A universal genotyping-microarray constructed by ligating a universal fluorescence-probe with SNP-encoded flaps cleaved from multiplex invasive reaction

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Experimental section

Reagents and materials

Aldehyde coated glass slides and spotting buffer were purchased from CapitalBio Corporation (Beijing, China). TIANamp Blood DNA Kit was purchased from Tiangen Biotech, Co, Ltd (Beijing, China). *rTaq* DNA polymerase was purchased from Takara Biomedical Technology (Dalian, China). QIAGEN Multiplex PCR Kit was purchased from QIAGEN (Düsseldorf, Germany). Ampligase DNA Ligase were purchased from Epicentre Biotechnologies (Chicago,USA). *Archaeoglobus fulgidus* (*Afu*) flap endonuclease was prepared in our laboratory [1, 2].

Sample collection and DNA extraction

Twenty healthy volunteers were recruited from our lab. Written informed consent was obtained from each person. Genomic DNA was extracted from peripheral blood samples following anticoagulation with EDTA using TIANamp Blood DNA Kit according to the manufacturer's protocol.

Multiplex PCR

14-plex multiplex PCR were performed in 10-μl reaction mixtures containing 2×Multiplex PCR Master Mix, 0.2 μM each primer, 50 ng of genomic DNA, and H_2O was added up to 10 μl. Amplification was achieved using the T100™ Thermal Cycler (Bio-Rad Laboratories, Inc., California, USA) with the following conditions: 5 min at 95 °C, followed by 25 cycles of 30 s at 95 °C, 30 s at 55 °C, and 30 s at 60 °C, then a final extension at 72 °C for 7 min.

Multiplex invasive reaction

14-plex multiplex invasive reaction were carried out in 10 μl reaction mixtures containing 10 mM MOPS (pH 7.5)，0.05% Tween 20, 0.05% Nonidet P 40, 12 ng/μl BSA, 3.5% PEG 8000, 7.5 mM Mg^{2+}，200 nM each upstream probe (UP), 20 nM each downstream probe (DP), 70 ng of *Afu* flap endonuclease, 1 μl of multiplex PCR

products, and H_2O was added up to 10 μl. This mixture was incubated at 94 °C for 3 min and then incubated at 63 °C for 2 h.

SNP typing by the proposed universal genotyping-microarray

Design and synthesis of flaps, oligonucleotide, and invasive probes

The flaps on the DPs were allele-specific and used to encode allele species. After invasive reaction, the cleaved flaps were hybridized with oligonucleotides immobilized onto the microarray surface with a preset address. In this study, 34 flap sequences were selected in a way to minimize secondary structures, and their Tm values were strictly controlled in a range of 28-37 °C with 45-65% GC%. The Tm values of the flaps were calculated by OligoAnalyzer 3.1 (Integrated DNA Technologies, Inc., Iowa, USA) at the condition of 0.0002 μM flaps，7.5 mM Na^+, 7.5 mM Mg^{2+}, 0 mM dNTPs. The oligonucleotides immobilized onto the microarray surface are designed to contain three parts, termed as "spacer" (10-mer poly T), "identifier" to capture flaps, and "reporter" to capture the universal tag (UT). All of the oligonucleotides were modified in 3' C6 terminus with an amino group, which was used to react with aldehyde coated on glass slides. Invader upstream probes (UP) and downstream probes (DP) for each SNP were designed by Universal Invader™ Design Software Version 1.2.4 (Third Wave Technologies, Inc., Wisconsin, USA). Then the flaps on the DPs were replaced by the designed allele-specific flaps. All of the flaps, primers, oligonucleotide and probes were synthesized and purified by Invitrogen Co. (Shanghai, China), and the sequences were listed in Table S1 and Table S3.

Microarray construction

NH_2-modified oligonucleotides and spotting buffer were equally mixed to obtain 30 μM spotting mixture. Then the mixture were spotted onto the 75-mm×25-mm aldehyde-coated glass slides using the PersonalArrayer™ 16 (CapitalBio Corporation, Beijing, China) following the manufacturer's protocol. The spotting process was performed at 25 °C and 60% humidity, and all oligonucleotides were spotted in

triplicate on the microarray. The arrays were kept at 25 °C in slide boxes before use. The arrays were incubated at 37 °C for 12 h in humid chambers to immobilize the oligonucleotides onto the slides. Then the uncoupled oligonucleotides were washed twice in 0.2% SDS for 5 min, followed by three additional washes in distilled water for 1 min. After drying, the slides were soaked in 250 ml of sodium borohydride solution (0.625 g of $NaBH_4$, 187.5ml of PBS and 62.5mL of ethanol) for 5 min. With two final washes in distilled water for 1.5 min, the slides were dried and labelled for using. All of the washing steps were carried out on the shaker at 80 rpm.

Hybridization, ligation and washing

The hybridization and ligation was simultaneously carried out in the same reaction solution. A final volume of 90 μl of reaction solution was prepared by adding 500 nM cy3 UT, 1×Ampligase buffer, 0.05×Standard Saline Citrate (SSC), 5×Denhardt solution, 5 U/40 μl Ampligase DNA Ligase, 10 μl of invasive reaction solution, and H_2O was added up to 90 μl. The hybridization-ligation reaction solution was added to the array on the slides. Then the slides were placed into a humid hybridization chamber (CapitalBio Corporation, Beijing, China). The hybridization-ligation reaction was carried out in the dark at 42 °C for 2 h in a 9105 Refrigerated Circulator (PolyScience Inc, Niles, Illinois, USA). After removal of the chamber, the slides were washed twice with pre warmed (about 42 °C) washing buffer I (0.3×SSC，0.1% SDS) for 3 min. Then the slides were further washed twice with pre-warmed (about 42 °C) washing buffer II (0.06×SSC) for 3 min. All of the washing steps were carried out on the shaker at 80 rpm.

Scanning and data-analysis

The dried slides were scanned with a LuxScan 10K Microarray Scanner (CapitalBio Corporation, Beijing, China). The fluorescent signals for Cy3 dye were detected at 532 nm, a photomultiplier tube (PMT) of 95%, a PMT Gain of 600, and a scan resolution of 10 μm. LuxScan 3.0 software (CapitalBio Corporation, Beijing, China) was used to measure the signal intensity and local background from each spot

on the microarray. Each spot's signal was normalized by subtracting the local background from the observed signal. A spot was defined as a positive signal only if fluorescence intensity is larger than 1000. When a sample was detected in more than one spot, the average values of these replicated spots were used as the signal value. Each SNP has two alleles, and a ratio of signal intensity between two alleles can be readily obtained. When the ratio was >2.5 or <0.4, the SNP was a homozygote, and when the ratio was among 0.4-2.5, the SNP was a heterozygote.

SNP typing by Sanger sequencing

Samples were also genotyped by Sanger sequencing for the comparisons with the results of microarray. The PCR was performed in a volume of 50 μl, containing 1× PCR buffer, 0.2 mM each dNTP, 2.0 mM $MgCl_2$, 0.3 μM each of primers, 1.25 U of *rTaq* DNA polymerase, 50 ng of genomic DNA, and H_2O was added up to 50 μl. Amplification was performed on the T100™ Thermal Cycler with the following conditions: 5 min at 95 °C, followed by 35 cycles of 30 s at 95 °C, 30 s at 55 °C, and 30 s at 72 °C, then a final extension at 72 °C for 7 min. The PCR products were sequenced by Beijing Genomics Institute (ShenZhen, China). The sequencing data were analyzed by Chromas 2.6 (Technelysium Pty Ltd, South Brisbane, Australia).

Table S1. The sequences of multiplex PCR primers and multiplex invasive reaction probes.

NO.	Name	Sequence (5'-3')
14 SNPs related to personalized medicine		
	CYP2C9 *2-F	ATGACGCTGCGGAATTTT
	CYP2C9 *2-R	CACCCACCCTTGGTTTTTCT
SNP1	CYP2C9 *2-UP	GGGAAGAGGAGCATTGAGGACA
	CYP2C9 *2-DP-W	*GCAATTCCCTGC*GTGTTCAAGAGGAAGC
	CYP2C9 *2-d-DP-M	*GCGTCTGACAGAT*GTGTTCAAGAGGAAGC
	CYP2C9 *3-F	CGTGTGATTGGCAGAAACC
	CYP2C9 *3-R	GGGGACTTCGAAAACATGG
SNP2	CYP2C9 *3-UP	GTGCACGAGGTCCAGAGATACT
	CYP2C9 *3-DP-W	*AGAGTCCACACA*TTGACCTTCTCCCCA
	CYP2C9 *3- DP-M	*CTAACGGTCAAC*TTGACCTTCTCCCCA
	VKORC1-F	GGGAAGTCAAGCAAGAGAAGACCT
	VKORC1-R	GCCTCCCAAAATGCTAGGATTAT
SNP3	VKORC1-UP	CGTGAGCCACCGCACCA
	VKORC1-DP-W	*ACCAGTTAGTGC*GGCCAATGGTTGT
	VKORC1-DP-M	*GATGAGACCGGAT*GGCCAATGGTTGTTT
	CYP2C19*2-F	CCAGAGCTTGGCATATTGTATCTA
	CYP2C19*2-R	CGCAAGCAGTCACATAACTAAGC
SNP4	CYP2C19*2-UP	CAAGGTTTTTAAGTAATTTGTTATGGGTTCCA
	CYP2C19*2-DP-W	*ACCATGTAGGAC*GGGAAATAATCAATGATAGTG
	CYP2C19*2-DP-M	*CACGGAGACCT*GGGAAATAATCAATGATAGTG
	CYP2C19*3-F	TGCAATGTGATCTGCTCCATTAT
SNP5	CYP2C19*3-R	AGCAAAAAACTTGGCCTTACCTG
	CYP2C19*3-DP-W	*GCTTTAGGAACG*ATCCAGGTAAGGCCA
	CYP2C19*3-DP-M	*CGCAAGTCCTA*ATCCAGGTAAGGCCA
	CYP19A1790-F	TTTTTCCAGCAAGGATTTGA
	CYP19A1790-R	ATGGGAATTACAGTTAGTTCAGGT
SNP6	CYP19A1790-UP	GTTTCTCTTCTGTGGAAATCCTGCT
	CYP19A1790-DP-W	*GTAACTTGCACG*TCTTTTTTCTGCTATCAGAA
	CYP19A1790-DP-M	*GACAACTGAGCTA*TCTTTTTTCTGCTATCAGAAC
	CYP19A1 1091-F	TTCTTTGTTCCTTTTATCTGTTTC
SNP7	CYP19A1 1091-R	AGGATAATGTTTGTCCCCTTT
	CYP19A1 1091-UP	GTGATGGAAAACTTCATTTATGAGAGCAA

	CYP19A1 1091-DP-W	*CGTTCGTCACAT*GCGGTACCAGCC
	CYP19A1 1091-DP-M	*AGTCCAACTGATC*GCGGTACCAGC
	CYP19A1 115-F	CCCTCTGAGGTCAAGGAAC
	CYP19A1 115-R	CAAAATCCCAAGTAAATAATCTC
SNP8	CYP19A1 115-UP	GCTCCTCACTGGCCTTTTTCTCA
	CYP19A1 115-DP-W	*CGATCTGGCTT*TGGTGTGGAATTATGAGG
	CYP19A1 115-DP-M	*GCACTTTGCAC*TGGTGTGGAATTATGAGG
	CDA*3-F	GGGGCAAACTTCTTACTCAA
	CDA*3-R	AACCTGGCTTTCCCACTCA
SNP9	CDA*3-UP	CTGAGACGGCCTTCTGGATAGA
	CDA*3-DP-W	*AACGACTCTACC*GGTCCGTTCAGC
	CDA*3-DP-M	*GTGACCAGTAGT*GGTCCGTTCAGCA
	CDA79-F	GGGGCAAACTTCTTACTCAA
	CDA79-R	AACCTGGCTTTCCCACTCA
SNP10	CDA79-UP	GCTCCCAGGAGGCCAAGT
	CDA79-DP-W	*AAGCGCGGTA*AGTCAGCCTACTGC
	CDA79-DP-M	*CATAACGGCGTC*AGTCAGCCTACTGC
	DPYD-F	ACGGCTGCATATTGGTGT
	DPYD-R	GCATCAGCAAAGCAACTGG
SNP11	DPYD-UP	ACTAAAGGCTGACTTTCCAGACAACT
	DPYD-DP-W	*GTCACAGGCAG*TAAGTGTGATTTAACATCTAAAAC
	DPYD-DP-M	*CGACACATGACA*TAAGTGTGATTTAACATCTAAAACA
	ALDH2-F	CCTTTGGTGGCTACAAGATGTCG
	ALDH2-R	CCCCCAACAGACCCCAATC
SNP12	ALDH2-UP	GGGCTGCAGGCATACACTT
	ALDH2-DP-W	*GCACACACGG*AAGTGAAAACTGTGAGTG
	ALDH2-DP-M	*TCAACAGACGGA*AAGTGAAAACTGTGAGTG
	MTHFR677-F	GACTGTCATCCCTATTGGCAGGTT
	MTHFR677-R	CCCTCACCTGGATGGGAAAGA
SNP13	MTHFR677-UP	AAAGCTGCGTGATGATGAAATCGT
	MTHFR677-DP-W	*CAGGTTGATAACG*CTCCCGCAGACA
	MTHFR677-DP-M	*ACAAGGCACGA*CTCCCGCAGACAC
	MDR1 2667-F	AATAGCAGGAGTTGTTGAAATGAA
	MDR12667-R	TCCAAGAACTGGCTTTGCTACT
SNP14	MDR1 2667-UP	GCACTGAAAGATAAGAAAGAACTAGAAGGTC
	MDR1 2667-DP-W	*GATTAAGGTCCAG*CTGGGAAGGTGAGT
	MDR1 2667-DP-M	*CTTGACGAACGT*CTGGGAAGGTGAGTC

14 SNPs in the *BRCA* gene

	BRCA1 188-F	GCTCTTCGCGTTGAAGAAGT
	BRCA1 188-R	TCCCAAATTAATACACTCTTGTGC
SNP1	BRCA1 188-UP	CAAAATGTCATTAATGCTATGCAGAAAATCTTAT
	BRCA1 188-DP-W	*GTAAAGCGCG*AGTGTCCCATCTGGTA
	BRCA1 188-DP-M	*GTCACATTCCAG*TGTCCCATCTGGTAAG

	BRCA1 IVS7-F	CATGGTGTCAAGTTTCTCTTCAGGA
	BRCA1 IVS7-R	GTATCCGCTGCTTTGTCCTCAGA
SNP2	BRCA1 IVS7-UP	CAGAACTGGCCAACAATTGCTTT
	BRCA1 IVS7-DP-W	*GTAACTTGCACG*ACTGTTCTTTACCATACTGT
	BRCA1 IVS7-DP-M	*ACAGCAGGCAG*ACCATACTGTTTAGCAGG
	BRCA1 589-F	CATGGTGTCAAGTTTCTCTTCAGGA
	BRCA1 589-R	GTATCCGCTGCTTTGTCCTCAGA
SNP3	BRCA1 589-UP	AAACCAGTCTCAGTGTCCAACTCC
	BRCA1 589-DP-W	*CGATCTGGCTT*CTAACCTTGGAACTGTG
	BRCA1 589-DP-M	*GTGACCAGTAGT*AACCTTGGAACTGTGAG
	BRCA2 6064-F	GGTTTTTGCTGACATTCAGAGTG
	BRCA2 6064-R	ACACTTGTCTTGCGTTTTGTAATG
SNP4	BRCA2 6064-UP	GAAGTTTCCAAACTAACATCACAAGGTT
	BRCA2 6064-DP-W	*GCACACACGG*TATATTTTAGAAACTTTCTCCAATC
	BRCA2 6064-DP-M	*GACGATTAGTGAG*ATATTTTAGAAACTTTCTCCAATC
	BRCA1 1081-F	TGTAATAAAAGCAAACAGCCTGG
	BRCA1 1081-R	CAGGGGATCAGCATTCAGATC
SNP5	BRCA1 1081-DP-UP	TTAGCAAGGAGCCAACATAACAGAC
	BRCA1 1081-DP-W	*CGTTCGTCACAT*GGGCTGGAAGTAAGG
	BRCA1 1081-DP-M	*CACGGAGACCT*GGCTGGAAGTAAGGA
	BRCA1 1100-F	TGTAATAAAAGCAAACAGCCTGG
	BRCA1 1100-R	CAGGGGATCAGCATTCAGATC
SNP6	BRCA1 1100-UP	CTGGGAGTCCGCCTATCATTAT
	BRCA1 1100-DP-W	*GCAATTCCCTGC*ATGTTTCCTTACTTCCAG
	BRCA1-1100-DP-M	*AGAACGAGGCAC*GTTTCCTTACTTCCAGC
	BRCA1 3232-F	GGGAAATGAGAACATTCCAAGTA
	BRCA1 3232- R	TTTTGGCCCTCTGTTTCTACCTA
SNP7	BRCA1 3232-UP	CTTCATTAATATTGCTTGAGCTGGCTA
	BRCA1 3232-DP-W	*GCGTCTGACAGAT*CTTTAAAAACATTTTCTCTAATGTTAT
	BRCA1 3232-DP-M	*CTAACGGTCAAC*CTTTAAAAACATTTTCTCTAATGTTA
	BRCA1 4446-F	ACAGCTACCCTTCCATCATAAGTGAC
	BRCA1 4446-R	AAGGGGAAGGAAAGAATTTTGCTTA
SNP8	BRCA1 4446-UP	TTCTGATGTGCTTTGTTCTGGATTTCT
	BRCA1 4446-DP-W	*CAGGTTGATAACG*CAGGTCCTCAAGG
	BRCA1 4446-DP-M	*TCAACAGACGGA*CAGGTCCTCAAGGG
	BRCA2 5803-F	CTGCATTTAGGATAGCCAGTGGT
	BRCA2 5803-R	ACCTTATGTGAATGCGTGCTAC
SNP9	BRCA2 5803-UP	TGAAACTGTCTGTAAATATGTCTTTCACTTTTC
	BRCA2 5803-DP-W	*GATGAGACCGGAT*TAATTGTTTCATGTGAAACAC
	BRCA2 5803-DP-M	*CTTGACGAACGT*TGTTTCATGTGAAACACAA
	BRCA1 5589-F	GACCCTGGAGTCGATTGATTAGAG
SNP10	BRCA1 5589-R	GGGATCTGGGGTATCAGGTAGG
	BRCA1 5589-UP	GCTTGTGTTCTCTGTCTCCAGCT
	BRCA1 5589-DP-W	*AAGCGCGGTA*ATTGGGCAGATGTGT

	BRCA1 5589-DP-M	*CGCAAGTCCTA*GATGTGTGAGGCAC
	BRCA1 5640-F	GACCCTGGAGTCGATTGATTAGAG
	BRCA1 5640-R	GGGATCTGGGGTATCAGGTAGG
SNP11	BRCA1 5640-UP	TGGCACTGGTAGAGTGCTACAT
	BRCA1 5640-DP-W	*CATAACGGCGTC*TGTCCAACACCCAC
	BRCA1 5640-DP-M	*AGTCCAACTGATC*GTCCAACACCCAC
	BRCA2 1342-F	GCAAACGCTGATGAATGTGAA
	BRCA2 1342-R	GCCAAAGACGGTACAACTTCCT
SNP12	BRCA2 1342-UP	TGATACTGATCCATTAGATTCAAATGTAGCAT
	BRCA2 1342-DP-W	*GACAACTGAGCTA*ATCAGAAGCCCTTTGAG
	BRCA21342-DP-M	*AACGACTCTACC*ATCAGAAGCCCTTTGA
	BRCA2 3109-F	AACCCATTTTCAAGAACTCTACCA
	BRCA2 3109-R	GCCCATTTGTTCATGTAATCATT
SNP13	BRCA2 3109-UP	ATTTTAAATCTTGACCTAGAGTCATTTTTATATGCTT
	BRCA2 3109-DP-W	*GTCACAGGCAG*CTTTACACTATTTTTGTTCTC
	BRCA2 3109-DP-M	*CGACACATGACA*CTTTACACTATTTTTGTTCTCC
	BRCA2 5911-F	CTGCATTTAGGATAGCCAGTGGT
	BRCA2 5911-R	ACCTTATGTGAATGCGTGCTAC
SNP14	BRCA2 5911-UP	AATATCCTCTGAATCATCCAATGCCTT
	BRCA2 5911-DP-W	*ACCAGTTAGTGC*GTAACAACCTGCCATA
	BRCA2 5911-DP-M	*GCTTTAGGAACG*GTAACAACCTGCCAT

Note: “F” means upstream primer used in PCR. “R” means downstream primer used in PCR. “DP” means downstream probe used in invasive reaction. “UP” means upstream probe used in invasive reaction. “W” means DP of allele 1 used in invasive reaction. “M” means DP of allele 2 used in invasive reaction. The sequences of flaps on the DPs were marked in italics.

Table S2. The clinical significance of the 14 SNPs related to personalized medicine and the 14 SNPs in the *BRCA* gene.

No.	Personalized medicine chip				*BRCA* chip			
	Gene	Accession number	Allele type	Clinical significance	Gene	Accession number	Allele type	Clinical significance
SNP1	*CYP2C9**2	NG_008385.1	C/T	Genetic factors were used to predict warfarin dose.	*BRCA*1	NG_005905.2	188delAG	Harmful mutation
SNP2	CYP2C9*3	NG_008385.1	A/C		*BRCA*1	NG_005905.2	IVS7-14del	Harmful mutation
SNP3	*VKORC1*	NG_011564.1	G/A		*BRCA*1	NG_005905.2	589delCT	Harmful mutation
SNP4	*CYP2C19**2	NG_008384.1	G/A	Mutant patients should increase the dosage of clopidogrel.	*BRCA*2	NG_012772.3	6064 ins A	Harmful mutation
SNP5	*CYP2C19**3	NG_008384.1	G/A		*BRCA*1	NG_005905.2	1081delG	Harmful mutation
SNP6	*CYP19A1* 790	NG_007982.1	C/T	Mutant patients have decreased aromatase activity; letrozole was preferable for these patients.	*BRCA*1	NG_005905.2	1100 del AT	Harmful mutation
SNP7	*CYP19A1* 1091	NG_007982.1	T/C		*BRCA*1	NG_005905.2	3232A〉G	Unknown meaning
SNP8	*CYP19A1* 115	NG_007982.1	T/C		*BRCA*1	NG_005905.2	4446C→T	Harmful mutation
SNP9	*CDA**3208	NC_000001.10	G/A	Mutant patients have decreased metabolic detoxification activity; side effects were increased when they use gemcitabine.	*BRCA*2	NG_012772.3	5803 del ATTA	Harmful mutation
SNP10	*CDA* 79	NC_000001.10	A/C		*BRCA*1	NG_005905.2	5589del 8	Harmful mutation
SNP11	*DPYD*IVS14+1	NG_008807.1	G/A	Mutant patients were poor metabolisms; they are not suited to use Fluorouracil.	*BRCA*1	NG_005905.2	5640delA	Harmful mutation
SNP12	*ALDH2*-504		G/A	Mutant patients should not drink.	*BRCA*2	NG_012772.3	1342C>A	Unknown meaning
SNP13	*MTHFR*677	NG_013351.1	C/T	Folic acid metabolism will be largely blocked in nutation patients.	*BRCA*2	NG_012772.3	3109C□T	Harmful mutation
SNP14	*MDR1*-2677	NG_011513.1	G/T/A	The mutations are associated with multidrug resistance.	*BRCA*2	NG_012772.3	5911G>C	Harmful mutation

Table S3. Sequences of UT, flaps, DPs and NH_2 modified oligonucleotides.

Name	Sequence (5'-3')	GC%	Tm (°C)
UT-9 bp	P-TATCCTTCC-cy3	44.4	31.0
UT-10 bp	P-TATCCTTCCC-cy3	50.0	38.8
UT-11 bp	P-TATCCTTCCCA-cy3	45.5	44.0
Oligo1-9bp UT	GGAAGGATATACCGCGCTTGGGACATGATTTTTTTTTTTTTT-NH_2		
Oligo1-10bp UT	GGGAAGGATATACCGCGCTTGGGACATGATTTTTTTTTTTTTT-NH_2		
Oligo1-11bp UT	TGGGAAGGATATACCGCGCTTGGGACATGATTTTTTTTTTTTTT-NH_2		
Flap1-7 bp	CGCGGTA	71.4	7.9
DP1-7 bp	CGCGGTAAGTCAGCCTACTGC		
Flap1-8 bp	GCGCGGTA	75	20.6
DP1-8 bp	GCGCGGTAAGTCAGCCTACTGC		
Flap1-10 bp	AAGCGCGGTA	60%	30.6
DP1-10 bp	AAGCGCGGTAAGTCAGCCTACTGC		
Flap1-12 bp	CCAAGCGCGGTA	66.7	40.8
DP1-12 bp	CCAAGCGCGGTAAGTCAGCCTACTGC		
Flap1-14 bp	TCCCAAGCGCGGTA	64.3	48.2
DP1-14bp	TCCCAAGCGCGGTAAGTCAGCCTACTGC		
Flap1-16 bp	TGTCCCAAGCGCGGTA	62.5	53.1
DP1-16 bp	TGTCCCAAGCGCGGTAAGTCAGCCTACTGC		
Flap1-20 bp	ATCATGTCCCAAGCGCGGTA	55.0	57.9
DP1-20 bp	ATCATGTCCCAAGCGCGGTAAGTCAGCCTACTGC		
UP1	GCTCCCAGGAGGCCAAGT		
Flap1	AAGCGCGGTA	60.0	30.6
Oligo1	*GGGAAGGATA*TACCGCGCTTGGGATTTTTTTTTTTT-NH_2		
Flap2	CATAACGGCGTC	58.3	35.7
Oligo2	*GGGAAGGATA*GACGCCGTTATGAGTTTTTTTTTTTT-NH_2		
Flap3	AGAACGAGGCAC	58.3	36.5
Oligo3	*GGGAAGGATA*GTGCCTCGTTCTCGTTTTTTTTTTTT-NH_2		
Flap4	GCGTCTGACAGAT	53.8	38.5
Oligo4	*GGGAAGGATA*ATCTGTCCGACGCGTTTTTTTTTTTT-NH_2		
Flap5	GTAAAGCGCG	60.0	28.2
Oligo5	*GGGAAGGATA*CGCGCTTTACTTTTTTTTTTTT-NH_2		
Flap6	CTAACGGTCAAC	50.0	31.2
Oligo6	*GGGAAGGATA*GTTGACCGTTAGTTATTTTTTTTTTTT-NH_2		
Flap7	CGTTCGTCACAT	50.0	34.6
Oligo7	*GGGAAGGATA*ATGTGACGAACGTATGTTTTTTTTTTT-NH_2		
Flap8	AGAGTCCACACA	50.0	33.2
Oligo8	*GGGAAGGATA*TGTGTGGACTCTCTCTTTTTTTTTTTT-NH_2		
Flap9	GTCACATTCCAG	50.0	30.7
Oligo9	*GGGAAGGATA*CTGGAATGTGACCGTTTTTTTTTTTTT-NH_2		
Flap10	ACCAGTTAGTGC	50.0	32.5
Oligo10	*GGGAAGGATA*GCACTAACTGGTCTGTTTTTTTTTTTT-NH_2		
Flap11	GACGATTAGTGAG	46.2	32.6
Oligo11	*GGGAAGGATA*CTCACTAATCGTCTGCTTTTTTTTTTTTT-NH_2		
Flap12	GATGAGACCGGAT	53.8	36.8
Oligo12	*GGGAAGGATA*ATCCGGTCTCATCGCTTTTTTTTTTTT-NH_2		
Flap13	CGCAAGTCCTA	54.5	29.5
Oligo13	*GGGAAGGATA*TAGGACTTGCGTCTCTTTTTTTTTTTT-NH_2		
Flap14	CACGGAGACCT	63.6	31.9
Oligo14	*GGGAAGGATA*AGGTCTCCGTGCGAATTTTTTTTTTTT-NH_2		
Flap15	GACAACTGAGCTA	46.2	34.4
Oligo15	*GGGAAGGATA*TAGCTCAGTTGTCCGTTTTTTTTTTTT-NH_2		
Flap16	ACCATGTAGGAC	50.0	30.7
Oligo16	*GGGAAGGATA*GTCCTACATGGTGCTTTTTTTTTTTTT-NH_2		
Flap17	GTCACAGGCAG	63.6	31.6
Oligo17	*GGGAAGGATA*CTGCCTGTGACTCGTTTTTTTTTTTTT-NH_2		
Flap18	GATTAAGGTCCAG	46.2	31.8
Oligo18	*GGGAAGGATA*CTGGACCTTAATCGTGTTTTTTTTTTTT-NH_2		
Flap19	CAGGTTGATAACG	46.2	33.8

Oligo19	*GGGAAGGATA*CGTTATCAACCTGGGTTTTTTTTTTTT-NH_2		
Flap20	GCACACACGG	70.0	30.8
Oligo20	*GGGAAGGATA*CCGTGTGTGCAGAGTTTTTTTTTTTT-NH_2		
Flap21	ACAGCAGGCAG	63.6	34.1
Oligo21	*GGGAAGGATA*CTGCCTGCTGTAGACTTTTTTTTTTTT-NH_2		
Flap22	CGACACATGACA	50.0	33.7
Oligo22	*GGGAAGGATA*TGTCATGTGTCGCACTTTTTTTTTTTT-NH_2		
Flap23	ACAAGGCACGA	54.5	33.1
Oligo23	*GGGAAGGATA*TCGTGCCTTGTCATTTTTTTTTTTTT-NH_2		
Flap24	GTAACTTGCACG	50.0	33.0
Oligo24	*GGGAAGGATA*CGTGCAAGTTACCGTTTTTTTTTTTT-NH_2		
Flap25	AGTCCAACTGATC	46.2	34.1
Oligo25	*GGGAAGGATA*GATCAGTTGGACTCGTTTTTTTTTTTT-NH_2		
Flap26	GCAATTCCCTGC	58.3	35.8
Oligo26	*GGGAAGGATA*GCAGGGAATTGCCGTTTTTTTTTTTT-NH_2		
Flap27	CTTGACGAACGT	50.0	34.5
Oligo27	*GGGAAGGATA*ACGTTCGTCAAGAGTTTTTTTTTTTTT-NH_2		
Flap28	GCTTTAGGAACG	50.0	31.7
Oligo28	*GGGAAGGATA*CGTTCCTAAAGCTGATTTTTTTTTTTT-NH_2		
Flap29	CGATCTGGCTT	54.5	30.0
Oligo29	*GGGAAGGATA*AAGCCAGATCGACCTTTTTTTTTTTT-NH_2		
Flap30	GCACTTTGCAC	54.5	31.4
Oligo30	*GGGAAGGATA*GTGCAAAGTGCCGTTTTTTTTTTTT-NH_2		
Flap31	TCAACAGACGGA	50.0	34.2
Oligo31	*GGGAAGGATA*TCCGTCTGTTGAGTTTTTTTTTTTTT-NH_2		
Flap32	AACGACTCTACC	50.0	31.3
Oligo32	*GGGAAGGATA*GGTAGAGTCGTTTTTTTTTTTTT-NH_2		
Flap33	GTGACCAGTAGT	50.0	30.9
Oligo33	*GGGAAGGATA*ACTACTGGTCACTTTTTTTTTTTT-NH_2		
Flap34	TGCCTATGACAG	50.0	31.6
Oligo34	*GGGAAGGATA*TGCCTATGACAGTTTTTTTTTTTT-NH_2		

Note: The sequences of "reporter" on the oligonucleotides were marked in italics. The sequences of "identifier" on the oligonucleotides were underlined. The Tm values of UT were calculated by OligoAnalyzer 3.1 (Integrated DNA Technologies, Inc., Iowa, USA) at the condition of 0.5 μM flaps，7.5 mM Na^+, 7.5 mM Mg^{2+} , 0 mM dNTPs. The Tm values of flaps were calculated by OligoAnalyzer 3.1 (Integrated DNA Technologies, Inc., Iowa, USA) at the condition of 0.0002 μM flaps，7.5 mM Na^+, 7.5 mM Mg^{2+} , 0 mM dNTPs.

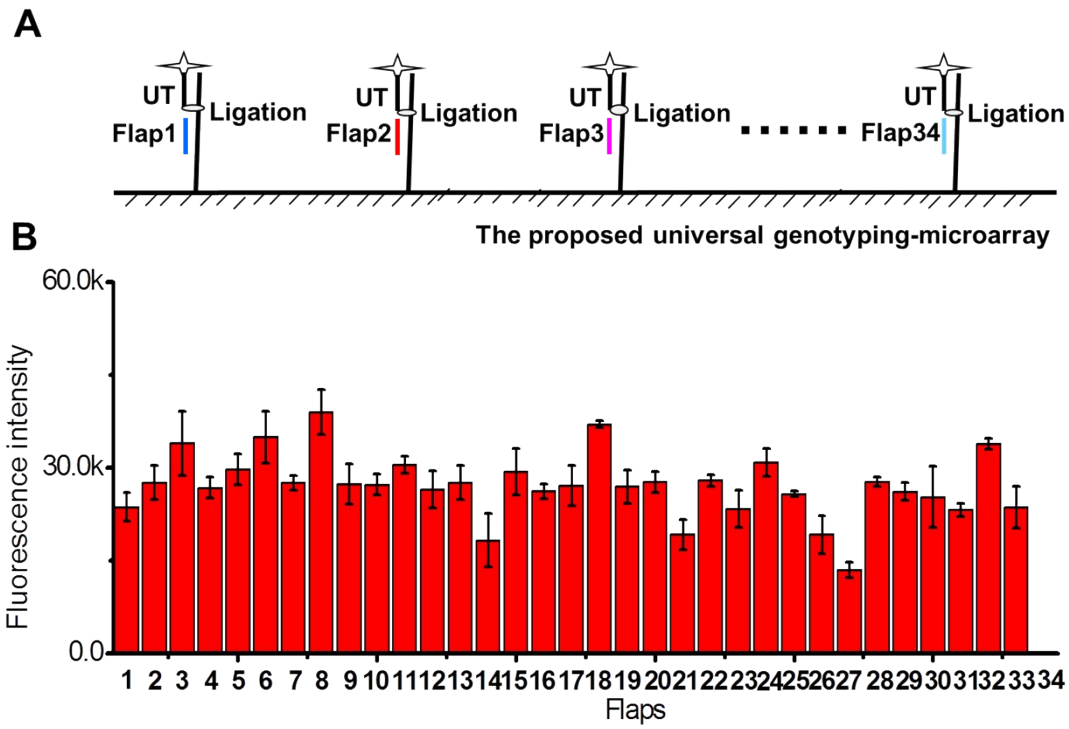

Fig. S1 34 equal amounts of flaps detected by the proposed universal genotyping-microarray through multiplex ligation reaction. (A) Schematic diagram of 34 flaps detected by the proposed universal genotyping-microarray through multiplex ligation reaction. (B) Fluorescence intensities of 34 flaps detected by the proposed universal genotyping-microarray through multiplex ligation reaction. Each sample was detected in triplicate.

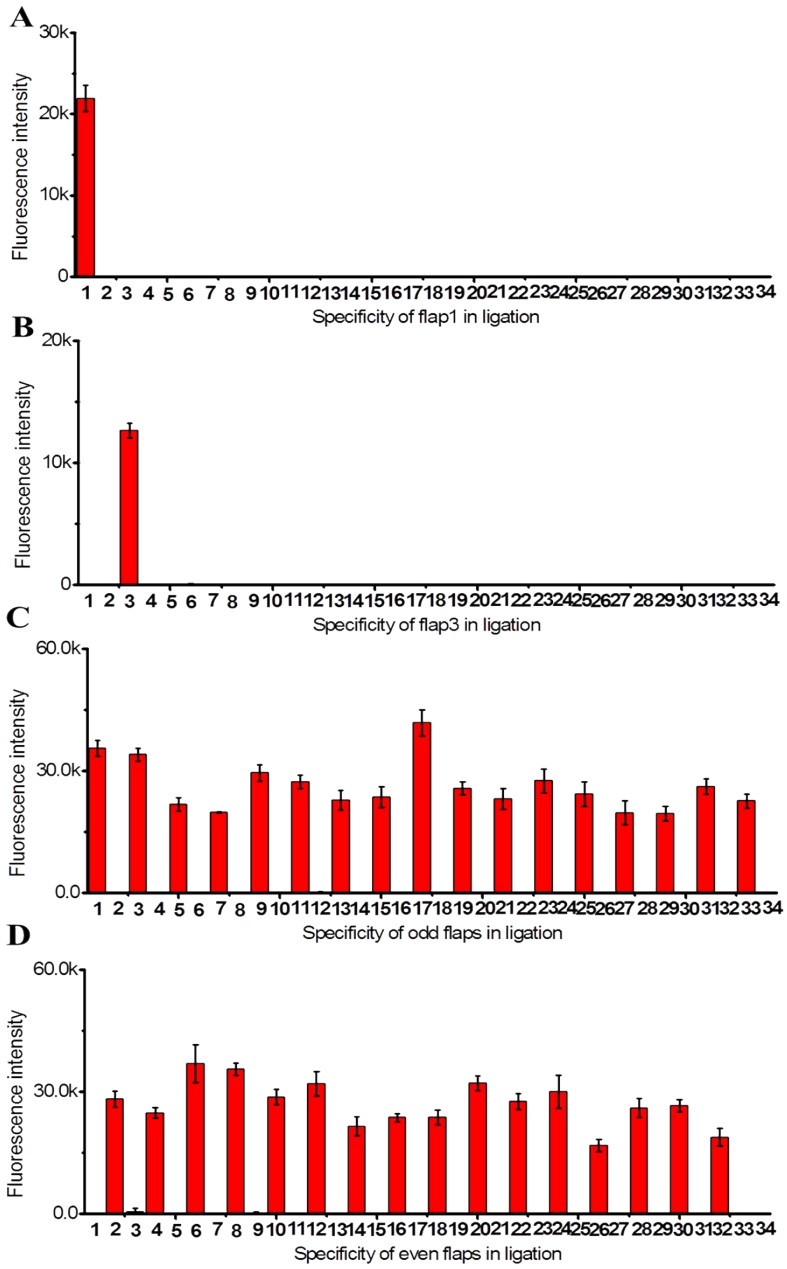

Fig. S2 The specificity of the multiplex ligation reaction on the proposed universal genotyping-microarray. (A) One flap (Flap1) detected by the proposed universal genotyping-microarray.(B) One flap (Flap3) detected by the proposed universal genotyping-microarray.(C) Odd flaps detected by the proposed universal genotyping-microarray.(D) Even flaps detected by the proposed universal genotyping-microarray. Each sample was detected in triplicate.

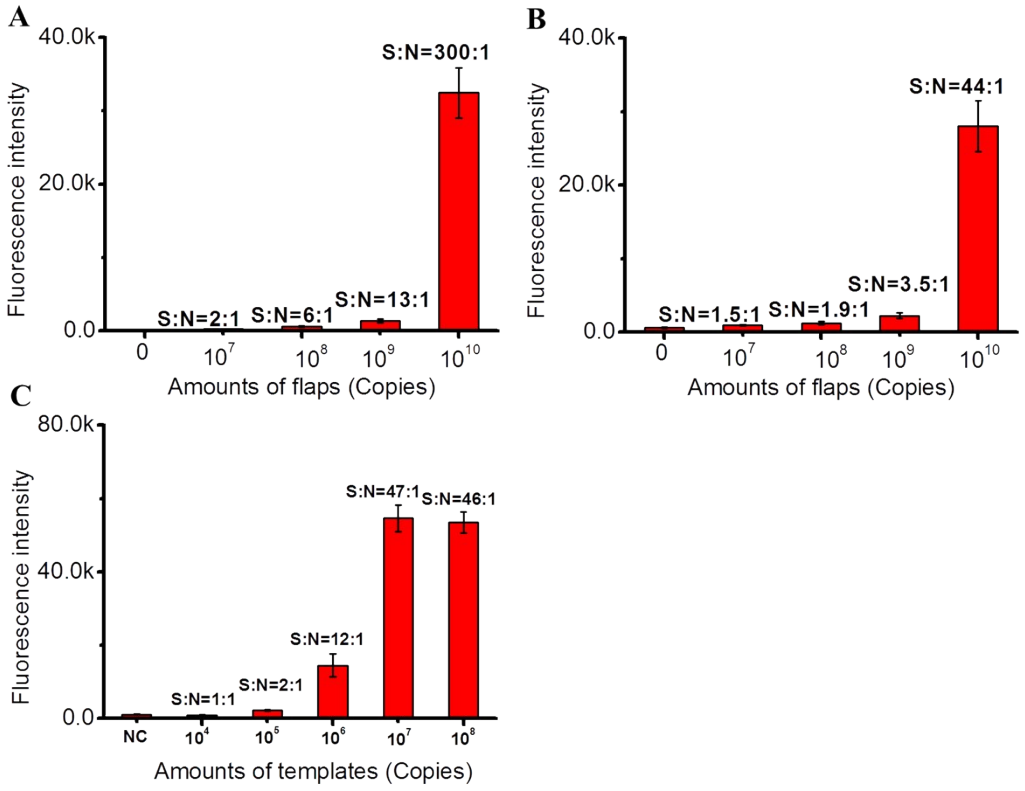

Fig. S3 Signal intensities and signal-to-noise ratios (S:N) from the proposed universal genotyping-microarray at various amounts of flaps in the absence of DP (A), amounts of flaps in the presence of 10^{13} copies of DP (B), and invasive reaction products from different amounts of PCR amplicons (C). Each sample was detected in triplicate.

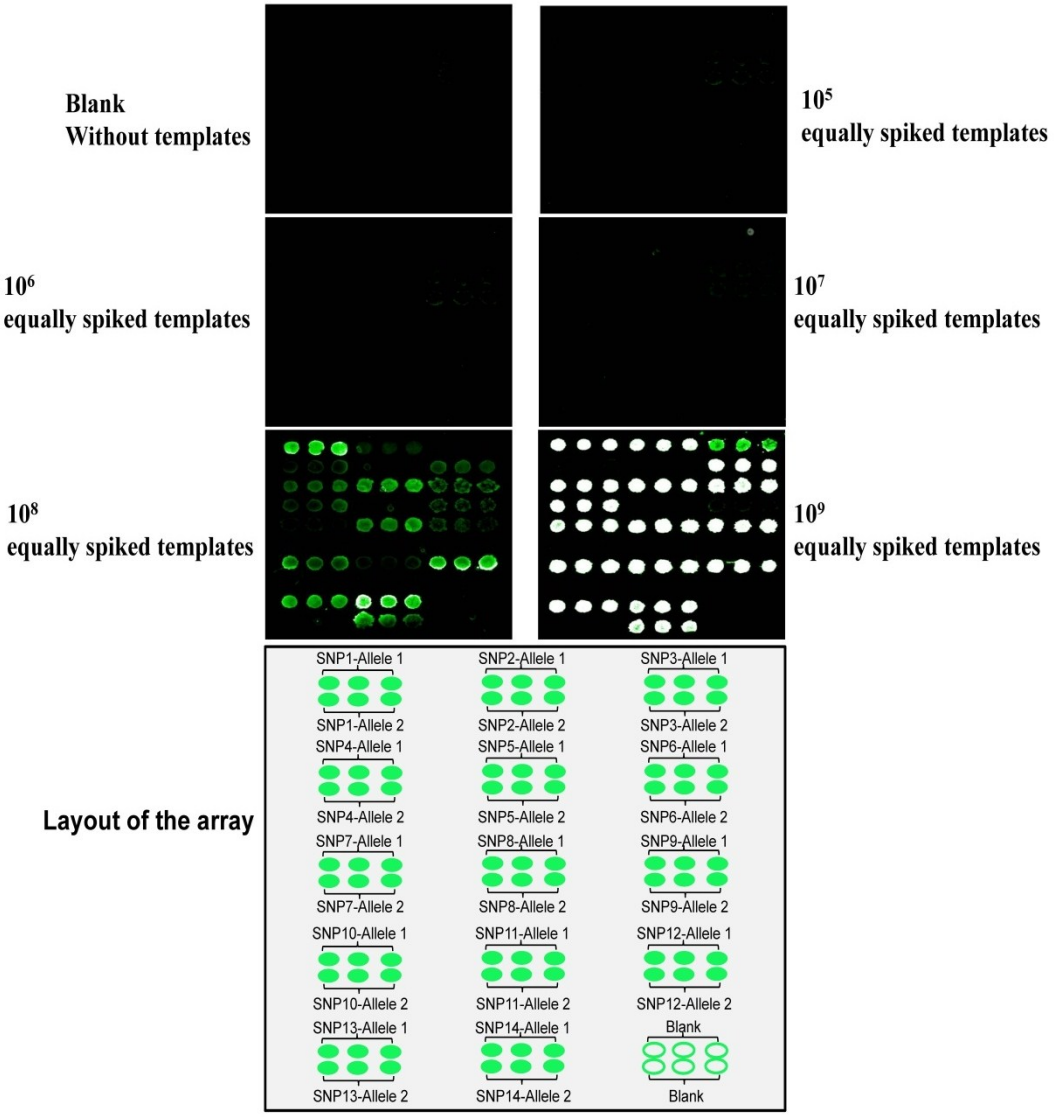

Fig. S4 Detection of the various amounts of equally spiked PCR amplicons by the proposed universal genotyping-microarray coupled with multiplex invasive reaction. The orders of the SNPs were marked in the layout of the array, and each sample was detected in three times.

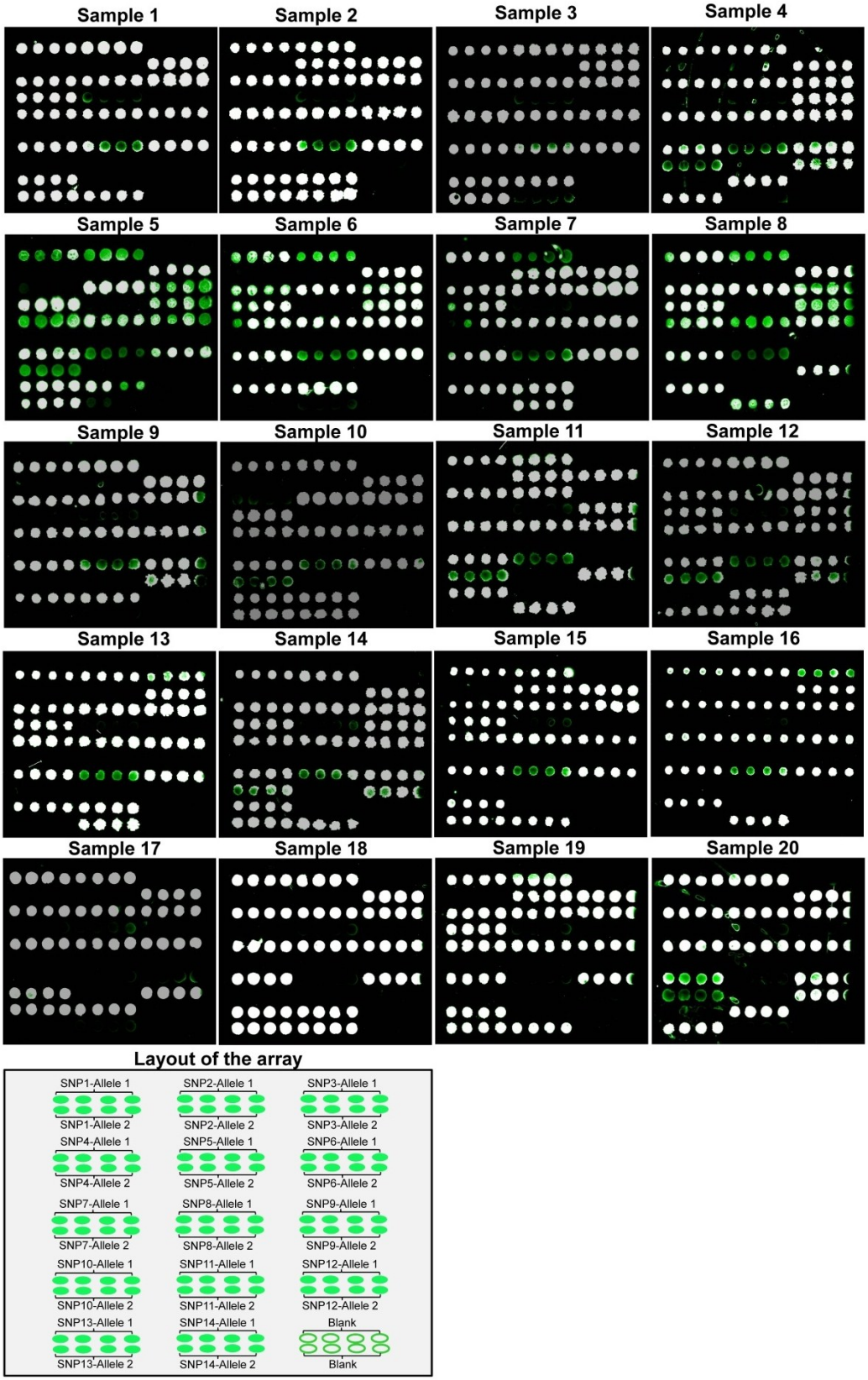

Fig. S5. Detection of 14 SNPs related to personalized medicine in a total of 20 clinical

samples by the proposed universal genotyping-microarray. The orders of the SNPs were marked in the layout of the array, and each sample was detected in four times.

Table S4. 14 SNPs related to personalized medicine in a total of 20 clinical samples genotyped by the proposed universal genotyping-microarray and Sanger sequencing.

Sample Number	Methods	SNPs													
		SNP1 (C/T)	SNP2 (A/C)	SNP3 (G/A)	SNP4 (G/A)	SNP5 (G/A)	SNP6 (C/T)	SNP7 (T/C)	SNP8 (T/C)	SNP9 (G/A)	SNP10 (A/C)	SNP11 (G/A)	SNP12 (G/A)	SNP13 (C/T)	SNP14 (G/T/A)
1	Sanger	C/C	A/A	A/A	G/A	G/G	C/C	T/T	T/T	G/G	A/A	G/G	G/G	C/T	T/A
	Chip	C/C	A/A	A/A	G/A	G/G	C/C	T/T	T/T	G/G	A/A	G/G	G/G	C/T	T/T
2	Sanger	C/C	A/C	A/A	G/G	G/G	C/C	T/T	T/T	G/G	A/A	G/G	G/G	C/T	G/T
	Chip	C/C	A/C	A/A	G/G	G/G	C/C	T/T	T/T	G/G	A/A	G/G	G/G	C/T	G/T
3	Sanger	C/C	A/A	G/A	G/G	G/G	C/C	T/T	T/T	G/G	A/A	G/G	G/G	C/T	G/G
	Chip	C/C	A/A	G/A	G/G	G/G	C/C	T/T	T/T	G/G	A/A	G/G	G/G	C/T	G/G
4	Sanger	C/C	A/A	A/A	G/G	G/G	C/T	T/T	T/T	G/G	A/C	G/G	G/A	T/T	G/A
	Chip	C/C	A/A	A/A	G/G	G/G	C/T	T/T	T/T	G/G	A/C	G/G	G/A	T/T	G/G
5	Sanger	C/C	A/A	A/A	A/A	G/G	C/T	T/T	T/T	G/G	A/C	G/G	G/G	C/T	G/G
	Chip	C/C	A/A	A/A	A/A	G/G	C/T	T/T	T/T	G/G	A/C	G/G	G/G	C/T	G/G
6	Sanger	C/C	A/A	A/A	G/A	G/G	C/T	T/T	T/T	G/G	A/A	G/G	G/G	C/C	G/G
	Chip	C/C	A/A	A/A	G/A	G/G	C/T	T/T	T/T	G/G	A/A	G/G	G/G	C/C	G/G
7	Sanger	C/C	A/C	A/A	G/A	G/G	C/C	T/T	T/T	G/G	A/A	G/G	G/G	C/C	G/T
	Chip	C/C	A/C	A/A	G/A	G/G	C/C	T/T	T/T	G/G	A/A	G/G	G/G	C/C	G/T
8	Sanger	C/C	A/A	A/A	G/A	G/G	C/T	T/T	T/T	G/G	A/A	G/G	A/A	C/C	A/T
	Chip	C/C	A/A	A/A	G/A	G/G	C/T	T/T	T/T	G/G	A/A	G/G	A/A	C/C	T/T
9	Sanger	C/C	A/A	A/A	G/G	G/G	C/C	T/T	T/T	G/G	A/A	G/G	G/A	C/C	G/A
	Chip	C/C	A/A	A/A	G/G	G/G	C/C	T/T	T/T	G/G	A/A	G/G	G/A	C/C	G/G
10	Sanger	C/C	A/A	A/A	A/A	G/G	C/C	T/T	T/T	G/G	A/C	G/G	G/G	C/T	G/T
	Chip	C/C	A/A	A/A	A/A	G/G	C/C	T/T	T/T	G/G	A/C	G/G	G/G	C/T	G/T

Sample Number	Methods	SNPs													
		SNP1 (C/T)	SNP2 (A/C)	SNP3 (G/A)	SNP4 (G/A)	SNP5 (G/A)	SNP6 (C/T)	SNP7 (T/C)	SNP8 (T/C)	SNP9 (G/A)	SNP10 (A/C)	SNP11 (G/A)	SNP12 (G/A)	SNP13 (C/T)	SNP14 (G/T/A)
11	Sanger	C/C	A/C	A/A	G/G	G/G	T/T	T/T	T/T	G/G	A/C	G/G	A/A	C/C	T/T
	Chip	C/C	A/C	A/A	G/G	G/G	T/T	T/T	T/T	G/G	A/C	G/G	A/A	C/C	T/T
12	Sanger	C/C	A/A	A/A	G/A	G/G	C/T	T/T	T/T	G/G	A/C	G/G	G/A	T/T	G/T
	Chip	C/C	A/A	A/A	G/A	G/G	C/T	T/T	T/T	G/G	A/C	G/G	G/A	T/T	G/T
13	Sanger	C/C	A/A	?	G/A	G/G	C/C	T/T	T/T	G/G	A/A	G/G	G/G	C/C	G/T
	Chip	C/C	A/A	G/A	G/A	G/G	C/C	T/T	T/T	G/G	A/A	G/G	G/G	C/C	G/T
14	Sanger	C/C	A/A	?	G/A	G/G	C/T	T/T	T/T	G/G	A/C	G/G	G/A	C/T	T/A
	Chip	C/C	A/A	A/A	G/A	G/G	C/T	T/T	T/T	G/G	A/C	G/G	G/A	C/T	T/T
15	Sanger	CC	A/C	A/A	G/A	G/G	C/C	T/T	T/T	G/G	A/A	G/G	G/G	C/T	T/A
	Chip	C/C	A/C	A/A	G/A	G/G	C/C	T/T	T/T	G/G	A/A	G/G	G/G	C/T	T/T
16	Sanger	C/C	A/A	G/A	G/G	G/G	C/C	T/T	T/T	G/G	A/A	G/G	G/G	C/C	T/T
	Chip	C/C	A/A	G/A	G/G	G/G	C/C	T/T	T/T	G/G	A/A	G/G	G/G	C/C	T/T
17	Sanger	C/C	A/A	A/A	G/G	G/G	C/C	T/T	T/T	G/G	C/C	G/G	A/A	C/C	G/G
	Chip	C/C	A/A	A/A	G/G	G/G	C/C	T/T	T/T	G/G	C/C	?	A/A	C/C	G/G
18	Sanger	C/C	A/A	A/A	G/G	G/G	C/C	T/T	T/T	G/G	A/A	G/G	G/G	C/T	G/T
	Chip	C/C	A/A	A/A	G/G	G/G	C/C	T/T	T/T	G/G	A/A	?	G/G	C/T	G/T
19	Sanger	C/C	A/C	A/A	G/A	G/G	C/C	T/T	T/T	G/G	A/A	G/G	G/G	C/T	T/A
	Chip	C/C	A/C	A/A	G/A	G/G	C/C	T/T	T/T	G/G	A/A	G/G	G/G	C/T	T/T
20	Sanger	C/C	A/A	A/A	G/G	G/G	C/C	T/T	T/T	G/G	A/C	G/G	G/A	T/T	G/G
	Chip	C/C	A/A	A/A	G/G	G/G	C/C	T/T	T/T	G/G	A/C	G/G	G/A	T/T	G/G

Note: Failed tests were marked by "? " in the Table, and inconsistent SNPs genotyped by the two methods were marked with red.

Table S5. 14 SNPs in a total of 20 clinical samples genotyped by the proposed universal genotyping microarray and Sanger sequencing.

	Total Number of SNPs	Number of successfully detected SNPs	Detection rate	Number of consistent SNPs genotyped by the two methods	Accuracy rate
The proposed universal genotyping microarray	280	278	99.3%	270	97.1%
Sanger sequencing	280	278	99.3%		100%

Table S6. Comparison of the proposed universal genotyping microarray with nicking endonuclease-based method and conventional allele-specific chips in terms of cost, complexity, multiplex levels, sensitivity and specificity.

Methods	Steps	Cost		Complexity of experiment setup	Multiplex levels	Sensitivity	Specificity
		Running cost	Setting-up cost				
Nicking endonuclease-based method[3, 4]	Circularization of the Padlock Probe Rolling Circle Amplification Nicking Endonuclease-Assisted Nanoparticles Amplification	***	***	**	*	*	*** (Nicking endonuclease - dependent)
Conventional allele-specific chips[5]	PCR (including preparation) PCR purification Microarray construction SNP typing by the conventional type of microarray (including hybridization, washing, and Scanning)	*	*	*	***	*** (PCR-depandent)	* (Allele-specific hybridization-dependent)
Proposed universal genotyping microarray	Multiplex PCR Multiplex invasive reaction Microarray construction SNP typing by the proposed universal genotyping-microarray(including ligation, washing, and Scanning)	**	***	**	***	*** (PCR-depandent)	*** (*Afu* endonuclease cleavage-dependent)

Note: The larger number of "*" indicates the superior performance of the method.

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