Electronic Supplementary Information (ESI) for:

DNA microarray-based resonance light scattering assay for multiplexed detection of DNA mutation in papillary thyroid cancer

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Contents

1 Additional Experimental Section

1.1 Preparation of DNA-modified GNPs

13 nm gold nanoparticles (GNPs) were synthesized by traditional ‘‘Turkevich-Frens’’ method.[S1, S2] The polyvalent ssDNAs modified GNPs (denoted as ssDNAs@GNPs) were prepared by previously reported procedure with slight modification.[S3-S5] Generally, 5 μL mixture of three label ssDNAs (named as, L1, L2 and L3, see Table S1 for details) with desired molar ratios were added to 300 μL 13 nm GNPs solution (8 nM). The total ssDNA concentration of the ssDNA mixture was 100 μM. After standing at room temperature for 12 h, 305 μL PBS (10 mM PB, 0.2 M NaCl, pH 7.5) were added dropwisely to the mixture and incubated for another 10 h. The solution was concentrated to 100 μL by a vacuum concentrator (Eppendorf AG Co., Germany). Subsequently, the polyvalent ssDNAs modified GNPs (denoted as ssDNAs@GNPs) were purified by repeated centrifugation (9000 rpm for 15 min, three times). Finally, the ssDNAs@GNPs were redispersed in probe buffer (5 nM in 1×SSC with 0.1% (w/v) SDS) for future use.

1.2 Preparation of genomic DNA and PCR amplification

K1 cells were cultured in fresh DMEM medium supplemented with 10% FBS in humidified air containing 5% CO_2 at 37 °C. Thyroid tissue specimens were obtained from patients who underwent thyroid surgery in the department of thyroid in the First Hospital of Jilin University and patient’s informed consent was obtained before surgery. Specimens were collected and rinsed with normal saline. The experiments were carried out within 30 min. The extraction of genomic DNAs was performed with

Cell/Tissue genomic DNA extraction Kits (Bioteke Inc., Beijing, China) according to the manufacturer's instruction. The extracted genomic DNAs were used as templates for the asymmetric overlap extension PCR experiment which consisted of two separate PCR steps. In the first PCR step, 10 μL of 2×One Taq Hot Start Master, 0.5 μM F2, 0.5 μM R2, and 1 μL template were mixed well in 20 μL reaction set, then heated at 94 °C for 3 min, followed by 30 cycles of 30 s at 94 °C, 20 s annealing at 53.2 °C, 20 s of primer extension at 68 °C, and a final extension at 68 °C for 5 min, then quickly chilled to 4 °C. The as-obtained PCR products from the first step were directly used as the second step's template. In the second PCR step, 10 μL 2×One Taq Hot Start Master Mix, 0.5 μM f2, 0.01 μM r2 and 5 μL template were mixed well in 20 μL reaction set, then heated at 94 °C, followed by 35 cycles of 30 s at 94 °C, 20 s annealing at 62 °C, 20 s of primer extension at 62 °C, and a final extension at 68 °C for 5 min, then quickly chilled to 4 °C. The raw asymmetric PCR products from genomic DNAs of these practical samples were diluted with hybridization buffer and directly analyzed by the DNA microarray-based RLS assay.

1.3 Fabrication of DNA microarray

Desired amounts of 8 ssDNA probes (named P1M, P1W, P2M, P2W, P31M, P31W, P32M and P32W) were dissolved by spotting buffer (3×SSC and 0.1% (w/v) SDS with 1.5 M betaine) and spotted onto the Aldehyde 3-D glass slide by Smart-Arrayer 136 system (CapitalBio Ltd Co., China) under contact-printing mode. After incubation at 37 °C with 75% humidity for 12 h, the microarrays were washed by washing buffer (1×SSC with 0.01% (w/v) SDS, 30 mL, three times) and Milli-Q

water (30 mL, three times), respectively. Subsequently, the microarrays were incubated with blocking solution (0.1 M ethanolamine in PBS (pH 7.5, 50 mM plus 0.15 M NaCl)) at 30 °C for 1 h to deactivate unreacted aldehyde groups on the slide. Then, the microarrays were washed with 30 mL PBS buffer (three times) and 30 mL Milli-Q (three times), dried by centrifugation (480 g for 1 min), and separated into 12 independent subarrays by the PTFE grid, respectively.

2. Additional Figures

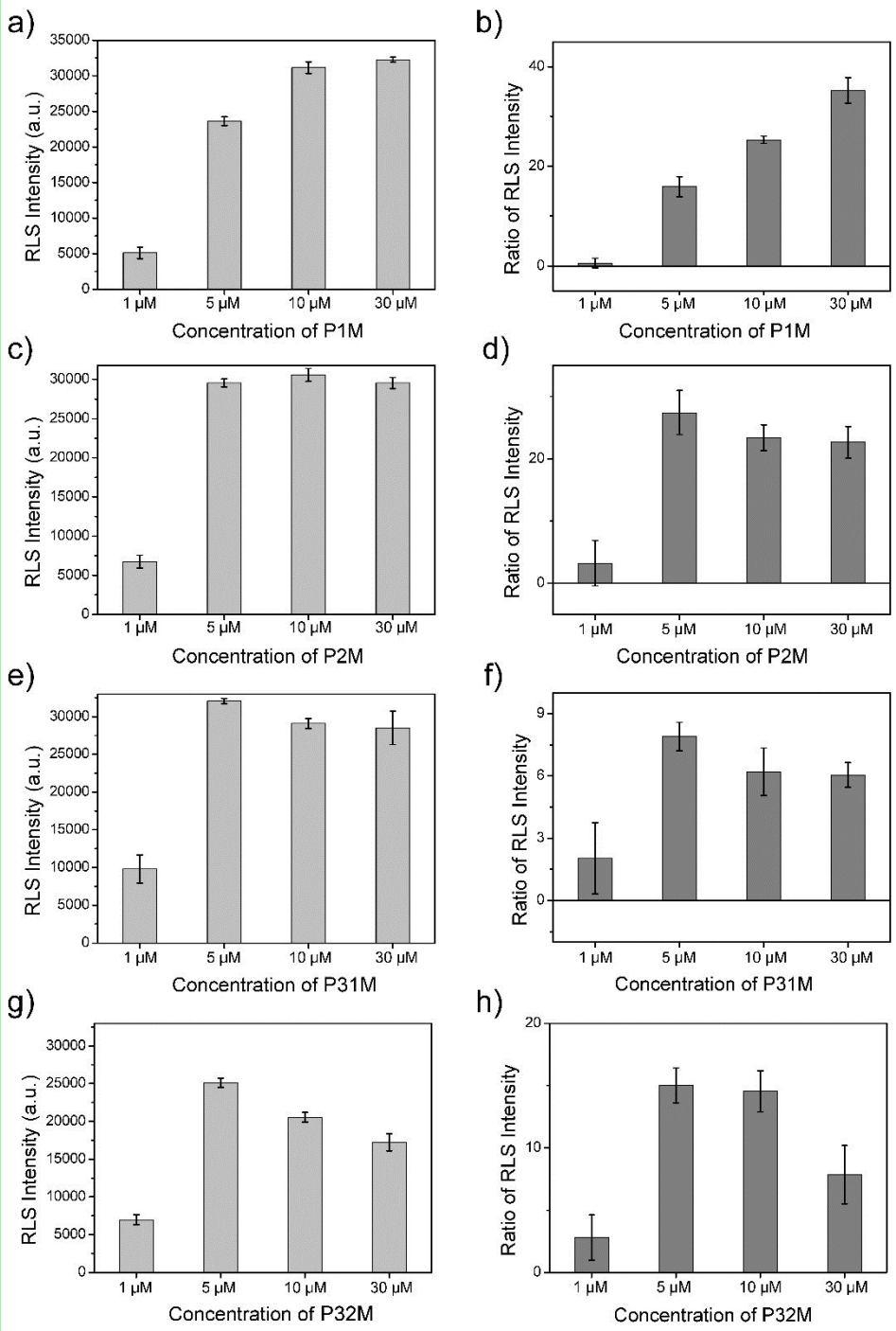

Fig. S1 The effect of probe ssDNA concentration in the spotting solution on the assay performance. The RLS intensities of (a) P1M hybridization with T1M, (c) P2M hybridization with T2M, (e) P31M hybridization with T3M and (g) P32M hybridization with T3M, respectively. Corresponding RLS intensity ratios of (b) P1M

hybridization with T1M to P1M hybridization with T1W, (d) P2M hybridization with T2M to P2M hybridization with T2W, (f) P31M hybridization with T3M to P31M hybridization with T3W, and (b) P32M hybridization with T3M to P32M hybridization with T3W, respectively. The concentrations of target ssDNAs are 100 nM while the concentration of ssDNAs@GNPs is 5 nM.

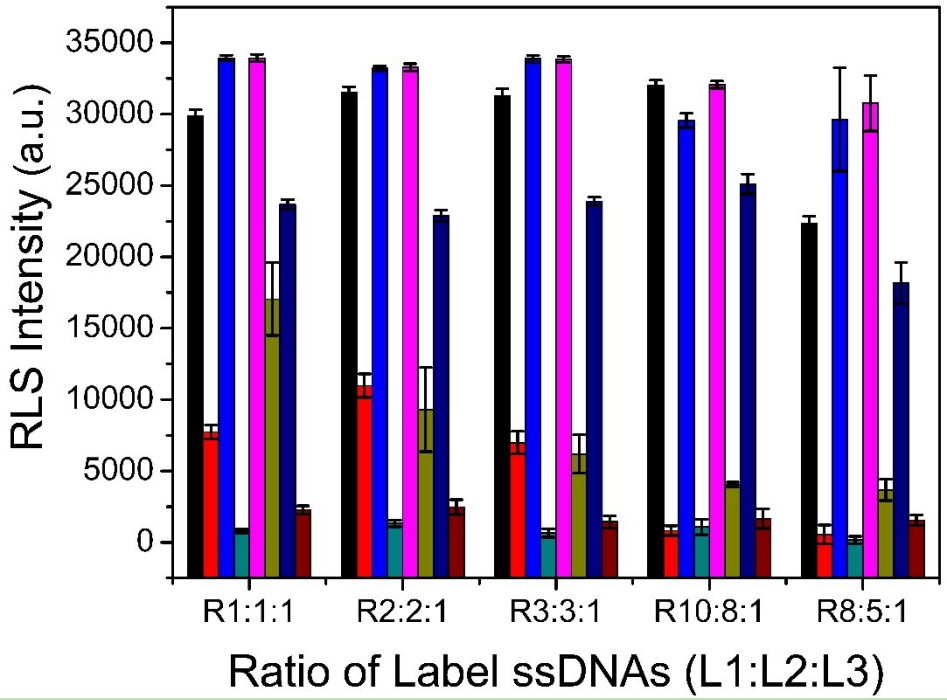

Fig. S2 The effect of molar ratio of label ssDNA on ssDNAs@GNPs. The total concentration of label ssDNAs is 5 nM. The concentrations of probe ssDNAs in the spotting solution are 30 μM for P1M and P1W, and 5 μM for P2M, P2W, P31M, P31W, P32M and P32W, respectively. The concentrations of target ssDNAs are 100 nM.

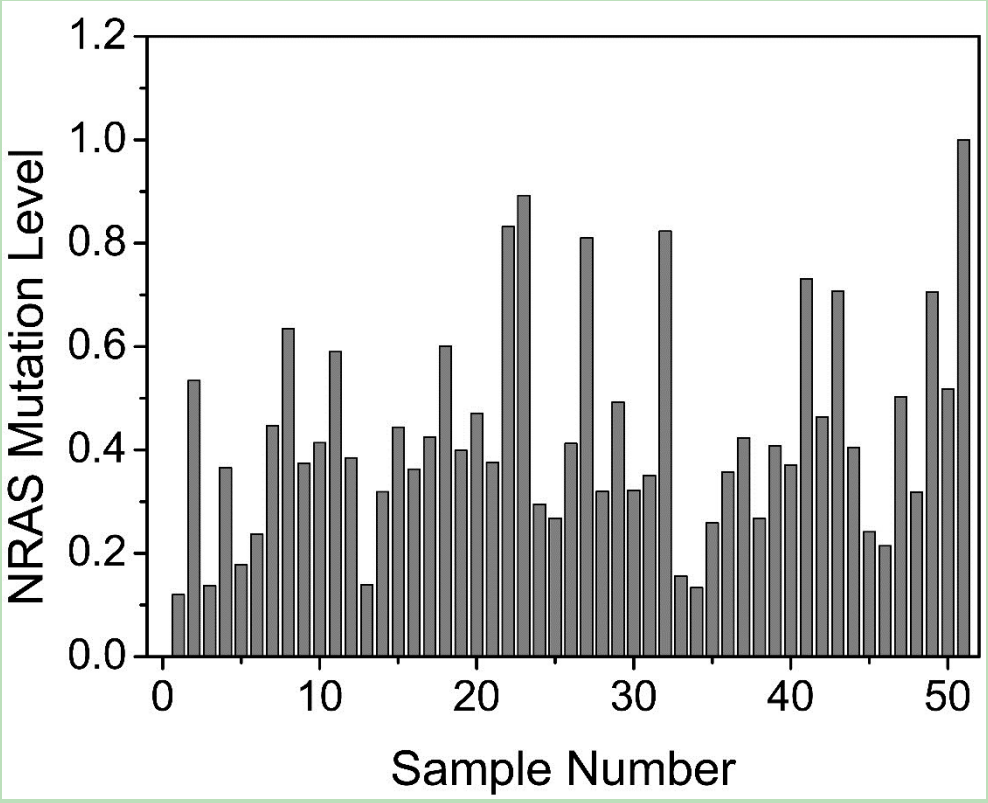

Fig. S3 The expression levels of t2m (mutant NRAS codon 61) in thyroid tissues of PTC patients by commercial Sanger sequencing assay (General Biosystems Ltd., Tuzhou, China). The K1 cell (no. 51) was employed as a positive control sample while the normal thyroid tissue was used as negative control sample (no. 0). The NRAS mutation level was defined by ratio of the amount of mutant NRAS codon 61 of thyroid tissue with the amount of mutant NRAS codon 61 of K1 cell. The amount of mutant NRAS codon 61 in negative control sample can not be detected by Sanger sequencing assay since it is lower than the detection limit of the used instrument.

3 Additional Tables

Table S1 Sequences of single stranded oligonucleotides (ssDNAs) in the assay

Name	Sequence（5’ to 3’）	Functionality	Mutation Point
L1	HS-SH-C_6-T_{10}GGATCCAGACAACTGT	ssDNA for GNP modification	$BRAF^{V600E}$ (t1m)
L2	HS-SH-C_6-T_{10}CGCCTGTCCTCAT	ssDNA for GNP modification	NRAS codon 61 (t2m)
L3	HS-SH-C_6-T_{10}AGGGGAGGGGCTG	ssDNA for GNP modification	TERT promoter (t31m and t32m)
P1M	GCGAGATTTCTCTGTAGT_{10}-C_6-NH_2	Probe ssDNA for T1M	$BRAF^{V600E}$ (t1m)
P1W	GAGATTTCACTGTAGT_{10}-C_6-NH_2	Probe ssDNA for T1W	$BRAF^{V600E}$ wild-type
P2M	ACTCTTCTCGTCCAGT_{10}-C_6-NH_2	Probe ssDNA for T2M	NRAS codon 61 (t2m)
P2W	TCTTCTTGTCCAGT_{10}-C_6-NH_2	Probe ssDNA for T2W	NRAS codon 61 wild-type
P31M	CCCGGAAGGGGCTGGT_{10}-C_6-NH_2	Probe ssDNA for T31M	TERT promoter g.1295228 (t31m)
P31W	CGGGAGGGGCTGGT_{10}-C_6-NH_2	Probe ssDNA for T31W	TERT promoter g.1295228 wild-type

P32M	CCCGGAAGGGGCTGGT$_{10}$-NH$_2$	Probe ssDNA for T32M	TERT promoter g.1295250 (t32m)
P32W	CGGAGGGGGCTGGT$_{10}$-NH$_2$	Probe ssDNA for T32W	TERT promoter g.1295250 wild-type
T1M	GATTTTGGTCTAGCTACAGAGAAATC TCGATGGAGTGGGTCCCATCAGTTTG AACAGTTGTCTGGATCC	Synthetic ssDNA for BRAF mutant	BRAF^{V600E} (t1m)
T2M	GACATACTGGATACAGCTGGACGAGA AGAGTACAGTGCCATGAGAGACCAAT ACATGAGGACAGGCGAA	Synthetic ssDNA for NRAS mutant	NRAS codon 61 (t2m)
T3M	CCCGCCCCGTCCCGACCCCTTCCGGG TCCCCGGCCCAGCCCCTTCCGGGCCC TCCCAGCCCCTCCCCT	Synthetic ssDNA for TERT mutant	TERT promoter g.1295228/1295250 (t31m/t32m)
T1W	GATTTTGGTCTAGCTACAGTGAAATC TCGATGGAGTGGGTCCCATCAGTTTG AACAGTTGTCTGGATCC	Synthetic ssDNA for BRAF wild-type	BRAF^{V600E} wild-type
T2W	GACATACTGGATACAGCTGGACAAGA AGAGTACAGTGCCATGAGAGACCAAT ACATGAGGACAGGCGAA	Synthetic ssDNA for NRAS wild-type	NRAS codon 61 wild-type
T3W	CCCGCCCCGTCCCGACCCCTCCCGGG	Synthetic ssDNA for	TERT promoter

	TCCCCGGCCCAGCCCCCTCCGGGCCC TCCCAGCCCCTCCCCT	TERT wild-type	g.1295228/12952 50 wild-type
F2	GACATAGTGGATACAGCTGGACG	Forward primer for NRAS mutant	NRAS codon 61 (t2m)
R2	GTGGTAACCTCATTTCCCCAT	Reverse primer for NRAS mutant	NRAS codon 61 (t2m)
f2	GACATACTGGATACAGCTGGACG	Forward primer for NRAS mutant	NRAS codon 61 (t2m)
r2	TTCGCCTGTCCTCATGTATTG	Reverse primer for NRAS mutant	NRAS codon 61 (t2m)

The letters with red color indicate the genetic mutation sites. The ssDNA probes were designed according our previous reports several criteria including immobilization efficiencies on GNPs/substrates, position of mutation site and melting pointing after hybridization with target ssDNA[S3-S7].

4. Additional References

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