

# Determination of *SMN1* and *SMN2* copy number using Multiplex Ligation-dependent Probe Amplification (MLPA) method

2006-10-18

臨床病理科

李建宏

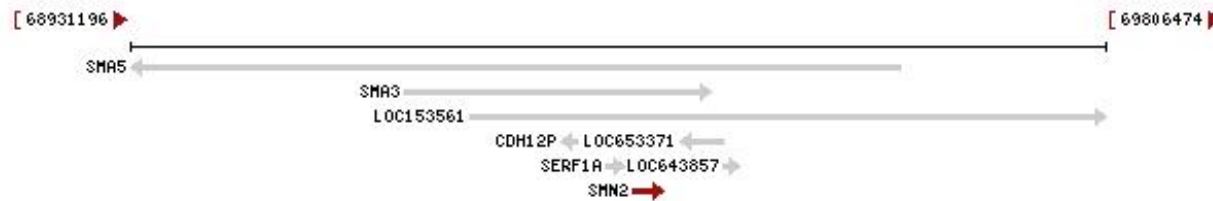
- SMA 基因簡介
- DHPLC偵測原理
- MLPA偵測原理
- MLPA評估結果
- DHPLC與MLPA的比較

# SMA

chromosome: 5; **Location:** 5q13



chromosome: 5; **Location:** 5q13



# SMN1/2

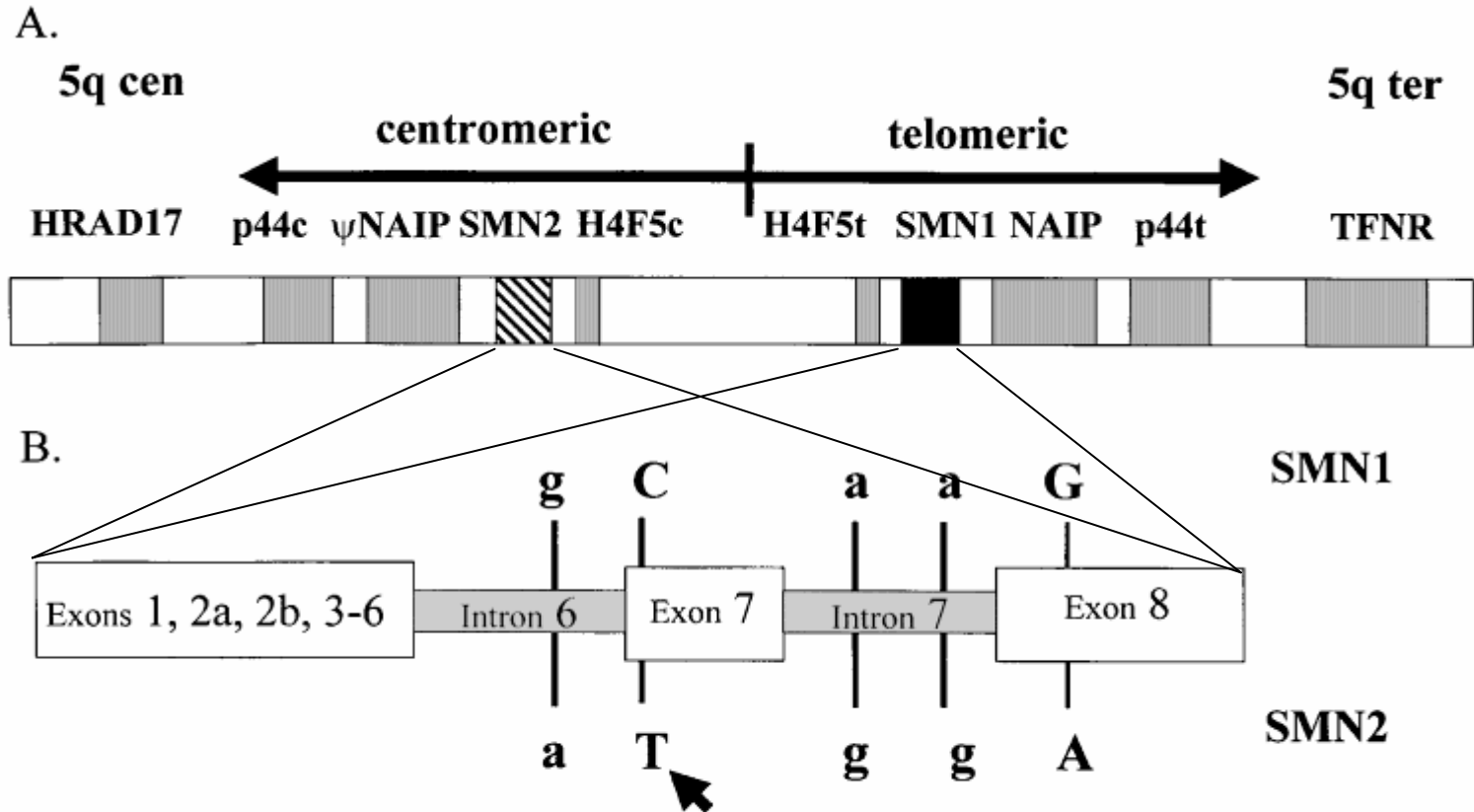
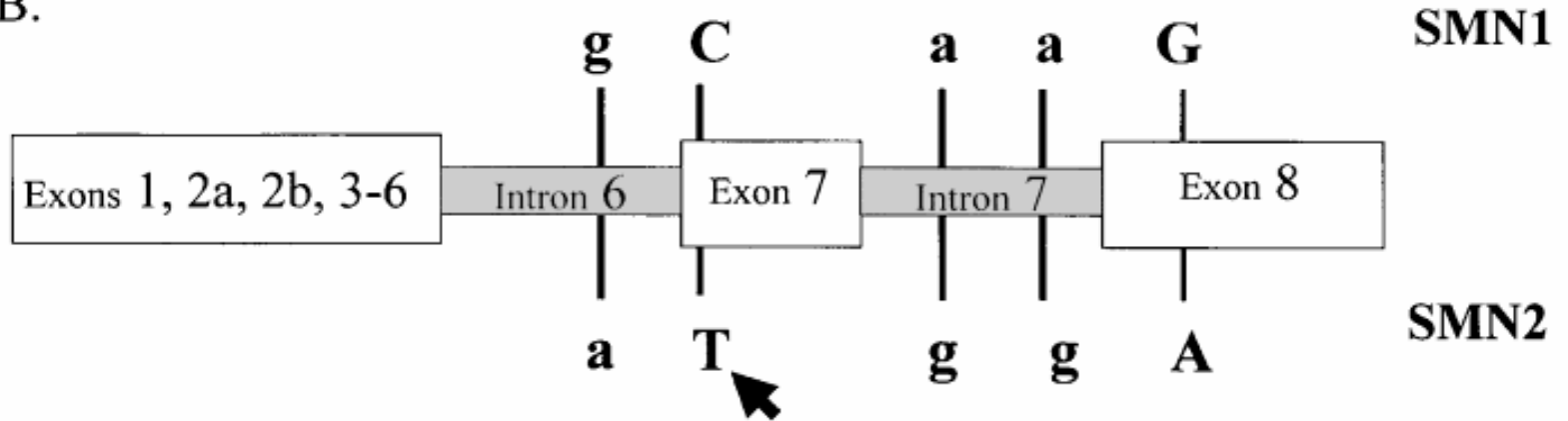


FIGURE 1. **A:** Schematic representation of the inverted and duplicated SMA region (5q13), including four duplicated genes: H4F5, SMN, NAIP, and BTFp44. The region is flanked by HRAD17 proximal [von Deimling et al., 1999] and TFNR (Kelter and Wirth, unpublished results). **B:** Localization of the nucleotides by which SMN1 can be distinguished from SMN2.

# SMN1/2

B.



# SMN1/SMN2

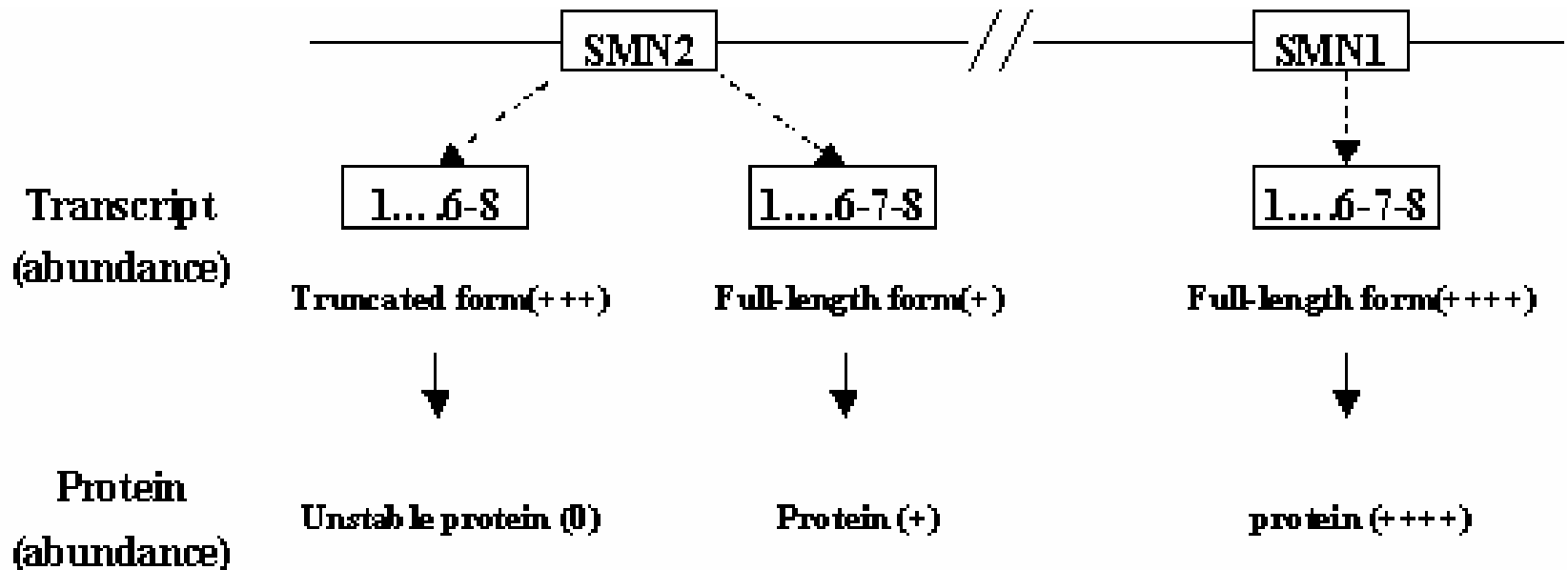
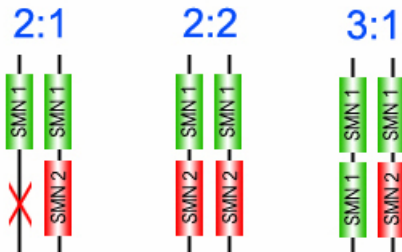


Figure 2. Genomic organization of the SMA locus in human healthy individual.

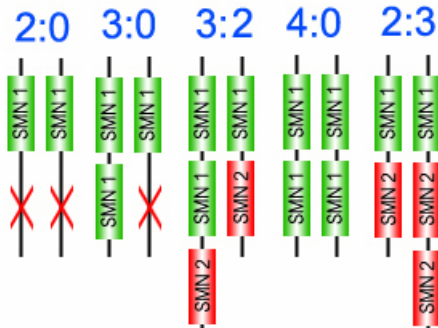
# SMN1基因及SMN2基因的排列方式

正常人之SMN1及SMN2基因可能之排列方式

常見之排列型

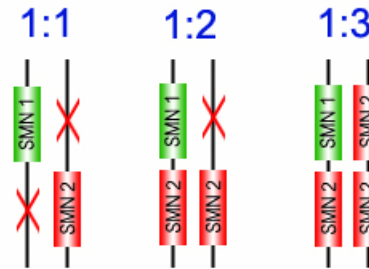


較少見之排列型

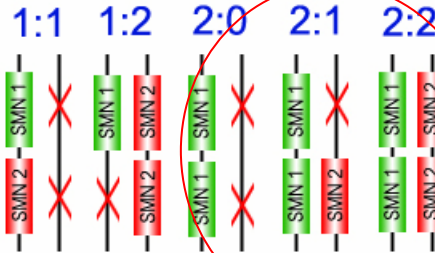


SMA帶因者之SMN1及SMN2基因可能之排列方式

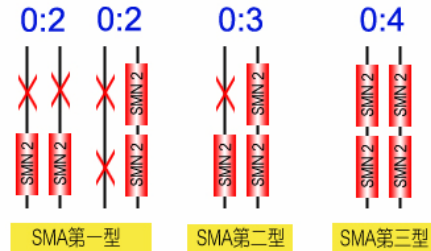
常見之排列型



較少見之排列型



SMA患者之SMN1及SMN2基因可能之排列方式



# DHPLC

HUMAN MUTATION 25:460–467 (2005)

## METHODS

### Quantitative Analysis of *SMN1* and *SMN2* Genes Based on DHPLC: A Highly Efficient and Reliable Carrier-Screening Test

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# DHPLC



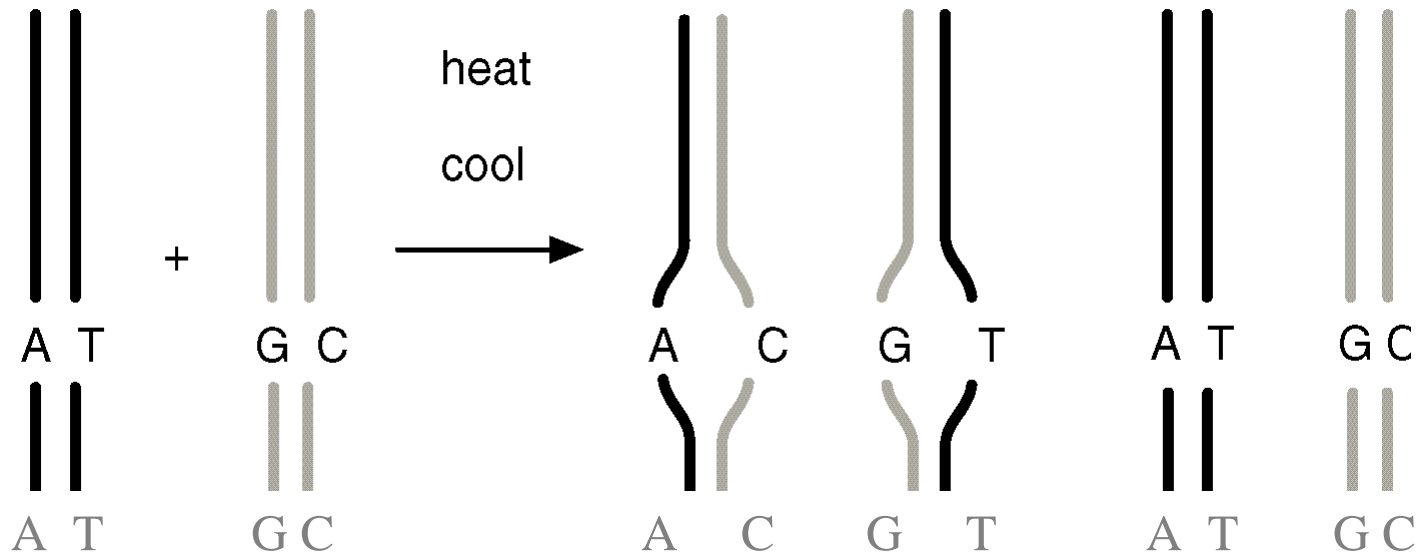
# Heteroduplex Analysis

wild type

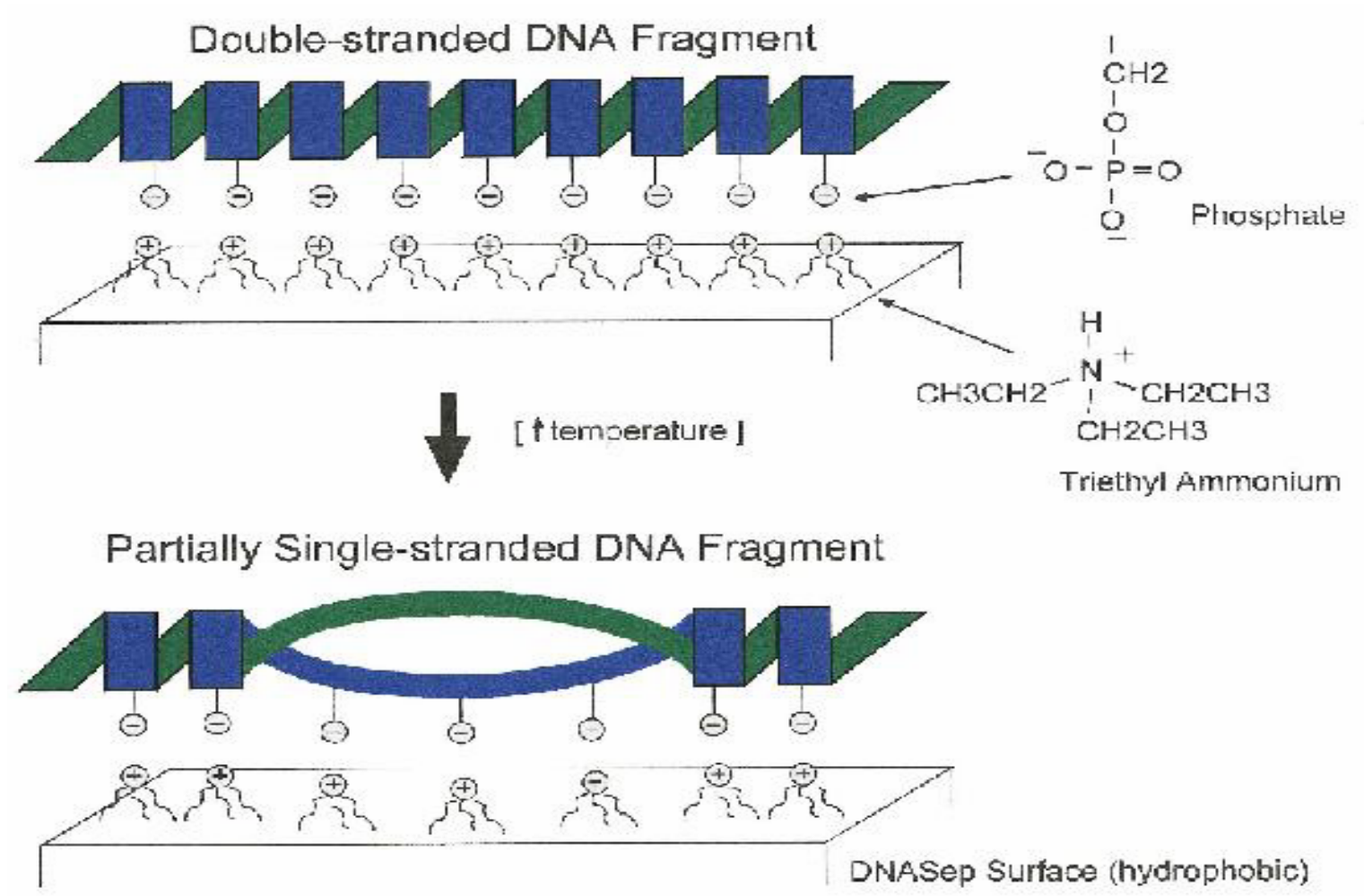
mutant

heteroduplexes

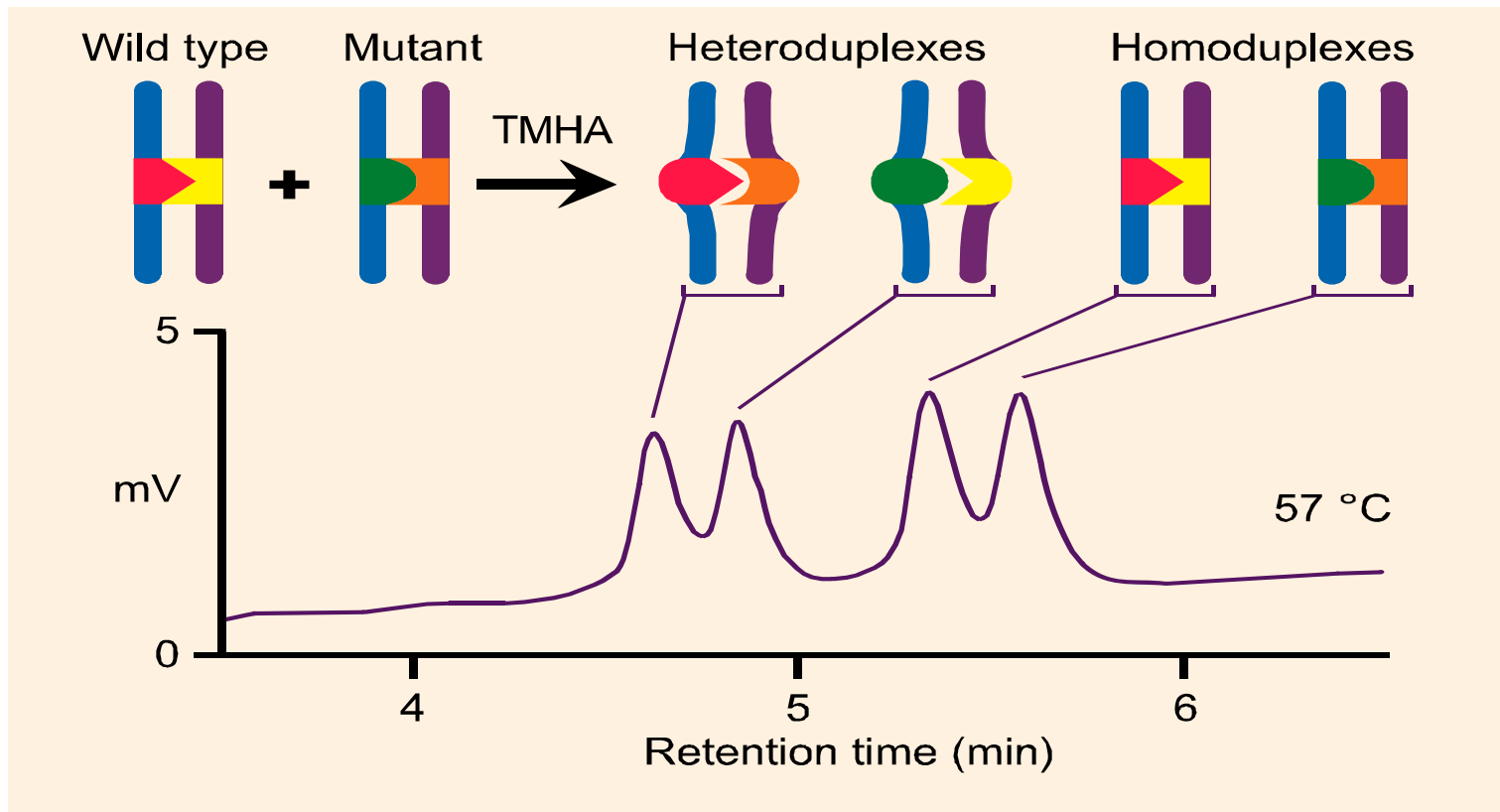
homoduplexes



# DHPLC



# Examples of Partial Denaturing Operation for Mutation Detection



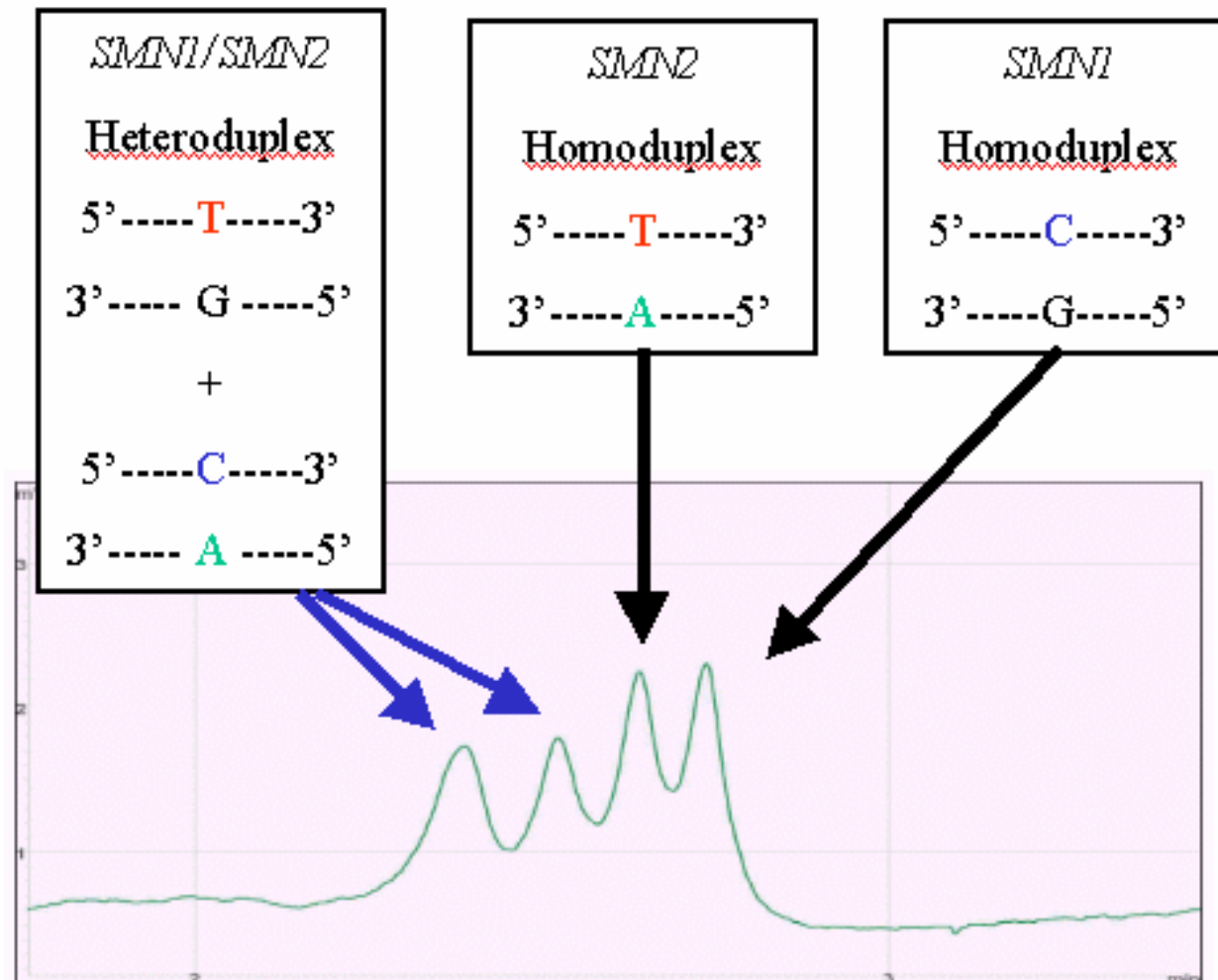
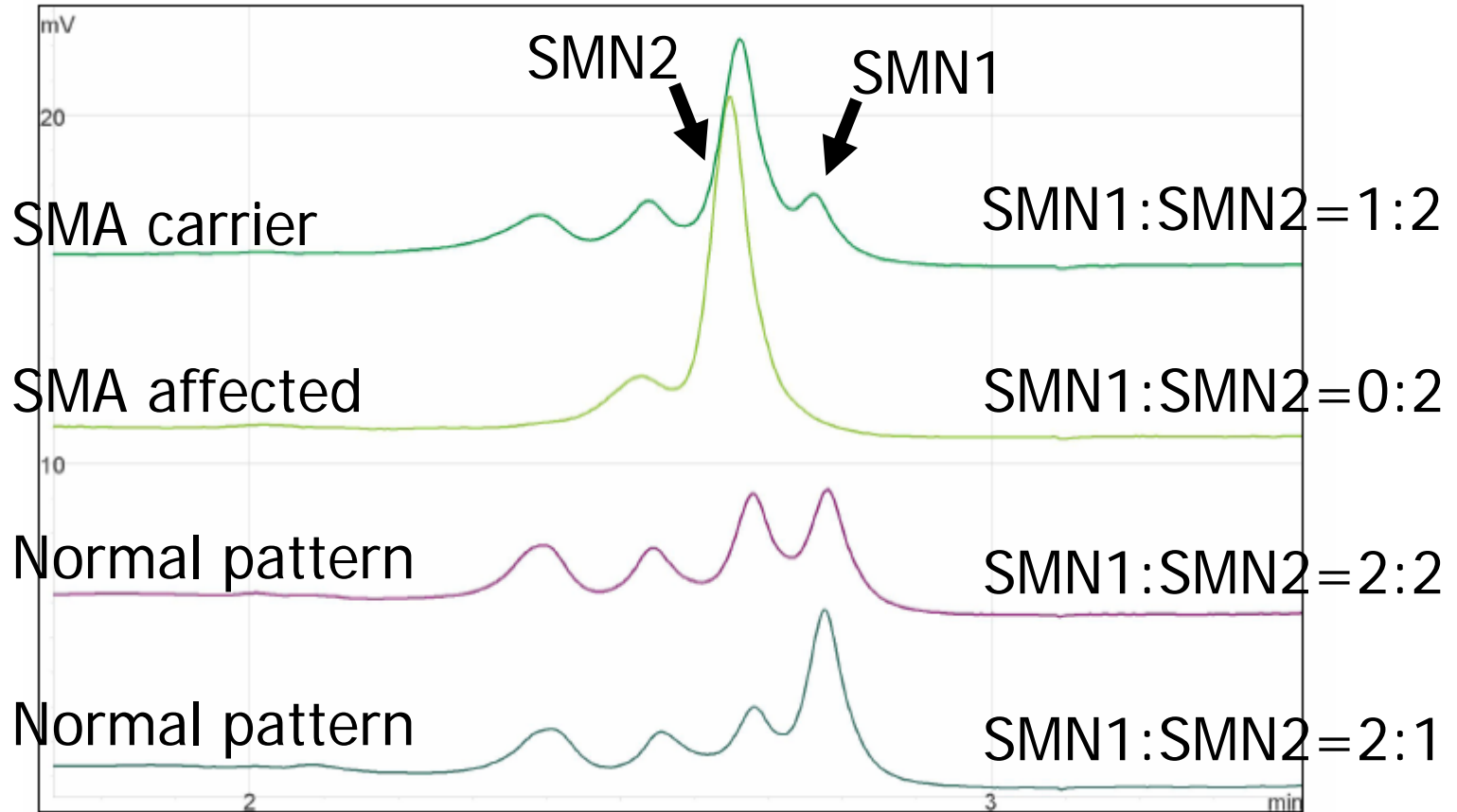
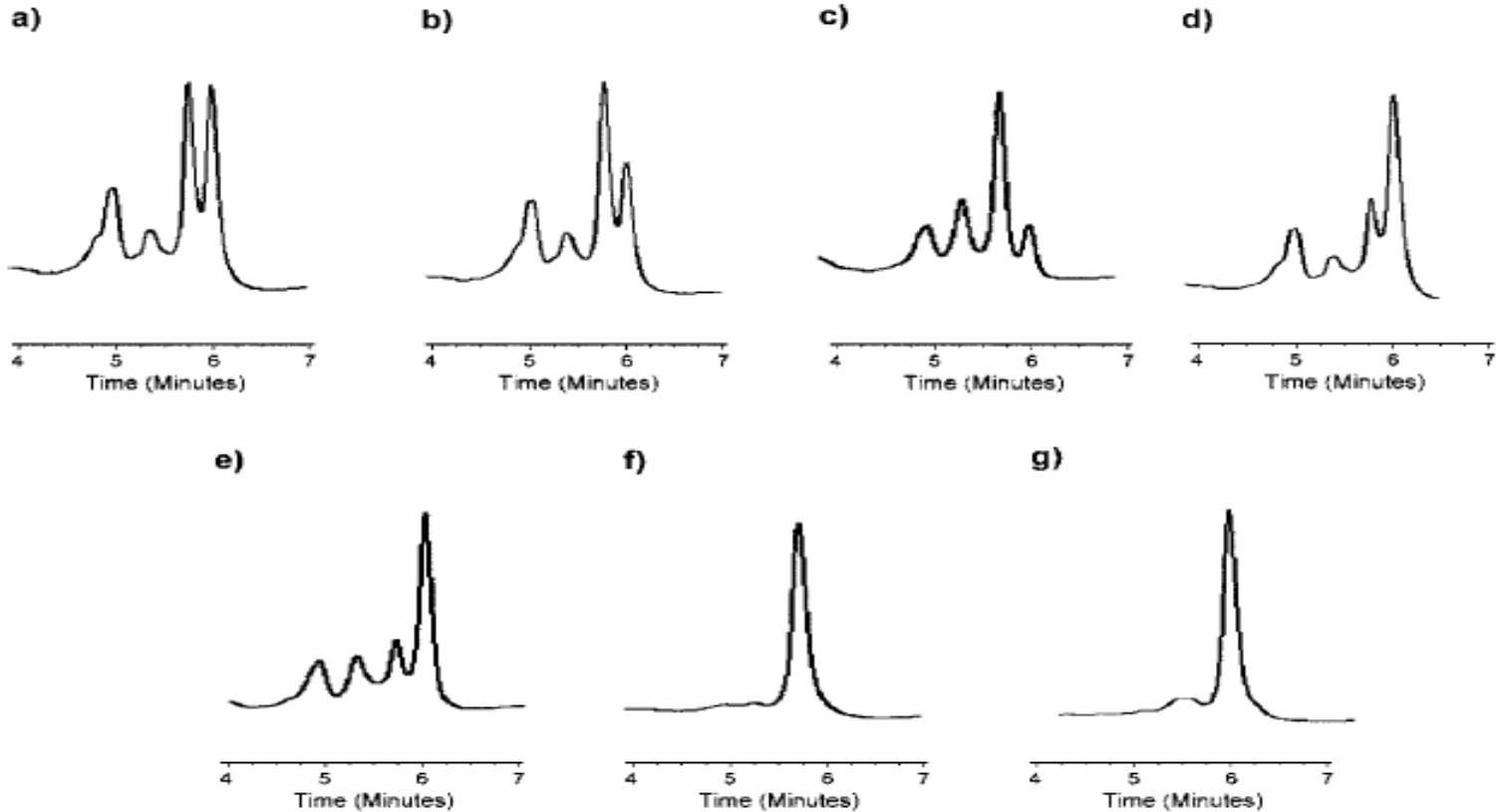


FIG. 1. DHPLC of an individual with an SMN1/SMN2 gene (wild type)

# Chromatography of DHPLC



# DHPLC of an individual with an SMN1/SMN2 gene ratio



(a) 2:2

(b) 1:2

(c) 1:3

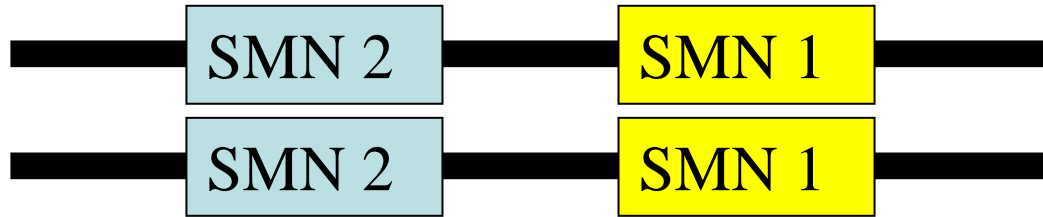
(d) 2:1

(e), 3:1

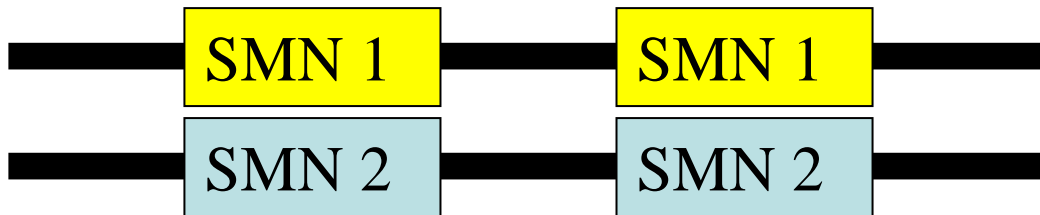
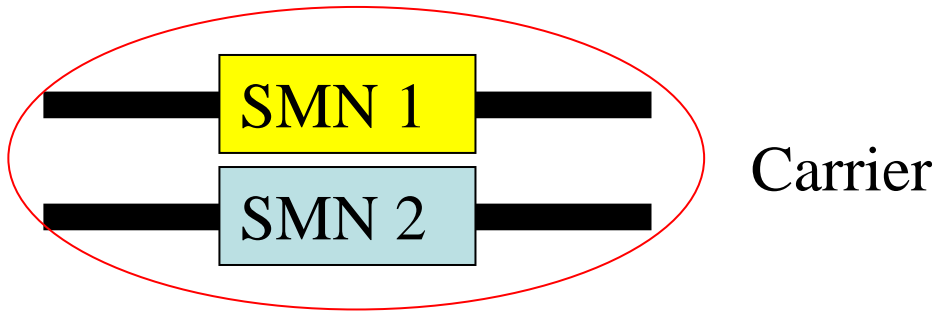
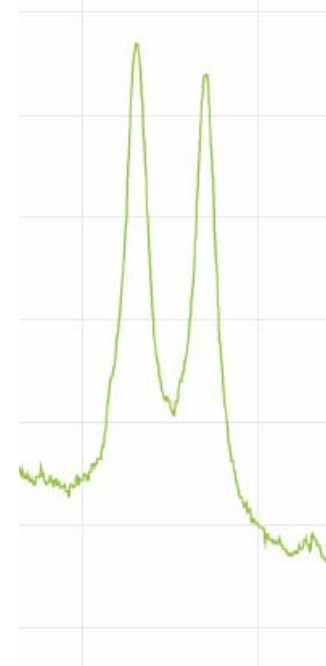
(f), SMN2 gene only

(g). SMN1 gene only

# Misdiagnosis of SMA carrier

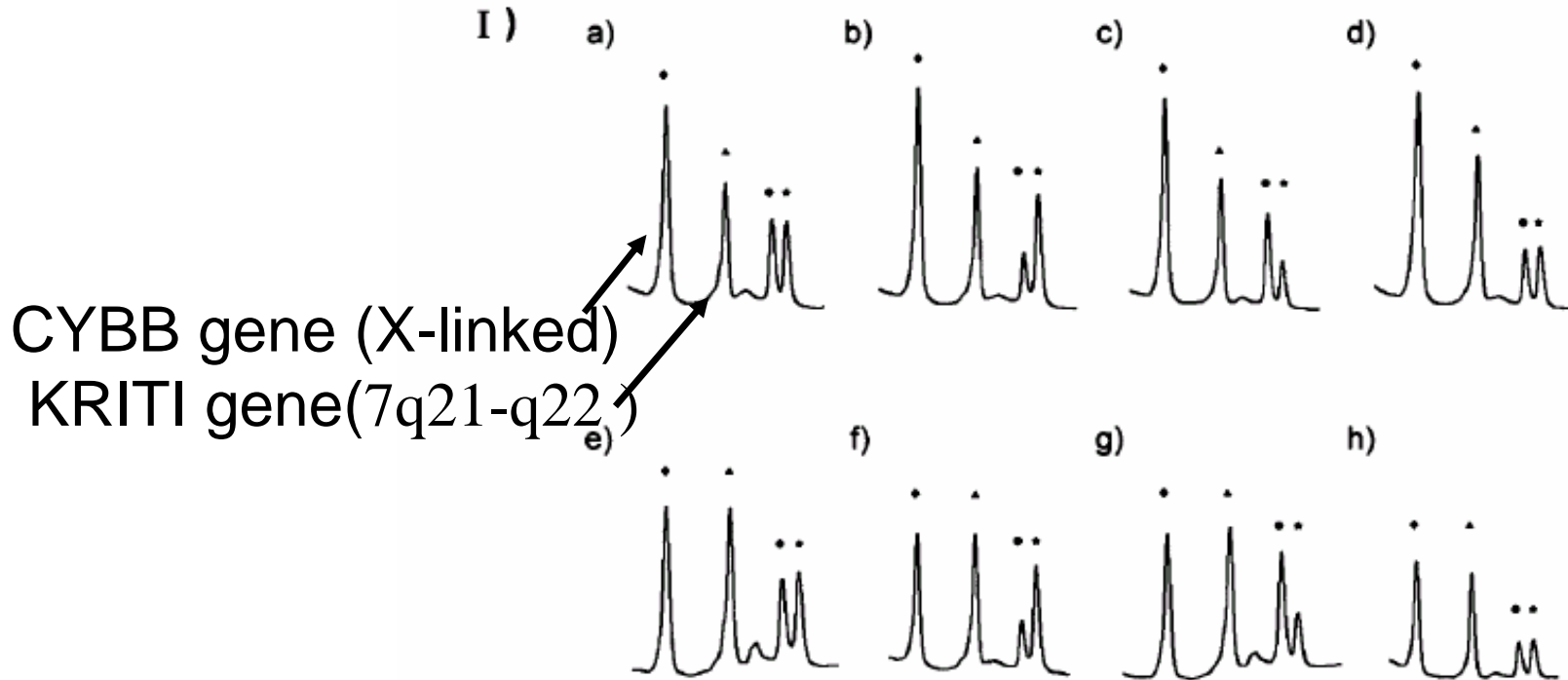


Wild Type





# DHPLC of multiplex PCR analysis with different SMN1/SMN2 ratios.



a~d: Female individuals with various SMN1/SMN2 ratios:

(a) 2:2

(b) 2:1

(c) 1:2

(d) 1:1

e~h: Male individuals with various SMN1/SMN2 ratios:

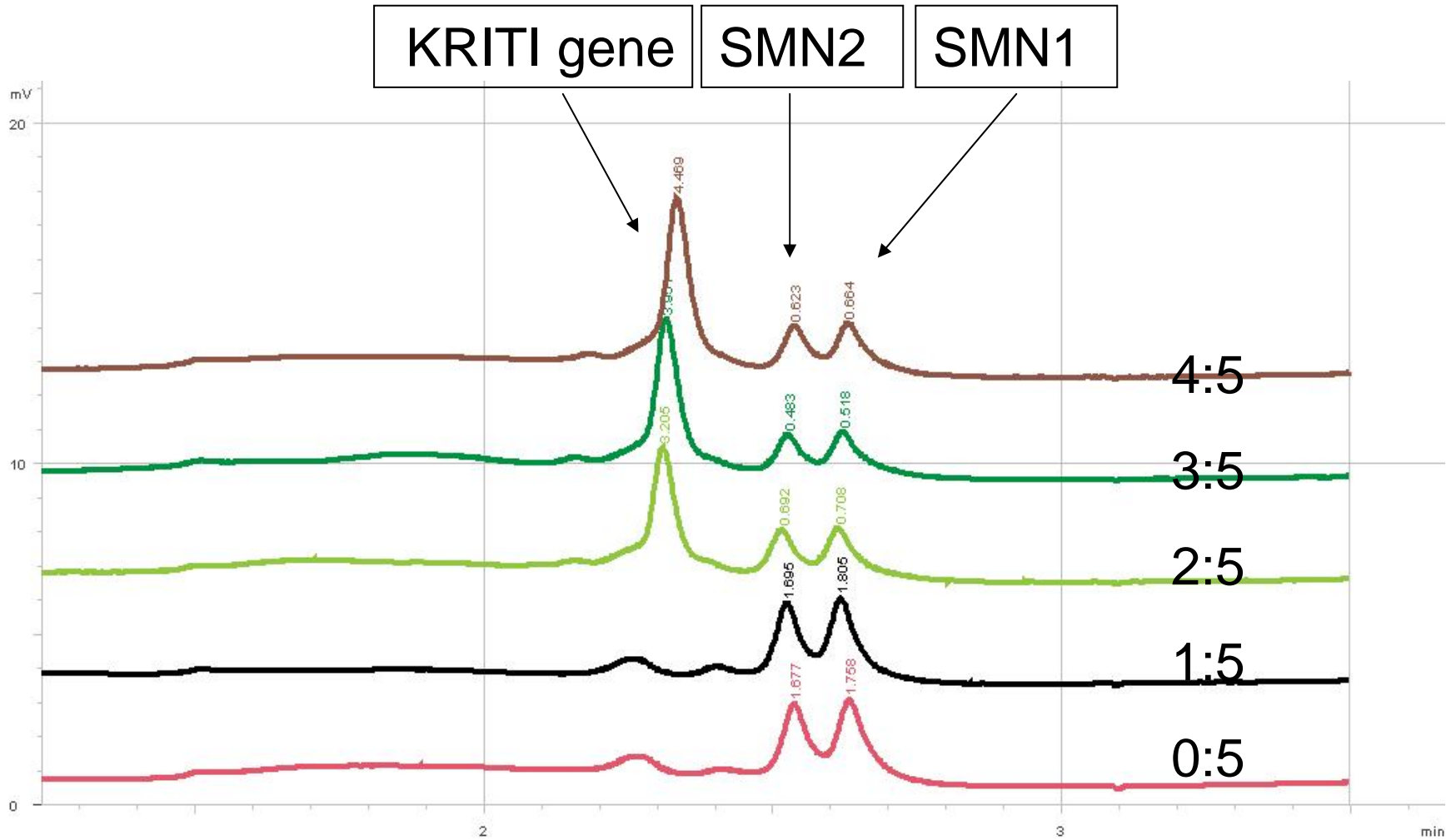
(e) 2:2

(f) 2:1

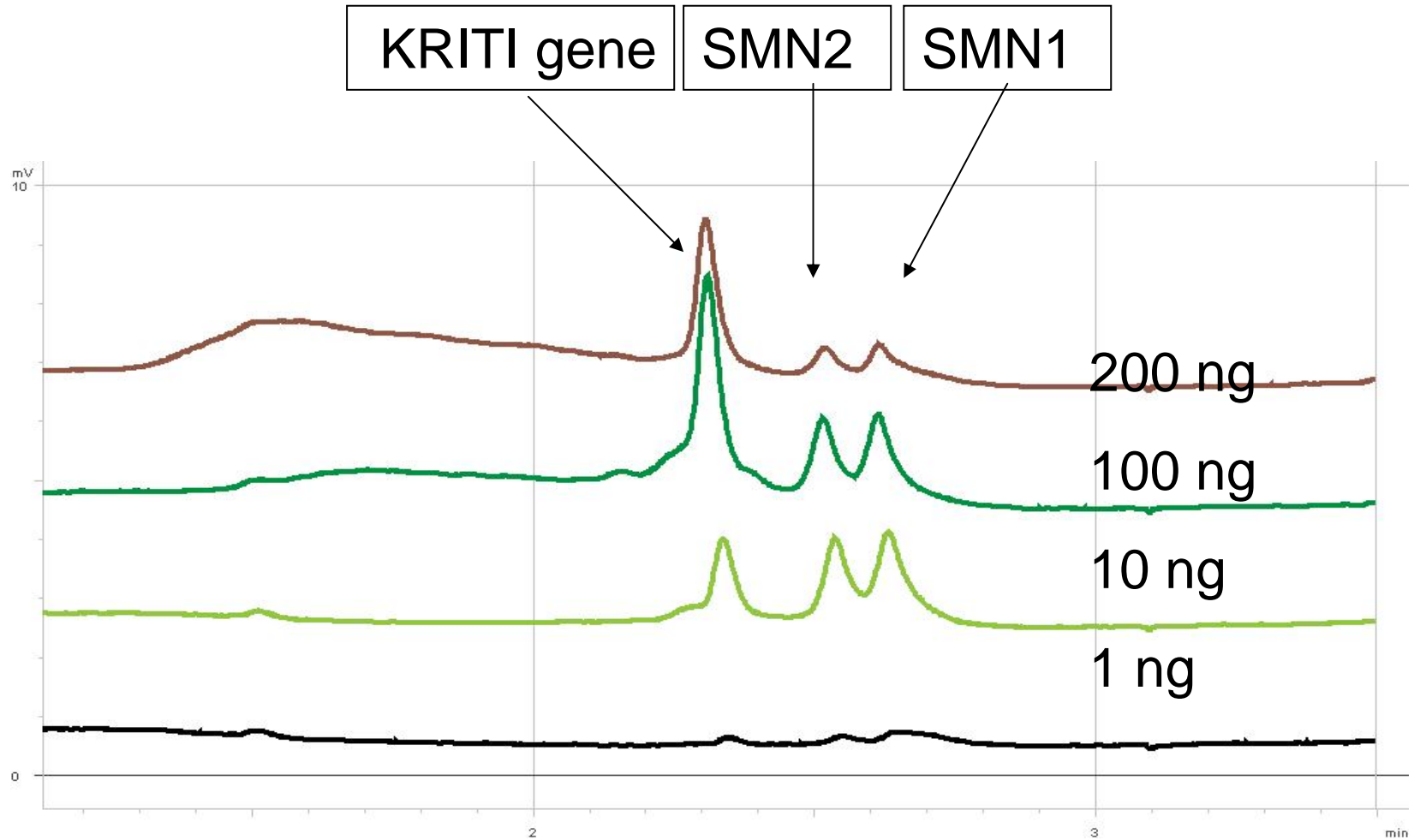
(g) 1:2

(h) 1:1

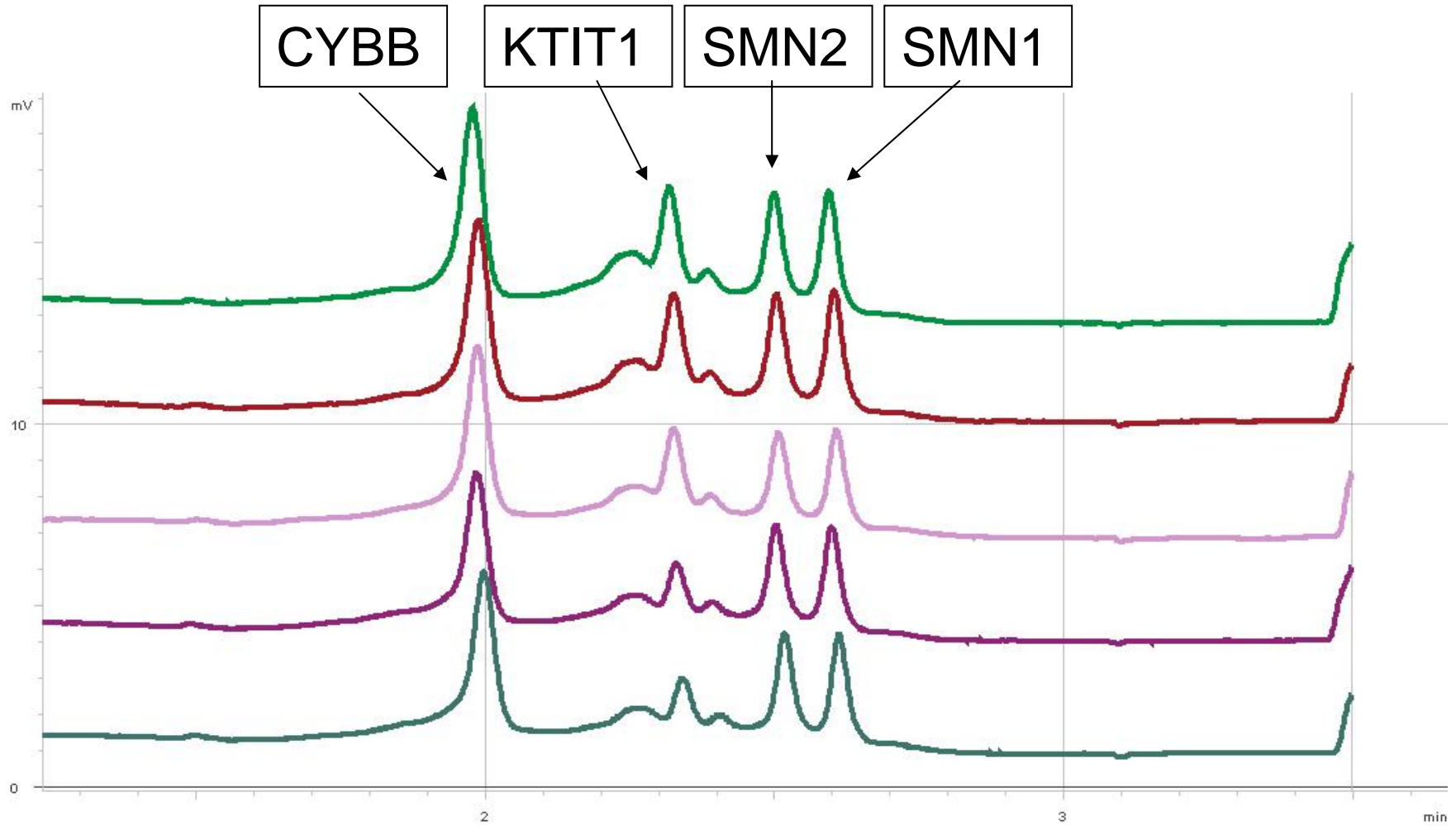
# Primer ratio



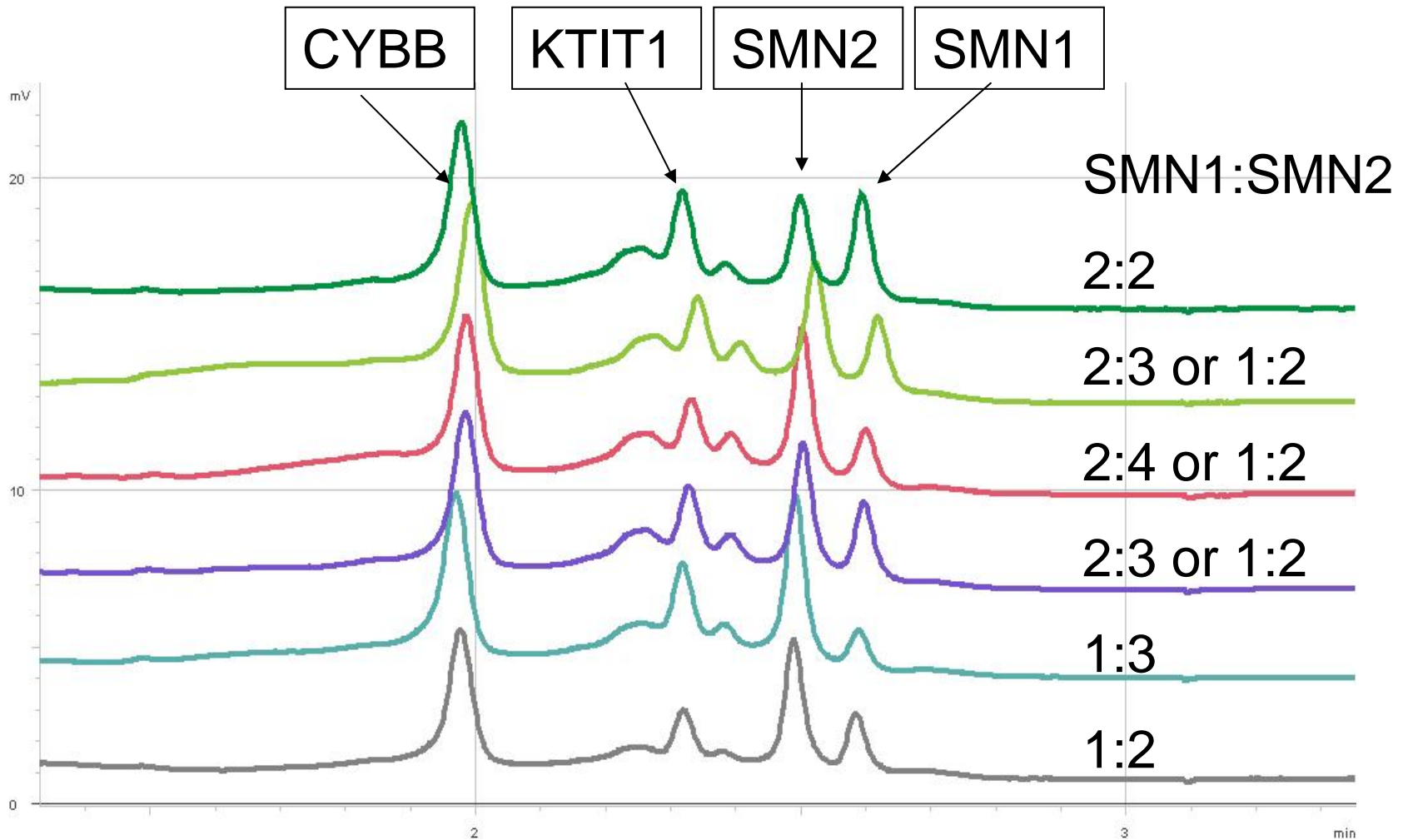
# DNA



# SMN1:SMN2=2:2



# SMN1:SMN2= 1:2 or 2:3



# 何謂"MLPA" ?

- MLPA是Multiplex Ligation-dependent Probe Amplification的簡稱，由荷蘭的Dr. Schouten JP所帶領的研發團隊於2002年發表，是一種靈敏度極高的相對定量技術，乃利用簡單的雜合(hybridization)、連接(ligation)及PCR增福(PCR amplification)反應，於單一反應管內可同時偵測最多40個不同的核苷酸序列的拷貝數變化。

# MLPA

- Detection of exon deletions/duplication
- Detection of trisomies such as Down's syndrome
- SNP and mutation detection
- DNA methylation
- Relative quantification of mRNAs

# MLPA

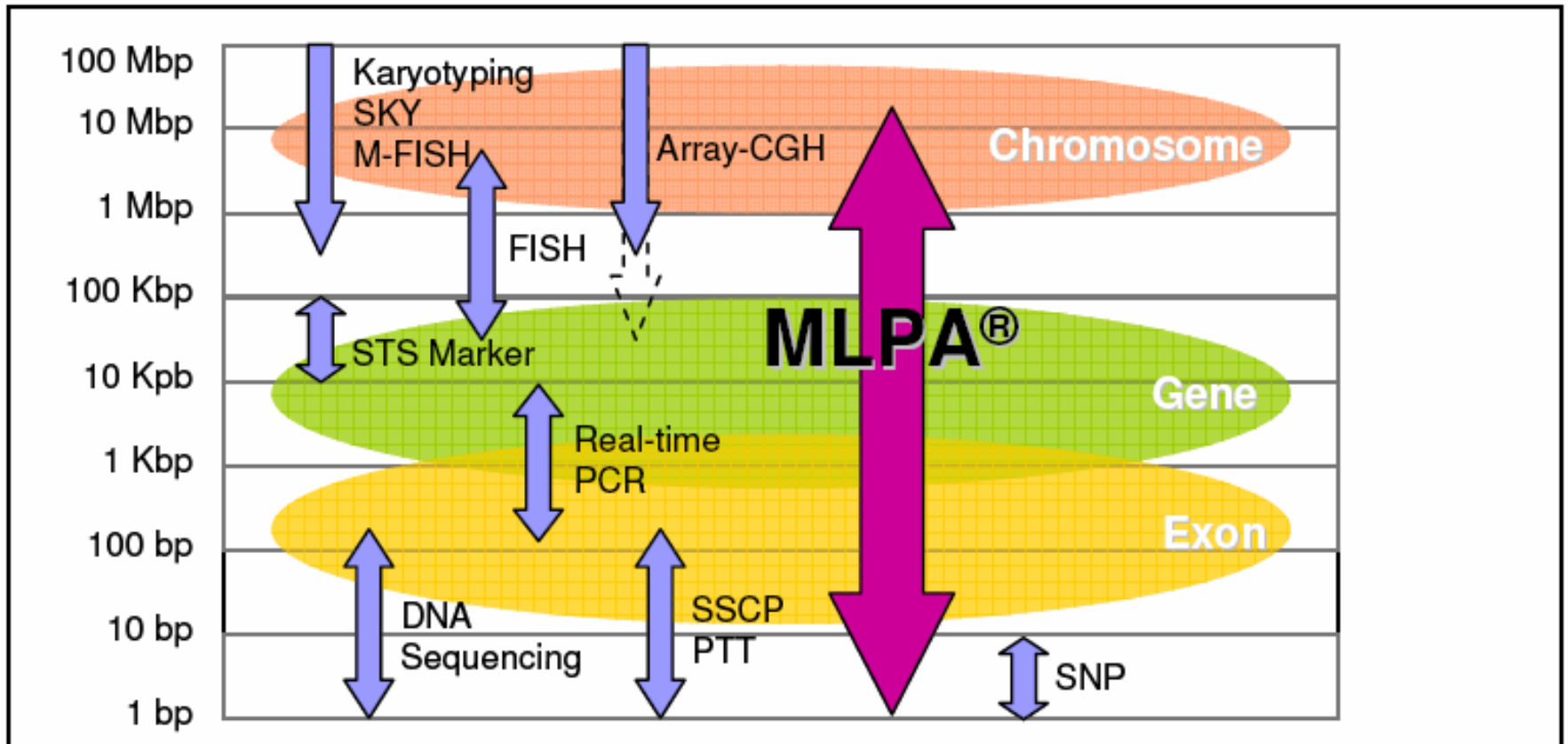
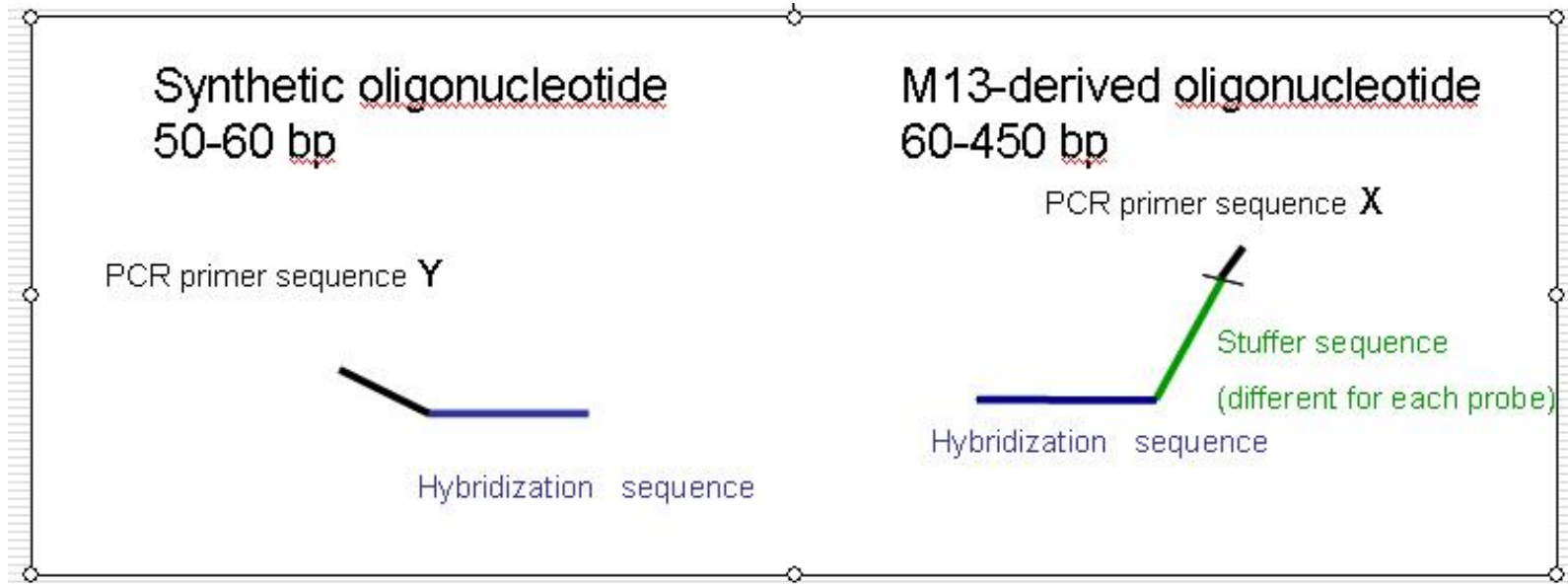


Figure 1 – MLPA compared to other techniques. MLPA can detect a wide range of genomic alterations, from single point mutations to large chromosomal deletions/duplications.

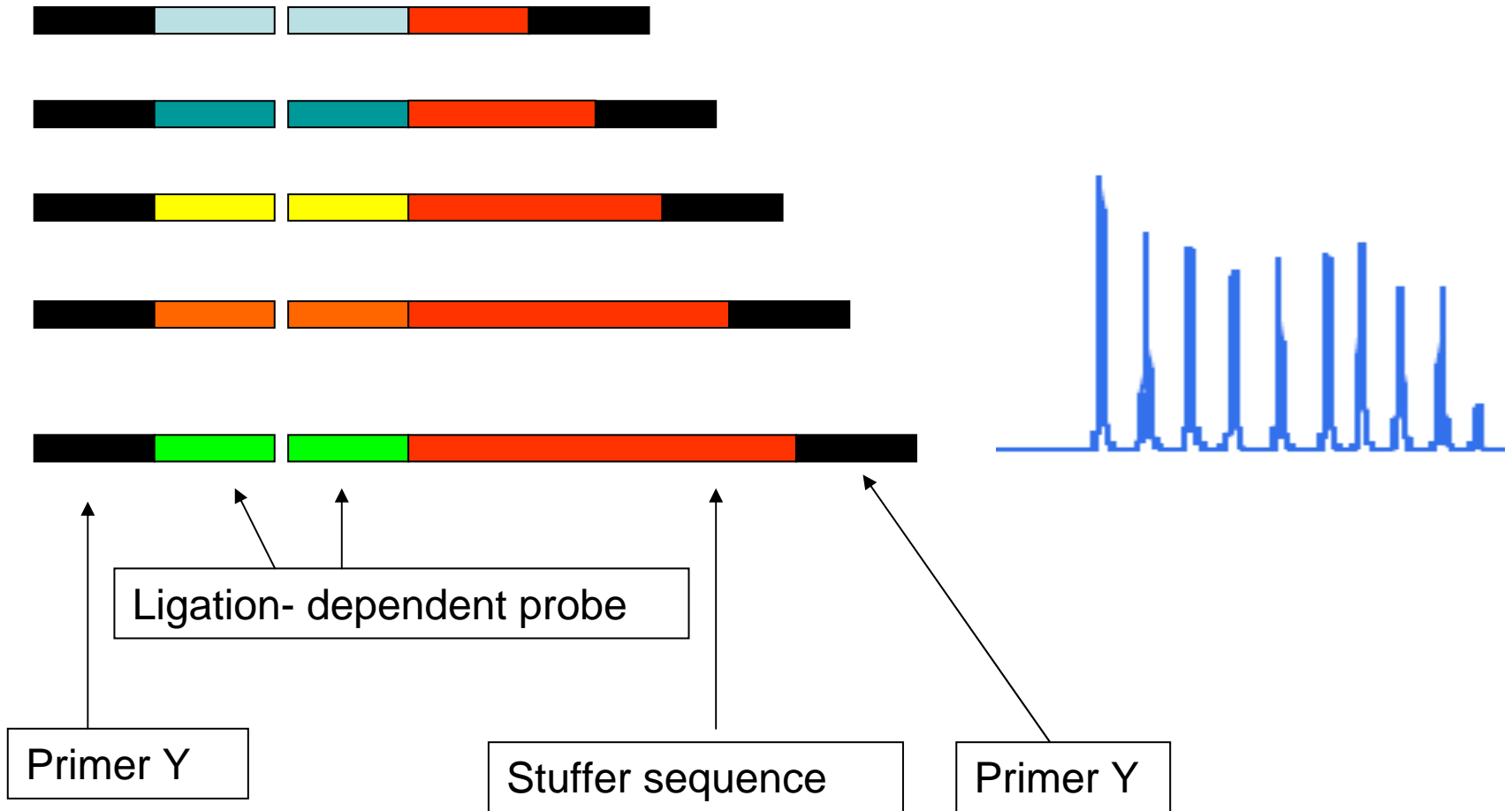


# MLPA



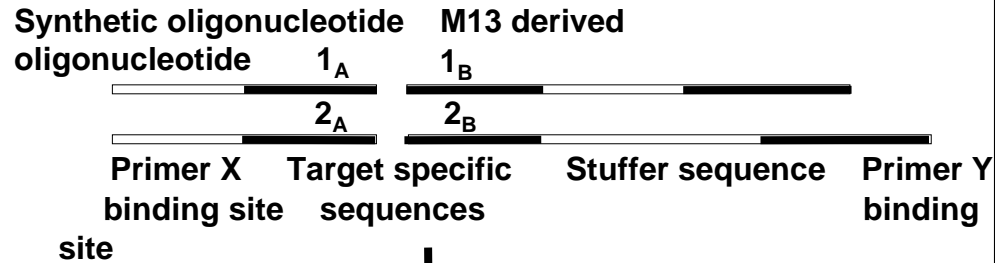
## Multiplex Ligation-dependent Probe Amplification

# MLPA

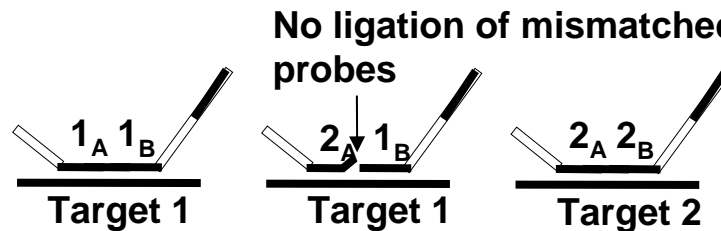




## Probe design



## Multiplex hybridisation and ligation

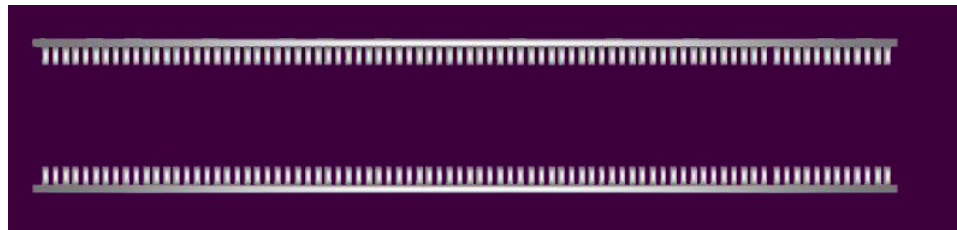
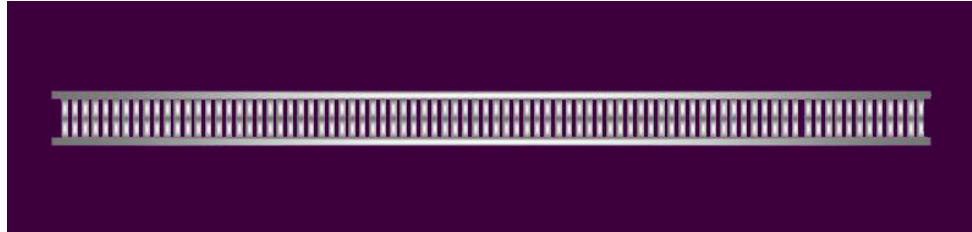


## PCR with universal primers X and Y

No exponential amplification of non ligated probes

## Fragment Analysis

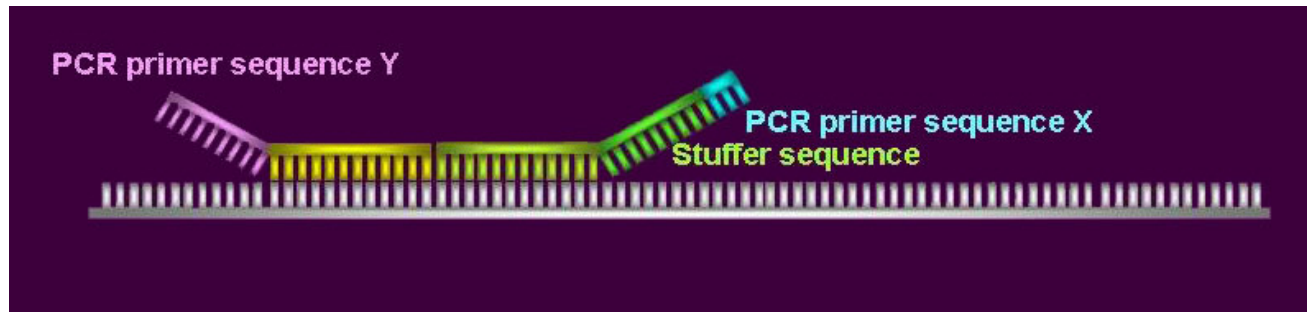
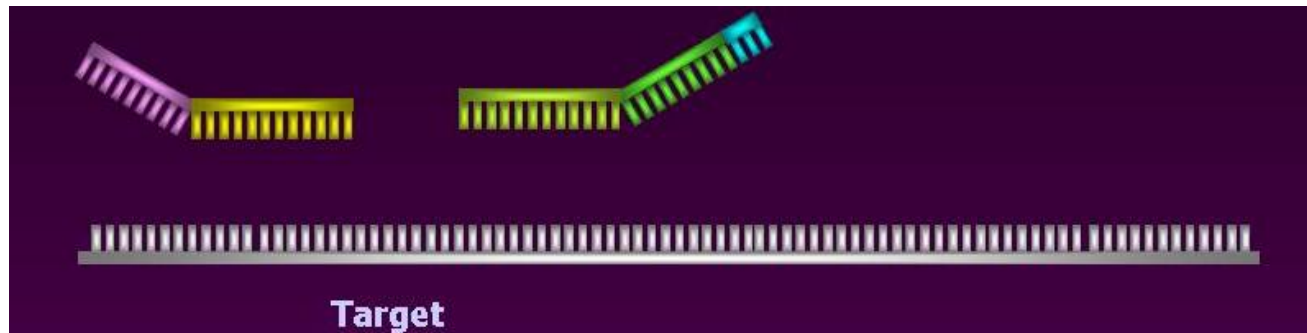
# DNA Denaturation



Dilute the DNA-sample (20-500 ng DNA) with TE to 5 ul.

Heat 5 minutes at 98°C; Cool to 25°C before opening the thermal cycler

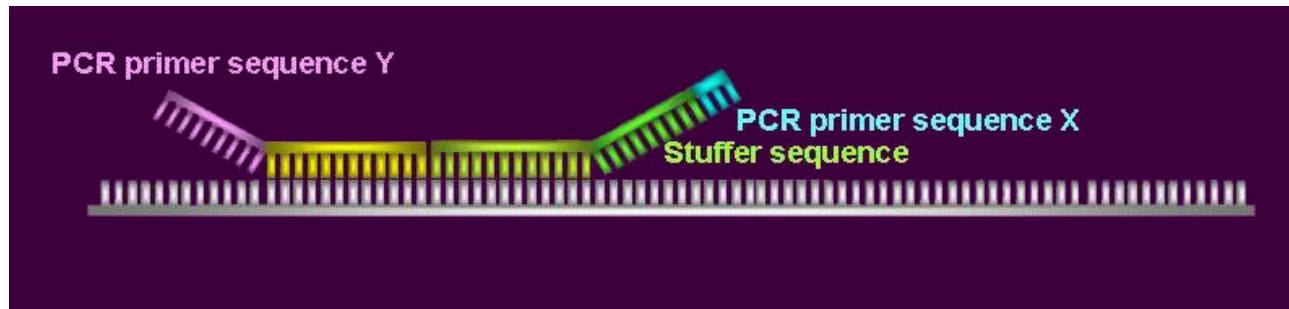
# Hybridisation of the SALSA-probes



Add: 1.5 -l SALSA Probe-mix (black cap) + 1.5 -l MLPA buffer (yellow cap).

Mix with care. Incubate 1 minute 95°C, then 16 hrs at 60°C.

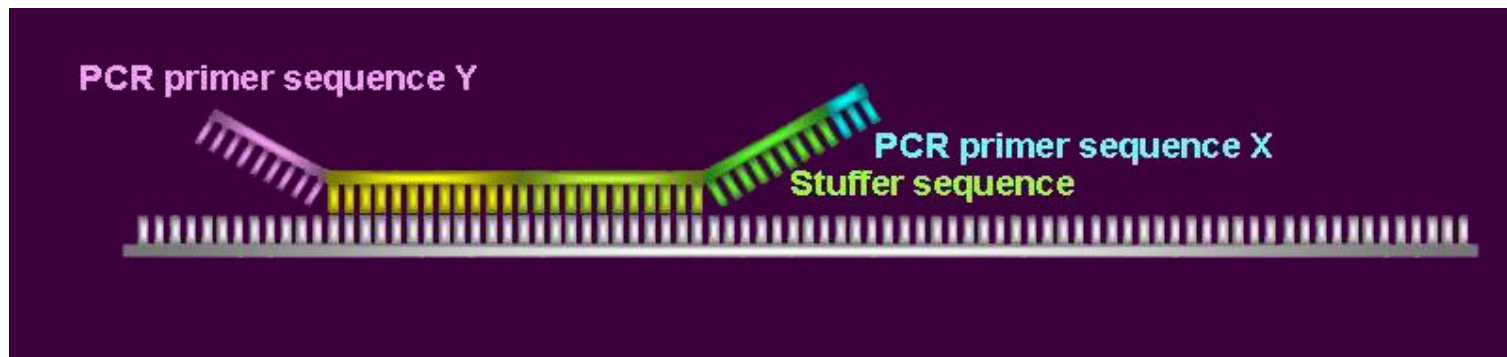
# Ligation reaction



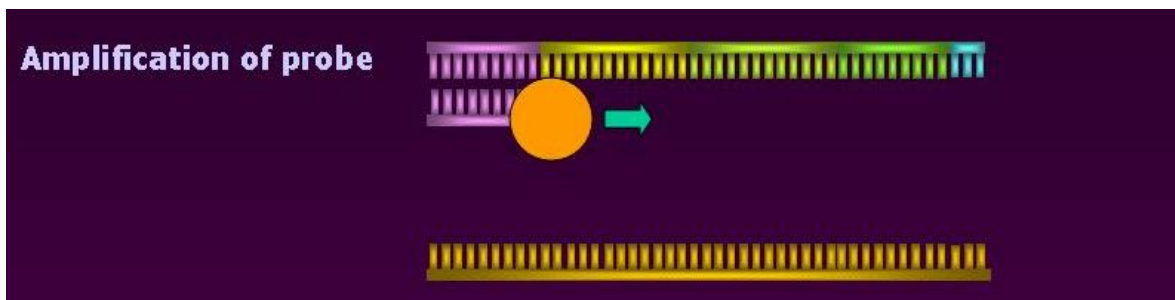
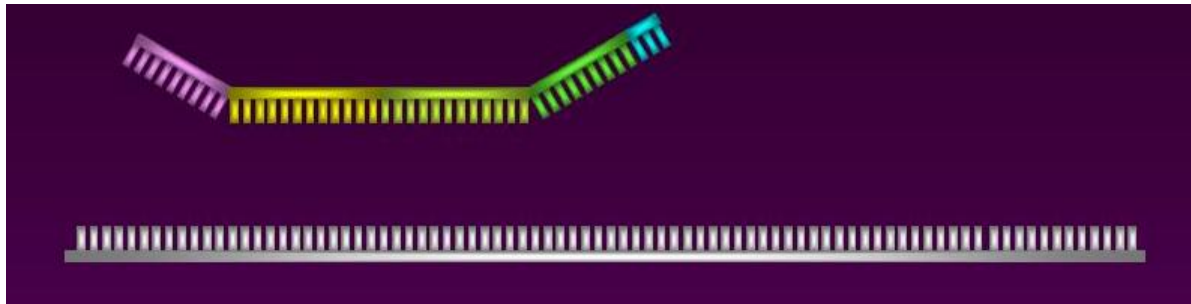
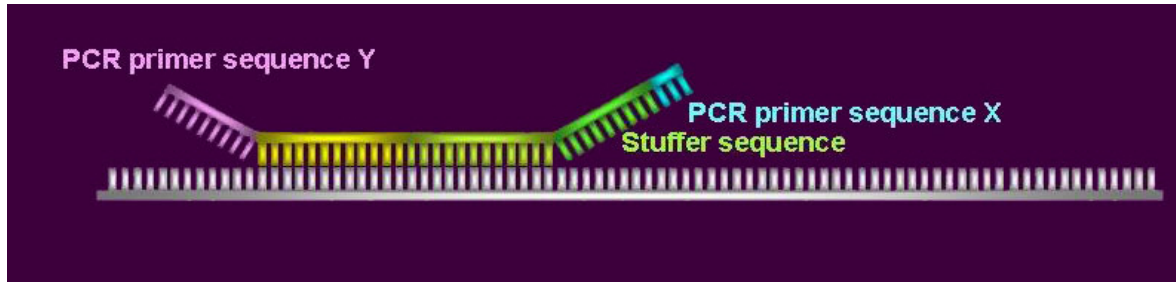
Reduce temperature of the thermal cycler to 54°C. While at 54°C, add 32 ul Ligase-65 mix to each sample and mix.

Incubate 15 minutes at 54°C, then heat 5 minutes at 98°C.

# Ligation

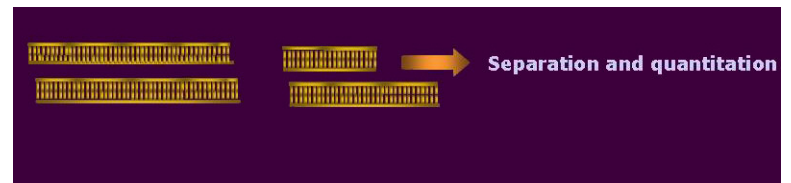
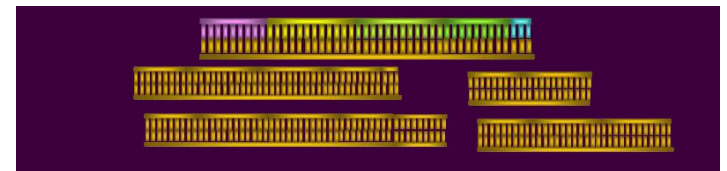
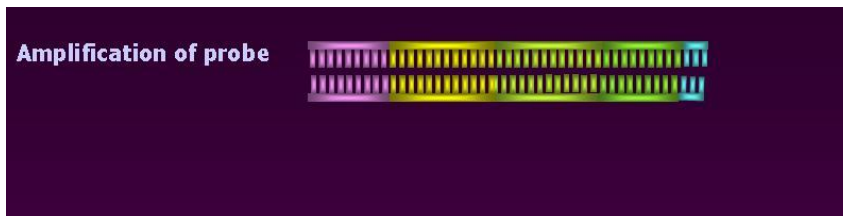
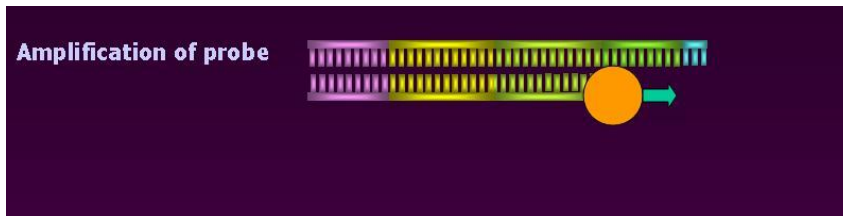
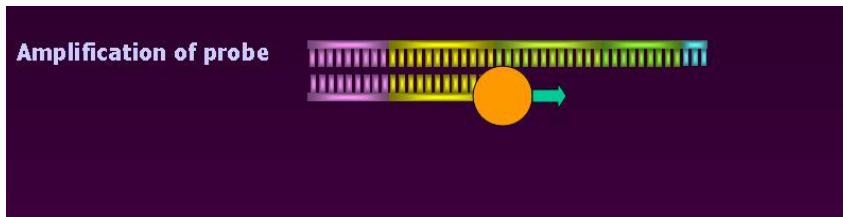
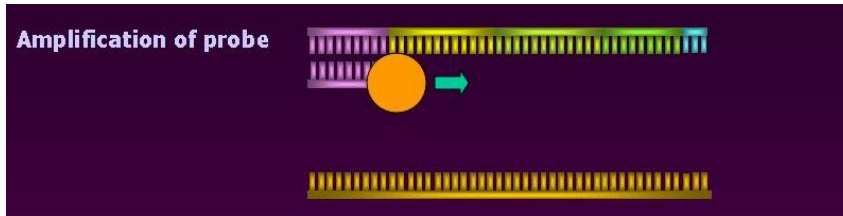


# PCR reaction

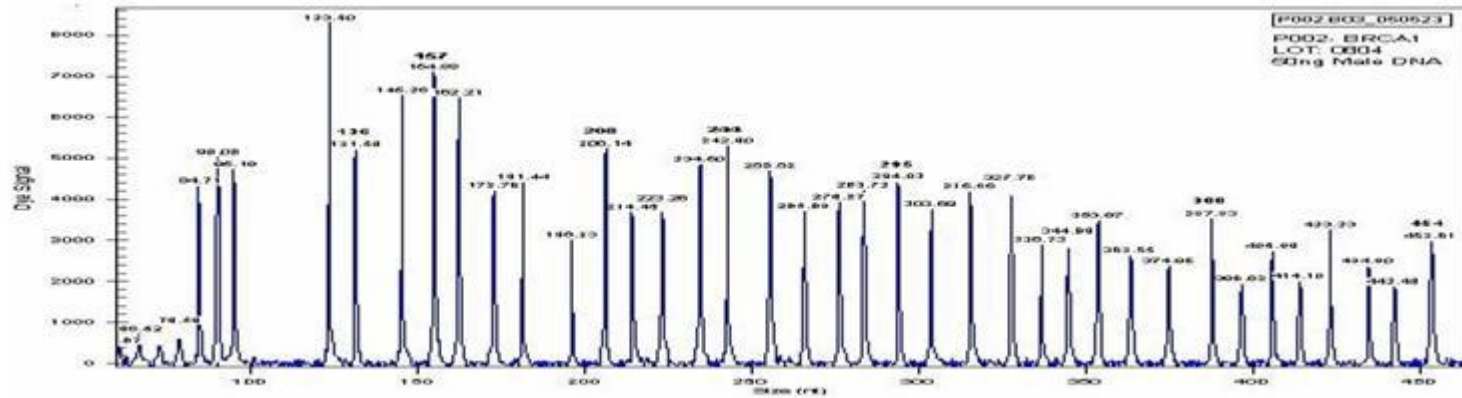
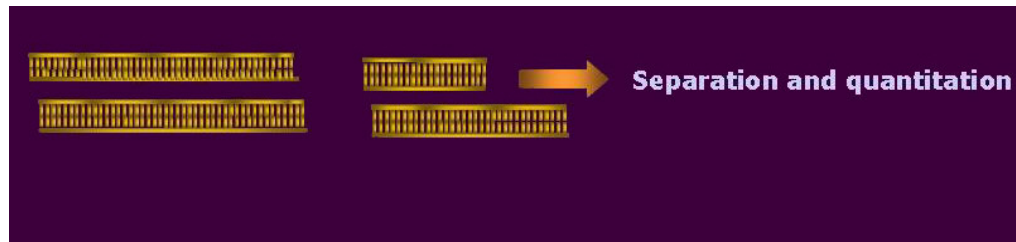
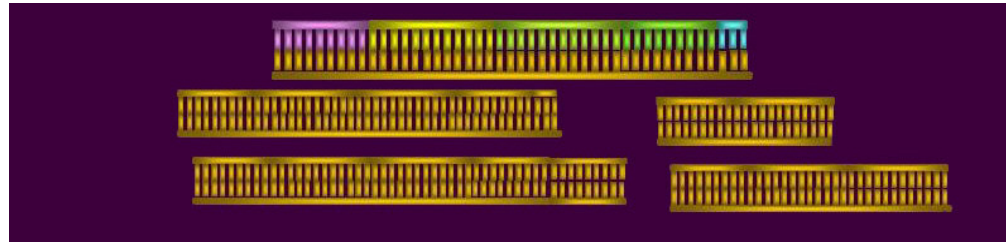




# PCR reaction



# PCR reaction



# P021 SMA-MLPA

- Synthetic control probe : 1
- Control probes : 21
- 5p15.1-14 : 1
- SMN1 cluster start : 2
- **SMN1 : 2(exon 7,exon 8)**
- SMN1 cluster end : 1
- SMN2 cluster start : 2
- **SMN2 : 2(exon 7,exon 8)**
- SMN1/2 cluster start : 2
- **SMN1/2 : 4(exon 1, exon 4, exon 6, exon 8)**

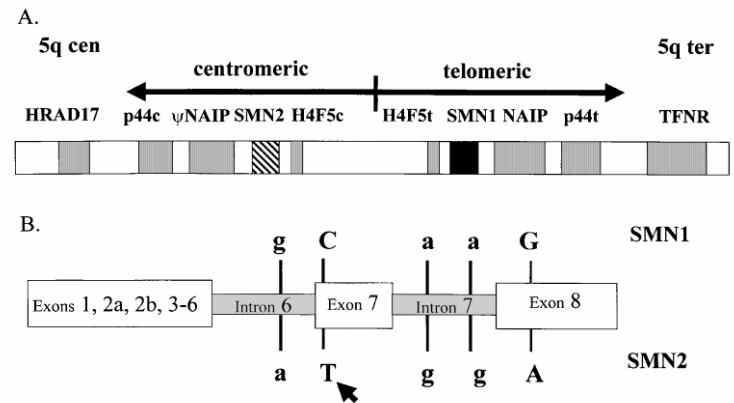
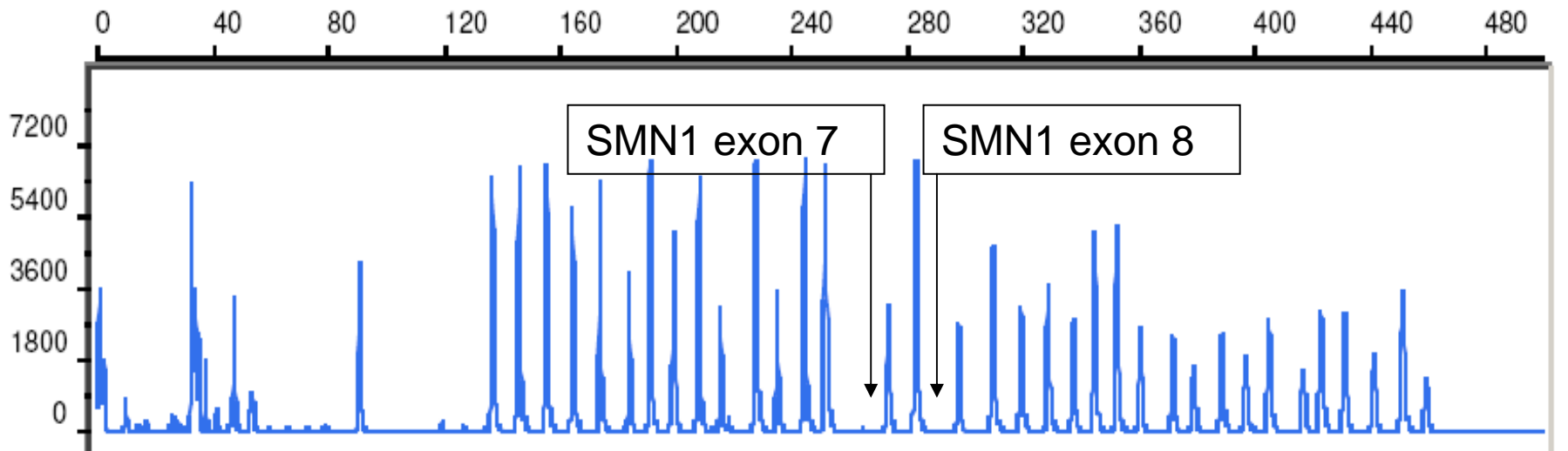
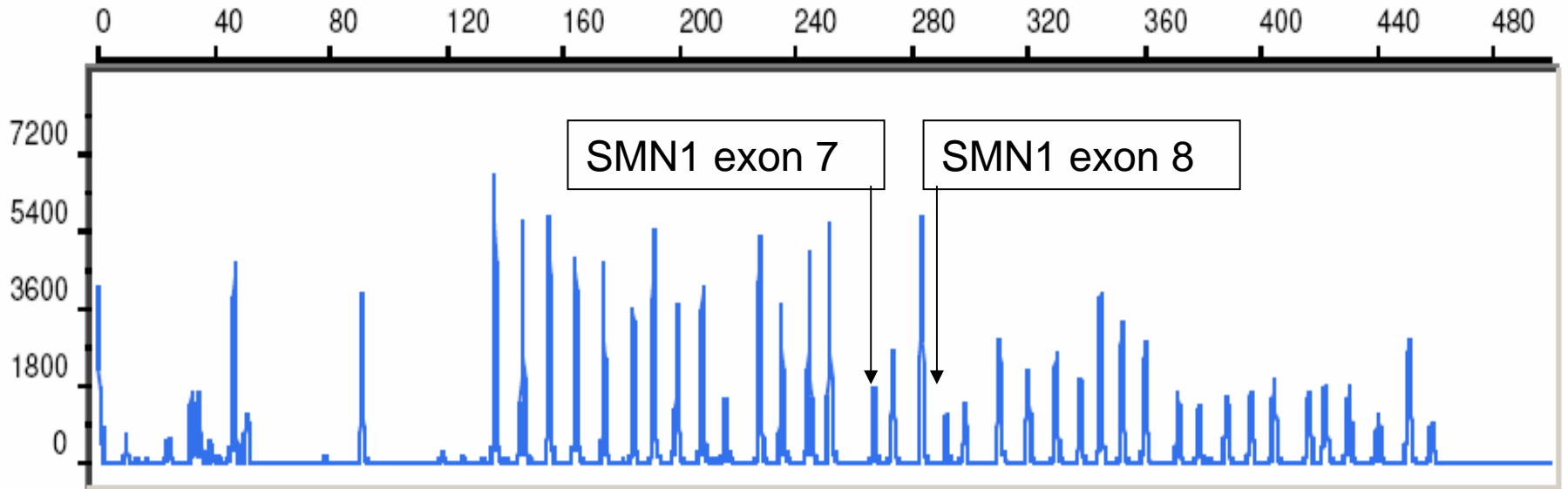


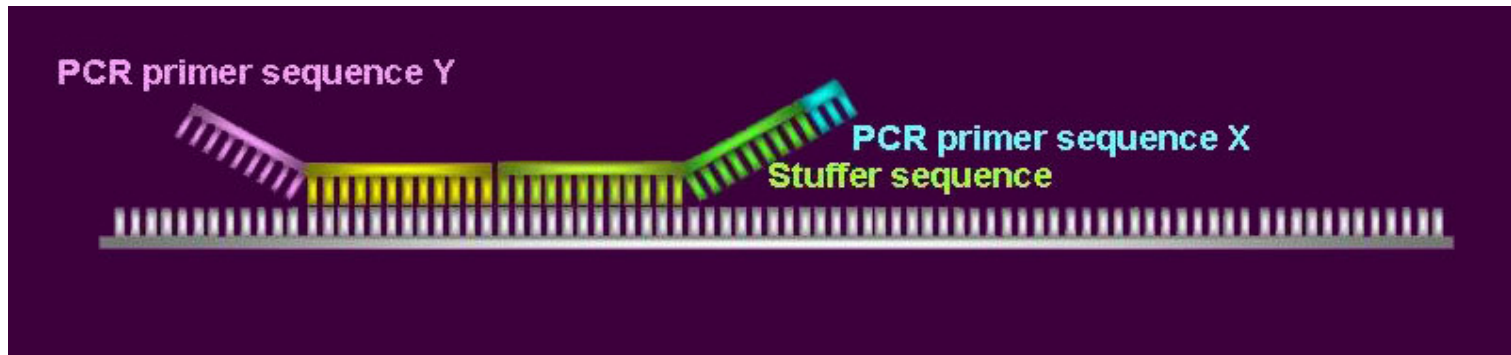
FIGURE 1. A: Schematic representation of the inverted and duplicated SMA region (5q13), including four duplicated genes: H4F5, SMN, NAIP, and BTfP44. The region is flanked by HRAD17 proximal [von Deimling et al., 1999] and TFNR (Kelter and Wirth, unpublished results). B: Localization of the nucleotides by which SMN1 can be distinguished from SMN2.

# MLPA result

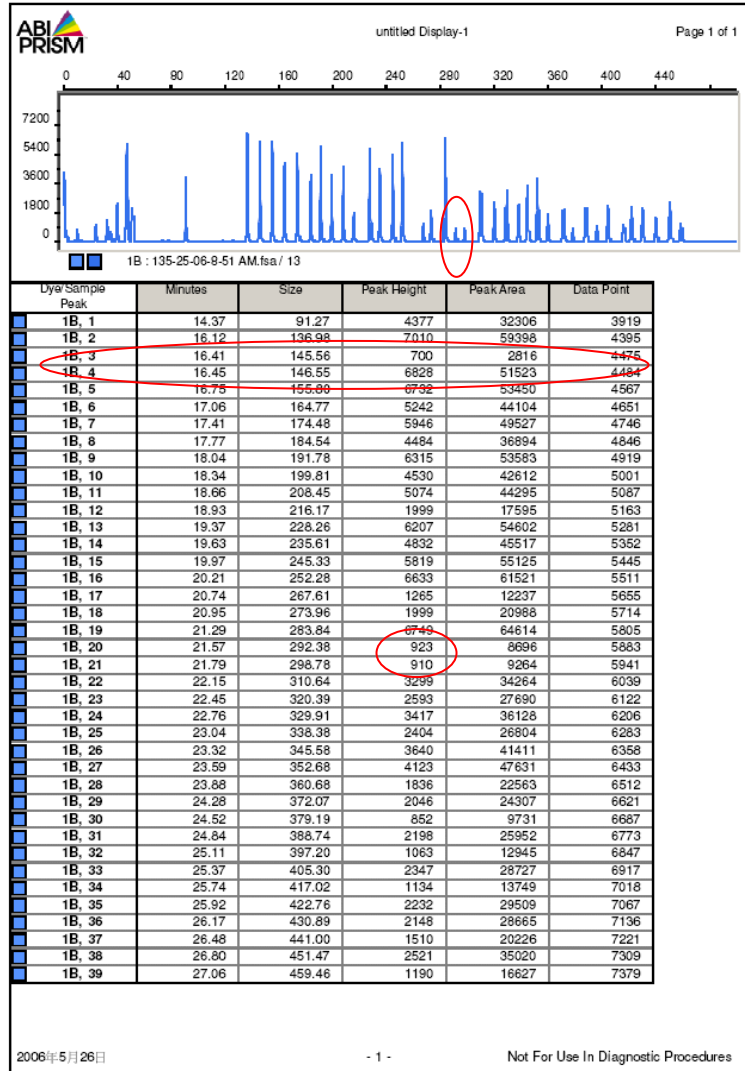


# Multiplex PCR vs MLPA

- Multiplex PCR
  - One pair of primers for each fragment to be amplified
- MLPA
  - All fragments are amplified with the use of a single PCR primer pair.



# Gene scan





Case: 1 Folder: 2006.5.23

Date: 2006/6/30

P021-vs02 SMA Spinal Muscular Atrophy 2:2 (SMN1 to SMN2 for exon 7 and 8) Female & male ref.: P021-Normlist-CGMH-2-2

Ver.: 4.66

----- Normalized Peak Area -----																	
no.	Label	Size	Ref. Size	Size diff.	Peak Height	Peak width	Area	Peak Area	Ref. Mean	Ref. SD	Ref. Weight	Position p-tel band	Ratio	Dist. in SD	low	1.0	high
	cm -		60.80									cm64					
	cm -		66.28									cm70					
	cm -		72.25									cm76					
	cm -		78.20									cm82					
<b>Control mix probes</b>				Mean							1.00	(CV: )					
1	2A	91.26	91.10	0.16	3254	7.9 *	25837	0.647	0.672	0.036	1.00	2q14 synt	0.96	-0.7			
<b>Synthetic control probe</b>				Mean	3254	7.9	25837	0.647	0.672	0.036	1.00	(CV: )	0.96				
2	12A	137.03	137.08	-0.05	6334	8.3	52559	1.316	1.323	0.068	0.84	66.8 12q14	0.99	-0.1			
5	3A	155.83	155.79	0.04	4983	8.7	43510	1.090	1.057	0.083	0.56	99.8 3q12	1.03	0.4			
6	17A	164.83	164.80	0.03	3040	9.3	28138	0.705	0.739	0.092	0.35	16.8 17p11.2	0.95	-0.4			
7	18A	174.46	174.38	0.08	3970	9.0	35696	0.894	0.879	0.042	0.91	55.7 18q21.2	1.02	0.4			
9	7A	191.74	191.73	0.01	4564	9.2	41795	1.047	1.090	0.049	0.96	86-9 7q21	0.96	-0.9			
11	10A	208.45	208.47	-0.02	3981	9.5	37858	0.948	0.911	0.038	1.04	1.45 10p15.1	1.04	1.0			
13	11B	228.22	228.19	0.03	4292	9.5	40676	1.005	0.989	0.045	0.95	65.2 11q13	1.02	0.4			
15	21B	245.20	245.23	-0.03	3961	10.4	41064	1.015	0.957	0.042	0.98	14.67 21q11	1.06	1.4			
16	18B	252.27	252.25	0.02	4492	10.1	45399	1.122	1.140	0.026	1.89	75.32 18q23	0.98	-0.7			
19	3B	283.66	283.61	0.05	4766	10.3	48934	1.210	1.246	0.032	1.69	156.3 3q25.1	0.97	-1.1			
22	13B	310.49	310.45	0.04	2324	11.3	26216	0.648	0.668	0.027	1.09	110.6 13q34	0.97	-0.8			
23	8C	320.21	320.17	0.04	1662	11.7	19497	0.971	0.944	0.043	0.96	141.9 8q24	1.03	0.6			
25	21C	338.36	338.20	0.16	1561	12.3	19164	0.955	0.919	0.045	0.88	26.3 21q21.3	1.04	0.8			
27	2C	352.64	352.74	-0.10	2480	12.7	31539	1.571	1.544	0.057	1.17	71.5 2p13.2	1.02	0.5			
29	8C	371.88	372.02	-0.14	1072	13.4	14318	0.713	0.755	0.047	0.70	38.4 8p11.2	0.94	-0.9			
31	3C	388.69	388.82	-0.13	1191	13.3	15857	0.790	0.837	0.059	0.62	10.2 3p25.3	0.94	-0.8			
33	2D	405.23	405.29	-0.06	1431	14.2	20310	1.195	1.147	0.044	1.13	55.2 2p14	1.04	1.1			
35	8D	422.73	422.99	-0.26	1296	15.1	19521	1.148	1.149	0.040	1.25	117.8 8q24.11	1.00	0.0			
36	17D	430.85	431.10	-0.25	1376	15.2	20917	1.230	1.214	0.035	1.51	35.0 17q12	1.01	0.5			
37	13D	440.95	441.20	-0.25	849	15.4	13052	0.768	0.819	0.038	0.93	110.2 13q34	0.94	-1.3			
39	12D	459.43	459.52	-0.09	690	16.2 *	11208	0.659	0.672	0.050	0.58	120.9 12q24.13	0.98	-0.2			
<b>Control probes</b>				Mean	2872	11.7	29868	1.000	1.000	0.048	1.00	(CV: 0.03)	1.00				



4	5 A	146.52	146.56	-0.04	4915	8.4	41345	1.036	1.034	0.045	1.00	31.3	CDH6-D01	1.00	0.0	.
<b>5p15.1-14</b>					Mean	4915	8.4	41345	1.036	1.034	0.045	1.00	(CV: )	1.00		
8	5 A	184.52	184.48	0.04	3176	8.9	28397	0.711	0.778	0.180	0.79	69.8	GTF2H2ex10"	0.91	-0.4	I.
14	5 B	235.58	235.58	0.00	2383	10.5	24998	0.618	0.625	0.094	1.21	70.3	BIRC1 ex 5'	0.99	-0.1	.
<b>SMN1 cluster start</b>					Mean	2780	9.7	26698	0.665	0.702	0.137	1.00	(CV: 0.05)	0.96		
20	5 B	292.22	292.18	0.04	1106	10.3	11339	0.280	0.304	0.028	1.08	69.4	SMN1 ex 8	0.92	-0.8	I.
17	5 B	267.55	267.58	-0.03	1624	10.3	16743	0.414	0.416	0.045	0.92	69.4	SMN1 ex 7	1.00	0.0	.
<b>SMN1</b>					Mean	1365	10.3	14041	0.347	0.360	0.036	1.00	(CV: 0.05)	0.96		
38	5 D	451.38	451.51	-0.13	2183	16.3 *	35507	2.088	2.220	0.124	1.00	69.3	N-Cadherin-lik	0.94	-1.1	I.
<b>SMN1 cluster end</b>					Mean	2183	16.3	35507	2.088	2.220	0.124	1.00	(CV: )	0.94		
10	5 A	199.81	199.85	-0.04	3281	9.7	31704	0.794	0.836	0.060	1.59	68.7	RAD17-D02	0.95	-0.7	.
12	5 B	216.18	216.13	0.05	1778	9.0	15916	0.393	0.281	0.078	0.41	68.9	GTF2H2ex7"	1.40	1.4	III
<b>SMN2 cluster start</b>					Mean	2530	9.3	23810	0.594	0.558	0.069	1.00	(CV: 0.25)	1.04		
21	5 B	298.63	298.53	0.10	1196	9.7	11642	0.288	0.340	0.035	0.93	69.4	SMN2 ex 8	0.85	-1.5	II.
18	5 B	273.93	273.94	-0.01	2485	11.0	27358	0.676	0.679	0.060	1.07	69.4	SMN2 ex 7	1.00	-0.1	.
<b>SMN2</b>					Mean	1841	10.4	19500	0.482	0.509	0.047	1.00	(CV: 0.11)	0.93		
24	5 C	329.80	329.64	0.16	2266	11.5	26163	1.303	1.287	0.125	0.76	70.3	GTF2H2ex 4'	1.01	0.1	.
26	5 C	345.47	345.47	0.00	3437	12.6	43246	2.154	2.002	0.120	1.24	70.3	BIRC1 ex 13	1.08	1.3	I.
<b>SMN1/2 cluster start</b>					Mean	2852	12.1	34705	1.729	1.644	0.122	1.00	(CV: 0.04)	1.05		
28	5 C	360.59	360.69	-0.10	1803	13.3	23933	1.192	1.188	0.143	0.75	69.4	SMN1/2 ex 8	1.00	0.0	.
34	5 D	416.86	417.00	-0.14	977	14.2	13851	0.815	0.905	0.109	0.75	69.4	SMN1/2 ex 6	0.90	-0.8	I.
32	5 D	397.02	397.19	-0.17	1354	14.1	19146	1.126	1.068	0.065	1.50	69.4	SMN1/2 ex 4	1.05	0.9	I.
30	5 C	379.13	379.26	-0.13	964	12.5	12052	0.600	0.690	0.063	0.99	69.4	SMN1/2 ex 1'	0.87	-1.4	I.
<b>SMN1/2</b>					Mean	1275	13.5	17246	0.933	0.963	0.095	1.00	(CV: 0.09)	0.97		

Total of all peaks except the Control Mix Probes, and except peaks marked by a "?":

Mean values	-0.03	2592	11.5	27274	0.951	0.956	0.063	0.99
Standard deviations	0.11	(Coef. of variance:	0.439 )	0.407	0.398			0.07
<b>Quality assessment</b>		<b>Quality limits</b>	<b>Quality</b>					Weighted mean ratios are tested for being outside ratio 1
38 peak areas and <2 primer peaks		>0.50 (1.50)						
38 peak areas and <2 control mix peaks		>0.65 (1.50)						
Mean height of probes AB		> 225 ( 400)	3424					
Mean height of probes CD		> 140 ( 250)	1564					Female & male ref.
Ratio of probe heights AB / CD		<3.00 (2.50)	2.19					Mean SMN1 to SMN2 copies suggest 2:2, if SMN1/2 is about 1.00
CV of Control Probes		<0.20 (0.15)	0.03					SMN1 to SMN2 copies look like: 2:2 for exon 7 and 2:2 for exon 8
1 unidentified peak area / 38 peak areas		< (0.02)	0.00					

\* marks: Size Diff.>0.5, Peak Height>7000, unexpected wide peaks and \*Dist. in SD\*>4.0

Ratio group mean and coefficient of variance (CV) are weighted by the ref. weights

(No Rox peaks are available in the raw data)

Unreliable GTF2H2, BIRC1 & SMN1/2 probes are marked by " or ' "

# MLPA SMA Kit結果判讀要點

Gene	Corresponded copy number	Mean of ratio (expected)	Ratio range
SMN1	0	0.0	0-0
	1	0.5	0.49-0.58
	2	1.0	0.84-1.17
	3	1.5	1.44-1.44
SMN2	0	0.0	0-0.14
	1	0.5	0.45-0.65
	2	1.0	0.82-1.13
	3	1.5	1.34-1.51
SMN1+SMN2	2	0.50	0.53-0.58
	3	0.75	0.68-0.84
	4	1.00	0.91-1.11
	5	1.25	1.23-1.41
CV of Control Probe		<0.1	0.03-0.10

# SMN1 only

8	5 A	184.52	184.48	0.04	5599	9.4	52675	1.015	0.778	0.180	0.79	69.8	GTF2H2ex10"	1.30	1.3	·
14	5 B	235.55	235.58	-0.03	3162	11.1	34944	0.661	0.625	0.094	1.21	70.3	BIRC1 ex 5'	1.06	0.4	·
<b>SMN1 cluster start</b>					Mean	4381	10.2	43810	0.838	0.702	0.137	1.00	(CV: 0.15)	1.15		
20	5 B	292.30	292.18	0.12	1464	11.5	16867	0.319	0.304	0.028	1.08	69.4	SMN1 ex 8	1.05	0.5	·
17	5 B	267.56	267.58	-0.02	2284	10.7	24547	0.464	0.416	0.045	0.92	69.4	SMN1 ex 7	1.12	1.1	·
<b>SMN1</b>					Mean	1874	11.1	20707	0.392	0.360	0.036	1.00	(CV: 0.04)	1.08		
37	5 D	451.38	451.51	-0.13	2245	16.2	36405	1.469	2.220	0.124	1.00	69.3	N-Cadherin-lik	0.66	-6.0*	·
<b>SMN1 cluster end</b>					Mean	2245	16.2	36405	1.469	2.220	0.124	1.00	(CV: )	0.66		
10	5 A	199.81	199.85	-0.04	4674	10.7	49912	0.962	0.836	0.060	1.59	68.7	RAD17-D02	1.15	2.1	·
12	5 B	216.16	216.13	0.03	2232	9.6	21394	0.405	0.281	0.078	0.41	68.9	GTF2H2ex 7"	1.44	1.6	·
<b>SMN2 cluster start</b>					Mean	3453	10.1	35653	0.683	0.558	0.069	1.00	(CV: 0.14)	1.21		
39	5 B	0.00	298.53	-----	0	0.0	0	0.000	0.340	0.035	0.93	69.4	SMN2 ex 8	0.00	-9.8*	·
18	5 B	273.95	273.94	0.01	786	12.3	9659	0.183	0.679	0.060	1.07	69.4	SMN2 ex 7	0.27	-8.3*	·
<b>SMN2</b>					Mean	393	6.1	4830	0.091	0.509	0.047	1.00	(CV: 1.31)	0.14		P= 5.81%
23	5 C	329.97	329.64	0.33	3742	12.1	45441	1.561	1.287	0.125	0.76	70.3	GTF2H2ex 4'	1.21	2.2	·
25	5 C	345.56	345.47	0.09	3101	13.2	40947	1.407	2.002	0.120	1.24	70.3	BIRC1 ex 13	0.70	-5.0*	·
<b>SMN1/2 cluster start</b>					Mean	3422	12.7	43194	1.484	1.644	0.122	1.00	(CV: 0.39)	0.90		
27	5 C	360.62	360.69	-0.07	1394	13.9	19348	0.665	1.188	0.143	0.75	69.4	SMN1/2 ex 8	0.56	-3.6	·
33	5 D	416.82	417.00	-0.18	830	14.3	11859	0.479	0.905	0.109	0.75	69.4	SMN1/2 ex 6	0.53	-3.9	·
31	5 D	397.01	397.19	-0.18	983	14.7	14460	0.584	1.068	0.065	1.50	69.4	SMN1/2 ex 4	0.55	-7.5*	·
29	5 C	379.13	379.26	-0.13	796	13.8	11021	0.379	0.690	0.063	0.99	69.4	SMN1/2 ex 1'	0.55	-4.9*	·
<b>SMN1/2</b>					Mean	1001	14.2	14172	0.526	0.963	0.095	1.00	(CV: 0.02)	0.55		P= 0.001%

Mean SMN1 to SMN2 copies suggest 2:0, if SMN1/2 is about 0.50  
 SMN1 to SMN2 copies look like: 2:1 for exon 7 and 2:0 for exon 8

# SMN2 only

7	5 A	184.25	184.48	-0.23	5589	7.7	42855	0.777	0.778	0.180	0.79	69.8	GTF2H2ex10"	1.00	0.0	.
13	5 B	235.54	235.58	-0.04	4179	8.8	36863	0.556	0.625	0.094	1.21	70.3	BIRC1 ex 5'	0.89	-0.7	I.
<b>SMN1 cluster start</b>					Mean	4884	8.2	39859	0.667	0.702	0.137	1.00	(CV: 0.08)	0.93		
38	5 B	0.00	292.18	-----	0	0.0	0	0.000	0.304	0.028	1.08	69.4	SMN1 ex 8	0.00	-10.8 *	.
37	5 B	0.00	267.58	-----	0	0.0	0	0.000	0.416	0.045	0.92	69.4	SMN1 ex 7	0.00	-9.3 *	.
<b>SMN1</b>					Mean	0	0.0	0	0.000	0.360	0.036	1.00	(CV: )	0.00		P= 0.003%
35	5 D	451.62	451.51	0.11	4737	13.5	63992	1.771	2.220	0.124	1.00	69.3	N-Cadherin-lik	0.80	-3.6	.
<b>SMN1 cluster end</b>					Mean	4737	13.5	63992	1.771	2.220	0.124	1.00	(CV: )	0.80		
9	5 A	199.90	199.85	0.05	5632	9.2	51855	0.940	0.836	0.060	1.59	68.7	RAD17-D02	1.13	1.7	-.
11	5 B	216.04	216.13	-0.09	4048	8.3	33739	0.509	0.281	0.078	0.41	68.9	GTF2H2ex7"	1.81	2.9	-.
<b>SMN2 cluster start</b>					Mean	4840	8.8	42797	0.725	0.558	0.069	1.00	(CV: 0.31)	1.27		
18	5 B	298.18	298.53	-0.35	3789	9.4	35637	0.538	0.340	0.035	0.93	69.4	SMN2 ex 8	1.58	5.7 *	-.
16	5 B	273.91	273.94	-0.03	5435	9.8	53069	0.801	0.679	0.060	1.07	69.4	SMN2 ex 7	1.18	2.0	-.
<b>SMN2</b>					Mean	4612	9.6	44353	0.669	0.509	0.047	1.00	(CV: 0.21)	1.37		P= 22.54%
21	5 C	329.15	329.64	-0.49	4825	10.3	49596	1.207	1.287	0.125	0.76	70.3	GTF2H2ex 4'	0.94	-0.6	. .
23	5 C	345.32	345.47	-0.15	6251	11.0	68573	1.669	2.002	0.120	1.24	70.3	BIRC1 ex 13	0.83	-2.8	.
<b>SMN1/2 cluster start</b>					Mean	5538	10.6	59085	1.438	1.644	0.122	1.00	(CV: 0.08)	0.87		
25	5 C	360.94	360.69	0.25	3737	11.5	43055	1.048	1.188	0.143	0.75	69.4	SMN1/2 ex 8	0.88	-1.0	. .
31	5 D	417.11	417.00	0.11	2474	11.9	29391	0.814	0.905	0.109	0.75	69.4	SMN1/2 ex 6	0.90	-0.8	. .
29	5 D	397.50	397.19	0.31	2667	12.1	32357	0.896	1.068	0.065	1.50	69.4	SMN1/2 ex 4	0.84	-2.7	.
27	5 C	379.51	379.26	0.25	2276	11.6	26323	0.641	0.690	0.063	0.99	69.4	SMN1/2 ex 1'	0.93	-0.8	. .
<b>SMN1/2</b>					Mean	2789	11.8	32782	0.849	0.963	0.095	1.00	(CV: 0.05)	0.88		

Mean SMN1 to SMN2 copies suggest 0:3, if SMN1/2 is about 0.75  
 SMN1 to SMN2 copies look like: 0:2 for exon 7 and 0:3 for exon 8

# SMN1:SMN2=1:1

8	5 A	185.23	184.48	0.75 *	1644	8.7	14383	0.377	0.778	0.180	0.79	69.8	GTF2H2ex10"	0.48	-2.2	
14	5 B	236.13	235.58	0.55 *	1406	9.5	13426	0.346	0.625	0.094	1.21	70.3	BIRC1 ex 5'	0.55	-3.0	
<b>SMN1 cluster start</b>					Mean	1525	9.1	13905	0.362	0.702	0.137	1.00	(CV: 0.09)	0.53	<b>P= 3.11%</b>	
20	5 B	293.67	292.18	1.49 *	626	11.0	6909	0.178	0.304	0.028	1.08	69.4	SMN1 ex 8	0.59	-4.5 *	
17	5 B	267.92	267.58	0.34	865	10.6	9129	0.235	0.416	0.045	0.92	69.4	SMN1 ex 7	0.57	-4.0 *	
<b>SMN1</b>					Mean	746	10.8	8019	0.207	0.360	0.036	1.00	(CV: 0.02)	0.58	<b>P= 1.05%</b>	
38	5 D	450.91	451.51	-0.60 *	1367	15.0	20556	1.341	2.220	0.124	1.00	69.3	N-Cadherin-lik	0.60	-7.1 *	
<b>SMN1 cluster end</b>					Mean	1367	15.0	20556	1.341	2.220	0.124	1.00	(CV: )	0.60		
10	5 A	199.80	199.85	-0.05	3257	10.0	32591	0.855	0.836	0.060	1.59	68.7	RAD17-D02	1.02	0.3	.
12	5 B	216.82	216.13	0.69 *	1496	9.4	14129	0.364	0.281	0.078	0.41	68.9	GTF2H2ex 7"	1.30	1.1	
<b>SMN2 cluster start</b>					Mean	2377	9.7	23360	0.609	0.558	0.069	1.00	(CV: 0.14)	1.08		
21	5 B	300.27	298.53	1.74 *	625	10.1	6342	0.163	0.340	0.035	0.93	69.4	SMN2 ex 8	0.48	-5.1 *	
18	5 B	274.40	273.94	0.46	1306	11.0	14335	0.369	0.679	0.060	1.07	69.4	SMN2 ex 7	0.54	-5.2 *	
<b>SMN2</b>					Mean	966	10.6	10339	0.266	0.509	0.047	1.00	(CV: 0.09)	0.51	<b>P= 2.78%</b>	
24	5 C	331.48	329.64	1.84 *	1735	11.1	19204	1.008	1.287	0.125	0.76	70.3	GTF2H2ex 4'	0.78	-2.2	
26	5 C	345.99	345.47	0.52 *	1754	12.2	21386	1.122	2.002	0.120	1.24	70.3	BIRC1 ex 13	0.56	-7.4 *	
<b>SMN1/2 cluster start</b>					Mean	1745	11.6	20295	1.065	1.644	0.122	1.00	(CV: 0.24)	0.65	<b>P= 14.25%</b>	
28	5 C	360.04	360.69	-0.65 *	1075	13.2	14235	0.747	1.188	0.143	0.75	69.4	SMN1/2 ex 8	0.63	-3.1	
34	5 D	416.75	417.00	-0.25	481	12.1	5811	0.379	0.905	0.109	0.75	69.4	SMN1/2 ex 6	0.42	-4.8 *	
32	5 D	396.35	397.19	-0.84 *	679	13.6	9261	0.604	1.068	0.065	1.50	69.4	SMN1/2 ex 4	0.57	-7.2 *	
30	5 C	378.64	379.26	-0.62 *	671	12.7	8545	0.448	0.690	0.063	0.99	69.4	SMN1/2 ex 1'	0.65	-3.8	
<b>SMN1/2</b>					Mean	727	12.9	9463	0.545	0.963	0.095	1.00	(CV: 0.16)	0.57	<b>P= 0.39%</b>	

Mean SMN1 to SMN2 copies suggest 1:1, if SMN1/2 is about 0.50

SMN1 to SMN2 copies look like: 1:1 for exon 7 and 1:1 for exon 8

# SMN1:SMN2=2:3

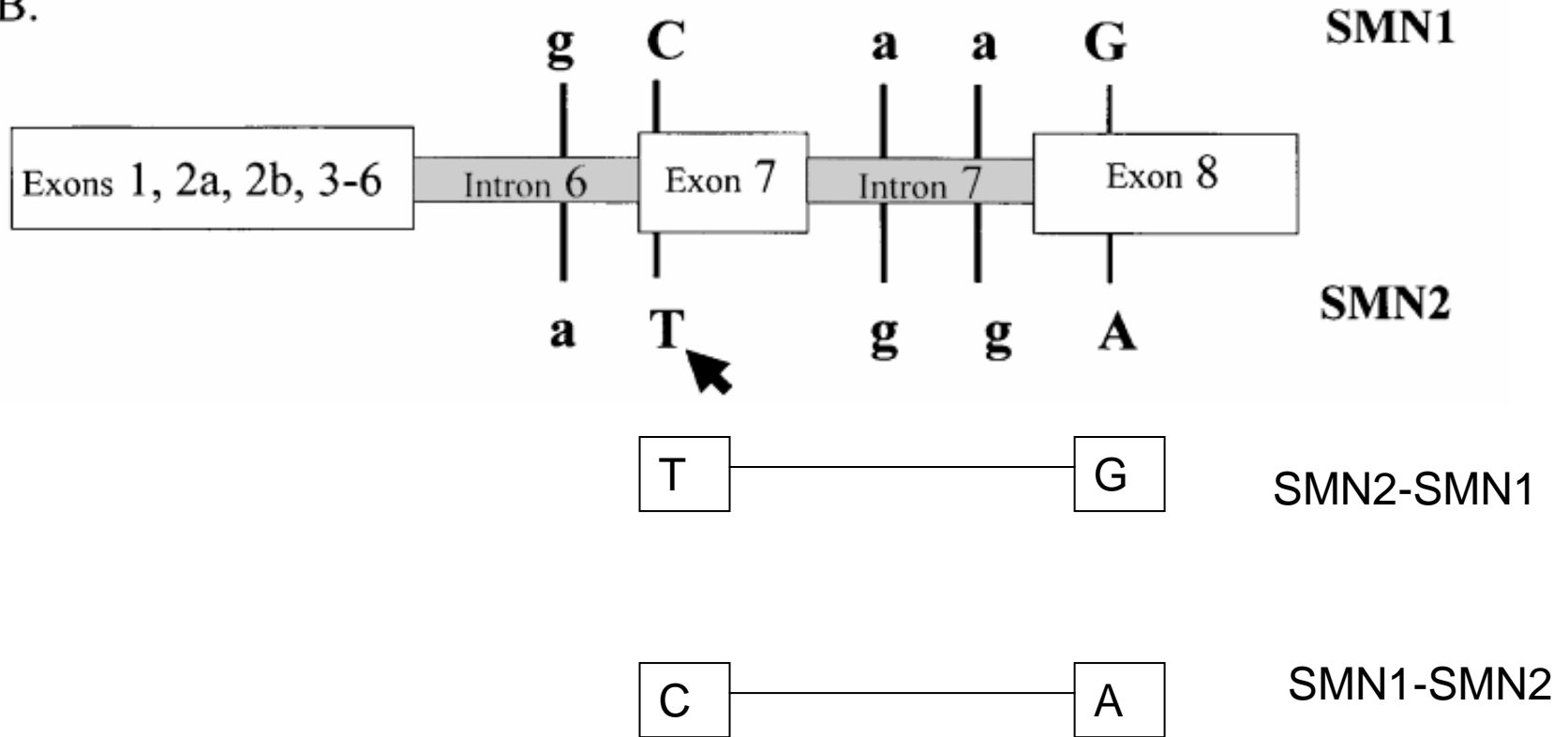
7	5 A	184.10	184.48	-0.38	5000	7.7	38385	1.024	0.778	0.180	0.79	69.8	GTF2H2ex10"	1.32	1.4	-
13	5 B	235.55	235.58	-0.03	3138	8.8	27671	0.576	0.625	0.094	1.21	70.3	BIRC1 ex 5'	0.92	-0.5	·
<b>SMN1 cluster start</b>					Mean 4069	8.2	33028	0.800	0.702	0.137	1.00		(CV: 0.25)	1.08		
19	5 B	291.69	292.18	-0.49	1667	9.4	15599	0.325	0.304	0.028	1.08	69.4	SMN1 ex 8	1.07	0.7	-
16	5 B	267.65	267.58	0.07	2008	9.0	18006	0.375	0.416	0.045	0.92	69.4	SMN1 ex 7	0.90	-0.9	·
<b>SMN1</b>					Mean 1838	9.2	16803	0.350	0.360	0.036	1.00		(CV: 0.12)	0.99		
37	5 D	451.62	451.51	0.11	5339	12.7	67542	2.433	2.220	0.124	1.00	69.3	N-Cadherin-lik	1.10	1.7	-
<b>SMN1 cluster end</b>					Mean 5339	12.7	67542	2.433	2.220	0.124	1.00		(CV: )	1.10		
9	5 A	199.89	199.85	0.04	4026	9.0	36394	0.971	0.836	0.060	1.59	68.7	RAD17-D02	1.16	2.3	-
11	5 B	215.94	216.13	-0.19	3049	8.5	25935	0.540	0.281	0.078	0.41	68.9	GTF2H2ex7"	1.92	3.3	-
<b>SMN2 cluster start</b>					Mean 3538	8.8	31165	0.756	0.558	0.069	1.00		(CV: 0.33)	1.32		
20	5 B	297.95	298.53	-0.58 *	2702	9.9	26646	0.555	0.340	0.035	0.93	69.4	SMN2 ex 8	1.63	6.2 *	-
17	5 B	273.87	273.94	-0.07	4367	9.9	43143	0.898	0.679	0.060	1.07	69.4	SMN2 ex 7	1.32	3.6	-
<b>SMN2</b>					Mean 3535	9.9	34895	0.726	0.509	0.047	1.00		(CV: 0.15)	1.47		P= 13.79%
23	5 C	328.69	329.64	-0.95 *	4230	10.1	42858	1.426	1.287	0.125	0.76	70.3	GTF2H2ex 4'	1.11	1.1	-
25	5 C	345.13	345.47	-0.34	6445	10.8	69499	2.313	2.002	0.120	1.24	70.3	BIRC1 ex 13	1.16	2.6	-
<b>SMN1/2 cluster start</b>					Mean 5338	10.5	56179	1.870	1.644	0.122	1.00		(CV: 0.03)	1.14		
27	5 C	361.11	360.69	0.42	4285	11.4	49060	1.633	1.188	0.143	0.75	69.4	SMN1/2 ex 8	1.37	3.1	-
33	5 D	417.29	417.00	0.29	3116	11.5	35854	1.292	0.905	0.109	0.75	69.4	SMN1/2 ex 6	1.43	3.5	-
31	5 D	397.71	397.19	0.52 *	3502	11.5	40271	1.451	1.068	0.065	1.50	69.4	SMN1/2 ex 4	1.36	5.9 *	-
29	5 C	379.78	379.26	0.52 *	2845	10.9	31000	1.032	0.690	0.063	0.99	69.4	SMN1/2 ex 1'	1.50	5.4 *	-
<b>SMN1/2</b>					Mean 3437	11.3	39046	1.352	0.963	0.095	1.00		(CV: 0.05)	1.41		P= 0.16%

Mean SMN1 to SMN2 copies suggest 2:3, if SMN1/2 is about 1.25

SMN1 to SMN2 copies look like: 2:3 for exon 7 and 2:3 for exon 8

# SMN1-SMN2 hybrid

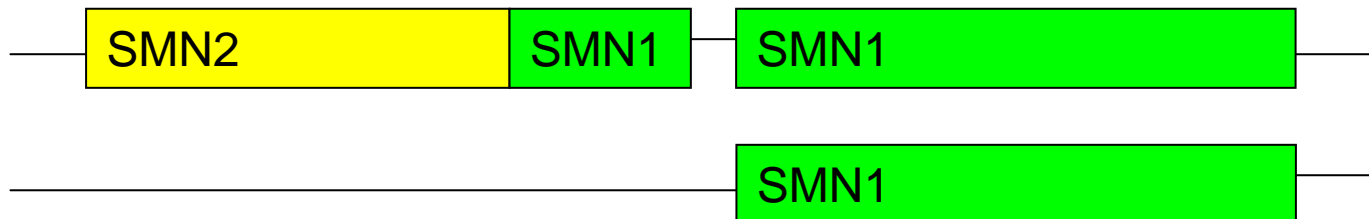
B.





# SMN1-SMN2 hybrid

9	5 A	184.53	184.48	0.05	4373	8.3	36221	0.697	0.778	0.180	0.79	69.8	GTF2H2ex10"	0.90	-0.5	I·
15	5 B	235.59	235.58	0.01	1789	10.0	17837	0.323	0.625	0.094	1.21	70.3	BIRC1 ex 5'	0.52	-3.2	IIII·
<b>SMN1 cluster start</b>					Mean 3081	9.1	27029	0.510	0.702	0.137	1.00	(CV: 0.39)	0.67	<b>P= 23.42%</b>		
21	5 B	292.34	292.18	0.16	2476	10.1	24912	0.451	0.304	0.028	1.08	69.4	SMN1 ex 8	1.48	5.2*	-IIII
18	5 B	267.60	267.58	0.02	2286	9.8	22457	0.407	0.416	0.045	0.92	69.4	SMN1 ex 7	0.98	-0.2	·
<b>SMN1</b>					Mean 2381	9.9	23685	0.429	0.360	0.036	1.00	(CV: 0.28)	1.25			
38	5 D	451.56	451.51	0.05	2849	14.7	41820	1.769	2.220	0.124	1.00	69.3	N-Cadherin-lik	0.80	-3.6	II·
<b>SMN1 cluster end</b>					Mean 2849	14.7	41820	1.769	2.220	0.124	1.00	(CV: )	0.80			
11	5 A	199.81	199.85	-0.04	4590	9.2	42325	0.814	0.836	0.060	1.59	68.7	RAD17-D02	0.97	-0.4	·
13	5 B	216.23	216.13	0.10	2300	9.1	20938	0.379	0.281	0.078	0.41	68.9	GTF2H2ex 7"	1.35	1.3	-IIII
<b>SMN2 cluster start</b>					Mean 3445	9.2	31632	0.597	0.558	0.069	1.00	(CV: 0.20)	1.05			
40	5 B	0.00	298.53	-----	0	0.0	0	0.000	0.340	0.035	0.93	69.4	SMN2 ex 8	0.00	-9.8*	IIIIIIII·
19	5 B	273.96	273.94	0.02	2276	10.7	24363	0.441	0.679	0.060	1.07	69.4	SMN2 ex 7	0.65	-4.0	IIII·
<b>SMN2</b>					Mean 1138	5.4	12182	0.221	0.509	0.047	1.00	(CV: 1.31)	0.35	<b>P= 17.69%</b>		
24	5 C	329.93	329.64	0.29	3282	11.1	36380	1.309	1.287	0.125	0.76	70.3	GTF2H2ex 4'	1.02	0.2	·
26	5 C	345.51	345.47	0.04	4075	11.8	48269	1.737	2.002	0.120	1.24	70.3	BIRC1 ex 13	0.87	-2.2	I·
<b>SMN1/2 cluster start</b>					Mean 3679	11.5	42325	1.523	1.644	0.122	1.00	(CV: 0.11)	0.92			
28	5 C	360.53	360.69	-0.16	2150	12.5	26954	0.970	1.188	0.143	0.75	69.4	SMN1/2 ex 8	0.82	-1.5	II·
34	5 D	416.96	417.00	-0.04	1263	13.5	17108	0.724	0.905	0.109	0.75	69.4	SMN1/2 ex 6	0.80	-1.7	II·
32	5 D	397.03	397.19	-0.16	1504	13.0	19562	0.828	1.068	0.065	1.50	69.4	SMN1/2 ex 4	0.77	-3.7	II·
30	5 C	379.18	379.26	-0.08	1139	11.5	13145	0.473	0.690	0.063	0.99	69.4	SMN1/2 ex 1'	0.69	-3.4	III·
<b>SMN1/2</b>					Mean 1514	12.7	19192	0.749	0.963	0.095	1.00	(CV: 0.07)	0.77	<b>P= 1.63%</b>		



# MLPA KIT P021 SMA評估結果

- SALSA MLPA KIT P021 SMA評估結果，其DNA量由100 ng~500ng皆可分析，precision結果一致(Within-technologist、Between-technologist、Within-run、Between-run)。
- 實驗室做交換檢體測試結果，與不同機器分析相同MLPA-PCR結果，皆有一致性，顯示此試劑分析結果不會因操作人員、儀器設備不同而有不同的結果。

# MLPA KIT P021 SMA評估結果

- MLPA同時可以偵測*SMN1* exon 7與exon 8，*SMN2* exon 7與exon 8與*SMN1+SMN2*的exon 1、exon 4、exon 6、exon 8，得到的結果與參考值比對後，可分別得到*SMN1*、*SMN2*與*SMN1+SMN2*的copy number，另外它提供21個位於不同chromosome上的copy number control probe，因此對於copy number的計算更準確且可靠。

# MLPA KIT P021 SMA評估結果

- DHPLC以competitive PCR分析SMN total copy的結果會受到primer的比例與DNA量的影響，因此不同次分析的結果並不完全一致，容易造成判讀上的困擾。尤其對於1:1的carrier檢體可能會誤判為正常的2:2，而正常的2:3的檢體可能會誤判成carrier (1:2)。而MLPA在分析1:1與2:3的檢體時，多次分析都出現一致明確的結果。

# 診斷之侷限性

- Cannot detect non-deletion or 2:0 carriers
  - 少數帶因者並不是缺失型*SMN1*基因突變，而是基因內的突變(intragenic mutation)，這些特殊的突變無法經由本方法檢驗。
  - 少數帶因者2套的*SMN1*基因皆位於同一條染色體上，另一條染色體上則沒有*SMN1*基因，此種特殊的基因型實際上是帶因者，但目前檢驗的方法無法檢出。

# Comparison of MLPA and DHPLC

	MLPA	DHPLC
主要設備	PCR + Sequencer	PCR+DHPLC
Turn around time	2~3 工作天	1~2 工作天
Reagent cost	Kit is expensive	PCR reagent
偵測基因位點	Exon 1,4,6,7,8....	Exon 7
DNA	100 ng~500 ng	須精確定量 100 ng
QC	內含 Internal control, 通常不需再加作其它 control	須多種不同比例 control 一起操作分析
Copy number 判讀	有明確數據提供判讀	1:1 與 2:2 以及 2:3 與 1:2 較難判讀
FDA prove	no	no