Electronic Supplementary Material (ESI) for Lab on a Chip
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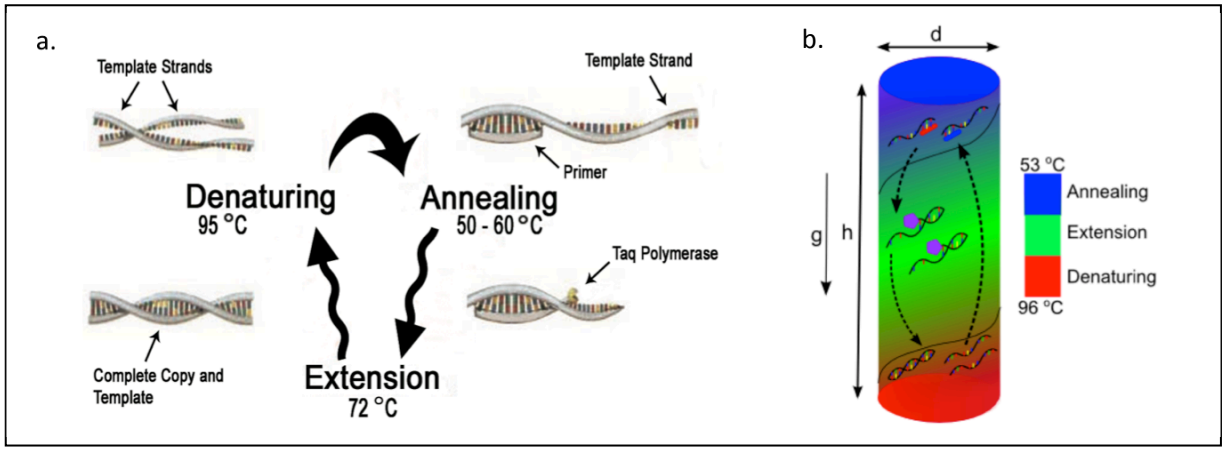
Convective PCR: Lab instruction manual

Introduction

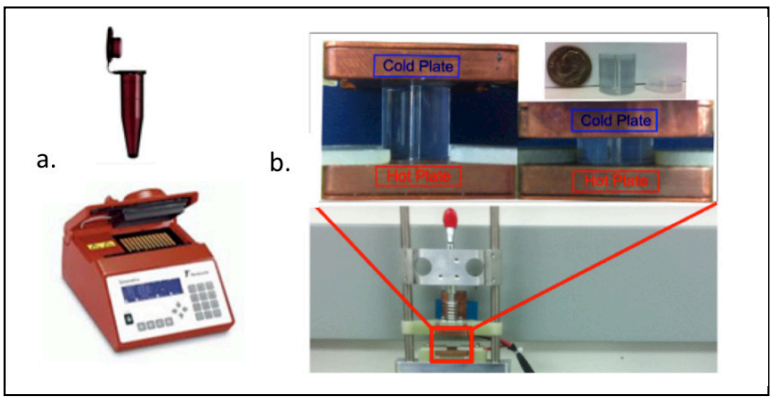
The polymerase chain reaction (PCR) is a very important tool in molecular biology because it enables a sequence of DNA to be replicated across several orders of magnitude, generating millions to billions of copies by cycling the reagents (DNA, Primers, dNTPs, enzymes, etc.) through the prescribed temperature regimes. The idea is very simple: DNA, which is double stranded helix of nucleotides (DNA building blocks), is heated to ~ 95°C where it unwinds into two single DNA strands. The temperature is then lowered to ~ 50 – 60 °C, where target specific DNA primers anneal themselves onto the two single stranded template DNA molecules. Finally the temperature is again raised to ~72 °C where DNA polymerase enzyme assembles a new double stranded DNA from nucleotides, by using the single stranded DNA as a template. After one cycle (see fig a. below), two DNA molecules are produced and in subsequent cycles the number of DNA molecules doubles in a geometric progression (2^N after N cycles), exponentially increasing the concentration of target DNA.

The process of heating and cooling the PCR solution is known as thermal cycling. Conventionally thermal cycling is performed by programmed heating and cooling of heavy metallic blocks containing the PCR reaction mixture in thermo-cyclers. These are not very efficient as they consume large reaction time, sample volume and energy. Our lab has developed a novel technique to perform PCR in microfluidic convective cells. It is a simple design where the PCR mixture is confined in a cylindrical enclosure whose bottom temperature is maintained at a higher temperature than the top. This causes the fluid to circulate through the cell; shuffling the PCR reagents through the optimum temperature regimes (see fig b. below).



Apparatus

a. Conventional thermo-cycler
b. Convective flow thermo-cycler



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Reagents

To make a 50 µL batch of PCR mixture

Reagents	Volume (µL)
Distilled water	27.2
Buffer (KOD kit)	5
$Mgcl_2$ (KOD kit)	2
dNTPs (KOD kit)	5
Primer 1 (237 bp)	4
Primer 2 (237 bp)	4
Template DNA (lambda DNA)	2
Polymerase Enzyme (KOD enzyme)	0.8
Total volume	50

Procedure

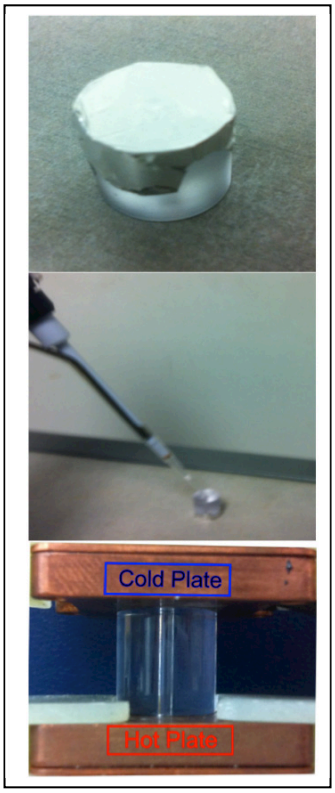
1a. Conventional thermo-cycler

1. After the reagents have been thawed from their frozen state, pipette appropriate amounts of each reagent in that sequence into a micro-tube. Add the polymerase enzyme at the very end.
2. Place the micro tubes with the reagent mixture in the thermo-cycler wells and follow the following thermo-cycling protocol:

Steps	Temperature °C	Hold time (s)	
1	96	10	
2	95	10	X 30
3	60	30	
4	72	30	
5	72	300	
6	4	Pause	

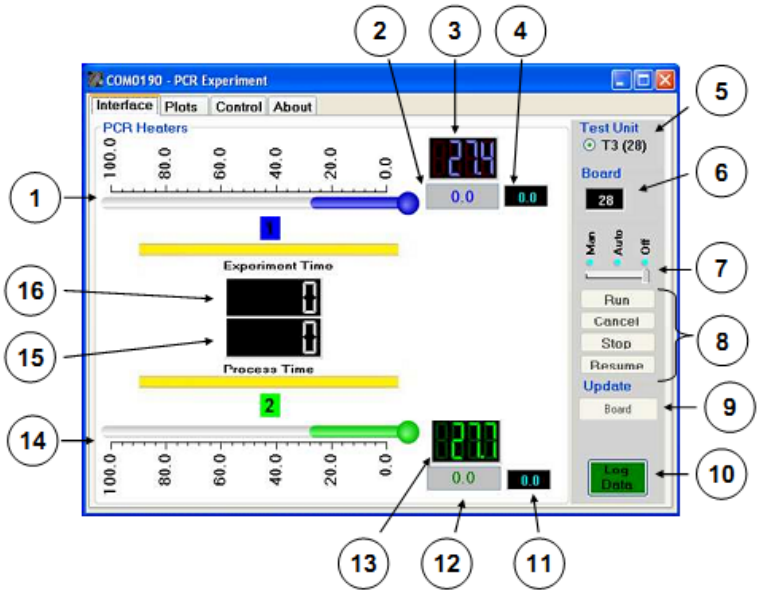
3. After the thermo-cycling, remove the micro-tubes from the thermo-cycler and pipette the products out for analysis using gel electrophoresis.

1b. Convective flow thermo-cycler



1. Before loading the reagent mixture, seal the bottom of the convective cells with thin aluminum sheets. Rinse the cell walls with a 10 mg/ml aqueous solution of bovine serum albumin followed by Rain-X Anti-Fog. This is done minimize adhesion of reagents on the cell walls.
2. After the reagents have been thawed from their frozen state, pipette appropriate amounts of each reagent in that sequence into a micro-tube. Add the polymerase enzyme at the very end.
3. Transfer the reagents with the help of a micro pipette into the convective cells. Seal the top of the cell with aluminum sheet and ensure that no air bubbles show up.
4. Clamp the convective cells between the top and the bottom plates of the convective device tightly and run the lynntech software through the computer interface to maintain the temperature of the top and bottom plate as 55 °C and 95 °C respectively. Refer to section 1c. below for software operation.
5. After running the reaction for 15 minutes, remove the top aluminum sheet and pipette the products out of the convective cells into another micro tube for analysis using gel electrophoresis.

1c. Operating the COM0190 software in manual mode



COM0190 Manual Mode

1. Graphical representation of top heater temperature (˚C).
2. User input box for setting top heater temperature (˚C).
3. Actual temperature (˚C) of top heater as measured by the system.
4. Temperature value (˚C) for top heater that the controller board has been programmed to achieve.
5. Test unit numbur with corresponding controller board ID number selected for data display and control.
by the software application.
6. Controller board ID number feedback from the controller board.
7. Operation Mode Selector.
8. Operation controls for running unit in Auto mode.
9. Operation controls for running unit in Manual mode.
10. Controls for logging temperature data.
11. Temperature value (˚C) for bottom heater that the controller board has been programmed to achieve.
12. User input box for setting bottom heater temperature (˚C).
13. Actual temperature (˚C) of bottom heater as measured by the system.
14. Graphical representation of bottom heater temperature (˚C).
15. Displays time of current process the instrument is performing.
16. Displays total time experiment has been running in seconds.

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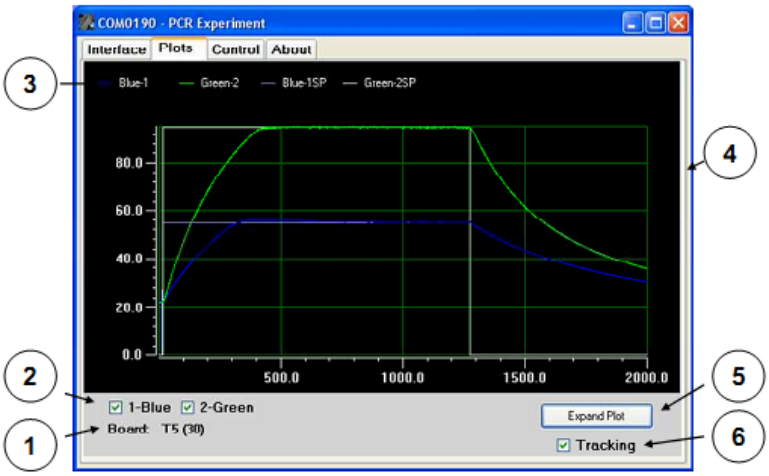
1. Set Operation Mode Selector to "**Man**" (7). In manual mode the user can set each heater temperature individually.
2. Input temperature (˚C) for single or both heaters in the heater input boxes (2, 12). Press "**Board**" (9) to update temperature for selected test unit. Heaters will heat to the input values and remain at that temperature until values are changed.
3. Logging temperature data can be performed by the user pressing "**Log Data**", the box will turn red, and temperature data will now be logged. To stop the data logging press "**Log Data**" again.
4. The temperature variation of the two heaters can be viewed under the "**Plots**" tab.

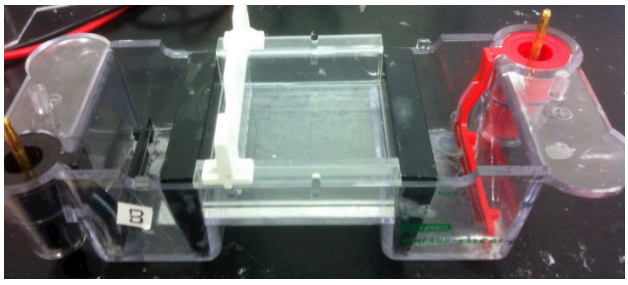


1. Test unit ID and board number ID for plot data.
2. Channel display selection.
3. Legend display.
4. Graph area.
5. Expand plot button.
6. Tracking enable.

5. When the operation is completed, set the Operation Mode Selector to "**Off**". This will disable the heaters.

Gel electrophoresis analysis

1. Prepare a 2 wt % agarose gel by heating 10 g of agarose with 500 ml of 1x buffer on a stirring hot plate until the solution becomes clear.
2. Load the agarose gel into the casting tray and insert the comb and let the gel set for 30 minutes.



3. Remove the comb and add 1x TAE buffer until the gel is submerged.
4. Prepare fluorescently stained DNA samples by mixing 2 µL 100x SYBR Green I solution, 2 µL PCR product from convective cells/thermo-cycler, 2 µL 6x orange loading dye and 4 µL TAE buffer.
5. Add DNA samples into the wells and run the separation at 60 V for 1 h with a 100 bp DNA ladder sizing marker.
6. Remove the gel and photograph it under UV light to view results.