Biomed Microdevices (2008) 10:539–546
DOI 10.1007/s10544-008-9163-x

Loop-mediated isothermal amplification of a single DNA molecule in polyacrylamide gel-based microchamber

Liza Lam · Shouichi Sakakihara · Koji Ishizuka · Shoji Takeuchi · Hideyuki F. Arata · Hiroyuki Fujita · Hiroyuki Noji

Published online: 27 February 2008

Abstract Loop-mediated isothermal amplification (LAMP) is an original nucleic acid amplification method established by Notomi et al. LAMP is performed under isothermal condition, employing only a basic reaction protocol and minimal supporting electronics. These requirements prove to be viable for exploring the avenues to down-scale this biological reaction for Lab-on-a-chip application. Hence here, we developed a novel technique for fluorescent imaging of LAMP at a single molecule level. The experiment was conducted in a polyacrylamide (PAA) gel-based microchamber where a single DNA template, freely suspended in a solution containing primers and polymerase was initially encapsulated. In order to activate the amplification reaction, a microheater regulated by an automatic computerized feedback system was used for localized heating. This microchamber-based approach for LAMP demonstrated the effective exploitation of minute amount of templates and primers, and the overall reduction in LAMP detection time. An average efficiency of 80% was evaluated for conducting DNA amplification after 50 min of incubation at 65°C. As the total time for reaction including detection can be completed in less than 1 h, this one-step, direct observation method displays the potential as a simple alternative to conventional techniques for genetic analysis and diagnosis in the clinical laboratory.

L. Lam (✉) · S. Sakakihara · K. Ishizuka · H. Noji (✉)
The Institute of Scientific and Industrial Research,
Osaka University,
Osaka, Japan
e-mail: lizalam@sanken.osaka-u.ac.jp
e-mail: hnoji@sanken.osaka-u.ac.jp

S. Takeuchi · H. F. Arata · H. Fujita
Center for International Research on MicroMechatronics,
Institute of Industrial Science, University of Tokyo,
Tokyo, Japan

Keywords DNA amplification · Microchamber · Polyacrylamide · Single molecule level

1 Introduction

The rapid and efficient detection of specific nuclei-acid sequences (full deoxyribonucleic acid (DNA) sequence), usually available in minute quantities, is a key element for a wide field of scientific applications such as genetic and medical research, biotechnology, molecular biology and forensic science. The identification of the DNA sequence proves to be the basis for understanding its role in the formation of life. However, current processing procedures of these biomolecules at low concentrations are complex and would often take up relatively long time to generate detectable levels for assays and analyses. Therefore, it is essential to develop methods to improve detection sensitivity and resolution such as nuclei amplification, which is an essential tool for many investigators to monitor infectious diseases, genetic disorders and traits. Polymerase chain reaction (PCR; Saiki et al. 1985), self-sustained sequence replication (Guatelli et al. 1990), nuclei acid sequence-based amplification (Compton 1991), strand displacement amplification (Walker 1992), rolling circle amplification (RCA; Lizardi et al. 1998; Nallur et al. 2003) and loop-mediated isothermal amplification (LAMP; Notomi et al. 2000) are examples of existing established DNA amplification methods. The most extensively used technique is PCR where the subsequent DNA synthesis is performed by heat denaturation of double-stranded DNA products. Although the PCR method is flexible and amplifies geometrically to achieve levels of 10^6 or more, it requires a temperature cycling protocol for the amplification process. This imposes constraints in terms of instrumentation for

PCR, hence making it more complex and limits its rate of amplification to the temperature cycling profile. In order to overcome these drawbacks, loop-mediated isothermal amplification (LAMP) offers the advantages of amplifying DNA under isothermal conditions using a non-denatured DNA template (Nagamine et al. 2001).

The LAMP reaction requires a DNA polymerase with strand displacement capability and a set of four specially designed primers, known as forward and backward inner primers (FIP and BIP) and outer (F3 and B3) primers for enhancing specificity. Large amount of amplified products is generally produced when subjected to a constant temperature between 60 and 65°C for approximately 1 h. For the starting material, a stem-loop DNA structure with the sequences of both DNA ends derived from the inner primer, is constructed. Next, an inner primer hybridizes to the loop on the product in the LAMP cycle and initiates strand displacement DNA synthesis, resulting in the original stem-loop DNA and a new stem-loop DNA with a stem double in length. The final amplified products are stem-loop DNAs, consisting of several inverted repetitions of the target DNA. These reactions occur in less than an hour to produce cauliflower-like configurations with multiple loops and amplify to 10^9 copies of the target.

Although conventional bulk measurements are currently still widely used, Hataoka et al. have been known to be the first to down-scale this method by integrating LAMP and the subsequent detection of amplification products on poly (methyl methacrylate) (PMMA) microchips at the microscale level (Hataoka et al. 2004). Nonetheless, ideal analyses of biological nature would be at a single molecule level (Ishii and Yanagida 2000; Dear 2003) where evaluations could minimize the degree of inaccuracy and complication. Advancement in engineering microscale structures has brought about vast developments in single molecule detection (SMD) techniques to enable in-depth study on a wide range of life sciences over the recent years. Many investigations once thought impossible, could now be explored with much ease and accuracy. Experiments on molecular motors, DNA transcription, enzyme reactions and protein dynamics have all benefited from the SMD technique (Weiss 1999; Fisher et al. 2000; Min et al. 2005).

In our previous studies (Rondelez et al. 2005), we have shown that the measurement of biomolecular reactions at the single molecule level without the need for immobilization was possible in femtoliter microchambers made of polydimethylsiloxine (PDMS). Recently, this fabrication technique was successfully adopted to produce polyacrylamide (PAA) gel-based microchambers for performing single-molecule restriction enzyme assay (Lam et al. 2007). Due to the porosity of PAA, ions were allowed to diffuse through its gel matrix to initiate the reaction within the microchamber. In addition, electric signals can penetrate the gel structure to manipulate the motion of encapsulated molecules. This advantageous feature has demonstrated the prospect of integrating conventionally independent assays and analyses. In this study, we further explored the versatility of this novel PAA gel-based microchamber by the direct observation of real-time LAMP reaction on a single encapsulated DNA template using optical microscopy.

2 Materials

2.1 Chemicals

LAMP primers were designed based on a 2 kbp target extracted from the λDNA sequence as given in the Appendix. Using the LAMP primer designing software (Primer Explorer, Japan), the following primers were generated—FIP contained F1c (25 nt), a tttt linker and the complementary sequence of F2c (22 nt): 5′-acaacgtcgt gactgggaaaaccct-tttt-gtgcgggcctcttcgctattac-3′; BIP contained the complementary sequence of B1 (24 nt), a tttt linker and B2 (24 nt): 5′-cgactctagaggatccccgggtac-tttt-tgttgtgtggaattgtgagcggat-3′; F3: 5′-gttgggaagggcgatcg-3′; and B3: 5′-actttatgcttccggctcgta-3′.

2.2 LAMP reaction

For the microchamber experiment, the mixture of sample solution consisted of 3 pM λDNA template stained with SYBR Green II, LAMP reaction mix (Loopamp DNA amplification kit, USA), 0.4 μM each FIP and BIP, and 0.05 μM each F3 and B3. For verifying the resultant LAMP products, the restriction enzyme *Bci* VI (New England Biolabs, USA) was employed using bulk gel electrophoresis.

2.3 Polyacrylamide (PAA) gel-based microchamber

The dimensions of the microchamber were approximately 1.5 μm in height and 17.5 μm in diameter. The molds were created on glass or silicon substrates using chromium patterned mask by photolithographic means. A mixture of 30%w/v acrylamide solution, containing *N*, *N*′-Methylenebisacrylamide (Wako, Japan), ammonium persulfate (APS; Wako, Japan), and *N*, *N*, *N*′, *N*′-tetramethylethylenediamine (TEMED; Wako, Japan) was poured on the mold and cured at room temperature for 40 min. The PAA gel-based microchambers for our purpose were fabricated using 14% acrylamide gel.

2.4 Microheater

The microheater system comprised of circular heater and a temperature sensor as shown in Fig. 1 (Arata et al. 2005).

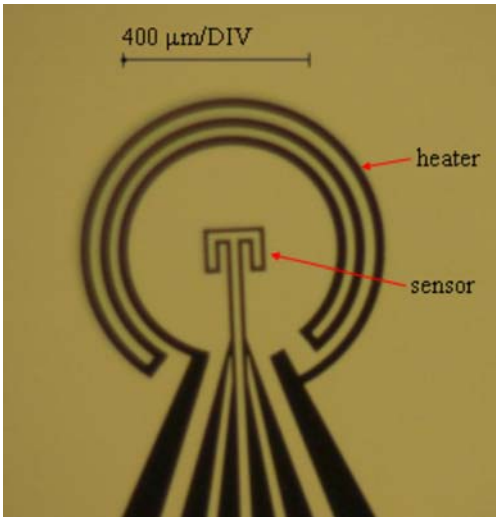

Fig. 1 Microheater pattern

The pattern was fabricated using 200-nm Titanium deposited on a microscope glass slide with a final overlying coat of methacryloyloxyethyl phosphorylcholine (MPC; present from Professor K. Ishihara, University of Tokyo, Japan). One pair of sensor electrodes was connected to a constant current source (Advantest R6240A, USA) set to an output of 0.1 mA. The other pair of sensor electrodes was connected to the Keithley 2000 multimeter (Keithley, USA) which measured the voltage across the sensor and the heater was connected to a constant voltage source (Advantest R6240A, USA) which provided the required voltage level to elevate the temperature to the desired value. Both the multimeter and constant voltage source were controlled by LabVIEW (National Instruments, USA) via the GPIB connections (National Instruments, USA) to automate the regulation of temperature.

3 Experiments

The image of molecular activities was obtained through a fluorescence microscope equipped with a mercury arc-lamp source, a 6% ND filter and an image intensifier unit (Hamamatsu Photonics C8600-05, Japan). Approximately 10 μl of sample solution was initially deposited on a glass slide, printed with the MPC-coated microheater. The PAA gel-based microchamber was then placed above the sample solution and a glass needle was lowered to press the upper surface of the gel against the glass slide as illustrated in Fig. 2. This way, only one or two λDNA templates would be trapped within a single microchamber. Finally, 30 μl of MiliQ added with SYBR green II dye was applied to cover the external of the PAA gel, keeping it hydrated in order to retain its structure and encapsulation capability. The microchambers located within the circumferential region bounded by the heater pattern were observed.

4 Results and discussions

4.1 Initial bulk LAMP analyses

Firstly, an initial study was carried out at the bulk level to identify possible reaction inhibiting factors before adapting LAMP to the microchamber system. Basic LAMP sample solutions contain λDNA templates, LAMP reaction mix (Loopamp DNA amplification kit, USA), 1.6 μM each FIP and BIP, and 0.2 μM each F3 and B3. Various types of fluorescent dyes such as SYBR gold, SYBR green I, SYBR green II and EVAGreen (Invitrogen, USA) were separately added to LAMP sample solutions and then incubated at 65°C for 1 h, after which analysis was done using 3% agarose gel electrophoresis as shown in Fig. 3.

From Fig. 3, lane 2 shows the amplified products resulted from LAMP reaction by subjecting basic LAMP sample solution to 65°C for 1 h. The lengths of these amplified fragments ranged from approximately 150 bp to 1.5 kbp, forming a ladder-like pattern (Notomi et al. 2000). When the basic LAMP solution was incubated at room temperature for 1 h, no amplified products was formed, indicating the absence of LAMP activity as depicted in lane 3. This is as expected since LAMP reaction only occurs at temperatures between 60°C and 65°C, and not at room temperature. The influences of each fluorescent dye (lanes 4 to 7) on the amplification process at 65°C were also examined and it was found that only SYBR green II (lane 7) did not inhibit LAMP reaction. SYBR green II is a sensitive nucleic acid stain, which is maximally excited at the wavelength of 497 nm. The maximum fluorescence emission of SYBR green II stained samples occurs at 520 nm.

For lanes 2 and 7, large numbers of amplified products with high molecular weight were also observed as denoted by the high intensity level at the comb areas (sample loading regions) of these lanes. The amplified products, especially those with high molecular weight were further digested using *Bci* VI at 37°C for 1 h. Theoretically, *Bci* VI

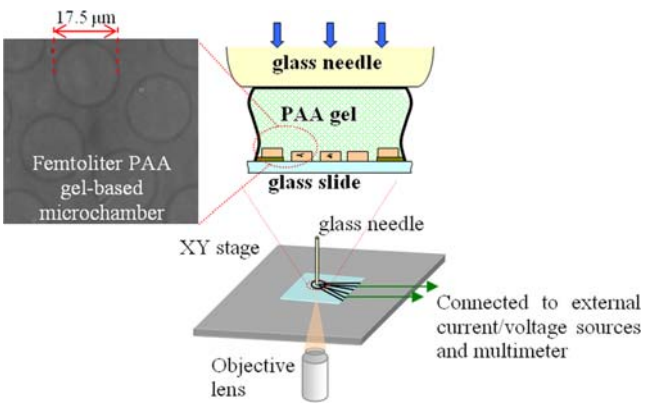

Fig. 2 Experimental setup with microscopic view of PAA gel-based microchamber

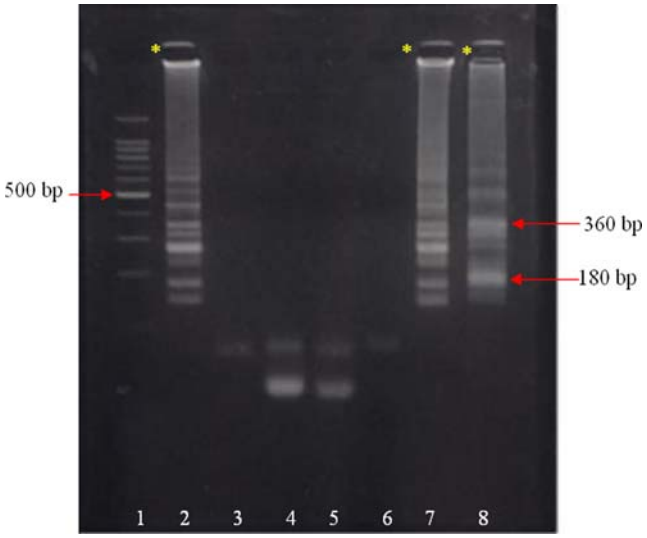

Fig. 3 Gel electrophoretic analysis. *Lanes 1*—100 bp ladder (Takara, Japan); *Lane 2*—the occurrence of LAMP reaction, resulting in LAMP amplified products; *Lane 3*—the absence of LAMP reaction by incubating sample solution at room temperature; *Lanes 4* to *7*—results of LAMP reaction on sample solutions, each containing SYBR green I, SYBR gold, EVAGreen and SYBR green II, respectively, at 65°C for 1 h; *Lane 8*—*Bci* VI-digested LAMP amplified products. *Asterisk* indicates the position of visible comb area of a column, which is also the loading area for the sample

cleaves the recognized site along F2 and the amplified products would be digested to 181 and 360 bp fragments. Lane 8 of the gel electrophoresis verified the structure as there were increases in the number of fragments at the corresponding bands of 180 and 360 bp; and a reduction of high molecular weight fragments as indicated by the lower intensity at the comb areas (Fujino et al. 2005).

4.2 LAMP in microchamber

With the information obtained from the initial study on LAMP bulk analysis, LAMP method was adapted for the PAA gel-based microchamber system. Figure 4 shows the images obtained from four adjacent microchambers, depicting the occurrence of LAMP when subjected to the constant temperature of 65°C at the start of the experiment (0 min) and at incubation intervals of 50 min.

In Fig. 4(a), a single λDNA template and two λDNA templates, suspended in LAMP sample solution were trapped in the bottom-left and top-right PAA gel-based microchambers, respectively. The other two microchambers did not contain any trapped templates. It was noted that the encapsulated fragments remained mobile and the initial background of each microchamber emitted relatively high levels of fluorescence. This background fluorescence was found to be due to the presence of primers which also experienced binding of the fluorescent dye. In such a case, the concentration of primers was minimized so that templates remained visible to facilitate the encapsulation process.

After 50 min of heating at 65°C, it was observed that there was an increase in the number of amplified products in the two microchambers, which initially contained templates, while no amplified products were found in the originally empty chambers, as shown in Fig. 4(b). These images evidently suggested the occurrence of LAMP reaction using the PAA gel-based microchambers. At this point, it was important to highlight that as our microscope system supported the imaging of fragments with sizes larger than 300 bp, the fragments clearly seen in Fig. 4(b) were DNA molecules with large molecular weight. These were equivalent to the amplified products residing at the comb area of the bulk gel electrophoresis (Fig. 3). The current experimental configuration of the microchamber approach could not display the amplified products of lower molecular weight, which corresponded to those observed within the ladder-like pattern (Fig. 3). However, this limitation could be overcome by incorporating other bioimaging techniques

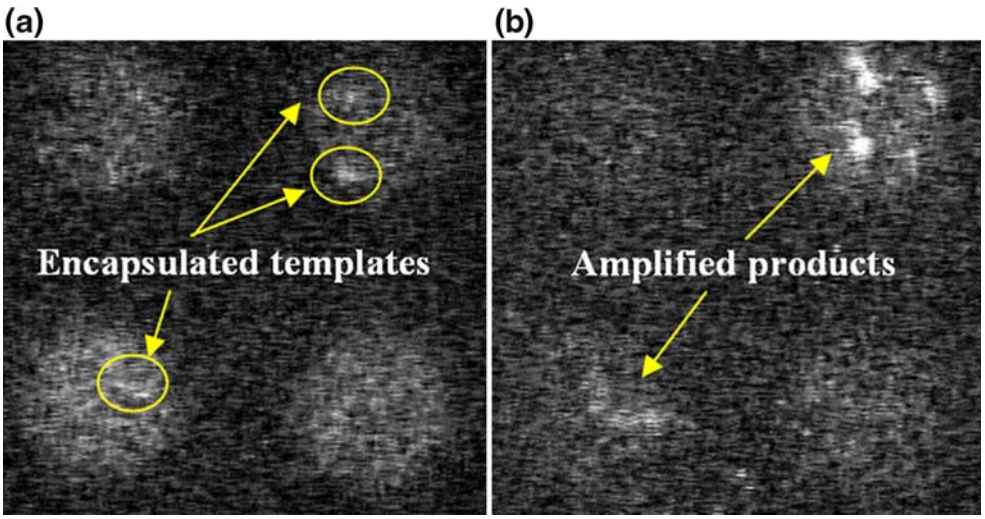

Fig. 4 Fluorescent images of LAMP reaction in PAA gel-based microchambers. (**a**) Four microchambers under observation at room temperature, 0 min; (**b**) Amplified products after 50 min of incubation at 65°C

such as Fluorescence Correlation Spectroscopy (Hess et al. 2002) or X-ray fluorescence microscopy (Ritman 2002), which enhance the visualization of submicron-order biological samples.

In order to further verify the results, three separate tests were conducted in the microchambers. Firstly, the λDNA templates suspended in the LAMP sample solution (similar to the LAMP mixture used for Fig. 4) were subjected to room temperature conditions. For the second test, the sample solution did not contain any primers and in the final experiment, the λDNA templates were replaced with 13.8 kbp fragments, obtained from *Nhe* I digestion on λDNA molecules as these resultant fragments do not contain the sequence for LAMP amplification using the designed primers. Both second and third sample solutions were incubated at 65°C. After 50 min, all three tests showed no amplified products as seen in Fig. 5, hence confirming the viability of LAMP reaction in microchambers.

From repeated experiments, it was noted that for the affirmative occurrence of LAMP reaction in microchambers, visible amplified products could be formed as quickly as 20 min after incubation, but sometimes it may take as long as 50 min after incubation. The exact causes for such irregularities in reactions were not known. However, we hypothesized several possibilities, which included the temporary adhesion of fragment surfaces to the walls of the microchambers and the non-uniformity of temperature distribution within the tested region. The circumferential heater element (Fig. 1) enclosing an approximate circular area of 400 μm in diameter, generated a radial heat distribution for microchambers within this region. In other words, microchambers located at various distances from the heater element would experience slight differences in temperature. Moreover, the temperature distribution may also be affected as a result of minute variations in size and shape of the PAA gel and individual microchamber as well as the actual contents encapsulated in each chamber. Therefore, although each experiment was conducted as consistently as possible to minimize variations in experimental conditions, the amplification rate in individual microchamber could not be accurately determined.

In our case, to statistically analyse the LAMP reaction in microchambers, we first monitored a larger sample size of 14 microchambers as shown in Fig. 6.

From Fig. 6(a), the number of templates in each microchamber could not be clearly visualized from the snapshot image due to the relatively high background fluorescence from the primers. By averaging over 30 frames, the influence of background fluorescence was effectively reduced (Fig. 6(b)) and it was then possible to determine that the sample solution was diluted down to an approximate ratio of 1:1 template per chamber, as shown by the colour map in Fig. 6(c). From repeated tests, the percentage of one microchamber encapsulating only one λDNA template was found to be approximately 72%. Figure 6(d) shows the snapshot image of the same microchambers as presented in Fig. 6(a) after subjecting to 65°C for 50 min. To improve the images for analysis, averaging was performed over 200 frames and distinct increases of

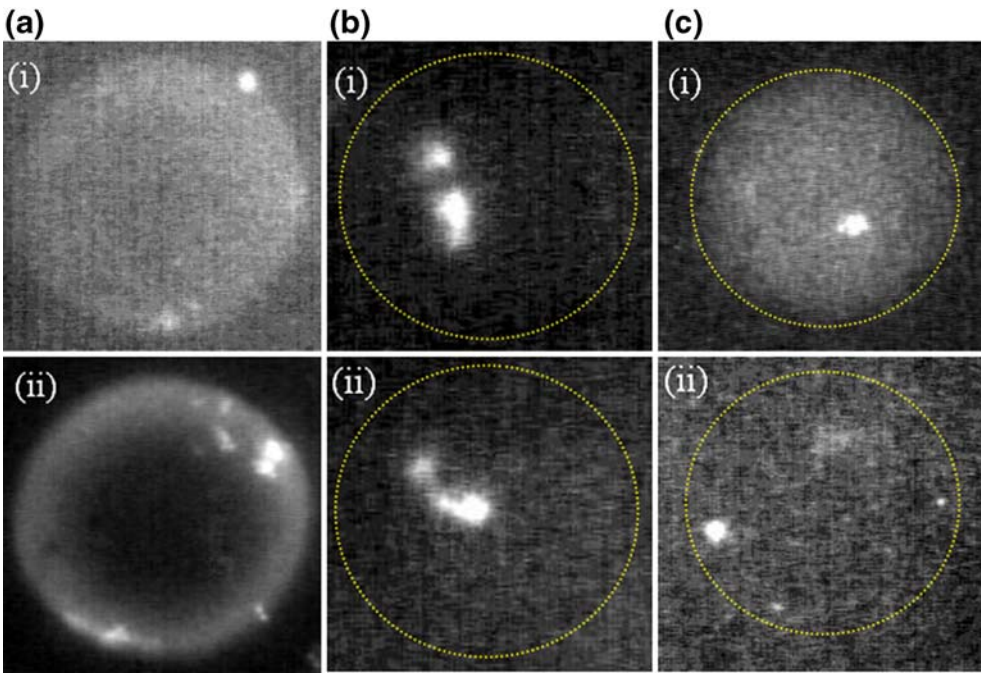

Fig. 5 Conditional tests of LAMP reaction in microchambers. Images subjected to averaging over 100 frames. (*i*) Encapsulated templates at 0 min and (*ii*) No amplified products at 50 min, respectively. (**a**) Sample solution with λDNA as templates, incubated under room temperature conditions; (**b**) Sample solution with λDNA as templates and did not contain primers, incubated at 65°C; (**c**) Sample solution with *Nhe* I-cleaved (13.8 kbp) fragments as templates, incubated at 65°C

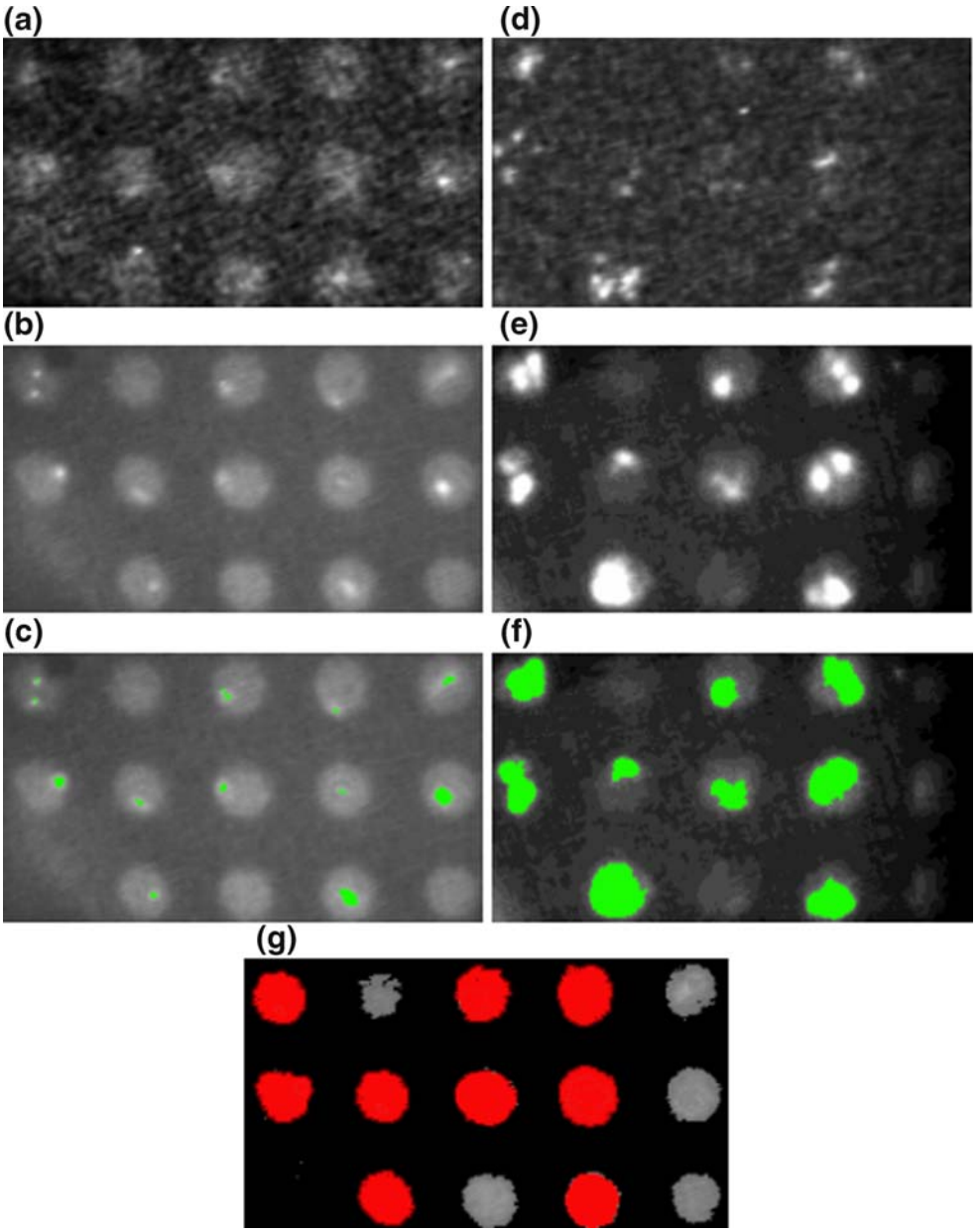

Fig. 6 Spatial observation of LAMP activities in PAA gel-based microchambers. Encapsulated templates at start of experiment, 0 min—(**a**) snapshot image, (**b**) averaged over 30 frames, (**c**) highlighting templates; Amplified products after 50 min of incubation at 65°C—(**d**) snapshot image, (**e**) averaged over 200 frames, (**f**) highlighting amplified products; (**g**) Quantized differences between end-products and initial sample in each microchamber

fluorescent intensity in microchambers experiencing the LAMP reaction were obtained as depicted in the resultant image (Fig. 6(e)) and its corresponding colour map (Fig. 6(f)). The difference between the images at 0 and 50 min after incubation was taken and quantized according to the presence or absence of amplified products in each microchamber. We then defined the efficiency of LAMP reaction in microchambers by evaluating the ratio of the number of microchambers containing amplified products and the total number of microchambers loaded with templates at the start of the experiment. It was essential to emphasize that the efficiency evaluated was based on the presence of amplified products with large molecular weight. For the case depicted in Fig. 6, the efficiency was estimated at 9 of 11 or 81.8%. Using the same methodology, another set of sample solutions was examined, and the results as well as the corresponding efficiencies are summarized in Table 1.

Sample A and B as denoted in Table 1, originated from the same prepared solution. In each case, comparable PAA-gel based microchambers with similar shape and dimensions were employed. Experimental conditions were kept similar using the same underlying microheater. Based on the observations of similar microchamber locations for the two sets of examples given in Table 1, it can be seen that the efficiencies for both cases at the same time of measurement differ significantly. After 20 min, the effi-

Table 1 LAMP reaction efficiency, calculated as the ratio between the number of PAA gel-based microchambers with amplified products and the sample size of 11

		Number of microchambers			Efficiency (%)
		With amplified products	Remaining unchanged	With reduction in number of fragments	
Sample A	After 20 min	7	2	2	63.6
	After 50 min	9	0	2	81.8
Sample B	After 20 min	3	6	2	27.3
	After 50 min	4	5	2	36.4
	After 80 min	9	1	1	81.8

ciency of LAMP reaction ranged from 27.3% to 63.6%; while after 50 min, the range of efficiency increased to 36.4–81.8%. The specific reasons for such variations could not be identified at this moment as we believed that there were several unknown variables and uncontrollable factors such as the exact content of the encapsulated sample in each microchamber, resulting in stochastic amplification which may differ for each case. Nonetheless, after repeating the same procedures for several more experiments, it was noted that beyond 50 min of incubation at 65°C, the efficiency of amplifying high-molecular-weight products usually reached over 80% for most cases.

In comparison, the previously mentioned cases of verifying LAMP reaction in the microchambers (Fig. 5) would correspond to the efficiency of 0% as no amplified products were observed when variations were made to the experimental conditions such as incubation temperatures, primer compositions or DNA templates. The contrast in efficiencies substantiated the high specificity of the single-molecule LAMP method.

It was important to note that the LAMP efficiency at the single molecule level was statistically analyzed and differently defined from that of conventional bulk LAMP assay, which reflected amplification rate or detection efficiency relating the total amount of amplified products.

5 Conclusion

In this investigation, LAMP reaction was performed and directly observed in the PAA gel-based microchamber using an integrated, localized heating system. One of the most attractive aspects of miniaturizing reaction chambers and down-scaling LAMP assay to a single molecule detection level using this novel approach was reflected in the required amount of template and primers, which has been significantly reduced as much as 75% required by standard protocols. Amplified products could be visualized after 20 min of incubation at 65°C, but often with relatively low repeatability at an averaged efficiency of 45.5%, based on the presence of high-molecular-weight products. By extending incubation time beyond 50 min, the reaction of LAMP in the microchambers was improved to an average of 59.1%. The efficiencies of 45.5% or 59.1% were indications to imply that approximately half of the total number of microchambers (50%) would contain amplified products. In such a case, the single-molecule LAMP method could be viewed as having a minimum of two-molecule detection limit. This would mean that by just observing the outcome of minimum two independent microchambers, each containing one DNA template, it would be statistically possible to equate that result to the case of all microchambers.

The obtained result verified the effectiveness of performing LAMP reaction in the PAA gel-based microchamber and demonstrated the simplicity of this one-step approach, which allowed assay and detection by direct observation to be completed within 50 min. This proved to be an advantageous improvement in detection time compared to a typical bulk LAMP assay where DNA amplification would take 30 to 60 min, followed by gel electrophoresis detection requiring an additional estimated time of 25 min. The microchamber system displayed potentials of performing several assays and it is anticipated to extend to other biological applications, hence offering a cost-effective and accurate alternative to conventional techniques.

Acknowledgement The authors would like to thank Professor K. Ishihara at University of Tokyo, Japan for providing the MPC samples, and acknowledge Dr K. V. Tabata, Dr R. Iino and Ms Y. Iko at Osaka University, Japan for their encouragement and valuable discussions. L. Lam is supported by a fellowship from the Japan Society for the Promotion of Science (JSPS). This work is supported by Grants-in-Aid from JSPS, Japan.

Appendix

Sequence of 2 kbp target

5′-ggcggcgacctcgcgggttttcgctatttatgaaaattttccggtt
taaggcgtttccgttcttcttcgtcataacttaatgtttttatttaaaatacccctctgaaaa
gaaaggaaacgacaggtgctgaaagcgaggctttttggcctctgtcgtttcctttctc
tgtttttgtccgtggaatgaacaatggaagtcaacaaaaagcagctggctga
cattttcggtgcgagtatccgtaccattcagaactggcaggaacagg
gaatgcccgttctgcgaggcggtggcaagggtaatgaggtgctttat
gactctgccgccgtcataaaatggtatgccgaaagggatgctgaaattgagaac
gaaaagctgcgccgggaggttgaagaactgcggcaggccagcgaggca
gatctccagccaggaactattgagtacgaacgccatcgacttacgcgtgcg
caggccgacgcacaggaactgaagaatgccagagactccgctgaagtggtg
gaaaccgcattctgtactttcgtgctgtcgcggatcgcaggtgaaattgccag
tattctcgacgggctccccctgtcggtgcagcggcgttttccggaactggaaaacc
gacatgttgatttcctgaaacgggatatcatcaaagccatgaacaaag
cagccgcgctggatgaactgataccggggttgctgagtgaatatatcgaacagt
caggttaacaggctgcggcattttgtccgcgccgggcttcgctcactgtt
caggccggagccacagaccgccgttgaatgggcggatgctaattactatctccc
gaaagaatccgcataccaggaagggcgctgggaaacactgcccctttcagcgggc
catcatgaatgcgatgggcagcgactacatccgtgaggtgaatgtggt
gaagtctgcccgtgtcggttattccaaaatgctgctgggtgtttatgcctactttata
gagcataagcagcgcaacacccttatctggttgccgacggatggtgatgccga
gaacttttatgaaaacccacgttgagccgactattcgtgatattccgtcgctgctggc
gctggccccgtggtatggcaaaaagcaccgggataacacgctcacca
tgaagcgtttcactaatgggcgtggcttctggtgcctgggcggtaaagcgg
caaaaaactaccgtgaaaagtcggtggatgtggcgggttatgat
gaacttgctgcttttgatgatgatattgaacaggaaggctctccgacgttcctgggt
gacaagcgtattgaaggctcggtctggccaaagtccatccgtggctccacgc
caaaagtgagaggcacctgtcagattgagcgtgcagccagtgaatccccgcattt
tatgcgttttcatgttgcctgcccgcattgcggggaggagcagtatcttaaatttggc
gacaaagagacgccgtttggcctcaaatggacgccggatgacccctc
cagcgtgttttatctctgcgagcataatgcctgcgtcatccgccagcaggagctg
gactttactgatgcccgttatatctgcgaaaagaccgggatctggacccgtgatgg
cattctctggttttcgtcatccggtgaagagattgagccacctgacagtgtgacctttt
cacatctggacagcgtacagcccgttcaccacctgggtgcagattgtcaaagactg
gatgaaaacgaaaggggatacgggaaaacgtaaaaccttcgtaaacac
cacgctcggtgagacgtgggaggcgaaaattggcgaacgtccggatgctgaagt
gatggcagagcggaaagagcattattcagcgcccgttcctgaccgtgtggct
tacctgaccgccggtatcgactcccagctggaccgctacgaaatgcgcgtatggg
gatgggggccgggtgaggaaagctggctgattgaccggcag-3′

References

H.F. Arata, Y. Rondelez, H. Noji, H. Fujita, Anal. Chem. **77**, 4810 (2005)

J. Compton, Nature **350**, 91 (1991)

P.H. Dear, Brief. Funct. Genomic. Proteomic. **1**(4), 397 (2003)

T.E. Fisher, P.E. Marszalek, J.M. Fernandez, Nat. Struct. Biol. **7**, 719 (2000)

M. Fujino, N. Yoshida, S. Yamaguchi, N. Hosaka, Y. Ota, T. Notomi, T. Nakayama, J. Med. Virol. **76**, 406 (2005)

J.C. Guatelli, K.M. Whitfield, D.Y. Kwoh, K.J. Barringer, D.D. Richman, T.R. Gingeras, Proc. Natl. Acad. Sci. USA **87**, 1874 (1990)

Y. Hataoka, L. Zhang, Y. Mori, N. Tomita, T. Notomi, Y. Baba, Anal. Chem. **76**, 3689 (2004)

S.T. Hess, S. Huang, A.A. Heikal, W.W. Webb, Biochemistry **41**(3), 697 (2002)

Y. Ishii, T. Yanagida, Single Molecules **1**, 5 (2000)

L. Lam, S. Sakakihara, K. Ishizuka, S. Takeuchi, H. Noji, Lab. Chip. **7**(12), 1738 (2007)

P.M. Lizardi, X. Huang, Z. Zhu, P. Bray-Ward, D.C. Thomas, D.C. Ward, Nat. Genet. **19**, 225 (1998)

W. Min, B.P. English, G. Luo, B.J. Cherayil, S.C. Kou, X.S. Xie, Acc. Chem. Res. **38**, 923 (2005)

K. Nagamine, K. Watanabe, K. Ohtsuka, T. Hase, T. Notomi, Clin. Chem. **47**, 1742 (2001)

G. Nallur, R. Marrero, C. Luo, R. Murli Krisnna, P.E. Bechtel, W. Shao, M. Ray, S. Wiltshire, L. Fang, H. Huang, C.G. Liu, L. Sun, J.R. Sawyer, S.F. Kingsmore, B. Schweitzer, J. Xia, Biomed. Microdevices **5**(2), 115 (2003)

T. Notomi, H. Okayama, H. Masubuchi, T. Yonekawa, K. Watanabe, N. Amino, T. Hase, Nucleic Acids Res. **28**(12), E63 (2000)

E.L. Ritman, J Cell Biochem Suppl **39**, 116 (2002)

Y. Rondelez, G. Tresset, K.V. Tabata, H. Arata, H. Fujita, S. Takeuchi, H. Noji, Nat. Biotechnol. **23**, 361 (2005)

R.K. Saiki, S. Scharf, F. Faloona, K.B. Mullis, G.T. Horn, H.A. Erlich, N. Arnheim, Science **230**, 1350 (1985)

G.T. Walker, M.S. Fraiser, J.L. Schram, M.C. Little, J.G. Nadeau, D.P. Malinowski, Nucleic Acids Res. **20**, 1691 (1992)

S. Weiss, Science **283**(5408), 1676 (1999)