

An integrated temporary negative pressure assisted microfluidic chip for DNA purification and digital PCR detection

Qingchang Tian^{#a}, Baodong Yu^{&b}, Ying Mu^{*a}, Yanan Xu^a, Congcong Ma^a, Tao Zhang^a, Wei Jin^a, Qinhan Jin^a

5

^aResearch Center for Analytical Instrumentation, Institute of CyberSystems and Control, State Key Laboratory of Industrial Control Technology, Zhejiang University, Hangzhou 310058, Zhejiang, P. R. China.

E-mail: muying@zju.edu.cn; Fax: +86 571-88208382;

Tel: +86 571-88208383

10 ^bChina-Japan Union Hospital of Jilin University, Changchun, 130021, Jilin, P. R. China.



FIG. S1. Reagent segments were loaded into Teflon tube with the help of pipettor.

5

Table S1. The statistical analysis result of the digital PCR

X_{dil}	Observed value			Average value	STDEV	$-\ln(1-f_0)$	$-\ln(1-STDEV/2560)$
0.0016	10	18	14	14	4	0.00548376	0.00156372
0.008	60	62	69	63.66666667	4.725815626	0.02518427	0.00184773
0.04	252	248	258	252.6666667	5.033222957	0.1039148	0.00196804
0.2	942	958	949	949.6666667	8.020806277	0.46356606	0.00313805

10

15

Table S2. The statistical analysis result of the real-time PCR

$\ln(X_{dil})$	Ct value			Average Ct value	STDEV
6.437752	36.62	37.05	37	36.89266667	0.234717561
4.828314	33.2	33.4	34.1	33.56866667	0.481049201
3.218876	30.4	30.38	30.5	30.43066667	0.073934656
1.609438	26.34	27.01	27.1	26.82233333	0.425483646
0	22.39	22.69	22.7	22.57733333	0.162666325

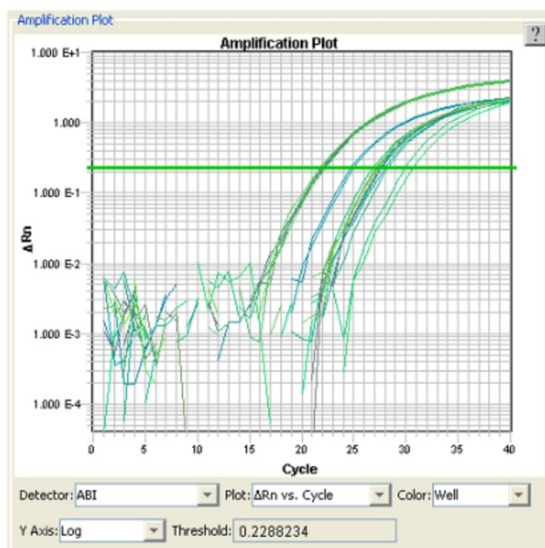
20

25

Table S3. The statistical analysis result of the real time PCR

Gradients	Samples	DNA concentration (ng/ μ L)	Average	Standard deviation	Results of Mann-Witney U statistical test
$WR=10^{-3}$	3	0.725	0.747	0.067	$P=0.127 > 0.05$
	3	0.823			
	3	0.694	0.628	0.119	
	4	0.494			
	4	0.671			
	4	0.72			

Amplification Plot



Standard Curve Plot

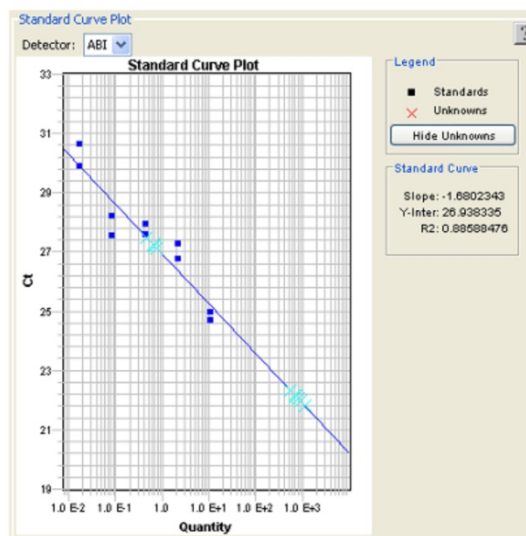


FIG. S2. Amplification plot and standard curve plot of qPCR

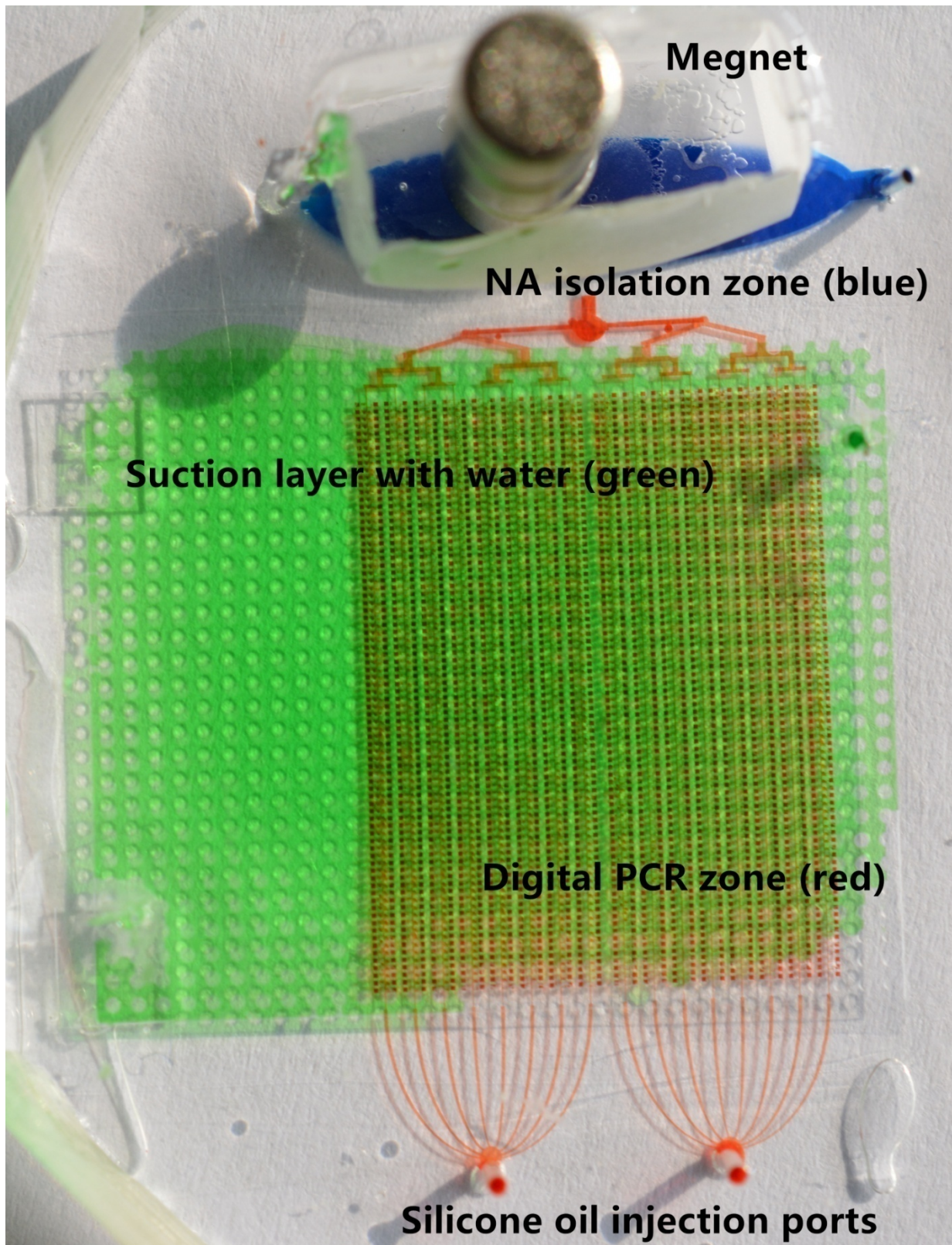
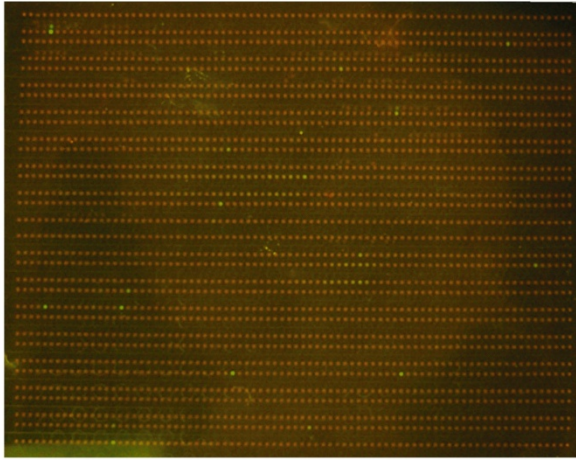
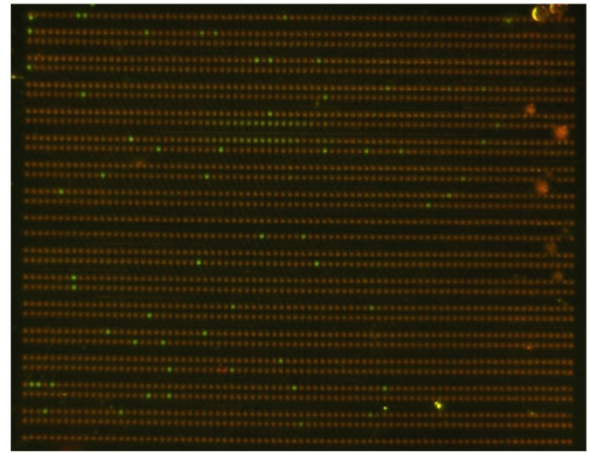


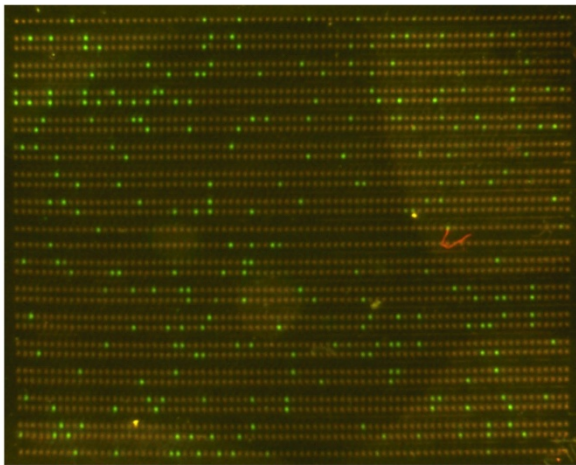
Fig. S3 Photograph of the microdevice. Reagent (blue) could pass through the NA isolation zone from inlet to outlet 1 and could not enter the digital PCR zone (red) under negative pressure from outlet 2. The area of suction layer (green) was larger than the digital PCR layer (red) which was loaded water to avoid evaporation and ensure the efficiency of PCR in each chamber.



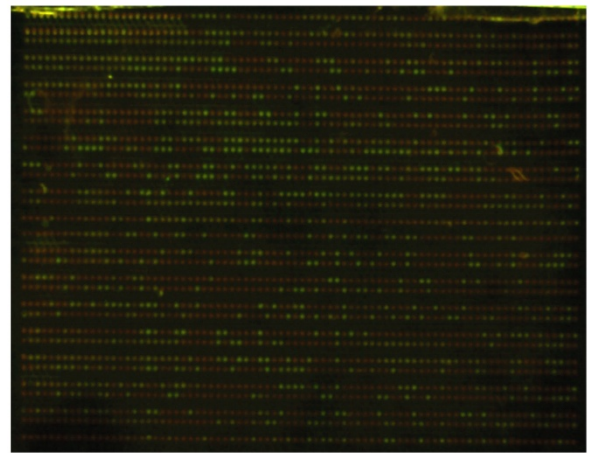
$X_{dil}=0.0016$



$X_{dil}=0.008$



$X_{dil}=0.04$



$X_{dil}=0.2$

5 **FIG. S4** Digital PCR fluorescent images with a serial dilution of target GAPDH DNA template ranging from 0.0016 to 0.2 dilutions.

10

15

Amplification Plot

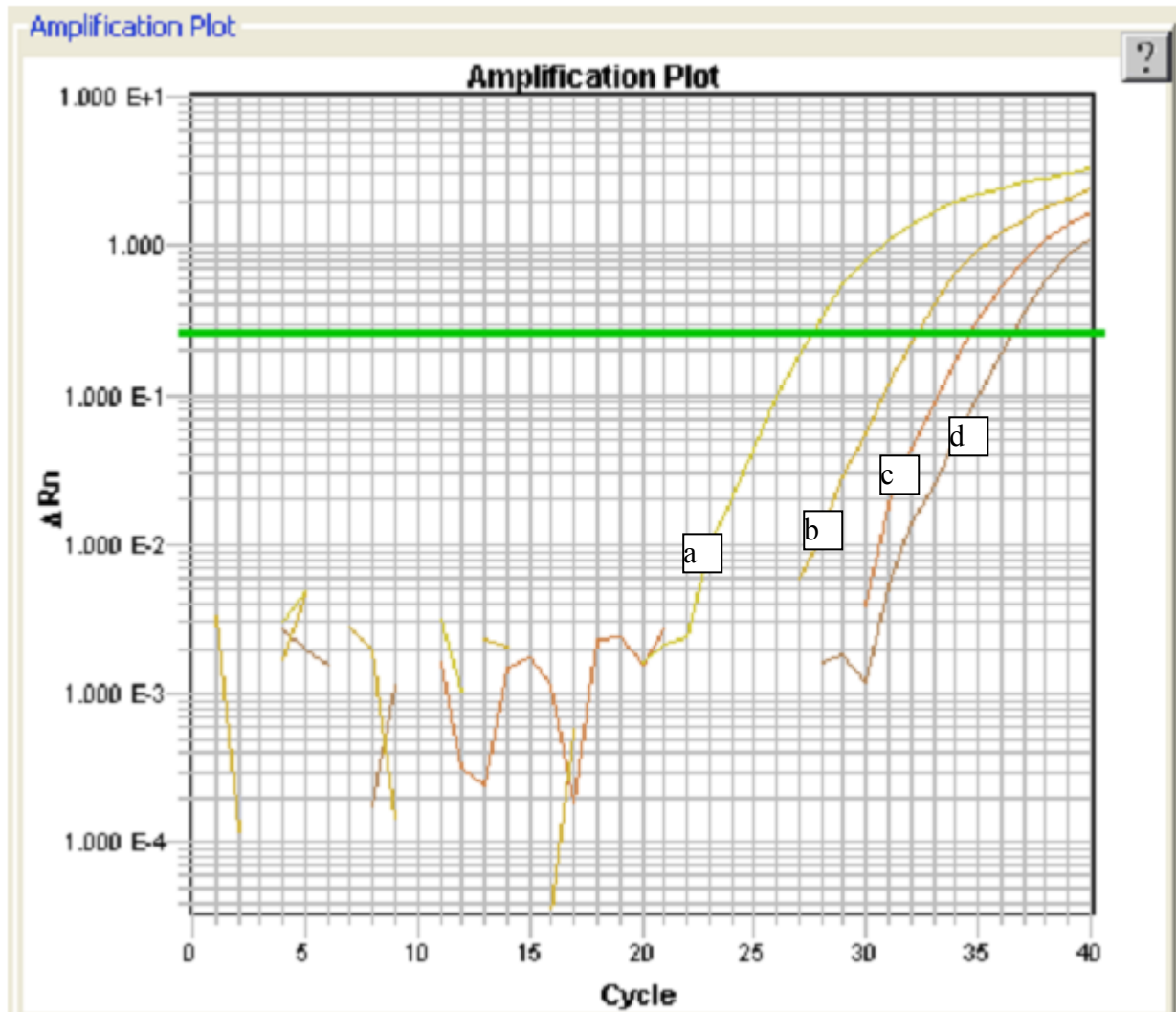


FIG. S5 Amplification plot of different concentrations of bovine lysate. a, $WR=10^{-7}$; b, $WR=10^{-8}$; c, $WR=10^{-9}$; d, $WR=10^{-10}$.

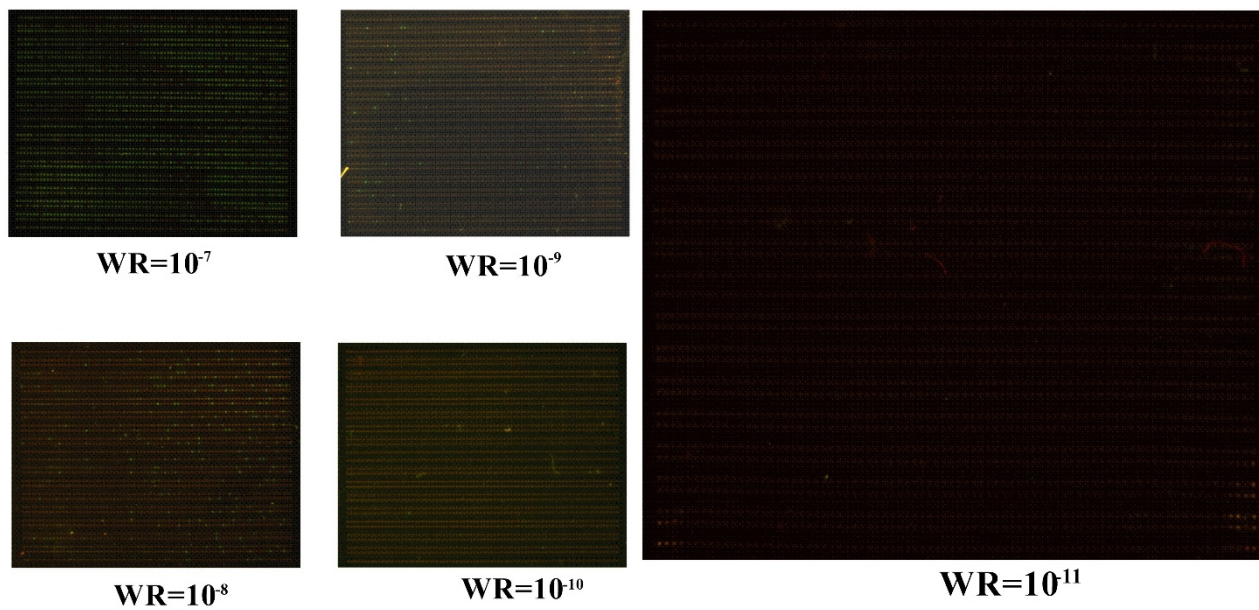


Fig. S6 Digital PCR fluorescent images (partly in Fig. 5) on the microdevice with different concentrations of bovine lysate.

