Supporting Information for:

Portable optical waveguide resonance light-scattering scanner for microarray detection

Xuefeng Xing[a], Wanyao Liu[b], Tao Li[a,*],Shu Xing[b], Xueqi Fu[b], Dongyang Wu[a], Dianjun Liu[a], and Zhenxin Wang[a,*]

[a]State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun, 130022, China,

[b]Edmond H. Fischer Signal Transduction Laboratory, School of Life Sciences, Jilin University, Changchun 130012, China.

[*]Corresponding author: Phone:(+86) 431-85262757. E-mail: litao@ciac.ac.cn.

Additional Experimental Section

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Additional Figures: S1-S13

Additional References

Additional Experimental Section

PCR amplification and sequencing analysis. Genomic DNAs were extracted from the whole blood with the Genomic DNA Extraction Kit (BioTeke). The DNA fragments from genomic DNAs containing 6 SNPs were amplified by polymerase chain reaction (PCR) with P_{fn} and P_{rn} (as shown in Table S1 and Table S2). The PCR was performed with Taq DNA polymerase (TaKaRa) for 35 cycles with 94 ℃ for 20 seconds, 58 ℃ for 20 seconds, and 72 ℃ for 20 seconds. The PCR products were purified from agarose gels with Multifunction DNA Purification Kit (Bioteke) and sequenced by use of the ABI 3730XL DNA analyzer at BioS Biotechnology (Harbin) Co., Ltd.

Preparation of DNA conjugated GNPs. Sigle-stranded (ss) DNAconjugated GNPs (ssDNA-GNPs) were prepared using a procedure as previously reported.[S1] Generally, the GNPs solution (8 nM, 600 μL) was incubated with 10 μL mixture of alkanethiol-modified ssDNA SP_1-SP_6 (the sequences were shown in Table S1, the ratio of GNPs and total ssDNA was 1:200; the proportion of SP_1-SP_6 in the ssDNA mixtures was 1.2:1.2:0.6:1:1.6:1.6, respectively) in aqueous solution overnight and diluted with equal volume PBS-Ⅰ (pH 7.5, 10 mM PB, 0.2 M NaCl). After further incubation for 10 h, the solution was evaporated to 100 μL by vacuum centrifugation. Excess ssDNA were removed by repeated centrifugation (9000 rpm, 3 times). Finally, the DNA-GNPs were dispersed in probe reaction buffer (1×SSC, 0.1% (w/v) SDS) and stored at 4 °C for further use.

Preparation of peptide-stabilized GNPs. The citrate stabilized 13 nm GNPs

were synthesized using the classical Turkevich-Frens method.[S2, S3] Peptide-stabilized GNPs were prepared using a peptide capping procedure as our previously reports.[S4-S6] Generally, an aqueous solution of peptide mixture (CALNN: CALNNGK(biotin)G) was added to the solution of 13 nm GNPs (2 mL, 3.8 nM) to give a final concentration of total peptide of 1.5 mM. The ratio of CALNN and CALNNGK(biotin)G in the mixture was 9:1. Excess peptides were removed by repeated centrifugation (8600 rpm, 3 times) using an Eppendorf centrifuge (Eppendorf, Germany). After centrifugation, the purified GNPs were re-suspended in PBS-Ⅱ (pH 7.5, 50 mM PB, 0.15 M NaCl) and stored at 4 °C.

Fabrication of peptide microarray. Peptide microarrays were manufactured by the standard procedure using a SmartArrayer 48 system (Capitalbio Ltd., China) according to the previously reported methods.[S4-S6] CALNNGK(biotin)G was spotted on aldehyde-treated glass microscope slides (Capitalbio Ltd., China) in a spotting buffer (PBS-Ⅲ (pH 8.5, 0.3M PB, 0.2M NaCl), 20 μg/mL BSA and 40% glycerol, v/v) at a series of desired concentrations. After an overnight incubation under vacuum at 30 ℃, the slide was quickly rinsed with 20 mL PBS-Ⅱ containing1%(w/v) BSA and 0.1% Tween-20 (v/v), and immersed in 30 mL blocking buffer (PBS-Ⅱ-containing 1% (w/v) BSA and 0.1 M ethanolamine) for 1 h to remove remaining free aldehyde groups. The array was incubated with avidin–fluorescein, which was diluted to the desired concentration with 20μL probe buffer (PBS-Ⅱ supplemented with 0.1% Tween-20 (v/v) and 1% BSA, w/v). Following 1 h incubation at 37 °C, the slide was rinsed with probe buffer and washed with 30 mL of

washing buffer-Ⅰ (PBS-Ⅱ supplemented with 0.1% Tween-20) for 3 min (3 times) and following rinsed with PBS-Ⅱ (3 times) and Milli-Q water (3 times) and dried by centrifugation.

Fabrication of protein microarray. 100 μg/mL protein A was spotted on aldehyde-treated glass microscope slides in a spotting buffer and processed as described for the peptide microarray. The slide was first incubated with biotin–IgG, which was diluted to the desired concentration with 20μL probe buffer. Following 1 h incubation at 37°C, the slide was subjected to a series of rinsing steps: (1) washing buffer-Ⅰ for 5 min (3 times); (2) PBS-Ⅱ for 5 min (3 times); (3) Milli-Q water for 5 min (3 times) and dried by centrifugation (480 g for 30 sec). The microarray was incubated with avidin–fluorescein and washed as described in the peptide microarray fabrication.

Attachment of ssDNA-GNPs. After the hybridization step, the DNA microarrays were incubated with 5 nM ssDNA-GNPs (20 μL hybridization buffer each subarray) at 30°C for 60 min and then washed with hybridization buffer, washing buffer and water as previously described.

Attachment of peptide-stabilized GNPs. After the incubation with avidin–fluorescein, the peptide microarrays and protein microarrays were treated with 200 μL peptide stabilized gold nanoparticles (5.0×10^{-9} M) solution in probe buffer for 1 h at 37 °C and washed as described before and dried by centrifugation (480 g for 30 sec).

Table S1 Oligonucleotide sequences of 6 SNPs associated with T2DM used in experiments. [a]

Name	Gene	SNP		Sequence(5'-3')
L1	TCF7L2	rs11196205	P_1	CTGCTCGTAGTTATAA(T_{10})-NH_2
			P_{1A}	CTGCTGGTAGTTATAA (T_{10})-NH_2
			T_1	TTATAACTACGAGCAGTATGTAAGAGAG
			T_{1A}	TTATAACTACCAGCAGTATGTAAGAGAG
			SP_1	(T_{10}) CTCTCTTACATA
L2	TCF7L2	rs11196218	P_2	TCGGGTGCTTATGAAA(T_{10})-NH_2
			P_{2A}	TCGGGTGTTTATGAAA(T_{10})-NH_2
			T_2	TTTCATAAGCACCCGAGAAGGTTTAAAT
			T_{2A}	TTTCATAAACACCCGAGAAGGTTTAAAT
			SP_2	(T_{10}) ATTTAAACCTTC
L3	TCF7L2	rs6585205	P_3	TCTAAGTCGTGGGGCA (T_{10})-NH_2
			P_{3A}	TCTAAGTAGTGGGGCA(T_{10})-NH_2
			T_3	TGCCCCACGACTTAGATGAAACCAGGAA
			T_{3A}	TGCCCCACTACTTAGATGAAACCAGGAA
			SP_3	(T_{10}) TTCCTGGTTTCA
L4	CDKAL1	rs10946398	P_4	GACAGCATAACGATAC(T_{10})-NH_2
			P_{4A}	GACAGCAGAACGATAC(T_{10})-NH_2
			T_4	GTATCGTTATGCTGTCATTGCATCAAGT
			T_{4A}	GTATCGTTCTGCTGTCATTGCATCAAGT
			SP_4	(T_{10}) ACTTGATGCAAT
L5	CDKM2A/2B	rs10811661	P_5	TCATGAGAAAACTAAA (T_{10})-NH_2
			P_{5A}	TCATGGGAAAACTAAA (T_{10})-NH_2
			T_5	TTTAGTTTTCCCATGACAGTAAGTCTAT
			T_{5A}	TTTAGTTTTCTCATGACAGTAAGTCTAT
			SP_5	(T_{10}) ATAGACTTACTG
L6	TCF7L2	rs7903146	P_6	ATATAGTATCTAAAAA(T_{10})-NH_2
			P_{6A}	ATATAATATCTAAAAA(T_{10})-NH_2
			T_6	TTTTTAGATACTATATAATTTAATTGCC
			T_{6A}	TTTTTAGATATTATATAATTTAATTGCC
			SP_6	(T_{10})GGCAATTAAATT

[a] P_n and P_{nA} were the probe ssDNAs immobilized on the microarray; T_{nA} was the risk target ssDNA and T_n was the wild target ssDNA; SP_n was the ssDNA modified GNPs.

Table S2. Oligonucleotide sequences of the primers of PCR amplification used in experiments.

Name	Sequence(5’-3’)
Pf_1	CCCAGGACAAAAGGTAGAAAAG
Pr_1	TAAAATCATTCCAACCATAACTCTC
Pf_2	GCCATTTCAATTTCGTACATTTTT
Pr_2	CAAACGAGGGCTAATTTATGAGAA
Pf_3	AGATTTTTAAGATTTGGGCATAATG
Pr_3	CGGCTTGGATGCTCTCCTCTTG
Pf_4	AGGTTGAACTGGTTTTTCCTCTTT
Pr_4	GACAAGTGTTCTGATATTAATGCAATTA
Pf_5	CCCATTTTCTTTGTCAATAAGCGTTC
Pr_5	CAGTAAAGTCAAAAACCTTCCCCATC
Pf_6	GCTTTCTCTGCCTCAAAACCTA
Pr_6	ACTTGCCTTCCCTGTAACTGTG

Table S3. Quantitative detection of 6 SNPs associated with T2DM by PW-RLS scanner.

Target ssDNA	Detection limit (nM)	dynamic range (nM)	R^2	Allele frequency
T_1	0.05	0.05-50	0.984	0.5%
T_2	0.05	0.05-50	0.990	0.5%
T_3	0.01	0.01-50	0.994	0.2%
T_4	0.01	0.01-50	0.990	0.2%
T_5	0.05	0.05-50	0.989	1%
T_6	0.05	0.05-50	0.991	1%
T_{1A}	0.01	0.05-100	0.995	0.2%
T_{2A}	0.01	0.05-100	0.998	0.5%
T_{3A}	0.005	0.01-100	0.990	0.5%
T_{4A}	0.005	0.05-100	0.980	0.2%
T_{5A}	0.05	0.05-100	0.997	1%
T_{6A}	0.05	0.05-100	0.999	1%

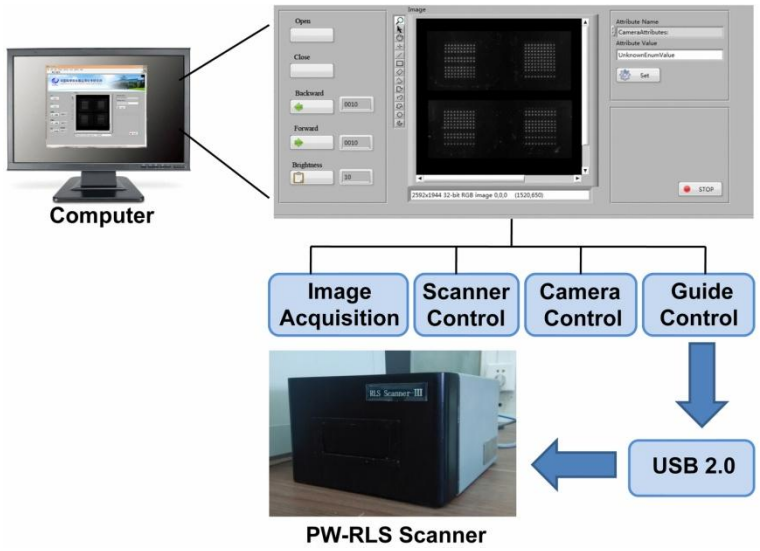


Fig. S1. The block diagram of soft ware and communication protocol.

A Home-written PC software including image acquisition, scanner control, camera control and guide control was used to operate the PW-RLS scanner. The image acquisition area was responsible for previewing microarray information and positioning specific area for high precision scanning. Scanner control area was used to definite CCD position and image acquisition. The camera control region was used to set the exposure time and gain compensation of the camera. The guide control area provides provided real-time control of the rail. In addition of automatic scanning function, the electric rail could also be moved according to the instructions of the users. USB2.0 was used as communication protocol to output signal and control the dialogue between software and hardware by the chip CY7C68013 in the First Input First Output (FIFO) mode.

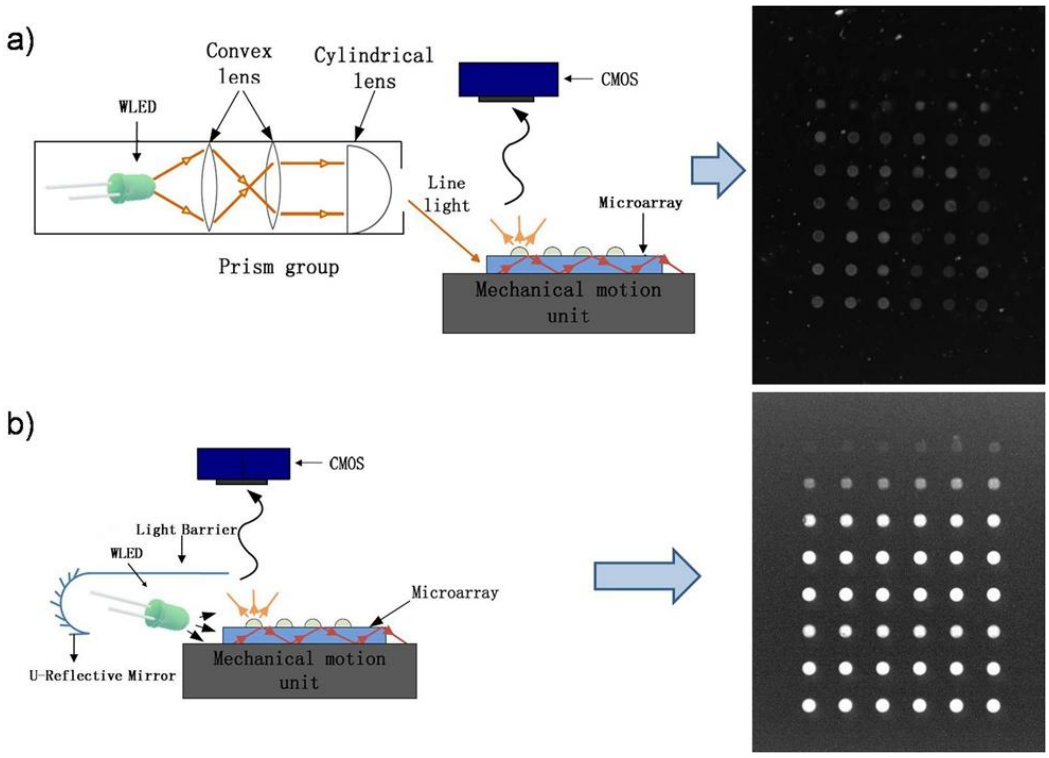


Fig. S2. RLS images and corresponding optical structure of PW-RLS scanner with (a) coupling lens and (b) U-reflective mirror and light barrier.

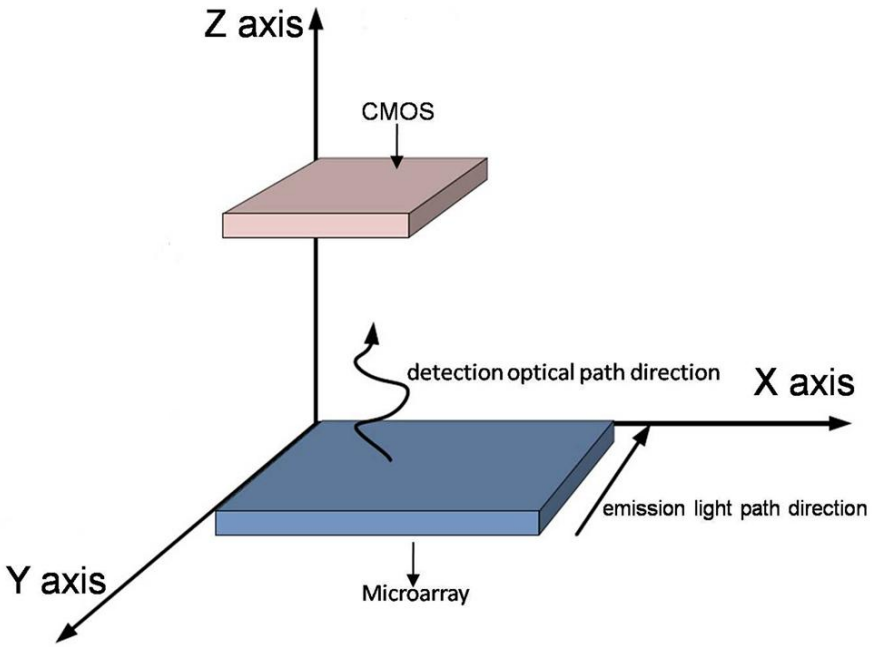


Fig. S3. The directions of the detection of optical path and the emission light path. The detection of optical path is perpendicular to the microarray and the emission light path is parallel to the microarray.

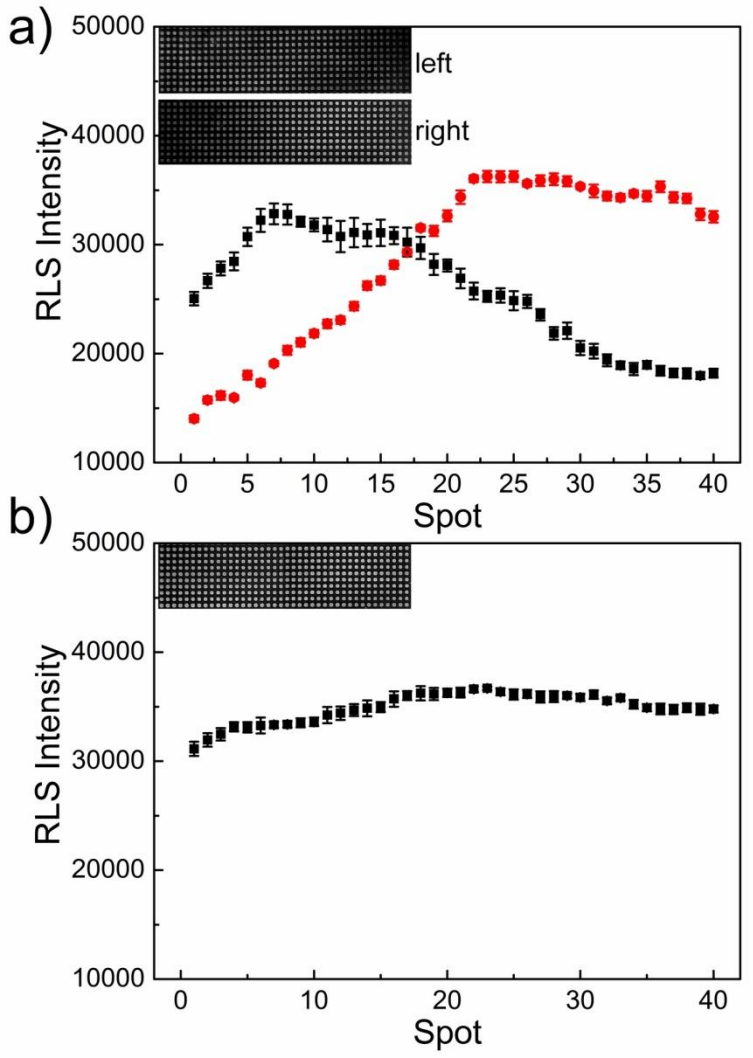


Fig. S4. RLS images and corresponding RLS intensities of 40×10 spots on the DNA microarray detected by PW-RLS scanner with (a) light incident from left (■) /right (●) side of the microarray slide and (b) light incident from both sides of the microarray slide.

According to the detection results, the non-uniform RLS intensity caused by one side incident can be minimized significantly by the way of both sides incident.

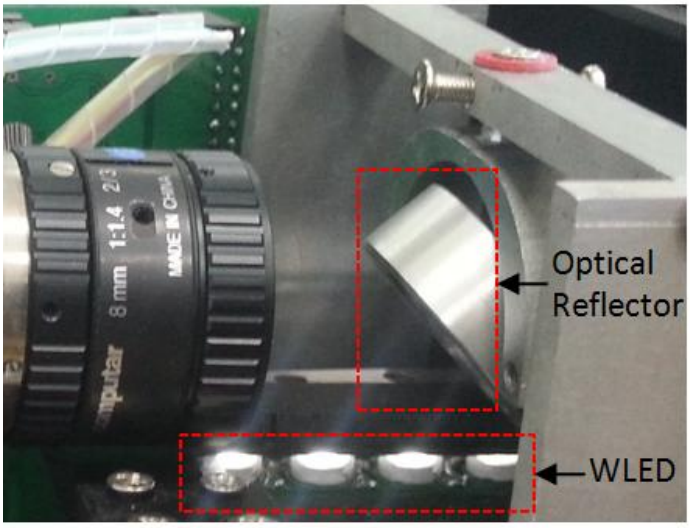


Fig. S5. The photograph of WLEDA assembled with optical reflector to generate the 90 °folded imaging optical path.

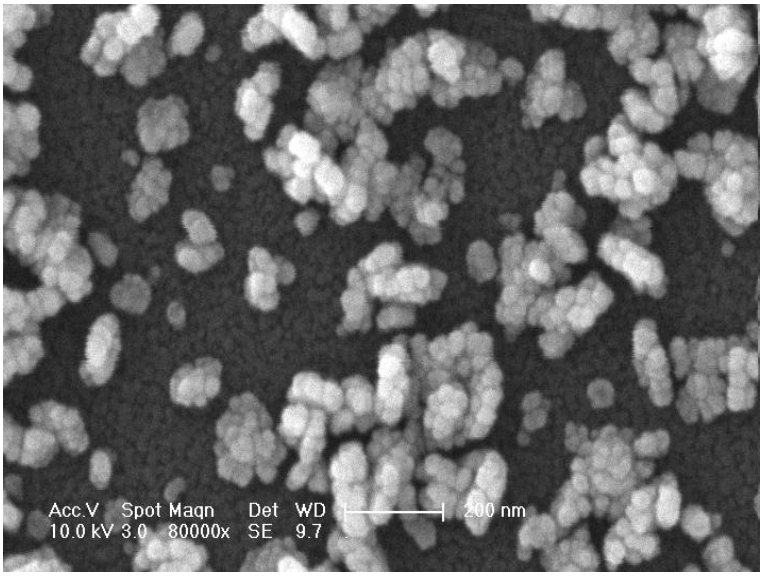


Fig. S6. Scanning electronic microscopy (SEM) micrographs of GNPs. The scale bar is 200 nm and the diameter of GNPs is about 60 nm.

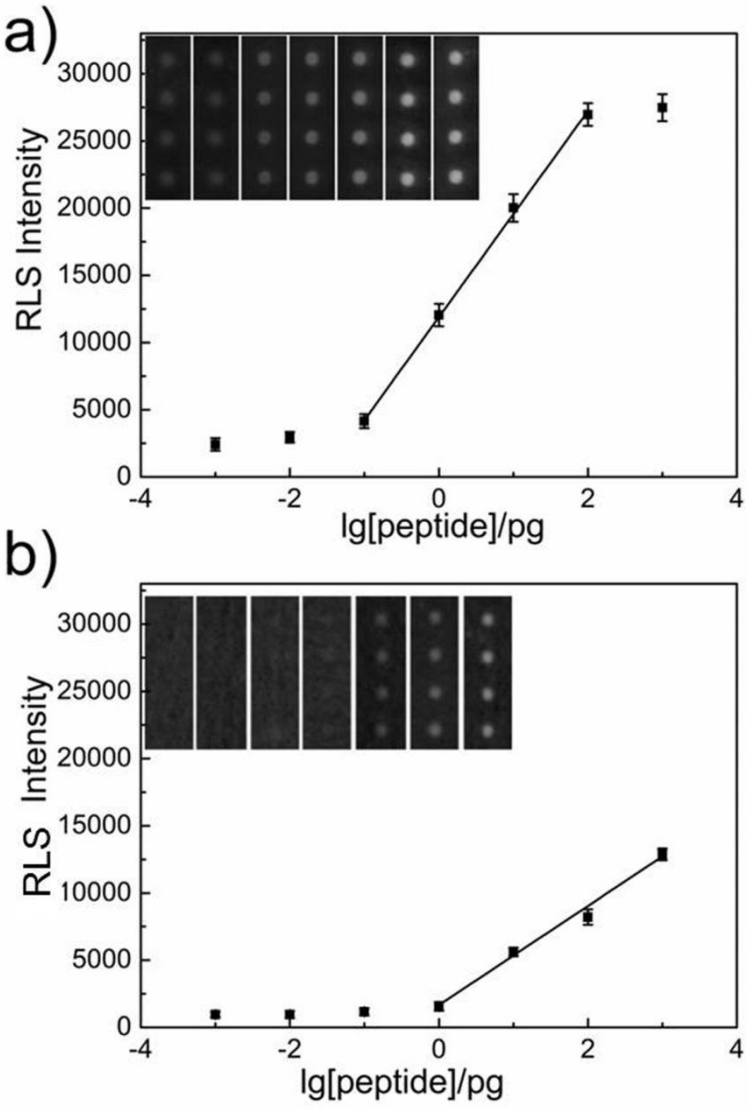


Fig. S7. Logarithmic plot of (a) PW-RLS scanner and (b) commercial scanner signal intensity as a function of the concentration of peptide on the microarray spots. The insets were corresponding images of microarrays.

The detection limit for the immobilized peptides of the PW-RLS scanner (100 fg) is one order of magnitude more sensitive than that of commercial scanner (1 pg).

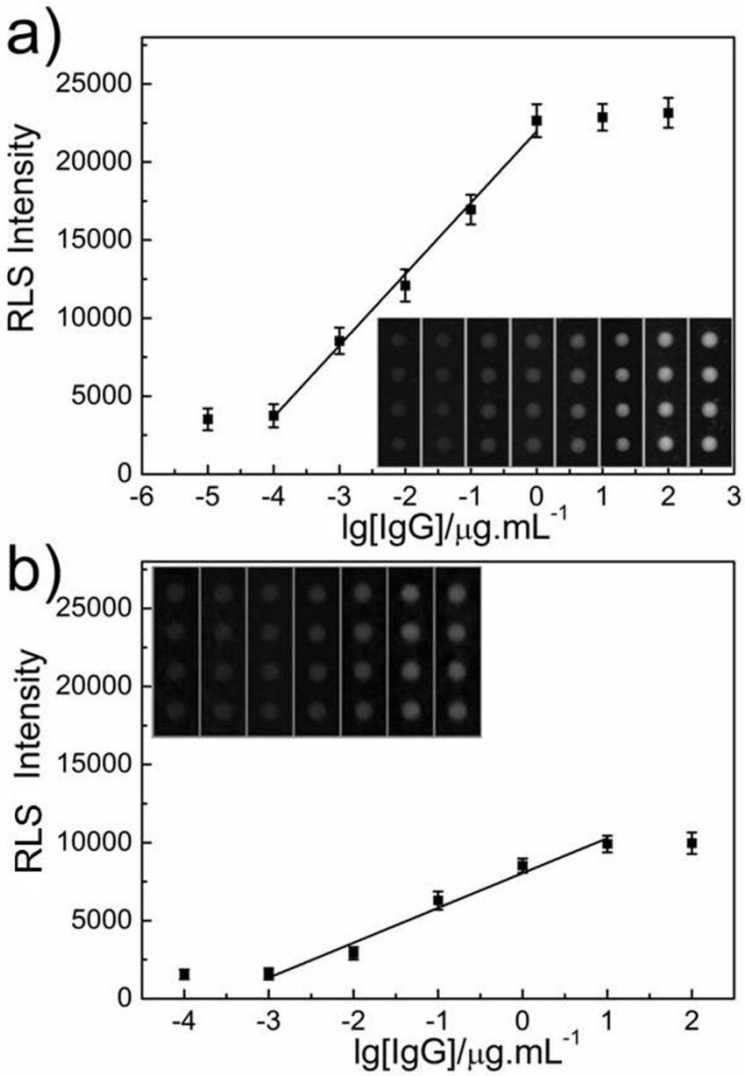


Fig. S8. Logarithmic plot of (a) PW-RLS scanner and (b) commercial scanner signal intensity as a function of the concentration of IgG in the probe solution. The insets were corresponding images of microarrays. The protein A concentration in the spotting solution was 100 μg/mL.

The detection limit for IgG in the solution of the PW-RLS scanner (0.1 ng/mL) was one order of magnitude more sensitive than that of commercial scanner (1ng/mL).

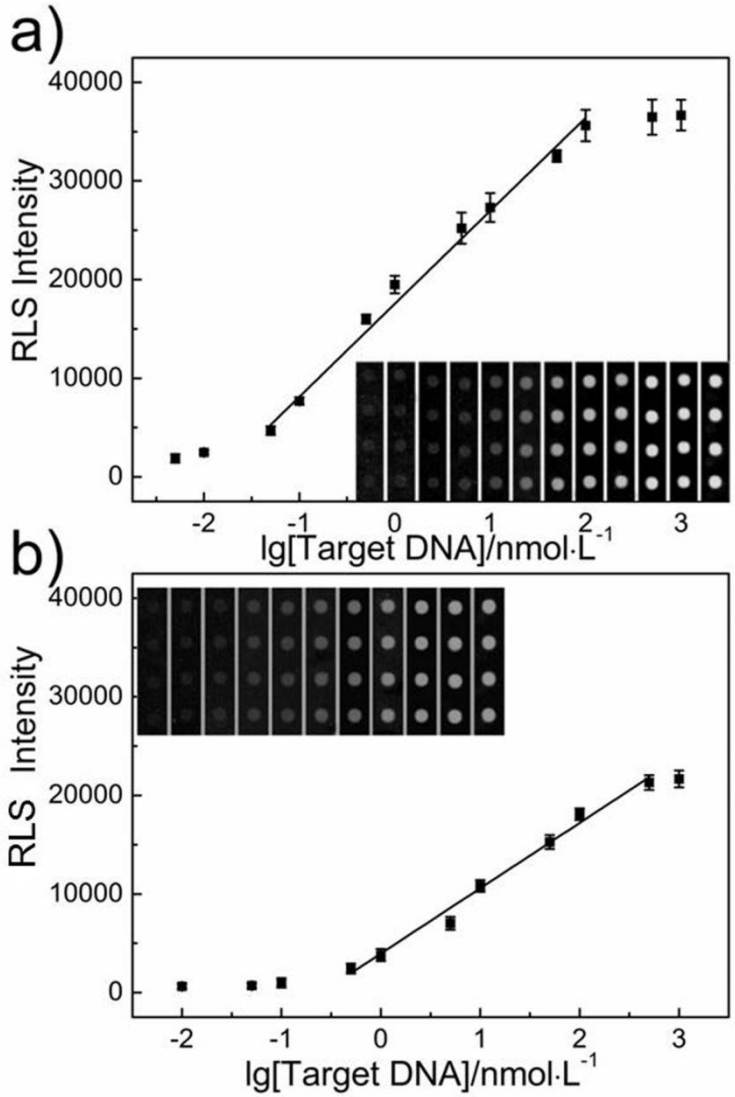


Fig.S9. Logarithmic plot of (a) PW-RLS scanner and (b) commercial scanner signal intensity as a function of the concentration of target ssDNA (T_{3A}) in the probe solution. The insets were corresponding images of microarrays. The probe ssDNA (P_{3A}) concentration in the spotting solution was 30μM.

The detection limit for target ssDNA in the solution of the PW-RLS scanner (0.005 nM) was two orders of magnitude more sensitive than that of commercial scanner (0.5 nM).

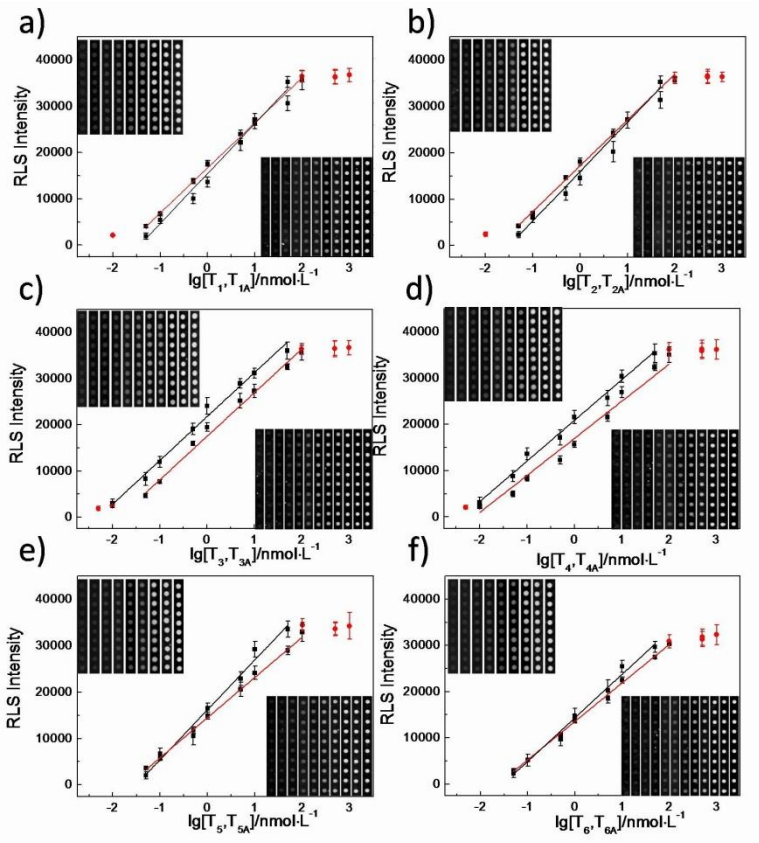


Fig. S10. RLS images and the corresponding logarithmic plots of the integrated RLS intensity as a function of the concentration of target ssDNAs T_n (●) and T_{nA} (■) in the solution. (a)-(f) represent 6 SNPs association for T2DM. The probe ssDNAs concentration in the spotting solution was 30 μM. The target ssDNAs concentration in the solution was 0.01 nM, 0.05 nM, 0.1 nM, 0.5 nM, 1 nM, 5 nM, 10 nM, 50 nM, 100 nM, 500 nM and 1000 nM, respectively.

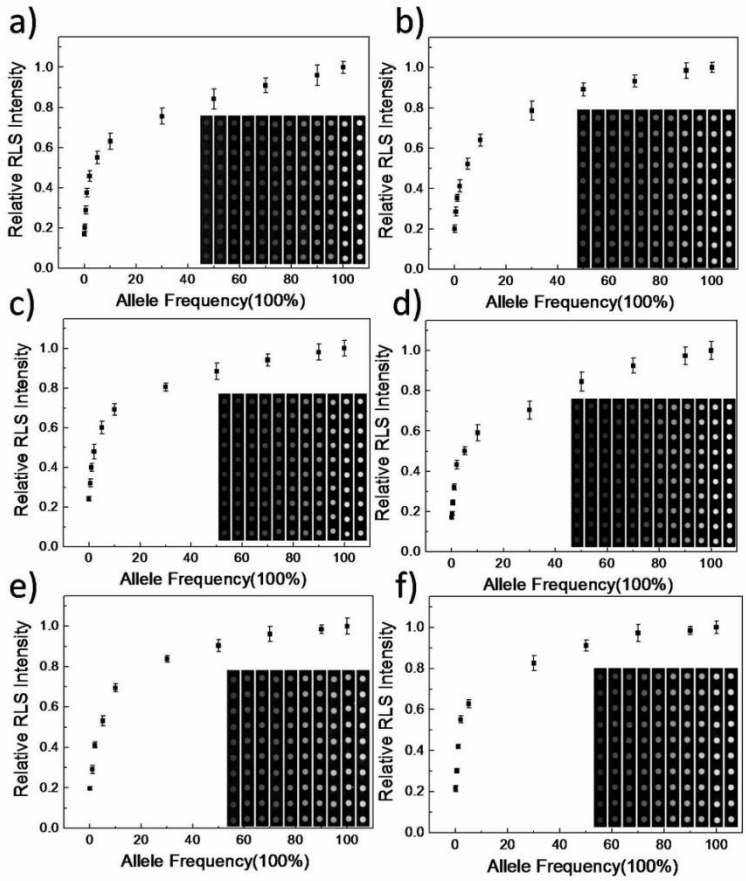
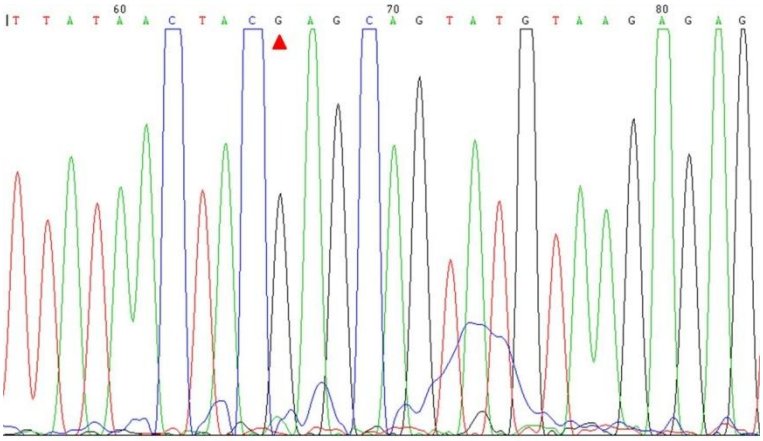
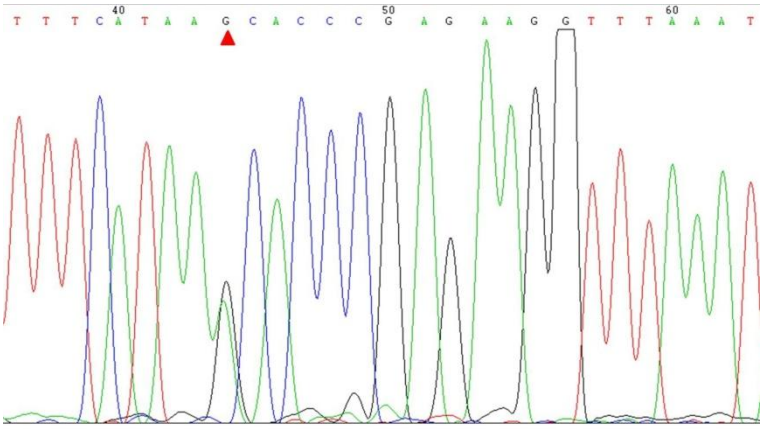


Fig. S11. The allele frequency and the corresponding RLS images of (a)-(f) 6 SNPs associated with T2DM. The probe ssDNAs concentration in the spotting solution was 30 μM. The proportion of T_{nA} in 2.5 pM mixture of T_n and T_{nA} was 2%, 5%, 10%, 30%, 50%, 70%, 90% and 100%, respectively.



S_1-L1



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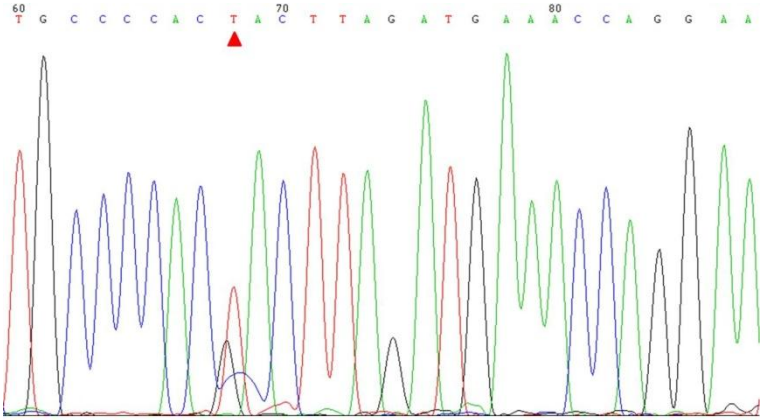

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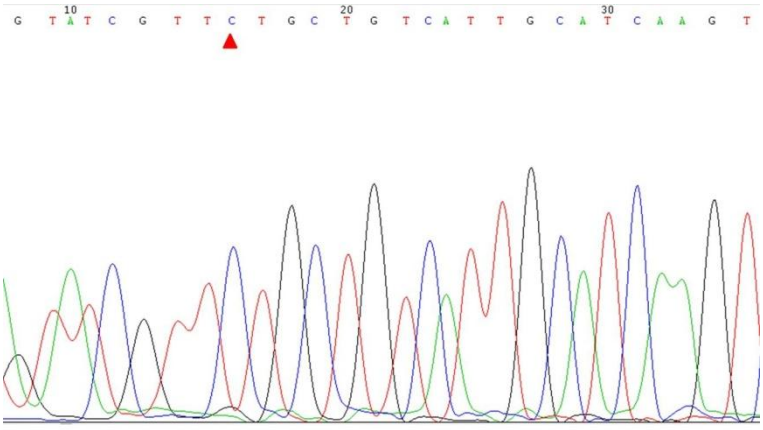

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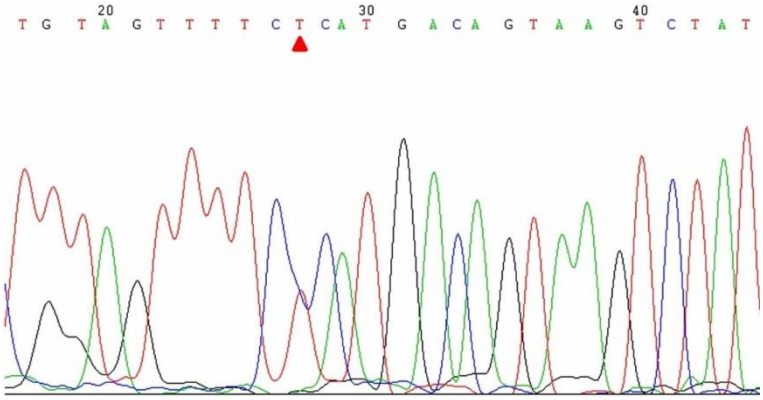

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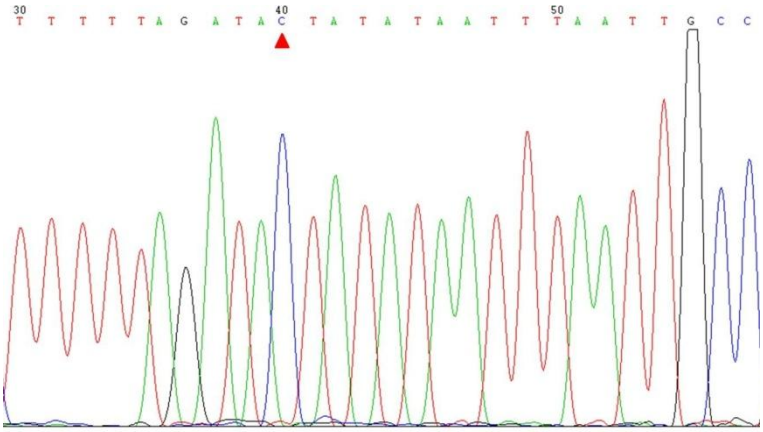

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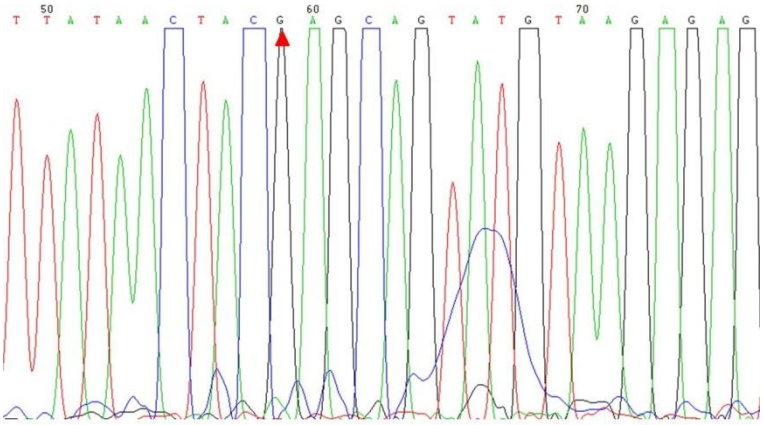

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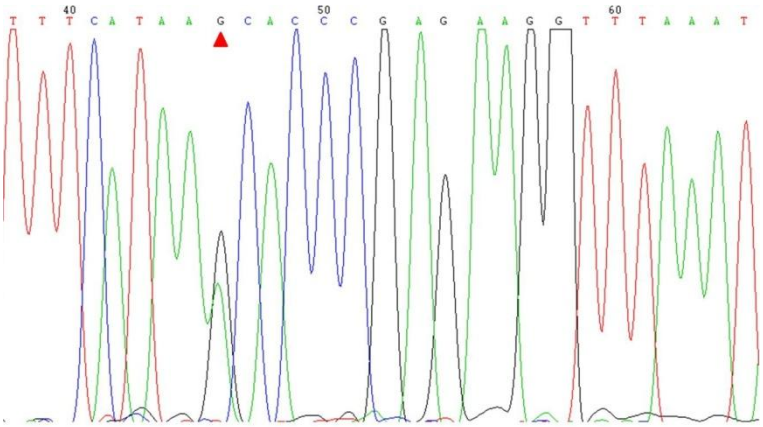

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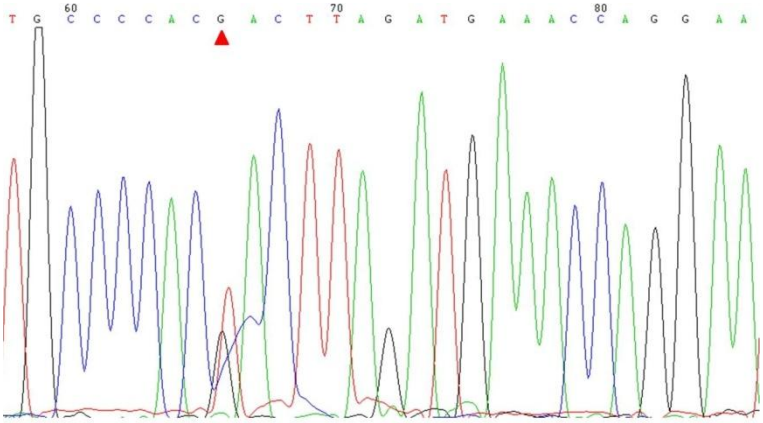

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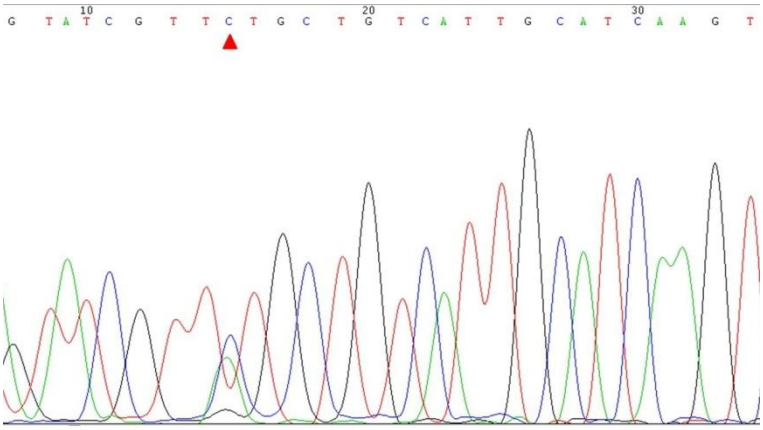

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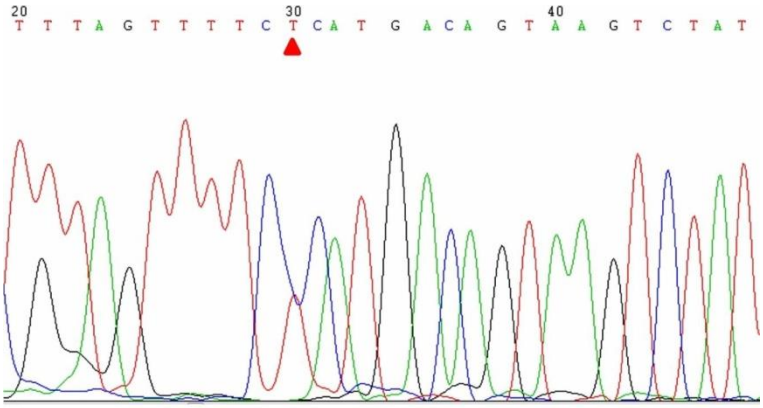

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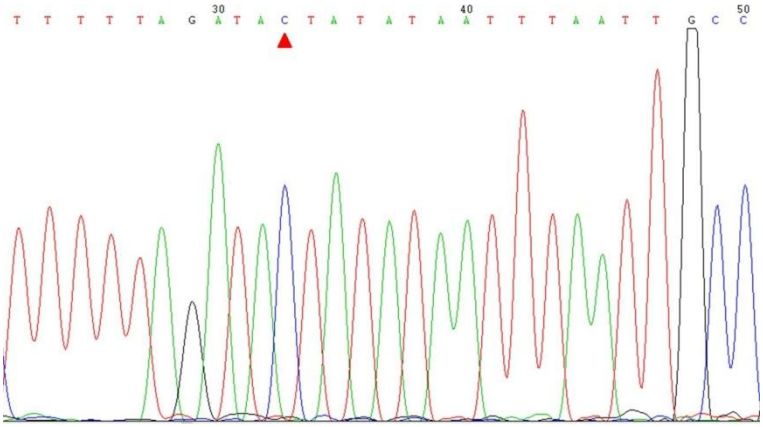

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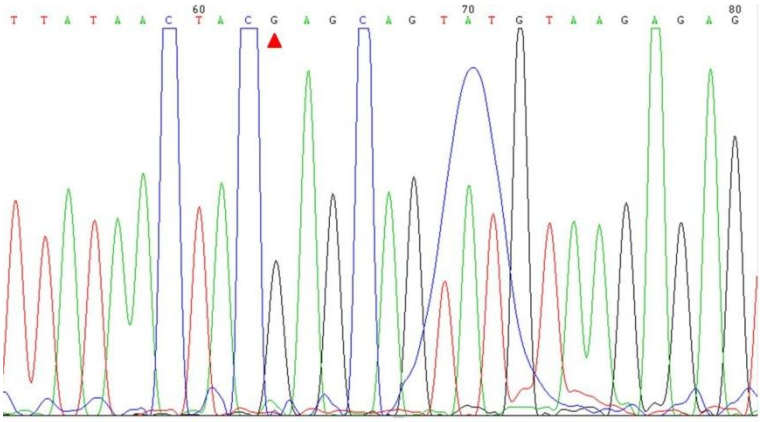

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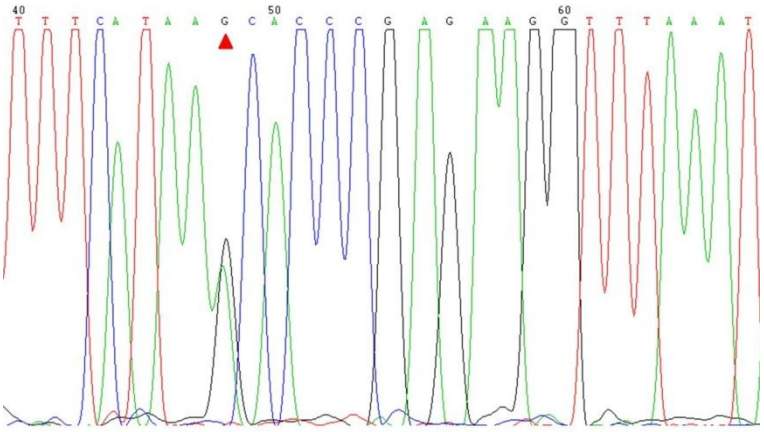

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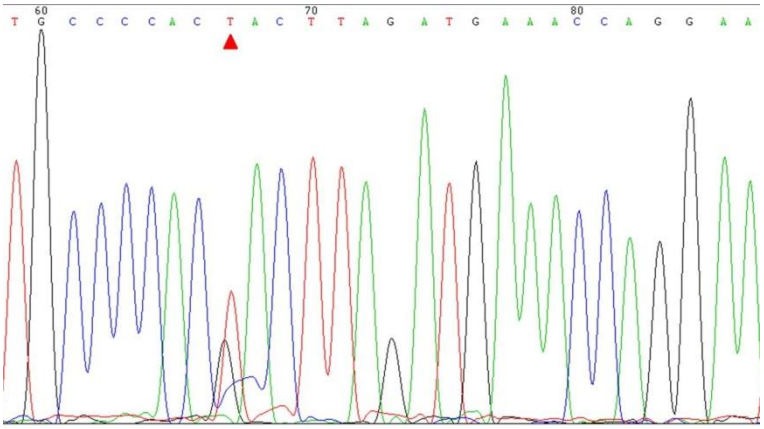

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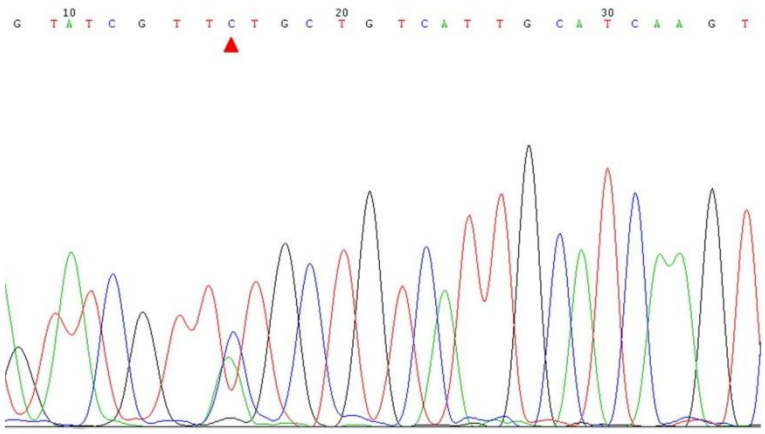

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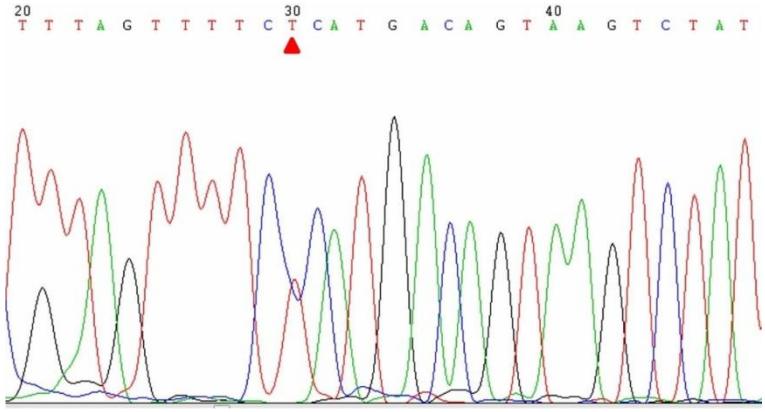

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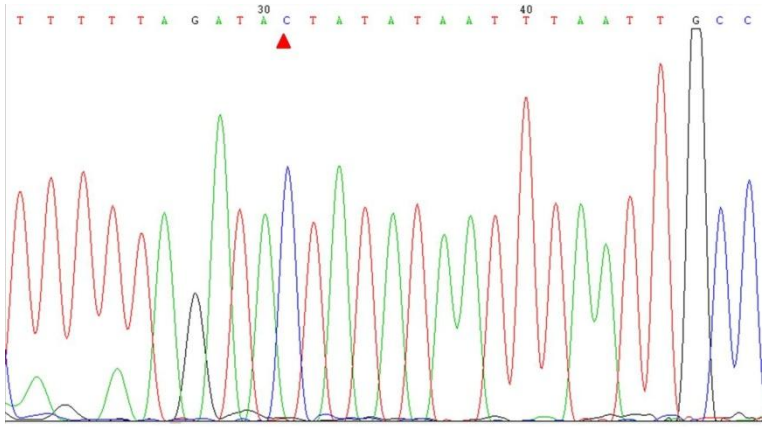

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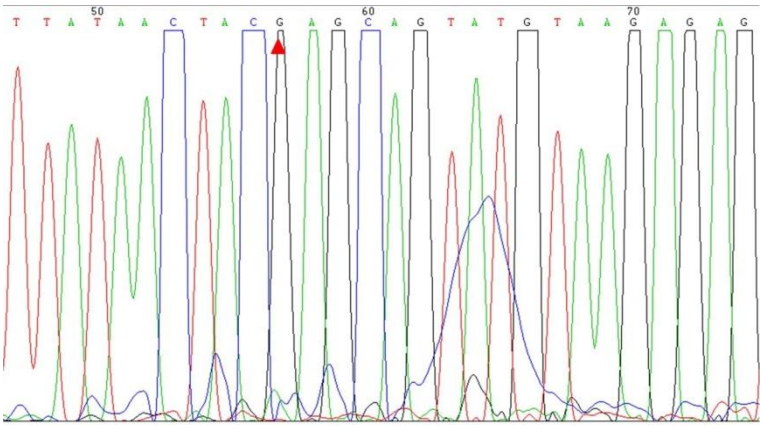

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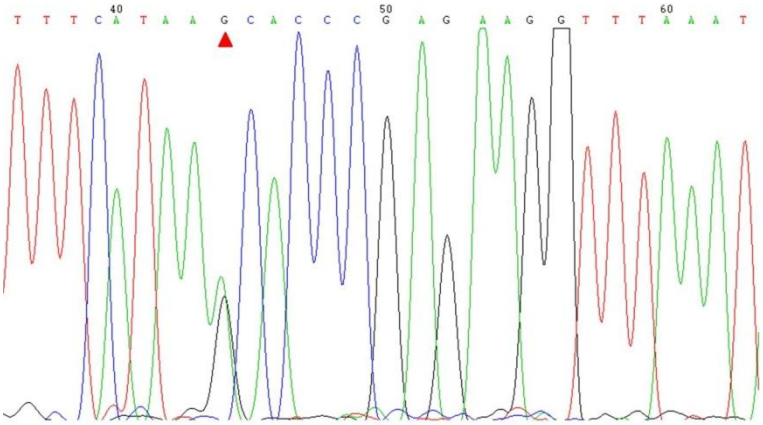
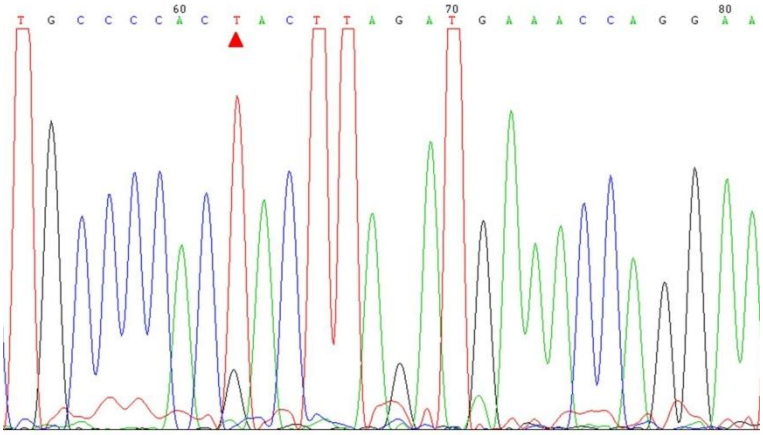


S_4-L2



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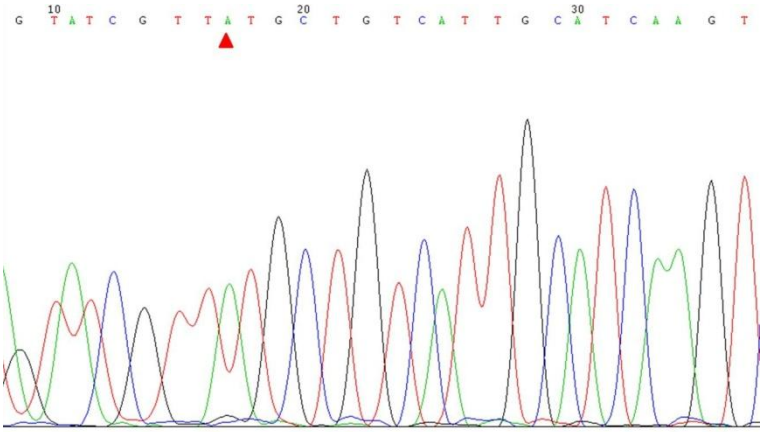
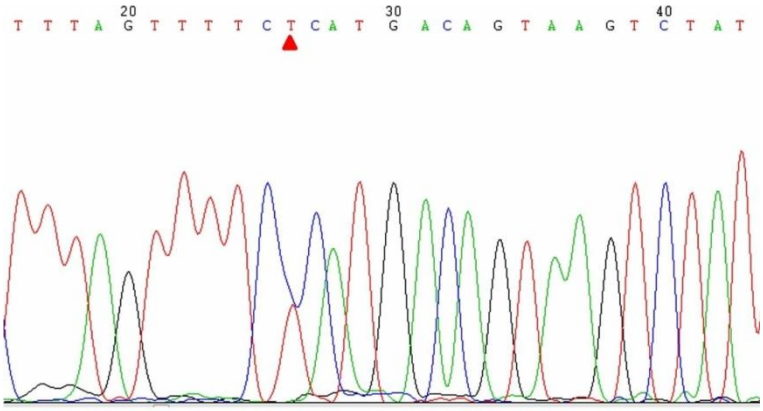
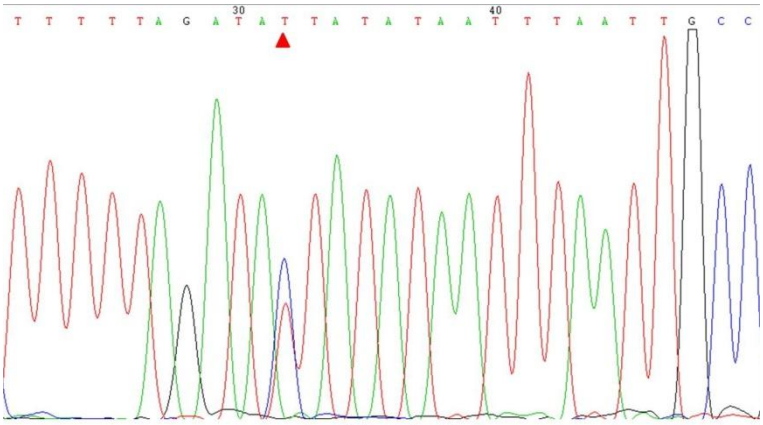


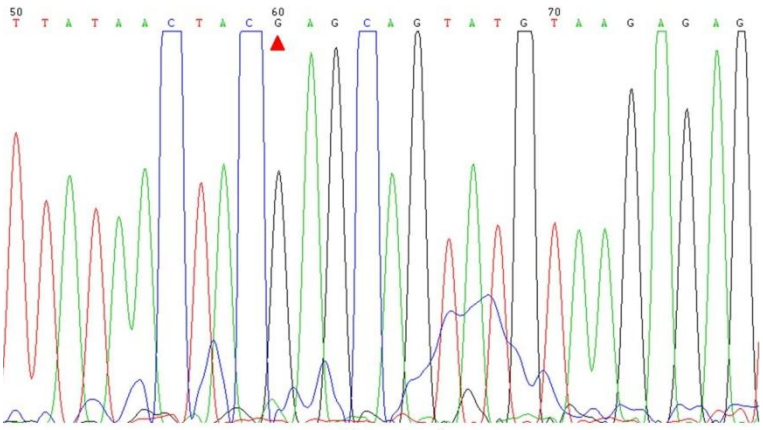
S_4-L4



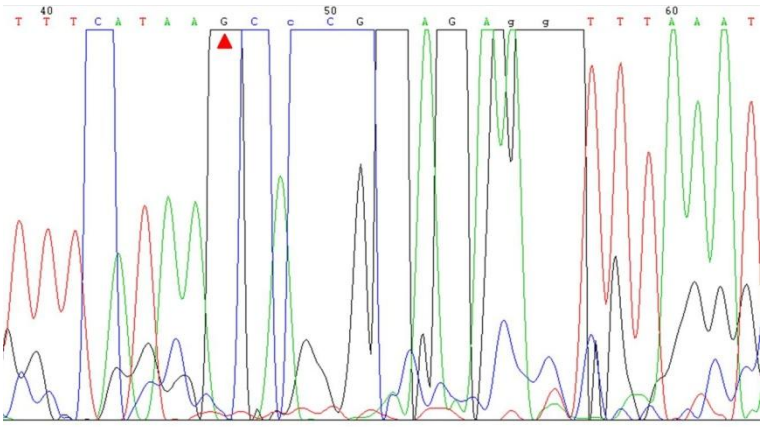
S_4-L5



S_4-L6



S_5-L1



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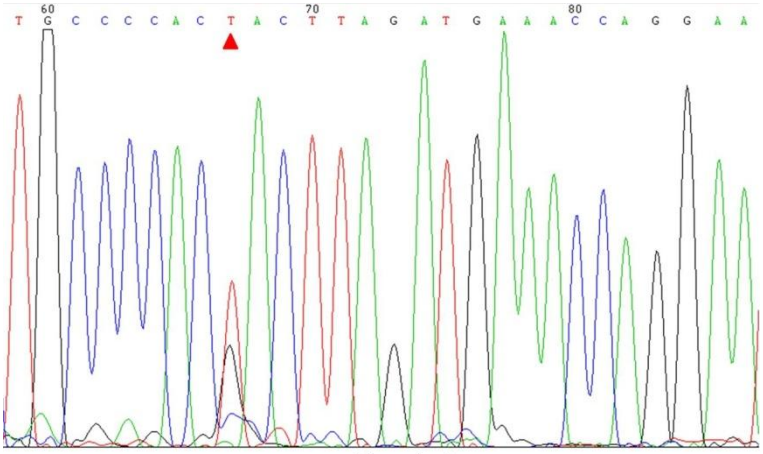

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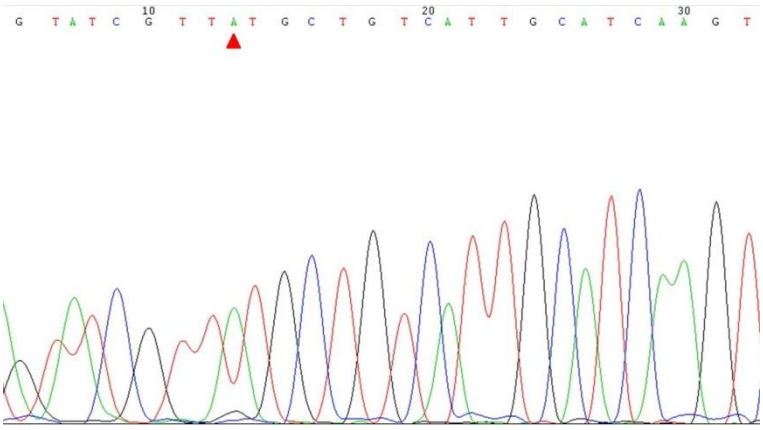

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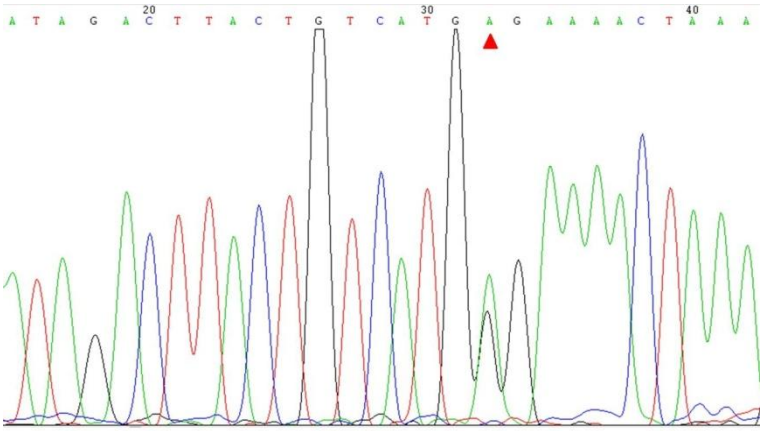
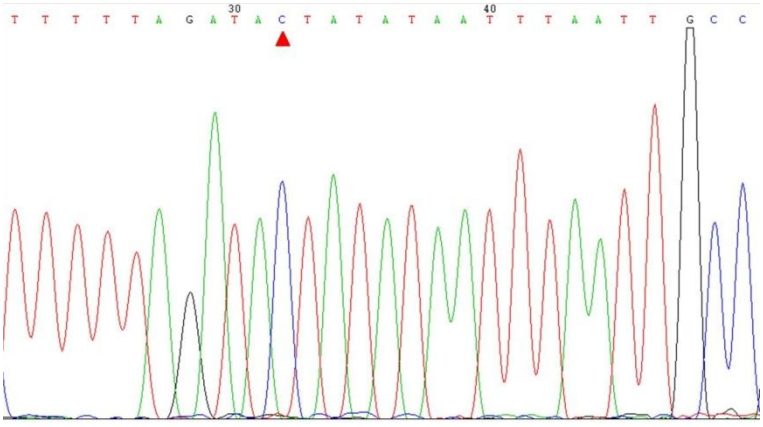
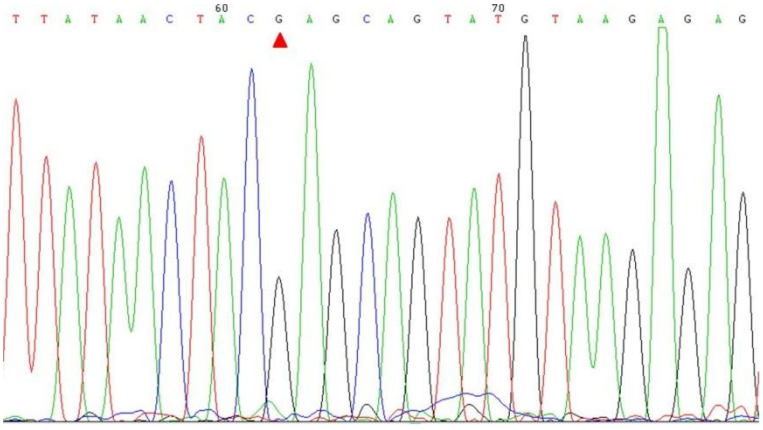


S_5-L5(R)



S_5-L6



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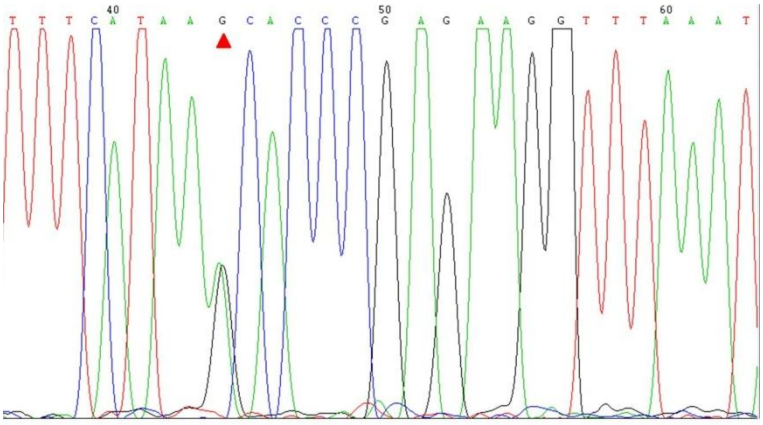

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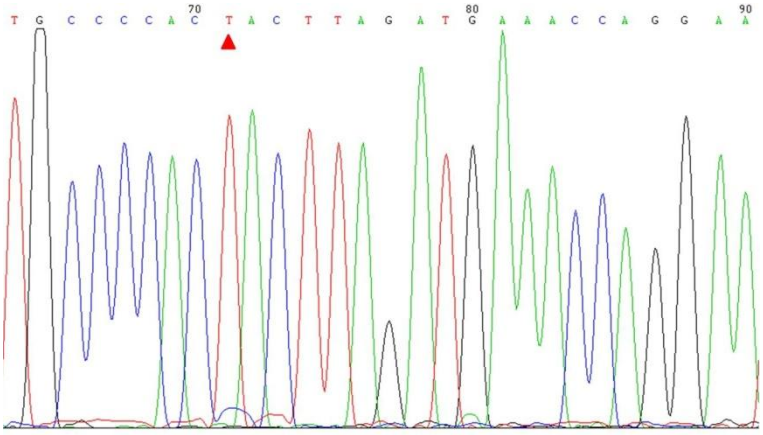

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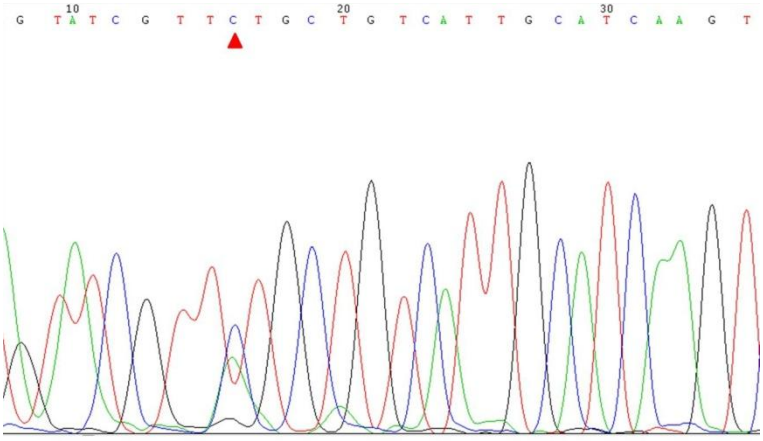

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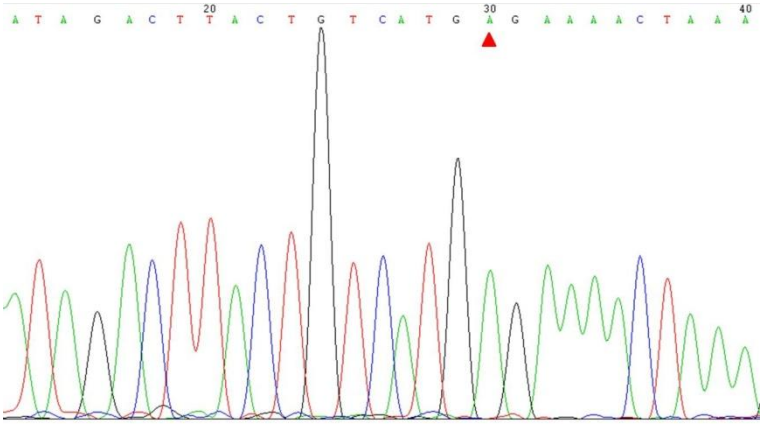
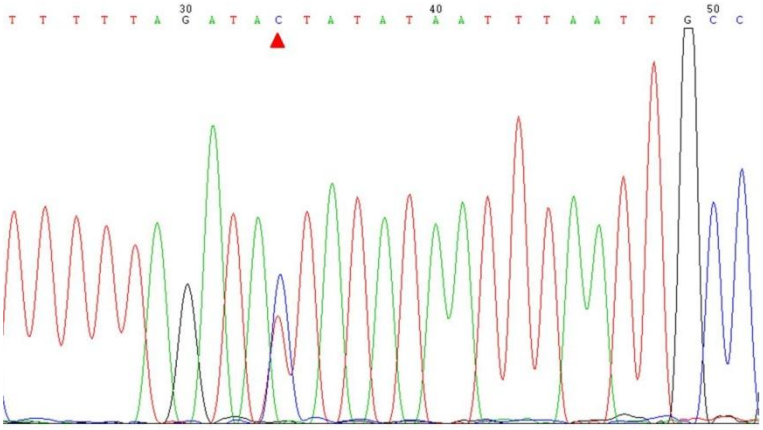


S_6-L5(R)



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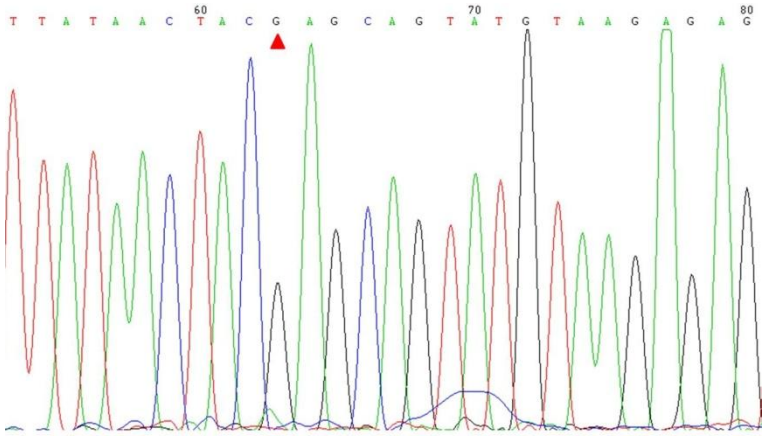

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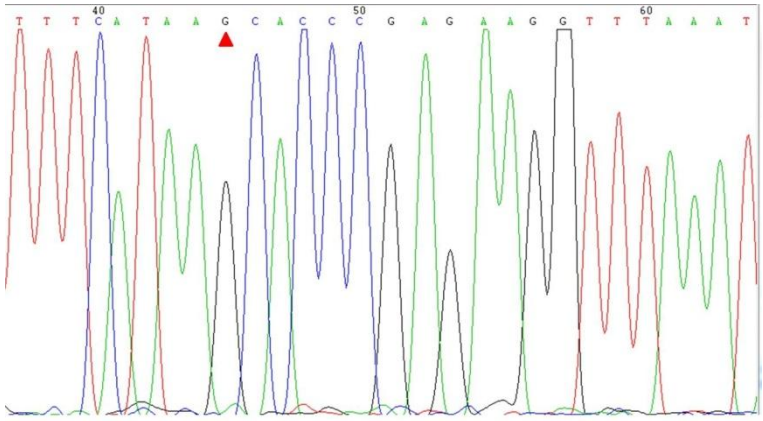

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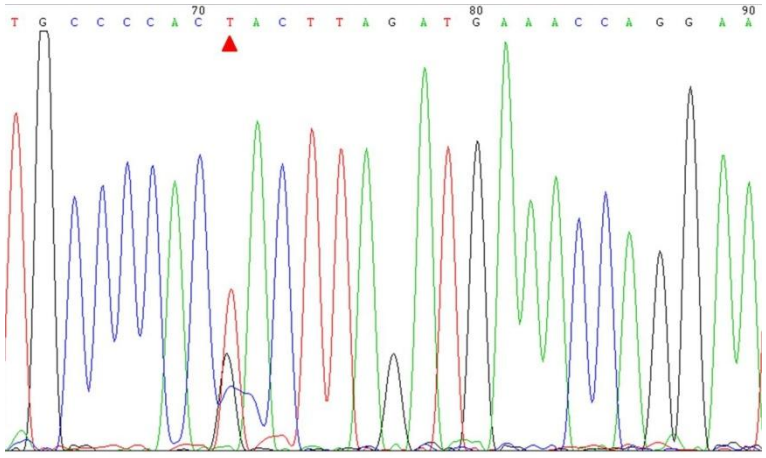

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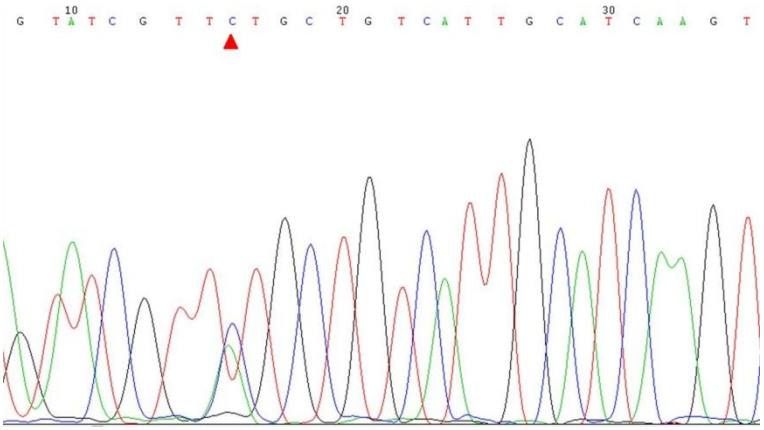

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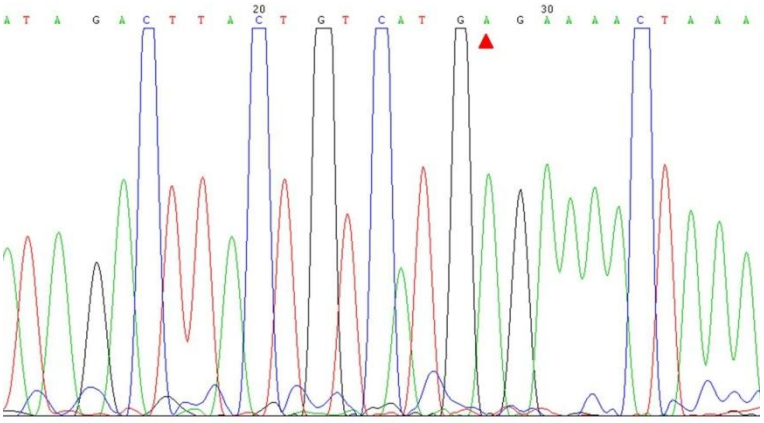

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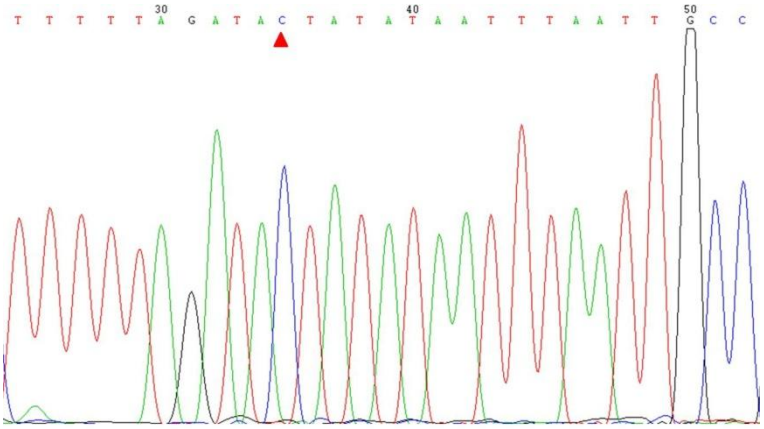
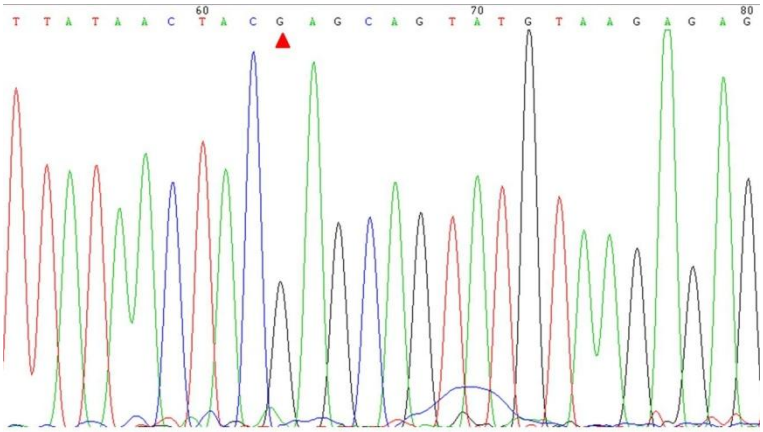


S_7-L6



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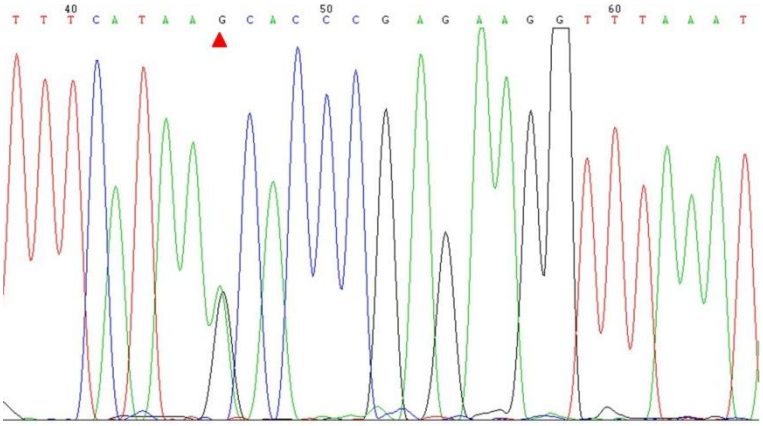

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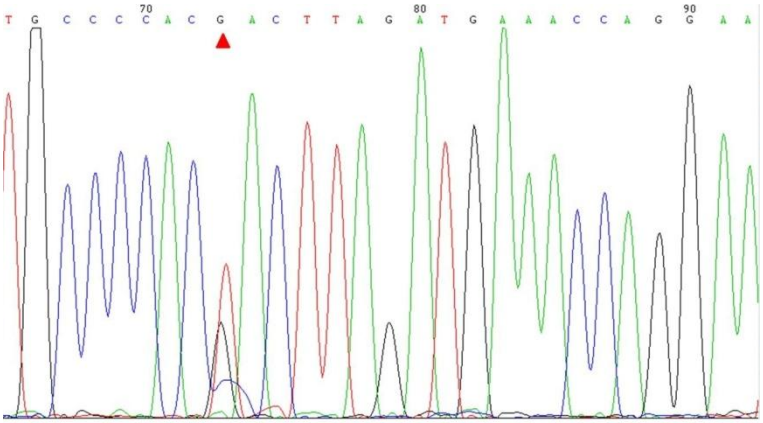

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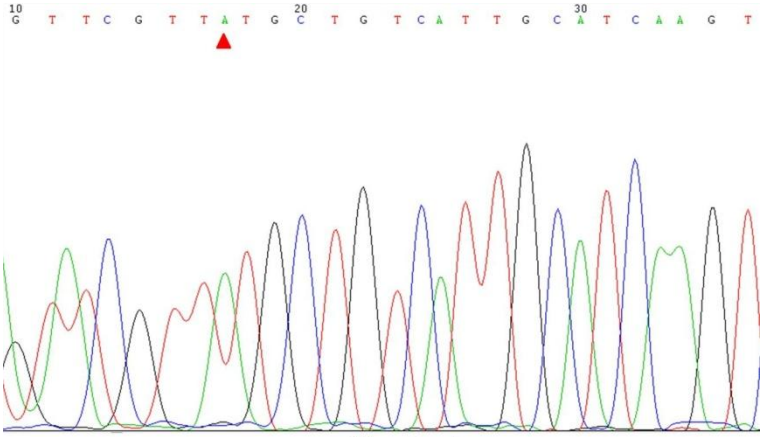

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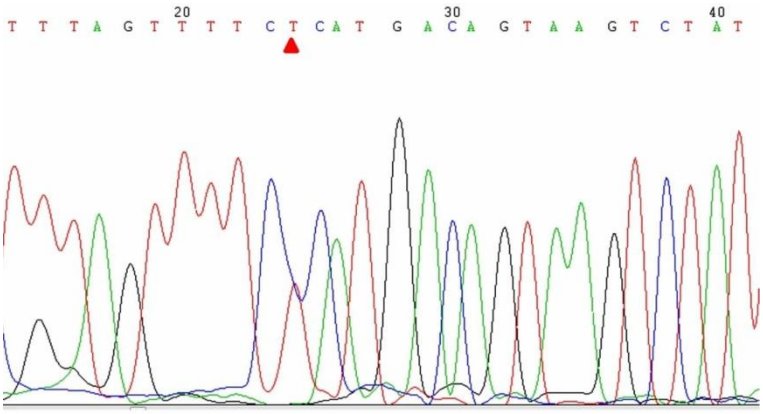

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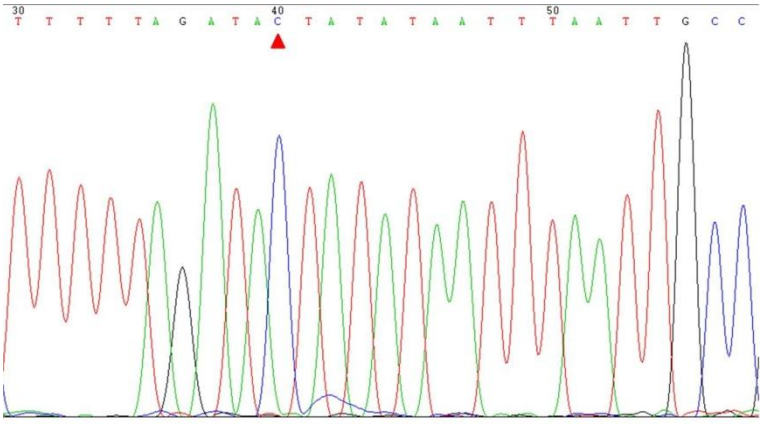

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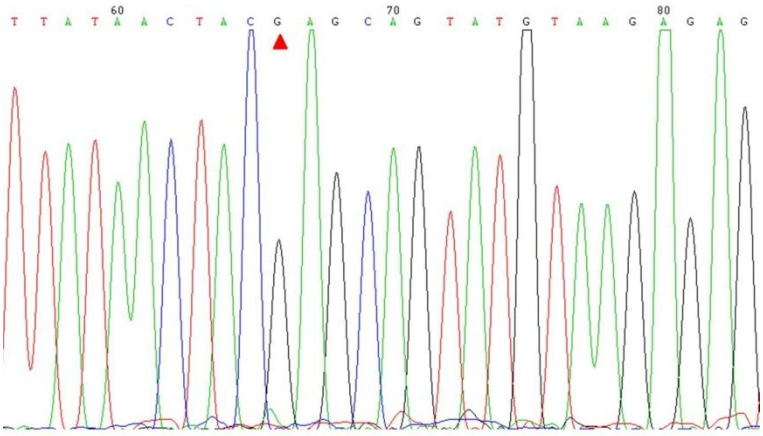
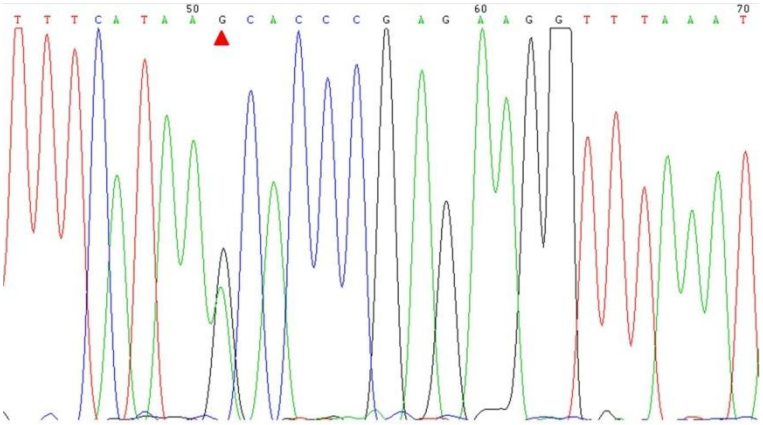


S_9-L1



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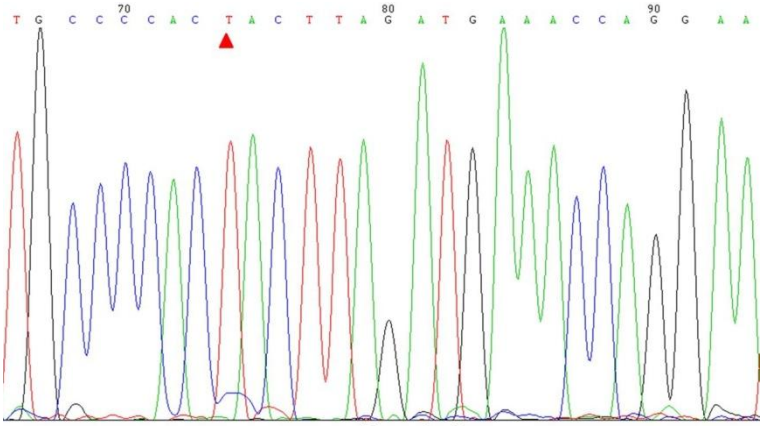

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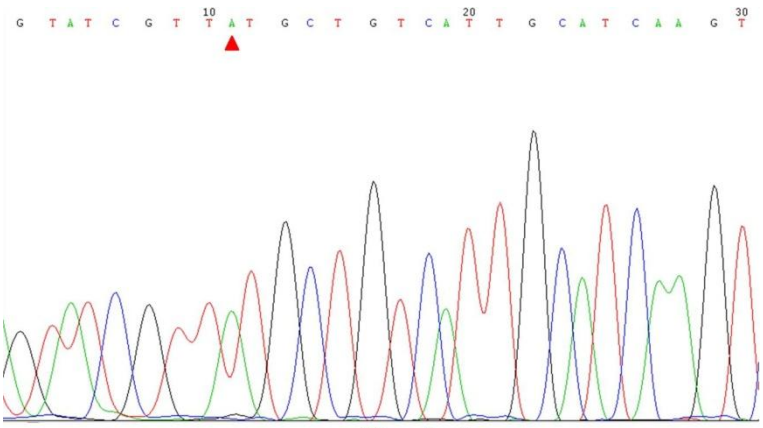

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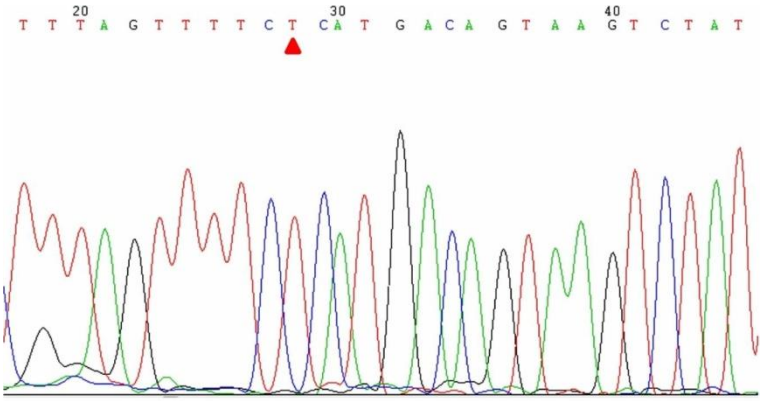

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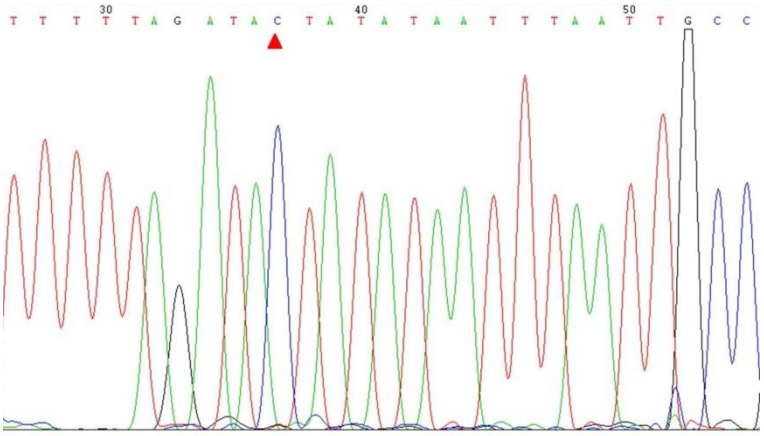
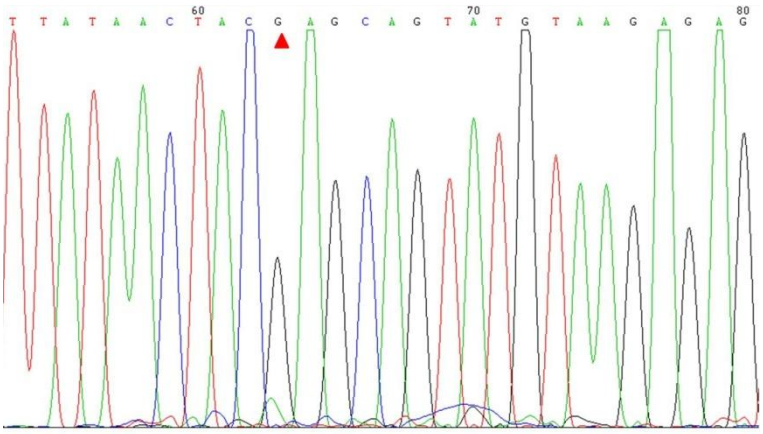
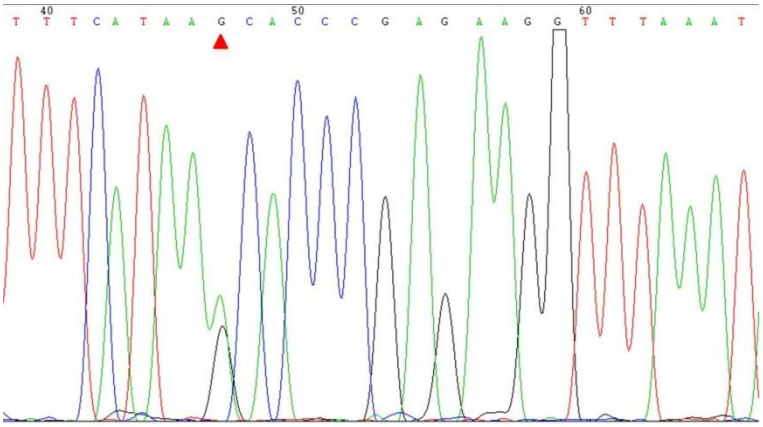


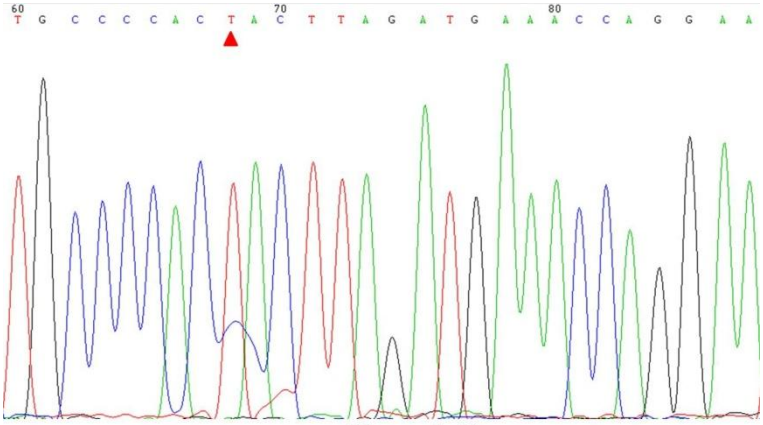
S_9-L6



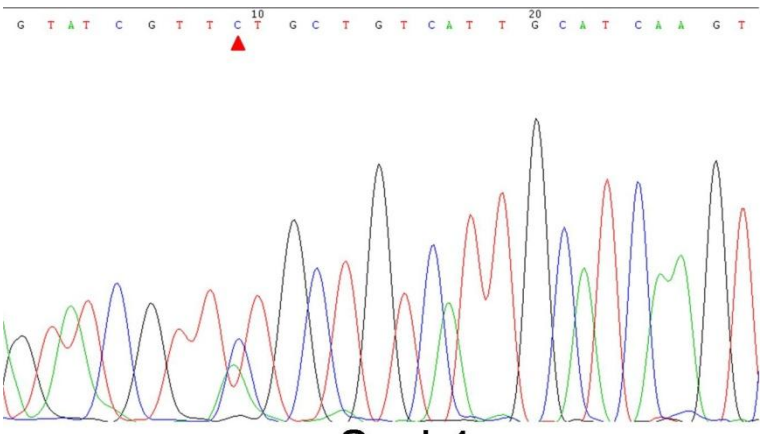
S_{10}-L1



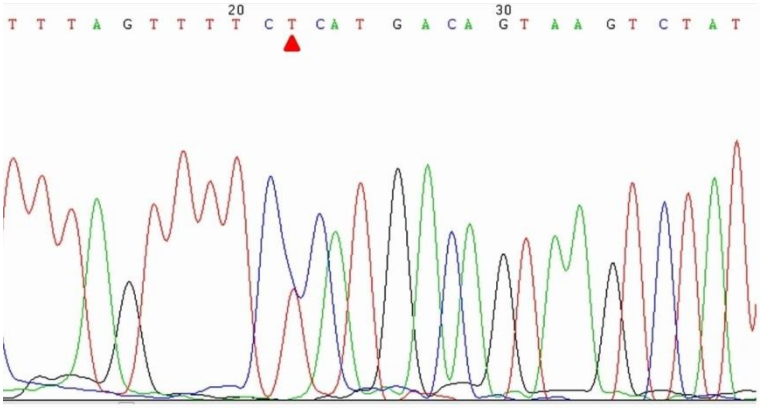
S_{10}-L2



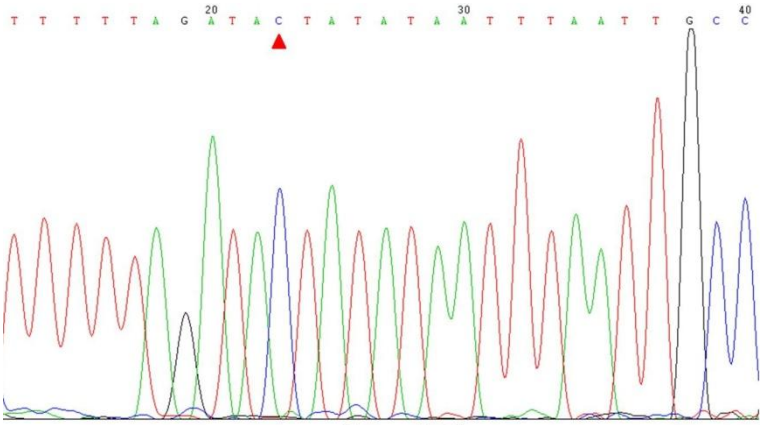
S_{10}-L3



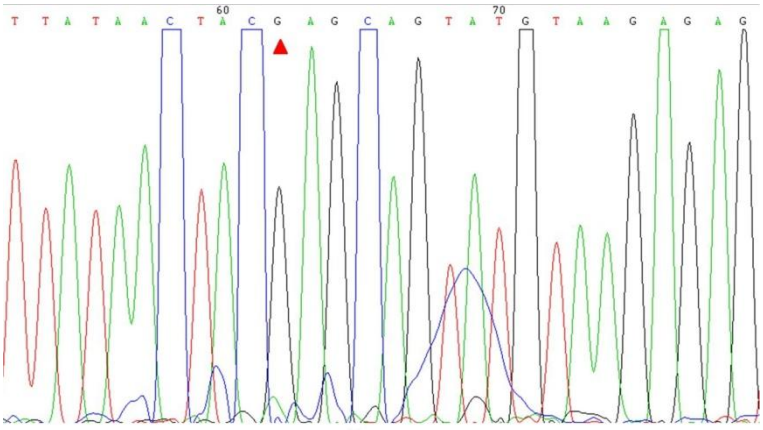
S_{10}-L4



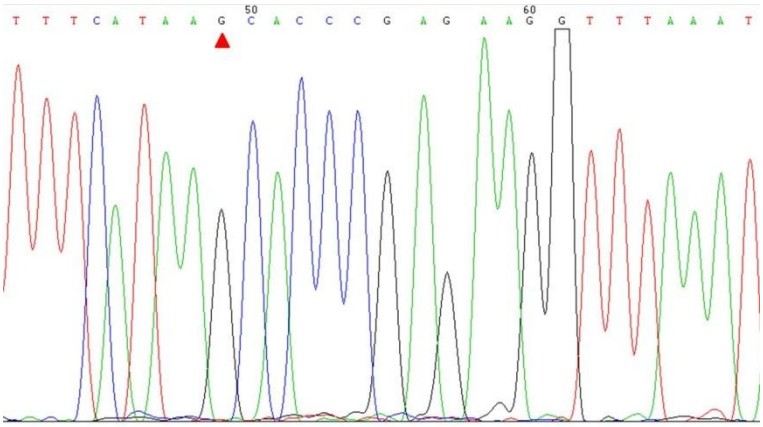
S_{10}-L5



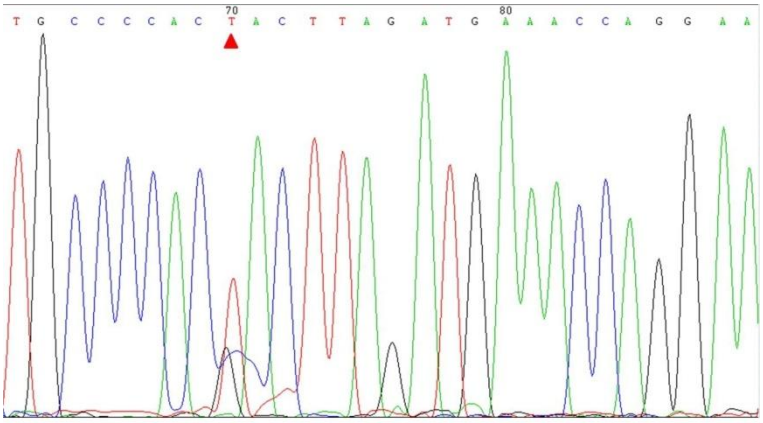
S_{10}-L6



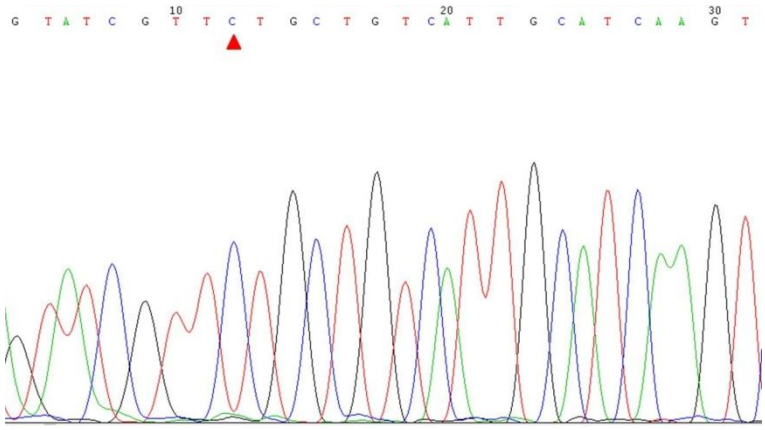
S_{11}-L1



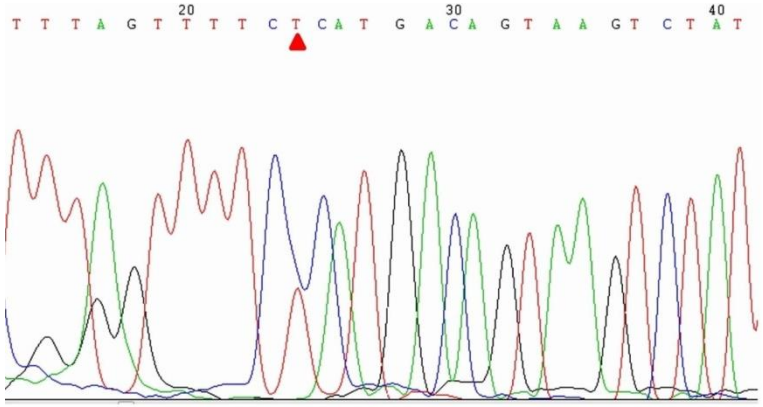
S_{11}-L2



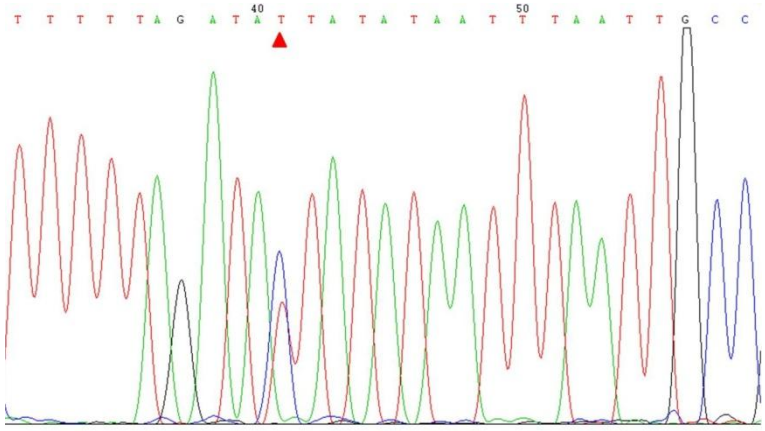
S_{11}-L3



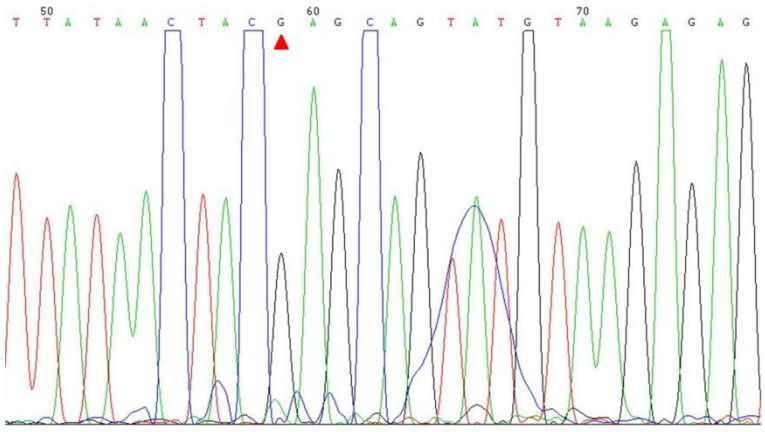
S_{11}-L4



S_{11}-L5



S_{11}-L6



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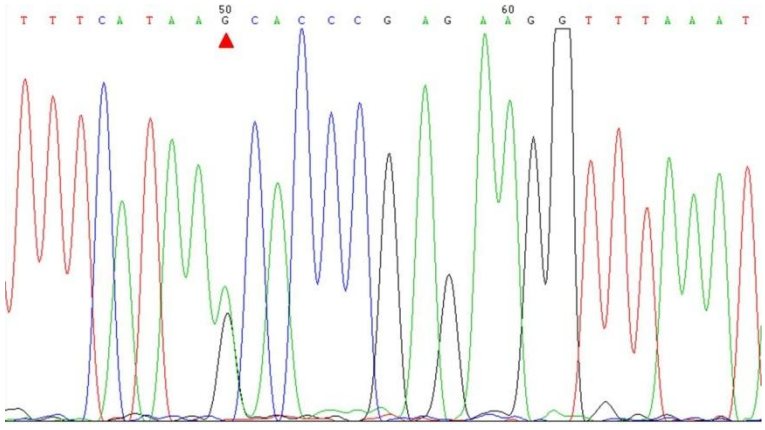
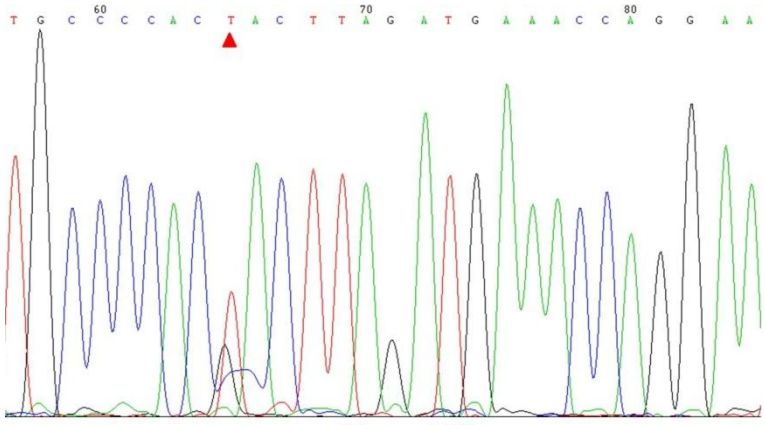
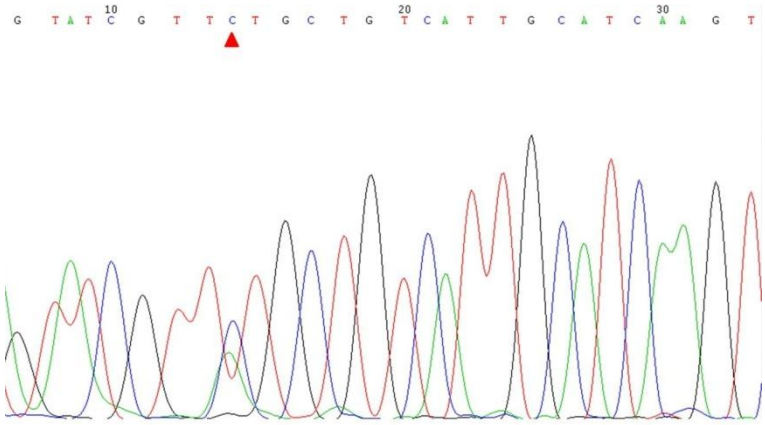


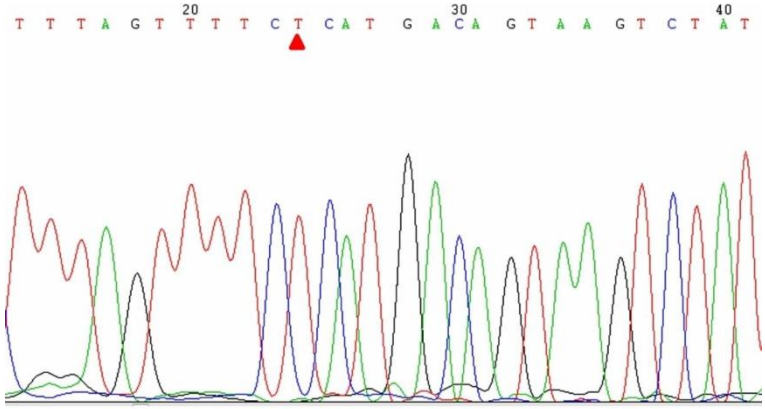
S_{12}-L2



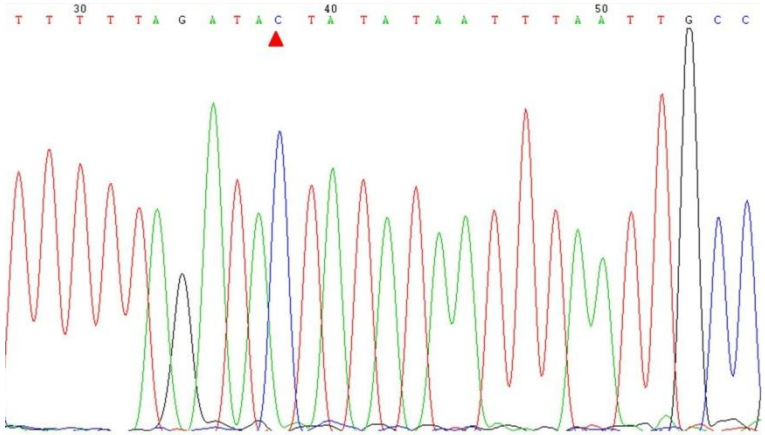
S_{12}-L3



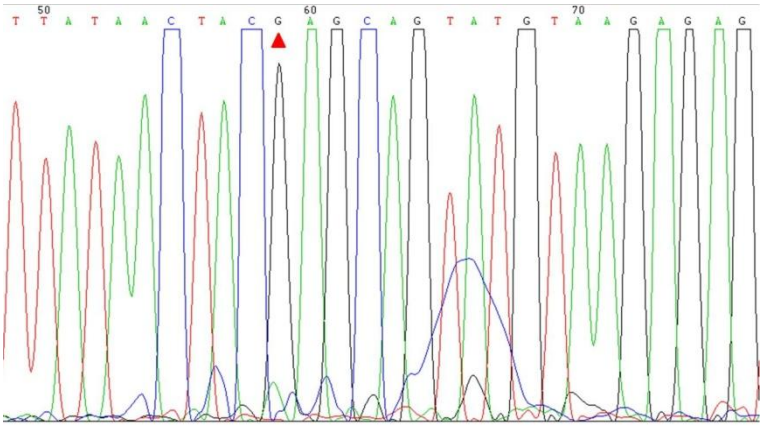
S_{12}-L4



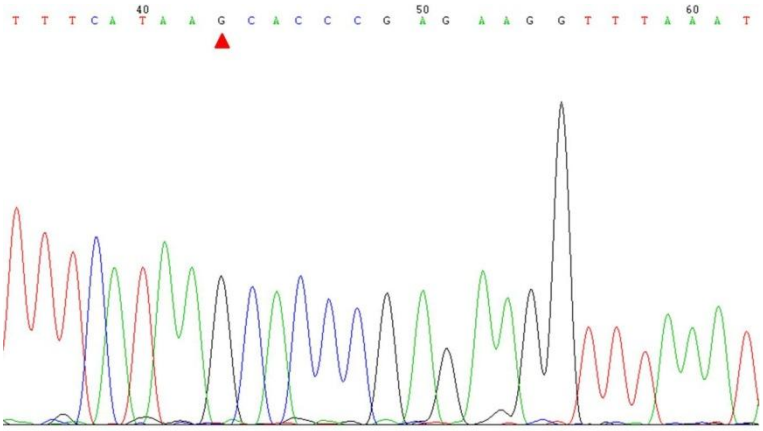
S_{12}-L5



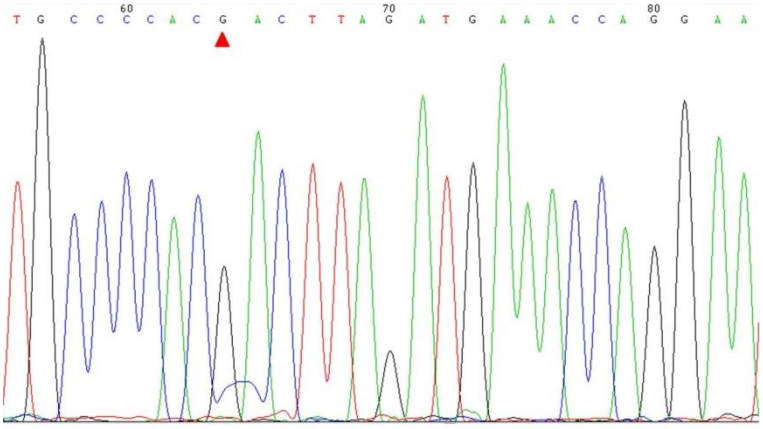
S_{12}-L6



S_{13}-L1



S_{13}-L2



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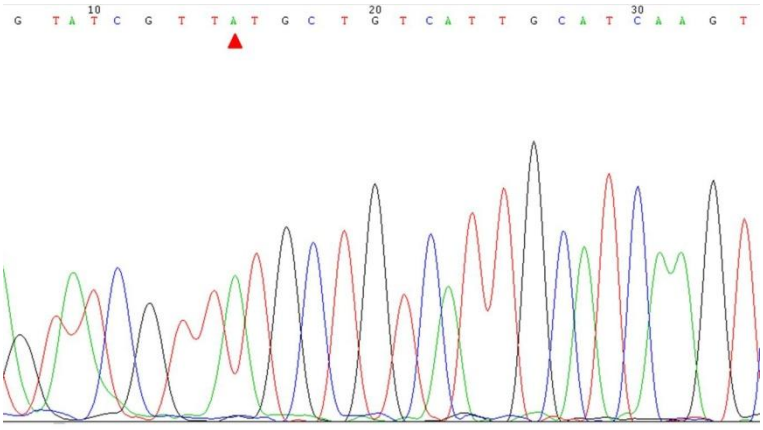

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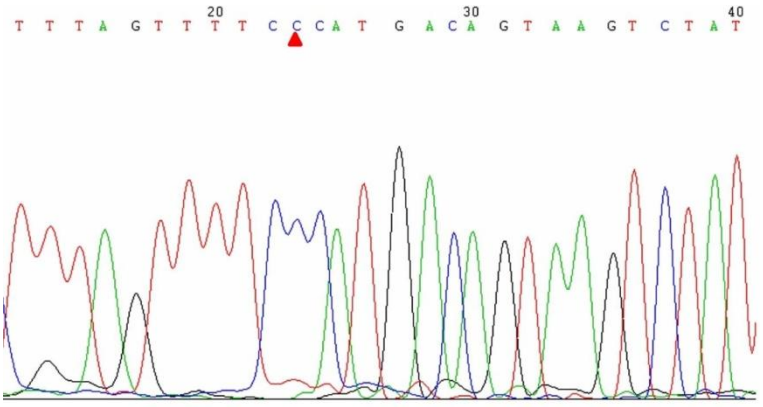
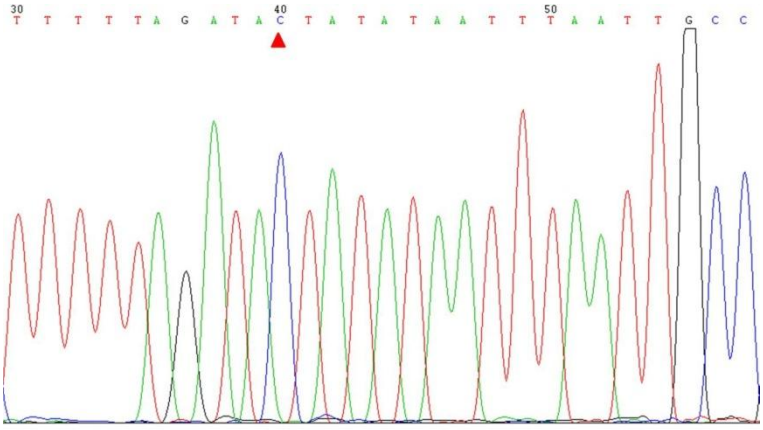
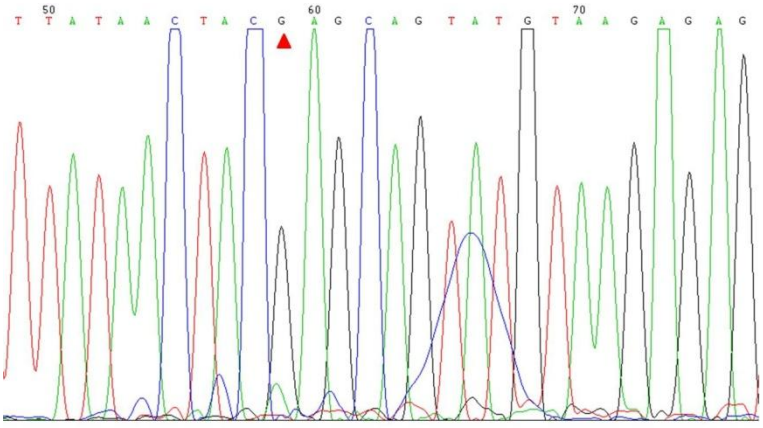


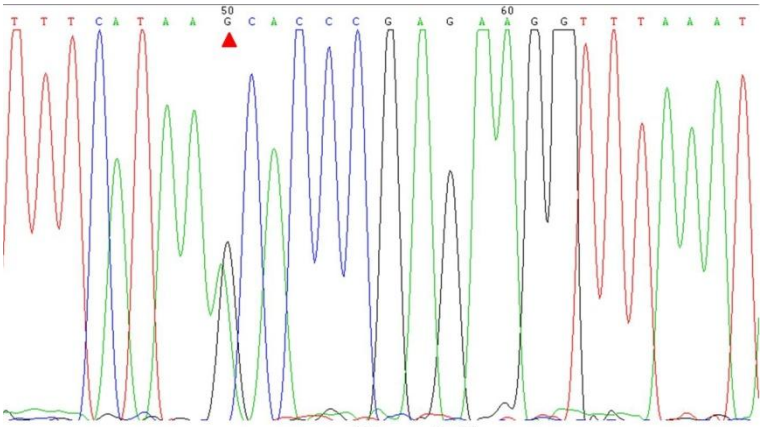
S_{13}-L5



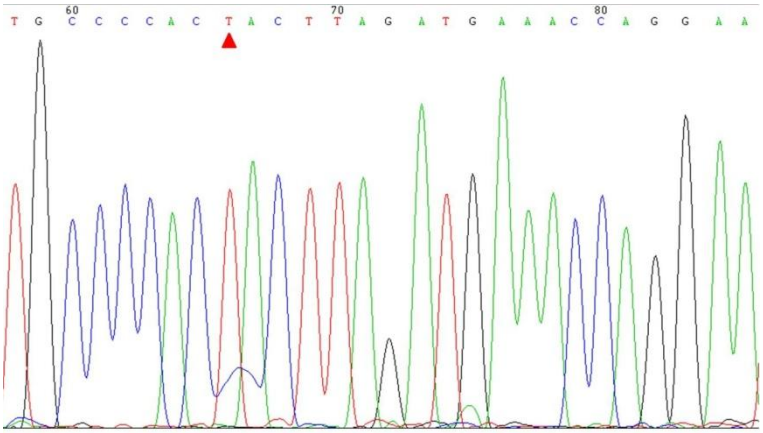
S_{13}-L6



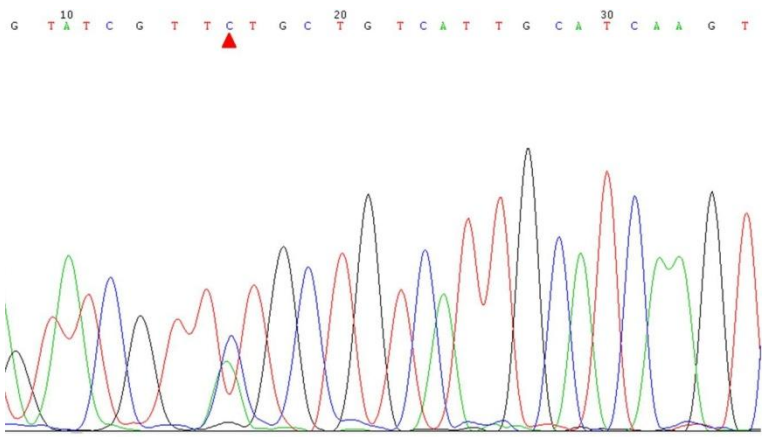
S_{14}-L1



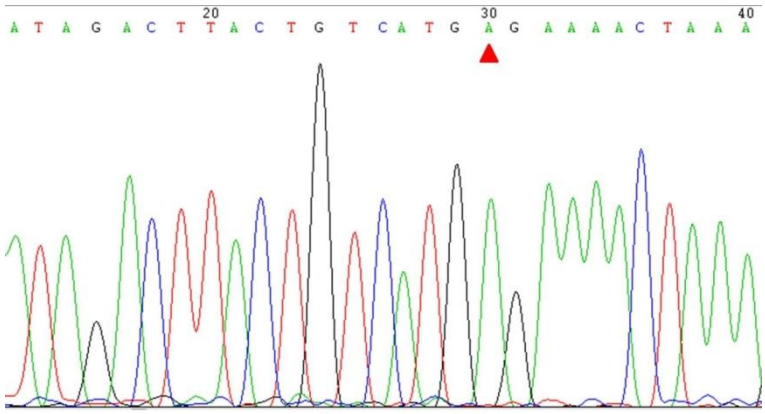
S_{14}-L2



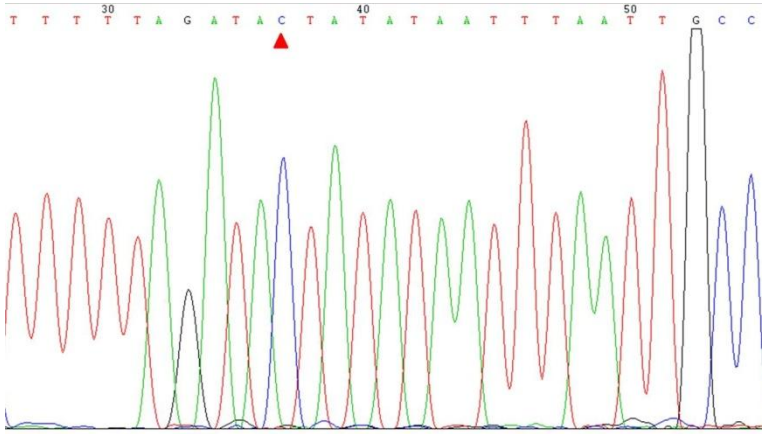
S_{14}-L3



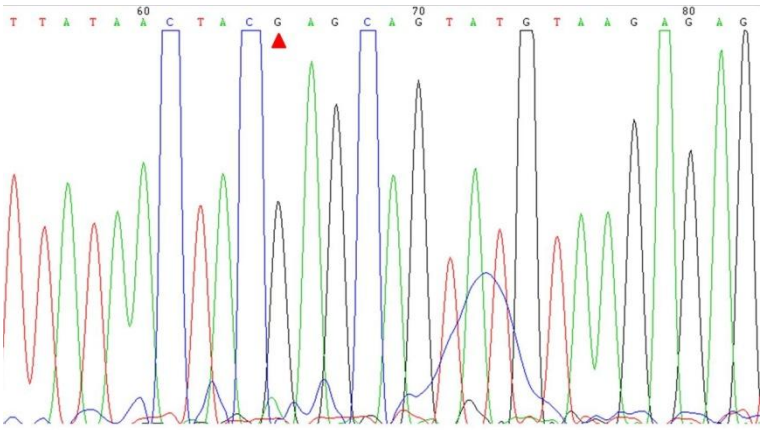
S_{14}-L4



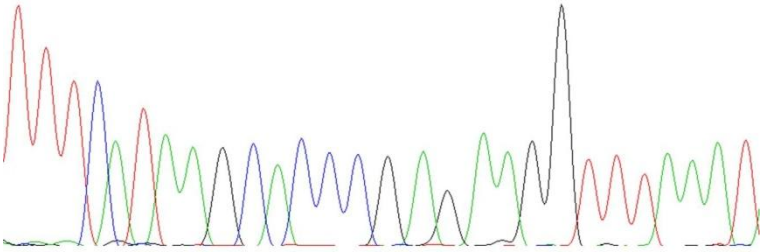
S_{14}-L5(R)



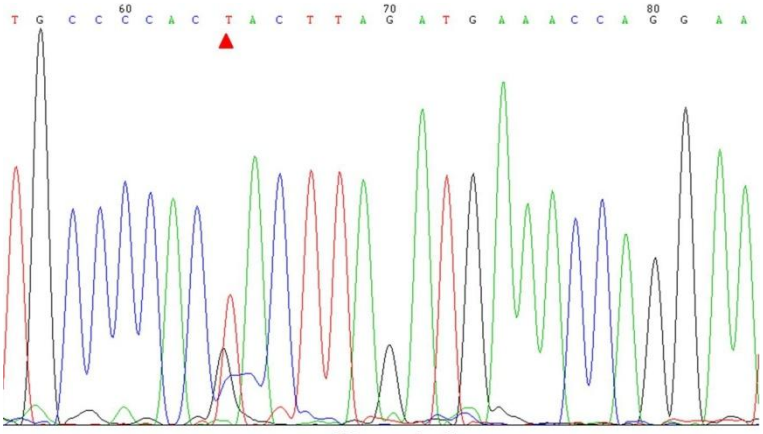
S_{14}-L6



S_{15}-L1



S_{15}-L2



S_{15}-L3

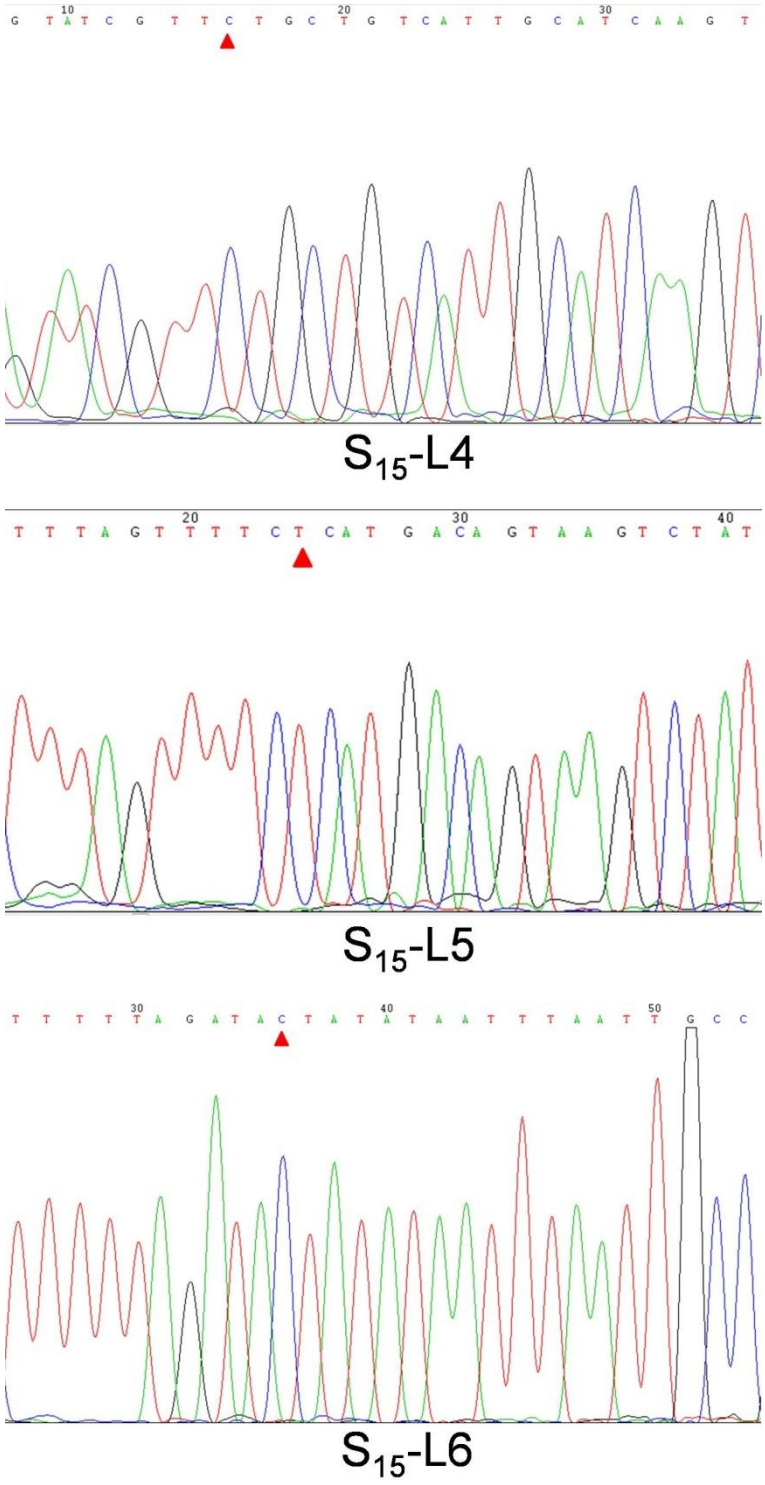


Fig. S12. Sequencing results of PCR products carrying 6 SNPs (L1-L6, marked by▲) from 15 T2DM patient blood samples (S_1-S_{15}) using Sanger chain termination method. The R in bracket represents the reverse sequencing.

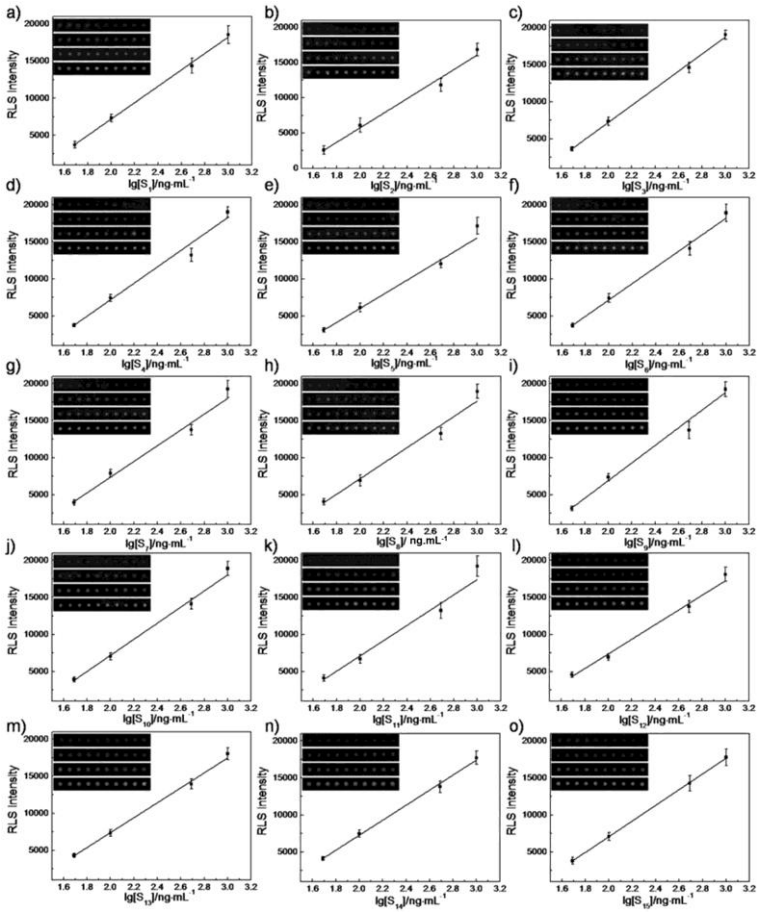


Fig. S13. RLS images and the corresponding logarithmic plots of the integrated RLS intensity as a function of the concentration of PCR products carrying rs6585205 amplified from genomic DNAs of (a)-(o) 15 T2DM patient blood samples. The concentrations of probe ssDNAs in the spotting solution were 30 μM. The PCR product concentration was diluted to1000 ng/mL, 500 ng/mL,100 ng/mL and 50 ng/mL, respectively.

Additional References

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