Supporting Information for

A "turn on/off" scorpion biosensor targeting point mutation of *SMN* gene for diagnosis of spinal muscular atrophy

Chung-An Chen[1], Chun-Chi Wang[1], Yen-Ling Chen[2],

Yuh-Jyh Jong[3,4,5], Shou-Mei Wu[1,6]*

[1] *School of Pharmacy, College of Pharmacy, Kaohsiung Medical University, Kaohsiung, Taiwan*

[2] *Department of Fragrance and Cosmetic Science, College of Pharmacy, Kaohsiung Medical University, Kaohsiung, Taiwan*

[3] *Graduate Institute of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan*

[4] *Department of Pediatrics, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan*

[5] *College of Biological Science and Technology, National Chiao Tung University, Hsinchu, Taiwan*

[6] *Department of Chemistry, College of Sciences, National Sun Yat-Sen University, Kaohsiung, Taiwan*

* *Corresponding author: Shou-Mei Wu, Fax: 886-7-3159597; Tel: 886-7-3121101 ext 2164; E-mail: shmewu@kmu.edu.tw*

Experimental Section

Materials and reagents: Hydroxypropylmethylcellulose (HPMC) (2% in H_2O ~15000 cps), Hydroxylethylcellulose (HEC) (1% in H_2O ~145 mPa.s) and poly(ethylene oxide) (PEO) (MW 8,000,000) were purchased from Aldrich (St. Louis, MO, USA). Methanol was purchased from Merck (Darmstadt, Germany). Tris-borate-EDTA (TBE) buffer (5X) was purchased from Protech Technology Enterprise and was diluted to an identical concentration with double distilled water before use. Oligonucleotide sequences are summarized in Table S1. All oligonucleotides were ordered from MD Bio, Inc (Taipei, Taiwan), and stored as 10 μM stocks in H_2O at -20℃. The double-distilled water was obtained from a Milli-Q water system (Millipore, Bedford, MA, USA).

Table S1. The sequence of primers used of *SMN* gene for this study.

Primer's name	Sequence (5'→ 3')	DNA Length (bp)
Scorpion primer	5-FAM-AGGGTTTTGTCTGAAACCCT-BHQ1-HEG (hexathylene glycol)-TAACTTCCTTTATTTTCCTTACAG	125 bp
3'-reverse primer	TCACT TTCAT AATGC TGGCA GAC	
Block probe	TTAAC TTCCT TTATT TTCCT TACAG GGTTT	30 mer

HEG (hexathylene glycol) is a blocker in PCR

Genomic DNA samples: DNA samples from SMA patients, carriers and normal individuals were obtained from Chung-Ho Memorial Hospital, Kaohsiung Medical University. Genomic DNA was collected from peripheral whole blood using Genomic

DNA purification kit (ZYMO RESEARCH, Quick-gDNA MiniPrep-D3024, USA), according to the standard protocol. The ethical approval for this study was obtained from the Institutional Review Board at Kaohsiung Medical University Hospital where participants were recruited and experiments on humans were conducted. Written informed consents were obtained from all participants.

Appartus: The PCR amplification was performed in a Tprofessional Thermocycler (Biometra, Göttingen, Germany). CE analysis was performed by using a Beckman Proteome Lab^{TM} PA800 system (Fullerton, CA, USA) equipped with a LIF detector which monitored by exciting the sampled at 488 nm and measuring the emission at 520 nm).

Assay procedures for PCR and hybridization reaction: The final volume of PCR was 25 μL containing a mixture of 50 ng of genomic DNA, 2.5 mM dNTP (TaKaRa, Shiga, Japan), 1× PCR buffer (TaKaRa), 200 nM of each primer and 2.5 U of e2TAK DNA polymerase (TaKaRa). The PCR amplification was performed in an initial denature step at 95℃ for 10 min, followed by 30 cycles of denaturing at 95℃ for 30 sec, annealing at 52℃ for 20 sec, extension at 72℃ for 30 sec, and a final extension step at 72℃ for 10 min. After PCR cycling was finished, the product was 5-fold diluted by adding dd-water for hybridization. The composition was 5 μL PCR products, 400 nM of block probe and the volume filled by dd-water to 25 μL, and the hybridization procedures were heating to 95℃ for 10 min, then incubated at 60℃ for 30 min and slowly cooled at 20℃ for 1 min. Finally, the hybridization products were directly analyzed by the CGE system.

CE assay: The separation matrix contained a solution of polymer powders prepared in 1x TBE buffer, of various concentrations. CE separations were performed in a coated DB-17 capillary (Agilent, California, USA) with 100 μm internal diameter and 30 cm effective length. A detection window was fabricated at a location 10 cm from the outlet. The new capillary was preconditioned with methanol for 10 min and dd-water for 10 min; before the first run, it was rinsed for 40 min with the polymer mixture, with a 20 min separation buffer rinse between runs. Sample injection was carried out at -10 kV for 20 sec and then the separation was accomplished at -6 kV, also in reverse polarity mode. The effect of capillary temperatures on the separation was also investigated, as reported in the results.

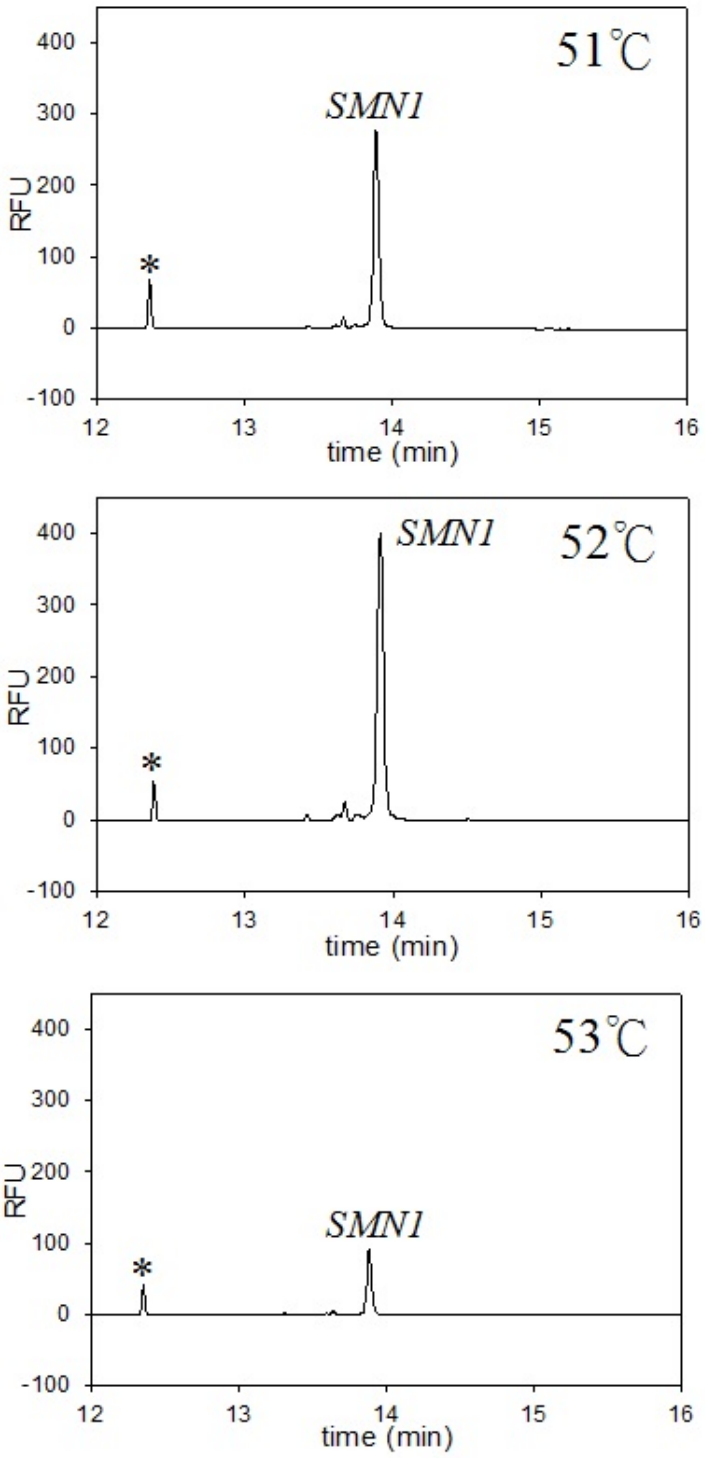

Figure S1. The effects of PCR annealing temperature which demo DNA samples for the ratio of *SMN1*/*SMN2* = 3:0, and the (*) was the peak of residues primer. The CE conditions were followed, sample loading, -10 kV for 20 s; separation voltage, -6 kV; capillary temperature, 25℃; separation matrix, 1.2% PEO added with YOPRO-1 dye in 1X TBE buffer; DB-17 coated capillary, 30.0 cm (effective length) × 100 μm id.

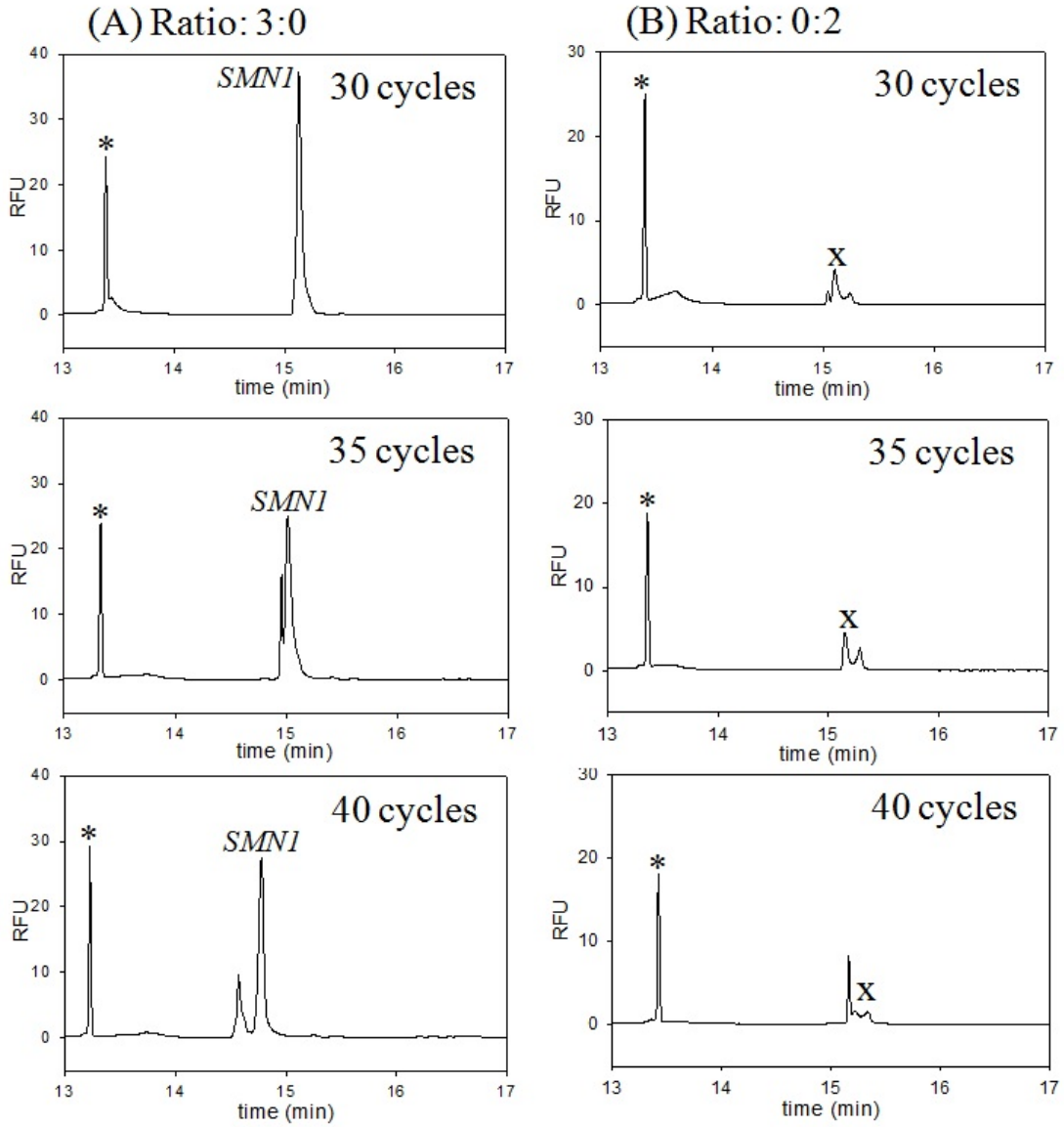

Figure S2. The effects of cycle numbers for PCR reaction which demonstrate DNA samples for (A) *SMN1*/*SMN2* = 3:0; (B) *SMN1*/*SMN2* = 0:2. The "x" was not identified as a substantial signal for this study, and the (*) was the peak of residues primer. The CE conditions followed were sample loading, -10 kV for 20 s; separation voltage, -6 kV; capillary temperature, 25℃; separation matrix, 1.2% PEO in 1X TBE buffer; DB-17 coated capillary, 30.0 cm (effective length) × 100 μm id.

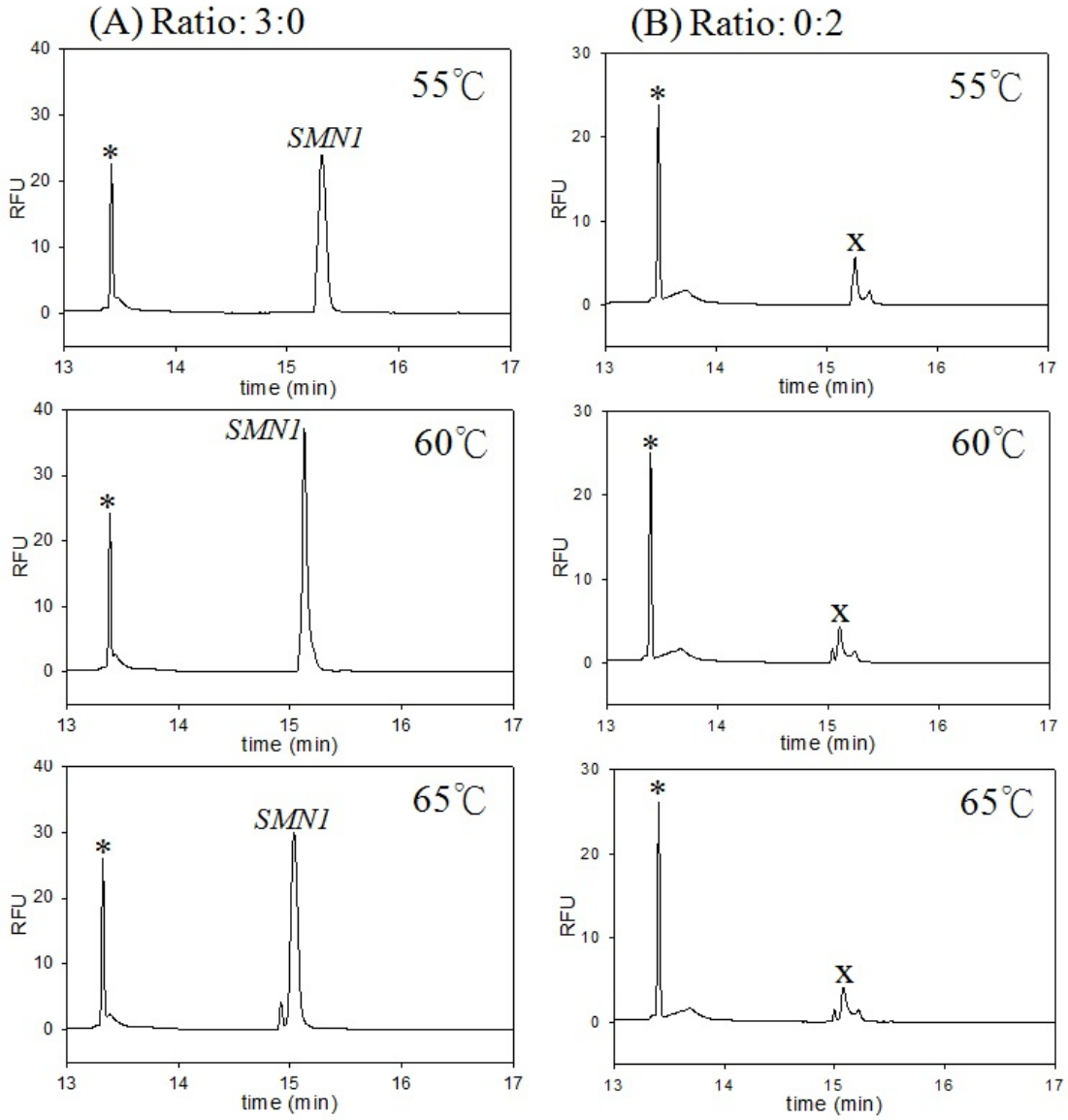

Figure S3. The effects of hybridization temperature which demo DNA samples for (A) *SMN1*/*SMN2* = 3:0; (B) *SMN1*/*SMN2* = 0:2. The "x" was not identified as a substantial signal for this study, and the (*) was the peak of residues primer. Other conditions were the same as in Fig. S2.

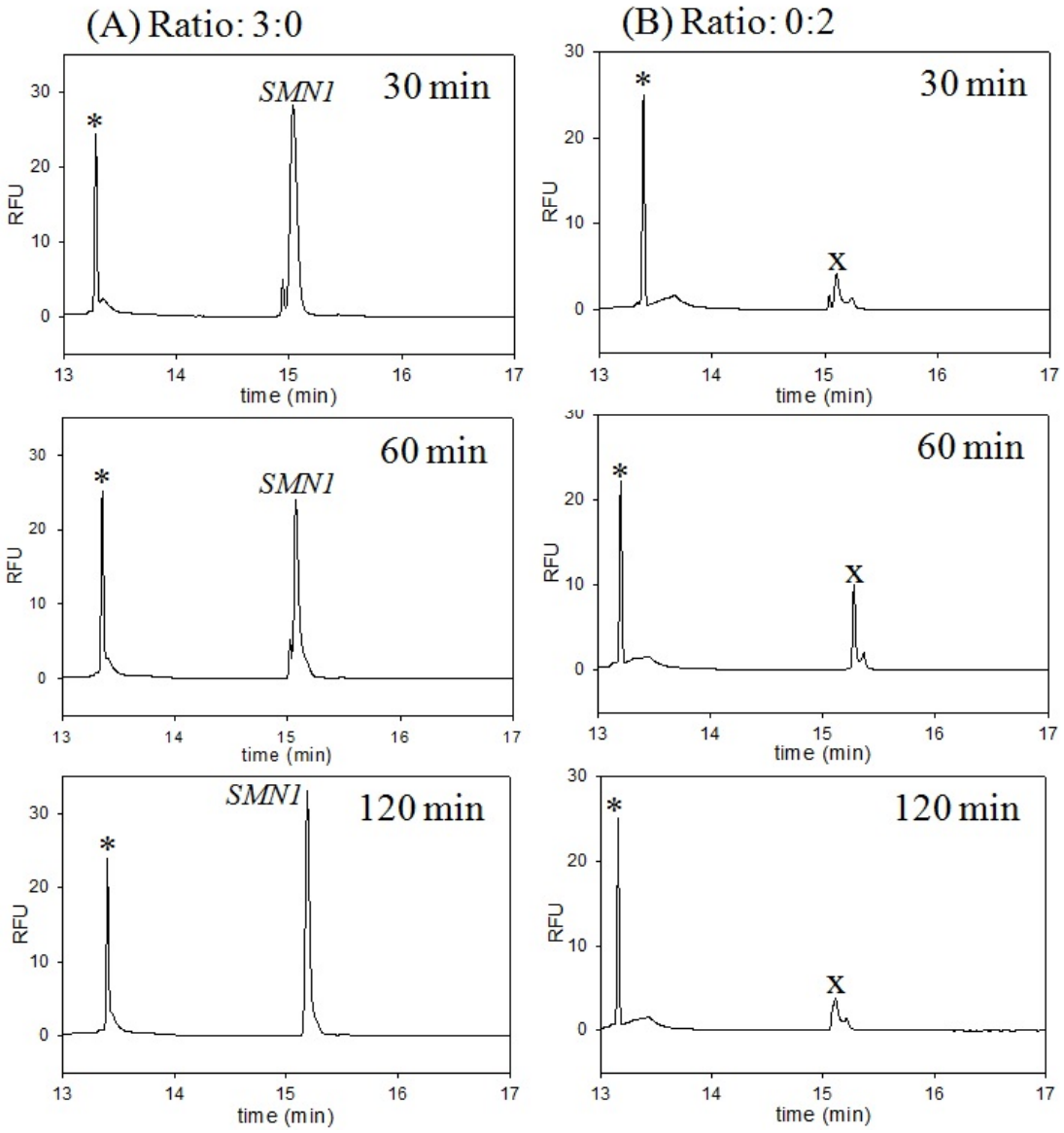

Figure S4. The effects of hybridization time which demo DNA samples for (A) *SMN1*/*SMN2* = 3:0; (B) *SMN1*/*SMN2* = 0:2. The "x" was not identified as a substantial signal for this study, and the (*) was the peak of residues primer. Other conditions were the same as in Fig. S2.

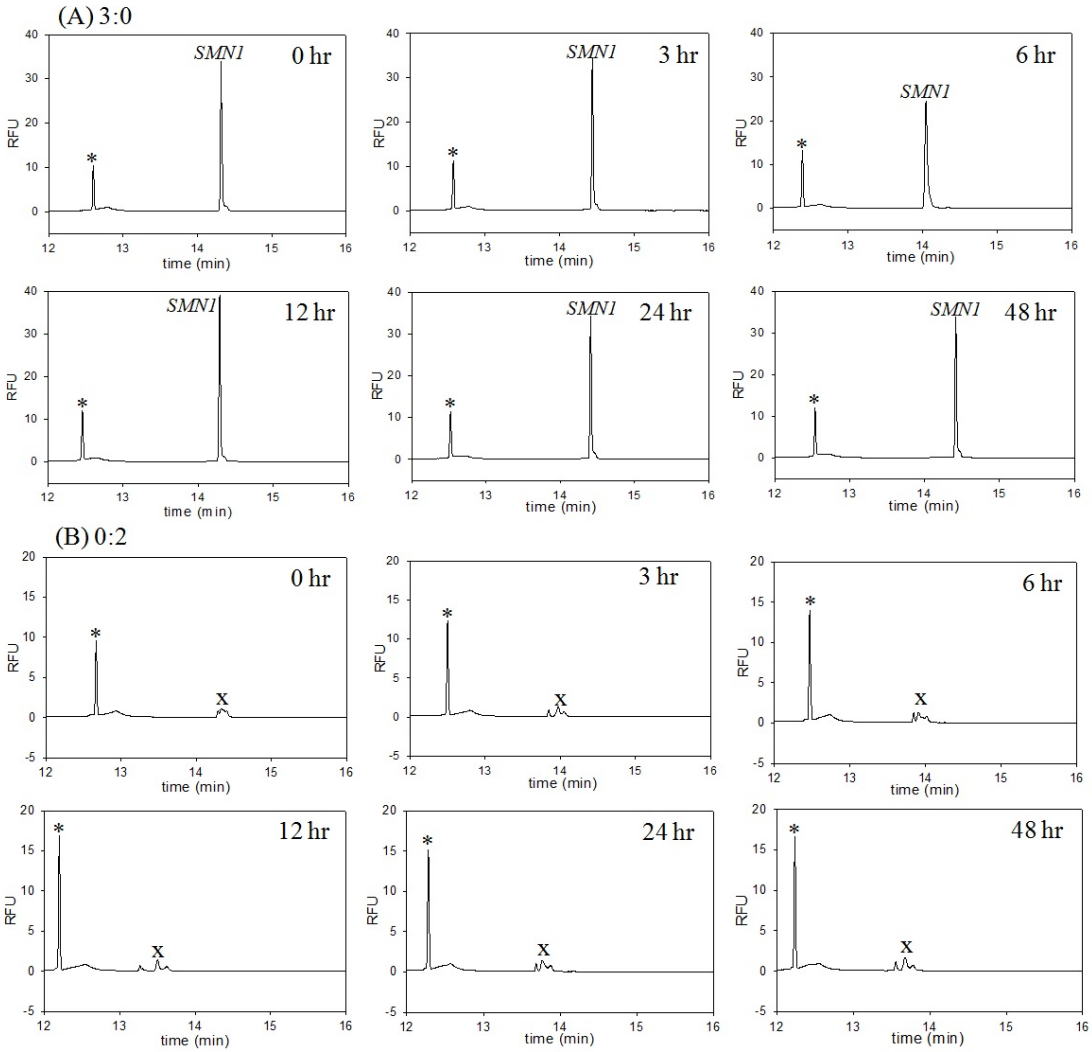

Figure S5. The stability test of DNA samples for (A) *SMN1*/*SMN2* = 3:0; (B) *SMN1*/*SMN2* = 0:2. The "x" was not identified as a substantial signal for this study, and the (*) was the peak of residues primer. Other conditions were the same as in Fig. S2.

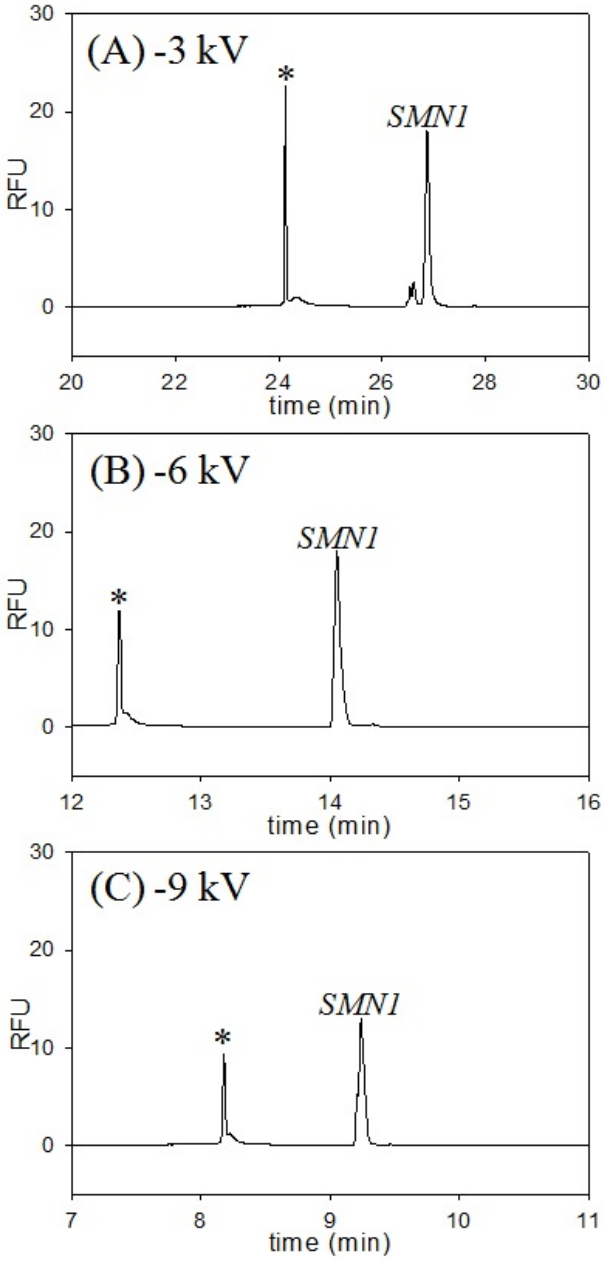

Figure S6. The effects of separation voltage which demo by *SMN1*/*SMN2* = 3:0 samples for (A) -3 kV; (B) -6 kV; (C) -9 kV, and the (*) was the peak of residues primer. Other conditions were the same as in Fig. S2.

References

1. C. T. Ng, C. A. Gilchrist, A. Lane, S. Roy, R. Haque and E. R. Houpt, *J. Clin. Microbiol.*, 2005, **43**, 1256-1260.