Electronic Supplementary Information (ESI) for:

Multiple Detection of Single Nucleotide Polymorphism by Microarray-Based Resonance Light Scattering Assay with Enlarged Gold Nanoparticle Probes

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Table S1. Sequences of oligonucleotides in the assay

Name	Sequence (5' to 3')	Functionality
P1	5'-NH_2-C_6-TTTTTTTTTTCTGTCGAATTATTTAGTAT	Probe ssDNA for T1
P2	5'-NH_2-C_6-TTTTTTTTTTGGTTAGCAGCAGC	Probe ssDNA for T2
P3	5'-NH_2-C_6-TTTTTTTTTTGAGTGACTGAGTACAG	Probe ssDNA for T3
P4	5'-NH_2-C_6-TTTTTTTTTTTTTAAATCACGTTTAACA	Probe ssDNA for T4
T1	CTGTCTTGCCAGTGATACTAAA**T**AATTCGACAG	Target ssDNA with 16q12.1 mutant
T2	AAGACCTGGCTTCTAGCTGCT**G**CTAACC	Target ssDNA with 1q32.1 mutant
T3	GTATCATTGCAAAACTCCTGTACT**C**AGTCACTC	Target ssDNA with 5q14.3 mutant
T4	AGGGTCAAGGTTTTCTGTTAAAC**G**TGATTTAAA	Target ssDNA with , 15q26.1 mutant
S1	CTGTCTTGCCAGTGATACTAAA**C**AATTCGACAG	Target ssDNA with 16q12.1wild type
S2	AAGACCTGGCTTCTAGCTGCT**A**CTAACC	Target ssDNA with 1q32.1wild type
S3	GTATCATTGCAAAACTCCTGTACT**T**AGTCACTC	Target ssDNA with 5q14.3wild type
S4	AGGGTCAAGGTTTTCTGTTAAAC**A**TGATTTAAA	Target ssDNA with 15q26.1wild type
L1	CACTGGCAAGACAGTTTTTTTTTT-C_6-SH-3’	ssDNA for GNP modification
L2	TAGAAGCCAGGTCTTTTTTTTTTTT-C_6-SH-3’	ssDNA for GNP modification

L3	GAGTTTTGCAATGATACTTTTTTTTTT-C_6-SH-3’	ssDNA for GNP modification
L4	GAAAACCTTGACCCTTTTTTTTTTT-C_6-SH-3’	ssDNA for GNP modification
P1w	5'-NH_2-C_6-TTTTTTTTTTCTGTCGAATTGTTTAGTAT	Probe ssDNA for S1
P2w	5'-NH_2-C_6-TTTTTTTTTTGGTTAGTAGCAGC	Probe ssDNA for S2
P3w	5'-NH_2-C_6-TTTTTTTTTTGAGTGACTAAGTACAG	Probe ssDNA for S3
P4w	5'-NH_2-C_6-TTTTTTTTTTTTTAAATCATGTTTAACA	Probe ssDNA for S4
F1	TCTGTCTTGCCAGTGATACTAAAT	Forward primer for 16q12.1
R1	GACTCAATGGGTGGAGGTGT	Reverse primer for 16q12.1
F2	GACCAGGCTTCTAGCTGCTG	Forward primer for 1q32.1
R2	TGGAGGACATTCCCGAACTC	Reverse primer for 1q32.1
F3	TATCATTCCAAAACTCCTGTACTC	Forward primer for 5q14.3
R3	CAGTTCGGACTTTGTACCTG	Reverse primer for 5q14.3
F4	GGTCAAGGTCTTCTGTTAAACG	Forward primer for 15q26.1
R4	GACGCAGGAATACCTATCAT	Reverse primer for 15q26.1
f1	TCTGTCTTGCCAGTGATACTAAAT	Forward primer for 16q12.1
r1	AGGGTGGTATGTGGTTTTGC	Reverse primer for

		16q12.1
f2	GACCTGACTTCTAGCTGCTG	Forward primer for 1q32.1
r2	GTTACAGTAGTAACAACTGAGTCC	Reverse primer for 1q32.1
f3	TATCATTCCAAAACTCCTGTACTC	Forward primer for 5q14.3
r3	CACATCCACCAGAACCTACAAG	Reverse primer for 5q14.3
f4	GGTCAAGGTCTTCTGTTAAACG	Forward primer for 15q26.1
r4	GGATTGTCAGCCCCTAAAGT	Reverse primer for 15q26.1

Highlighted bases are the SNP sites.

Table S2. Experimental conditions of the gold enlargement.

Subarray Number	**1**	**2**	**3**	**4**	**5**	**6**	**7**
$HAuCl_4$/ mM	0.05	0.2	0.5	0.5	0.2	0.2	0.2
H_2O_2/ M	0.1	0.1	0.1	0.05	0.05	0.5	2.5

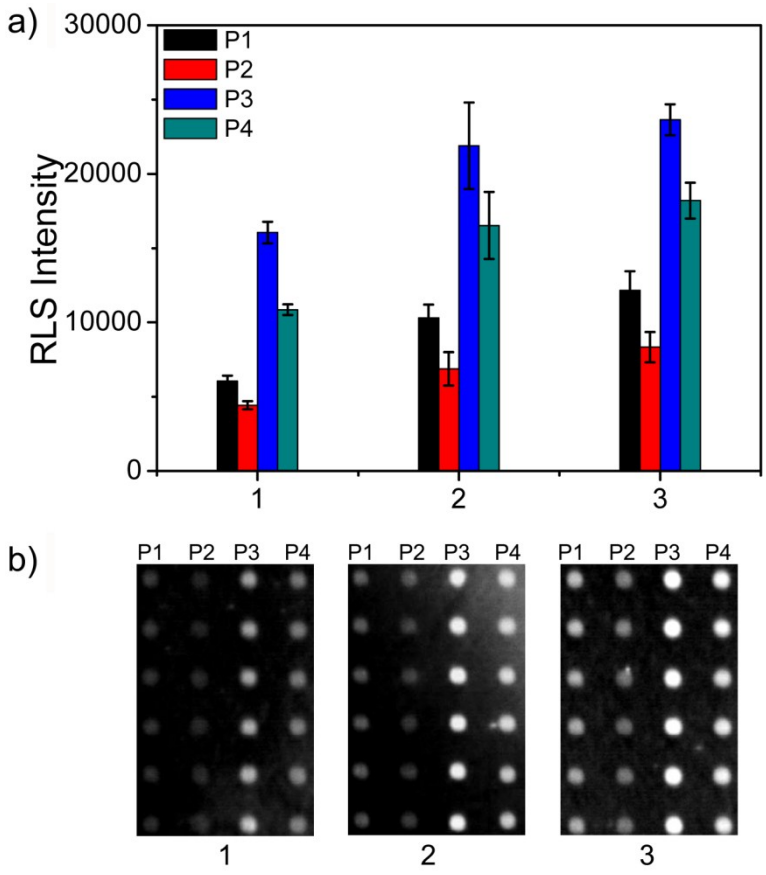

Fig. S1. RLS intensities (a) and corresponding images (b) of subarrays. The various concentrations of $HAuCl_4$ are used for enlargement of GNPs. (as shown in Table S2). The concentrations of probe ssDNAs are 10 μM in spotting solution which are immobilized on the subarrays as indicated. The concentration of total DNAs in the target ssDNA cocktail (T1:T2:T3:T4=1:1:1:1) is 10 nM, respectively. The concentration of DNA-GNPs is 5 nM.

Relative high RLS intensity and S/N have been obtained at 0.5 mM $HAuCl_4$ in the reaction mixture.

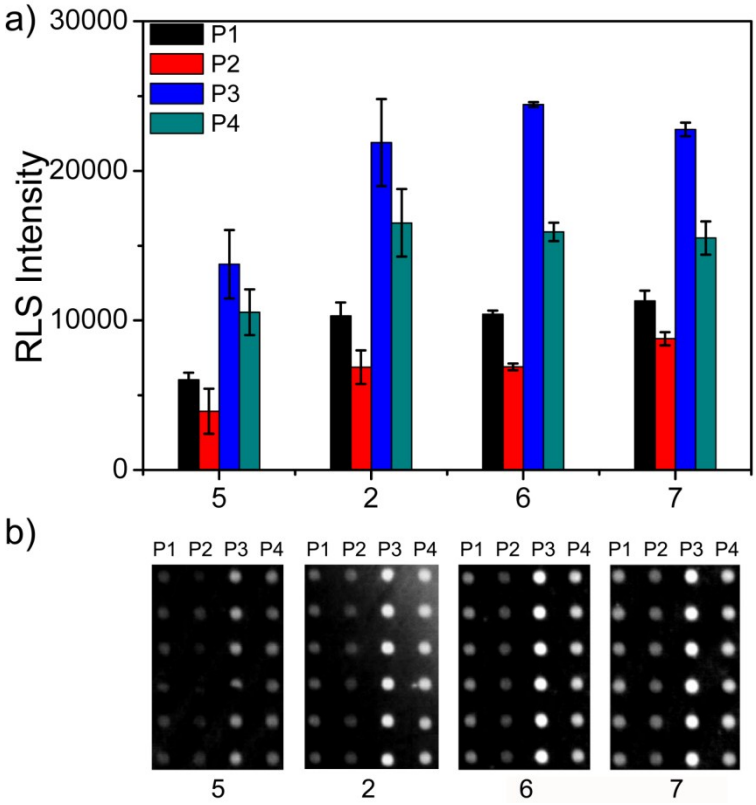

Fig. S2. RLS intensities (a) and corresponding images (b) of subarrays. The various concentrations of H_2O_2 are used for enlargement of GNPs. (as shown in Table S2). The concentrations of probe ssDNAs are 10 μM in spotting solution which are immobilized on the subarrays as indicated. The concentration of total DNAs in the target ssDNA cocktail (T1:T2:T3:T4=1:1:1:1) is 10 nM, respectively. The concentration of DNA-GNPs is 5 nM.

In this case, relative high RLS intensity and S/N have been obtained at 0.5 M H_2O_2 in the reaction mixture. However, there are significant differences among RLS signals from different target ssDNAs.

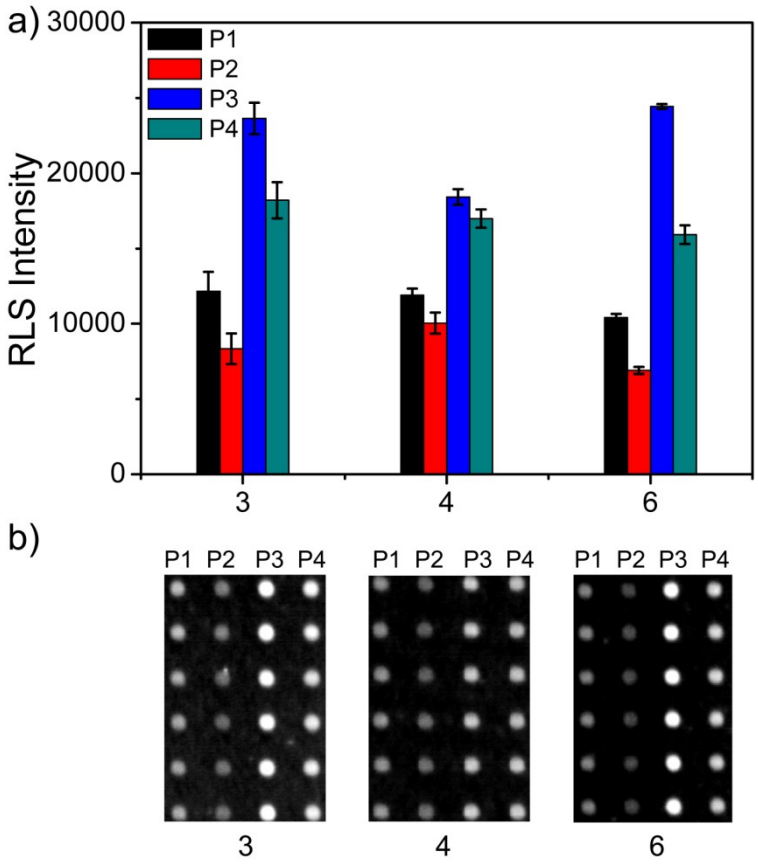

Fig. S3. RLS intensities (a) and corresponding images (b) of subarrays. The various concentrations of H_2O_2 and $HAuCl_4$ are used for enlargement of GNPs (as shown in Table S2). The concentrations of probe ssDNAs are 10 μM in spotting solution which are immobilized on the subarrays as indicated. The concentration of total DNAs in the target ssDNA cocktail (T1:T2:T3:T4=1:1:1:1) is 10 nM, respectively. The concentration of DNA-GNPs is 5 nM.

Because relative homogenous RLS intensity and S/N have been obtained at 0.5 mM $HAuCl_4$ and 0.05 M H_2O_2 in the reaction mixture (No. 4), the following experiments are performed in this experimental condition (0.5 mM $HAuCl_4$ and 0.05 M H_2O_2).

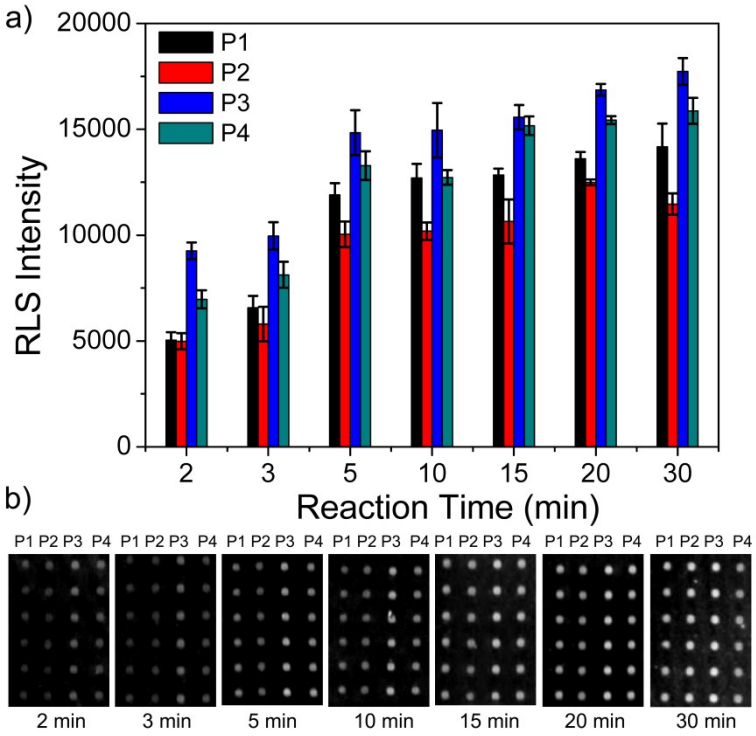

Fig. S4. RLS intensities (a) and corresponding images (b) of subarrays. The various gold deposition times are employed for enlargement of GNPs. The concentrations of H_2O_2 and $HAuCl_4$ are 0.05 M and 0.5 mM, respectively. The concentrations of probe ssDNAs are 10 μM in spotting solution which are immobilized on the subarrays as indicated. The concentration of total DNAs in the target ssDNA cocktail (T1:T2:T3:T4=1:1:1:1) is 10 nM, respectively. The concentration of DNA-GNPs is 5 nM.

Both of the RLS intensity of subarray (S) and background noise (N) are increased with increasing of gold deposition time. Consideration of S/N and assay time, 5 minutes gold deposition is used in the following experiments.

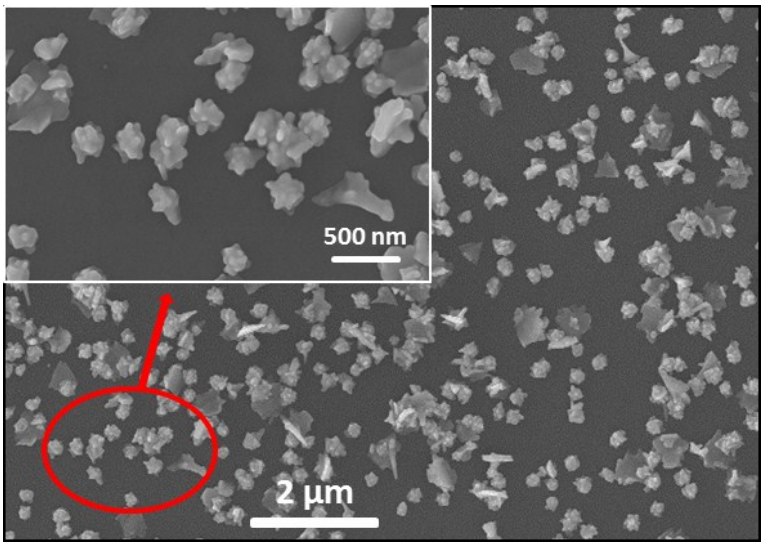

Fig. S5. SEM micrograph of enlarged GNPs.

The average size of GNPs is increased from 13 nm to 200 nm and the morphology of GNP change from sphere to irregular shape with rough surface.

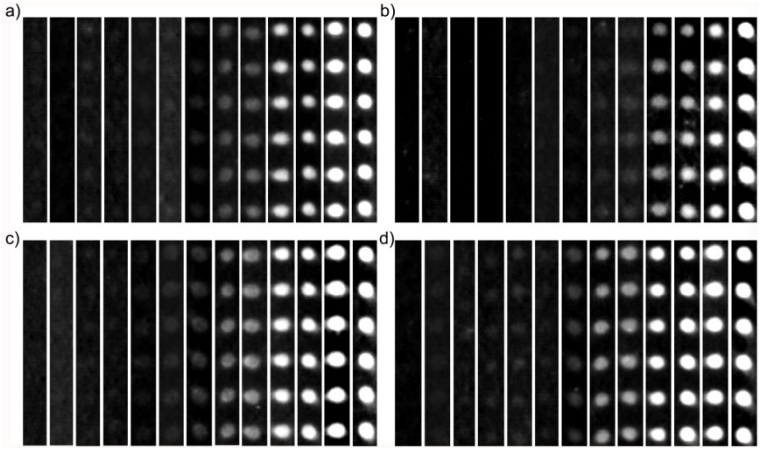

Fig. S6. RLS images as a function of allele frequency. (a) T1, (b) T2, (c) T3, (d) T4, respectively. The total concentration of mutant (T1 to T4) and wild-type (S1 to S4) target ssDNAs is 0.3 pmol in the 30 μL hybridization buffer. The concentrations of probe ssDNAs (P1 to P4) are 10 μM in spotting solution, respectively. The concentration of DNA-GNPs is 5 nM.

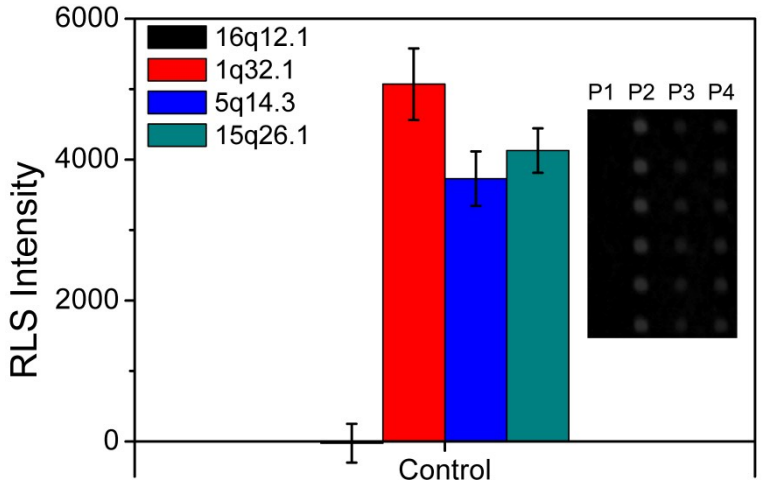

Fig. S7. The RLS images (inset) and corresponding data analysis of subarray. The subarray is hybridized with blank control (asymmetric PCR solution without template). The concentrations of probe ssDNAs (P1 to P4) are 10 μM in spotting solution, respectively. The concentration of DNA-GNPs is 5 nM.

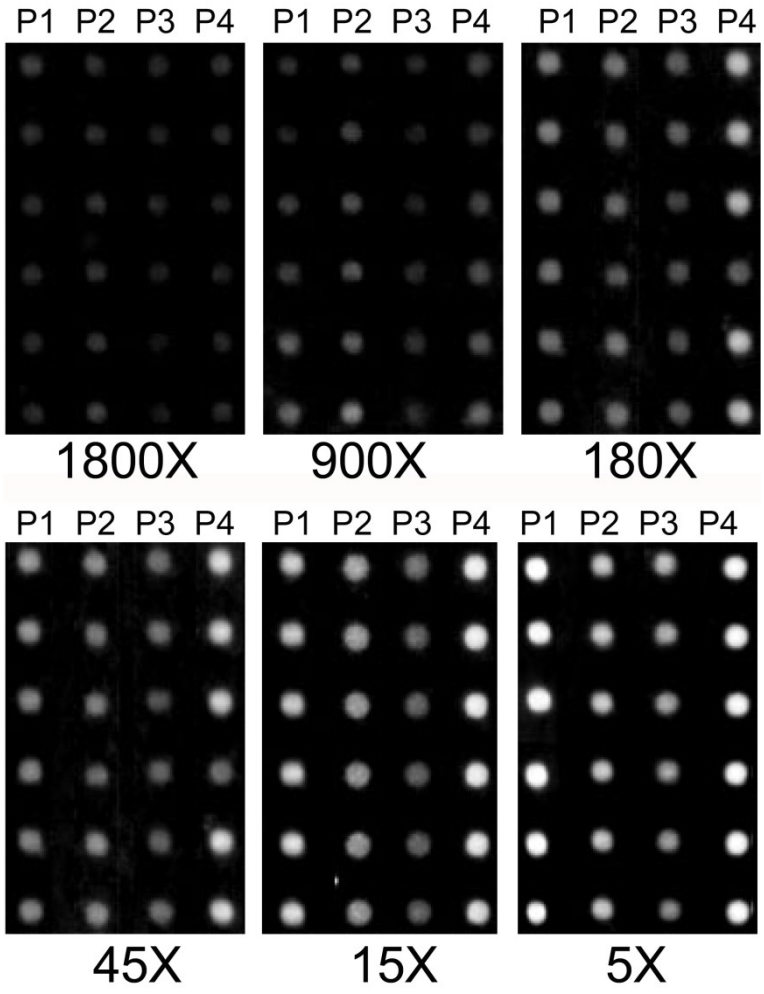

Fig. S8. The RLS images subarrays. The subarrays are hybridized with various times diluted asymmetric PCR products of MCF-7. The concentrations of probe ssDNAs (P1 to P4) are 10 μM in spotting solution, respectively. The concentration of DNA-GNPs is 5 nM.

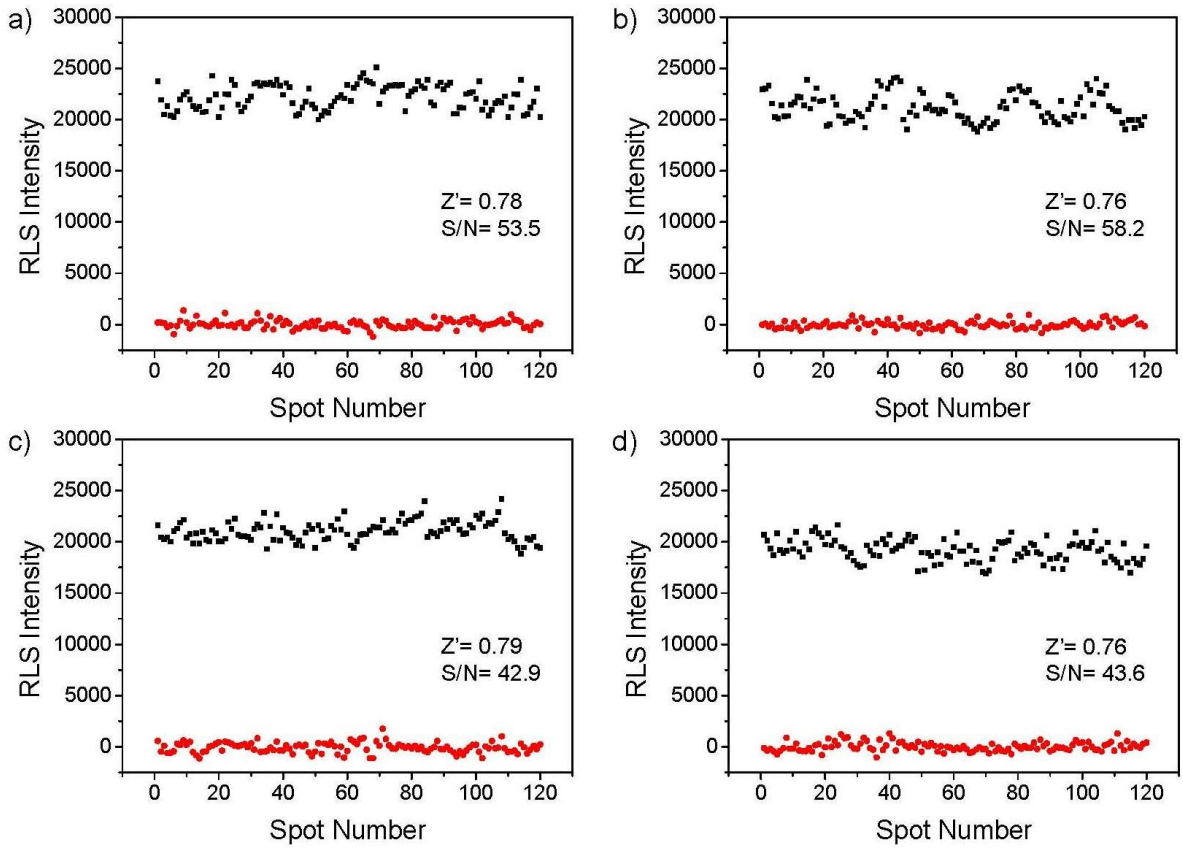

Fig. S9. Evaluation of the assay performance. RLS intensities (a) P1 hybridized with T1, (b) P2 hybridized with T2, (c) P3 hybridized with T3 and (d) P4 hybridized with T4 (black squares) are shown in comparison with control measurements (red dots), respectively. The concentrations of probe ssDNAs are 10 μM in spotting solution. The concentration of total DNAs in the target ssDNA cocktail (T1:T2:T3:T4=1:1:1:1) is 10 nM, respectively. The concentration of DNA-GNPs is 5 nM. The mean values and standard deviations extracted from the data were used to calculate the S/N ratio and Z' factor. The hybridization buffer without target ssDNAs is used as control sample.