

Selection of Aptamers Based on Protein Microarray Integrated with Microfluidic Chip

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Table S1

	Round 1	Round 2	Round 3	Round 4	Round 5	Round 6	Round 7	Round 8
Circle-1	1000 pmol	48.60 pmol	53.13 pmol	43.86 pmol	52.19 pmol	59.51 pmol	53.85 pmol	-
Circle-2	1000 pmol	87.83 pmol	88.38 pmol	96.48 pmol	89.34 pmol	94.13 pmol	92.27 pmol	88.75 pmol
Circle-3	1000 pmol	69.92 pmol	76.76 pmol	71.10 pmol	64.02 pmol	66.77 pmol	71.37 pmol	58.40 pmol

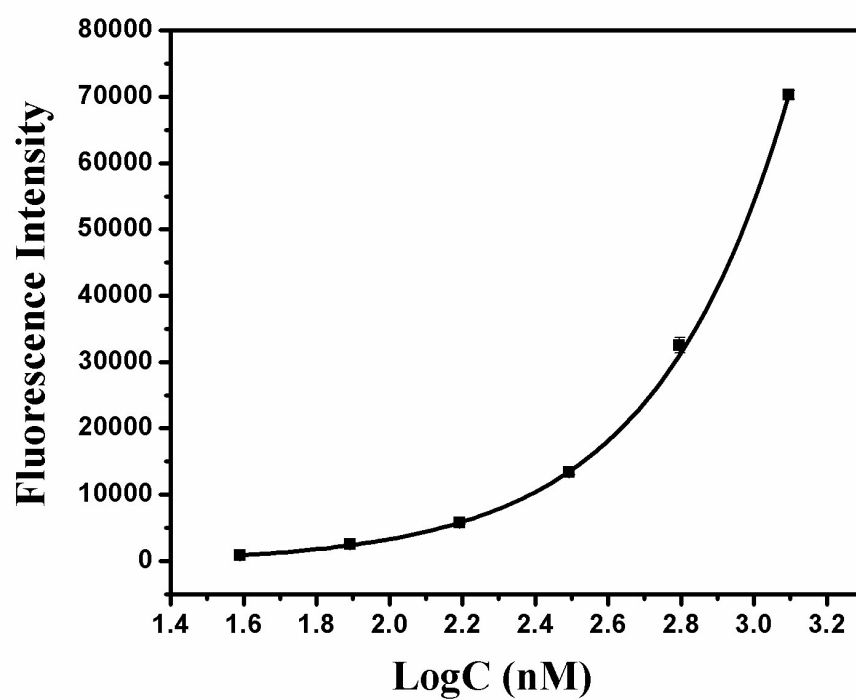


Figure S1. The fluorescence intensity of lib with different concentration.

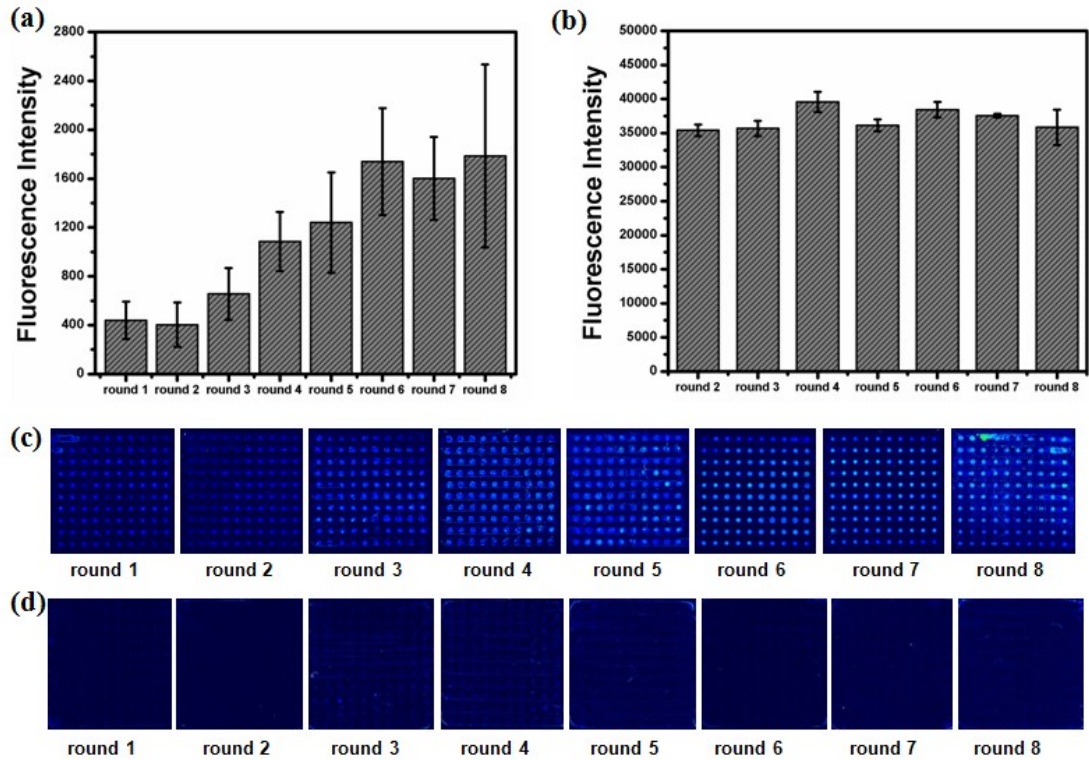


Figure S2. (a)The fluorescence intensity of evolved ssDNA pool in every round of selection. (b)The fluorescence intensity of enriched aptamers on target microarrays in every round of selection.(c) The fluorescence images of enriched aptamers on target microarrays in every round of selection before (d) and after elution (e).

Table S2 The selected sequences in the second time of PMM-SELEX.

Aptamer-ID	Sequences of N40 from 5' to 3'	Repeated times			Total times
		The fifth round	The sixth round	The seventh round	
Lac-14	CCTAACACGTACGGGGCATTATGGCATAGCTCTTCCTCC	18	16	8	42
Lac-6a	CGGTGCATCTATGGCTACTAGCTTTCTCTGCCTATACTAC	7	9	7	23
Lac-9	GGGCAATCTTGCTCTTATTTTACACATTGATAAATATGTC	1	2	1	4
Lac-5	GGGCTTATGCTCTTAAAAATCCTGAGCGACTTTTATGTA	2	0	2	4
Lac-3a	AATACTCCTGTTACCGTGCATCTATGGCCATTGGCTTTTC	1	1	1	3
Lac-6b	TCGGGCAAAGCTCTGAATAATGTCCAACCAATATCTGTGC	0	1	3	4
Lac-A1	ACGGGCTGATGCTCTTTATTTTACCTAAATAAAGTGTC	2	4	3	9
Lac-A2	ATTTGAGCAGACGGGAGACTTTTAGAGTTGTAACCTTGAGT	2	2	8	12
Lac-A3	CGGGCATTTGCTCTCAATTTAGTCTCAAATTGGCCCTGC	0	6	1	7
Lac-A4	CGGTGCATCTATGGCTACTAGCTCTTCTGCCTATACTAC	2	4	7	13
Lac-A5	GGGCTATGCTCTAAATTCTTCTACTGACGCAATTTTGGA	7	3	1	11
Lac-A6	GGGTGCACACTTATTTTACACGAGCCAAAAATATGTC	2	1	2	5
Lac-A7	TCATCCCAAGCTCCGGTGCATCTATGGGCTTCGCTTTTC	1	2	3	6
Lac-A8	CCCTAGTTCCTGGTGCAATTTATGGCAAAGCTTTCTTGCC	4	2	1	7
Lac-A9	ACCGGTGCGTCCCACGGCTCCAGCTCTTCTGCCTATTTTC	3	0	0	3
Lac-A10	ACTGCTTTATCCCCGTCGGCTTGCTCTTCGACAGTGTGG	12	27	13	52
Lac-A11	GGGCGAACGCTCTTTATTTTGGAAACCCACAAAATAATGTT	1	0	4	5
Lac-A12	GGGCGGAGCTCTTCCATTTCAGATTGTAAATGGATGTCA	0	2	2	4
Lac-A13	GGTGCAATTATTGGTGAACACTCTTCCTTAGCCTGCTACT	2	2	3	7
Lac-A14	TGAACGGTCTTGGCTACTGCACATTTTCTCTGCCTTTGTT	1	6	1	8

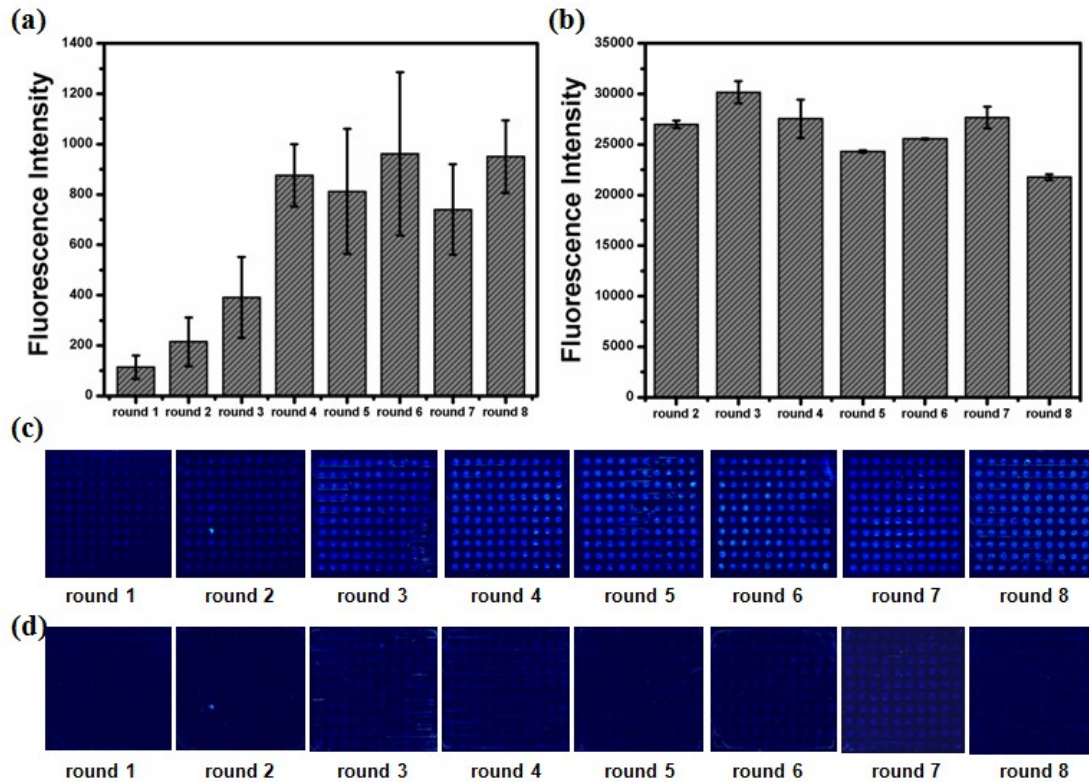


Figure S3. (a)The fluorescence intensity of evolved ssDNA pool in every round of selection.(b)The fluorescence intensity of enriched aptamers on target microarrays in every round of selection.(c) The fluorescence images of enriched aptamers on target microarrays in every round of selection before (d) and after elution (e).

Table S3 The selected sequences in the third time of PMM-SELEX.

Aptamer-ID	Sequences of N40 from 5' to 3'	Repeated times			Total times
		The fifth round	The sixth round	The seventh round	
Lac-14	CCTAACACGTACGGGGCATTTATGGCATAGCTTTCCTCC	12	5	8	25
Lac-6a	CGGTGCATCTATGGCTACTAGCTTTCTGCTATACTAC	4	2	4	10
Lac-9	GGGCAATCTTGCTCTTATTTTACACATTGATAAATATGTC	0	2	0	2
Lac-5	GGGCTTATGCTCTTAAAAATCCTGAGCGACTTTTATGTA	2	0	0	2
Lac-3a	AATACTCCTGTTACCGTGCATCTATGGCATTGGCTTTTC	2	2	0	4
Lac-A1	ACGGGCTGATGCTCTTTATTTTACCTAAATAAAGTGTC	8	10	5	23
Lac-A2	ATTTGAGCAGACGGGAGACTTTTAGAGTTGTAACCTGAGT	2	1	0	3
Lac-A3	CGGGCATTGCTCTCAATTTAGTCTCAAATTTGGCCCTGC	2	5	4	11
Lac-A4	CGGTGCATCTATGGCTACTAGCTTCTGCTATACTAC	8	7	12	27
Lac-A5	GGGCTATGCTCTAAATTCTTCTACTGACGCAATTTTGA	8	5	2	15
Lac-A6	GGGTGCACACTCTTATTTTACACGAGCAAAAAATATGTC	7	1	4	12
Lac-A7	TCATCCCAAGCTCCGGTGCCATCTATGGGCTTCGCTTTTC	3	3	4	10
Lac-A8	CCCTAGTTCCTGGTGCATTTATGGCAAAGCTTTCTCTGCC	3	2	2	7
Lac-B1	AACAACCTTCGTATCCGGTGCATTTATGGCGAATGCTTTTC	0	0	3	3
Lac-B2	ACTACCTCTTTTCGGTGCGATCCATTTCGGCTTTGCTTTTC	2	16	5	23
Lac-B3	ACTGCTTTATCCCGTCCGGCTTGGCTCTTCGACAGTGTTG	1	1	1	3
Lac-B4	ACTGCTTTATCCCGTCCGGCTTGTCTCTTCGACAGTGTTG	0	2	1	3
Lac-B5	ATTTGAGCAGACGGGAGACCTTTAGAGTTGTAACCTGAGT	7	7	5	19
Lac-B6	CAGTATAGGTGCATTTTGGCGCAAGCTTCTCTGCCCTG	2	0	2	4
Lac-B7	CATCGACGGTGCCGGTCTCGGGCATTTTGCTCTTCTGCC	5	1	0	6
Lac-B8	CCCACTCGAGGTGCAATTTTGGCGTGAGCTCTTCTGCCG	0	1	3	4
Lac-B9	CCCTAGTTCCTGGTGCATCTATGGCAAAGCTTCTCTGCC	1	2	0	3
Lac-B10	CCCTAGTTCCTGGTGCATTTATGGCAAAGCTTCTCTGCC	1	3	0	4
Lac-B11	CTACAACGGTGCCATCTATGGGCTTTGCTTTTCTGCTC	4	2	2	8
Lac-B12	GGTGCATCCATGGCTTTTAGCTCTTCTGAACTGTCACAC	2	4	7	13
Lac-B13	GGTGCATCTATGGCTTTGCTCTTCTACCTGTTCTACGAG	0	3	5	8
Lac-B14	GGTGGGTGTACACGGCTCTGCTTTTCTGCTCTGTTCAC	6	1	5	12

Table S4 The association time (K_{on}), dissociation time (K_{off}) and dissociation constant (K_d) of selected aptamers.

Aptamer-ID	$K_{on} (M^{-1}s^{-1})(\times 10^6)$	$K_{off} (s^{-1})(\times 10^{-2})$	K_d (nM)
Lac-14	2.01 ± 0.39	1.06 ± 0.15	5.48 ± 1.79
Lac-6a	11.66 ± 5.19	1.04 ± 0.11	1.04 ± 0.50
Lac-9	3.42 ± 0.98	3.43 ± 0.02	1.07 ± 0.34
Lac-5	1.20 ± 0.03	0.11 ± 0.00	0.92 ± 0.02
Lac-3a	4.60 ± 0.88	0.29 ± 0.04	0.63 ± 0.06

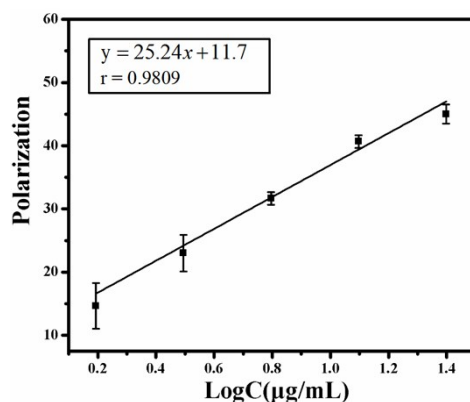


Figure S4. The polarization of Lac-6a-FITC at different five different concentrations (25 $\mu\text{g/mL}$, 12.5 $\mu\text{g/mL}$, 6.25 $\mu\text{g/mL}$, 3.12 $\mu\text{g/mL}$, 1.56 $\mu\text{g/mL}$ of Lactoferrin in Meadjohnson Enfamil (100-fold diluted) sample. $\mu\text{g/mL}$, 6.25 $\mu\text{g/mL}$, 3.12 $\mu\text{g/mL}$, 1.56 $\mu\text{g/mL}$ of Lactoferrin in Meadjohnson Enfamil (100-fold diluted) sample.

Table S5 Analytical recovery of infant formula (Meadjohnson Enfinitas, 100-fold diluted, 4.5 mg/mL Lactoferrin) sample with with fluorescence polarization method.

Concentration [$\mu\text{g/mL}$]	Recovery in infant formula
	[%]
4.5	111

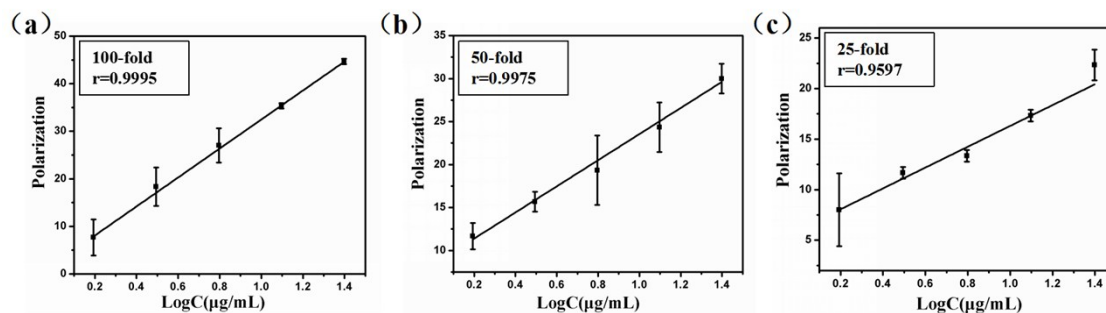


Figure S5. The linear correlation between polarization signal and Lactoferrin concentrations in the range of 1.56 - 25 $\mu\text{g/mL}$ in (a) 100-fold, (b) 50-fold, (c) 25-fold diluted mike (Telunsu) samples.