Supplemental Information

A single-round selection of selective DNA aptamers for mammalian cells by polymer-enhanced capillary transient isotachophoresis

Kazuki Hirose,[1] Maho Tsuchida,[1] Hinako Asakura,[1] Koji Wakui,[2] Keitaro Yoshimoto,[2] Keisuke Iida,[1] Makoto Sato,[3] Masami Shibukawa,[1] Masami Suganuma,[1,4] Shingo Saito[1]*

[1] Graduate School of Science and Engineering, Saitama University, 255 Shimo-Okubo, Sakura-ku, Saitama 338-8570, Japan. E-mail: shingo@mail.saitama-u.ac.jp; Tel: +81 48 858 3559

[2] Department of Life Sciences, Graduate School of Arts and Sciences, The University of Tokyo, 3-8-1 Komaba, Meguro-ku, Tokyo, 153-8902, Japan

[3] Sagamihara R & D Center, Shino-Test Corporation, 2-29-14 Oonodai, Minami-ku, Sagamihara 252-0331, Japan

[4] Research Institute for Clinical Oncology, Saitama Cancer Center, Ina, Kitaadachi-gun, Saitama 362-0806, Japan

Table of Contents

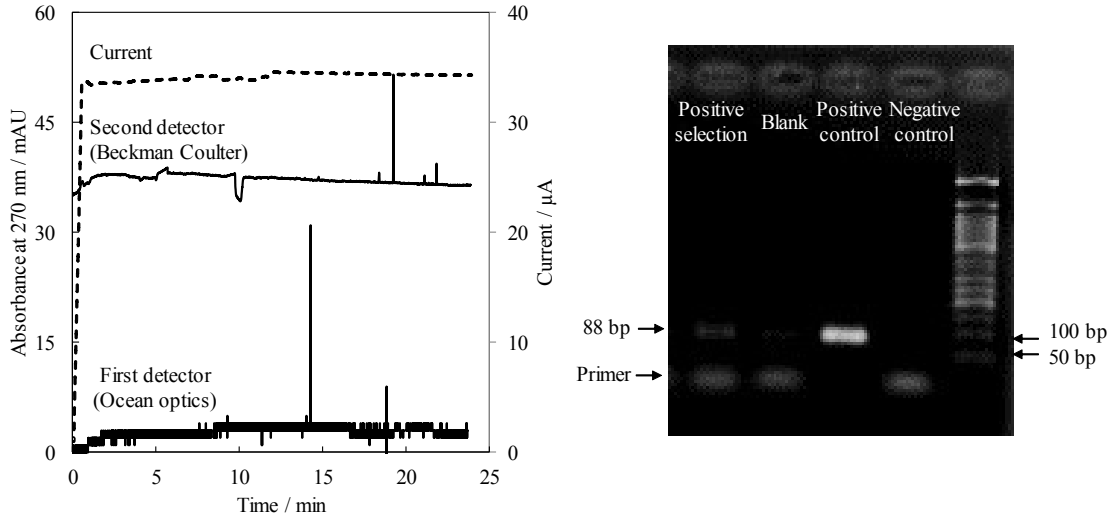

Figure S-1. Left: typical electropherogram of P1 pool (one round of positive PectI selection), as obtained using the dual-detector system. Right: corresponding agarose gel electropherogram, with lanes corresponding to samples as indicated on the figure.

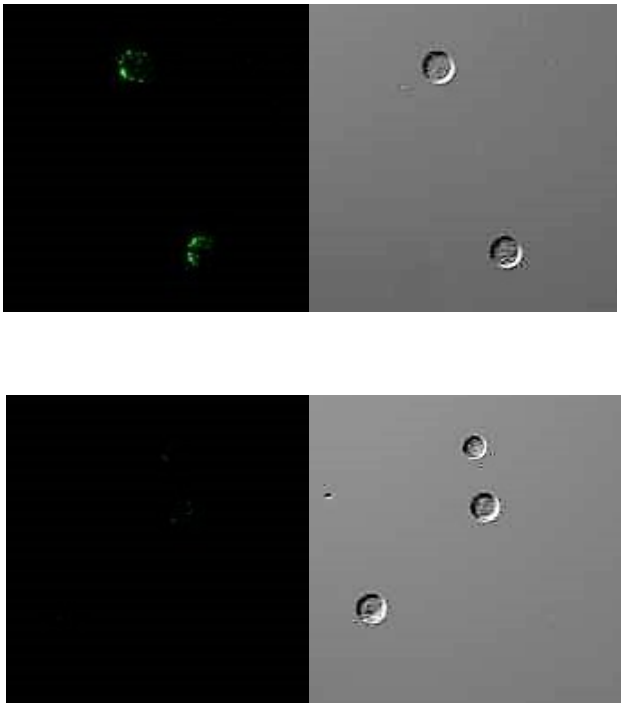

Figure S-2. Fluorescence (left) and differential interference contrast image (right) for mixtures of PC-9 cells with P1 pool (top) and randomized DNA library (bottom). Sample preparation: a mixture of 3×10^6 cells/mL of PC-9, 1.0×10^{-6} M of DNA and 20 mM/5 mM/5 mM/2.5 mM/290 mM

Tris-HCl/NaCl/$MgCl_2$/Glc was incubated for 2 hours on ice, followed by centrifugation at 200g for 60 s to remove the supernatant. This washing sequence was repeated three times before fluorescence microscopy.

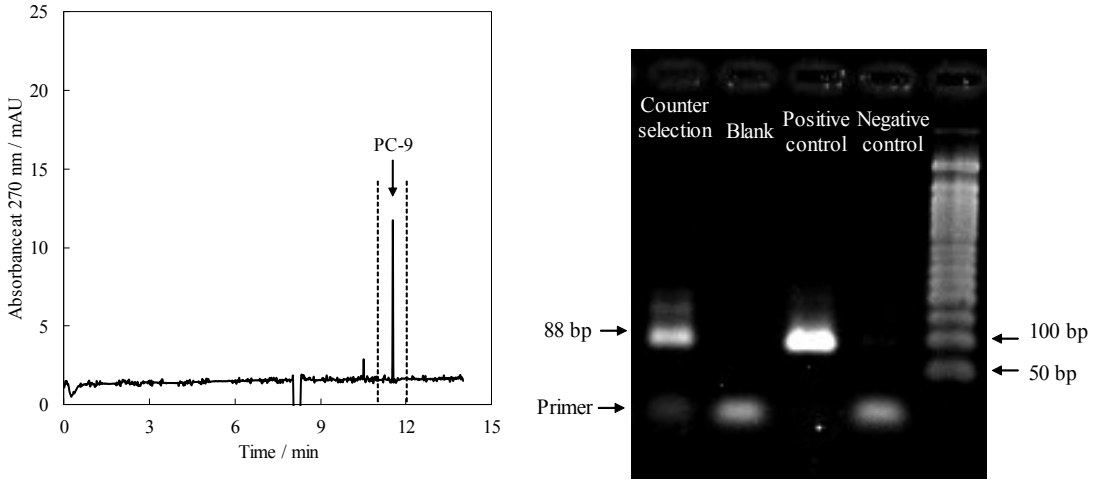

Figure S-3. Typical electropherogram of positive PectI selection after a simplified counter selection using HL-60 cells, to partition PC-9–DNA complexes using a dual-detector UV system (left) and gel electrophoresis of the obtained C1 pool (right). The zone between the dotted lines in the left panel shows the partitioning interval.

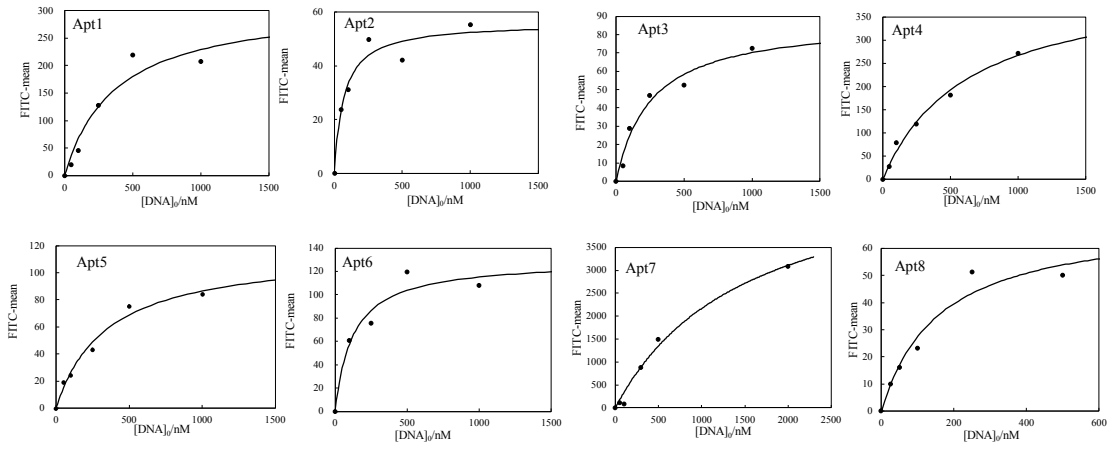

Figure S-4. Binding curves of DNA aptamers for PC-9 cells.

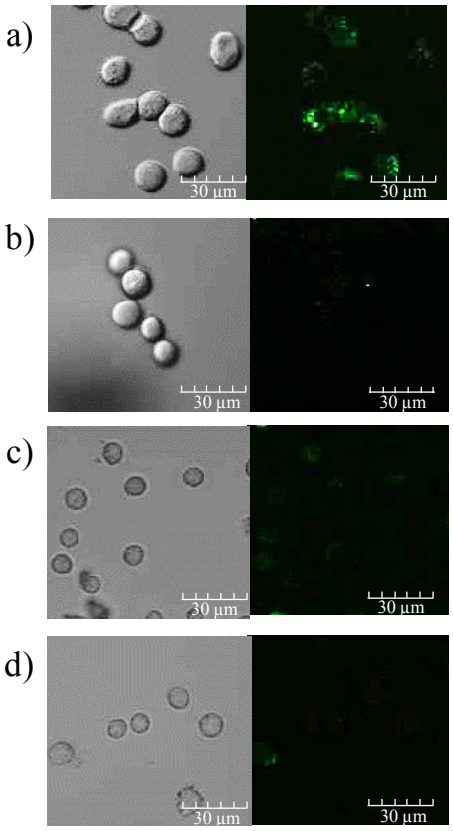

Figure S-5. Fluorescence (right) and differential interference contrast image (left) for mixtures of PC-9 cells with *Apt6* (a) and randomized DNA library (b), HL-60 cells with Apt6 (c) and randomized DNA library (d). 1000 nM DNA, 3×10^6 PC-9 or HL-60 cells/mL, λ_{em} = 490 nm. The procedure for the sample preparation was the same as in Fig. S-2

Table S-1. Results of NGS analysis.

	One round of PectI positive selection with simplified counter selection	One round of PectI positive selection
Read number of sequences	19612	171245
Number of families (A)	366	11
Number of sequences forming families (B)	1965	56
Rate of sequences forming families in all sequences (A/B)	0.100	0.00033

Table S-2. Families found by cluster analysis.

	Number of sequences composing family	Total counts	Typical sequence (name of aptamer)
Family1	8	12	*Apt1*
Family2	8	10	*Apt2*
Family3	8	10	*Apt3*
Family4	8	10	*Apt4*
Family5	7	20	
Family6	7	19	
Family7	7	17	
Family8	7	16	
Family9	7	15	
Family10	7	15	
Family11	7	15	
Family12	7	14	*Apt5*
Family13	7	14	
Family14	7	14	
Family15	7	13	
Family16	7	12	
Family17	7	12	*Apt6*
Family18	7	12	
Family19	7	10	
Family20	7	10	
Family21	7	10	
Family22	7	10	
Family23	7	9	
Family24	7	9	
Family25	7	8	
Family26	6	21	*Apt7*
⋮	⋮		
Family107	5	20	*Apt8*
⋮	⋮		