

Supplementary information

**Detection of prostate-specific antigen in semen using DNA aptamers: an application of
nucleic acid aptamers in forensic body fluid identification**

Tetsuya Satoh^{a,*}, Seiya Kouroki^a, Yusuke Kitamura^b, Toshihiro Ihara^b, Kazutoshi Matsumura^a and
Susumu Iwase^a

^a*Forensic Science Laboratory, Kumamoto Prefectural Police Headquarters, 6-18-1 Suizenji, Chuo-ku,
Kumamoto 862-8610, Japan*

^b*Division of Materials Science and Chemistry, Faculty of Advanced Science and Technology, Kumamoto
University, 2-39-1 Kurokami, Chuo-ku, Kumamoto 860-8555, Japan*

Tetsuya Satoh, Seiya Kouroki, Kazutoshi Matsumura, Susumu Iwase: kasoken@police.pref.kumamoto.jp

Yusuke Kitamura: ykita@kumamoto-u.ac.jp

Toshihiro Ihara: toshi@chem.kumamoto-u.ac.jp

*To whom correspondence should be addressed.

Forensic Science Laboratory, Kumamoto Prefectural Police Headquarters, 6-18-1 Suizenji, Chuo-ku,
Kumamoto 862-8610, Japan

Tel: +81 96 381 0110

Fax: +81 96 381 0110

E-mail: kasoken@police.pref.kumamoto.jp

Hemoglobin-binding DNA Aptamer

The specificity of two 72-mer DNA oligonucleotides for blood from body fluids (blood, semen, saliva, urine, sweat, vaginal secretion) was examined. The oligonucleotides were biotin-labeled at their 5'-end, and the specificity was examined by ELONA. The sequences are as follows.

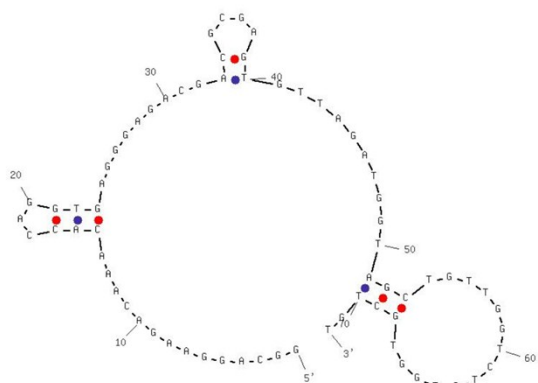
Hb aptamer No.1

5' – GGCAGGAAGACAAACACCAGGTGAGGGAGACGACGCGAGTGTTAGATGGTAGCTGTTGGTC-
TGTGGTGCTGT – 3'

Hb aptamer No.2

5' – ACAGCACACAGACCAACAGCTACCATCTAACACTCGCGTCGTCTCCCTCACCTGGTGTTG-
TCTTCCTGCC – 3'

(A)



(B)

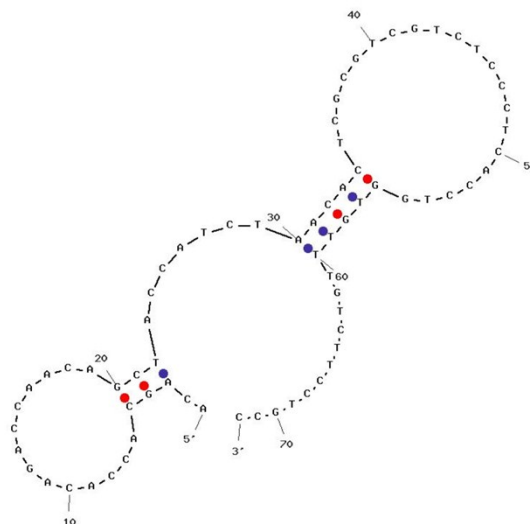


Fig. S1 Predictions of the most stable secondary structures of 72mer DNA oligonucleotides (A) Hb aptamer

No.1 (B) Hb aptamer No.2. The secondary structures were predicted by Oligo Analyzer Version 3.1 software,

and their ΔG values were $-5.08 \text{ kcal mol}^{-1}$ and $-4.09 \text{ kcal mol}^{-1}$, respectively.

Ct							Δ Ct								
Blood							Blood								
Dilution Factor	1	2	3	4	5	Average	Dilution Factor	1	2	3	4	5	Average	SD	
× 100	30.59859	30.72241	29.08351	30.34104	31.29961	30.40903	× 100	-1.69713	-1.57331	-3.21221	-1.95468	-0.99611	1.934078	0.819893	
× 1000	30.36125	29.69796	30.62374	30.16388	30.18003	30.20537	× 1000	-1.93447	-2.59776	-1.67198	-2.13184	-2.11569	2.129318	0.338807	
× 10000	31.04502	29.63845	31.2708	30.31689	30.81842	30.61792	× 10000	-1.2507	-2.65727	-1.02492	-1.97883	-1.4773	1.78458	0.651699	
× 100000	31.33663	31.09942	31.30963	30.29636	31.55151	31.11871	× 100000	-0.95909	-1.1963	-0.98609	-1.99936	-0.74421	1.23149	0.486797	
Semen							Semen								
Dilution Factor	1	2	3	4	5	Average	Dilution Factor	1	2	3	4	5	Average	SD	
× 100	21.45375	21.00876	20.04816	19.88708	20.36861	20.55327	× 100	-10.842	-11.287	-12.2476	-12.4086	-11.9271	11.74245	0.661699	
× 1000	24.1754	24.2114	22.89834	22.60442	23.37691	23.45329	× 1000	-8.12032	-8.08432	-9.39738	-9.6913	-8.91881	8.842426	0.729821	
× 10000	27.22483	29.64789	28.61595	28.8323	29.12061	28.68832	× 10000	-5.07089	-2.64783	-3.67977	-3.46342	-3.17511	3.607404	0.904906	
× 100000	32.2838	32.64747	31.62076	31.952	31.62977	32.02676	× 100000	-0.01192	0.35175	-0.67496	-0.34372	-0.66595	0.26896	0.441412	
Saliva							Saliva								
Dilution Factor	1	2	3	4	5	Average	Dilution Factor	1	2	3	4	5	Average	SD	
× 100	24.9094	30.28649	28.34942	27.02665	28.84188	28.62611	× 100	-7.38632	-0.25792	-2.19499	-3.51776	-1.70253	1.9183	2.709135	
× 1000	29.67179	28.1325	29.02339	29.41941	30.93997	29.43741	× 1000	-2.62393	-2.41191	-1.52102	-1.125	0.39556	1.165593	1.206221	
× 10000	30.46093	31.48419	31.05487	30.80034	28.09174	30.37841	× 10000	-1.83479	0.93978	0.51046	0.25593	-2.45267	0.186625	1.521405	
× 100000	31.61162	31.01933	28.39113	30.24556	30.61406	30.37634	× 100000	-0.6841	0.47492	-2.15328	-0.29885	0.06965	0.47689	1.010072	
Urine							Urine								
Dilution Factor	1	2	3	4	5	Average	Dilution Factor	1	2	3	4	5	Average	SD	
× 100	31.68953	29.09391	31.55892	29.17027	27.5362	29.80977	× 100	1.14512	-1.4505	1.01451	-1.37414	-3.00821	0.734644	1.780699	
× 1000	31.42474	32.03217	28.15218	29.953	32.14045	30.74051	× 1000	0.88033	1.48776	-2.39223	-0.59141	1.59604	-0.1961	1.68938	
× 10000	31.33521	30.73311	30.44569	31.14104	30.80018	30.89105	× 10000	0.7908	0.1887	-0.09872	0.59663	0.25577	-0.34664	0.350471	
× 100000	29.78504	33.29053	28.16635	30.98774	30.82074	30.61008	× 100000	-0.75937	2.74612	-2.37806	0.44333	0.27633	-0.06567	1.872453	
Sweat							Sweat								
Dilution Factor	1	2	3	4	5	Average	Dilution Factor	1	2	3	4	5	Average	SD	
× 100	30.06103	33.41594	31.8965	30.01217	30.66107	31.20934	× 100	-0.48338	1.12022	-0.39922	-2.28355	-1.63465	0.736116	1.306261	
× 1000	33.14231	30.94997	32.10131	31.19628	29.40065	31.3581	× 1000	2.5979	-1.34575	-0.19441	-1.09944	-2.89507	0.587354	2.028743	
× 10000	32.72204	31.64239	31.71271	32.00256	31.5049	31.91692	× 10000	2.17763	-0.65333	-0.58301	-0.29316	-0.79082	0.028538	1.246603	
× 100000	33.13022	31.77835	31.98372	26.52201	30.82317	30.84749	× 100000	2.58581	-0.51737	-0.312	-5.77371	-1.47255	1.097964	3.021917	
Vaginal secretion							Vaginal Secretion								
Dilution Factor	1	2	3	4	5	Average	Dilution Factor	1	2	3	4	5	Average	SD	
× 100	26.85601	29.66229	30.73665	26.27146	25.36695	27.77867	× 100	-3.6884	-0.88212	0.19224	-6.02426	-6.92877	3.466262	3.108218	
× 1000	28.84622	31.81331	32.59149	29.45574	28.5008	30.24151	× 1000	-1.69819	1.2689	2.04708	-2.83998	-3.79492	1.003422	2.555246	
× 10000	32.45535	33.20383	32.80976	31.95367	30.66546	32.21761	× 10000	1.91094	2.65942	2.26535	-0.34205	-1.63026	-0.97268	1.864151	
× 100000	37.00951	34.34871	35.02484	30.87988	35.16357	34.4853	× 100000	6.4651	3.8043	4.48043	-1.41584	2.86785	-3.24037	2.918635	
No body fluid															
Plate 1	32.29572														
Plate 2	30.54441														

Sheet 1 C_t and ΔC_t values of blood, semen, saliva, urine, sweat, and vaginal secretion