

Manual PCR

Low Budget iGEM challenge entry by Aalto-Helsinki and ETH Zürich

Aim

To perform a low budget manual PCR and compare it to the PCR on a thermocycler.

Materials required

- Microwave oven
- 2 microwave safe beakers (1L and 100 ml)
- Heat resistant rubber gloves
- Water resistant tape
- 3 waste styrofoam boxes
- PCR tubes
- PCR mix (M0273S)
- DNA of interest
- 5L tap water
- Tweezers
- Thermometer
- 3 Timers

Procedure

1. Prepare 2 x triplicates of the sample. Here, the sample volume was 50 μ L.
2. Run one set of samples on the thermocycler (only for comparison).
3. Label the styrofoam boxes as 95 C, 60 C, and 68 C respectively.
4. Fill the boxes with water heated to the corresponding temperatures. Use the thermometers to constantly measure the temperatures.
5. Prepare a frame using the tape and punch holes for the tubes.
6. Start the PCR by placing the frame with the tubes in the 95 C box for 1 min. For the remaining cycles the tubes are incubated at 95 C only for 30 sec.
7. Then transfer the frame with the tweezers to the 60 C box for 45 sec.
8. Then transfer the frame to the 68 C box for 50 sec.
9. Repeat steps 5, 6, and 7 29 times.
10. During each cycle, constantly heat water in the microwave to maintain temperatures.
11. At the end of cycle 30 place the tubes on ice.

Results

The triplicates were loaded on the gel for comparing the two methods (Fig 1 and Fig 2).

Further, we performed a gel clean up of the samples and measured the amount of DNA using NanoDrop 200. The results are summarized in the Table 1.

Samples	Conc. of DNA (ng/ml)
A1	43.5
M1	30.8
A2	40.4
M2	24
A3	38.1
M3	27.1

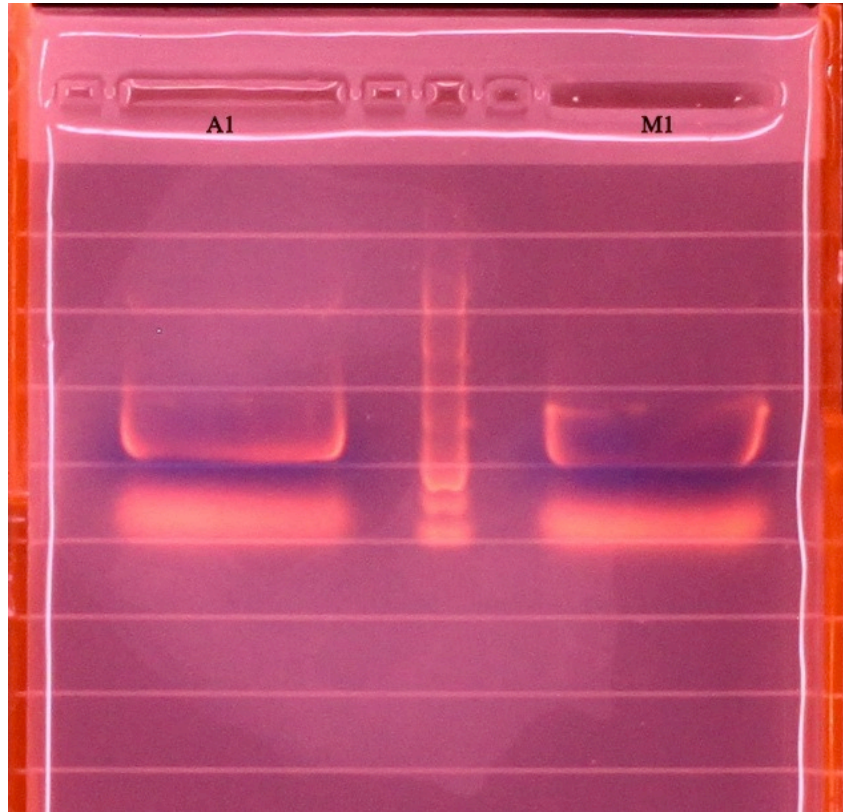


Figure 1 A1: Sample 1 run on the thermocycler. M1: Sample 1 run manually.

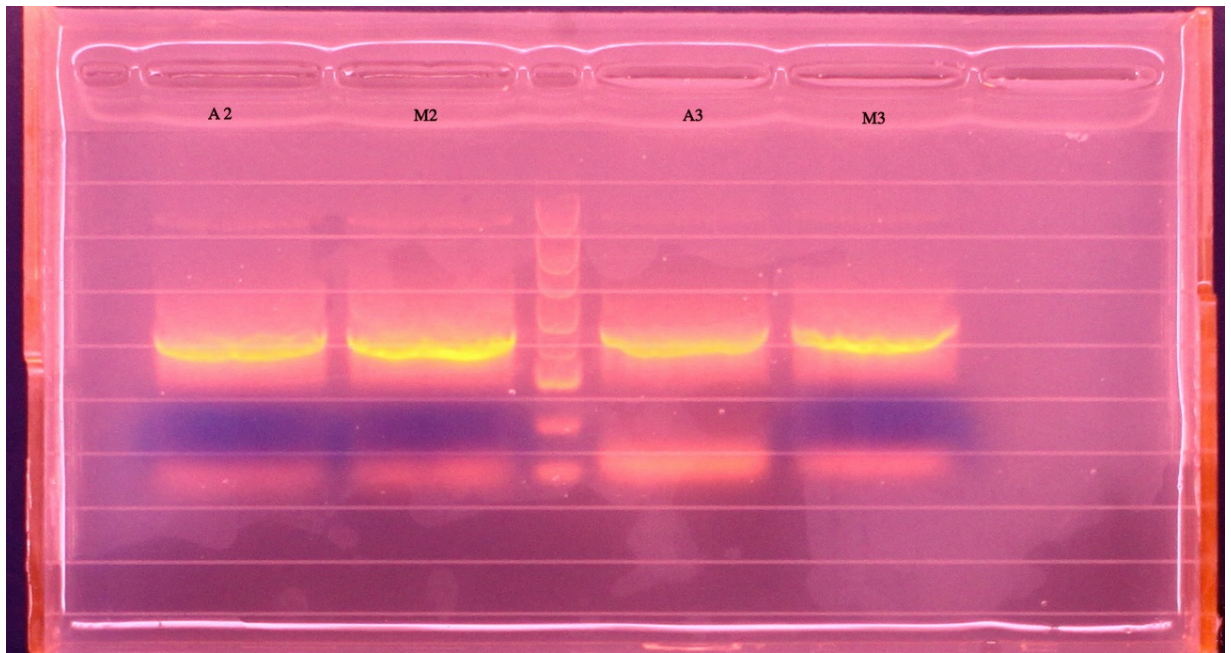


Figure 2: A2 and A3: Sample 2 and 3 run on the thermocycler.
M2 and M3: Sample 2 and 3 run manually

Expenses

Fixed costs

Items	Price in \$	Price in CHF
3 polystyrene boxes with lids	Reusable waste	Reusable waste
Heat insulated rubber glove	19.5	18.5
Microwave resistant 1L container	3	4.95
Microwave	69	55
Thermometer	15.77	15
Knife	0.799	0.76
3 Timers	35.94	34.2
Total:	144.009	128.41

Running costs/(sample and 30 cycles)

Items	Price in \$	Price in CHF
PCR mix	0.21	0.1296
10L of water (per run)	0.02	0.02
Electricity (per run)	0.1	0.1
PCR tube	0.044	0.0425
Tape	0.003	0.003
Total:	0.377	0.2951

Advantages of the method:

- The protocol although straightforward, is easy and can be performed in any lab.
- It is reproducible.
- The average yield is about 68% of the average yield produced by the automated method.
- Technology wise it is simple and feasible.

Link to our instruction video:

<https://www.youtube.com/watch?v=icPgP3OOVOQ>

ETH Zürich wiki:

http://2014.igem.org/Team:ETH_Zurich

Aalto-Helsinki wiki:

<http://2014.igem.org/Team:Aalto-Helsinki>