

Third Wave Technologies Universal Invader™ Chemistry Tutorial

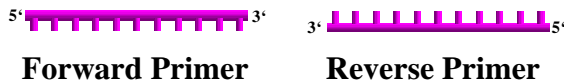
This Tutorial has been designed in order to assist you in understanding the Third Wave Technologies (TWT) Universal Invader™ chemistry.

Oligonucleotides Involved In Universal Invader™ Chemistry

Target Nucleic Acid Sequence - dsDNA



Primer Pair

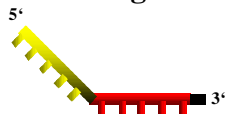


Invading Oligo





!NOTE: The 3' base of the invading oligo is mismatched to the target nucleic acid to prevent extension during amplification.


Probe Oligo



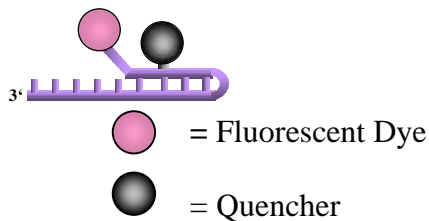
Parts of probe oligo

 = Non-target specific “arm” region of probe

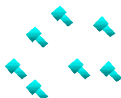
 = Target specific region of probe

 = 3' blocking group

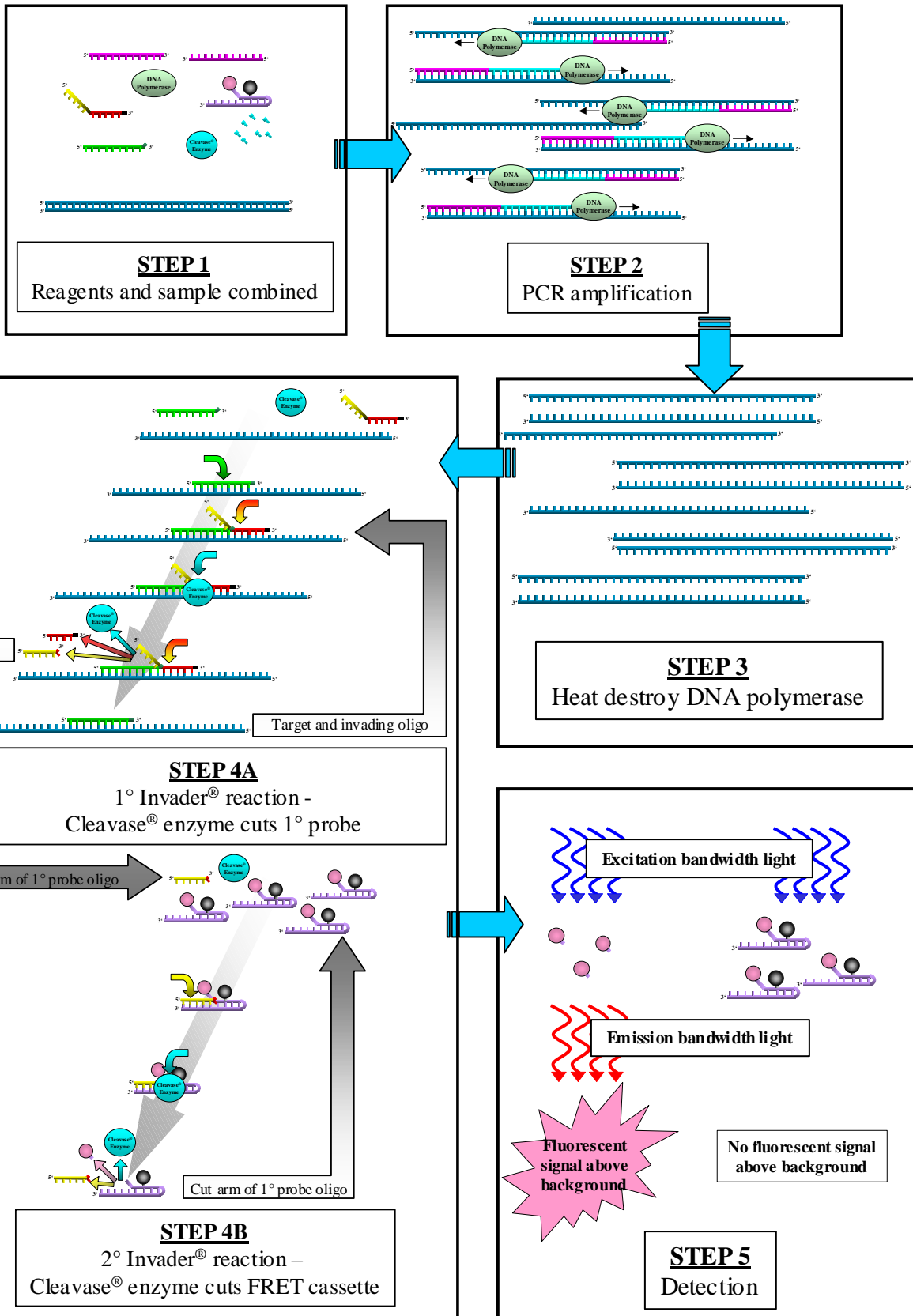
FRET Cassette



dNTPs



Universal Invader™ Reaction Overview



Universal Invader™ Reaction Basic Steps

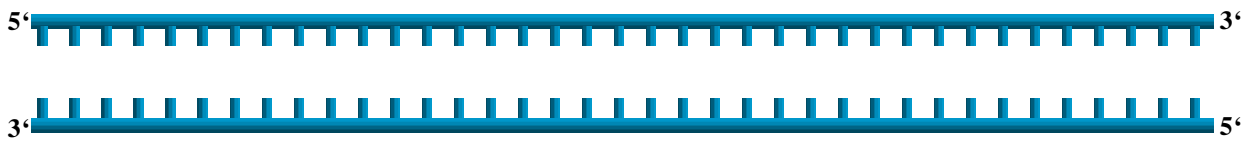
STEP 1: Reaction Setup

STEP 2: PCR Amplification

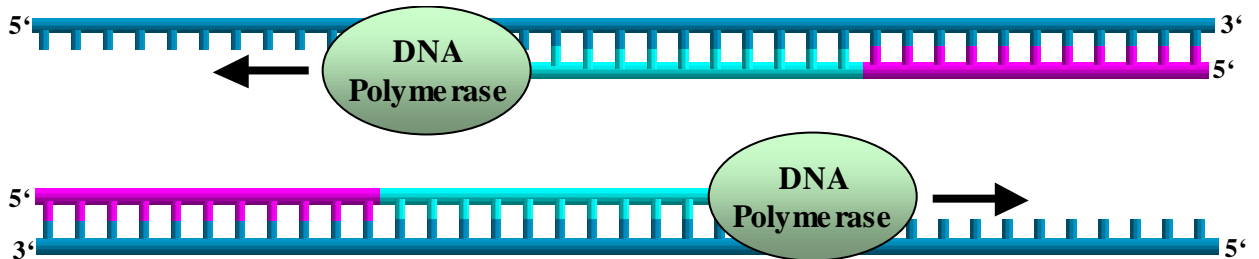
1. Reaction starting material dsDNA.



2. Denature DNA.



3. Anneal and extend primers.

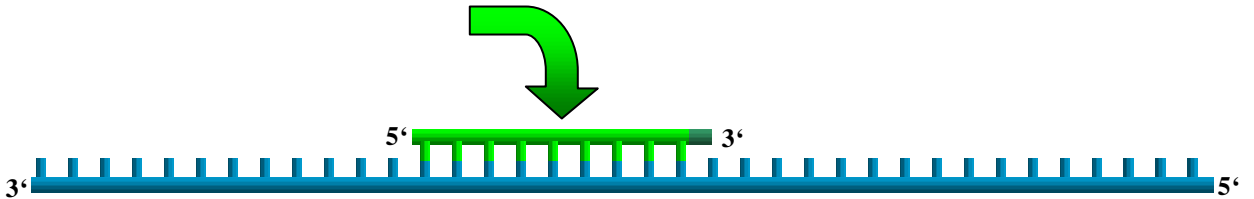


4. Repeat steps 1 through 3.

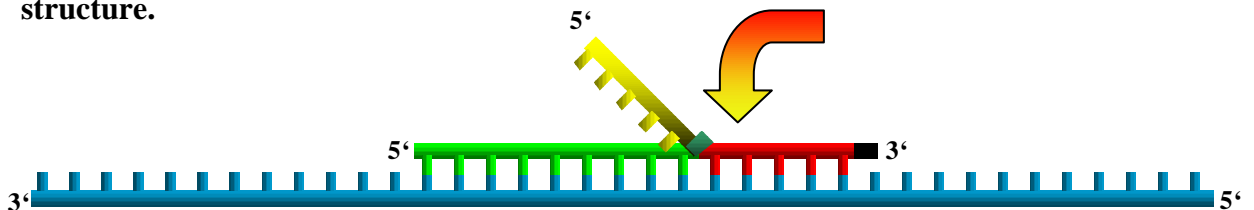
STEP 3: Heat Destruction of DNA Polymerase at 99°C for 10 minutes

STEP 4: Invader® Reaction

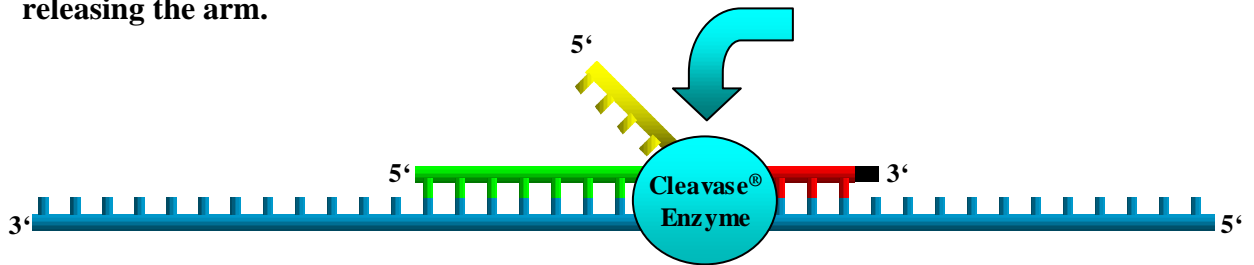
1. Invading oligo binds to amplified DNA target.



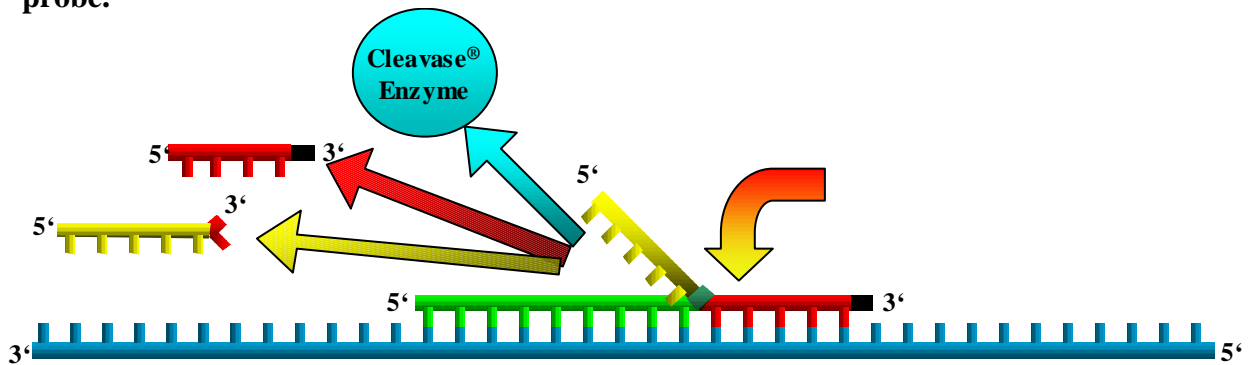
2. 1° probe oligo binds to amplified DNA target forming invasive cleavage structure.



3. Cleavase[®] enzyme recognizes invasive cleavage structure and cuts 1^o probe releasing the arm.

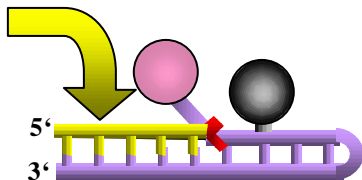


4. Cut 1^o probe oligo cycles off amplified DNA and is replaced by a new uncut 1^o probe.

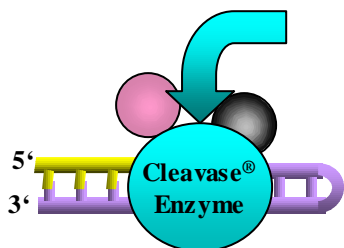


!NOTE: One target has several 1^o probes bind to it throughout the course of the reaction.

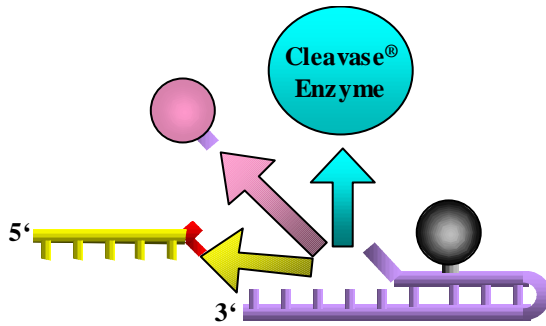
5. Arm sequence of cut 1^o probe binds to complementary sequence of FRET cassette forming invasive cleavage structure.



6. Cleavase[®] enzyme recognizes invasive cleavage structure and cuts the FRET cassette releasing the fluorescent dye.



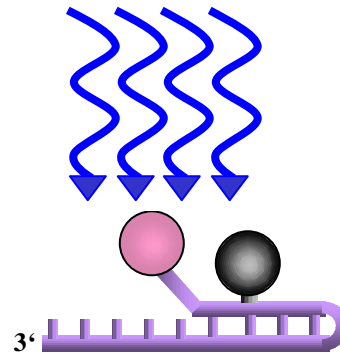
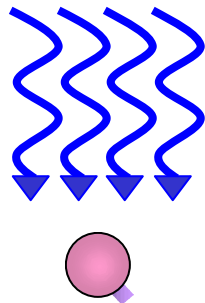
7. 1° probe arm cycles off cut FRET cassette.



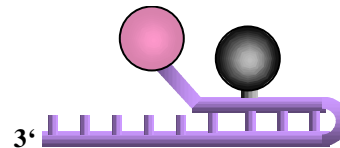
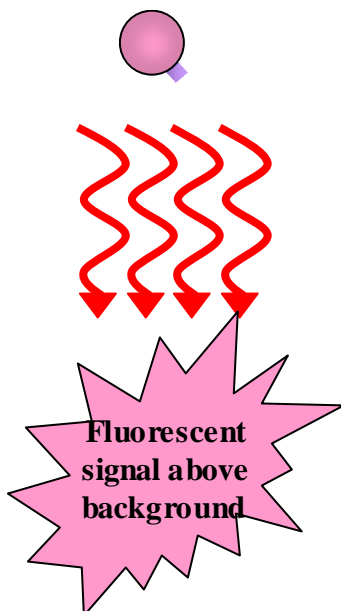
!NOTE: One 1° probe arm can bind to several FRET cassettes throughout the course of the reaction.

STEP 5: Detection

1. Expose reactions to appropriate excitation wavelength of light.



2. Dye that was cut from FRET cassette is excited and emits light of a different wavelength. Uncut FRET cassette gives no additional signal at emission wavelength above background.



No fluorescent signal above background