

Table S1 Experimental conditions for PCR-RFLP**Table S1-1. Conventional PCR****Table S1-1-1. Conventional PCR reaction mixture**

Components	Volume (μL)
Distilled water	10.5
2×PCR Buffer for KOD FX Neo	25.0
KOD FX Neo (1 U/μl)	1.0
dNTPs mixture (A, C, G, T each 2 mM)	10.0
R111 (8 pmol/mL)	1.5
X7-Dra (8 pmol/mL)	1.5
DBS circle (1.2 mm in diameter)	1 piece
Total	50.0

Table S1-2. Restriction enzyme digestion**Table S1-2-1. Restriction enzyme mixture**

Components	Volume (μL)
Distilled water	6.0
10×M Buffer	3.0
Dra I (15 U/μl)	3.0
PCR products	18.0
Total	30.0

Table S1-1-2. Conventional PCR conditions

	Step	Temperature	Time
1	Initial denaturation	94°C	7 min
2	Denaturation	94°C	1 min
3	Annealing	56°C	1 min
4	Elongation	72°C	1 min
	(30 cycles of steps 2 to 4)		
5	Final elongation	72°C	7 min
6	Hold or storage	15°C	∞

Table S1-2-2. Restriction enzyme treatment conditions

	Step	Temperature	Time
1	Restriction enzyme treatment	37°C	12 hrs
2	Hold or storage	15°C	∞

Table S2 Experimental conditions for real-time PCR**Table S2-1. Real-time PCR reaction mixture**

Components	Volume (μL)
Distilled water	3.0
2×PCR Buffer for KOD FX Neo	12.5
KOD FX Neo (1 U/μl)	0.5
dNTPs mixture (A, C, G, T each 2 mM)	5.0
cenSMNex7forw (10 pM)	0.25
cenSMNint7rev (10 pM)	0.25
20x EvaGreen®	2.5
ROX reference dye	0.5
DBS circle (1.2 mm in diameter)	1 piece
Total	25.0

Table S2-2. Real-time PCR conditions

	Step	Temperature	Time
1	Initial denaturation	94°C	7 min
2	Denaturation	94°C	1 min
3	Annealing	56, 58, 60, 62°C	1 min
4	Elongation	72°C	1 min
	(45, 55 cycles of steps 2 to 4)		
5	Final elongation	72°C	7 min
6	Storage	20°C	∞

Table S3. Characteristics of the healthy control

RBC (x10⁶/μL)	4.55
Hemoglobin (g/dL)	14.3
Hematocrit (%)	42.0
WBC (/μL)	4700

Table S4. Characteristics of artificial blood samples

Table S4-1. Different hematocrits

	Ht 40%	Ht 60%	Ht 80%
RBC ($\times 10^6/\mu\text{L}$)	4.55	7.30	9.63
Hemoglobin (g/dL)	14.3	22.7	29.0
Hematocrit (%)	42.0	66.3	84.7
WBC ($/\mu\text{L}$)	4700	4800	3200
Estimated DNA amount in a DBS circle (ng)	~70	~70	~50

Table S4-2. Different WBC counts

	Normal WBC count	Low WBC count	Very low WBC count
RBC ($\times 10^6/\mu\text{L}$)	5.93	6.33	6.21
Hemoglobin (g/dL)	18.5	20.0	19.3
Hematocrit (%)	53.2	56.7	55.1
WBC ($/\mu\text{L}$)	4100	700	300
Estimated DNA amount in a DBS circle (ng)	~60	~10	~5

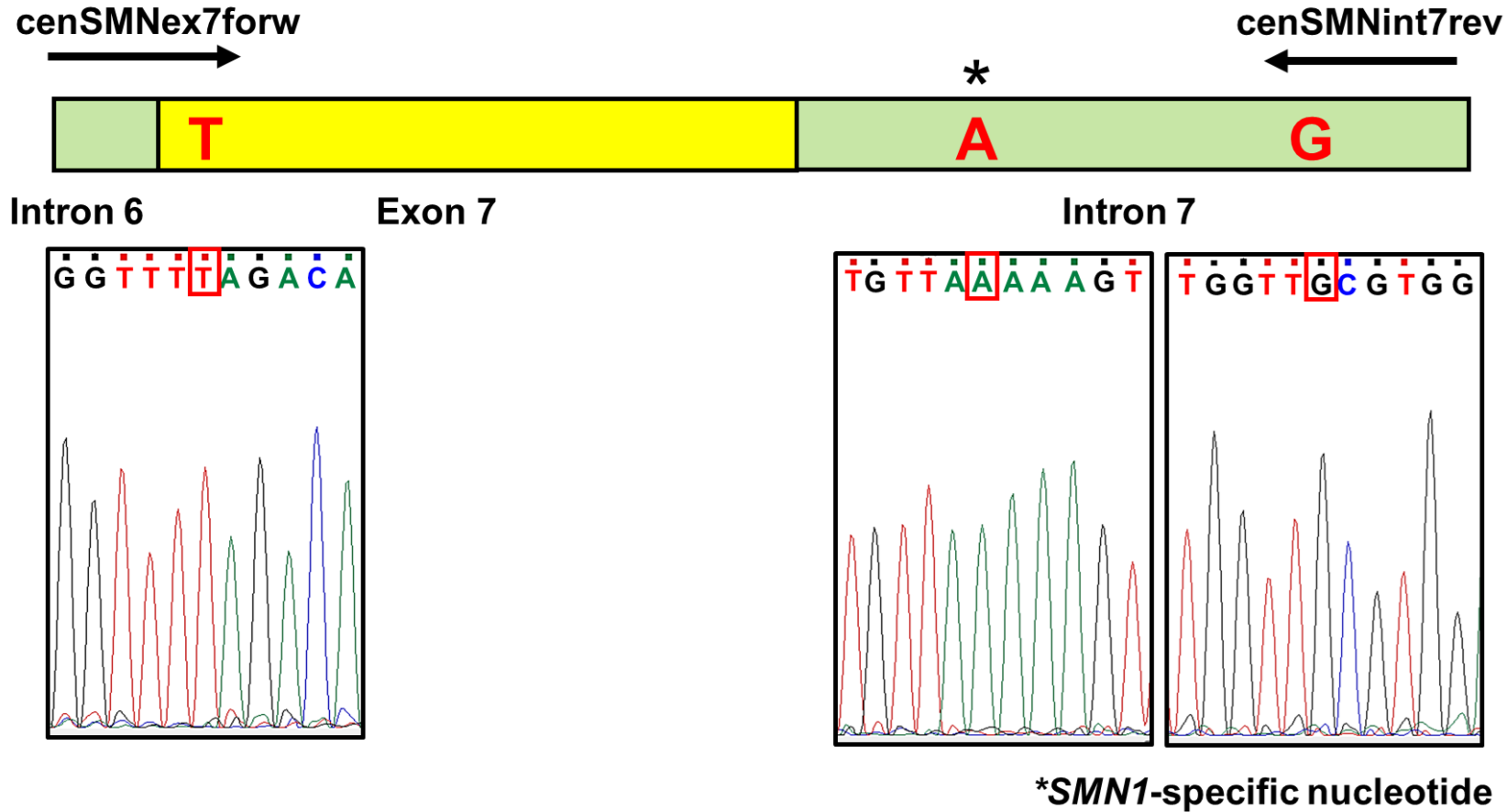
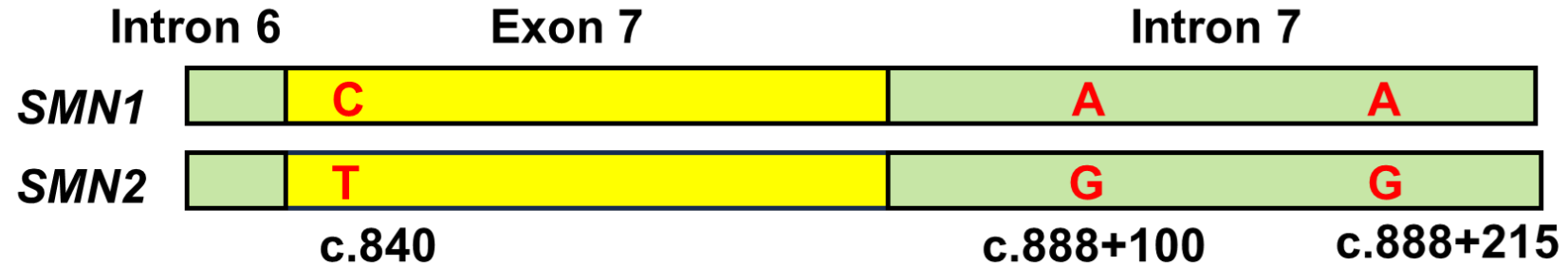


Figure S1. Sequencing analysis of amplified PCR products from DBS with *SMN2* deletion. PCR product from DBS with *SMN2* deletion showed the presence of an *SMN1*-specific nucleotide.

(1) Real-time PCR: Nucleotide sequences of *SMN1* and *SMN2*



(2) Real-time PCR: Amplified PCR products from DBS with *SMN2* deletion

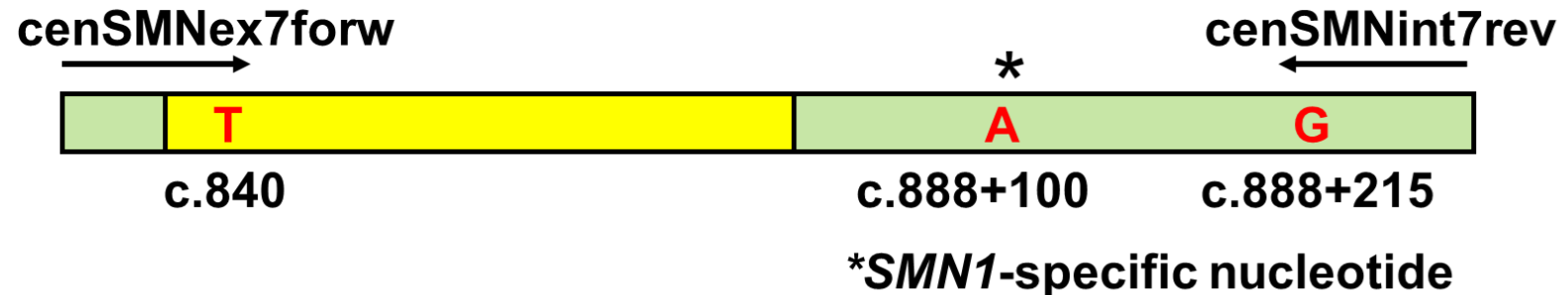


Figure S2. An *SMN1*-specific nucleotide identified in false-negative PCR products. *SMN2*-specific primers can amplify *SMN1* sequences by mis-annealing of the primers to *SMN1* sequence under some conditions including low annealing temperature.