Supplementary Material

Ligase chain reaction-based electrochemical biosensor for the ultrasensitive and specific detection of single nucleotide polymorphisms

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Table S1 Sequences of the oligonucleotides in this work.

Name	Sequence (5' – 3')
WtDNA	CCTCCACCGTGCAGCTCATCA**C**GCAGCTCATGCCCTTCGGC
MutDNA	CCTCCACCGTGCAGCTCATCA**T**GCAGCTCATGCCCTTCGGC
Probe A	P-TGATGAGCTGCACGGT
Probe A′	GACTACATAAGCTGGCGTTGG ACCGTGCAGCTCATCA
Probe B-mut	AGGGCATGAGCTGCA
Probe B′-mut	P-TGCAGCTCATGCCCT GGATGTCAGTCTGTCTCGTGG
Probe B-wt	AGGGCATGAGCTGCG
Probe B′-wt	P-CGCAGCTCATGCCCT GGATGTCAGTCTGTCTCGTGG
SP-DNA	CCAACGCCAGCTTATGTAGTCAAAAA-NH_2
Stem-loop DNA	CCAACGCCACGAGACAGACTGACATCCCGTTGG

Note: probe A and probe A′ are universal for MutDNA and WtDNA LCR-based electrochemical biosensor.

Functional modifications of GCE

Before modification, the bare GCE was polished thoroughly with 1.0, 0.3, and 0.05 μm alumina slurry and rinsed with distilled water. The GCE was then ultrasonically cleaned in ethanol, water, and acetone sequentially for 5 min. Finally, the GCE electrode was dried using N_2 at RT.

Afterwards, 4 mg of cMWCNTs were dispersed in 1 mL of DMF (4 mg·mL^{-1}) and sonicated for 60 min to form a black and well-dispersed suspension. Next, 10 μL of the cMWCNTs suspension was drop-coated onto the surface of the polished bare GCE and subsequently dried at RT (cMWCNTs/GCE). Then, 10 μL of EDC/NHS solution was dropped onto the surface of the cMWCNTs/GCE for 2 h to activate the carboxyl groups of cMWCNTs. Subsequently, the modified and activated cMWCNTs/GCE was immersed in capture probe SP-DNA solution (10 μM) for 2 h at RT to immobilize the probe via amide bonds (SP-DNA/cMWCNTs/GCE). Before the electrochemical measurements, the electrode was rinsed with purified water to eliminate nonspecific adsorption and stored in N_2 atmosphere at 4 °C.

The relationship between peak current and scanning rate

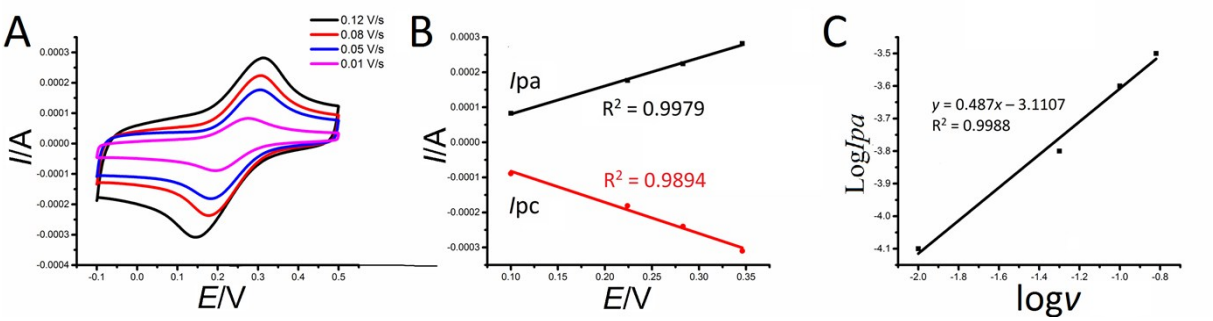

Fig. S1 (A) CV curves of SP-DNA/cMWCNTs/GCE at different scan rates in the range of 0.01 ~ 0.12 V/s. (B) linear calibration plots for the peak current versus square root of scan rate. (C) linear calibration plots for the logarithm of peak current versus logarithm of scan rate.

Optimization the concentration of SP-DNA

The concentration of the SP-DNA immobilized on the surface of the cMWCNTs/GCE was evaluated. As shown in Fig. S1, the peak current values of the SP-DNA/cMWCNTs/GCE slightly decreased with increasing SP-DNA concentrations (from 0 μM to 10 μM) and reached a stable electrochemical signal at 10 μM, demonstrating that the carboxyl groups of cMWCNTs modified on the surface of the bare GCE were almost completely bound to SP-DNA at 10 μM. Moreover, we investigated the electrochemical response of the SL-DS-DNA/cMWCNTs/GCE changed with SP-DNA concentrations, when the concentration of MutDNA is 0.1 fM in LCR. The electrochemical signal fell to its lowest value at 10 μM, and slightly increased at higher concentrations due to steric effect. Therefore, the optimal concentration of SP-DNA was observed to be 10 μM.

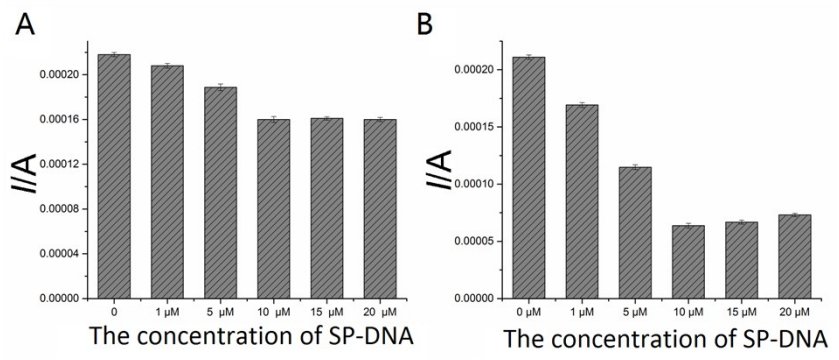

Fig. S2 Dependence of the peak currents on the concentrations of SP-DNA (A) in the absence and (B) presence of the products of LCR (0.1 fM Mut DNA). Error bars show the standard deviation of three experiments.

The repeatability and reproducibility of MutDNA LCR-based electrochemical biosensor

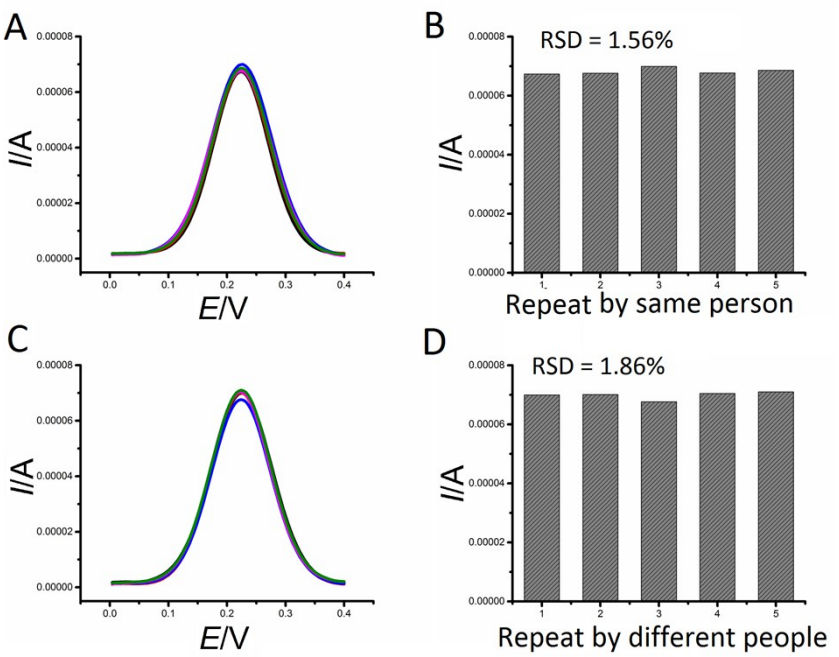

Fig. S3 (A) The DPV curves of developed MutDNA LCR-based electrochemical biosensor obtained by the same person, (B) histogram of DPV peak current in (A). (C) The DPV curves of developed MutDNA LCR-based electrochemical biosensor obtained by five persons, (D) histogram of DPV peak current in (C).

The repeatability and reproducibility of WtDNA LCR-based electrochemical biosensor

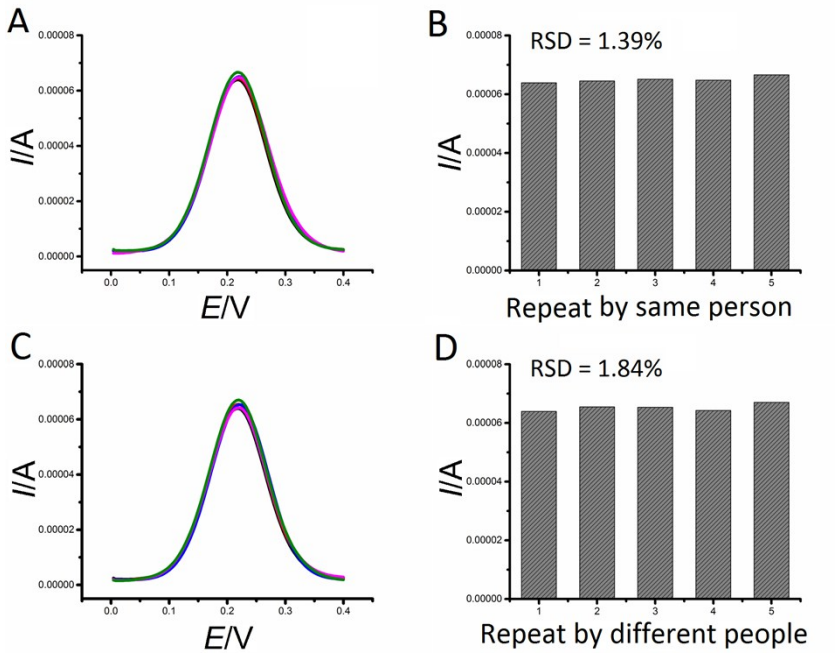

Fig. S4 (A) The DPV curves of developed WtDNA LCR-based electrochemical biosensorr obtained by the same person, (B) histogram of DPV peak current in (A). (C) The DPV curves of developed WtDNA LCR-based electrochemical biosensor obtained by five persons, (D) histogram of DPV peak current in (C).

The influence of different modified electrodes to WtDNA LCR-based electrochemical biosensor

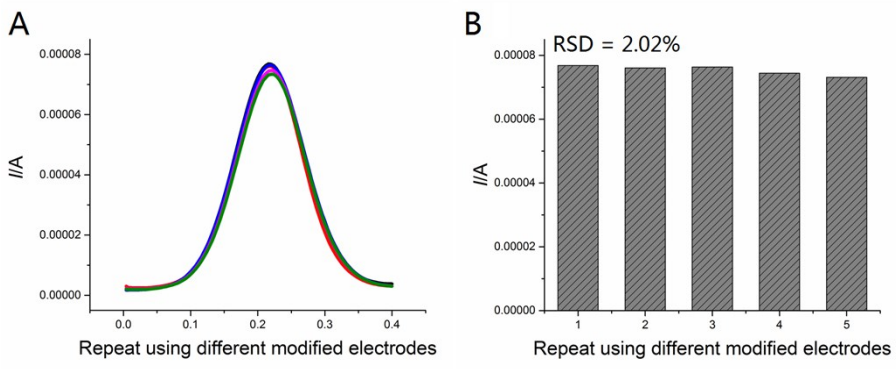

Fig. S5 (A) The DPV curves of developed WtDNA LCR-based electrochemical biosensor obtained by different modified electrodes, (B) histogram of DPV peak current in (A).

Table S2 The LOD, linear range, specificity, reproducibility and stability of various methods for SNPs.

Approaches	LOD	Linear range	Specificity	Reproducibility [a]	Stability [a]	Reference
MNP-enzyme	50 fM	5 pM to 200 nM	10%	–	–	1
ligation-LCR	0.1 nM	1 nM to 0.6 pM	0.05%	general	general	2
encoded probes	25 fmol	500 pM to 10 nM	0.5%	–	–	3
LCR-CCPs	1 fM	1 fM to 10 pM	1.0%	–	–	4
LCR-CRET	0.86 fM	1 fM to 10 pM	0.01%	–	–	5
Real-time LCR	10 aM	10 aM to 1 pM	0.1%	–	–	6
Electrochemistry-LCR	1.18 aM	10 aM to 10 pM	0.1%	good	good	This work

"–": The proper data was not provided.

[a] "General" and "good" represent the reproducibility and stability of the SNPs detection approach is general or good.

TableS3 The accuracy of the developed LCR-based electrochemical biosensor (n = 3).

Biosensor	Added (fmol)	found (fmol)	Recovery (%)	RSD (%)
MutDNA biosensor	50	47.9	95.8	2.9
	200	192.8	96.4	1.7
WtDNA biosensor	50	51.7	103.4	3.2
	200	196.8	98.4	2.1

Reference

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