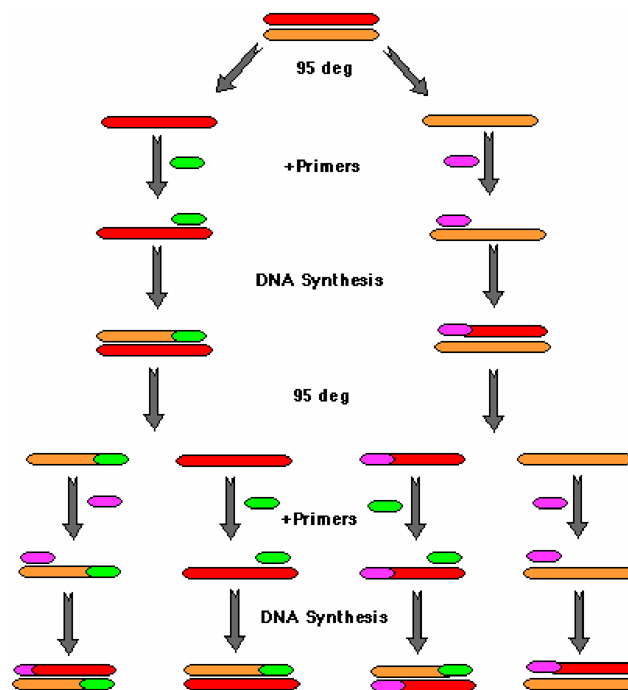
DFS Taq Polymerase
PCR Kit with DFS Taq DNA Polymerase

Code	**Component**
N224-500U	DFS Taq Polymerase *Includes: DFS Taq Buffer, 10X* Sufficient material for 200 x 50 µl reactions
N555-KIT	PCR Kit with DFS Taq DNA Polymerase *Includes:* *10 mM dNTPs, 50X* *10X Reaction buffer with $MgCl_2$* *10X Reaction Buffer without $MgCl_2$* *25 mM $MgCl_2$* *Taq DNA Polymerase, 500U* *Sterile, Ultrapure Water, 2 x1.5 ml* Sufficient material for 200 x 50 µl reactions

Principle:

The PCR (Polymerase Chain Reaction) is a widely used molecular biology application that allows for the rapid amplification (duplication) of specific gene sequences. The DNA to be amplified is added to a solution containing the following components: primers, dNTP's, DNA Polymerase, and reaction buffer containing divalent cation (Mg^{2+}, Mn^{2+}). A typical PCR reaction consists of three steps that combine to form one doubling cycle. In the first step, denaturation, the reaction is heated to 95ºC to relax the DNA template and separate the double-stranded helix. The reaction is then cooled to 35-72ºC for the second Annealing (binding) step which allows the primers to bind to the recognition sequence of the template. In the final, Elongation step, Taq polymerase extends from the 3' end of the primer at 72ºC to form a newly synthesized "daughter" strand. The cycle then repeats itself for a total of 25 – 50 times and results in an exponential increase in the concentration of double-stranded DNA template.

Fig. 1



Storage/Stability:

Taq DNA Polymerase is stable for 1 year when stored frozen (-15 to -22ºC).

Application Disclaimer

For Research Use Only.
Not for Therapeutic or Diagnostic Use.

Protocol:

To a thin-walled PCR tube or to each well of a 96 well plate, add the following:

Reaction Component	Volume
Template DNA	1-3µl (50-150ng)
DFS Taq Buffer, 10X	5µl
dNTPs (10mM)	1µl
Primer A (sense, 10µM)	4µl
Primer B (anti-sense, 10µM)	4µl
Sterile deionized water	As needed to bring final volume to 50µl
DFS Taq polymerase	0.5µl

- To perform multiple, parallel reactions, it is recommended that a master mix consisting of dNTPs, primers, buffer, and template DNA is prepared and aliquotted to multiple tubes or wells. The polymerase should be added last. This will alleviate the potential for any error and will save time. At least 20% more master mix should be prepared than actually needed to prevent the possibility of running short.

- To prevent the occurrence of non-specific amplification, PCR reactions should be prepared on ice. Taq polymerase should always be added to the reaction immediately before it is due to begin.

- Once the reactions have been completely set up, gently vortex the samples and briefly centrifuge to insure that all of the solution is collected in the bottom of the tube.

- Place samples in a thermocycler and begin PCR.

Temperature Cycling:

Step	Temperature (ºC)	Time (min.)	Number of cycles
Initial Denaturation	94 – 95	1 – 5	1
Denaturation	94 – 95	0.5	
Annealing	55 – 65 (depends on Tm of primers)	0.5 – 2.0	25 -35
Elongation	72	0.5 – 2.0	
Final Elongation	72	5 – 10	1

- The number of cycles required for optimal amplification depends strongly on the starting concentration of DNA template.

- During the final elongation step, the terminal transferase activity of DFU Taq polymerase will incorporate an additional A nucleotide to the 3' ends of the resulting amplimers. If the PCR fragments are to be T/A cloned, this extension step can be extended for up to 30 minutes.

Troubleshooting:

Symptoms	Possible Causes	Possible Remedy
Low or no yield	Insufficient number of cycles	Replace PCR vials in thermocycler and run an extra 5 cycles
	DNA template denatured	Check DNA quality on agarose gel
	Thermocycler program not correct	Check temp. and cycle times
	Inhibitors present in reaction	Precipitate the original samples or purify over columns. Ions such as EDTA may inhibit the PCR reaction.
	Missing reaction component	Check components and set up new reactions.
	Unsuitable reaction conditions	Decrease annealing temperature and/or increase elongation time.
	Evaporation	Cover reaction with high quality mineral oil or use a thermocycler with a heated lid.
	Bad primers	Insure that 5' and 3' ends of primers are not complementary. A high GC content or extremely mismatched Tm's may also have an effect on binding efficiency.
	Incorrect primer specificity	Check primer sequences
	Primer concentration to low	Check concentration and increase if necessary.
	Bad dNTPs	dNTP solutions should be stored frozen with limited freeze/thaw cycles. Replace nucleotide solution if necessary.
	Target not present in DNA template	Try another region in DNA template.
Non-specific Amplification	Sub-optimal reaction conditions	Optimize MgCl concentration, annealing temperature, elongation time, and number of cycles. Keep reactions on ice when all reagents are mixed.
	Badly defined primers	Re-design primers. Insure ends are not complementary and do not containg 3 successive G's or C's at 3' end.
	Primer concentration to high	Reduce primer concentration
	Contamination with other template	Use dedicated pipettes and tips. Work in separate rooms or clean areas.
	Several targets present with same target sequence	Develop new primers and run BLAST search in public database to insure primer specificity.

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