

Supplementary Materials

Nanobiosensor for detection and quantification of swine DNA in degraded mixed meats

M. E. Ali¹, U. Hashim^{1*}, S. Mustafa², Y. B. Che Man², M. H. M. Yusop², M. Kashif¹, Th. S. Dhahi¹, M. F. Bari³, M. A. Hakim⁴
and M. A. Latif⁵

¹Institute of Nano Electronic Engineering (INNE), Universiti Malaysia Perlis, 01000 Kangar, Perlis, Malaysia

²Halal Products Research Institute, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

³School of Materials Engineering, Universiti Malaysia Perlis, 01000 Kangar, Perlis, Malaysia

⁴Institute of Tropical Agriculture, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

⁵Department of Crop Science, Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

*Corresponding author: U. Hashim; E-mail: uda@unimap.edu.my

Table S-1: Data used in the generation of standard curve (figure 6 (a)) with synthetic oligo target (27 nt)

FI-1	FI-2	FI-3	± SD	Used TDNA (pmol/ml)	Av. FI	TDNA Copy N./ml	P/T
0.09	0.13	0.2	0.055677644	0	0.14	0	
0.38	0.5	0.65	0.135277493	0.00366	0.51	2.20E+09	4096:1
0.35	0.55	0.71	0.18036999	0.007324	0.536666667	4.40E+09	2048:1
0.49	0.72	0.85	0.182300119	0.01465	0.686666667	8.80E+09	1024:1
0.76	0.95	1.08	0.160934769	0.0293	0.93	1.76E+10	512:1
0.89	1.29	1.31	0.236924742	0.0586	1.163333333	3.52E+10	256:1
1.04	1.66	1.85	0.423595719	0.1172	1.516666667	7.04E+10	128:1
1.28	2.13	2.25	0.528803681	0.234375	1.886666667	1.41E+11	64:1
1.51	2.69	2.53	0.640104158	0.46875	2.243333333	2.82E+11	32:1
2.16	3.33	3.28	0.661538611	0.9375	2.923333333	5.63E+11	16:1
3.23	3.95	4.73	0.750199973	1.875	3.97	1.13E+12	8:1
4.33	5.73	6.02	0.903714557	2.8125	5.36	1.68E+12	6:1
6.01	7.62	7.77	0.975721955	3.75	7.133333333	2.25E+12	4:1
8.44	9.91	10.26	0.965729431	5.6	9.536666667	3.38E+12	3:1
11.47	12.7	13.56	1.05044435	7.5	12.57666667	4.50E+12	2:1
14.84	16.33	17.32	1.248372274	11.25	16.16333333	6.75E+12	3:2
18.98	20.06	22.33	1.709863542	15	20.45666667	9.01E+12	1:1
23.14	24.44	26.44	1.662327685	22.5	24.67333333	1.35E+13	2:3
27.66	28.88	30.38	1.362399843	30	28.97333333	1.80E+13	1:2
32.45	33.68	35.59	1.582224173	45	33.90666667	2.70E+13	1:3
37.88	39.25	41.22	1.67895801	60	39.45	3.60E+13	1:4
40.05	41.09	41.98	0.965971014	90	41.04	5.40E+13	1:6
40.99	41.73	42.54	0.775263396	120	41.75333333	7.20E+13	1:8

FI-1, 2, 3: Fluorescence intensity of triplicates; SD: standard deviation of triplicates; TDNA: Synthetic target DNA; Av. FI:

Average fluorescence intensity of the triplicates, TDNA Copy N.: target DNA copy number and P/T: Probe/target ratio.

Table S-2: Data used in the generation of standard curve (figure 6 (b)) with *AluI* digested swine DNA extracted from raw pork.

FI-1	FI-2	FI-3	± SD	^a TDNA (µg/ml)	Av. FI	Copy N./ml	P/T	^c Relative accuracy (%)	Ref. FI (table S-1)
0.45	0.53	0.62	0.085049005	0	0.533333333				
0.42	0.57	0.78	0.180831413	0.0585937	0.59	4.76E+09	1907:1	14.23	0.54
0.52	0.74	0.84	0.163707055	0.1171875	0.7	8.92E+09	1010:1	1300%	0.68
0.97	1.3	1.45	0.245560583	0.234375	1.24	5.78E+10	155.9:1	43.64	1.51
1.39	1.67	1.96	0.28501462	0.46875	1.673333333	1.25E+11	72:1	46.64	1.88
1.78	2.48	2.51	0.413077878	0.9375	2.256666667	2.80E+11	32.2:1	52.31	2.24
2.56	3.02	3.59	0.515978036	1.875	3.056666667	8.60E+11	10.3:1	87.64	3.97
4.02	4.3	5.19	0.610928255	2.81	4.503333333	1.40E+12	7.16:1	87.2	5.36
5.67	6.76	6.93	0.683690963	3.75	6.453333333	2.04E+12	4.41:1	95.22	7.13
7.88	8.56	9.53	0.829236597	5	8.656666667	3.05E+12	3.32:1	94.9	9.53
10.77	11.48	12.68	0.965418735	7.5	11.64333333	4.16E+12	2.16:1	97.08	12.58
14.03	14.99	16.27	1.123803067	11.25	15.09666667	6.29E+12	3.2:2	97.83	16.16
18.49	19.56	20.54	1.025329215	^b 15	19.53	8.57E+12	1.05:1	100	20.46
23.18	23.35	25.32	1.189495691	22.5	23.95	1.31E+13	2:2.9	98.1	24.67
27.55	28.45	29.88	1.175003546	30	28.62666667	1.78E+13	1:2	96.15	28.97
32.12	33.78	34.08	1.055714608	45	33.32666667	2.65E+13	1:3	96.93	33.9
37.68	37.92	39.72	1.114988789	60	38.44	3.50E+13	1:3.9	97.9	39.45
39.87	40.55	40.95	0.546015873	90	40.45666667	5.31E+13	1:5.9	96.74	41.04
40.55	41.19	41.33	0.415852538	120	41.02333333	5.39E+13	1:6	78.58	41.04

^a*AluI* digested swine DNA extracted from raw pork; ^bTaken as internal standard to calculate relative accuracy.

^cS-2: Calculation of relative accuracy

As copy number of mitochondrial DNA varies significantly from species to species and even within the species [47], we could not calculate absolute copy number. We found in triplicate experiments that 15 µg/ml *AluI*-cut genomic DNA produced 19.53 units of fluorescence on an average. It was close to 15 nM synthetic target which produced fluorescence of 20.46 units (table S-1) in triplicate experiments. We used the formula, $C_2 = C_1 I_2 / I_1$, described in section 3.1 to quantify targets and found 15 µg/ml *AluI*-cut genomic DNA contained 8.57×10^{12} copies of cytb gene. We took it as 100% accurate. Using the same quantification formula, we calculated the copy number of targets in each preparation using closely matched fluorescence in table S-1 as a reference. We compared it with the known values and expressed it as relative accuracy in percentage. For example, 7.5 µg/ml *AluI*-cut genomic DNA should contain 4.285×10^{12} copies of cytb. However, experimentally determined value using 12.58 in table S-1 as reference fluorescence was 4.16×10^{12} . Thus the calculated relative accuracy was $4.16/4.285 \times 100 = 97.08\%$.

Table S-3: Quantification of swine DNA from *AluI* digested mixed DNAs extracted from raw pork-beef mixture.

Pork (%)	FI-1	FI-2	FI-3	± SD	^a Calc. TDNA in µg/ml	^b Estd. in µg/ml	Av. FI	T. Copy N./ml	P/T	^c Rel. Accuracy (%)	Ref. FI (table S-2)
0	0.27	0.42	0.5	0.116761866	0	0	0.396666667				
0	0.45	0.5	0.63	0.092915732		0	0.526666667				
0.1	0.48	0.62	0.72	0.120554275	0.06	0.055	0.606666667	4.50E+10	1998:1	92.5	0.59
0.5	1.15	1.32	1.44	0.14571662	0.3	0.25	1.303333333	6.10E+10	147.7:1	82.5	1.24
1	1.66	1.98	2.15	0.248797106	0.6	0.54	1.93	1.44E+11	62.57:1	90	1.67
5	3.89	4.28	4.89	0.504017195	3	2.72	4.353333333	1.35E+12	6.67:1	90.75	4.5
10	8.35	8.74	9.68	0.683690963	6	5.82	8.923333333	3.15E+12	2.86:1	97	8.65
25	17.75	18.47	19.63	0.948542742	^d 15	14.33	18.61666667	8.19E+12	1.1:1	95.53	19.53
50	26.56	27.46	28.66	1.053565375	30	29	27.56	1.72E+13	1:1.9	96.65	28.62
100	35.86	36.89	38.08	1.110960545	60	57.63	36.94333333	3.36E+13	1:3.7	96.05	38.44

^aDNA calculated from the assumption that 100% pork contained 60 µg/ml swine DNA; ^bEstimated swine DNA calculated from closely matched reference fluorescence (Ref. FI in table S-2) from standard curve using experimental fluorescence (Av. FI); ^cInternal standard used in calculating relative accuracy.

^cS-3: Calculation of relative accuracy

In this calculation closely matched fluorescence in table S-2 was taken as a reference and 15 µg/ml *AluI*-cut swine DNA was taken as internal control. We used the formula, $C_2 = C_1 I_2 / I_1$, described in section 3.1 as quantification formula and 14.33 µg/ml DNA was found in place of 15 µg/ml. Thus the calculated relative accuracy was $14.33/15 \times 100 = 95.53\%$.

Table S-4: Quantification of swine DNA from *AluI* digested mixed DNAs extracted from autoclaved pork-beef mixture.

Pork (%)	FI-1	FI-2	FI-3	± SD	Estd. TDNA (µg/ml)	Av. FI	TDNA Copy N./ml	P/T	Relative Accuracy (%)	Ref. FI (table S-2)
0	0.11	0.16	0.22	0.05507570	0	0.163333333	0			
0	0.13	0.18	0.26	0.06557438		0.19	0			
0.1	0.44	0.52	0.68	0.12220201	0.055	0.54666666	4.50E+10	2002:1	77.39	0.59
0.5	0.58	0.79	0.93	0.17616280	0.129	0.76666666	9.80E+10	919:1	96.6	0.7
1	1.26	1.49	1.74	0.24006943	0.42	1.49666666	1.12E+11	80.4:1	42.69	1.67
5	2.13	2.69	2.94	0.41476901	1.08	2.58666666	3.23E+11	27.9:1	80.89	3.05
10	3.03	3.68	4.05	0.51636550	2.205	3.58666666	1.09E+12	8.26:1	83.7	3.05
25	8.77	9.16	9.92	0.58483615	5.99	9.28333333	3.25E+12	2.77:1	89.66	8.66
50	14.78	15.84	16.96	1.09013760	11.8275	15.86	6.61E+12	1.36:1	90	15.1
100	24.03	25.44	26.68	1.32590849	^a 26.7	25.3833333	1.58E+13	1:1.8	100	23.95

^aInternal standard used for calculating relative accuracy.

^aS-4: Calculation of relative accuracy

Prolong (2.5-h) autoclave cooking degraded many targets. Consequently, fluorescence was significantly reduced (figure 5). Table S-2 was used as a reference and it was estimated that 100% autoclaved pork contained 26.7 µg/ml *AluI*-cut swine DNA with 1.58×10^{13} copies of *cytb*. We considered it as 100% accurate. We used the formula, $C_2 = C_1 I_2 / I_1$, described in section 3.1 to quantify potential targets in each preparation and expressed as relative accuracy.

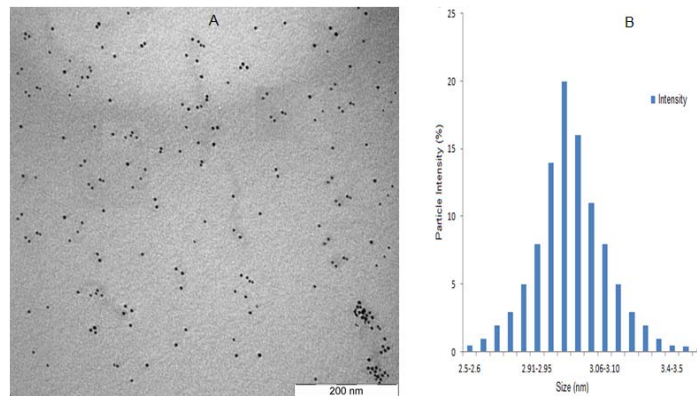


Figure S-1: TEM image of citrate-tannate coated gold nanoparticles (A) and size distribution chart (B) generated from the analysis of five hundred particles. The average diameter of the particles was found to 3 ± 0.2 nm.

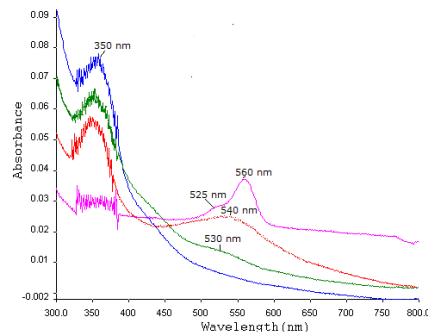


Figure S-2: UV-vis spectra of citrate-tannate-coated gold nanoparticles (10 nM) and TMR-labeled thiolated oligo-probe under various conditions. For illustration; thiolated oligo-TMR (15 nM) in water (pink curve), 10 nM GNPs incubated with 3-fold molar excess of thiolated oligo-TMR before purification (green curve) & after purification (red curve) and the supernatant (citrate-tannate) after removal of gold particles (blue curve). Please note the masked shoulder at 540 nm by citrate-tannate before purification.

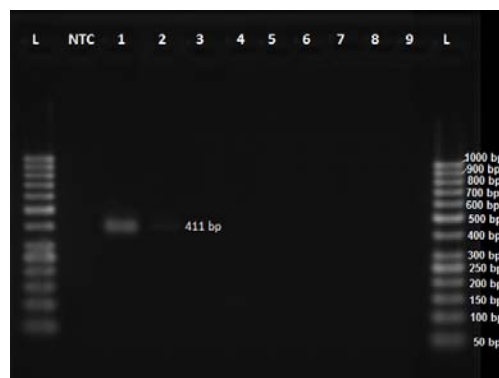


Figure S-3: Gel image of 411 bp swine PCR products amplified from swine DNA extracted from pure raw pork and 2.5-h autoclaved pork-beef mixtures. L: 50 bp ladder; NTC: negative template control; lane 1: pure raw pork; lanes 2-9: 100%, 50%, 25%, 10%, 5%, 1%, 0.5% and 0.1% pork in 2.5-h autoclaved pork-beef admixtures.