

Fluorescent detection of *Apolipoprotein E* gene polymorphisms based on oligonucleotide ligation and magnetic separation

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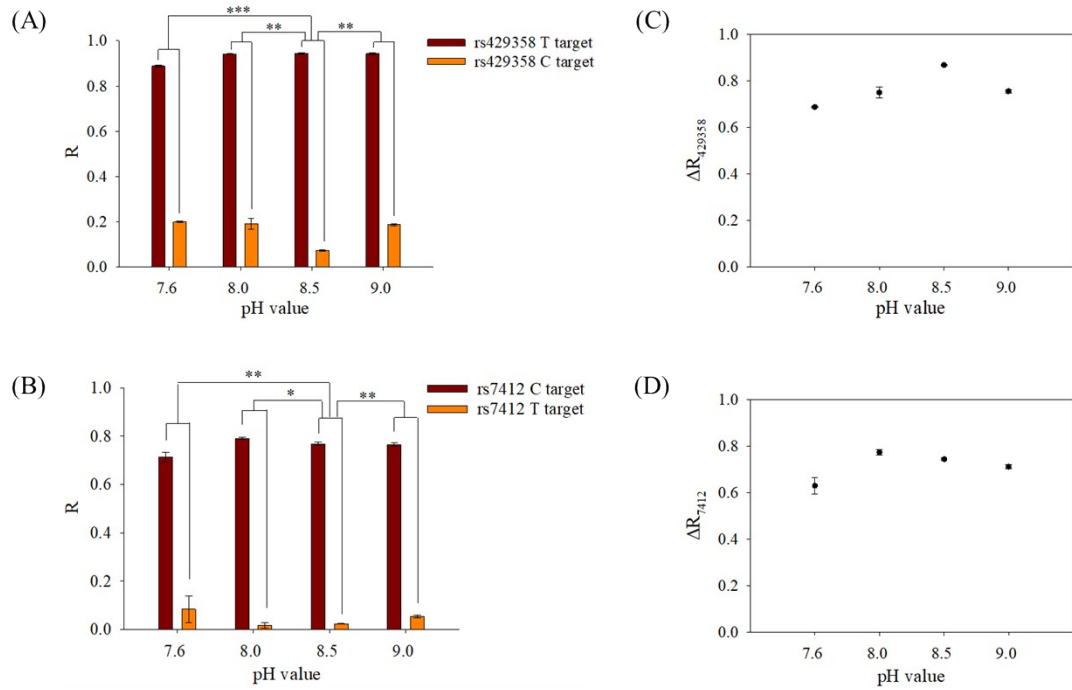


Fig. S1 Effect of pH value of ligase reaction buffer. (A) R-value of rs429358 (B) R-value of rs7412 (C) ΔR -value of rs429358 (D) ΔR -value of rs7412. R-value: $F_0 - F_1 / F_0$. F_0 : Fluorescence intensity in biosensor system without DNA target, F_1 : Fluorescence intensity in biosensor system with DNA target. ΔR -value_{rs429358}: $R_{T \text{ target}} - R_{C \text{ target}}$. ΔR -value_{rs7412}: $R_{C \text{ target}} - R_{T \text{ target}}$. "****": $P < 0.001$, "***": $P < 0.01$, "**": $P < 0.05$.

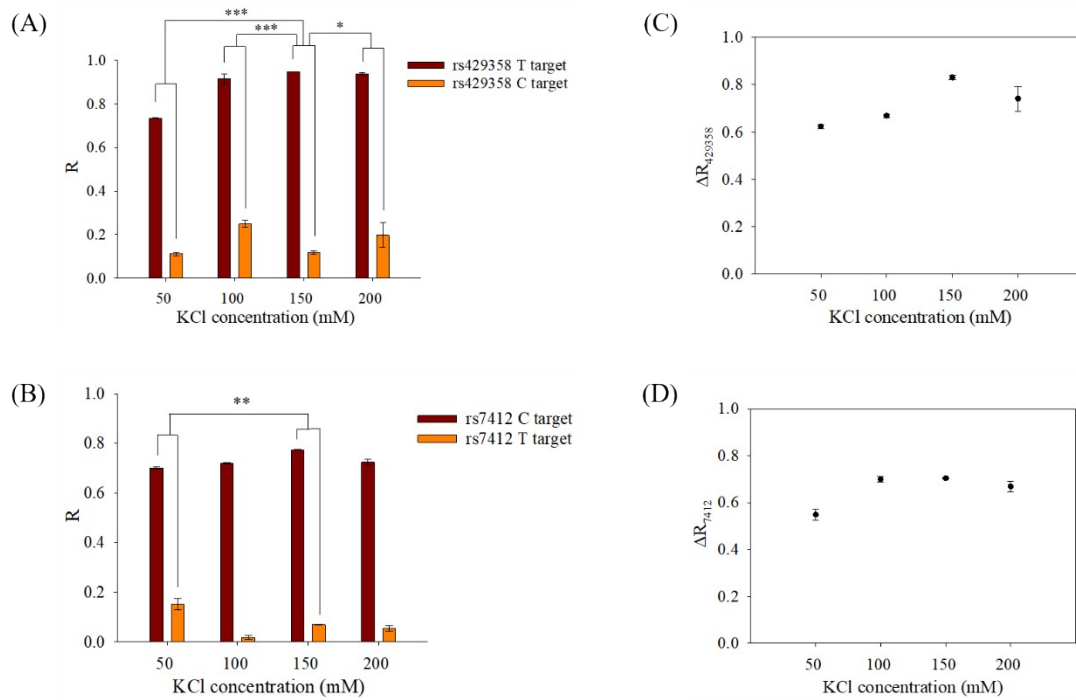


Fig. S2 Effect of KCl concentration on ligase reaction buffer. (A) R-value of rs429358 (B) R-value of rs7412 (C) ΔR -value of rs429358 (D) ΔR -value of rs7412. R-value: $F_0 - F_1 / F_0$. F_0 : Fluorescence intensity in biosensor system without DNA target, F_1 : Fluorescence intensity in biosensor system with DNA target. ΔR -value_{rs429358}: $R_{T \text{ target}} - R_{C \text{ target}}$. ΔR -value_{rs7412}: $R_{C \text{ target}} - R_{T \text{ target}}$. "****": $P < 0.001$, "***": $P < 0.01$, "**": $P < 0.05$.

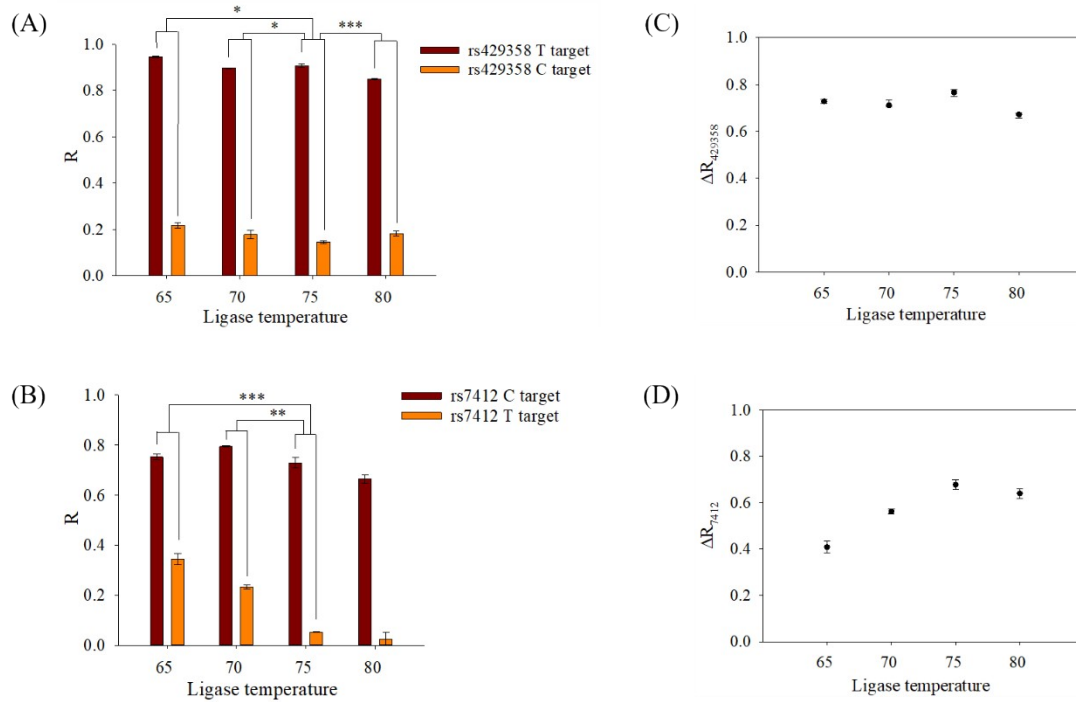


Fig. S3 Effects of temperature on the ligase detection reaction. (A) R-value of rs429358 (B) R-value of rs7412 (C) ΔR -value of rs429358 (D) ΔR -value of rs7412. R-value: $F_0 - F_1 / F_0$. F_0 : Fluorescence intensity in biosensor system without DNA target, F_1 : Fluorescence intensity in biosensor system with DNA target. ΔR -value_{rs429358}: $R_{T \text{ target}} - R_{C \text{ target}}$. ΔR -value_{rs7412}: $R_{C \text{ target}} - R_{T \text{ target}}$. "****": $P < 0.001$, "***": $P < 0.01$, "**": $P < 0.05$.

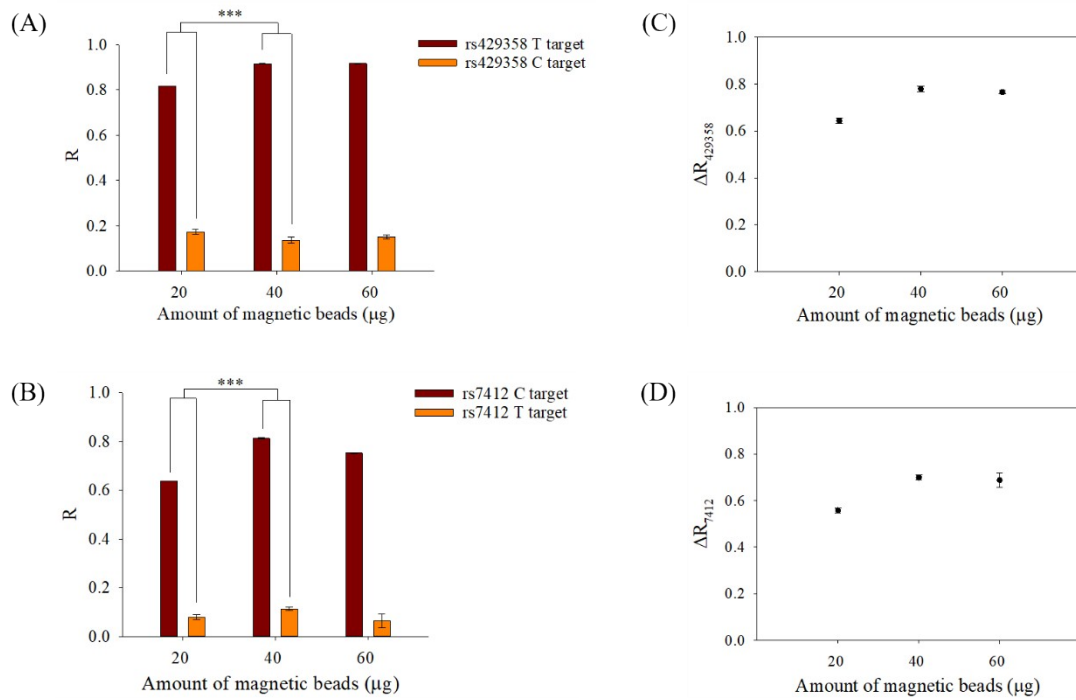


Fig. S4 Effects of magnetic beads amount on oligonucleotide ligation-fluorescent biosensor system. (A) R-value of rs429358 (B) R-value of rs7412 (C) ΔR -value of rs429358 (D) ΔR -value of rs7412. R-value: $F_0 - F_1 / F_0$. F_0 : Fluorescence intensity in biosensor system without DNA target, F_1 : Fluorescence intensity in biosensor system with DNA target. ΔR -value_{rs429358}: $R_{T \text{ target}} - R_{C \text{ target}}$. ΔR -value_{rs7412}: $R_{C \text{ target}} - R_{T \text{ target}}$. "****": $P < 0.001$, "***": $P < 0.01$, "**": $P < 0.05$.

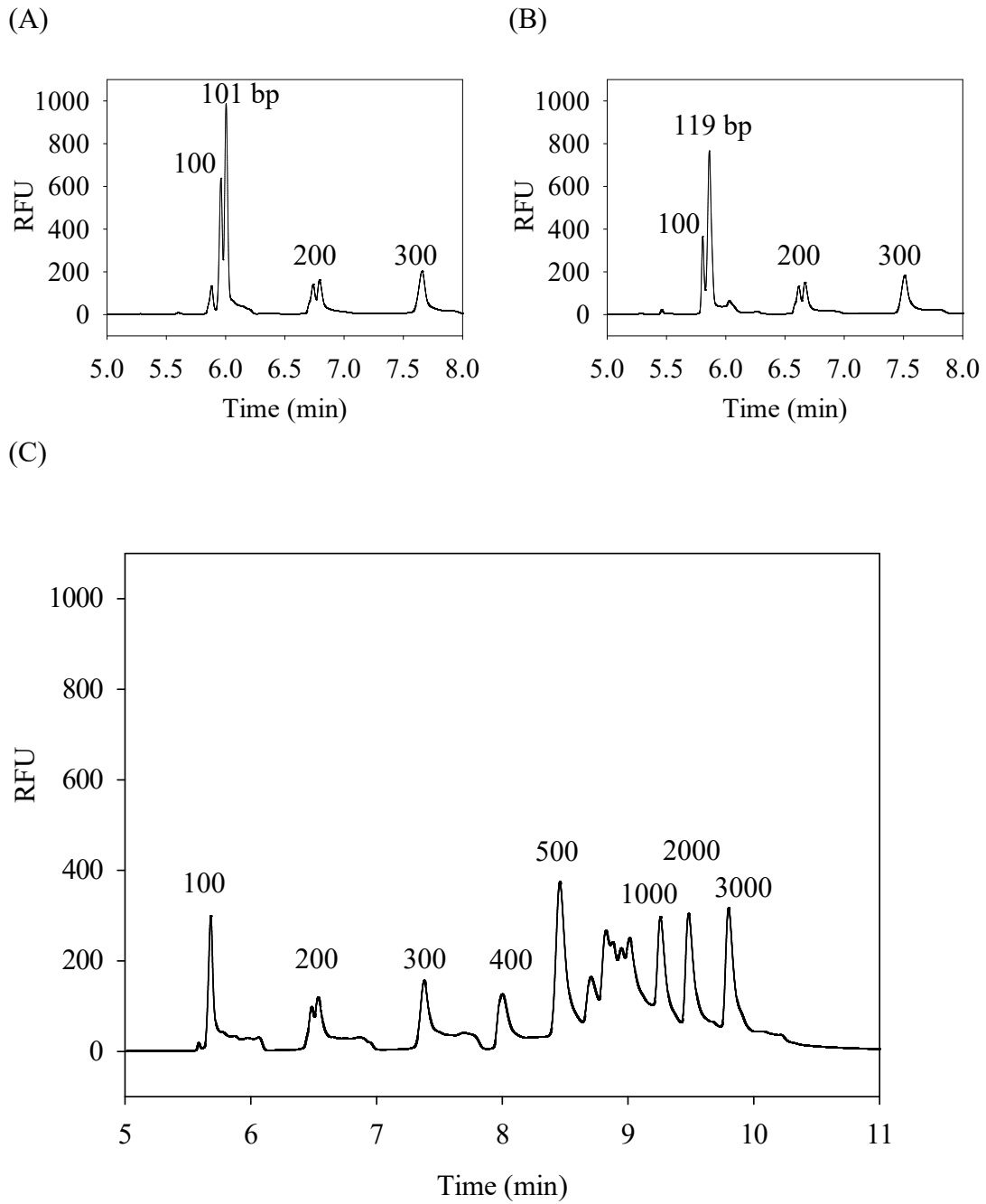


Fig. S5. (A) The electropherogram of PCR product of rs429358 (101 bp) with DNA ladder. (B) The electropherogram of PCR product of rs7412 (119 bp) with DNA ladder. (C) The electropherogram of DNA ladder. CE condition: DB-17 capillary, 30 cm (effective length) \times 100 μ m I.D., filled with 0.6% (w/v) PEO in 1 \times TBE containing 1 mM YOPRO-1 before sample injection. Injection -10 kV, 30 s; temperature, 25 $^{\circ}$ C.

Table S1 Probe design for discriminating allele-specific sites

Name	Sequence (5'-3')	Tm (°C)
rs429358-P1 probe (5'PN)	p- <u>A</u> CACGT CCTCC ATGTC CGCGC TTT TTT TTT -Biotin	61.6
rs429358-F1 probe	FAM-TACTG CACCA GGCGG CCGC	59.7
rs429358-P2 probe	p-CACGT CCTCC ATGTC CGCGC TTT TTT TTT -Biotin	61.5
rs429358-F2 probe (3'N)	FAM-TACTG CACCA GGCGG CCGC <u>A</u>	60.0
rs7412-P1 probe (5'PN)	p- <u>G</u> CCTTCT GCAGG TCATC GGCAT TTT TTT TTT -Biotin	58.9
rs7412-F1 probe	FAM-GCCTG GTACA CTGCC AGGC	57.6
rs7412- P2 probe	p-CTTCT GCAGG TCATC GGCAT TTT TTT TTT -Biotin	57.3
rs7412-F2 probe (3'N)	FAM-GCCTG GTACA CTGCC AGGC <u>G</u>	60.0