Supporting information for the article: **"Improving Aptamer Selection Efficiency through Volume Dilution, Magnetic Concentration, and Continuous Washing in Microfluidic Channels."** by Seung Soo Oh, Kareem M. Ahmad, Minseon Cho, Seon Kim, Yi Xiao* and H. Tom Soh*

Table S1: Sequences of DNA molecules used in these experiments

Name	Sequence	Description
ssDNA library	5'-AGCAGCACAGAGGTCAGATG-N_{60}-CCTATGCGTGCTACCGTGAA-3'	Random sequence in N_{60} flanked by primer sites
FP	5'-AGCAGCACAGAGGTCAGATG-3'	Forward primer
RP	5'-TTCACGGTAGCACGCATAGG-3'	Reverse primer
FP-FAM	5'FAM-AGCAGCACAGAGGTCAGATG-3'	FAM-labeled forward primer used for fluorescence based binding assay
RP-P	5'P-TTCACGGTAGCACGCATAGG-3'	Phosphorylated reverse primer for lambda exonuclease digestion

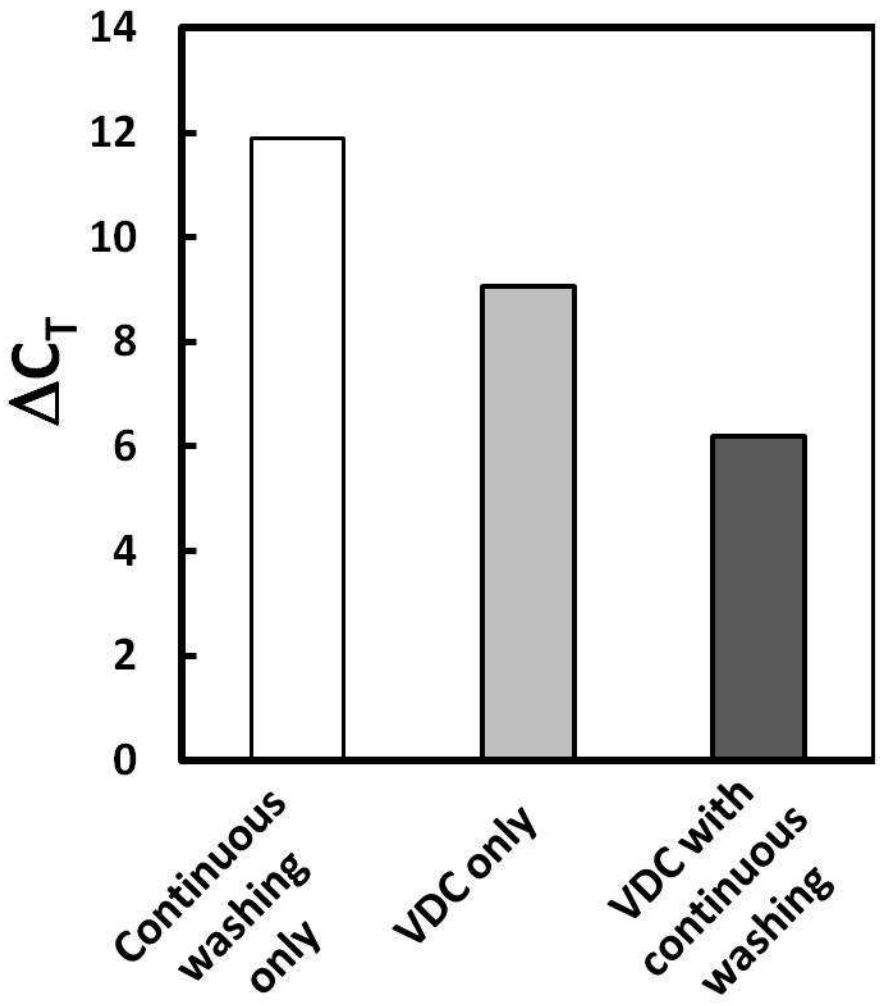

Fig S1. Comparison of washing efficiency with RT-PCR. We compared the efficiencies of three different washing methods: 1) continuous washing only (30 minutes), 2) VDC only (30 minutes) and 3) VDC with continuous washing (25+5 minutes). We used ΔC_T (difference in threshold cycle value between the sample and negative control with no template DNA) to quantify differences in the template copy number among samples obtained from the three methods. The combination of VDC with continuous washing showed that the smallest $\Delta C_T = 6.2$, indicating the most efficient removal of ssDNA. Continuous washing yielded $\Delta C_T = 11.9$ and VDC showed $\Delta C_T = 9.1$. VDC with continuous washing effectively removes ~30- and ~10-fold more ssDNA than using continuous washing or VDC alone, respectively.

Clone name	Core region of the cloned sequences
Clone #1	GTTTTTCTTGAGGGGTTTGAGGGCTTGCGTTTGATCCAACTCGTGCATTTCGGTCTCGTA
Clone #2	CATCAGTTTTCGGTTCAGGACATTGTGTCCCTCGCAAGTGACATTCAGGCACGTTTGTCAC
Clone #3	CGTTCCCTCTTCATCGGCATTACGTTTAGCGCTCCACGGATATTAGCTTTAGCAGACGGC
Clone #4	AGGAATGTATTCTTCTTTGAGGGTCTCATTATGAGTACCCGTGGAGACGTAGAGCAGTCT
Clone #5	GCTATAATCTTGGGCGTGTCTTCGGTAGTGCATTTGGTGTGTAATATTGCAATTGACAGA
Clone #6	TGGTACGCATGTTCAGACTTGTTGGACAGCGTTATGATTTCGTTTATGAGGCTCGAAAT
Clone #7	GTTACATGGATTTGTTTCTGTGTCTATATTCGATAAGCACTTACATGAGGATGTTCCAC
Clone #8	TGCACGTCTTGANAGGCTCATATGTGGTCATAAGGGACGTGTCACTTAGAACAGGGCGAA
Clone #9	CAAAATGTTCAGGGTTGGAAGGGCGTGAATCGCGGGATTCAGTAGAAAAGATGAAGTGTG
Clone #10	AGCGTTAAGGAGTTTCCCGATTTGGAGTTGGCCTAGAAAAGGTCGAAATTCGACGTGGC
Clone #11	GCCTTTCACCGCCAGTAGCAATGCTTAAATTCCACTTCGTATGCGTGTCTCAGCGGCGTA
Clone #12	TCGTCCGGGCTAGCTCTTAAGGTACTGTTAGGGTGTGTAAATCCCATTGGAAACAATGTA
Clone #13	CCCTATTCTCGTTGCTTGGATCAACTGAGACACTAGTGAGGGAATCACTCCCGAGAATG
Clone #14	GAAAGCGCTGCGTTAGCTTTTAGTATATGGTCATCACTTAATGGGATTTTCCCGGTAAAT
Clone #15	AACTTGAAAGCAGGTCGCCACCACCGGGCTTGCGATCGGGCTGAGGAAGGCAGGGCACTC
Clone #16	TTGGCTAAGCCGGGGCTCTAAAGCGTCTGTTTACCATTTTAAATGTTATAATGTAACTCGA
Clone #17	GAGCCCAGGCAAACGTCATGATCGATGGCCATTCGTCCTTAGTGTGAAAATCTAGACGTA
Clone #18	TAGAATATGGCGGTTTGACGGCATCACGCCACCGTTATGGGGTAGGGCGCCAAATTTTAA
Clone #19	TATTAGGTCGGGCTTGAAACAGCGAAGAAGGTTCCAAGGGTGTTGTGCTCTTCTAGATGA
Clone #20	TAAGGCGCCTCTCAAGCGGATGGGAACAAGGGATGACGCCCTCTAAGTAGCTATAATAGT

Fig S2. 20 cloned sequences from the R2 pool. Primer sites are not shown. Sequence alignment reveals no consensus.

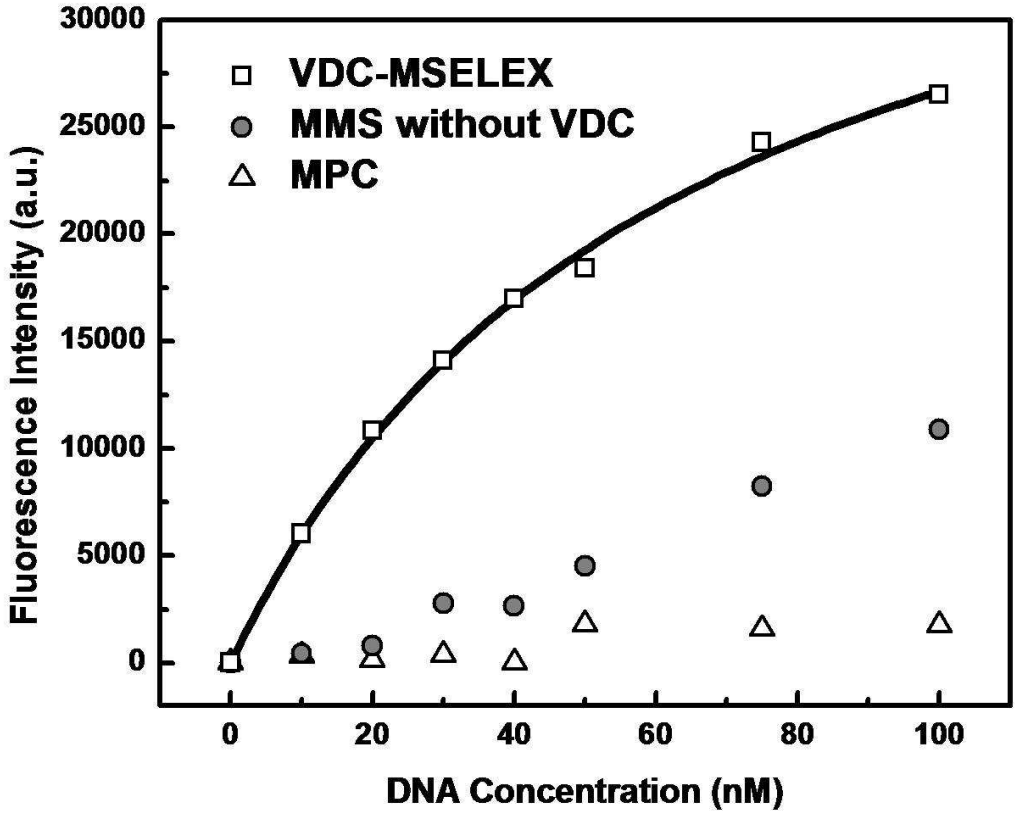

Fig S3. Comparative measurement of selection efficiencies: We compared the selection efficiency of VDC-MSELEX method with those of conventional magnetic selection using the MPC apparatus (Invitrogen), and the MMS chip (without volume dilution). For the MPC selection, we manually performed the buffer exchange with 1 mL of fresh buffer every 5 min. After three rounds of selections under the same experimental conditions including target concentration and the washing time, we observe that the bulk affinity of the final pool obtained with the VDC-MSELEX (square) ($K_d = 62.5 \pm 5.1$ nM) is significantly higher than those obtained with the MPC (Fig S3, triangle) and MMS without VDC (Fig S3, circle).