Development and validation of a one-step *SMN* assay for genetic testing in spinal muscular atrophy via MALDI-TOF MS

1. Supplementary Tables

Table S1 Amplification primers for *SMN* copy number quantification by MALDI-TOF MS

Primers	Sequence
SMN exon7_F	ACGTTGGATGCTTCCTTTATTTTCCTTAC
SMN exon7_R	ACGTTGGATGAATGCTGGCAGACTTACTCC
SMN exon8_F	ACGTTGGATGGGACTCTATTTTGAAAAACC
SMN exon8_R	ACGTTGGATGTTTCTCAACTGCCTCACCAC
*RPPH1*_F	ACGTTGGATGTGTCACTAGGCGGGAACAC
*RPPH1*_R	ACGTTGGATGAAATATTGCAGGGCGCCAC
SMN exon7_C	CTTCCTTTATTTTCCTTACAGGGTTT**G**AGACAAAATCAAAAAGAA GGAAGGTGCTCACATTCCTTAAATTAAGGAGTAAGTCTGCCAGCA
SMN exon8_C	GGACTCTATTTTGAAAAACCATCTGTAAAAGACTG**C**GGTGGGGGT GGGAGGCCAGCACGGTGGTGAGGCAGTTGAGAAA
*RPPH1*_C	AAATATTGCAGGGCGCCACTCCCCTGTCCCTCACAGCCATCTTCCT GCCAGGGCG**G**ACGCGCGCTGGGTGTTCCCGCCTAGTGACA

Note: *RPPH1* was used as an internal calibration in the panel of *SMN* copy number. *SMN*: survival motor neuron.

Table S2 Extension primers for *SMN* copy number quantification by MALDI-TOF MS

Primers	Sequence
SMN exon7_E	TTATTTTCCTTACAGGGTTT
SMN exon8_E	GTGCTGGCCTCCCACCCCCACC
*RPPH1*_E	GAACACCCAGCGCGCGT

Note: *RPPH1* was used as an internal calibration in the panel of *SMN* copy number. *SMN*: survival motor neuron.

Table S3 Amplification primers for *SMN* pathogenic variant detection by MALDI-TOF MS

Primers	Sequence
T835-5G_F	ACGTTGGATGGCTATTTTTTTTAACTTCC
T835-5G_R	ACGTTGGATGCATTAACCTTTCAACTTTT
27706-27707delAT_F	ACGTTGGATGGGAAGTGGAATGGGTAACTC
27706-27707delAT_R	ACGTTGGATGCCCCAGTCTTTTACAGATG
C689T_F	ACGTTGGATGGGTCTAAAATTCAATGGCCC
C689T_R	ACGTTGGATGTGGTGGTCCAGAAGGAAATG
T683A_F	ACGTTGGATGGGTCTAAAATTCAATGGCCC
T683A_R	ACGTTGGATGTGGTGGTCCAGAAGGAAATG
G400A_F	ACGTTGGATGGTGGTTTACACTGGATATGG
G400A_R	ACGTTGGATGGTCTCTGCTTCCAGAAATTG
T27134G_F	ACGTTGGATGGCTATTTTTTTTAACTTCC
T27134G_R	ACGTTGGATGCATTAACCTTTCAACTTTT

G863T_F	ACGTTGGATGGCTATTTTTTTTAACTTCC
G863T_R	ACGTTGGATGCATTAACCTTTCAACTTTT
22dupA_F	ACGTTGGATGTTTGCTATGGCGATGAGCAG
22dupA_R	ACGTTGGATGCTAATAGGGAGACTGCACTG
A815G_F	ACGTTGGATGGATGCTGATGCTTTGGGAAG
A815G_R	ACGTTGGATGGTCAGGAAAAGATGCTGAG

SMN: survival motor neuron.

Table S4 Extension primers for *SMN* pathogenic variant detection by MALDI-TOF MS

Primers	**Sequence**
T835-5G_E	ACTTCCTTTATTTTCCT
27706-27707delAT_E	ATATTTTACTGGACTCT
C689T_E	ATGGAGGCAGCCAGCAT
T683A_E	CCACCACCACCACCCCACT
G400A_E	ATCGGACAGATTTTGCTCCT
T27134G_E	TTTAACATCTGAACTTTTTAA
G863T_E	TAATTTAAGGAATGTGAGCAC
22dupA_E	TGCTCCGGGACGCCGCCACCACT
A815G_E	CTTACCATATAATAGCCAGTATGA

SMN: survival motor neuron.

Table S5 Comparative analysis of *SMN* copy numbers in 13 DNA samples between MALDI-TOF MS and MLPA.

Copy Number			MALDI-TOF MS				MLPA			
SMN1*:*SMN2	***SMN1*:*SMN2***	**N**	***SMN1*：*SMN2***		***SMN1*:*SMN2***		***SMN1*：*SMN2***		***SMN1*:*SMN2***	
E7	**E8**		**E7**		**E8**		**E7**		**E8**	
2:0	2:0	1	1.67±0.09	0.00±0.01	2.08±0.30	0.03±0.03	2.44±0.12	0	2.34±0.12	0
2:1	2:1	3	1.97±0.04	1.10±0.13	1.86±0.04	1.00±0.01	2.09±0.07	1.06±0.02	2.00±0.07	1.08±0.02
2:2	2:2	5	2.00±0.18	2.03±0.27	2.03±0.08	2.01±0.13	2.09±0.05	1.99±0.05	2.02±0.09	2.02±0.04
2:3	2:3	1	1.91±0.05	2.66±0.14	1.71±0.12	2.53±0.15	1.96±0.10	3.14±0.16	1.86±0.10	2.62±0.14
0:3	0:3	NA03813*	0.01±0.01	2.97±0.04	0	2.54±0.04	0	3	0	3
1:5	1:5	NA03814*	1.26±0.08	4.4±0.90	1.05±0.04	4.93±0.30	1	5	1	5
3:2	3:2	NA12548*	2.61±0.26	1.83±0.24	2.70±0.19	1.89±0.11	3	2	3	2
Total		13								

*Sample from Coriell biobank tested by MRC Holland with P021-B1 SMA for *SMN1* and *SMN2* copy number. The *SMN* copy number corresponding to Coriell DNA is provided in the kit instruction (P060-B2 SMA Carrier, P021-B1 SMA). MALDI-TOF MS, Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; MLPA, multiple ligation-dependent probe amplification; SMA, spinal muscular atrophy; *SMN*, survival motor neuron. SMA specifically denotes 5q SMA resulting from the pathogenic variation of *SMN1* located at 5q13.

Table S6 Kappa consistency assessment for the detection of *SMN* copy number in 13 DNA samples

Copy number		MLPA						Total
		0	1	2	3	4	5	
MALDI-TOF MS	0	4	0	0	0	0	0	4
	1	0	8	0	0	0	0	8
	2	0	0	31	1*	0	0	32
	3	0	0	0	6	0	0	6
	4	0	0	0	0	0	1Δ	1
	5	0	0	0	0	0	1	1

Total	4	8	31	7	0	2	52

*The copy number of *SMN1* E7 was determined to be 1.67±0.09 by MALDI-TOF MS and 2.44±0.12 by MLPA (gray value). The latter was classified as 3 copies in the kappa consistency test. ΔThe copy number of *SMN2* E7 was determined to be 4.4±0.90 by MALDI-TOF MS and 5 by MLPA. When conducting kappa tests, the total number of tests (total) was weighted on a case-by-case basis. The kappa value 0.935 (with a progressive standard error of 0.045, $P < 0.001$) suggests a strong agreement between the two test methods. MALDI-TOF MS, Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; MLPA: multiple ligation-dependent probe amplification; *SMN*: survival motor neuron.

Table S7 Comparative assessment of *SMN* copy numbers in one clinical cohort between MALDI-TOF MS and MLPA.

Copy Number			**MALDI-TOF MS**				**MLPA**			
SMN1*:*SMN2	***SMN1*:*SMN2***	**N**	***SMN1*：*SMN2***		***SMN1*:*SMN2***		***SMN1*：*SMN2***		***SMN1*:*SMN2***	
E7	**E8**		**E7**		**E8**		**E7**		**E8**	
0:2	0:2	2	0.09±0.13	1.99±0.36	0.00±0.00	2.05±0.27	0.00±0.00	2.27±0.16	0.00±0.00	2.26±0.00
0:2	0:3	1	0.00	2.34	0.00	2.89	0.00±0.00	2.58±0.14	0.00±0.00	2.44±0.14
0:3	0:3	12	0.11±0.15	2.90±0.33	0.00±0.00	3.04±0.24	0.00±0.00	3.06±0.21	0.00±0.00	3.06±0.22
0:3	1:2	2	0.12±0.00	2.73±0.07	1.07±0.06	2.24±0.04	0.00±0.00	2.86±0.31	1.02±0.08	1.92±0.14
0:3	2:1	1	0.05	3.12	2.04	1.16	0.00±0.00	3.24±0.06	2.04±0.04	1.21±0.02
0:5	0:4	1	0.00	4.39	0.00	4.35	0.00±0.00	4.40±0.14	0.00±0.00	4.06±0.14
1:2	1:2	3	1.00±0.12	1.77±0.31	0.95±0.04	1.90±0.09	1.05±0.12	1.99±0.02	1.00±0.09	2.07±0.14
1:3	1:3	2	1.17±0.18	2.71±0.15	0.92±0.06	2.98±0.05	1.01±0.13	3.13±0.18	1.12±0.08	3.06±0.37
1:3	2:2	1	1.04	2.84	2.19	2.21	1.04±0.04	3.16±0.14	2.22±0.10	2.28±0.10
2:1	2:1	10	1.98±0.37	1.07±0.19	2.10±0.20	1.09±0.11	1.96±0.21	1.04±0.09	2.08±0.25	1.10±0.09
2:1	3:1	1	2.42	1.04	3.07	1.12	2.54±0.06	0.96±0.02	2.76±0.08	1.06±0.02
2:2	1:3	1	2.13	1.99	0.98	2.70	2.00±0.06	1.94±0.06	1.10±0.04	3.04±0.08
2:2	2:2	38	1.88±0.17	1.90±0.17	1.94±0.14	1.93±0.13	1.93±0.18	1.95±0.13	1.97±0.18	1.99±0.15
2:2	3:1	1	1.93	2.16	2.79	1.15	2.04±0.04	1.92±0.04	2.90±0.06	1.06±0.02
3:1	3:1	1	2.48	1.03	2.49	1.15	2.68±0.06	0.88±0.02	2.74±0.08	1.04±0.02

3:2	3:2	1	2.96	2.18	2.55	1.88	2.68±0.10	1.86±0.08	2.78±0.10	1.86±0.08
Total		78								

MALDI-TOF MS, Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; MLPA, multiple ligation-dependent probe amplification; *SMN*, survival motor neuron.

Table S8 The results of the samples with ambiguous copy numbers in two detection methods

Sample ID	Type	MALDI-TOF MS				MLPA			
		SMN1*：*SMN2		***SMN1*:*SMN2***		***SMN1*：*SMN2***		***SMN1*:*SMN2***	
		E7		**E8**		**E7**		**E8**	
Fig 3A/C, #061	Normal	1.67	1.83	1.93	2.00	1.50	1.66	1.52	1.74
Fig 3A, #072*	Normal	2.53	1.37	2.46	1.17	1.62	0.88	1.74	0.96
Fig 3A, #071*	Normal	2.42	1.04	3.07	1.12	2.54	0.96	2.76	1.06
Fig 3B/D, #021	Patient	0.00	2.34	0.00	2.89	0.00	2.58	0.00	2.44
Fig 3B, #008	Patient	0.00	3.08	0.00	3.08	0.00	3.46	0.00	3.32
Fig 3B, #006	Patient	0.00	4.39	0.00	4.35	0.00	4.40	0.00	4.06
Fig 3D, #009	Patient	0.00	3.22	0.00	3.05	0.00	3.28	0.00	3.52

*Potential gene conversion sample. The yellow-highlighted data indicates its placement within the ambiguous range (i.e., gray value); the data highlighted in green can serve as the viable alternative to the ambiguous copy number (yellow). MALDI-TOF MS, Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; MLPA: multiple ligation-dependent probe amplification; *SMN*: survival motor neuron.

2. Supplementary Figures

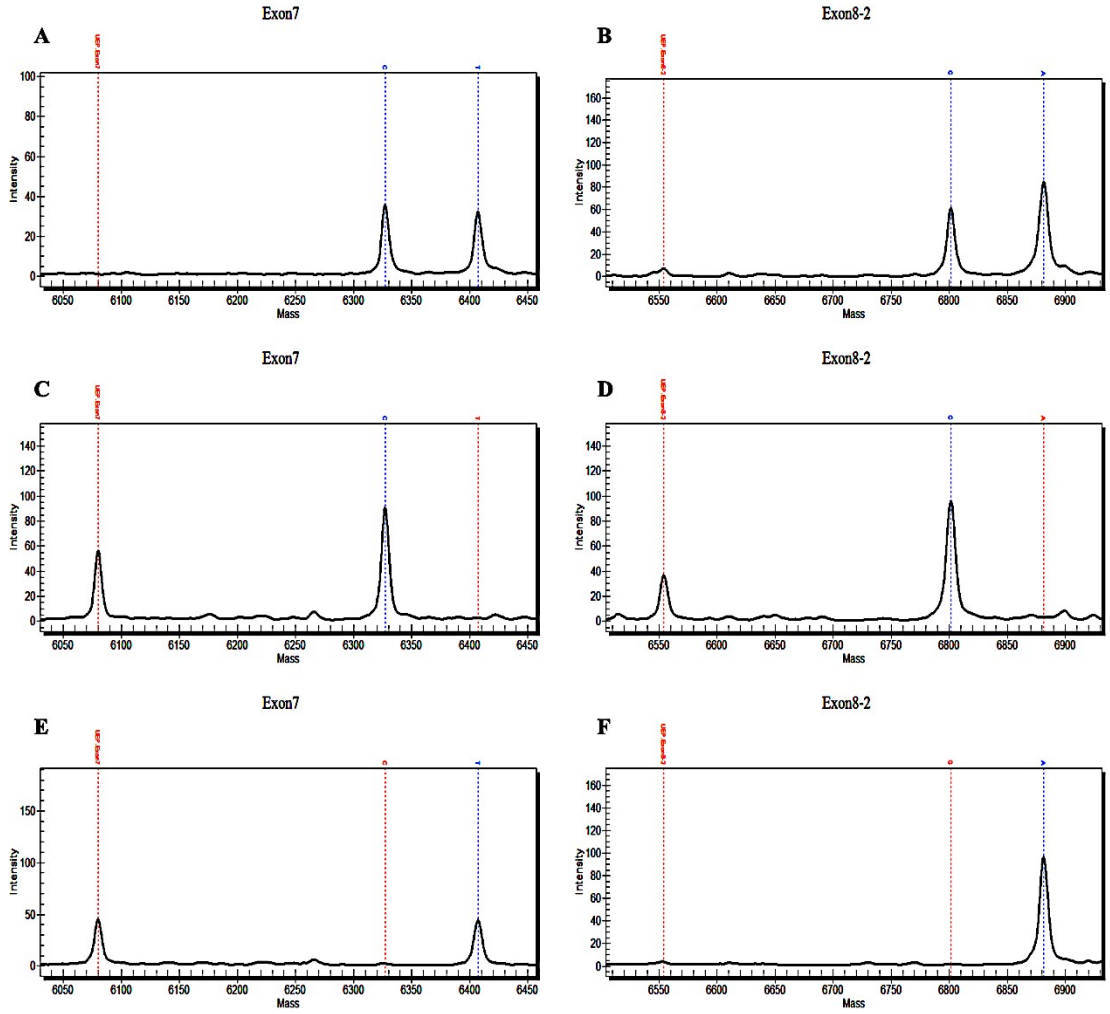

Fig. S1 DNA mass spectrometry of *SMN* gene from MALDI-TOF MS (without competitive templates).

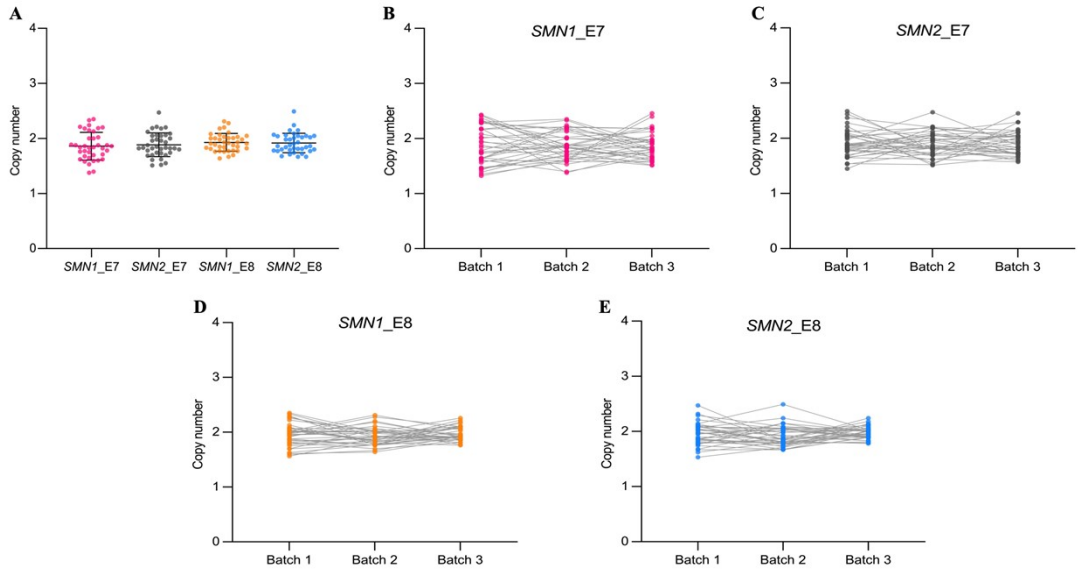

Fig. S2 Samples with normal copy numbers were detected through MALDI-TOF MS. (A) The copy numbers of *SMN* E7 and E8 in normal samples (2N) were detected with 20 ng DNA (n = 38). Detection results of copy number variations in different batches of normal samples, including *SMN1* Exon7 (B), *SMN2* Exon7 (C), *SMN1* Exon8 (D), and *SMN2* Exon8 (E).

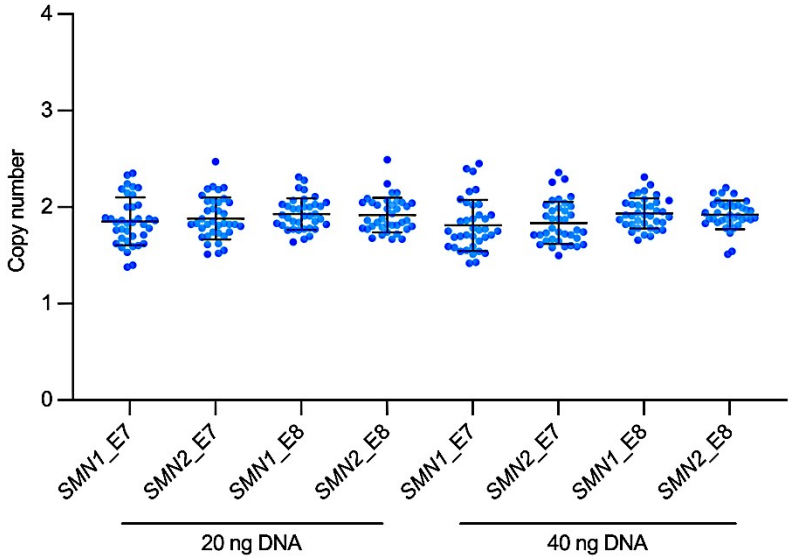

Fig. S3 Samples with normal copy numbers for two different quantities of DNA were identified through MALDI-TOF MS.

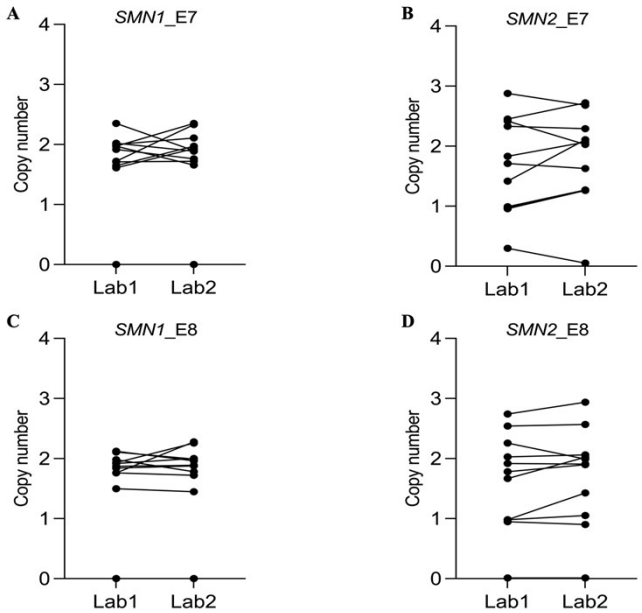

Fig. S4 Quantitative assessment of *SMN* copy number via MALDI-TOF MS conducted in two independent laboratories. The copy numbers of *SMN1* E7 (A), *SMN2* E7 (B), *SMN1* Exon8 (C), and *SMN2* Exon8 (D) were detected with 20 ng DNA (n = 11).

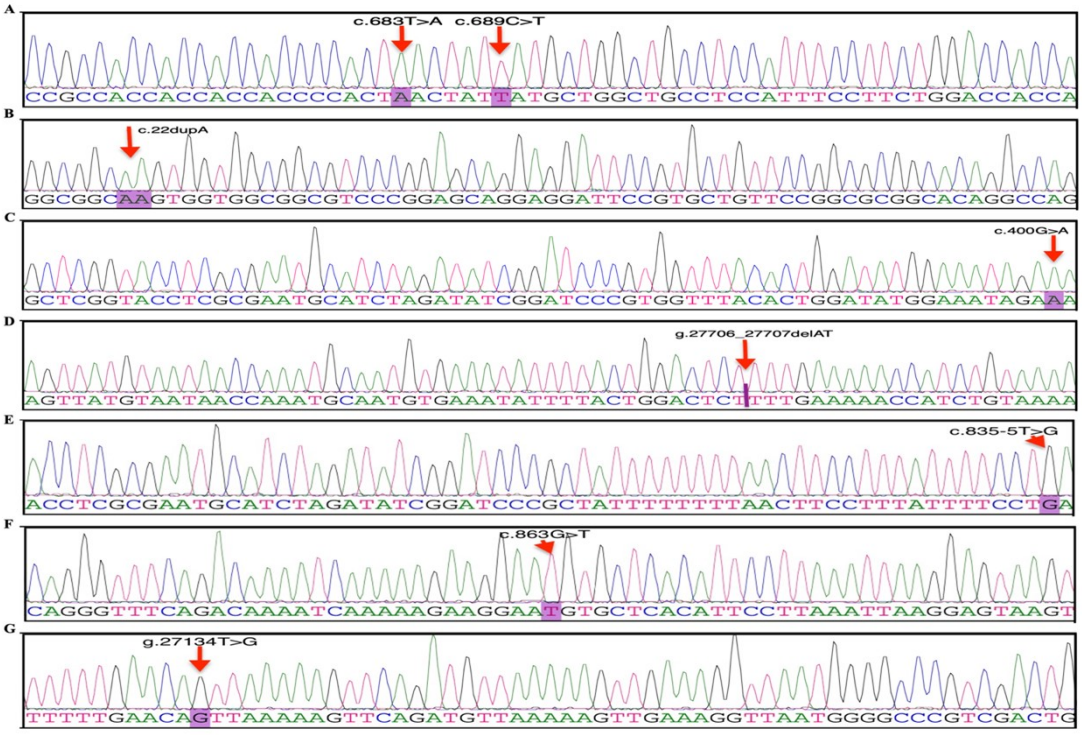

Fig. S5 Representative Sanger sequencing peck maps of the DNA extracted from the agarose gel of PCR product that amplified from pathogenic and sequence variants.

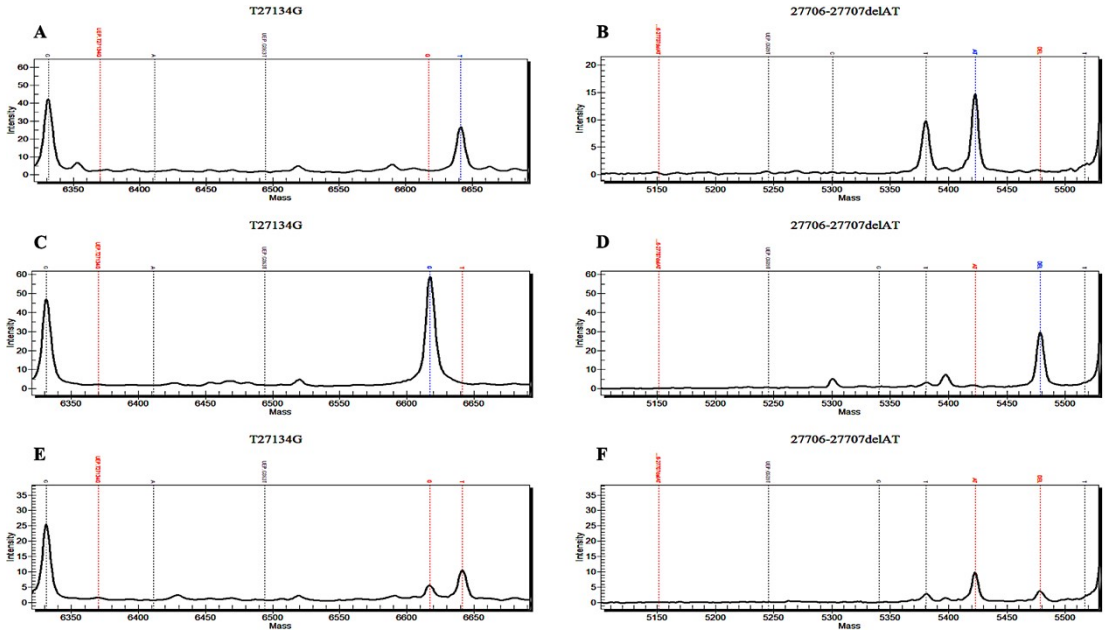

Fig. S6 DNA mass spectrometry of detection of sequence variants in the *SMN1* gene from MALDI-TOF MS. T27134G represents g.27134T>G in intron 7 and 27706-27707delAT represents g.27706_27707delAT in exon 8. The wild type (A, B) and the mutant type (C, D) of g.27134T>G and g.27706_27707delAT detected via MALDI-TOF MS. (E, F) Concurrent mutations of g.27134T>G and g.27706_27707delAT in one sample with 3 copies of *SMN1*.