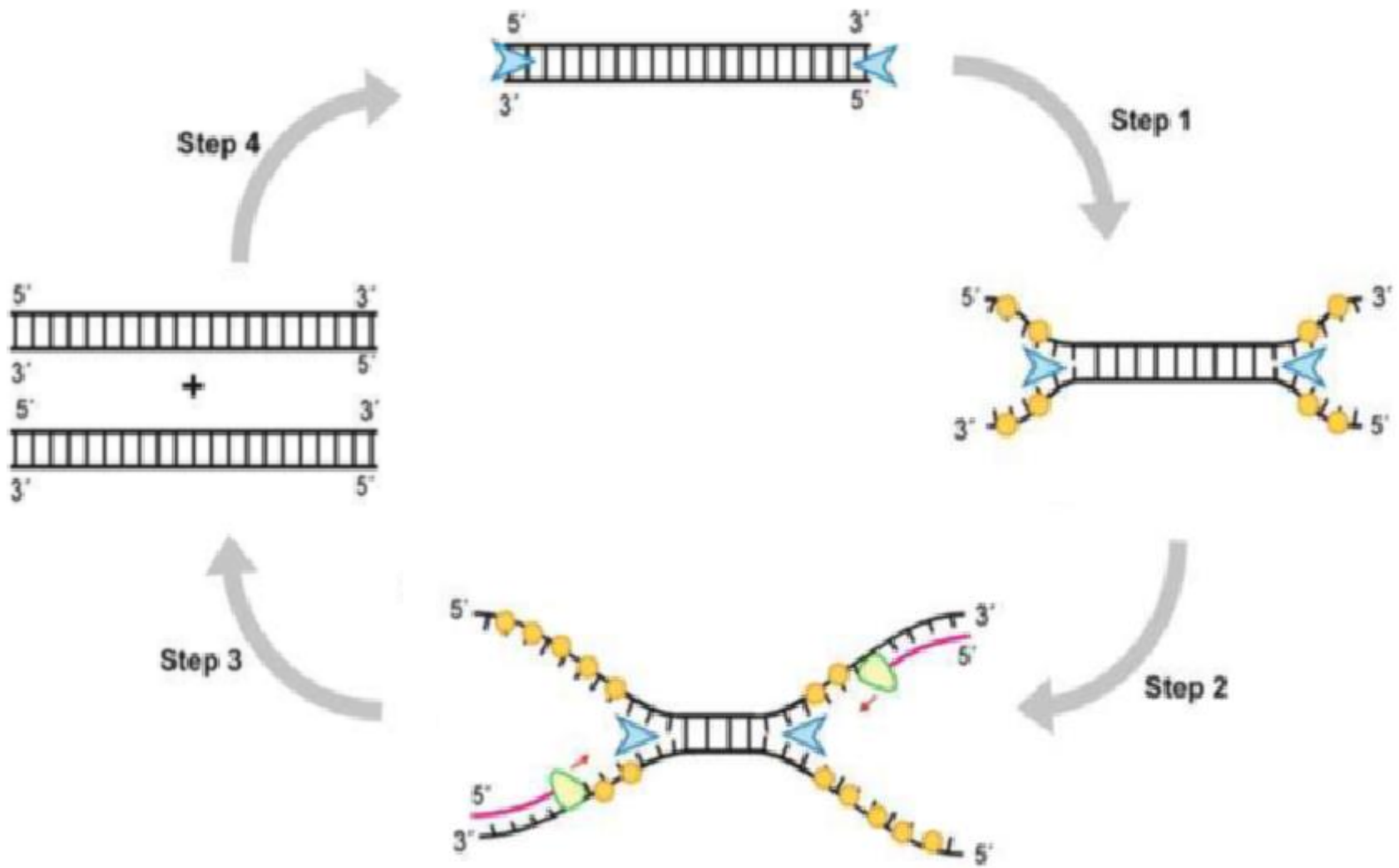
MANUAL DETECTION



SOLANA




Helicase Dependent Amplification





SSB ● Helicase ➤ Polymerase ◉ Primers — RNaseH2 ⚡ RNA probe ● F

3X



SSB 

Helicase 

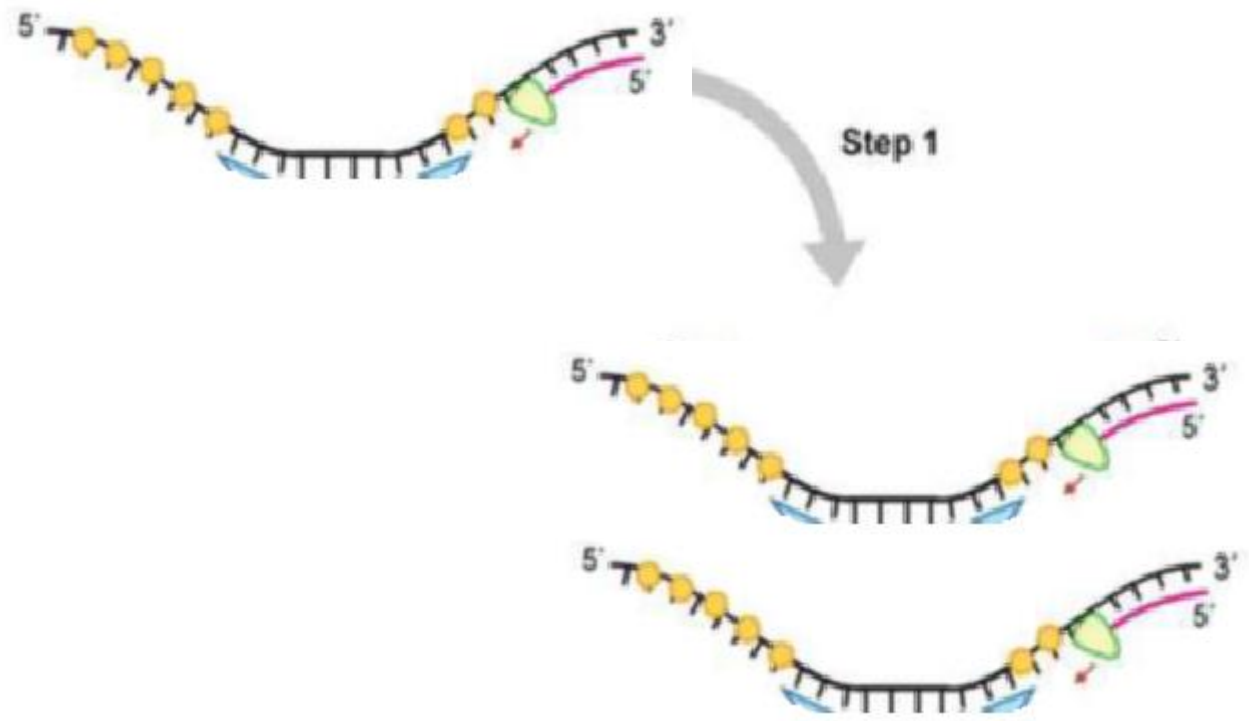
Polymerase 

Primers 

3X

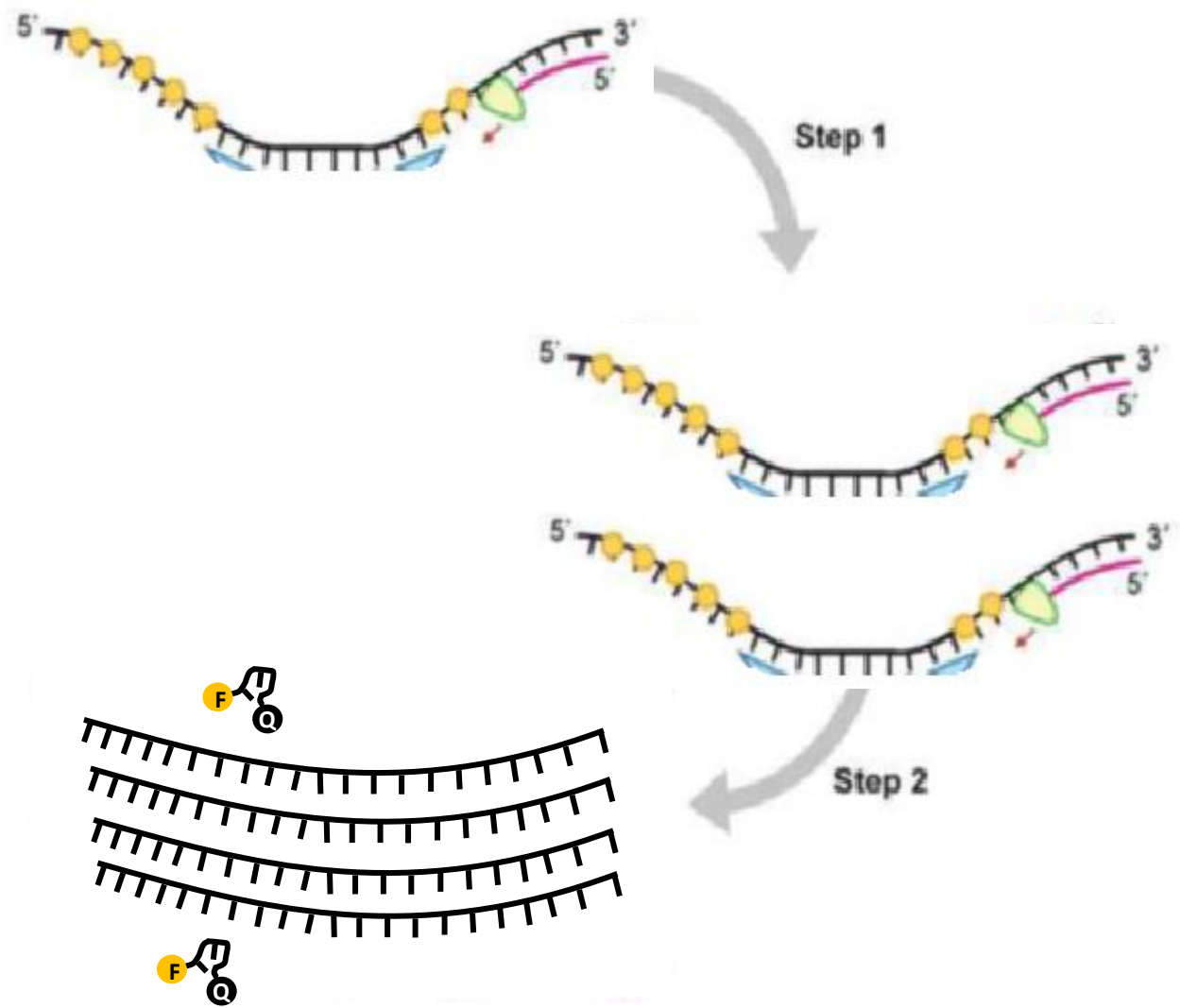
RNAseH2 

RNA probe 

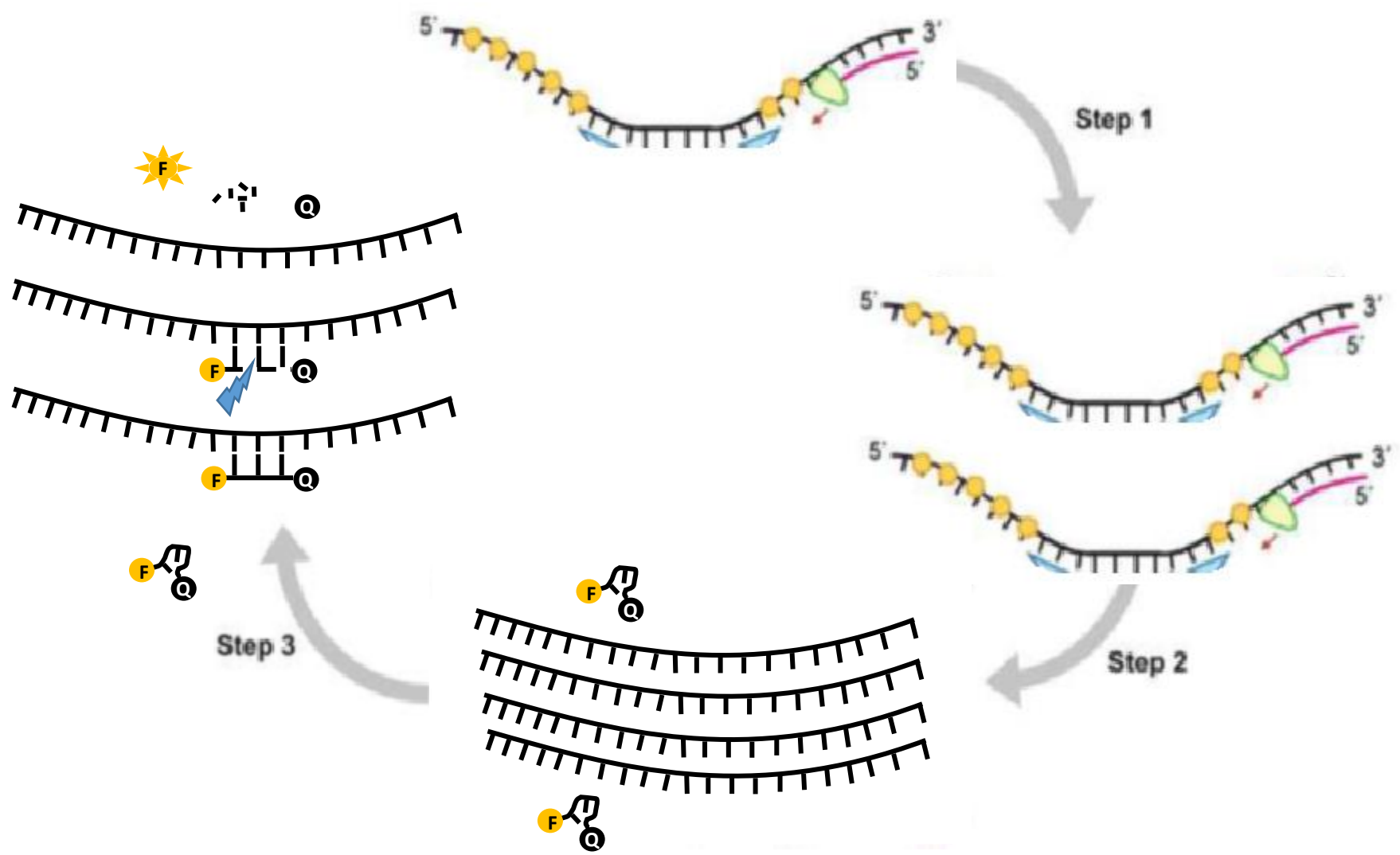


SSB 
 Helicase 
 Polymerase 
 Primers 
 RNaseH2 
 RNA probe 

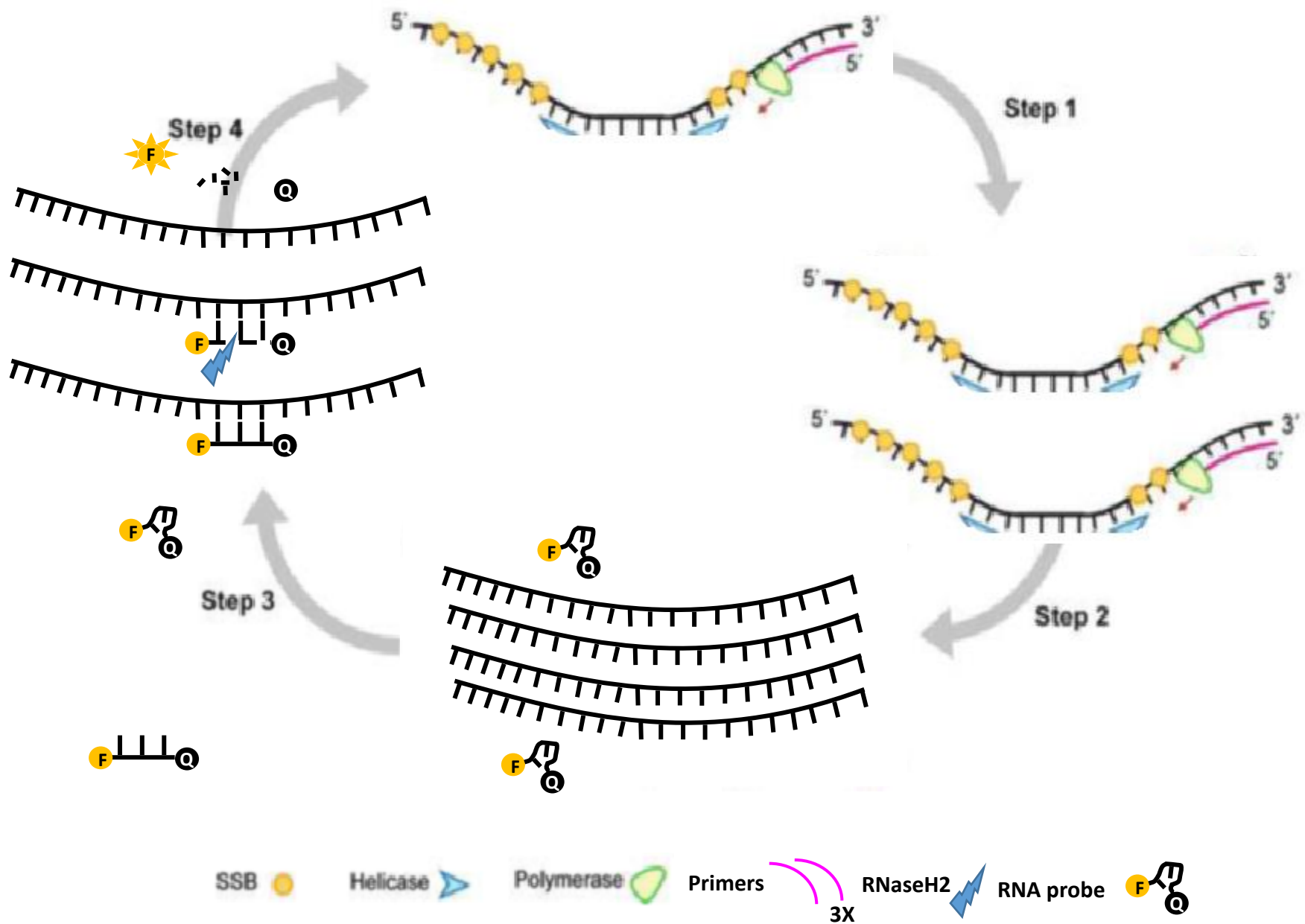
3X



SSB ● Helicase ▲ Polymerase ● Primers 3X RNaseH2 ⚡ RNA probe ● F



SSB 
 Helicase 
 Polymerase 
 Primers 
 RNaseH2 
 RNA probe 



SOLANA



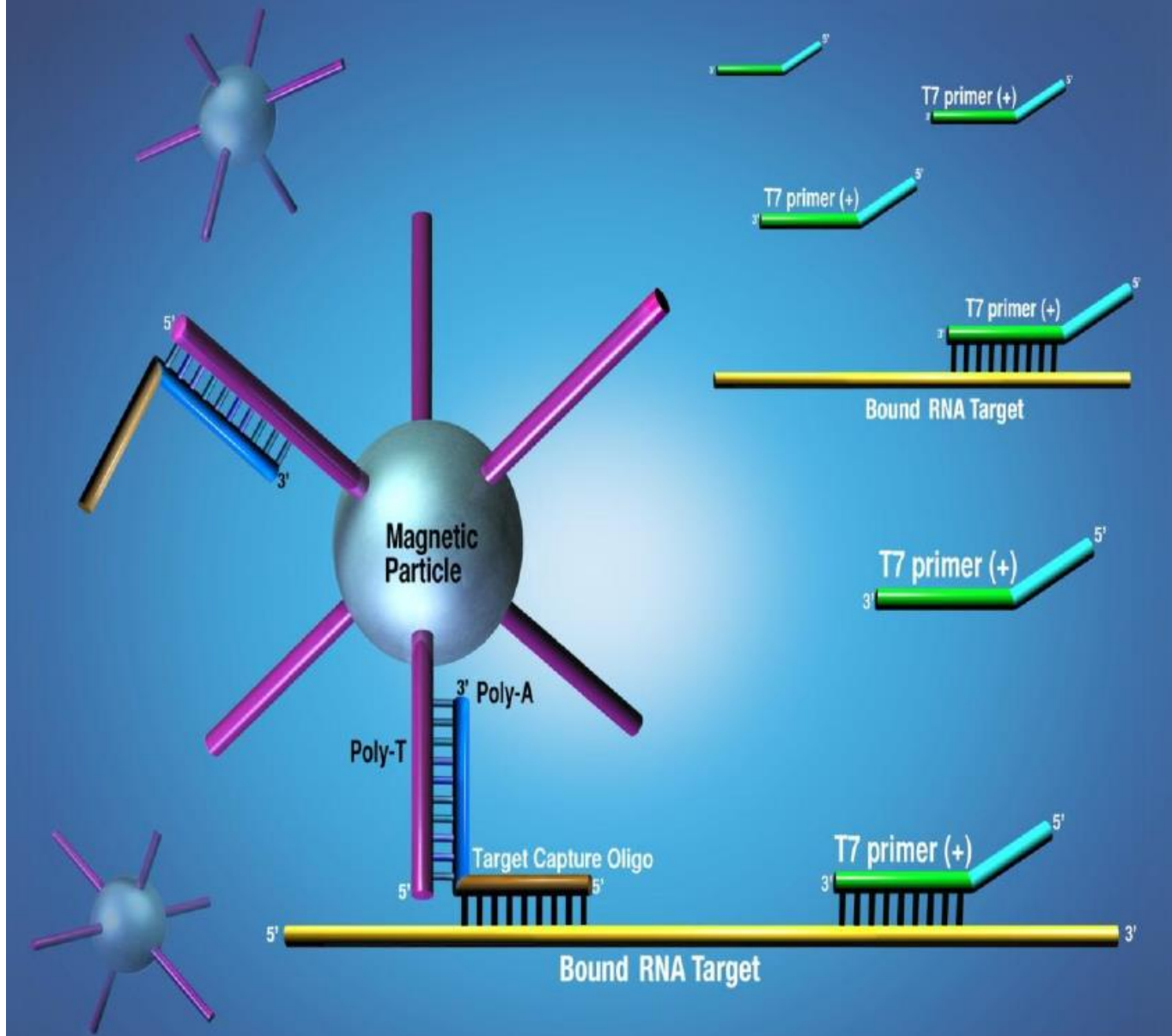
Helicase Dependent Amplification

PANTHER

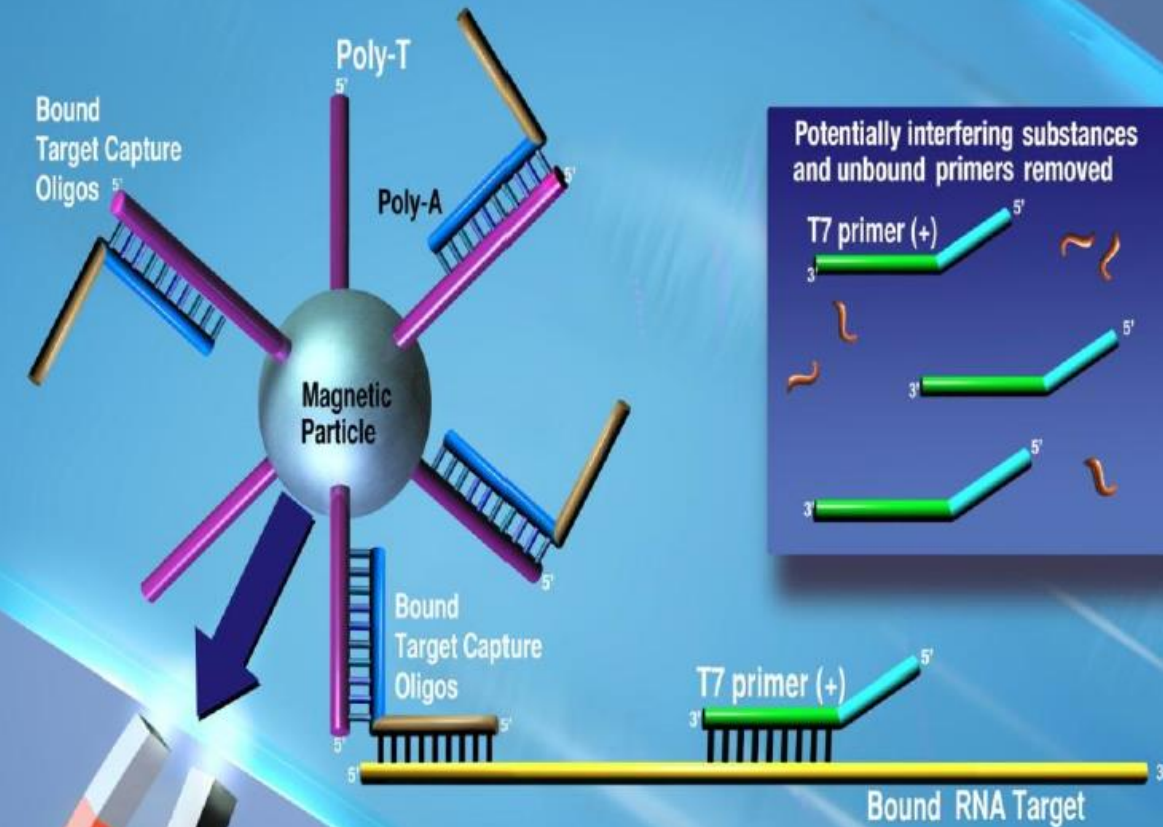


Transcription Mediated Amplification

Target Capture: Sample addition, annealing, and capture



Target concentration and wash steps



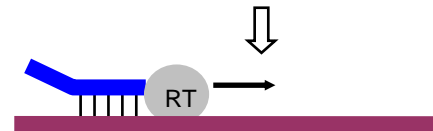
Magnet holds magnetic particles to the side of reaction tube during wash step

TRANSCRIPTION MEDIATED AMPLIFICATION

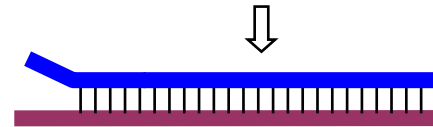
1: Primer 1 incl. T7-promotor sequence binds to target



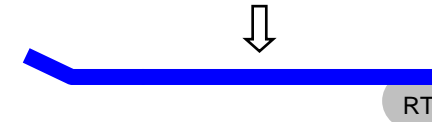
2: Rev. Transcriptase (RT) creates first strand of cDNA



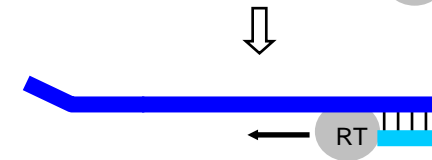
3: RNA:cDNA duplex is completed



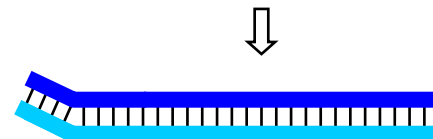
4: RNase H activity of RT degrades the target RNA



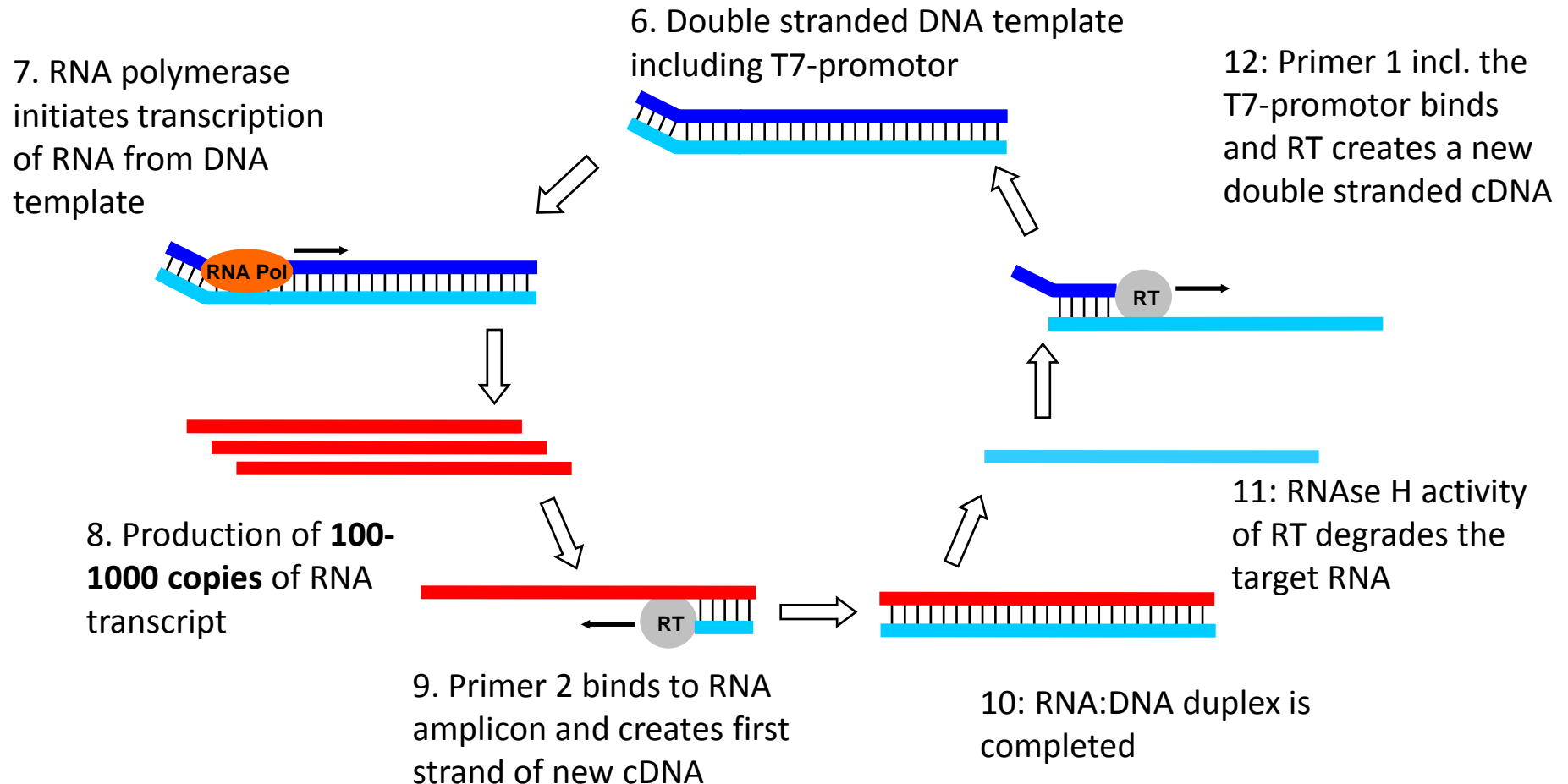
5: Primer 2 binds to cDNA and creates the second strand cDNA



6: Double stranded DNA template including T7-promotor sequence generated

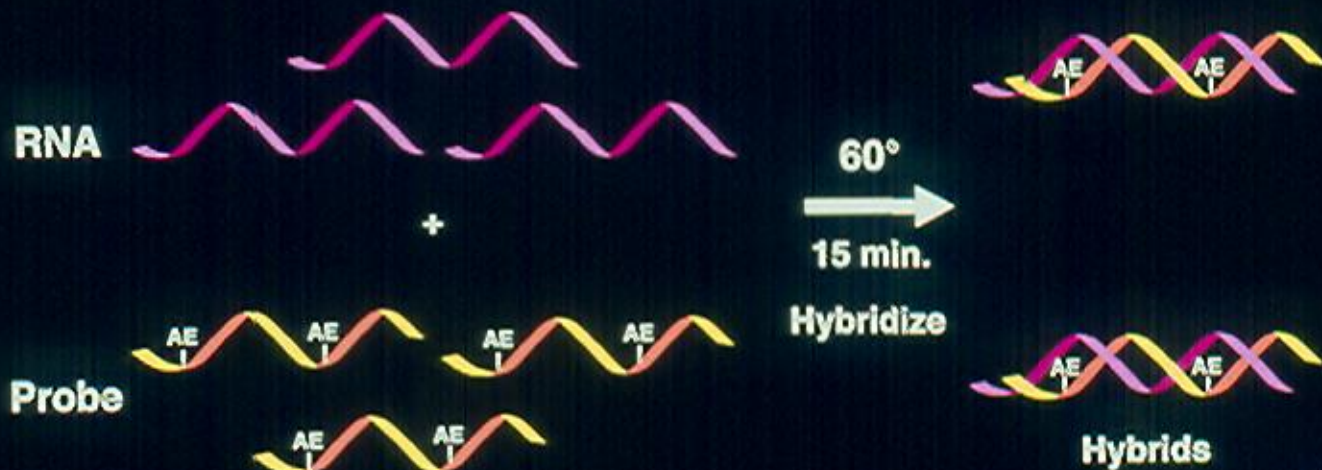


TRANSCRIPTION MEDIATED AMPLIFICATION



HPA Hybridization Protection Assay

Hybridization



HPA

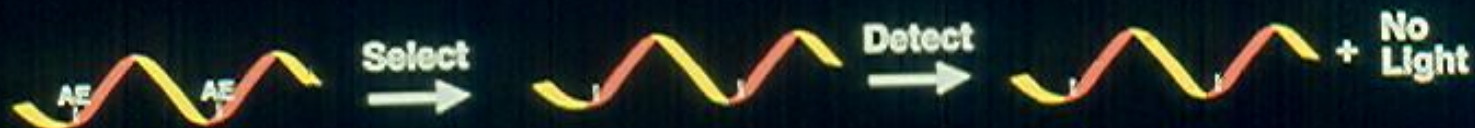
Hybridization Protection Assay

Selection/Detection

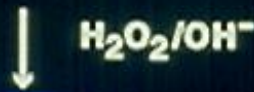
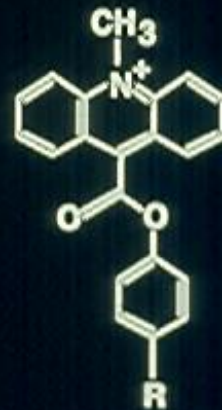
HYBRIDIZED PROBE



UNHYBRIDIZED PROBE



Chemiluminescence from an Acridinium Ester



PANTHER - FUSION

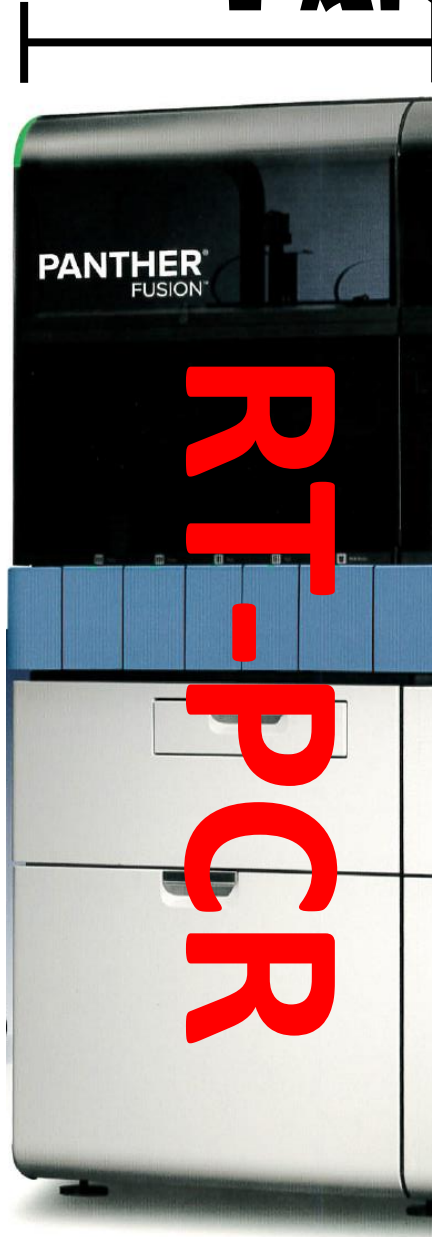


RT-PCR

TMA

HOLOGIC

PANTHER - FUSION



ALERE i

Consumables:



Test Base



Sample Receiver



Transfer Cartridge

Device:

Test Base
Holder

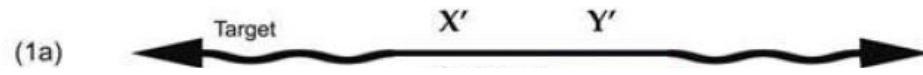
Sample Receiver
Holder

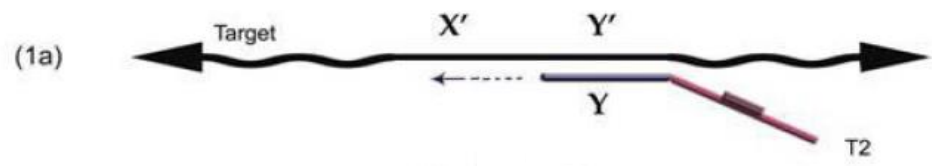
Lid

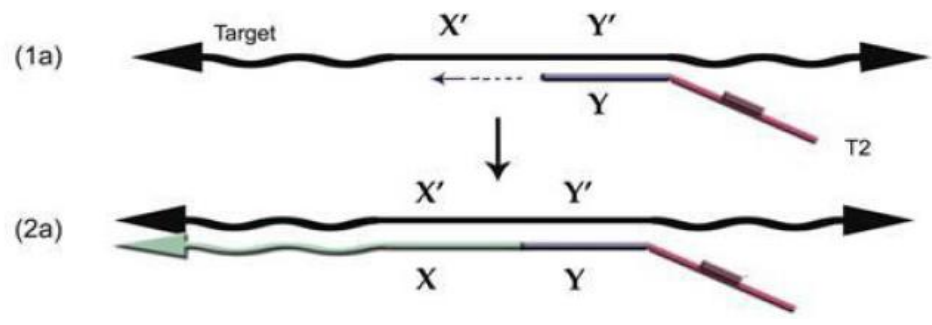
Display

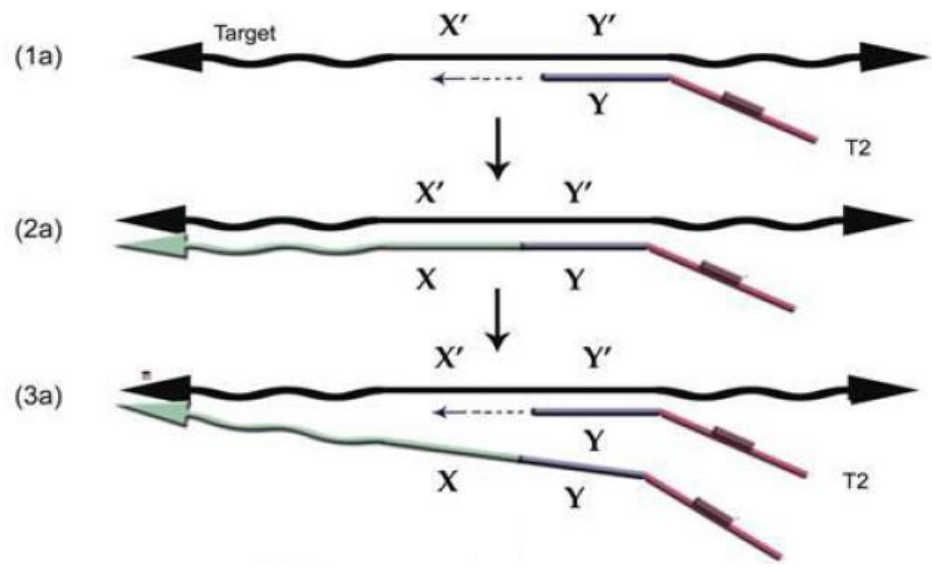


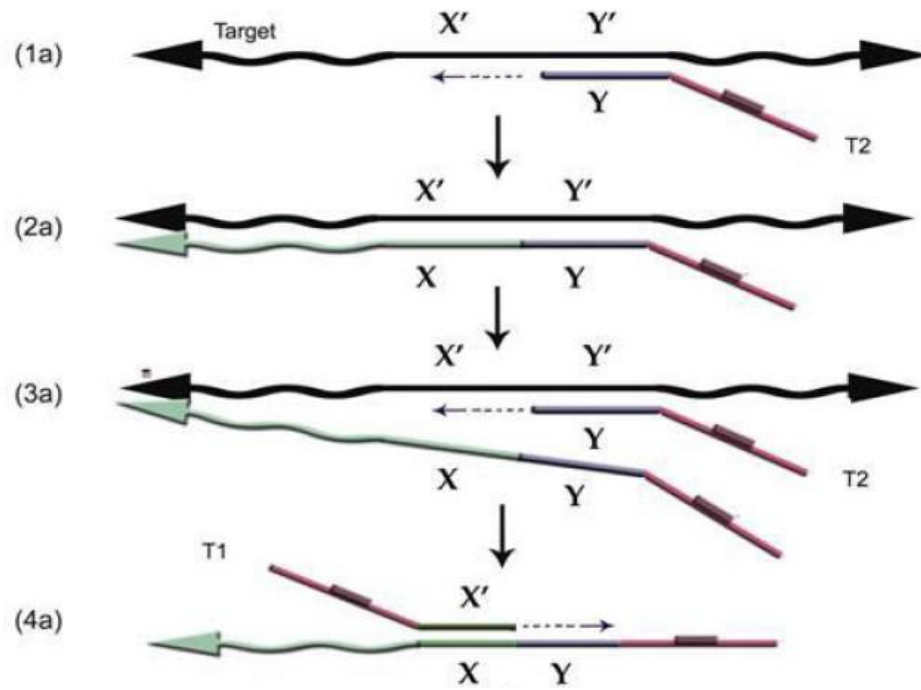
Nicking Enzyme Amplification Reaction



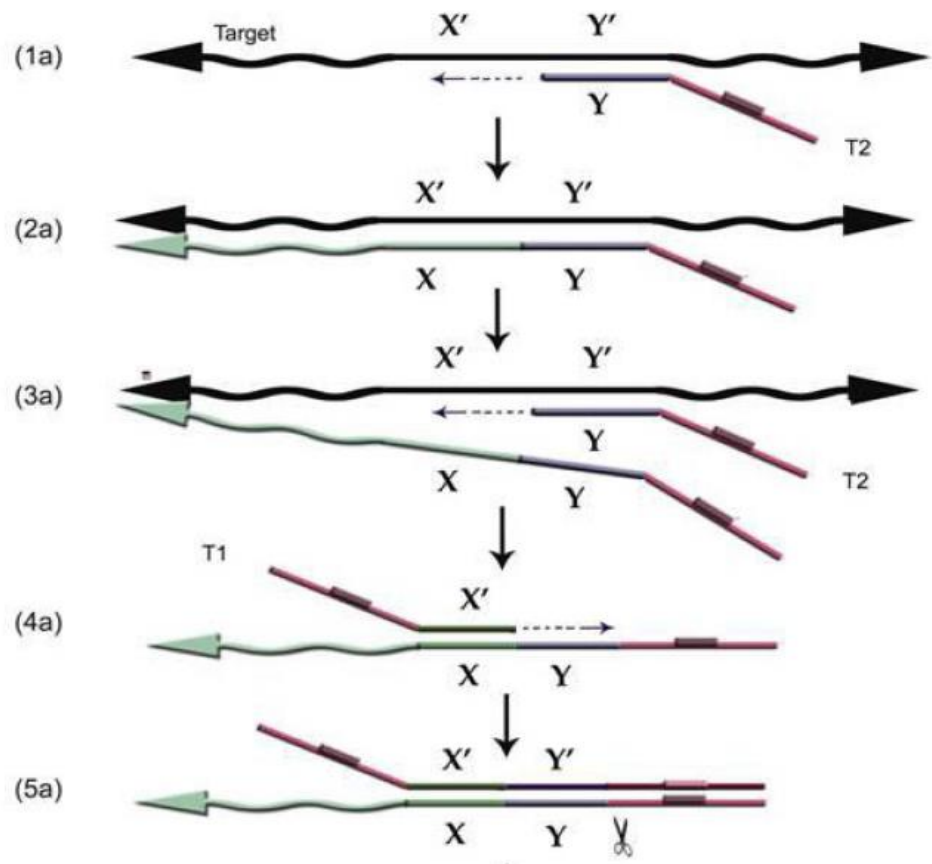




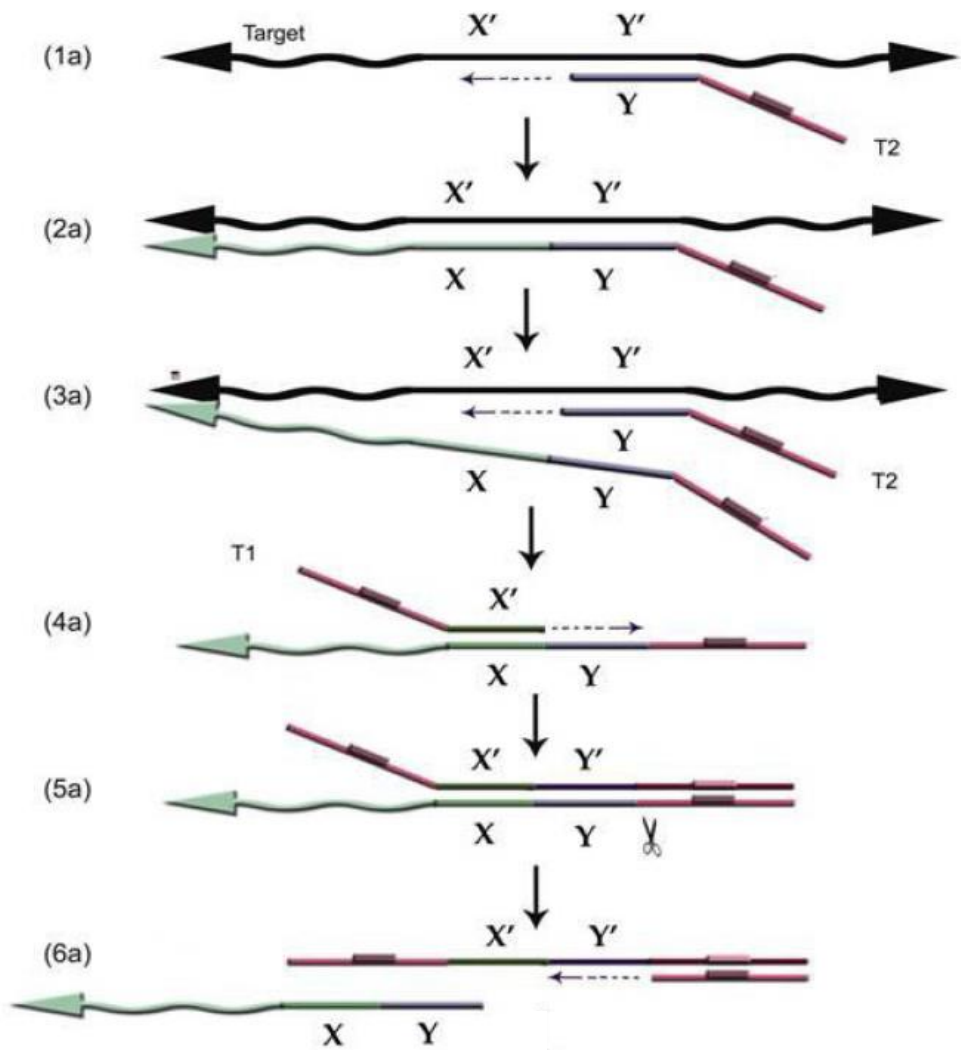




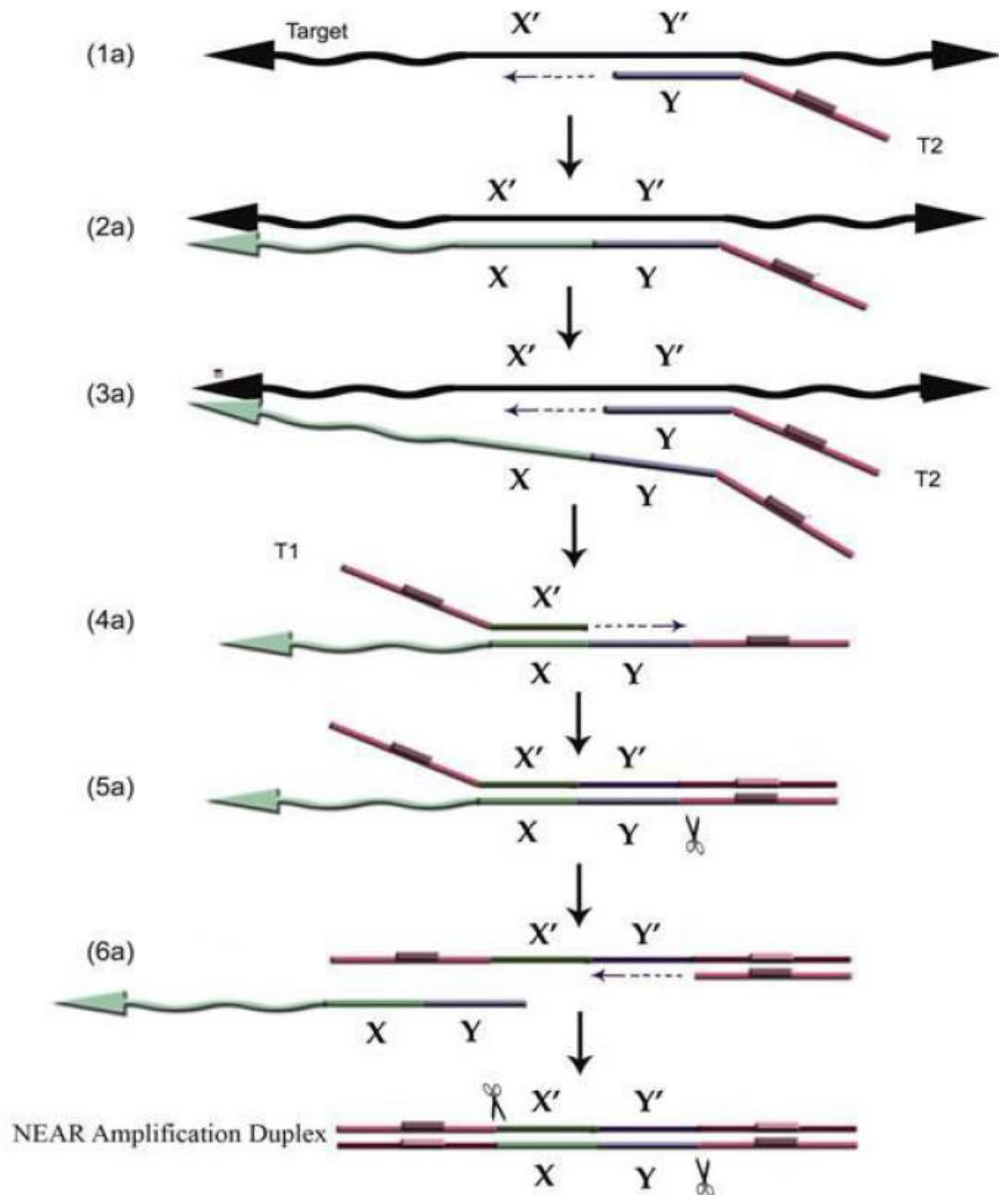
One template is in a higher concentration to drive asymmetric product formation.



One template is in a higher concentration to drive asymmetric product formation.



One template is in a higher concentration to drive asymmetric product formation.



One template is in a higher concentration to drive asymmetric product formation.

ALERE i

Consumables:



Test Base



Sample Receiver



Transfer Cartridge

Device:

Test Base
Holder

Sample Receiver
Holder

Lid

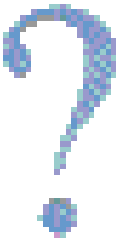
Display



Nicking Enzyme Amplification Reaction

THE END

HDA



NASBA

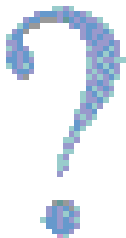
SDA



PCR

NEAR

TMA



LCR

LAMP