Electronic Supplementary Information

Fast magnetic isolation of simple sequence repeat markers in microfluidic channels

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Supplementary figures and tables

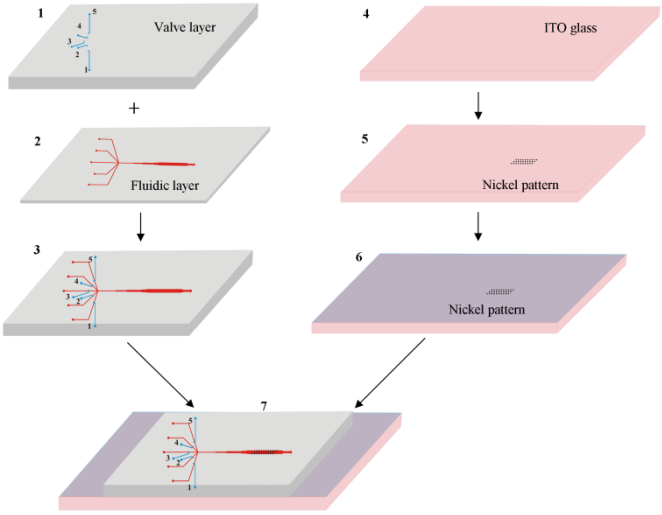

Figure S1. The MFCM-Chip device fabrication process: 1. Valve control channel fabrication. 2. Fluidic channel fabrication. 3. The valve and fluidic layers were bound together to form a "push-down" valve-controllable structure. 4. ITO glass. 5. Nickel pattern fabrication process. 6. PDMS-encapsulated nickel pattern. 7. Bonding of the nickel pattern layer and upper layers to generate the integrated magnetic field controllable microfluidic chip (MFCM-Chip).

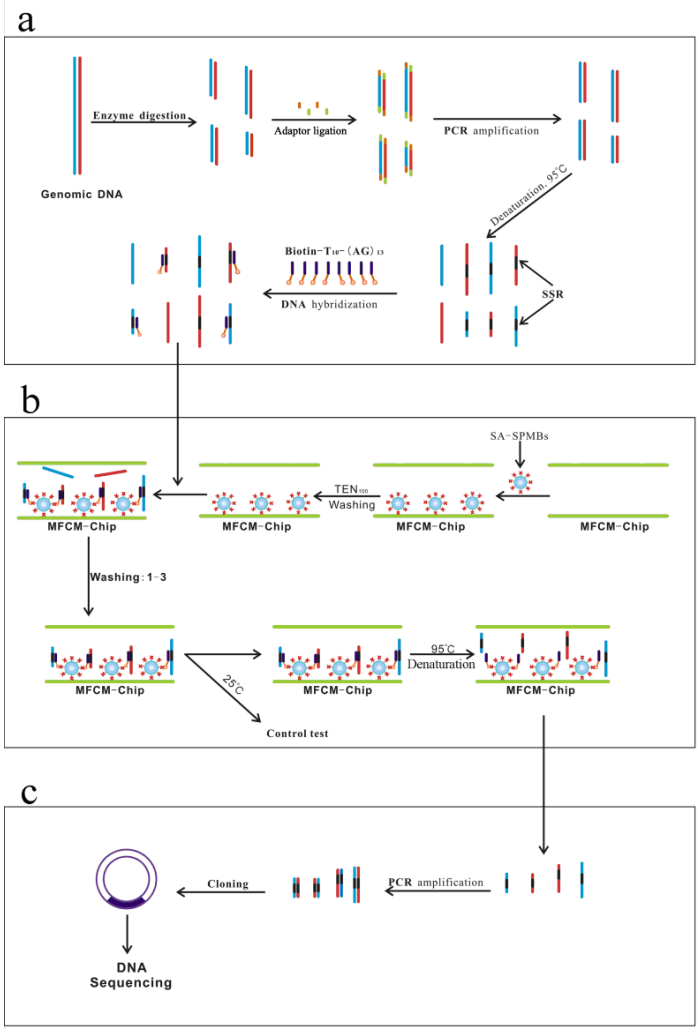

Figure S2. The SPMB-based FIASCO method in an MFCM-Chip. a) Preparation of DNA-probe complexes. The genomic DNA was digested with a restriction enzyme and ligated to adaptors. The ligation mixture was then amplified, and the PCR products were selectively hybridized to biotin-T_{10}-$(AG)_{13}$. b) The magnetic separation of SSR-containing fragments in the MFCM-Chip. The SA-SPMBs were loaded into the MFCM-Chip to generate the SA-SPMB patterns and were washed with TEN_{100}. The DNA-probe complexes were then loaded into the MFCM-Chip and incubated with SA-SPMBs, followed by washing with TEN_{1000}, 0.2×SSC, 0.1% SDS, and TE (1-3). Finally, the DNA-probe complexes were washed with TE at 95 °C, and the SSR-containing fragments were separated from the biotin probe and collected. c) The collected fragments were amplified, cloned, and sequenced.

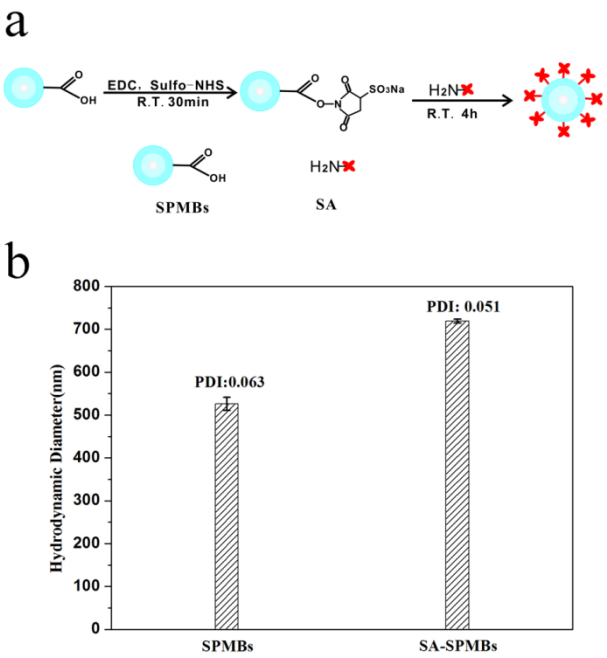

Figure S3. Preparation and characterization of SA-SPMBs. a) Scheme of preparation of SA-SPMBs. The carboxyl groups of the SPMBs were activated with EDC and sulfo-NHS, and the SPMBs were coupled with SA by the amino group and carboxyl group. b) Dynamic light scattering (DLS) measurements of the hydrodynamic diameters of the SPMBs and SA-SPMBs. The error bars represent the standard deviations from multiple trials.

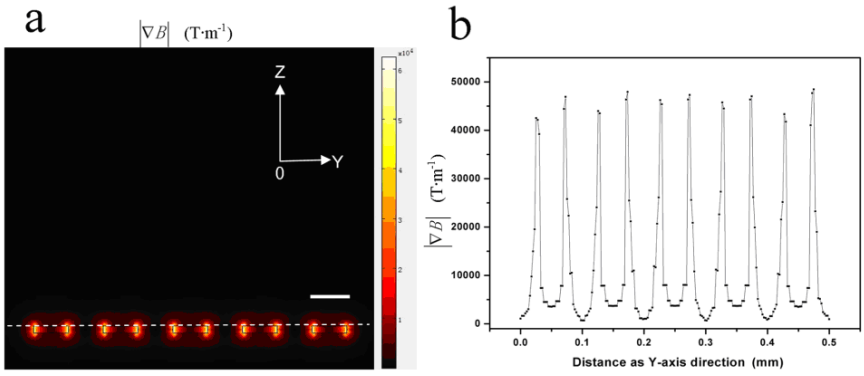

Figure S4. Simulation of the magnetic field gradient ($|\nabla B|$) at the nickel pattern area. a) Simulated absolute value of the magnetic field gradient in the ZY-axis. High magnetic field gradients were induced around the nickel pattern arrays. b) The absolute value of the magnetic field gradients along the white dotted line in (a). The maximum value of ($|\nabla B|$) reached 5×10^4 T·m^{-1}.

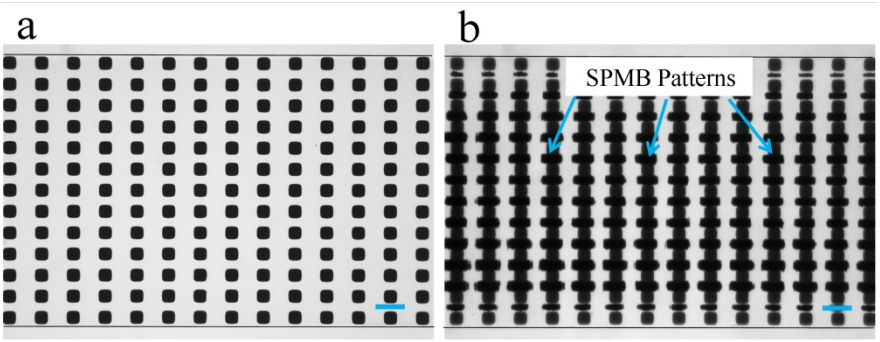

Figure S5. Generation of SA-SPMB patterns. a) Image of the nickel pattern under the fluidic channel. b) Capture of SA-SPMBs at the nickel pattern area and generation of SA-SPMB patterns. Scale bars are 100 μm.

Table S1. Comparison of the conventional FIASCO method and the SPMB-based FIASCO method in an MFCM-Chip.

Step No.	Name of the step	Conventional FIASCO method		SPMB-based FIASCO method in an MFCM-Chip		
		Vol	Time	Vol	Flow rate	Time
1	Generation of SA-SPMB patterns	NA		<0.1 mg magnetic beads	20 μL/min	5 min
2	Washing SA-SPMBs with TEN_{100}	300 μL×3 (1 mg magnetic beads)	5 min×3	40 μL	20 μL/min	2 min
3	DNA-probe complex	100 μL (1000 ng DNA, 250 nM probe)	NA	5 μL (50 ng DNA, 25 nM probe)	5 μL/min	1 min
4	Incubation	NA	30 min	NA	No flow	2 min 30 s
5	Non-stringent washing with TEN_{1000}	400 μL×3	5 min×3	40 μL	20 μL/min	2 min
6	Stringent washing with 0.2×SSC+0.1% SDS	400 μL×3	5 min×3	40 μL	20 μL/min	2 min
7	Washing with TE	400 μL×2	2 min×2	10 μL	20 μL/min	30 s
8	Denaturation at 95 ºC	100 μL	5 min	25 μL	5 μL/min	5 min
Total reagent volume and time		4300 μL solution and 1mg magnetic beads	84 min	160 μL solution and <0.1mg magnetic beads	NA	20 min

Table 2. Characteristics of the clones containing SSRs isolated from Herba Leonuri. The positive clones are arrayed from 1-24 in the vertical column, and the numbers in the horizontal column represent the number of repeats.

Clone	Number of repeats													Total
No.	3	4	5	6	7	8	9	10	11	12	13	14	15	
1	1													1
2	1	1		1	1									4
3	1	1		1	1									4
4	1	1		1	2									5
5		3			1									4
6	1		1										1	3
7	1	1			2									4
8	2													2
9	1	1			2									4
10		2												2
11		2												2
12	1	1		1	1									4
13				2										2
14				1	1									2
15		1												1
16	1		1											2
17		2												2
18		2												2
19	1	1		1				1						4
20												1		1
21	2													2
22	1		1											2
23	1		1		1									3
24	1	1		1				1						4

Supplementary methods

***MFCM-Chip design and fabrication*:** The MFCM-Chip was fabricated using the electroplate process and soft lithography methods [1, 2] as shown in Figure S1. First, a square-profile channel mould was fabricated using a 40-μm-thick positive photoresist (AZ50XT, AZ Electronic Materials USA Corp., NJ, USA) to form the valve control channel (Fig. S1(1)). Then, a poly(dimethylsiloxane) prepolymer (PDMS, GE Toshiba Silicones Co. Ltd., Tokyo, Japan, RTV615A: RTV615B = 10:1 (w/w)) was poured on the channel pattern. After baking in an oven (75 °C, 4 h), the solid PDMS was peeled off from the mould, and inlets were punched to allow gas to enter. The fluidic channel was fabricated using the same process (Fig. S1(2)). A 40-μm positive photoresist mould was baked on a 105 °C hot plate for 5 min to obtain a round-profile channel. Then, the bubble-free PDMS prepolymer (RTV615A: RTV615B = 15:1 (w/w)) was spin-coated on the photoresist mould at a speed of 1250 rpm/min to fabricate the fluidic channel. The PDMS encapsulated mould was placed in a 75 °C oven for 30 min to bake. The valve control layer and fluidic layer were then bound to gether after being treated with O_2 plasma (Fig. S1(3)). The two layers were baked in the oven for an additional 30 min. The two layers were then peeled off from the photoresist mould, and inlets and outlets were punched for fluids.

The fabrication of the nickel pattern on the indium tin oxide (ITO) glass (Yuguang Co., Hangzhou, China, Fig.S1(4)) was performed as previously described [3, 4]. In brief, a AZ9260 photoresist (AZ Electronic Materials USA Corp, NJ, USA) was used to fabricate the mould, followed by an electroplating process to fabricate the nickel pattern (13 rows ×81 columns, 10 μm high, Fig. S1(5)). After the lift-off process, the nickel pattern was encapsulated in a layer of thin PDMS (Fig. S1(6)), which was spin-coated at a speed of 4500 rpm/min. The valve control layer, the fluidic layer, and the PDMS-encapsulated nickel pattern layer were then bound together to obtain the integrated MFCM-Chip (Fig. S1(7)).

***Preparation and characterization of streptavidin-coated superparamagnetic beads*:** The superparamagnetic beads (SPMBs) with carboxyl groups (500 nm in diameter, 02150, Ademtech SA, Pessac, France) were conjugated with streptavidin (SA, AMERCO, Nevada, USA) according to the reported method [4]. The SPMBs were mixed with N-ethyl-N'-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and N-hydroxysuccinimidesodium salt (sulfo-NHS) dissolved in a 2-(N-morpholino) ethanesulphonicacid hydrate (MES) buffer to activate the carboxyl groups at room temperature. SA was added to the activated SPMBs and allowed to react for 4 h. The SA-SPMBs were preserved in 1 × PBS containing 1% (w/v) BSA and 0.05% (w/v) NaN_3 at 4 ºC for future use. The size of the SPMBs and the SA-SPMB conjugates was measured by dynamic light scattering (DLS, Zetasizer Nano ZS90, Malvern InstrumentsLtd., Malvern, UK).

Preparation of DNA-probe complexes: Genomic DNA was extracted from young Herba Leonuri leaves by the cetyltrimethylammnonium bromide (CTAB) method [5]. According to the conventional FIASCO procedure [6], the extracted genomic DNA was digested with the restriction enzyme *Mse*I (New England BioLabs, MA, USA), and fragments ranging from 200 to 800 bp were ligated to adaptors (*Mse*I A: 5'-TACTCAGGACTCAT-3', *Mse*I B: 5'-GACGATGAGTCCTGAG-3') using T4 DNA ligase (New England BioLabs, MA, USA). The ligation mixture was then amplified with specific primer (*Mse*I-N: 5'-GATGAGTCCTGAGTAAN-3'), and the PCR conditions were as follows: 1) 94 °C for 4 min; 2) 20 cycles of 94 °C for 30 s, 53 °C for 30 s, and 72 °C for 1 min; and 3) 72 °C for 10 min. The amplified DNA was analyzed by electrophoresis and hybridized to a biotin-T_{10}-$(AG)_{13}$ probe. The adaptors, primers, and probes were synthesized by Sangon Biochemistry Company (Shanghai, China).

***Cloning, sequencing, and SSR analysis*:** The DNA that was separated from the bead-probe complexes in the MFCM-Chip was precipitated with ethanol, re-suspended in ddH_2O, and amplified with primer (MseI-N: 5'-GATGAGTCCTGAGTAAN-3'). The PCR products were purified with an AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, CA, USA) and cloned into the Promega pGEM-T vector (Promega Corp., WI, USA). The clones were sequenced at GENEWIZ Inc. (Beijing, China), and the sequences were searched to determine the distribution and frequency of SSRs using an online tool (http://gramene.org/db/markers/ssrtool).

References

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