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Supporting information

A fast nucleic acid extraction system for point-of-care and integration of digital PCR

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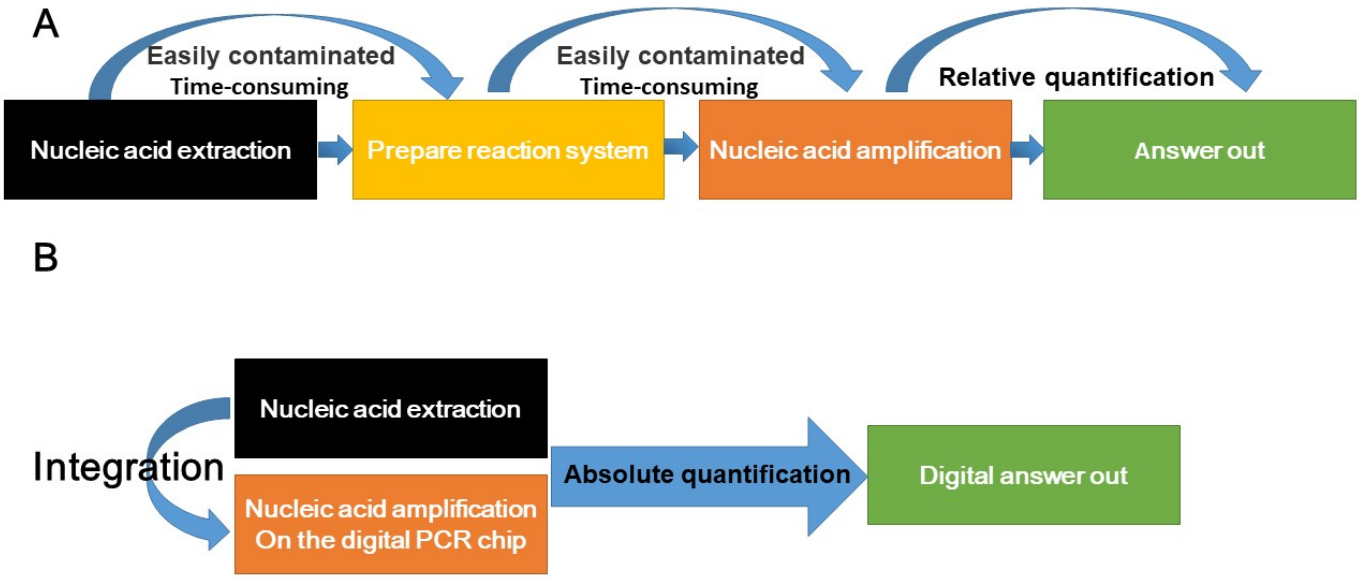
DNA extraction procedure using traditional method

1. Add 1μL, 3μL,5μL,7μL sample to a nuclease-free 1.5 mL tube. Add 10 mM Tris-HCl, PBS, or Elution Buffer to bring the volume up to 200 μL.
2. Add 200 μL Buffer MSL and 20 μL OB Protease to the cube. Mix throughly by vortexing 30 seconds.
3. Incubate sample at 65℃for 15 min. Briefly vortex the tube during incubation.
4. Add 300 μL absolute ethanol and 20 μL Mag-Bind Particles CND to the lysate. Vortex 30 seconds to mix reagents.
5. Place the tube on a magnetic separation device to magnetize the magnetic particles. Incubate at room temperature for 10 minutes.
6. Completely remove and discard the cleared supernatant. Remove any droplets of liquid from the wall of the tube.
7. Remove the tube from the magnetic separation device. Then add 600 μL Buffer MP/Ethanol mixture to the sample.
8. Resuspend the Mag-Bind particles pellet by vortexing 30 seconds.
9. Place the tube onto the magnetic separation device to magnetize the Mag-Bind particles. Completely remove and discard the cleared supernatant.
10. Remove the tube containing the Mag-Bind particles from the magnetic separation device. Add 700 μL SPM Buffer to the sample.
11. Resuspend the Mag-Bind particles pellet by vortexing.
12. Place the tube onto the magnetic separation device to magnetize the magnetic particles. Completely remove and discard the cleared supernatant.
13. Remove the tube containing the Mag-Bind particles from the magnetic separation device. Add 700 μL SPM Buffer to the sample.
14. Resuspend the Mag-Bind particles pellet by vortexing.
15. Place the tube onto the magnetic separation device to magnetize the magnetic particles. Completely remove and discard the cleared supernatant.
16. Air drying the tube on the magnetic separation device for 10 minutes. And remove any residue liquid with a pipettor.
17. Remove the tube from the magnetic separation device. Resuspend the Mag-Bind particles pellet by vortexing.
18. Add 40 μL Elution Buffer to the sample. Incubate 10 minutes at 65℃.
19. Resuspend the Mag-Bind particles by vortexing.
20. Place the tube onto a magnetic separation device to magnetize the Mag-Bind particles.
21. Transfer the cleared supernatant containing purified DNA to a new 1.5 mL tube.

Traditional RNA extraction procedure

1. Add 20 μL 2-mercaptoethanol per 1 ml of TRK lysis Buffer before use. Then add 400 μL of prepared TRK lysis Buffer to 1μL, 3μL,5μL,7μL sample.
2. Add 300 μL absolute ethanol and 20 μL Proteinase K to the sample. Shaking to mix for 1 minutes.
3. Add 10 μL magnetic Particles and shaking for 5 minutes.
4. Place the tube on a magnetic separation device to magnetize the magnetic particles.
5. Discard the cleared supernatant.
6. Remove the tube containing the magnetic particles from the magnetic separation device. Add 600 μL of MBW Wash Buffer. Shaking the tube at maximum speed for 1 minutes to resuspend magnetic particles pellet.
7. Place the tube onto a magnetic separation device to magnetize the magnetic particles. Aspirate the cleared supernatant.
8. Remove the tube from the magnetic separation device. Add another 600 μL of SPR Wash Buffer and resuspend magnetic particles pellet by shaking for 1 minutes.
9. Place the tube onto a magnetic separation device to magnetize the magnetic particles. Aspirate the cleared supernatant and leave the tube to air dry for 10 minutes.
10. Leave the tube on the magnetic separation device. Prepare 100 μL DNase I digestion mix by adding 10 μL DNase Digestion Buffer, 88.5 μL DEPC-Water and 1.5 μL DNase I.
11. Add 100 μL of DNase I digestion mix and resuspend the magnetic particles by pipetting up and down for 20 times. Incubate at room temperature for 10-15 minutes.
12. Add 400 μl SPR Wash Buffer to the sample and mix throughly by shaking for 5 minutes.
13. Place the tube onto a magnetic separation device to magnetize the magnetic particles. Aspirate the cleared supernatant.
14. Add 500 μL of SPR Wash Buffer and resuspend magnetic particles pellet by vortexing for 30 seconds.
15. Place the tube onto a magnetic separation device to magnetize the Mag-Bind particles.
16. Aspirate and discard the cleared supernatant and remove any liquid drop from tube. Leave the tube on the device to air dry the magnetic particles at room temperature for 7-10 minutes.
17. Add 50μl DEPC Treated water and resuspend magnetic particles pellet by vortexing and down for 20 times. Incubate at room temperature for 3 minutes.
18. Place the tube onto a magnetic separation device to magnetize the Mag-Bind particles.
19. Transfer the cleared supernatant contains purified RNA into a new 1.5 ml tube.



Schematic diagram S1. The advantage of the integration of the nucleic acid extraction and digital PCR. A: Traditional nucleic acid detection process. The transfer of the obtained nucleic acid and the preparation of the nucleic acid amplification system are easily contaminated and time consuming. Meanwhile, the results rely on the standard curve and cannot give accurate results. B: The process of the integration of nucleic acid extraction and digital PCR. The system can reduce the detection process and avoid the pollution. It can give a numerically absolute quantitative result.

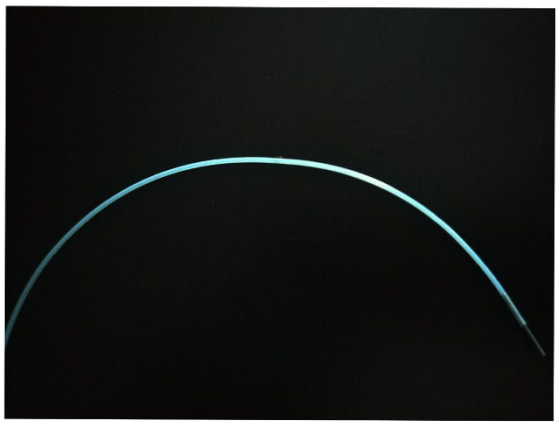


Figure S1 PTFE tubing for the nucleic acid extraction and integration of digital PCR chips in this research.

Table S1 Length corresponding to different volumes in PTFE tubing

Volumes(μL)	Length(cm)
5	1.25
10	2.5
15	3.75
20	5

Table S2. DNA concentration and purity values of the HepG2 samples using different approaches.

	Conventional approach			PTFE system		
Sample volumes(μL)	Concentration （ng/μL）	Total content(ng)	Purity (A260/280)	Concentration （ng/μL）	Total content	Purity (A260/280)
1	0.531±0.12	21.24	1.81	1.14±0.23	22.8	1.97
3	0.534±0.27	21.36	1.85	1.39±0.21	27.8	1.95
5	0.667±0.25	26.68	2.01	1.55±0.53	31	1.98
7	1.293±0.33	51.72	1.99	3.33±0.71	66.6	1.97

Sample is HepG2 cells and C = 1×10^6/ mL, n=3.

Table S3. DNA concentration and purity values of the blood samples

	Conventional approach			PTFE system		
Sample ID	Concentration （ng/μL）	Total content(ng)	Purity (A260/280)	Concentration （ng/μL）	Total content	Purity (A260/280)
1	0.16±0.01	6.5	1.81	0.31±0.03	6.2	1.78
2	0.28±0.03	11.1	1.90	0.53±0.02	10.6	1.83
3	0.25±0.01	10.0	1.91	0.42±0.02	8.4	1.80
4	0.27±0.02	10.8	1.91	0.45±0.02	9	1.93

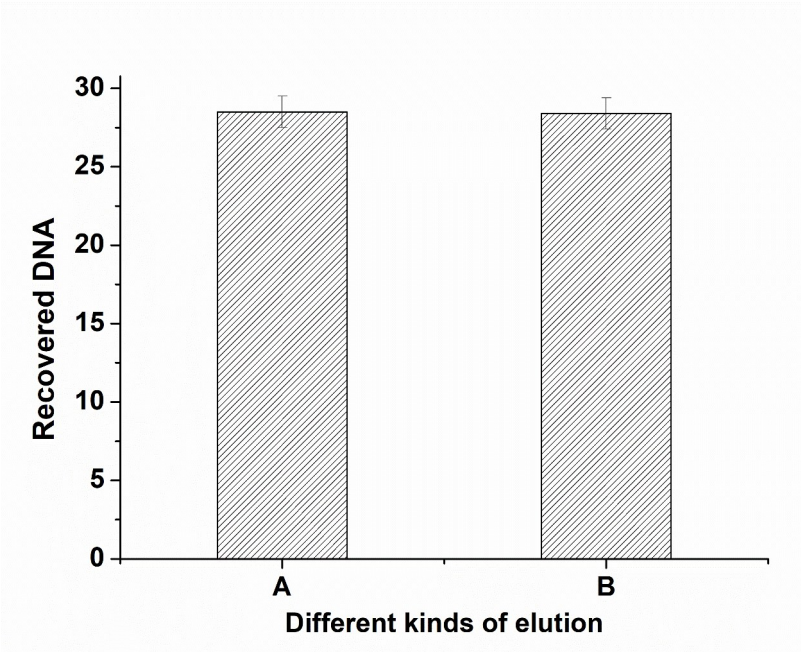


Figure S2 Comparison of elution effects using different lysis buffer. A: elution buffer from the kit. B：PCR mix

Table S4 RNA concentration and purity values from HepG2 samples using different approach

	Conventional approach			PTFE system		
Sample volumes(μL)	Concentration （ng/μL）	Total content(ng)	Purity (A260/280)	Concentration （ng/μL）	Total content	Purity (A260/280)
1	1.38±0.5	69	1.99	16.3±1.7	326	2.00
3	4.64±1.1	232	1.82	20.2±2.3	404	1.94
5	6.04±0.7	302	1.86	28.2±2.8	564	1.93
7	16.73±2.1	836.5	1.85	34.9±3.6	698	1.94

Sample is HpG2 cells and C = 1×10^6/ mL, n=3.

Table S5 Volumetric comparison of reagents required by different methods in different steps

Method	lysing(μL)	washing(μL)	elution(μL)
Commercial method(CM)	720	700	50
PNE system(PS)	10	10	20
CM/PS	72	70	20

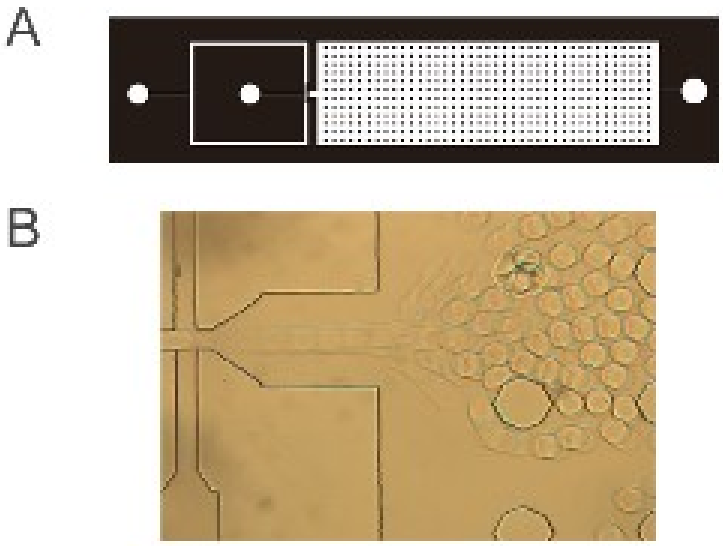


Figure S3 Schematic diagram of droplet digital PCR (ddPCR) chip used in this study. A: the design of ddPCR chip. B: Using a PTFE tube loaded with the reaction solution to generate droplets.