Electronic Supplementary Information (ESI)

## Fabrication of 3D continuous-flow reverse-transcription polymerase chain reaction microdevice integrated with on-chip fluorescence detection for semi-quantitative assessment of gene expression

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References	Chip design	Integration	Accessories
This study	Continuous-flow	- Amplification - Detection	<ul><li>A plastic syringe</li><li>A hotplate</li><li>A fluorescence microscopy</li></ul>
Obeid et al. (2003)	Continuous-flow	Amplification	- Two syringe pumps - Four copper heat blocks
Hartung et al. (2009)	Continuous-flow	Amplification	<ul><li>Two syringe pumps</li><li>Two copper cylindrical heaters</li></ul>
Felbel et al. (2008)	Continuous-flow	Amplification	<ul> <li>A four-channel syringe pump</li> <li>A heating plate with thin film heaters, temperature sensors, and an electronic controller</li> </ul>
Tsai et al. (2006)	Continuous-flow	Amplification	<ul> <li>Sample actuation was not mentioned</li> <li>Temperature control system consisting of heaters, thermal sensors, amplifiers, a microprocessor, and the control circuits</li> </ul>
Obeid et al. (2003)	Continuous-flow	- Amplification - Detection	<ul><li>Two syringe pumps</li><li>Four copper heat blocks</li><li>A laser-induced fluorescence system</li></ul>
Li et al. (2011)	Continuous-flow	- Amplification - Detection	<ul><li>One syringe pump</li><li>Two cylindrical copper heaters</li><li>A fluorescence microscopy</li></ul>
Yamanaka et al. (2011)	Continuous-flow	- Amplification - Detection	<ul> <li>One microsyringe pump</li> <li>Three heaters</li> <li>One disposable electrical printed chip (for electrochemical detection)</li> </ul>
Cao et al. (2012)	Continuous-flow	- Amplification - Detection	<ul> <li>Two syringe pumps</li> <li>Two thin film heaters</li> <li>A commercialized capillary electrophoresis system</li> </ul>
Lien et al. (2007)	Chamber	- Sample concentration - Amplification	<ul> <li>Micropumps and microvalves</li> <li>Microheaters, a micro temperature sensor, and an integrated circuit controller</li> </ul>
Lee et al. (2008)	Chamber	- Amplification - Detection	<ul> <li>Syringe pump</li> <li>Temperature control system with three infrared halogen lamps</li> <li>Micro pinch valves</li> <li>Image laser scanner and optical detection system with photodiodes and high-gain amplifier for chemiluminescence detection</li> </ul>
Saunders et al. (2013)	Chamber	- Amplification - Detection	<ul> <li>Pump was not mentioned</li> <li>Infrared laser system for temperature control</li> <li>Inverted microscope</li> </ul>
Liao et al. (2005)	Chamber	Amplification	- Micropumps and valves - Microheaters
Kaigala et al. (2008)	Chamber	- Amplification - Detection	<ul> <li>Pneumatically-actuated pumps and valves</li> <li>Thin-film resistive element acting as a heater and a temperature sensor</li> <li>Optical detection system including a laser diode and a charged coupled device camera for capillary electrophoresis</li> </ul>
Hagan et al. (2011)	Chamber	- Sample purification - Amplification	<ul> <li>Syringe pump</li> <li>Infrared-mediated heating for temperature control</li> </ul>

## Table S1. Comparison of chamber-type and CF RT-PCR microdevices



**Fig. S1** Time-dependent temperature measurement of the 3D CF RT-PCR microdevice in 15, 45, and 90 min. The microdevice quickly reached the desired temperature within 15 min, and the designated temperature was stably maintained up to 90 min.



Fig. S2 Miniaturization of the heater used for the 3D CF RT-PCR. A lab-made rubber heater was used as the temperature source in replacement of the hot plate to enhance the portability of the entire system.

## References

C. Zhang, D. Xing, Nucleic Acids Res., 2007, 35, 4223.

N. M. Toriello, E. S. Douglas, N. Thaitrong, S. C. Hsiao, M. B. Francis, C. R. Bertozzi, R. A. Mathies, *Proc. Natl. Acad. Sci. U. S. A.*, 2008, **105**, 20173.

I. Gustavsson, M. Lindell, E. Wilander, A. Strand, U. Gyllensten, J. Clin. Virol., 2009, 46, 112.

H. Laurell, J. S. Iacovoni, A. Abot, D. Svec, J.-J. Maoret, J.-F. Arnal, M. Kubista, *Nucleic Acids Res.*, 2012, 40, e51.

P. J. Obeid, T. K. Christopoulos, Anal. Chim Acta, 2003, 494, 1.

R. Hartung, A. Brösing, G. Sczcepankiewicz, U. Liebert, N. Häfner, M. Dürst, J. Felbel, D. Lassner, J. M. Köhler, *Biomed. Microdevices*, 2009, **11**, 685.

J. Felbel, A. Reichert, M. Kielpinski, M. Urban, T. Henkel, N. Häfner, M. Durst, J. Weber, *Chem. Eng.* J., 2008, **135S**, S298.

N.-C. Tsai, C.-Y., Sue, Biosens. Bioelectron., 2006, 22, 313.

P. J. Obeid, T. K. Christopoulos, H. J. Crabtree, C. J. Backhouse, Anal. Chem., 2003, 75, 288

Y. Li, C. Zhang, D. Xing, Microfluid. Nanofluid., 2011, 10, 367.

K. Yamanaka, M. Saito, K. Kondoh, M. M. Hossain, R. Koketsu, T. Sasaki, N. Nagatani, K. Ikuta, E. Tamiya, *Analyst*, 2011, **136**, 2064.

Q. Cao, M. Mahalanabis, J. Chang, B. Carey, C. Hsieh, A. Stanley, C. A. Odell, P. Mitchell, J. Feldman, N. R. Pollock, C. M. Klapperich, *PLoS ONE*, 2012, 7, e33176.

K.-Y. Lien, W.-C. Lee, H.-Y. Lei, G.-B. Lee, Biosens. Bioelectron., 2007, 22, 1739.

S. H. Lee, S.-W. Kim, J. Y. Kang, C. H. Ahn, Lab Chip, 2008, 8, 2121.

D. C. Saunders, G. L. Holst, C. R. Phaneuf, N. Pak, M. Marchese, N. Sondej, M. McKinnon, C. R. Forest, *Biosens. Bioelectron.*, 2013, 44, 222.

C.-S. Liao, G.-B. Lee, H.-S. Liu, T.-M. Hsieh, C.-H. Luo, Nucleic Acids Res., 2005, 33, e156.

G. V. Kaigala, V. N. Hoang, A. Stickel, J. Lauzon, D. Manage, L. M. Pilarski, C. J. Backhouse, *Analyst*, 2008, **133**, 331.

K. A. Hagan, C. R. Reedy, M. L. Uchimoto, D. Basu, D. A. Engel, J. P. Landers, *Lab Chip*, 2011, 11, 957.