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

Supplementary information

Fully automated, on-site isolation of cfDNA from whole blood for cancer therapy monitoring

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Supplementary Movies

Movie S1: Visualization of the cfDNA isolation process on a disc. Fluidic motion on the disc during cfDNA isolation was visualised using a built-in spinning disc imaging machine equipped with a strobe light and a camera. Images at specific disc positions were captured at 2 frames/sec during rotation for the blood separation, 4 frames/sec for the sample transfer, 6 frames/sec for drying, and 17 frames/sec for the mixing mode. The obtained images were utilised to generate a movie.

Supplementary Figures

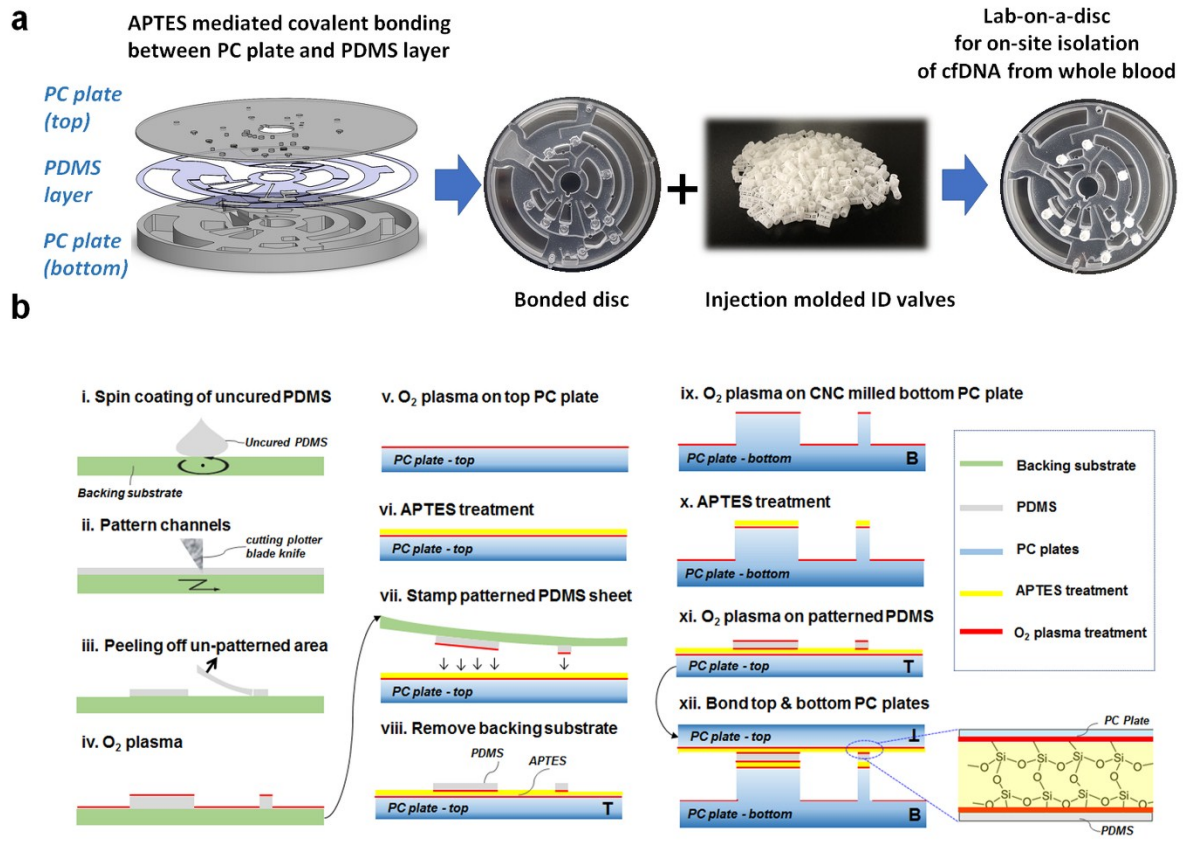

Fig. S1. Schematic illustration of disc assembly and APTES-mediated covalent bonding. a) Lateral view of the disc showing top and bottom plates made of polycarbonate that are assembled by APTES-mediated covalent bonding with the PDMS layer; the injection-molded ID valves are assembled on the bonded disc to prepare the lab-on-a-disc **b)** (i) Uncured PDMS was uniformly deposited onto a backing substrate by spin coating (700 rpm, 60 sec) and then cured in a drying oven (65 °C, 4 h). (ii) The cured thin PDMS sheet was patterned according to the design by using a plotter. (iii) Peeling off the un-patterned area. (iv) The patterned PDMS layer was modified by O_2 plasma treatment. (v-vi) The top PC plate was modified by O_2 plasma treatment and APTES treatment. (vii) The patterned PDMS layer was stamped to the modified PC plate. (viii) Removal of the backing substrate. (ix-x) The milled bottom PC plate was modified by O_2 plasma treatment and APTES treatment. (xi) The surface of the stamped PDMS layer was modified by O_2 plasma treatment. (xii) Finally, the two surface-modified PC plates were assembled together by PDMS adhesive support.

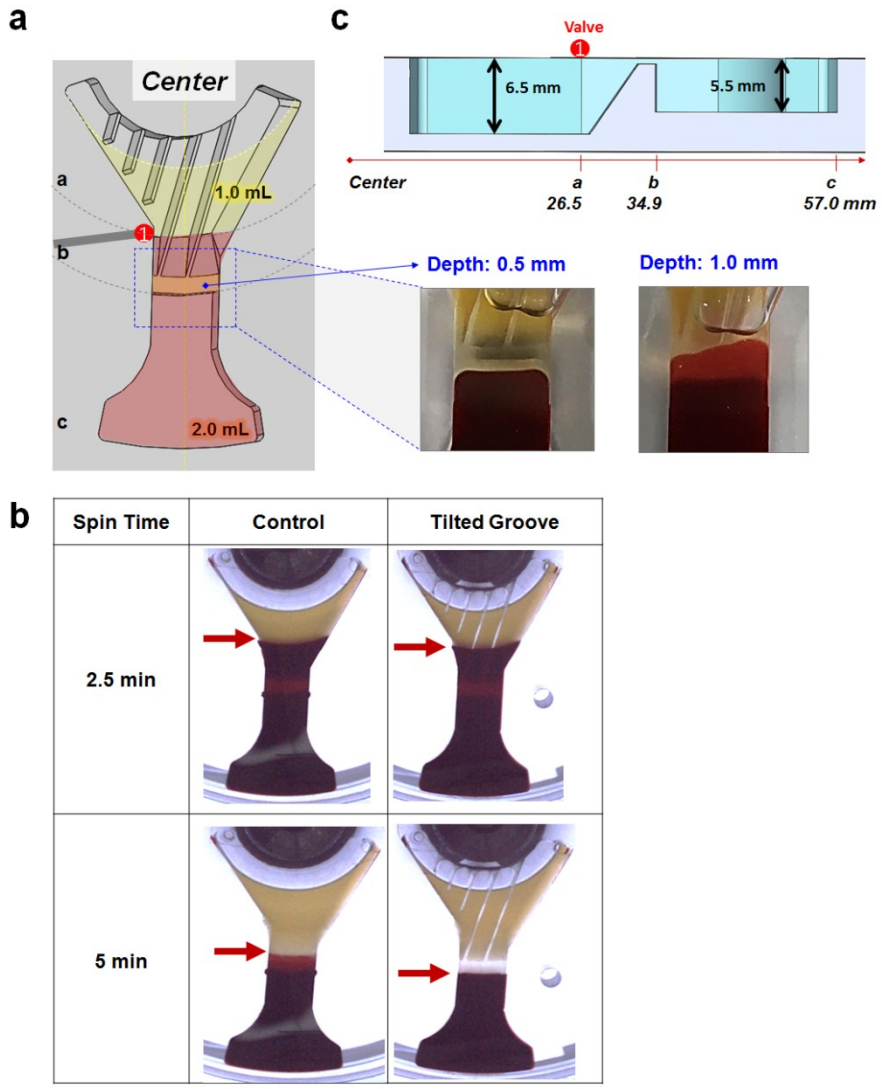

Fig. S2. Geometric configurations for effective plasma separation. **a)** Top view of the blood chamber showing the embankment positioned between the plasma storage chamber (volume: 1.0 ml) and the blood cell storage chamber (volume: 2.0 ml), and tilted groove walls with slanted angle; **b)** Upper: lateral view of the blood chamber indicating the dimensions with each radial position; bottom: photographs of the interface between the plasma storage chamber and the blood cell storage chamber taken immediately after the plasma separation process, showing that the narrower depth of interface prevents contaminants (blood cells); **c)** Still images at 5 min during the plasma separation process showing that tilted groove walls expedite blood cell sedimentations.

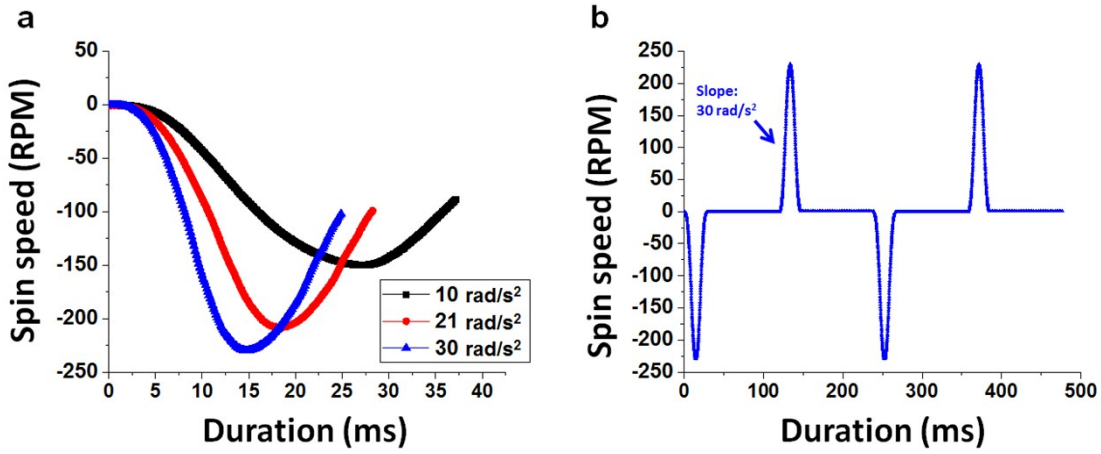

Fig. S3. Detailed spinning profiles of the mixing mode on the disc. a) Short-term measurement of the spin speed for the different angular accelerations; **b)** Long-term measurement of the spin speed for the mixing mode with 30 rad/s^2. The spin speed was obtained from the encoder connected to the servo motor of the spinning device.

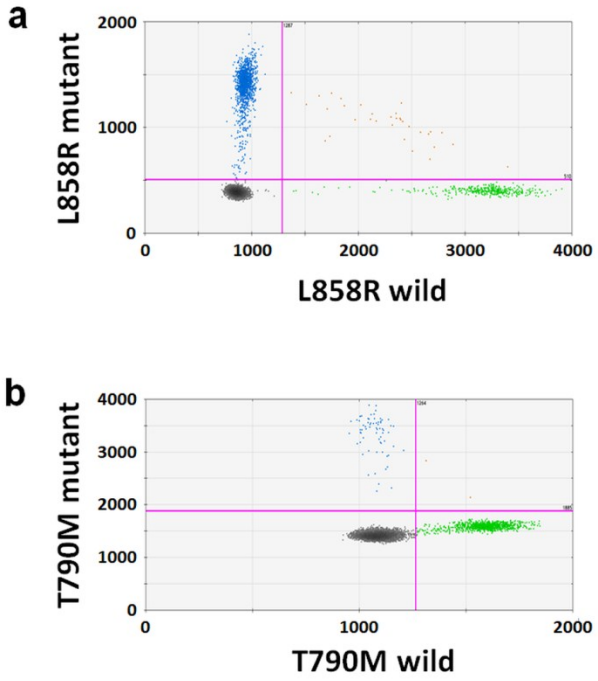

Fig. S4. Two-dimensional histograms of the ddPCR assay. Representative positive plots of L858R mutation. **a)** and T790M mutation; **b)** detected in the cfDNA sample purified from the blood of a patient with lung cancer. Each dot of different colours indicates negative (black), mutant (blue), double positive (red) and wild-type (green).

Table S1. Description of the reagents loaded in 11 chambers on the disc

Chamber	Reagents	Reagent volume (µl)
C1	Blood	3000
C2	Proteinase K	100
C3	Lysis buffer	800
C4	Binding buffer (BB)	1800
C5	Silica beads	400 mg
C6	Waste storage	5800
C7	Washing buffer 1 (WB1)	600
C8	Washing buffer 2 (WB2)	750
C9	Ethanol	750
C10	Elution buffer	200
C11	Eluent	200

Table S2. Operation steps for fully automated cfDNA purification

Step		Spin speed (rpm)	Time (s)	Operation
1	Plasma separation	3600	300	Plasma separation from whole blood
2		2400	20	Open valve #1 and #2 to transfer the plasma sample (C1) to lysis buffer chamber(C3) through the chamber containing proteinase K (C2)
3		Mix	20	Mix plasma sample with lysis buffer
4	Lyse	*	300	Incubate at 60 °C
5		2400	10	Open valve #3 to transfer the binding buffer (C4) into lysis buffer chamber (C3)
6		Mix**	300	Mix lysate with binding buffer (BB) and incubate at RT
7		2400	10	Open valve #4 to transfer the lysate-BB mixture into silica beads (C5)
8		Mix	60	Mix the lysate-BB mixture with silica beads
9	Bind	2400	10	Close valve #4 and open valve #5 to remove the lysate-BB mixture to waste chamber (C6) Repeat step 7 ~ 9 for 6 times
10		2400	10	Close valve #5 and open valve #6 to transfer washing buffer 1 (WB1, C7) into beads chamber (C5)
11		Mix	10	Mix beads with WB1
12		2400	10	Open valve #5 to transfer WB1 into waste chamber (C6)
13		2400	10	Close valve #5 and open valve #7 to transfer washing buffer 2 (WB2, C8) into beads chamber (C5)
14	Wash	Mix	10	Mix beads with WB2
15		2400	10	Open valve #5 to transfer WB2 into waste chamber (C6)
16		2400	10	Close valve #5 and open valve #8 to transfer ethanol (C9) into beads chamber (C5)
17		Mix	10	Mix beads with ethanol
18		3600	180	Open valve #5 to transfer ethanol into waste chamber (C6) and dry the beads
19		2400	10	Close valve#5 and open valve #9 to transfer elution buffer (EB, C10) into beads chamber (C5)
20	Elute	Mix	60	Mix beads with EB
21		3600	10	Open valve #10 to transfer eluted DNA sample into eluent chamber (C11)
		< 30 min		

* The temperature of the stage beneath the disc were increased to 60 °C.

* Mix is the spin program with acceleration rate of 30 rad/s^2 as shown in Figure S3.

Table S3. Comparison between disc and manual protocol

	Disc			Commercial kit (QIAamp Circulating Nucleic Acid Kit, Qiagen).		
Step	**Operation**	**Time (min)**	**Reagent: volume**	**Operation**	**Time (min)**	**Reagent: volume**
Plasma separation	Spin (3600 rpm)	5	Plasma: 1 mL	Centrifuge (1,900 g, 16,000g, 4 °C)	20	Plasma: 1 mL
Lyse	Spin (2400 rpm) Mix, & Incubation (60 °C, 5 min)	10.8	Proteinase K: 0.1 mL Lysis buffer: 0.8 mL	Pipetting & Incubation (60 °C, 30 min)	31	Proteinase K: 0.1 mL Lysis buffer: 0.8 mL
Bind	Spin (2400 rpm) & Mix	8	Binding buffer: 1.8mL	Pipetting & Incubation (on ice, 5 min) & Centrifuge (10,000 g)	5.5	Binding buffer: 1.8mL
Wash	Spin (2400 rpm, 3600 rpm) & Mix	4.3	Washing buffer 1 (ACW1): 0.6 mL Washing buffer 2 (ACW2): 0.75 mL EtOH: 0.75 mL	Centrifuge (6,000 g), Dry at 56 °C for 10min	20	Washing buffer 1 (ACW1): 0.6 mL Washing buffer 2 (ACW2): 0.75 mL EtOH: 0.75 mL
Elute	Spin (2400 rpm) & Mix	1.3	Elution buffer: 0.2 mL	Centrifuge (20,000 g)	1	Elution buffer: 0.2 mL
		29.4 min			**77.5 min**	

Table S4. Characteristics and clinical data of patients

Pt ID	Disease	Sex	Age	Stage	TNM	EGFR mutation	TKI type (first trial)	Metastasis localization
LP5	ADC	M	49	IV	T1bN1M1a	L858R	erlotinib	pleura
LP6	ADC*	M	60	IV	TxNxM1b	L858R	erlotinib	bone
LP13	ADC	F	54	IV	T4N0M1b	L858R	afatinib	brain, pleura
LP16	ADC	F	50	IV	T4N2M1b	L858R	afatinib	omentum, pleura, lung to lung
LP36	ADC	M	66	IV	T2aN0M1b	L858R	afatinib	brain, bone, lung to lung, pleura, abdominal lymph node
LP37	ADC	M	53	IV	T3N3M1b	L858R	afatinib	bone, liver
LP42	ADC	F	79	IV	T4N3M1b	L858R	**	brain, bone, pleura, adrenal gland
LP46	ADC	F	68	IV	T2aN3M1b	L858R	afatinib	brain, bone, pleura, lung to lung, adrenal gland
LP47	ADC	F	83	IV	T2N3M1b	L858R	gefitinib	bone, pleura
LP49#	SCC	F	65	IIIA	T4N0M0	L858R	erlotinib	no

ADC: Adenocarcinoma, **SCC:** Squamous cell carcinoma,
*** Recurrence, **Drop out, #Serial monitoring**