Electronic Supplementary Material (ESI) for Lab on a Chip.

Supporting Information

A point-of-care testing platform for on-site identification of genetically modified crops

Yangyang Wang, Furui Yang, Yingyi Fu, Xin He, Haowei Tian, Lili Yang, Mengxi Wu,*

Jijuan Cao and Junshan Liu**

*Corresponding author. Email: mengxiwu@dlut.edu.cn (M. Wu), caojijuan@dlnu.edu.cn (J. Cao), liujs@dlut.edu.cn (J. Liu)

This PDF file includes:

Note S1. Materials and Methods

Chip preparation. Microfluidic chips were manufactured by CNC engraving and milling machine (Beijing Jingdiao Group, JDPMT 400) using a standard milling process. Milling out each reaction chambers on a 3mm thick PC plate and drilling through ventilation holes on a 0.5mm thick COC material. A pressure-sensitive adhesive (PSA) is used to (Adhesive Research, Beijing, China) bond the PC and COC plate. For each experiment, 3 microfluidic chips were utilized to ensure the reproducibility of the results.

Paraffin preparation. There were two kinds of paraffin. The sterile liquid paraffin wax was used in the contact area of slide plate and substrate plate (GS070, Qingdao Hope Bio-Technology Co. Ltd., China). The other was a low-melting paraffin wax used for primer sealing, which used a mixed mass ratio of 1:2.2 between the solid and the above liquid paraffin. The melting point was about 38 degrees, and the solid paraffin was sliced paraffin with the original melting point of 48-52 degrees Celsius (69018961, Sinopharm Chemical Reagent Co. Ltd., China).

Plasmid. A virus DNA/RNA extraction kit (Magnetic Beads Based) by Vazyme company (Nanjing, China) was used to extract the DNA from the sample. A Warmstart colorimetric LAMP 2X Master Mix (BioLabs, New England) was used to perform the amplification reaction. WSSV and CGMMV positive plasmid were synthesized by TaKaRa (Dalian, China). All assays were prepared using Dnase/Rnase-free water (Aladdin).

Primer Design. The primers were designed by Dalian Minzu University, and synthesized by TaKaRa (Dalian, China), and their sequences are listed in Table 1, Supporting Information. Primers and probes for the specific amplification of CGMMV and WSSV were designed using Primer 5.0 software, and their specificity was evaluated with Basic Local Alignment Search Tool (BLAST) available at the NCBI website.

Assay preparation. For LAMP assays, a standard reaction volume of 25µL was used. This volume consisted of primer mix (10×), master mix (2×), Dnase/Rnase-free water (dH_2O) and DNA sample. The assays were incubated at 65°C for different times to observe the color change of different samples over time. The platform reaction was performed in a final volume of 50µL including, 25µL WarmStart Colorimetric LAMP 2X Master Mix, 5µL LAMP Primer Mix and 16µL dH_2OThe nucleic acid extraction part includes 2µL proteinase K, 2µL magnetic bead, 60µL lysis solution, 2µL target sample, 70µL washing buffer 1, 70µL washing buffer 2 and 4µL elution buffer.

Figure S1

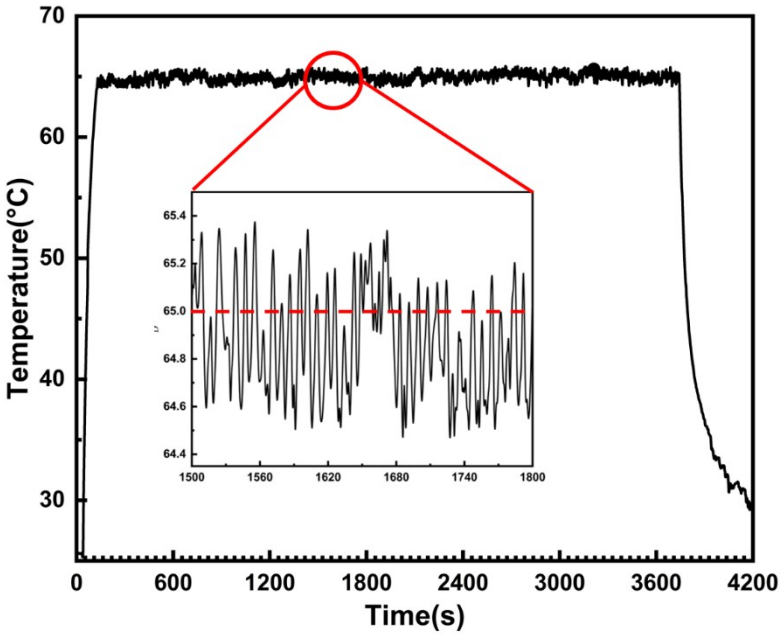

Figure. S1. Temperature control module stability test

Table. 1. The List of LAMP primer sequences.

ID	Target	Name	Primers (5'--3')
1	Bt-11	F3	GCTGTAGCTGGCCTAATC
		B3	GGCCAAGGTATCTAATCAGC
		FIP	TATCTGTCTCAGGGGCAGACTCTCAACTGGTCTCCTCTCC
		BIP	GCCAAGAAGGCGCAAGTCCATCCCATTTGTGATCTTTGTC
		Loop F	GTGTTCCCTCGGATCTCG
		Loop B	ACCGCGAGTTGTTGTATCATA
2	Bt176	F3	CATGACGTGGGTTTCTGG
		B3	GCGAGAACACGAGAAGAG
		FIP	CCAAGGCTTCAAGGCCATTGACCGAGATCTGATGTTCTCT
		BIP	GCTCCCTCTCTCTCCCTCTCATGTGGGAGGGAGAACTC
		Loop F	ATGGCGTGCATCAATGGA
		Loop B	TCCTATAAAGTCGATACCACGC
3	GA21	F3	GCTGTAGTTGTTGGCTGT
		B3	CCTTTTAACTGATGTTTTCACTT
		FIP	TGGGGGATCCACTAGTTCTAGAG-GTGGAAAGTTCCCAGTTGA
		BIP	GCAGGTCGAGGTCATTCATATG-GACCAGGTAATCTTACCTTTG
		Loop F	CTGCACTTCCTCTTTAGCATCC
		Loop B	CTTGAGAAGAGAGTCGGGATAGTC
4	MIR162	F3	TGATTAGAGTCCCGCAATT
		B3	TCATACAAAAAGGCCCAGT
		FIP	AACATAGATGACACCGCGCGGCGATAGAAAACAAAATATAGCG
		BIP	ATTCAGTACATTAAAAACGTCCGCCAAAACAACTACCACAAGGC
5	MIR604	F3	AATCTGCCTATCGAGAAGA
		B3	CGGTTCTGTCAGTTCCAA
		FIP	GTGTGATCTGGCGTCCAGGAACCAGTGAATGGAGATG
		BIP	CGCACGCAATTCAACAGAACCTTAATTCTCCGCTCATG
		Loop B	CGGGAAACGACAATCTGA
6	MON810	F3	GGGCTACATCGAAGACAGC
		B3	GCAAGCAAATTCGGAAATGA
		FIP	GCCAGAGGGAACCAGTACCGATTTACCTGATCCGCTACAA
		BIP	AAGTGTGCCCACCACAGCGAAAGTCCTCGTTCAGGTC
		Loop F	TTGACGGTCTCGTGCTTG
		Loop B	CACCACTTCTCCTTGGACAT
7	MON863	F3	CCTTTTGATGAAGTGACAGG
		B3	AAGTCCAGGTTGGTTGGT
		FIP	ACTATTGACCCTACTTGTTCGGA-TAGGATCGGAAAGCTTGGTA
		BIP	TAGGCCGTAACATTTAGCAAAAAACAGTGATAGGAGACTATCTAGCT
		Loop F	TGGGTGTTCACCCCAAAGTG
8	MON88017	F3	GCTAGCTTGATGGGGATCAG
		B3	CTTGTAGATGGCACCGCG
		FIP	GGCAGTATGCCGGAGTTGACC-TCGTTTCCCGCCTTCAGT
		BIP	CTGGCCGCACGCAGGAAAAATA-CTGTCGTGTCTGACCAAGG

		Loop B	GGGCGAATCAGAAAGGGCGT
9	**MON89034**	**F3**	TTGCTTTCGCCTATAAATACG
		B3	GAAACTTTGGGTTGAAATGAAAT
		FIP	GTAGATGTCCGCAGCGTTATTATAAACGGATCGTAATTTGTCGT
		BIP	ATTGACCATCATACTCATTGCATCCCCAATACTCAAAAAATAAC
10	**T25**	**F3**	GGAACGACTCAATGACAAGA
		B3	AGAGGCATCTTCAACGATG
		FIP	GGAATCCGAGGAGGTTTCCG-ATCTTCGTCAACATGGTGG
		BIP	TTGCCCAGCTATCTGTCACTTT-GCAATGATGGCATTTGTAGG
		Loop F	TTGGAGTAGACAAGCGTGTC
		Loop B	TAGTGGAAAAGGAAGGTGGC
11	**3272**	**F3(PMI)**	TCCCGATTCCAGTGGATGA
		B3(PMI)	ACAGTCACCGGTGATTCGT
		FIP(PMI)	ACAAAATGGCGGCACTCTGCTGCCTTCTCGCTGCATGAC
		BIP(PMI)	CGTCGAAGGCGATGCAACGTGGCGGCAATAAACGCTGAT
		F3(797E)	AGGCATCTGGTGGGACAC
		B3(797E)	TCCTGCTTGGACCCGAAG
		FIP(797E)	GGAAGCTGGCGGTATCCAGATCGCCAGAAGATCCCCGAGT
		BIP(797E)	CTCGATGGGCTACGACCCGTACCGTGCCCTTCTGGTAG
12	**59122**	**F3**	CGAACGATTCAGATGGCA
		B3	TTGCGGTTCTGTCAGTTC
		FIP	CCTTCACTCTTTCTTCCGTCCCTTCTCGTACTCACCAACATTG
		BIP	TTAAACTGAAGGCGGGAAACGAGTCATAACGTGACTCCCTTAA
		Loop F	TGAGCCAATCACAGGTGC
		Loop B	CAATCTGATCATGAGCGGAGA