Supplementary information

A fully integrated SNP genotyping system for hereditary hearing-loss detection

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Supporting information contents:

On-cassette DNA extraction process. First, when the rotary valve was switched to the lysis chamber, lysis buffer was mixed with the blood sample by the rapid liquid transfer between the lysis chamber and the syringe barrel for 20 minutes. Then, the lysed sample (800 μL) was transferred into the binding chamber and completely mixed with ChargeSwitch Purification Buffer (200 μL) containing ChargeSwitch Magnetic Beads for 5 minutes. Subsequently, the mixture was aspirated back into the syringe barrel and then slowly pushed into the binding chamber through the channel inside the rotary valve to collect magnetic beads in the bottom of the rotary valve using the magnet located close to the bottom of the rotary valve. After binding the DNA to magnetic beads, the rotary valve was switched to connect the inlet of the first washing chamber, with the magnet driven away from the rotary valve. Then, the syringe plunger was quickly pushed and pulled several times to resuspend and wash the magnetic beads in 500 μL of ChargeSwitch Wash Buffer for 3 minutes. After the first washing step, the magnet was pulled up again to collect the magnetic beads, and the waste buffer was discarded into the first washing chamber. The second and third washing steps followed a similar process. After the washing process, the magnet was moved away from the rotary valve, and the magnetic beads were suspended by 100 μL of KASP Master mix for 6 minutes to elute the bound DNA. Afterwards, the mixture of DNA template and the KASP Master mix was aspirated into the syringe barrel with the magnetic beads collected again.

Figure S1. (A) Schematic depiction of the optical module. (B) Validation of the real-time detection ability of the dual-channel fluorescence detection system. In comparison with the Bio-Rad CFX96 qPCR instrument, LAMP reactions were used to verify the performance of real-time fluorescence detection of the processing instrument. (B1) A real-time fluorescence curve in a FAM channel was obtained by the Bio-Rad qPCR instrument. (B2) A real-time fluorescence curve in blue was obtained by the dual-channel fluorescence detection system and a fitting curve in red was obtained from the blue one. The red curve in Figure B had the same curving shape and the peaking time with the blue curve in Figure A, which proved the real-time detection ability of the dual-channel fluorescence detection system.

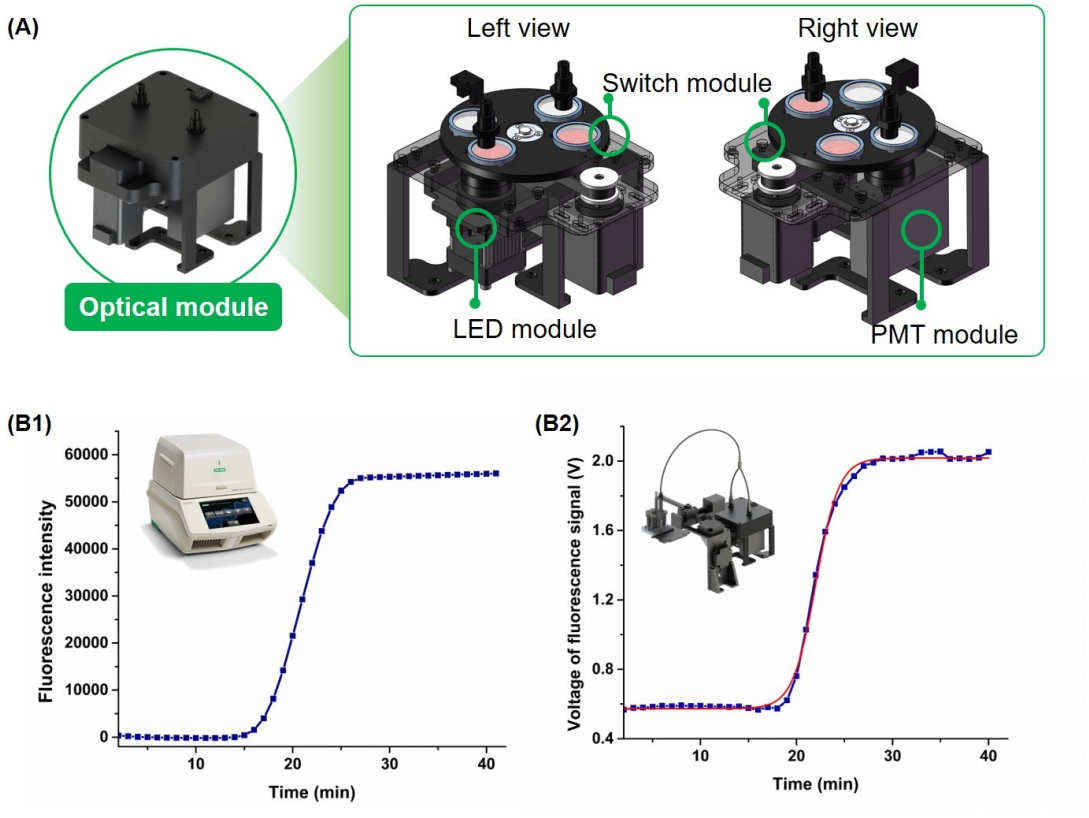

Figure S2. Verification of the PCR temperature control system. (A) Thermal images of the heating plate without and with the cassette. The heating area for eight reaction chambers is circled by a red box. (B) Thermal images of the heating plate without the cassette at 95℃. (C) The calibration curve between the temperature of the heating plate and the temperature of the chamber. The curve was well fitted to a linear equation with an R^2 equal to 0.9998. Cross-sectional schematic of the heating plate with an inserted reaction chamber of the cassette and two thermal sensors embedded into the chamber and the heating plate. (D) Thermal cycling profile. The black line shows the temperature of the heating plate, and the blue dotted line is the set temperature as a function of time. The temperature ramp rates were 2.5℃/s for heating and 1.4℃/s for cooling. (E) FAM and HEX fluorescence signals of eight reaction chambers. (F) Gel electrophoresis of KASP products in eight reaction chambers. (Lane M: DL2000 DNA marker; P: Positive control; N: Negative control.)

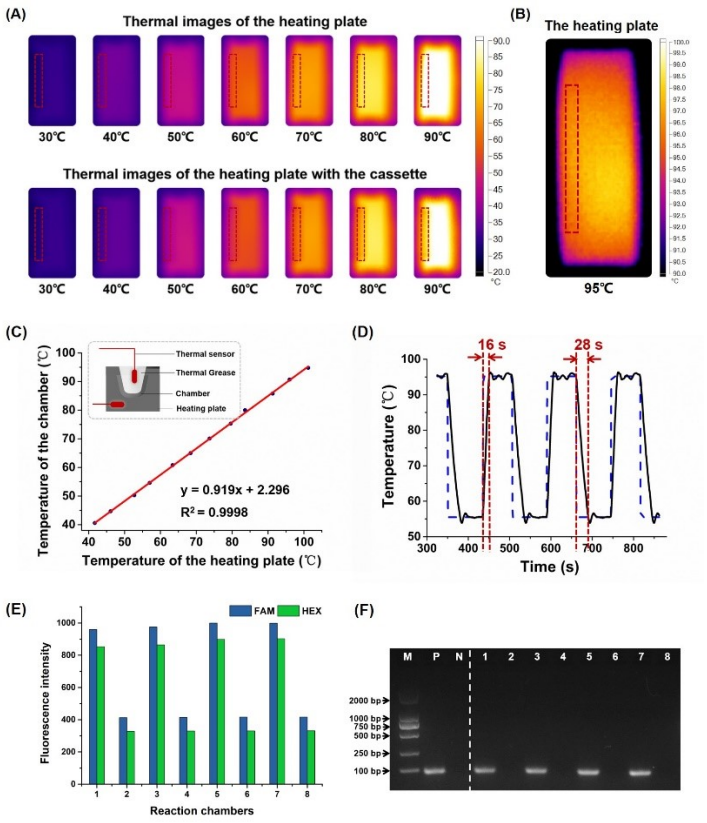

Figure S3. The results of the additional sensitivity tests of the cassette system.

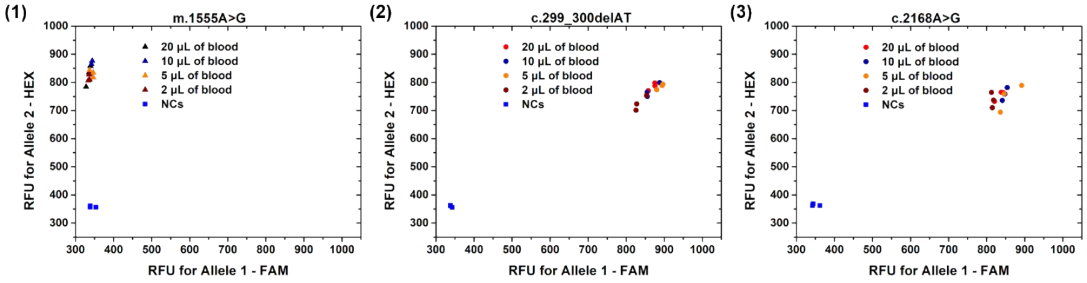

Figure S4. Detection results of 13 clinical samples using our cassette system. All the samples were tested in triplicate.

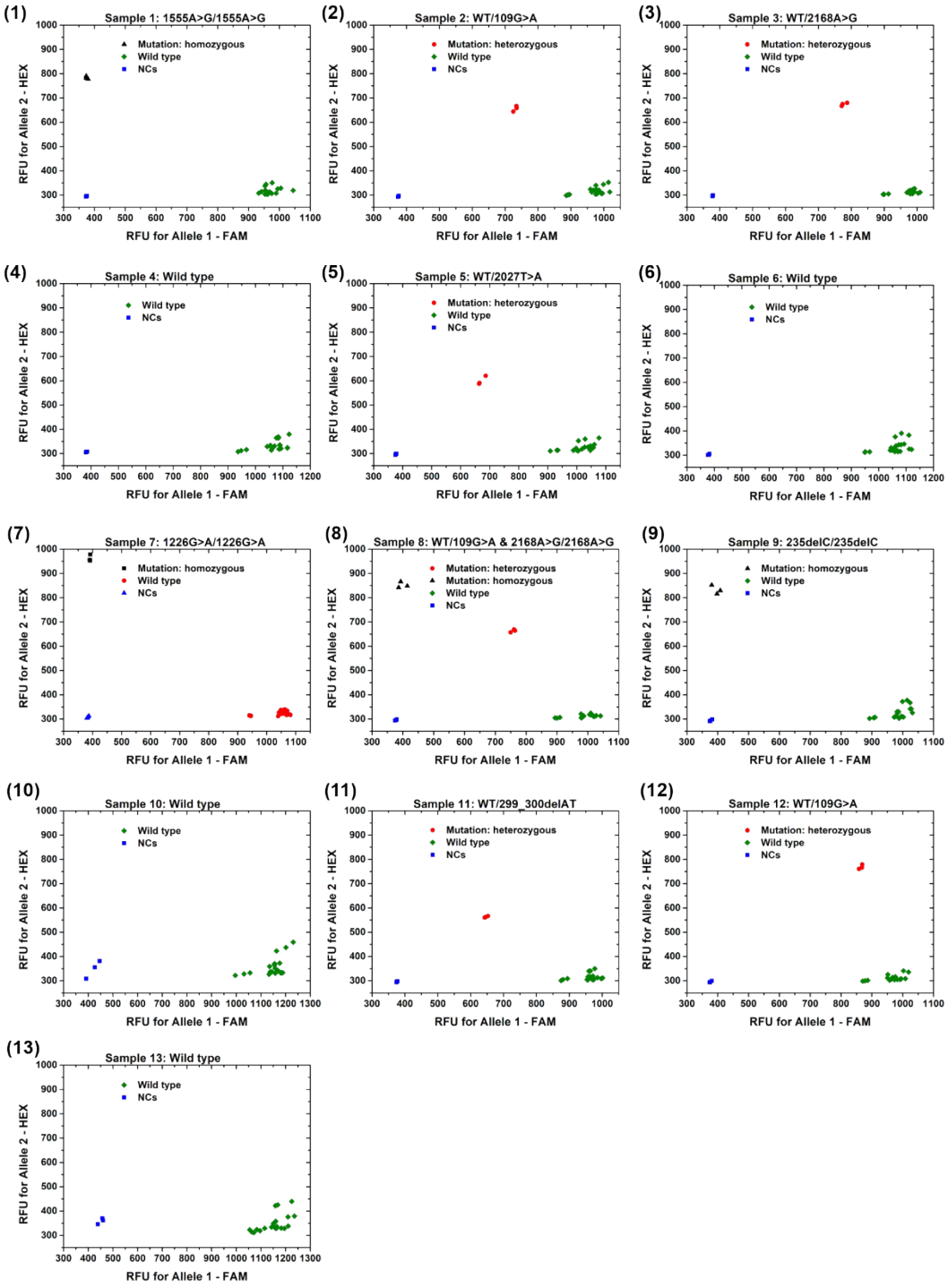

Figure S5. Sanger sequencing results of 13 clinical samples. The mutation sites are circled by a red box.

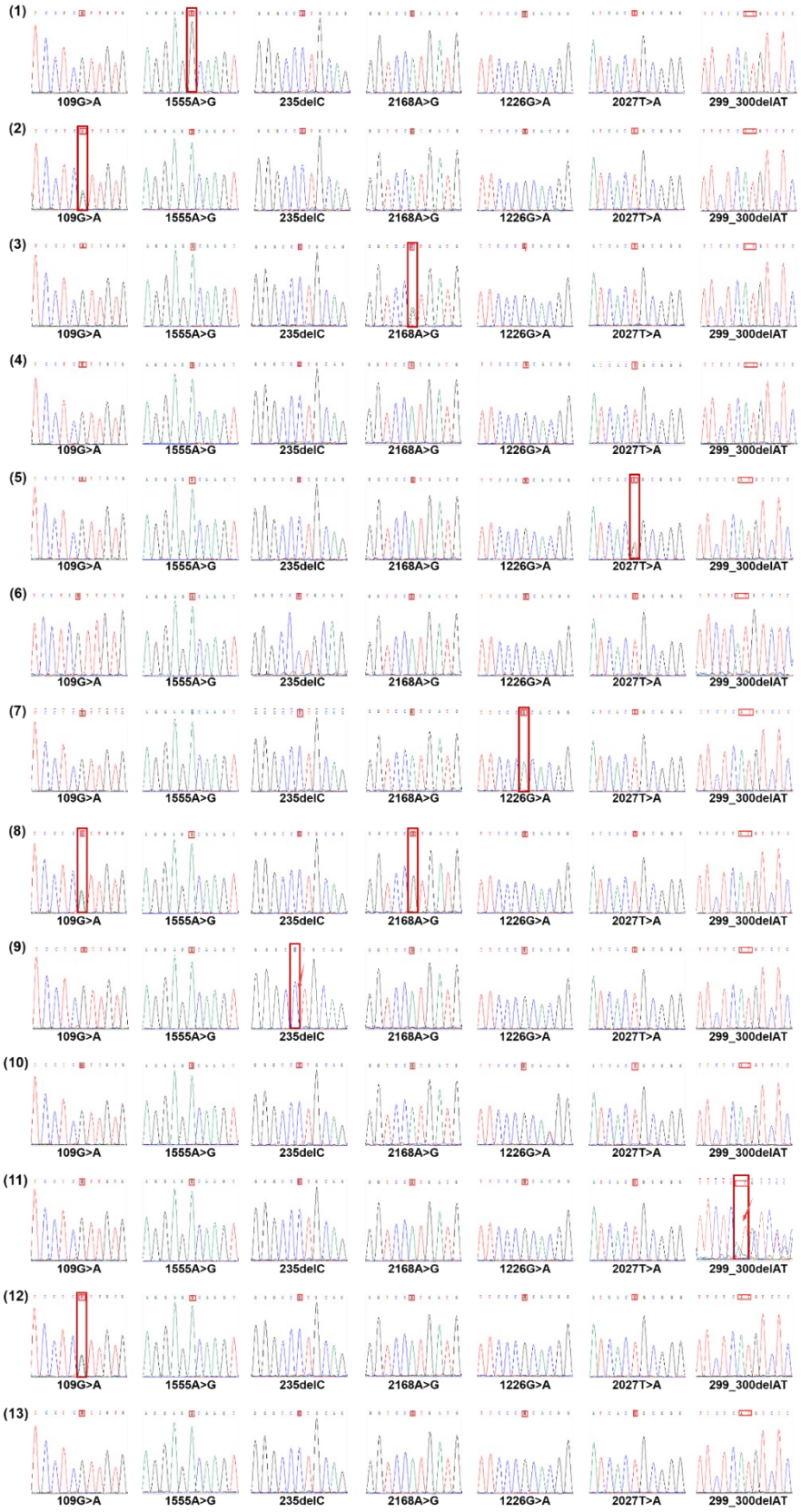

Table S1. Basic information of 13 clinical samples.

Clinical samples	Gender	Age	Hearing condition
1	Female	25	Deafness
2	Female	41	Healthy
3	Male	36	Poor hearing
4	Female	19	Healthy
5	Female	6	Deafness
6	Male	23	Healthy
7	Male	20	Healthy
8	Male	25	Deafness
9	Male	37	Deafness
10	Female	32	Healthy
11	Male	30	Healthy
12	Female	29	Healthy
13	Female	26	Healthy

Table S2. Sequences of cloning primers of 7 SNPs/InDels.

Gene	Detection site	RS ID	Sequence of the primers (5'-3')
MT-RNR1	m.1555A>G	rs267606617	F: GAAGGTGGATTTAGCAGTAA R: GGGTAAATGGTTTGGCTAAG
GJB2	c.109G>A	rs72474224	F: ACACGAAGATCAGCTGCAGG R: CTGTCCTAGCTAGTGATTCC
	c.235delC	rs80338943	F: GCCTTCGATGCGGACCTTCT R: ACAAACACTCCACCAGCATT
	c.299_300delAT	rs111033204	
SLC26A4	c.2168A>G	rs121908362	F: CCTAGCTAATTGGGAGGG R: TGAGGCTCCATGAAGTTA
	c.2027T>A	rs111033318	F: TGACAGTGTTTTCTTCGTTT R: AAGCCCATGTATTTGCCCTG
	c.1226G>A	rs111033305	F: GCGTCCAAACTCCTGATGTC R: GAATAGGCCTGAGGTGAATC

"RS ID" represents the reference SNP identification number, "F" represents the forward primer, "R" represents the reverse primer, "m." stands for mitochondrial mutation and "c." stands for chromosome mutation.

78 **Table S3.** Specificity of on-cassette tests for the detection of seven SNPs/InDels.

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Sample genotype	**Primers preloaded in reaction chambers**							
	m.1555A>G	c.109G>A	c.235delC	c.299_300delAT	c.2168A>G	c.2027T>A	c.1226G>A	NC
m.1555A>G/ m.1555A>G	△	◇	◇	◇	◇	◇	◇	□
WT/c.109G>A	◇	○	◇	◇	◇	◇	◇	□
WT/c.235delC	◇	◇	○	◇	◇	◇	◇	□
WT/c.299_300delAT	◇	◇	◇	○	◇	◇	◇	□
WT/c.2168A>G	◇	◇	◇	◇	○	◇	◇	□
WT/c.2027T>A	◇	◇	◇	◇	◇	○	◇	□
c.1226G>A/ c.1226G>A	◇	◇	◇	◇	◇	◇	△	□

80 Note: "△" indicates genotype with homozygous alleles, "○" indicates heterozygote, "◇" indicates

81 wild-type genotype, and "□" indicate NC.

Table S4. Sensitivity of on-cassette tests for the detection of seven SNPs/InDels.

ID	Sample genotype	The amount of whole blood samples			
		20 μL	10 μL	5 μL	2 μL
1	m.1555A>G/m.1555A>G	√	√	√	√
2	WT/c.109G>A	√	√	√	√
3	WT/c.235delC	√	√	√	√
4	WT/c.299_300delAT	√	√	√	√
5	WT/c.2168A>G	√	√	√	×
6	WT/c.2027T>A	√	√	√	×
7	c.1226G>A/c.1226G>A	√	√	√	×
8	m.1555A>G/m.1555A>G	√	√	√	√
9	WT/c.299_300delAT	√	√	√	√
10	WT/c.2168A>G	√	√	√	√

Note: "√" indicates the successful amplification fit the criteria for genotyping results, "×" indicates the failure amplification that could not fit the criteria for genotyping results.

Video S1. Automated processing on the cassette system for SNP/InDel genotyping. The video shows the workflow of on-cassette magnetic bead-based nucleic acid extraction, distribution of the reaction mixture into eight chambers, thermal sealing of chambers, PCR thermal cycling and dual-channel fluorescence detection steps, and the sample and reagents are represented by different coloured dyes.