

Supporting Information

Simultaneous Direct Detection of Shiga-toxin Producing *Escherichia coli* (STEC) Strains by Optical Biosensing with Oligonucleotide-functionalized Gold Nanoparticles

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Supplementary Information 1

Table1. Optimization of functionalized AuNP by using various concentration and volume ratios of oligonucleotide probes and AuNP solution during immobilization step. Absorbance at 520 nm of each functionalized AuNP solution is also indicated.

Solution	Concentration and volume of functionalized AuNP components	Volume Ratio	Absorbance at 520 nm
1	20 μ M, 2 μ l thiol-modified oligonucleotide + 20 nM, 998 μ l AuNP	1:500	0.55
2	20 μ M, 10 μ l thiol-modified oligonucleotide + 20 nM, 990 μ l AuNP	1:100	0.63
3	20 μ M, 20 μ l thiol-modified oligonucleotide + 20 nM, 980 μ l AuNP	1:50	1.089

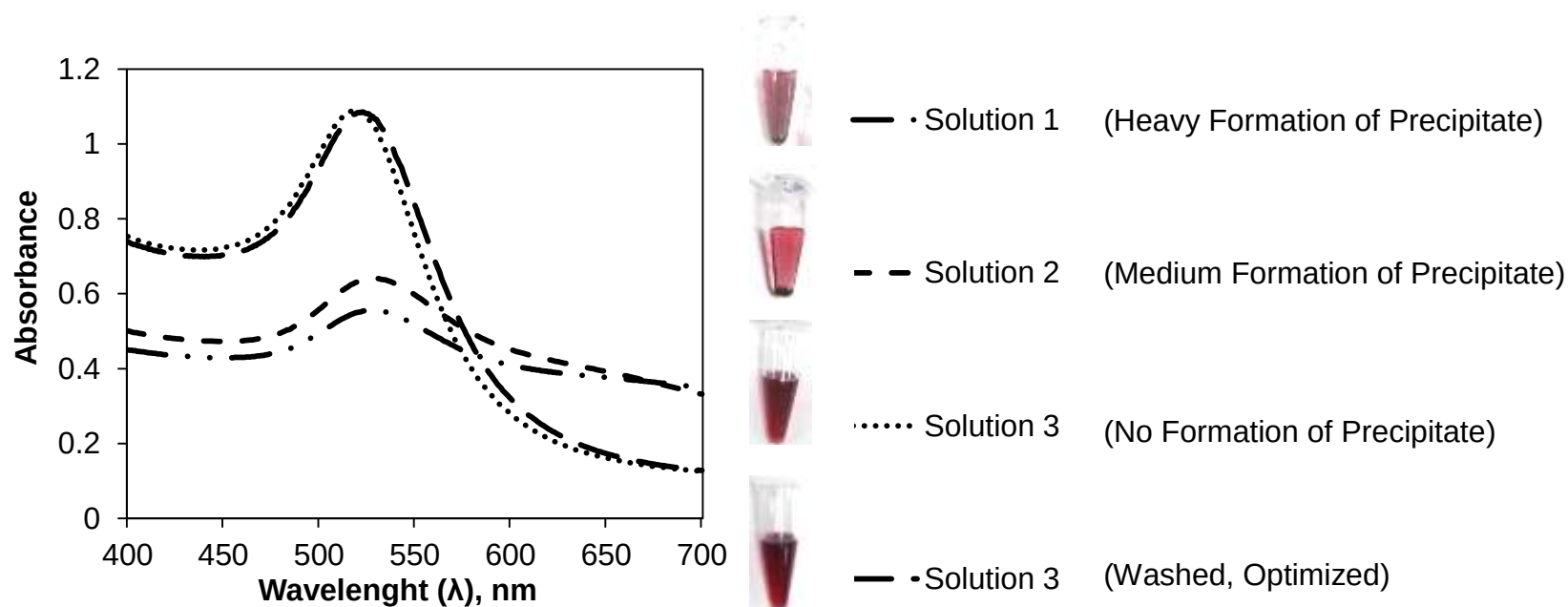


Figure 1. Absorbance (nm) and appearance of each functionalized AuNP solution during optimization process. Solution 3 had the highest absorbance at 520 nm; original dark red color was retained and no precipitation was observed after immobilization step. Both solutions 1 and 2 had lower absorbance at 520 nm; turned to lighter red color, developed grayish precipitate either dispersed throughout the solution or accumulated at the bottom of the tube. Solution 3 had the most stable condition even after washing.

Supplementary Information 2

Table 2. DNA concentration of bacterial samples presented in Fig. 3, 4, and 5 after asPCR and its absorbance ratio (625 nm /525 nm) after AuNP optical biosensing. Log CFU/ml refers to initial bacterial concentration used for genomic DNA extraction prior to asPCR. Data are expressed as mean $\pm$ standard deviation. na = not applicable.

Figure Number	Target Gene	Samples	1 log CFU/ml of bacteria		2 log CFU/ml of bacteria		3 log CFU/ml of bacteria	
			DNA conc (ng/ μ l) after asPCR	Absorbance Ratio (625 nm/525 nm)	DNA conc (ng/ μ l) after asPCR	Absorbance Ratio (625 nm/525 nm)	DNA conc (ng/ μ l) after asPCR	Absorbance Ratio (625 nm/525 nm)
3	stx1	STEC O26:H11	413.07 $\pm$ 19.40	0.66 $\pm$ 0.02	420.67 $\pm$ 15.66	0.75 $\pm$ 0.02	424.70 $\pm$ 19.82	0.70 $\pm$ 0.04
	stx2	STEC O111:H8	422.60 $\pm$ 30.19	0.79 $\pm$ 0.04	425.87 $\pm$ 29.72	0.80 $\pm$ 0.09	444.27 $\pm$ 12.09	0.77 $\pm$ 0.02
		<i>S. Typhimurium</i>	80 $\pm$ 10.00	1.12 $\pm$ 0.04	na	na	na	na
		Nuclease-free H ₂ O	na	1.20 $\pm$ 0.01	na	na	na	na
4	stx1	STEC O145:H-	425.57 $\pm$ 32.61	0.78 $\pm$ 0.10	430.27 $\pm$ 25.65	0.73 $\pm$ 0.01	438.77 $\pm$ 9.15	0.85 $\pm$ 0.03
		<i>S. Typhimurium</i>	85 $\pm$ 20.00	1.13 $\pm$ 0.02	90 $\pm$ 18.00	1.13 $\pm$ 0.01	80 $\pm$ 5.00	1.12 $\pm$ 0.02
		Nuclease-free H ₂ O	na	1.22 $\pm$ 0.08	na	na	na	na
	stx2	STEC O145:H-	378.60 $\pm$ 43.40	0.6 $\pm$ 0.11	359.50 $\pm$ 54.10	0.69 $\pm$ 0.11	356.20 $\pm$ 92.04	0.58 $\pm$ 0.14
		<i>S. Typhimurium</i>	81.63 $\pm$ 19.36	1.05 $\pm$ 0.08	85 $\pm$ 10.00	1.03 $\pm$ 0.11	90 $\pm$ 10.00	1.06 $\pm$ 0.09
		Nuclease-free H ₂ O	na	1.18 $\pm$ 0.03	na	na	na	na
5	stx1	STEC O45:H2	381.60 $\pm$ 28.46	0.68 $\pm$ 0.38	na	na	na	na
		STEC O103:H11	409.97 $\pm$ 15.88	0.66 $\pm$ 0.02	na	na	na	na
		STEC O121:H19	405.13 $\pm$ 7.46	0.68 $\pm$ 0.02	na	na	na	na
		STEC O157:H7	416.60 $\pm$ 10.81	0.55 $\pm$ 0.12	na	na	na	na
	stx2	STEC O45:H2	404.07 $\pm$ 7.88	0.73 $\pm$ 0.04	na	na	na	na
		STEC O103:H11	400.53 $\pm$ 6.80	0.67 $\pm$ 0.02	na	na	na	na
		STEC O121:H19	417.23 $\pm$ 16.97	0.68 $\pm$ 0.04	na	na	na	na
		STEC O157:H7	458.77 $\pm$ 35.67	0.59 $\pm$ 0.07	na	na	na	na
		<i>S. Typhimurium</i>	150 $\pm$ 0.01	1.05 $\pm$ 0.08	na	na	na	na
		Nuclease-free H ₂ O	na	1.13 $\pm$ 0.07	na	na	na	na