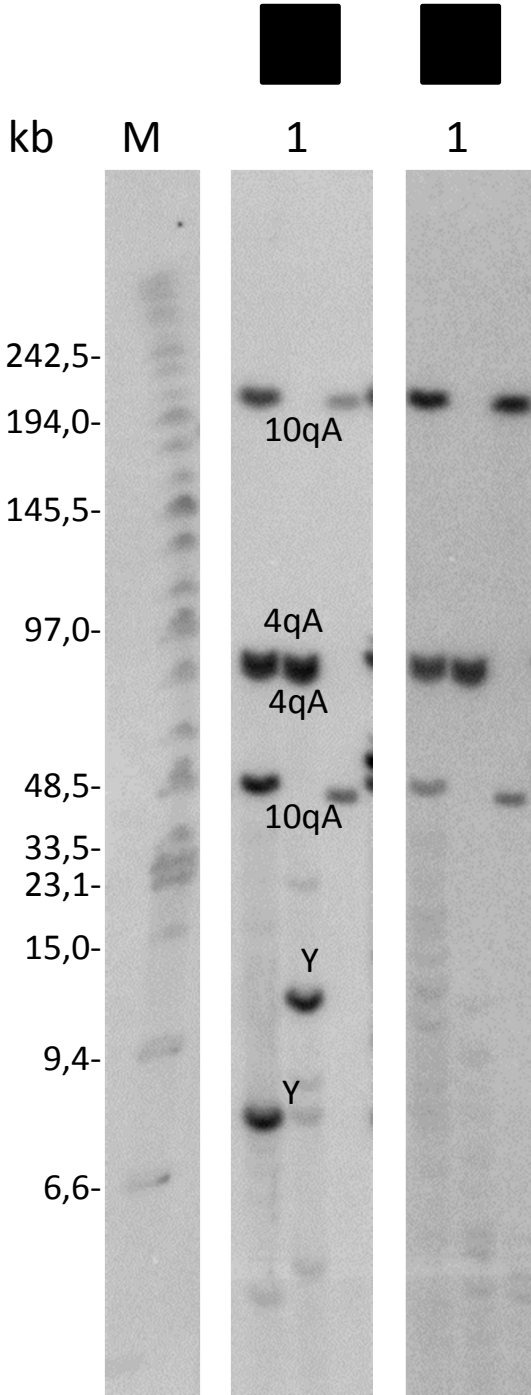


RF	Country	M/F	SMCHD1 variant				Methylation (%)				Southern blot			
			NM_015295.2	gene (hg19)	position	type	FseI	delta1	4q-1	4q-2	p13E-11 (+A/B)	10q-1	10q-2	D4Z4 (+A/B)
RI691.1	Canada	M	c.186+1G>A	g.2656261G>A	intron 1	splice	10	-35	21U (4A161S)	22U (4A161S)	11U (10A166)	55U (10A166)	no	
RI1637.2	France	M	c.2088_2138del	g.2707585_2707635del	exon 16	deletion	10	-34	28U (4A161S)	46U (4A161S)	10U (10A166)	12U (10A166)	no	
RI110.3	France	F	c.3048+2T>C	g.2729409T>C	intron 24	splice	10	-41	20U (4B163)	62U (4A161S)	20U (10A166)	28U (10A166H1)	no	
RI021.2	France	F	c.1580C>T	g.2700849C>T	exon 12	missense	24	-25	37U (4B163)	50U (4A161S)	11U (10A166)	19U (10A166)	75kb+8kb (10qA)	
RI1666.1	UK	F	c.2468delC	g.2722526delC	exon 20	deletion	12	-33	23U (4A161S)	100U (4A161S)	7U (10A166)	7U (10A166)	31kb (4q, no A or B)	
RI696.1	USA	M	c.1647+3A>G	g.2700919A>G	intron12	splice	12	-40	26U (4A161L)	38U (4A166H1)	32U (10A166)	52U (10A166)	35kb+25kb (4qA)	
RI392.2	Netherlands	M	c.4566G>A	g.2762234G>A	exon 36	splice	23	-27	29U (4A161L)	50U (4A161S)	18U (10A166)	24U (10A166)	80kb+19kb (4qA)	
RI1727.2	France	F	c.3631C>T	g.2740817C>T	exon 28	missense	10	-34	14U (4B163)	46U (4A161L)	6U (10A166)	38U (10A166)	25kb (4qA)	
RI975.3	France	F	c.24del	g.2656098del	exon 1	deletion	15	-38	40U (4A161S)	74U (4A161L)	14U (10A166)	20U (10A166)	25kb (4qA)	
RI878.2	USA	F	c.3276+4_3276+7del	g.2732494_2732497del	intron 25	splice	16	-35	47U (4A161S)	72U (4A161L)	3U (10A166)	33U (10A166)	24kb (4qA)	
RI874.3	USA	F	c.3274_3276+1del	g.2732488_2732491del	intron 25	splice	12	-35	14U (4B163)	69U (4A161L)	7U (10A166)	38U (10A166)	25kb (4qA)	
RI844.1	USA	M	c.3274_3276+1del	g.2732488_2732491del	intron 25	missense	7	-36	14U (4A161S)	68U (4A161L)	5U (10A166)	20U (10A166)	24kb (4qA)	

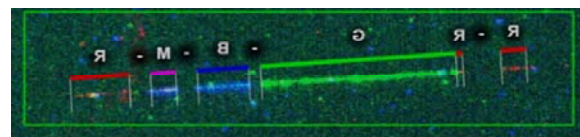
Supplemental Table 1

Overview of genetic and methylation data on the FSHD2 patients with SPA>20 units alleles in bold). The family number, country of origin and gender are indicated in columns 1-3. Information on the *SMCHD1* variant is indicated in columns 4 (cDNA position), 5 (genomic position), 6 (delta1 value), and 7 (predicted consequence). Columns 8 and 9 provide CpG methylation information: 8 (*FseI* methylation) and 9 (delta1 value). Columns 10-14 provide detailed information about D4Z4: chromosome 4 and 10 haplotypes and repeat sizes (p13E-11) and extra fragments observed upon hybridizations with D4Z4, 4qA and 4qB. The duplication alleles that have been identified after Southern blotting experiments are indicated in white (4A161L), dark grey (4A161S) and black (10A166).

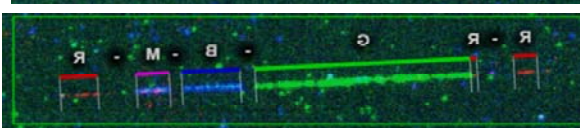


p13E-11 D4Z4

No duplication (4A161S+4A161L):

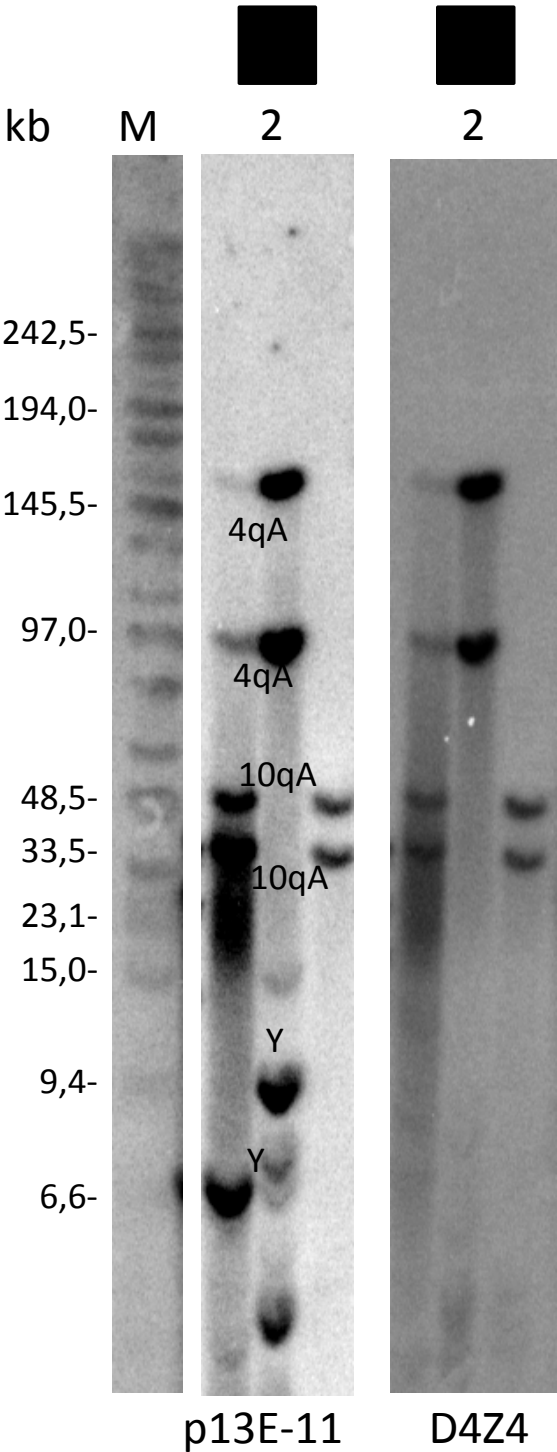


MC: 21U

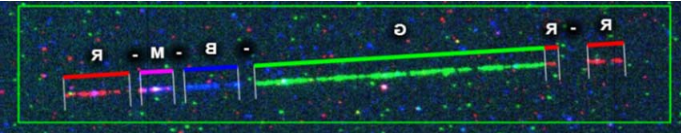


MC: 22U

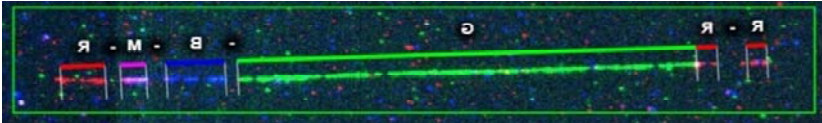
Rf1637



No duplication (2x 4A161S):

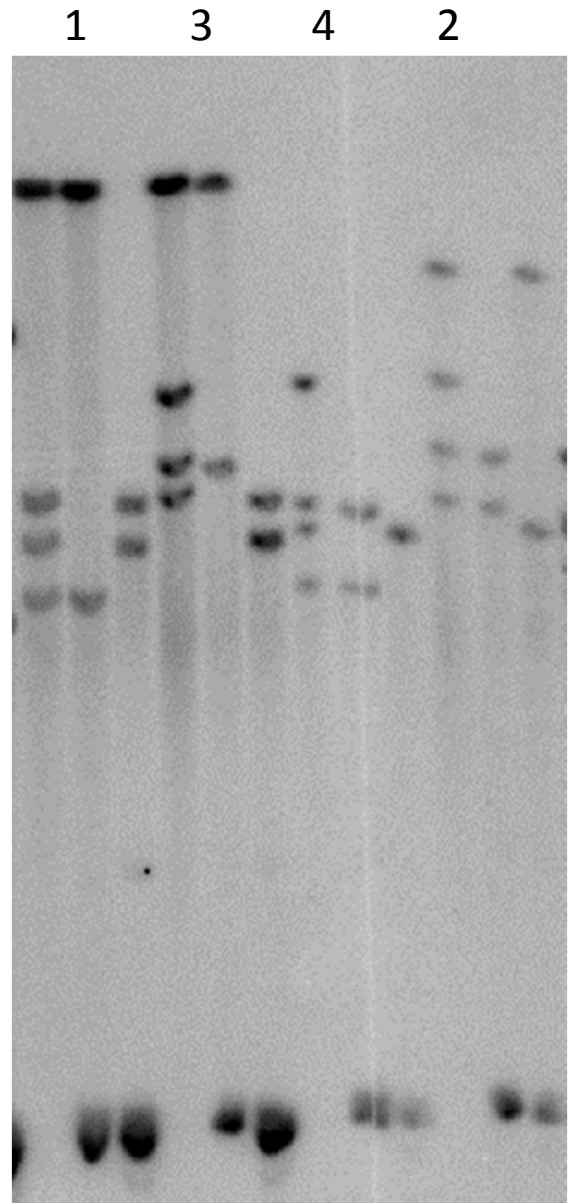
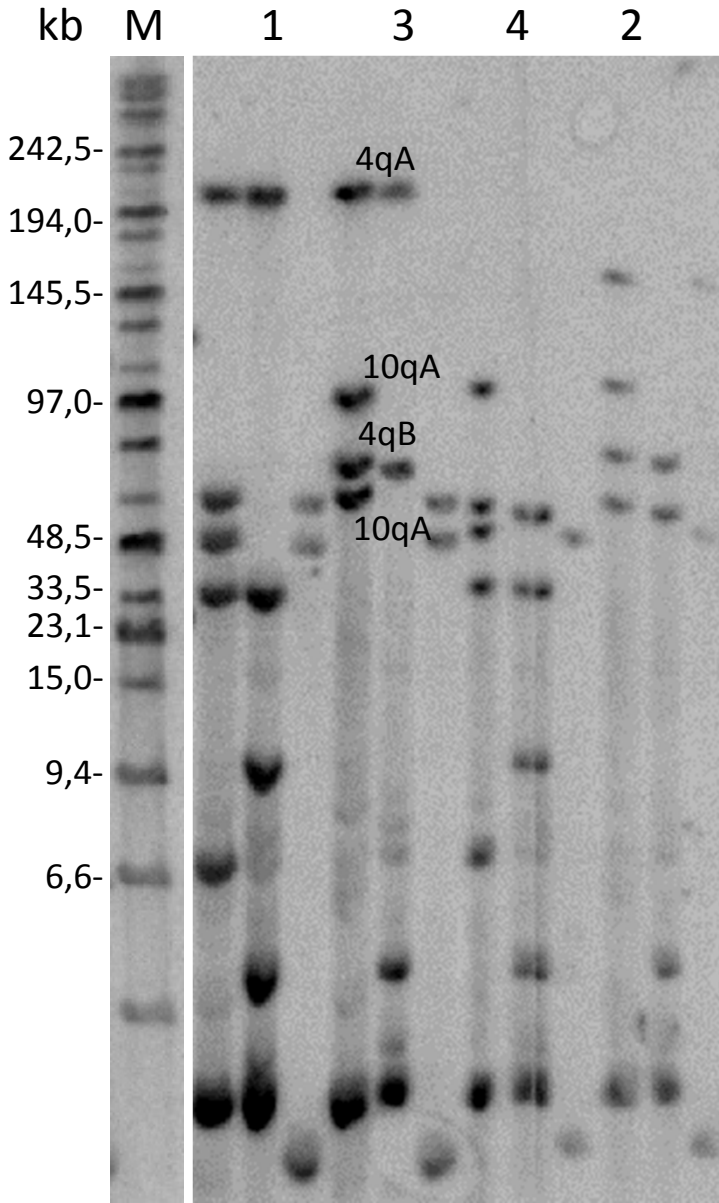
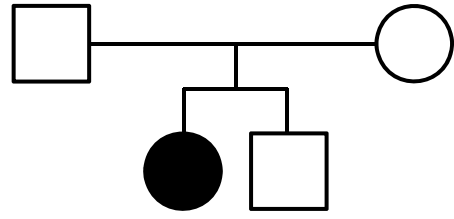
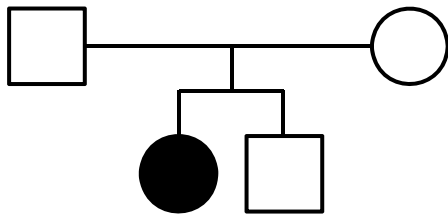


MC: 28U

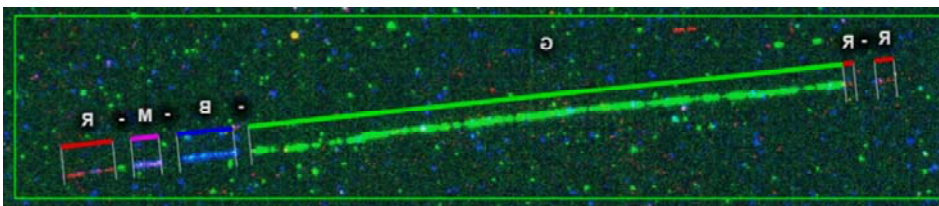


MC: 46U

Rf1110

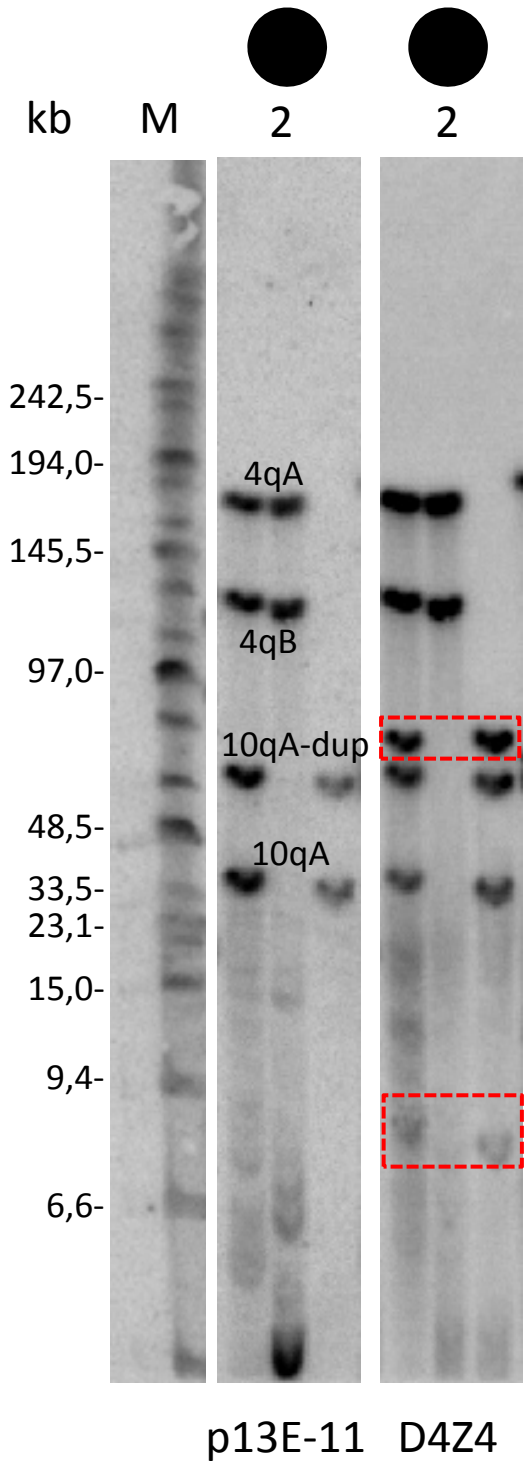


No duplication (4A161+ 4B163):



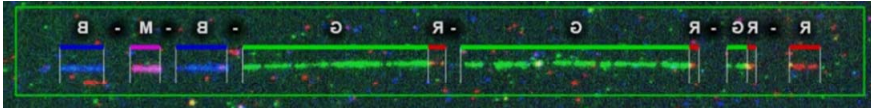
MC: 62U

Rf1021

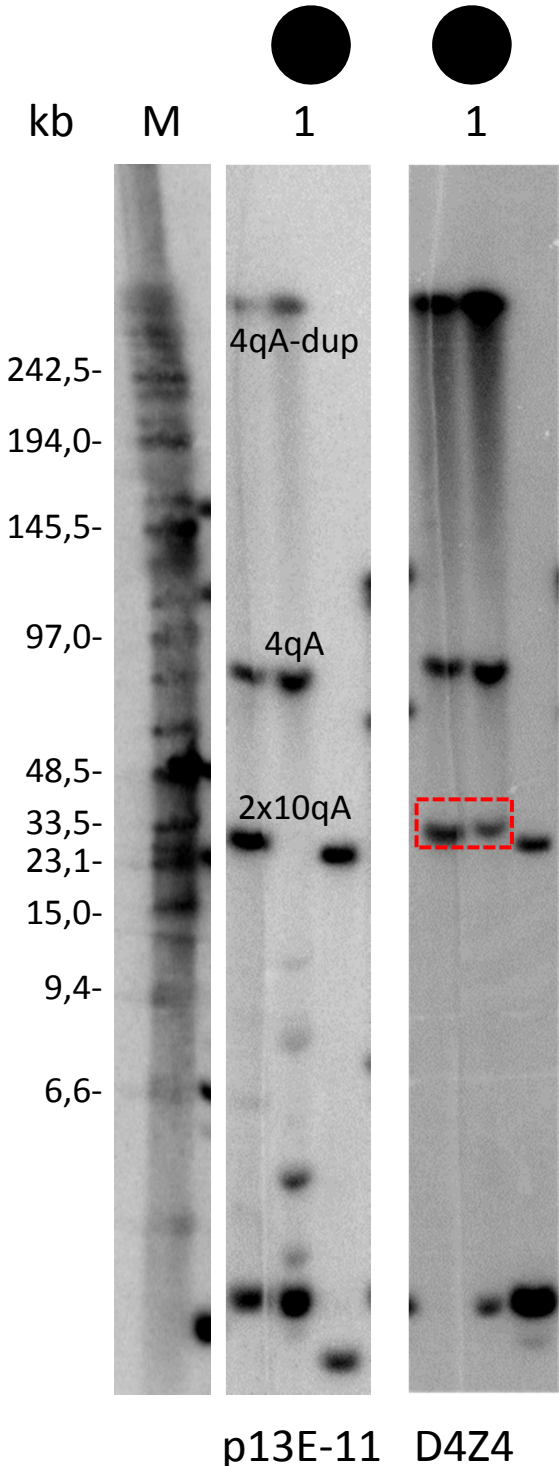


SB: extra 75kb+8kb D4Z4 (10A166)

MC: 19U+21U+2U



Rf1666

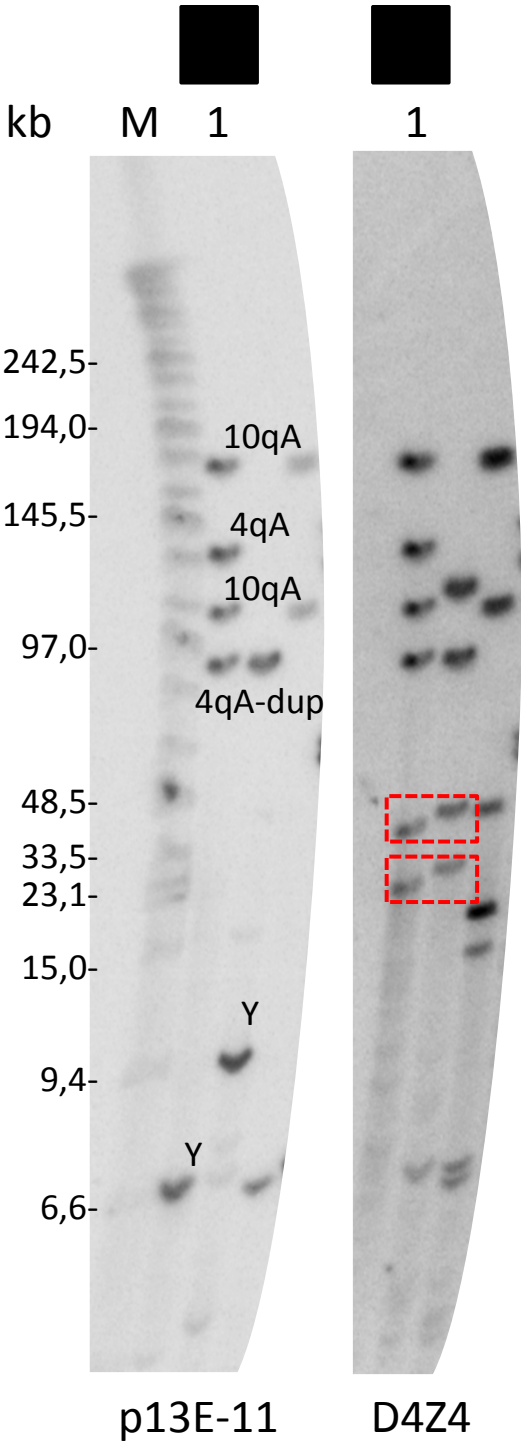


SB: extra 31kb D4Z4 (4A161S)

MC: 100U+ 5U

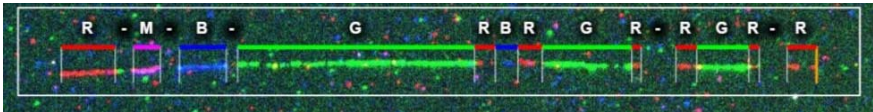


Rf696

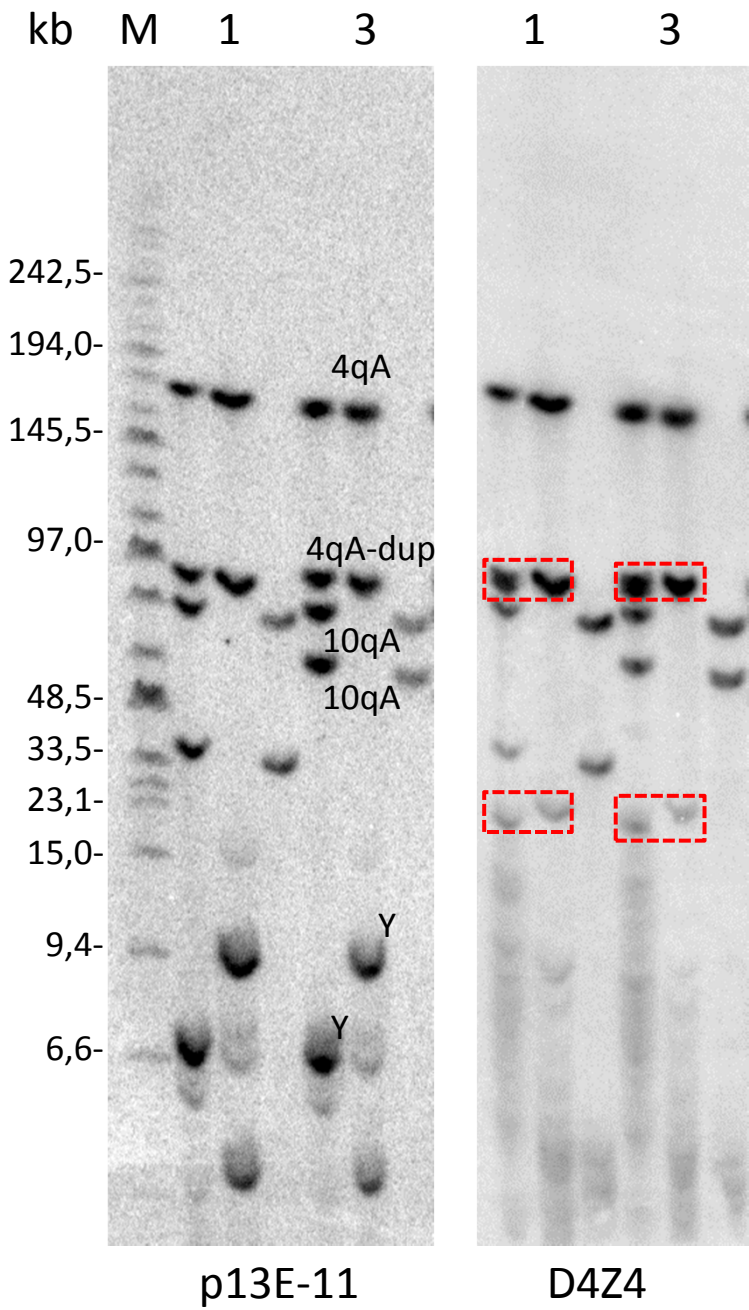
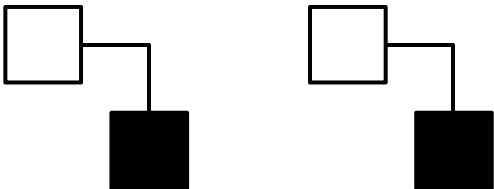


SB: extra 35kb + 25kb D4Z4 (4A161L)

MC: 26U+10U+5U

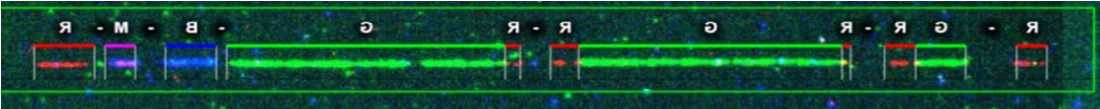


Rf392

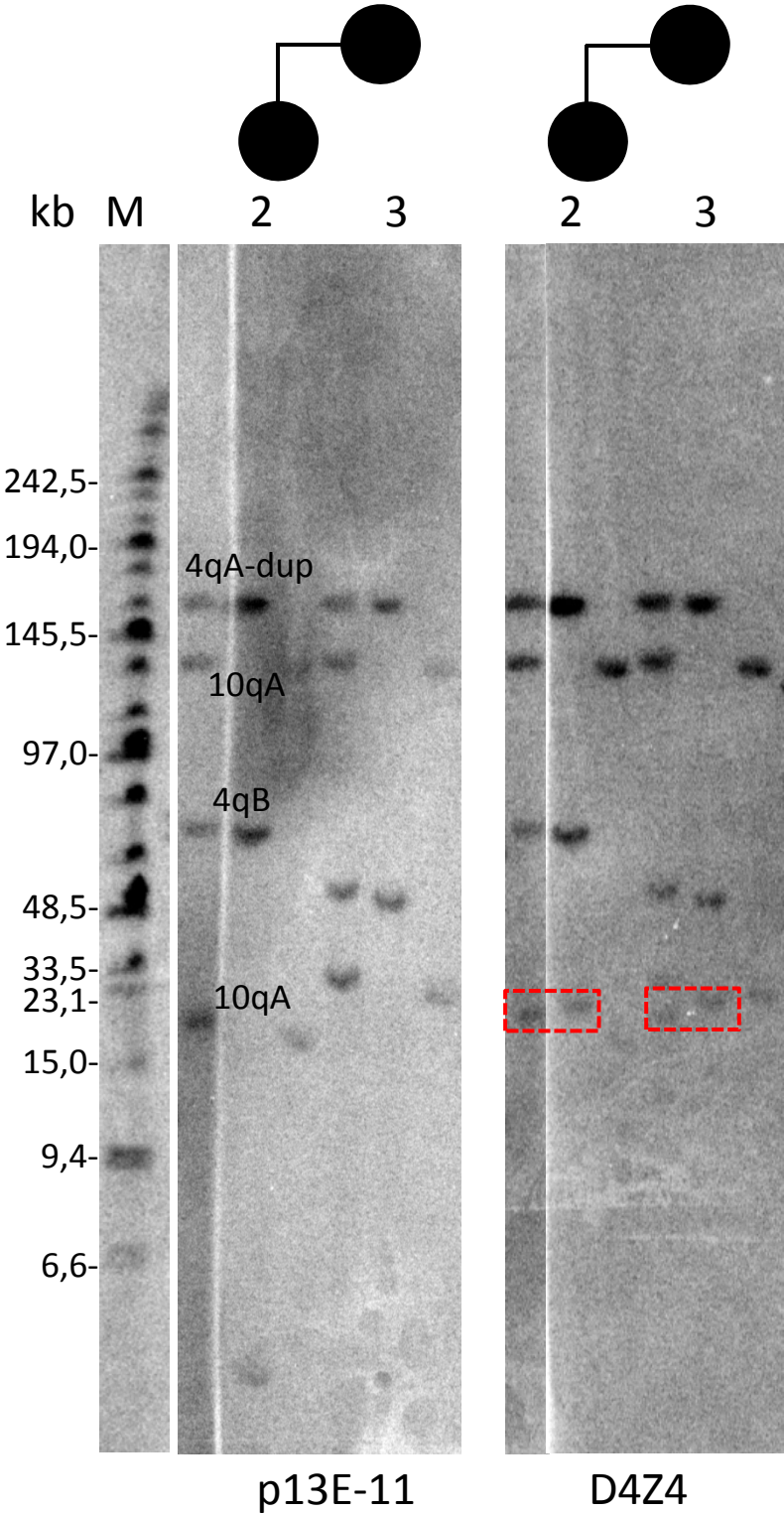


SB: extra 80kb + 19kb D4Z4 (4A161L)

MC: 29U+28U+5U

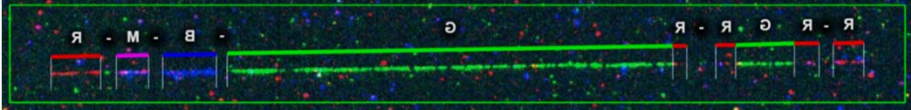


Rf1727

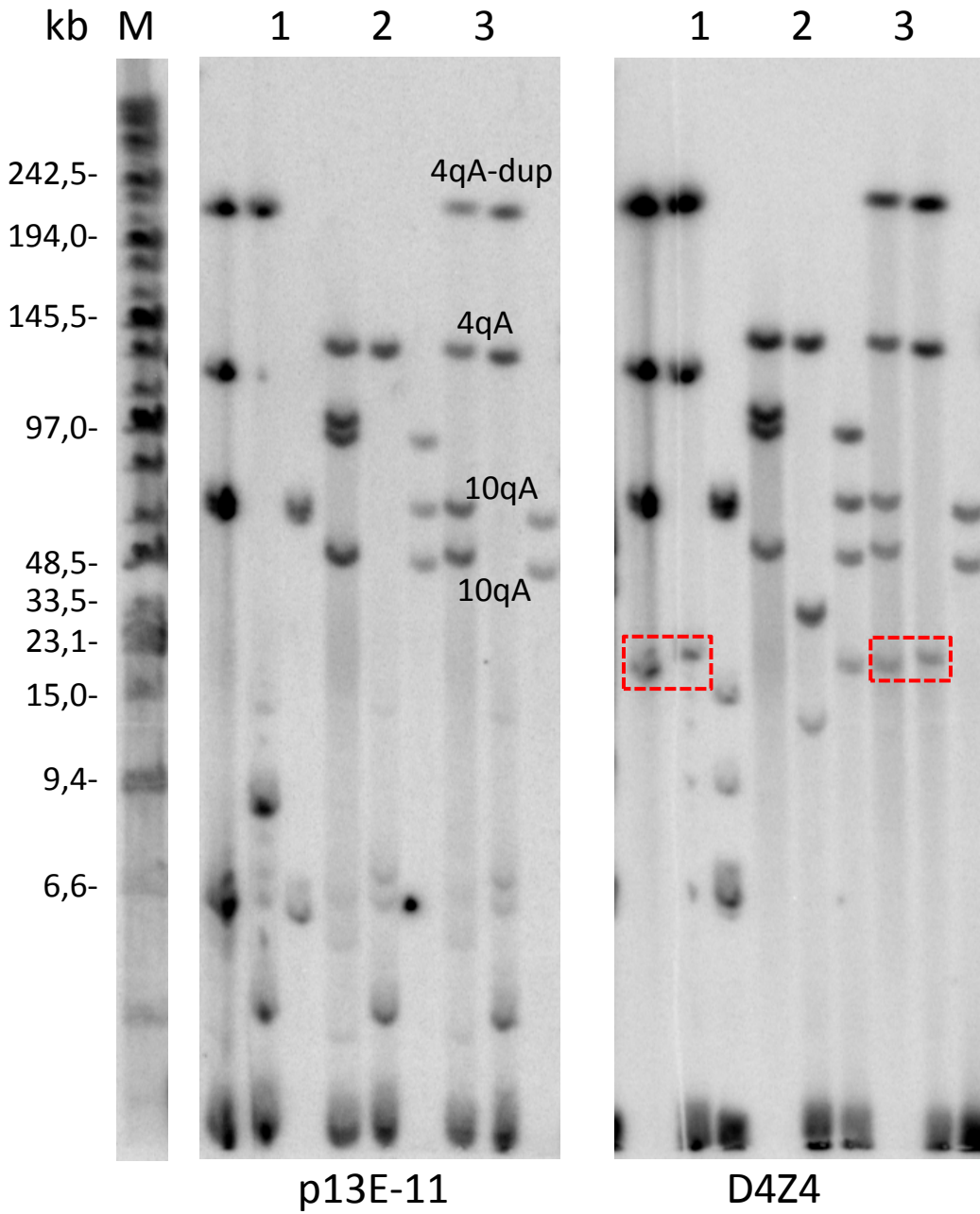
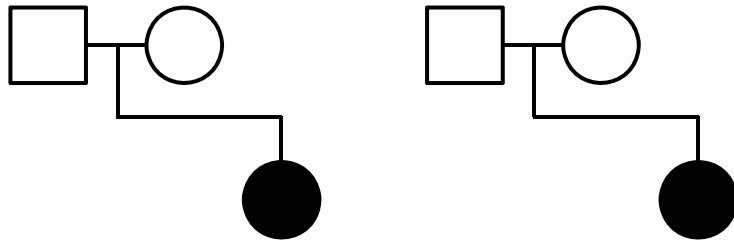


SB: extra 25kb D4Z4 (4A161L)

MC: 46U+6U

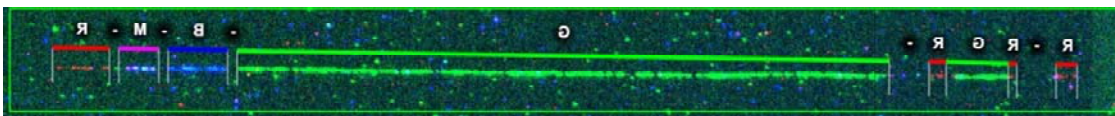


Rf975

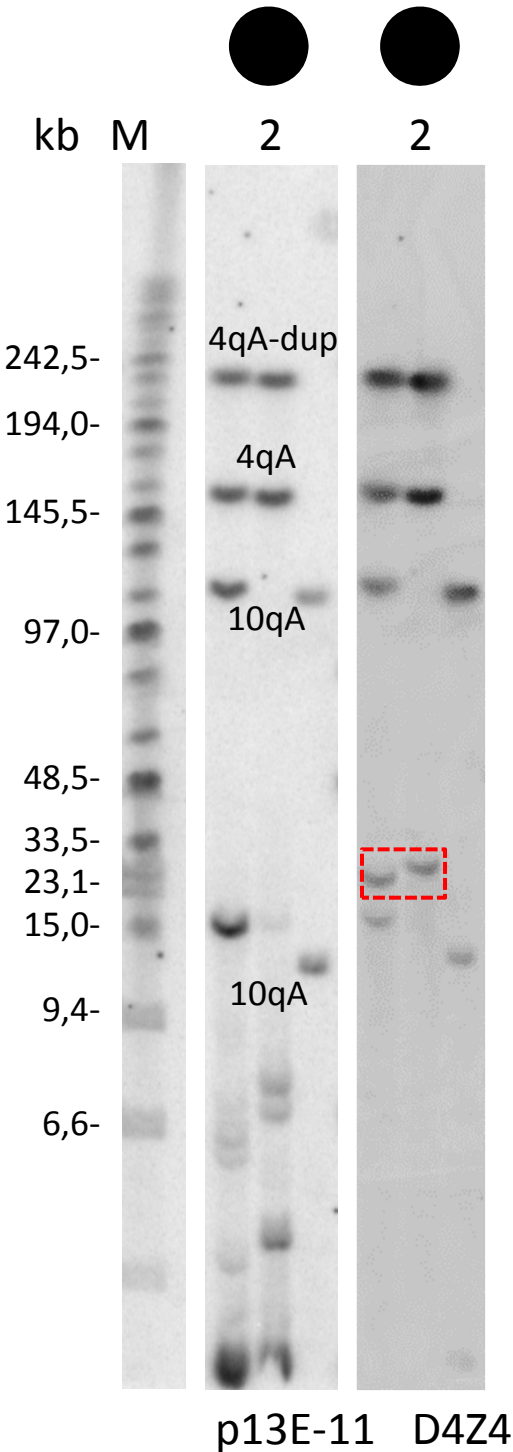


SB: extra 25kb D4Z4 (4A161L)

MC: 74U+6U

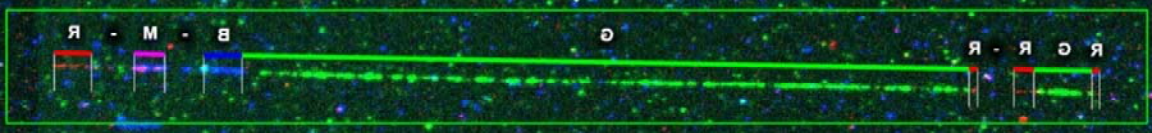


Rf878

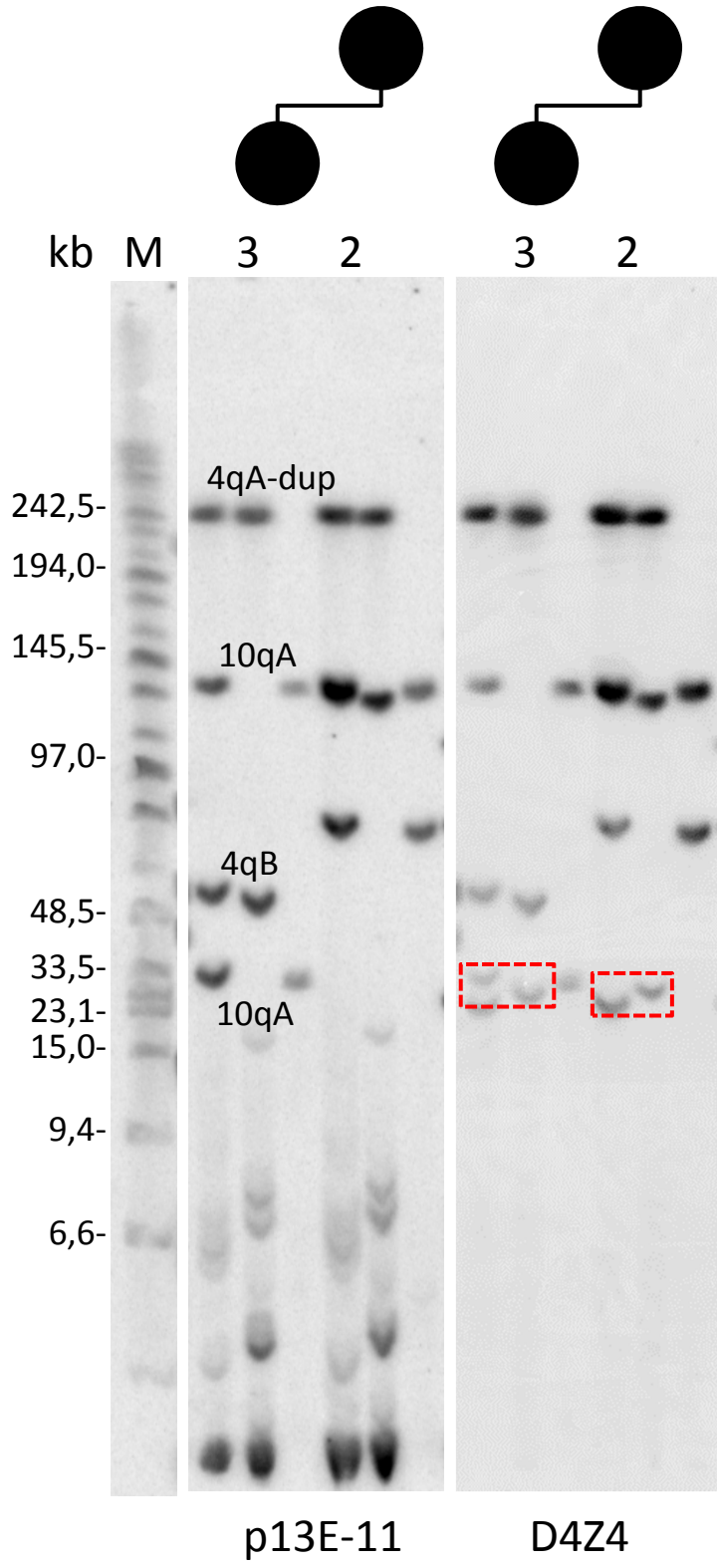


SB: extra 22kb D4Z4 (4A161L)

MC: 72U+6U

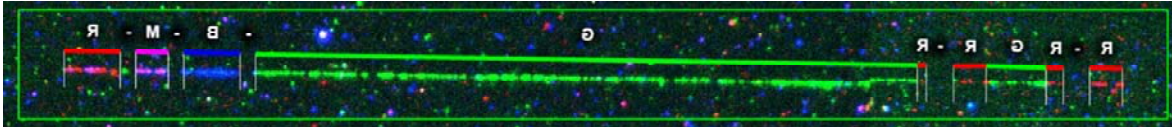


Rf874

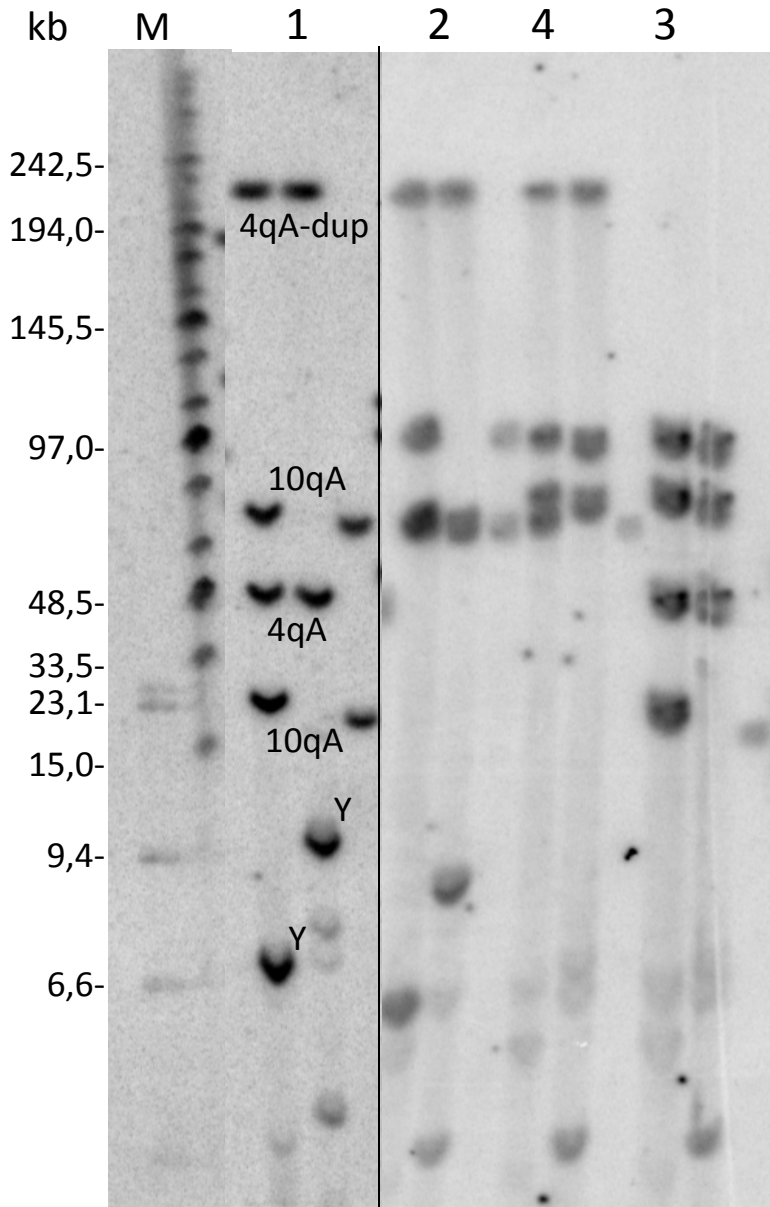
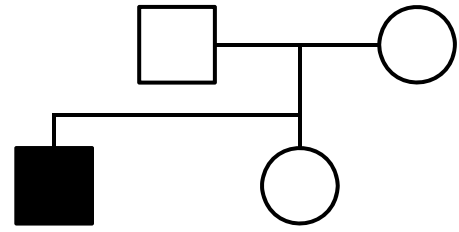
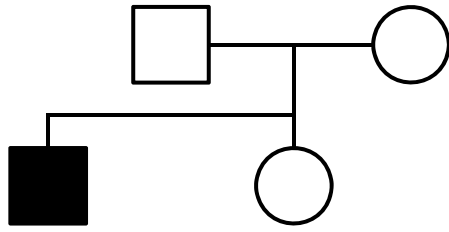


SB: extra 25kb D4Z4 (4A161L)

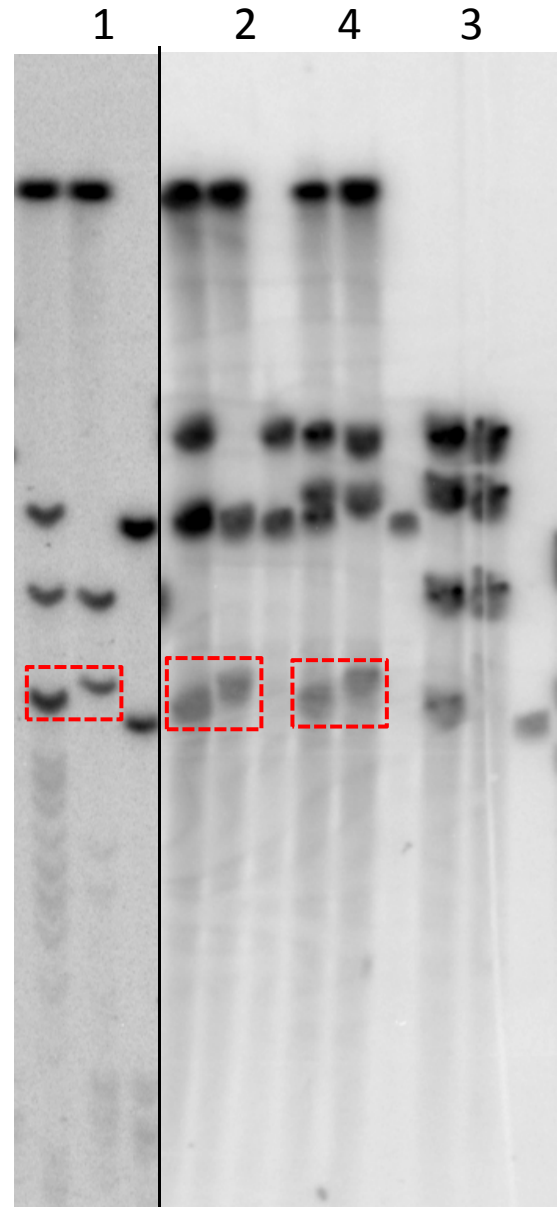
MC: 69U+6U



Rf844



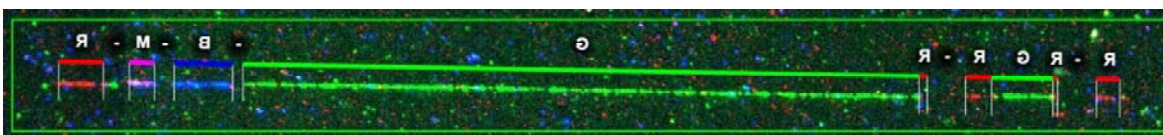
p13E-11



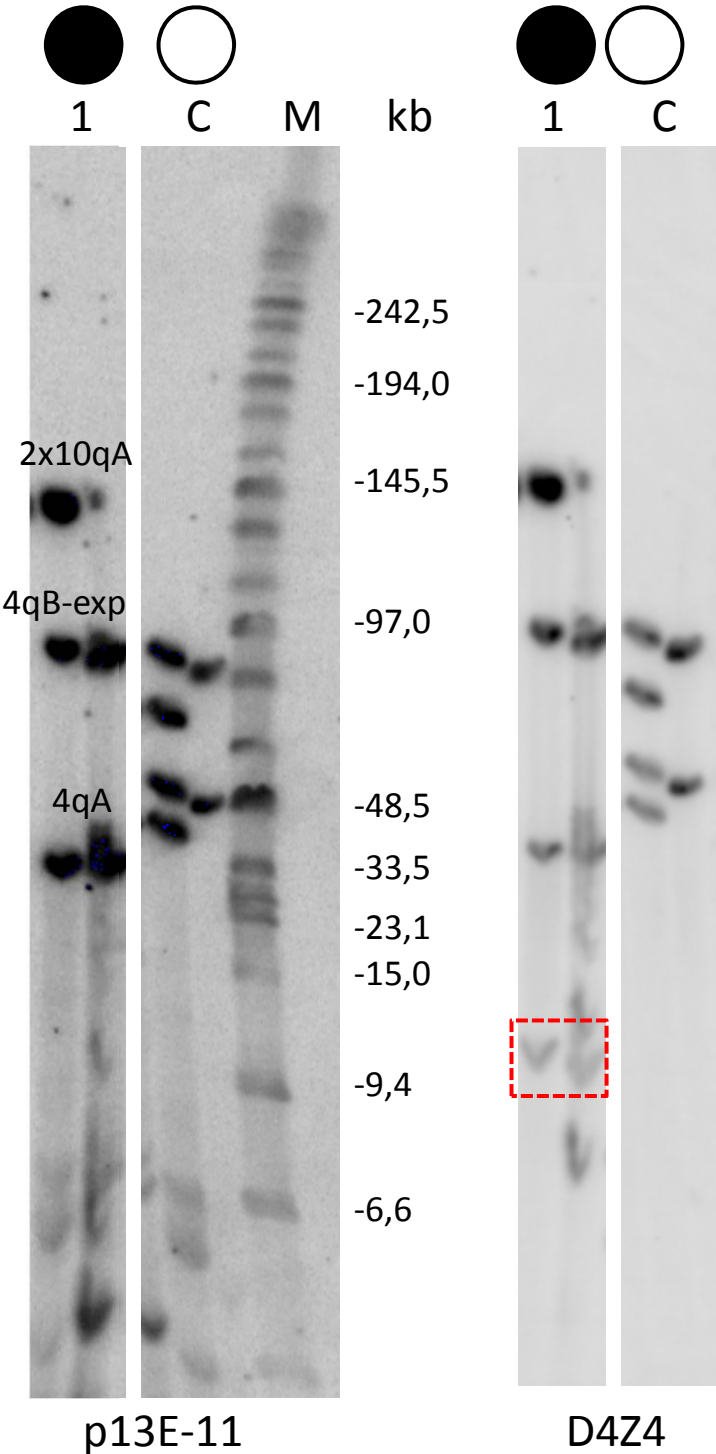
D4Z4

SB: extra 33kb D4Z4 (4A161L)

MC: 68U+6U

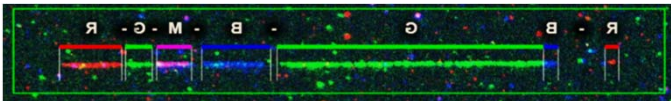


Rf887

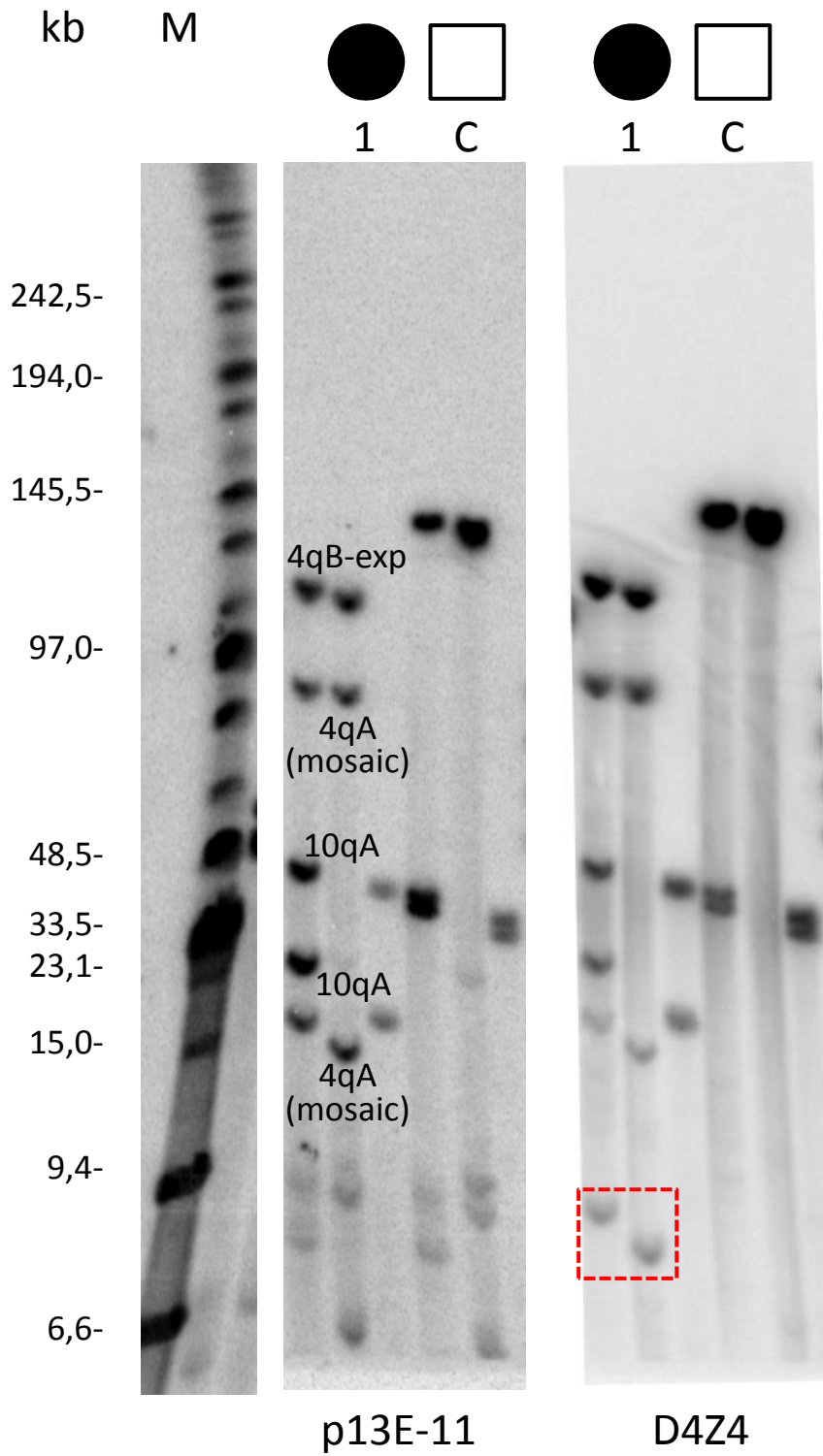


SB: extra 12kb D4Z4 (4B163)

MC: 3U+23U (expansion inverted D4Z4)

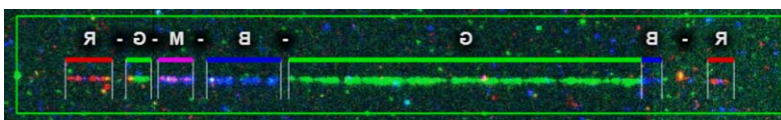


Rf903

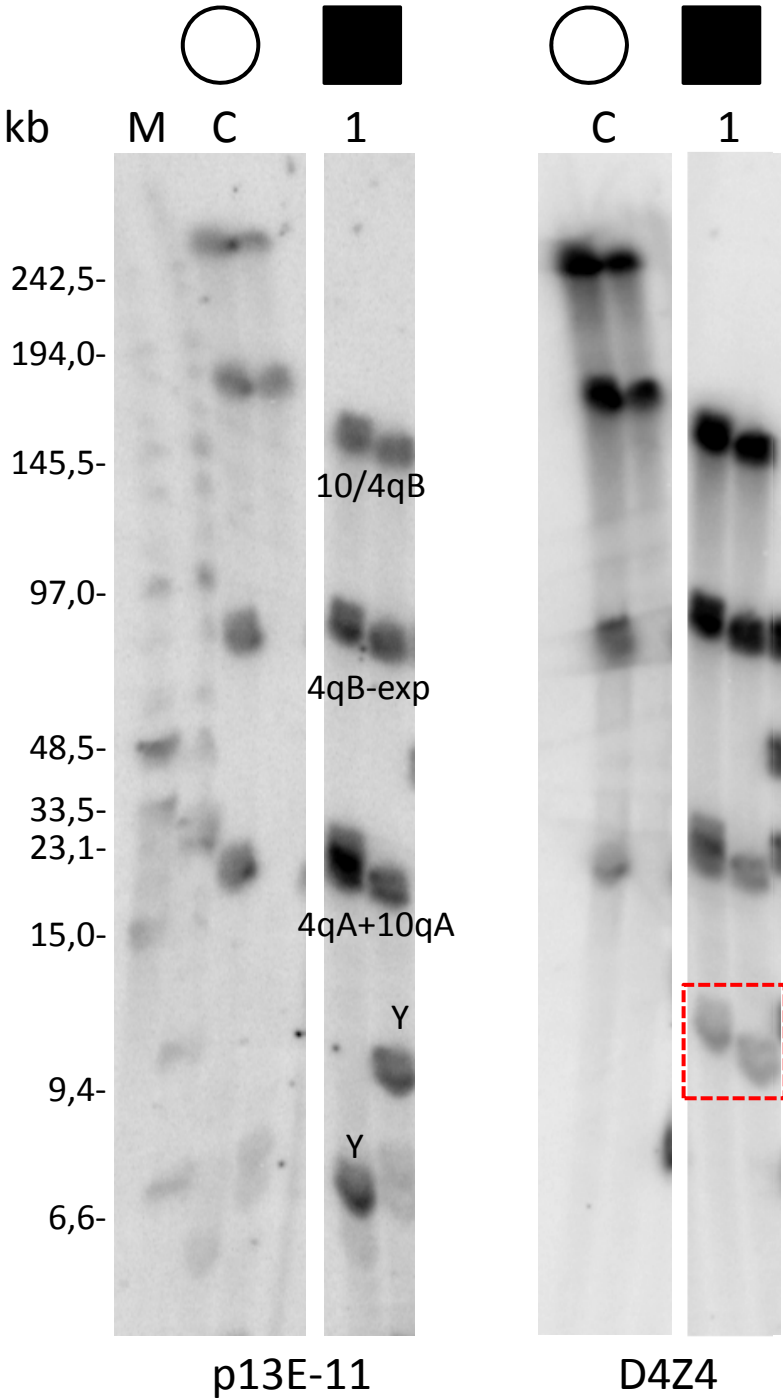


SB: extra 12kb D4Z4 (4B163)

MC: 3U+32U (expansion inverted D4Z4)

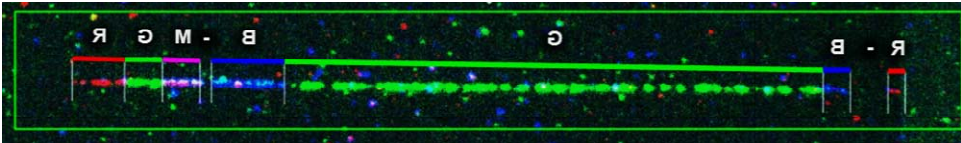


Rf897



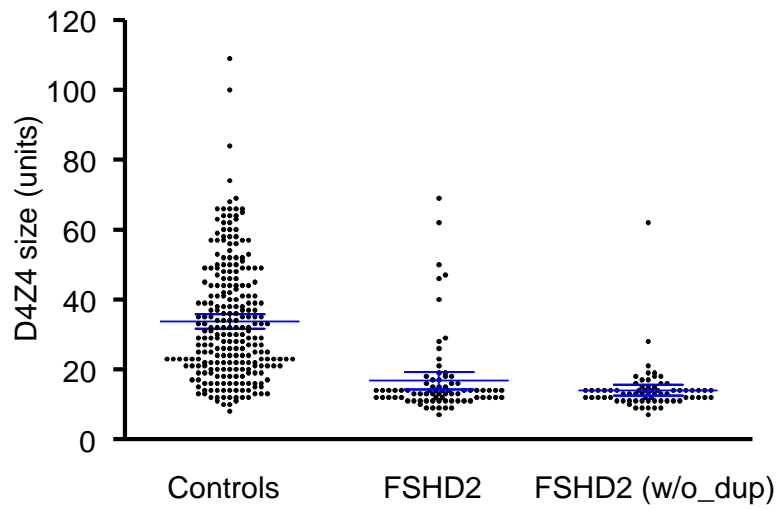
SB: extra 12kb D4Z4 (4B163)

MC: 3U+46U (expansion inverted D4Z4)



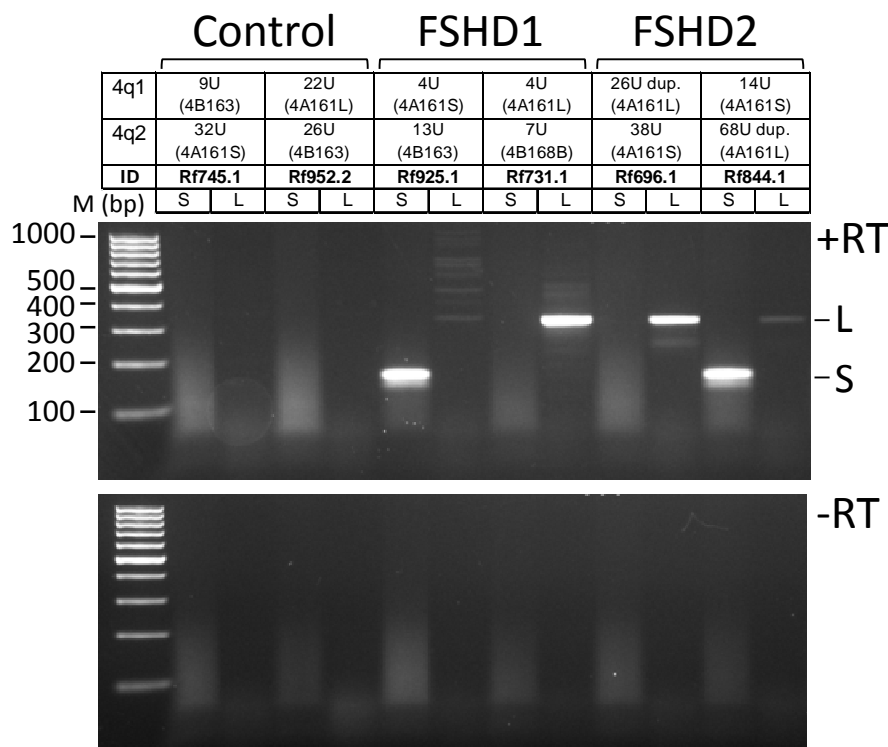
Supplemental Figure 1

Pedigrees and representative images of the Southern blot analysis and MC images of all FSHD2 patients (and family members) shown in supplementary figure 1 and FSHD1 patients (Rf887.1; Rf897.1 and Rf903.1) carrying an 4qB allele with an expansion of the inverted D4Z4 repeat unit. In each case the extra D4Z4 fragment, not visible by probe p13E-11, and hybridizing to the D4Z4 probe is boxed. For the Southern blots, DNA was digested by EcoRI and HindIII (E); EcoRI and BlnI (B) and XapI (X) (samples Rf887.1 and Rf897.1 are digested by EcoRI and HindIII (E); EcoRI and BlnI (B)) and hybridized with probes p13E-11 and D4Z4, respectively. Clinically affected individuals are indicated in black. For the cases discussed in the paper, the standard chromosome 4 and 10 alleles (4qA, 4qB or 10qA) are indicated, as well as the unusual duplication or expansion alleles (10qA-dup, 4qA-dup and 4qB-exp). The marker lane (M) in kb is indicated. Representative MC images of the unusual alleles or the relevant standard alleles (Rf691.1; Rf1637.1 and Rf1110.3) are shown below.



Supplemental Figure 2

D4Z4 array size of the SPA in controls and 79 FSHD2 patients from figure 1A and in the FSHD2 patients after elimination of the duplication alleles. We observe a decline in the standard deviation and mean D4Z4 size in FSHD2 patients after the elimination (from 16,8 to 14.1 units).



Supplemental Figure 3

DUX4 transcription analysis from 4A161S (S) and 4A161L (L) alleles in differentiated myoblasts. No expression was detected in individuals carrying a normal-sized 4A161S (Rf745.1) or 4A161L (Rf952.2) allele. FSHD1 samples with short 4A161S (Rf925.1) or short 4A161L (Rf731.1) show the expected DUX4 expression. FSHD2 patients (Rf696.1 and Rf844.1) display expression from their 4A161L-type duplication allele. (figure adapted from Lemmers *et al.* Eur J Hum Genet; 2018).