Electronic Supplementary Material (ESI) for Analyst

Electronic Supplementary Information

Flow-through PCR on a 3D qiandu-shaped polydimethylsiloxane (PDMS) microdevice employing a single heater: Toward microscale multiplex PCR

Wenming Wu,[a] *Kieu The Loan Trinh*[b] *and Nae Yoon Lee*[*b]

Gachon BioNano Research Institute, Gachon University, 1342 Seongnam-daero, Sujeong-gu, Seongnam-si, Gyeonggi-do, 461-701, Korea

Division of BioNano Technology and College of BioNano Technology, Gachon University, 1342 Seongnam-daero, Sujeong-gu, Seongnam-si, Gyeonggi-do, 461-701, Korea

Temperature stabilization tendencies observed on the slanted surface of the qiandu-shaped PDMS microdevice

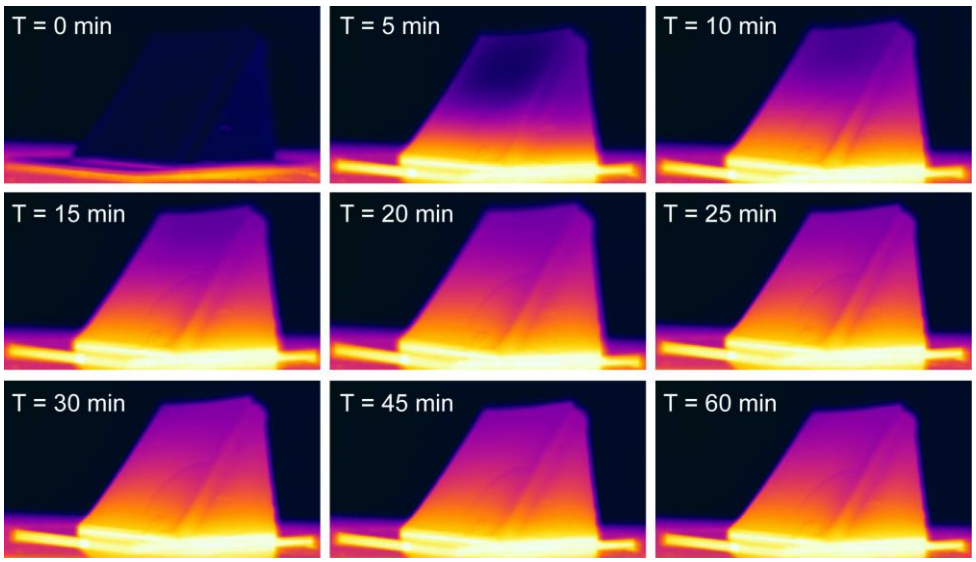

Fig. S1. Time-dependent temperature stabilization tendencies observed on the slanted surface of the qiandu-shaped PDMS microdevice over 1 h time course using the IR camera.

Some formulas for the calculation of the optimum T_m values

The sequences of the primers used in this study are as follows.

- Forward primer: 5'-CCG GCG AAC GTG GCG AGA AAG GAA GGG AAG AAA GC-3' (35-mer)
- Reverse primer: 5'-TCG CCT TGC AGC ACA TCC CCC TTT CGC CAG C-3' (31-mer)

For longer (> 14 bases) primers, the following two formulas were used.

i) Basic T_m calculation

T_m = 64.9°C + 41°C × (number of G's and C's in the primer – 16.4)/N (N: length of the primer)

Forward: 15G & 6C (N = 35)

Reverse: 5G & 15C (N = 31)

Therefore,

T_m (forward) = 64.9 + 41 × (21-16.4)/35 = 70.3°C

T_m (reverse) = 64.9 + 41 × (20-16.4)/35 = 69.1°C

ii) Salt-assisted T_m calculation (References [1] ~ [2])

$T_m = 81.5°C + 16.6°C \times (\log_{10}[Na^+] + [K^+]) + 0.41°C \times (\%\ GC) - 675/N$

Forward: GC 60% (N = 35)

Reverse: GC 64% (N = 31)

$[Na^+]$ = 0.05 M (for both cases)

Therefore,

T_m (forward) = $81.5 + 16.6 \times (\log_{10}[0.05]) + 0.41(60) - 675/35 = 65.2°C$

T_m (reverse) = $81.5 + 16.6 \times (\log_{10}[0.05]) + 0.41(64) - 675/31 = 64.6°C$

→ Although the resulting T_m values obtained in methods i) and ii) were slightly different, the differences were within the ranges of ±5°C.

References

[1] W. Rychlik, R. E. Rhoads, *Nucleic Acids Res.* 1989, **17**, 8543-8551.

[2] J. Sambrook, E. F. Fritsch, T. Maniatis, *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor 1989.

Comparison of target amplicon intensity under varying amplification conditions

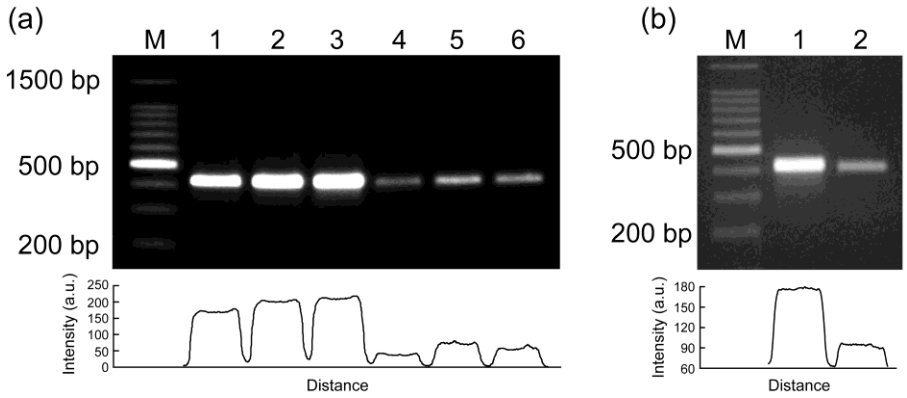

Fig. S2. Amplification results of 409 bp gene fragments. (a) Lanes 1 – 3 represent typical three-temperature PCR results with 30 – 40 s of residence time at each temperature regime, when annealing temperatures were 55.0, 57.0, and 60.0°C, respectively. Lanes 4 – 6 represent two-temperature PCR results with no residence time, when annealing/extension temperatures were 55.0, 57.0, and 60.0°C. (b) Lane 1 represents two-temperature PCR results with 30 s of residence time at each temperature regime. Lane 2 represents two-temperature PCR with no residence time. Annealing/extension temperatures were set to 57.0°C for both cases. M is a 100 bp DNA size marker. Relative intensity scales of the target amplicons were shown below the gel image.

Thermocycler-based gradient multiplex PCR

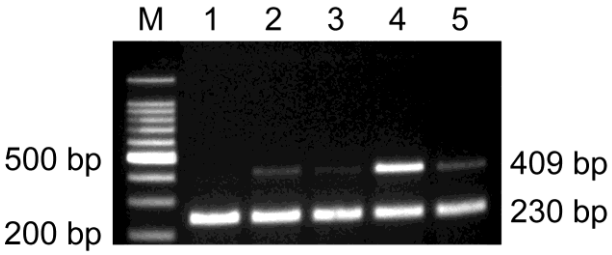

Fig. S3. Result of a gradient PCR employing two templates simultaneously using a thermocycler. Two-temperature PCR was performed with no residence time at each temperature regime. Lanes 1 – 5 represent multiplex PCR results when the annealing/extension temperatures were 51.0, 53.0, 55.0, 57.0, and 59.0 °C, respectively.