Automating the Promega GoTaq® PCR Core System II Reaction

Using the Gilson PIPETMAX® 268

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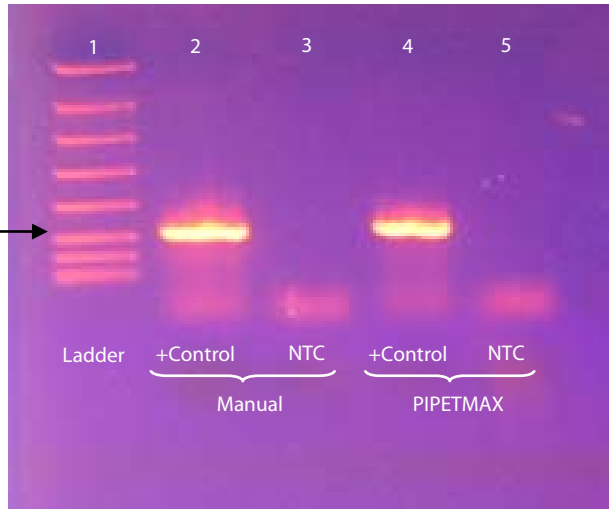

Figure 1. GoTaq PCR Core System II products were separated on a 1% Agarose gel, stained with ethidium bromide, and viewed under UV light. Lane 1 contains the 100bp DNA ladder. Lanes 2 and 3 contain the PCR products from the manually prepared reactions. Lanes 4 and 5 contain products from the reactions prepared on the PIPETMAX. The 323 bp product can be seen in both of the Positive Control (+Control) lanes, while it is absent from the No Template Control (NTC) lanes.

Table 1. GoTaq PCR Core System II 50 µL Reaction. PIPETMAX was used to 1) prepare a 196 µL Master Mix, 2) dispense 49 µL of the Master Mix into PCR tubes, and 3) add 1 µL plasmid DNA.

Component	Volume/ Reaction	Volume/ Master Mix	Final Concentration
M_gCl_2, 25 mM Solution	3.0 µL	12 µL	1.5 mM
5X Green GoTaq Flexi Buffer	10 µL	40 µL	1.0X
PCR Nucleotide Mix, 10 mM ea	1 µL	4 µL	200 µM ea
Upstream primer, 15µM	3.3 µL	13.2 µL	1.0 µM
Downstream primer 15µM	3.3 µL	13.2 µL	1.0 µM
GoTaq DNA Polymerase, 5 units/µl	0.25 µL	1 µL	1.25 units
Nuclease-Free H_2O	28.15 µL	112.6 µL	n/a
Positive Control Plasmid DNA	1 µL	-	1 ng

Table 2. Thermal Cycling Conditions

Step	Temperature	Time	Number of Cycles
Initial Denaturation	95°C	2 min	1 cycle
Denaturation	95°C	1 min	30 cycles
Annealing	60°C	1 min	
Extension	72°C	2 min	
Final Extension	72°C	5 min	1 cycle
Final Hold	4°C	Indefinite	1 cycle

The Polymerase Chain Reaction (PCR) is one of the most commonly performed techniques in research, clinical, and forensic laboratories around the world. Similarities in PCR protocols and simple addition steps make the pipetting steps for different reactions amenable to automation. The compact Gilson PIPETMAX 268, a reproducible set-up, run and walk-away sample prep assistant, was used to prepare PCR reactions with the GoTaq PCR Core System II, and to compare its effectiveness versus a manually pipetted procedure (Table 1). Positive and negative control reactions were set up and amplified using the manufacturer's recommended guidelines (Table 2), and analyzed by agarose gel electrophoresis (Figure 1). As shown, the PIPETMAX successfully generated the expected 323 bp product in the positive control reactions, and showed no contamination or carryover in the No Template Control.

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