SUPPLEMENTARY MATERIAL

A blinded international study on the reliability of genetic testing for GGGGCC-repeat expansions in *C9orf72* reveals marked differences in results among 14 laboratories

Chizuru Akimoto, Alexander E Volk *et al.*

Supplementary table 1. The results of 78 samples.

RP-PCR results		
All laboratories same results	50 samples (64.1%)	
1 laboratory different result	20 samples (25.6%)	13 samples (16.7%): 1 laboratory Q result 5 samples (6.41%):1 laboratory FP result 2 samples (2.56%): 1 laboratory FN result
2 laboratories different results	5 samples (6.41%)	4 samples (5.12%): 2 laboratories Q results 1 sample (1.28%): 1 laboratory FN and 1 laboratory Q results
6 laboratories different results	2 samples (2.56%)	1 sample (1.28%): 1 laboratory FN and 5 laboratories Q results 1 sample (1.28%): 6 laboratories Q results
7 laboratories different results	1 sample (1.28%)	1 sample (1.28%): 1 laboratory FN and 6 laboratories Q results
Amplicon-length analysis results		
All laboratories same results	72 samples (92.3%)	
1 laboratory different result	2 samples (2.56%)	1 sample (1.28%): 1 laboratory different peak number 1 sample (1.28%): 1 laboratory Q result
2 laboratories different results	1 sample (1.28%)	1 sample (1.28%): 1 laboratory different peak number and 1 laboratory Q result
3 laboratories different results	1 sample (1.28%)	1 sample (1.28%): 1 laboratory different peak number and 2 laboratories Q results
5 laboratories different results	2 samples (2.56%)	2 samples (2.56%): 4 laboratories different peak number and 1 laboratory Q result
RP-PCR + amplicon-length analysis results		
All laboratories same results	53 samples (67.9%)	
1 laboratory different result	19 samples (24.4%)	16 samples (20.5%): 1 laboratory Q result 2 samples (2.56%): 1 laboratory FP result 1 sample (1.28%): 1 laboratory FN result
2 laboratories different results	3 samples (3.85%)	2 samples (2.56%): 2 laboratories Q results 1 sample (1.28%): 1 laboratory FP and 1 laboratory Q results
5 laboratories Different results	2 samples (2.56%)	1 sample (1.28%): 1 laboratory FN and 4 laboratories Q results 1 sample (1.28%): 5 laboratories Q results
6 laboratories Different results	1 sample (1.28%)	1 sample (1.28%): 1 laboratory FN and 5 laboratories Q results

FP: False positive, FN: False negative, Q: Undecided or unclassified sample.

Supplementary table 2. The GGGGCC-repeat numbers on 78 samples from 32 families.

Sample	Allele 1	Allele 2
1.1	7	Expanded
1.2	6	Expanded
2.1	2	Expanded
2.2	2	Expanded
3.1	5	12
3.2	2	Expanded
4.1	4	Expanded
4.2	4	Expanded
4.3	8	Expanded
4.4	4	Expanded
5.1	2	2
5.2	2	2
6.1	5	5
6.2	2	Expanded
6.3	2	Expanded
6.4	2	Expanded
6.5	23-24	Expanded
6.6	8	Expanded
6.7	8	Expanded
6.8	2	2
7.1	2	6
7.2	2	5
8.1	2	6*
8.2	2	6*
9.1	2	10
9.2	2	8
10.1	5	Expanded
10.2	2	Expanded
11.1	2	Expanded
11.2	2	Expanded
12.1	2	5
12.2	2	8
12.3	2	5
13.1	2	Expanded
13.2	2	Expanded
14.1	2	2
14.2	2	8
15.1	2	6
15.2	6	10
16.1	2	Expanded
16.3	2	Expanded
17.1	2	6
17.2	2	5
18.1	5	Expanded
18.2	2	Expanded
19.1	2	2
19.2	2	5
20.1	5	13
20.2	5	6
21.1	5	Expanded
21.2	5	Expanded
22.1	11	Expanded
22.2	2	Expanded
23.1	8	Expanded
23.2	8	Expanded
24.1	2	Expanded
24.2	2	Expanded
25.1	2	2
25.2	2	8
26.1	2	Expanded
26.2	2	Expanded
26.3	23-25	Expanded
27.1	2	Expanded
27.2	8	Expanded
27.3	5	Expanded
27.4	5	Expanded
28.1	2	7
28.2	5	Expanded
29.1	2	5
29.2	2	6
30.1	2	6
30.2	2	5
31.1	7	Expanded
31.2	2	2
31.3	2	Expanded
32.1	6*	Expanded
32.2	2	Expanded
32.3	28-32	Expanded

*: mutant allele.

Supplementary table 3A. Total methods of 14 laboratories.

Laboratory	A	B	C	D	E	F	G	H	I	J	K	L	M	N
RP-PCR method														
Primers*	P1, 2, 3	P7, 8, 9	P1, 2, 3	P4, 13	P4, 5, 6	P10, 11, 12	P4, 5, 6	P1, 2, 3	P4, 5, 6	P4, 5, 6	No	P1, 2, 3	P4, 5, 6	P1, 2, 3
PCR reaction*	PR1	PR2	PR1	PR3	PR4	PR5	PR6	PR1	PR7	PR8	No	PR1	PR6	PR9
Primer 1 dose (μM)	1.4	0.2	1.4	0.2	0.4	0.33	1.0	1.4	1.4	2.5	No	1.4	1.0	1.4
Primer 2 dose (μM)	0.7	0.2	0.7	0.2	0.2	0.33	1.0	0.7	0.7	2.5	No	0.7	1.0	0.7
Primer 3 dose (μM)	1.4	0.2	1.4	-	0.4	0.33	1.0	1.4	1.4	0.25	No	1.4	1.0	1.4
Deaza-GTP (mM)	0.18	0.2	0.18	0.2	0.0375	0	0.25	0.18	0.18	0	No	0.18	0.25	0.16
DNA dose (ng/μl)	7.1	5.0	2.14	2.0	20	10.0	12.5	7.1	12.0	3.3	No	7.1	5.0	3.3
PCR protocol*	PP1	PP2	PP3	PP4	PP5	PP6	PP7	PP8	PP7	PP9	No	PP1	PP7	PP1
Extension time (min)	3	8-18	3	3	40 sec	2-10	8-16	3	8-16	8-16	No	3	8-16	3
Total cycle	40	45	40	32	65	35	35	43	35	34	No	40	35	40
Analysis machine*	M1	M2	M2	M1	M1	M1	M1	M1	M3	M1	No	M1	M1	M2
PCR product conc.	10%	10%	25%	20%	0.47%	16.2%	3.3%	0.94%	8.8%	10%	No	21%	4.8%	13.3%
Analysis software*	A1	A2	A1	A1	A1	A5	A2	A2	A2	A2	No	A1	A1	A3
Sensitivity	100%	100%	100%	100%	91.3%	100%	97.8%	93.5%	93.5%	93.5%	71.7%	95.7%	89.1%	93.5%
Specificity	100%	100%	100%	100%	96.8%	87.5%	100%	100%	100%	93.8%	96.9%	100%	100%	87.5%
Unclassified	0%	0%	0%	0%	5.1%	2.6%	0%	3.8%	3.8%	3.8%	14.1%	2.6%	5.1%	9.0%
Amplicon length analysis method														
Primers*	P14, 16	P14, 16	P14, 16	P15, 16	P14, 16	P17, 18	P14, 16	P14, 16	P15, 16	P14, 16	No	N.D.	N.D.	N.D.
PCR reaction*	PR10	PR11	PR12	PR13	PR10	PR14	PR15	PR16	PR13	PR17	No	N.D.	N.D.	N.D.
DNA dose (ng/μl)	2.2	5.0	2.4	4.2	2.0	3.3	3.35	7.1	2.0	3.3	No			
PCR protocol*	PP10	PP11	PP12	PP13	PP14	PP15	PP16	PP17	PP18	PP19	No	N.D.	N.D.	N.D.
Extension time (min)	1.5	1	2	2	1.5	1	1	3	2	8-16	No			
Total cycle	34	38	30	35	35	33	36	40	35	34	No			
Analysis machine*	M1	M2	M2	4% gel	M1	M1	M1	M1	3% gel	M1	No	N.D.	N.D.	N.D.
PCR product conc.	10%	0.5%	25%		0.47%	16.2%	0.17%	0.47%		10%	No			
Analysis software*	A1	A2	A1		A1	A5	A2	A2		A2	No			

*: Listed in supplementary table 3B and 3C, N.D.: not done. No: no method disclosed.

Supplementary table 3B. List of products and equipment.

Primers for RP-PCR	
P1	FAM-AGTCGCTAGAGGCGAAAGC
P2	tacgcatcccagtttgagacgGGGGCCGGGGCCGGGGCCGGGG
P3	tacgcatcccagtttgagacg
P4	FAM-tgtaaaacgacggccagtCAAGGAGGGAAACAACCGCAGCC
P5	caggaaacagctatgaccGGGCCCGCCCCGACCACGCCCCGGCCCCGGCCCCGG
P6	caggaaacagctatgacc
P7	FAM-CAAGGAGGGAAACAACCGCAGCC
P8	ggataacaatttcacacaggGGGCCCGCCCCGACCACGCCCCGGCCCCGGCCCCGG
P9	ggataacaatttcacacagg
P10	FAM-AGTACTCGCTGAGGGTGAAC
P11	cgtacgcatcccagtttgagaGCCCCGGCCCCGGCCCCGG
P12	cgtacgcatcccagtttgaga
P13	cacgacgttgtaaaacgaCCCCGGCCCCGGCCCCGG

Primers for amplicon-length analysis	
P14	FAM-CAAGGAGGGAAACAACCGCAGCC
P15	CAAGGAGGGAAACAACCGCAGCC
P16	GCAGGCACCGCAACCGCAG
P17	FAM-CAGGTGTGGGTTTAGGAGGT
P18	CCAGCTTCGGTCAGAGAAAT
Size standard	
S1	GeneScan 500XL ROX and 500ROX Size Standard (Applied Biosystems, USA)
S2	GeneScan 500LIZ Size Standard (Applied Biosystems, USA)
S3	GeneScan 400HD Rox Size Standard (Applied Biosystems, USA)
S4	GeneScan 600LIZ Size Standard (Applied Biosystems, USA)
Analysis machine	
M1	3730 and 3730*xl* DNA Analyzer (Applied Biosystems, USA)
M2	3130 and 3130*xl* Genetic Analyzer (Applied Biosystems, USA)
M3	3500 Genetic Analyzer (Applied Biosystems, USA)
Analysis software	

A1	Peak Scanner Software v1.0 or v2.0 (Applied Biosystems, USA)
A2	GeneMapper Software v4.0 or v4.1 (Applied Biosystems, USA)
A3	Geotyper v4 (Applied Biosystems, USA)
A4	Agarose gel
A5	In-house developed Tracl genotyping software

Supplementary table 3C-1. Concentration of ingredients in RP-PCR.

PCR reaction number	PR1	PR2	PR3	PR4	PR5	PR6	PR7	PR8	PR9
Laboratory	ACHL	B	D	E	F	GM	I	J	N
Qiagen buffer *1	-	-	-	1x	-	1x	-	-	-
FastStart PCR Master *2	1x	-	-	-	-	-	-	-	-
Taq DNA polymerase (units)	-	-	1.8	0.75	-	2.5	1.0	-	-
Other buffer and polymerase	-	*4	*5	-	*6	-	*7	*8	*9
Primer 1 (μM)	1.4	0.2	0.2	0.4	0.33	1.0	1.4	2.5	1.4
Primer 2 (GGGGCC repeat) (μM)	0.7	0.2	0.2	0.2	0.33	1.0	0.7	2.5	0.7
Primer 3 (μM)	1.4	0.2	-	0.4	0.03	1.0	1.4	0.25	1.4
dCTP, dATP, dTTP (each) (mM)	-	0.2	0.2	0.05	-	0.25	0.18	-	-
7-deaza-2-deoxy GTP (mM)	0.18	0.2	0.2	0.0375	-	0.25	0.18	-	0.16
dGTP (mM)	-	-	-	0.0125	-	-	-	-	-
dNTPs (each) (mM)	-	-	-	-	0.5	-	-	-	-
DMSO	7%	5%	5%	-	-	5%	-	-	7%
Betaine (M)	-	-	1.0	-	2.0	1.0	-	1.75	0.83
Q solution *3	1x	-	-	1x	-	-	-	-	-
$MgCl_2$ (mM)	0.89	2.0	-	-	-	-	-	-	0.83

*1: *Taq* DNA polymerase (QIAGEN, Germany)
*2: FastStart PCR Master mix (Roche, Switzerland)
*3: Q solution (QIAGEN, Germany)
*4: *Taq* Robust (Kapa Biosystems, USA), Kapa Enhancer 1x.
*5: RG25 buffer (160mM NH4SO4, 670mM Tris-HCl (pH 8.8), 1% Tween, 25mM MgCl2)
*6: Expand Long Template PCR system (Roche, Switzerland), Buffer 2 1x, Titanium *Taq* polymerase 2x.
*7: AccuPrime GC-rich DNA polymerase (Invitrogen, USA), Buffer A 1x.
*8: Extensor Mastermix (Thermo Scientific, USA)
*9: True Allele PCR Premix (Applied Biosystems, USA), PCR master mix 1x.

Supplementary table 3C-2. Concentration of ingredients in amplicon-length analysis PCR.

PCR reaction number	PR10	PR11	PR12	PR13	PR14	PR15	PR16	PR17
Used laboratory	AE	B	C	DI	F	G	H	J
Primer 4 (μM)	0.4	0.2	0.4	0.2	0.3	1.0	0.5	0.1
Primer 5 (μM)	0.4	0.2	0.4	0.2	0.3	1.0	0.5	0.1
dCTP, dATP, dTTP (each) (mM)	-	0.2	-	-	-	0.25	-	-
7-deaza-2-deoxy GTP (mM)	-	0.2	0.2	-	-	0.25	0.18	-
dNTP (each) (mM)	-	-	0.25	-	0.3	-	-	-
DMSO (%)	-	5.0	4.0	-	-	5.0	7.1	1.0
Betaine (M)	-	-	1.0	-	1.0	1.0	-	-
Q solution *1	-	-	-	-	-	-	1x	-
MgCl2 (mM)	-	2.0	-	-	-	-	0.89	-
Kit buffer	1x	1x	1x	1x	1x	1x	1x	1x
Kit polymerase (U)	-	0.2	1.25	0.4	0.3	2.5	-	-
Kit and polymerase	*2	*3	*4	*5	*6	*7	*8	*9

*1: Q solution (QIAGEN, Germany)
*2: One *Taq* 2X Master Mix with GC Buffer (New England BioLabs, UK)
*3: KAPA Enhncer and Taq Robust (Kapa Biosystems, USA)
*4: AmpliTaq and GeneAmp PCR buffer (Roche, Switzerland)
*5: AccuPrime GC-Rich DNA Polymerase (Invitrogen, USA)
*6: Kapa HiFi Hotstart PCR kit, GC buffer (Kapa Biosystems, USA)
*7: *Taq* DNA Polymerase (QIAGEN, Germany)
*8: FastStart PCR Master mix (Roche, Switzerland)
*9: Megamix Blue (Microzone, UK)

Supplementary table 3C-3. PCR protocols for RP-PCR (PP1-9).

PP1, Laboratory A, L, N

Temp	Time	Cycle
95°C	15 min	1
94°C 70°C 72°C	1 min 1 min 3 min	2
94°C 68°C 72°C	1 min 1 min 3 min	3
94°C 66°C 72°C	1 min 1 min 3 min	4
94°C 64°C 72°C	1 min 1 min 3 min	5
94°C 62°C 72°C	1 min 1 min 3 min	6
94°C 60°C 72°C	1 min 1 min 3 min	7
94°C 58°C 72°C	1 min 1 min 3 min	8
94°C 56°C 72°C	1 min 1 min 3 min	5
72°C	10 min	1
4°C	hold	1

PP2, Laboratory B

Temp	Time	Cycle
98°C	10 min	1
98°C 65-58°C 72°C	35 sec 2 min 8 min	13 Every cycle -1°C
97°C 58°C 72°C	35 sec 2 min 8-18 min	32 Every cycle +20 sec
72°C	10 min	1
4°C	hold	1

PP3, Laboratory C

Temp	Time	Cycle
95°C	8 min	1
95°C 70-56°C 72°C	1 min 1 min 3 min	8 Every cycle -2°C
95°C 56°C 72°C	1 min 1 min 3 min	32
72°C	10 min	1
4°C	hold	1

PP4, Laboratory D

Temp	Time	Cycle
95°C	5 min	1
95°C 68°C	30 sec 3 min	32
72°C	7 min	1
12°C	hold	1

PP5, Laboratory E

Temp	Time	Cycle
95°C	5 min	1
95°C 70-53.2°C 68°C	30 sec 30 sec 40 sec	49 Every cycle -0.35°C
95°C 58°C 68°C	30 sec 30 sec 40 sec	16
68°C	10 min	1
12°C	hold	1

PP6, Laboratory F

Temp	Time	Cycle
98°C	10 min	1
97°C 53°C 68°C	35 sec 2 min 2 min	10
97°C 53°C 68°C	35 sec 2 min 2-10 min	25 Every cycle +20 sec
68°C	10 min	1
4°C	hold	1

PP7, Laboratory G, I, M

Temp	Time	Cycle
98°C	10 min	1
97°C 64°C 68°C	35 sec 2 min 8 min	10
97°C 64°C 68°C	35 sec 2 min 8-16 min	25 Every cycle +20 sec
16°C	hold	1

PP8, Laboratory H

Temp	Time	Cycle
95°C	5 min	1
95°C 70-56°C 72°C	30 sec 30 sec 3 min	8 Every cycle -2°C
95°C 56°C 72°C	30 sec 30 sec 3 min	35
72°C	20 min	1
4°C	hold	1

PCR protocols for RP-PCR (continued).

PP9, Laboratory J

Temp	Time	Cycle
95°C	5 min	1
95°C 70-56°C 72°C	30 sec 30 sec 8 min	8 Every cycle -2°C
95°C 56°C 72°C	30 sec 30 sec 8-16 min	26 Every cycle +20 sec
72°C	7 min	1
4°C	hold	1

Supplementary table 3C-4. PCR protocols for amplicon-length analysis (PP10-19).

PP10, Laboratory A

Temp	Time	Cycle
98°C	5 min	1
97°C 65-56°C 68°C	30 sec 30 sec 1.5 min	10 Every cycle -1°C
97°C 55°C 68°C	30 sec 30 sec 1.5 min	24
68°C	10 min	1
12°C	hold	1

PP11, Laboratory B

Temp	Time	Cycle
97°C	5 min	1
97°C 60°C 72°C	30 sec 30 sec 1 min	10
97°C 60°C 72°C	10 sec 15 sec 30 sec	28
72°C	10 min	1
4°C	hold	1

PP12, Laboratory C

Temp	Time	Cycle
95°C	5 min	1
95°C 56°C 72°C	1 min 1 min 2 min	30
72°C	10 min	1
4°C	hold	1

PCR protocols for Amplicon length analysis (continued).

PP13, Laboratory D

Temp	Time	Cycle
95°C	3 min	1
95°C 68°C 72°C	30 sec 30 sec 2 min	35
72°C	10 min	1
12°C	hold	1

PP14, Laboratory E

Temp	Time	Cycle
98°C	5 min	1
97°C 65-55°C 68°C	30 sec 30 sec 1.5 min	11 Every cycle -1°C
97°C 55°C 68°C	30 sec 30 sec 1.5 min	24
68°C	10 min	1
12°C	hold	1

PP15, Laboratory F

Temp	Time	Cycle
95°C	5 min	1
98°C 59°C 72°C	20 sec 15 sec 1 min	33
72°C	5 min	1
4°C	hold	1

PP16, Laboratory G

Temp	Time	Cycle
94°C	4 min	1
94°C 62°C 72°C	30 sec 30 sec 1 min	36
72°C	10 min	1

PP17, Laboratory H

Temp	Time	Cycle
95°C	4 min	1
95°C 70-57°C 72°C	30 sec 30 sec 3 min	14 Every cycle -1°C
95°C 56°C 72°C	30 sec 30 sec 3 min	26
72°C	10 min	1
4°C	hold	1

PP18, Laboratory I

Temp	Time	Cycle
95°C	3 min	1
95°C 63°C 72°C	30 sec 30 sec 2 min	35
72°C	10 min	1
16°C	hold	1

PP19, Laboratory J

Temp	Time	Cycle
95°C	5 min	1
95°C 70-56°C 72°C	30 sec 30 sec 8 min	8 Every cycle -2°C
95°C 56°C 72°C	30 sec 30 sec 8-16 min	26 Every cycle +20 sec
72°C	7 min	1
4°C	hold	1

Supplementary table 3D. Methods of each laboratory.

Laboratory A

RP-PCR

	List	
Primer 1	P1	FAM-AGTCGCTAGAGGCGAAAGC
Primer 2 (with GGGGCC repeat)	P2	tacgcatcccagtttgagacgGGGGCCGGGGCCGGGGCCGGGG
Primer 3	P3	tacgcatcccagtttgagacg
Size standard	S2	GeneScan 500LIZ Size Standard (Applied Biosystems, USA)
Analysis machine	M1	3730*xl* DNA Analyzer (Applied Biosystems, USA)
Analysis software	A1	Peak Scanner Software v1.0 (Applied Biosystems, USA)

PCR reaction (PR1)

Products	Final conc.
FastStart PCR Master	1x
Primer 1	1.4 µM
Primer 2	0.7 µM
Primer 3	1.4 µM
7-deaza-2-deoxy GTP	0.18 mM
DMSO	7 %
Q solution	1x
$MgCl_2$	0.89 mM
DNA	7.1 ng/µl
Total	14.0 µl

FastStart PCR Master (Roche, Switzerland)
Q solusion (QIAGEN, Germany).

PCR protocol (PP1)

Temp	Time	Cycle
95°C	15 min	1
94°C 70-58°C 72°C	1 min 1 min 3 min	2-8 *
94°C 56°C 72°C	1 min 1 min 3 min	5
72°C	10 min	1
4°C	hold	1

* Every 2°C decrease add 1 cycle.

Capillary electrophoresis

PCR product	1.0 µl
Size standard	0.5 µl
Formamide	8.5 µl
Total	10.0 µl

95°C 5min and on ice. Analyse with DNA analyzer.

Amplicon length analysis

	List	
Primer 4	P14	FAM-CAAGGAGGGAAACAACCGCAGCC
Primer 5	P16	GCAGGCACCGCAACCGCAG
Size standard	S2	GeneScan 500LIZ Size Standard (Applied Biosystems, USA)
Analysis machine	M1	3730*xl* DNA Analyzer (Applied Biosystems, USA)
Analysis software	A1	Peak Scanner Software v1.0 (Applied Biosystems, USA)

PCR reaction (PR10)

Products	Final conc.
Primer 4	0.4 µM
Primer 5	0.4 µM
Kit buffer	1x
DNA	2.2 ng/µl
Total	15 µl

Kit buffer: One *Taq* 2x Master Mix with GC Buffer (New England BioLabs, UK).

PCR protocol (PP10)

Temp	Time	Cycle
98°C	5 min	1
97°C 65-56°C 68°C	30 sec 30 sec 1.5 min	10 Every cycle -1°C
97°C 55°C 68°C	30 sec 30 sec 1.5 min	24
68°C	10 min	1
12°C	hold	1

Capillary electrophoresis

PCR product	1.0 µl
Size standard	0.5 µl
Formamide	8.5 µl
Total	10 µl

95°C 5min and on ice. Analyse with DNA analyzer.

Supple table 3D. Methods of each laboratory

Laboratory B

RP-PCR

	List	
Primer 1	P7	FAM-CAAGGAGGGAAACAACCGCAGCC
Primer 2 (with GGGGCC repeat)	P8	ggataacaatttcacacaggGGGCCCGCCCCGACCACGCCCCGGC CCCGGCCCCGG
Primer 3	P9	ggataacaatttcacacagg
Size standard	S2	GeneScan 500 LIZ Size Standard (Applied Biosystems, USA)
Analysis machine	M2	3130*xl* Genetic Analyzer (Applied Biosystems, USA)
Analysis software	A2	GeneMapper Software v4.0 (Applied Biosystems, USA)

PCR reaction (PR2)

Products	Final conc.
KAPA Enhancer	1x
Primer 1	0.2 µM
Primer 2	0.2 µM
Primer 3	0.2 µM
dCTP, dATP, dTTP (each)	0.2 mM
7-deaza-2-deoxy GTP	0.2 mM
DMSO	5%
$MgCl_2$	2.0 mM
Taq Robust	0.2 U
DNA	5 ng/µl
Total	20 µl

KAPA Enhancer and *Taq* Robust (Kapa Biosystems, USA).

PCR protocol (PP2)

Temp	Time	Cycle
98°C	10 min	1
98°C 65-58°C 72°C	35 sec 2 min 8 min	13 Every cycle -1°C
97°C 58°C 72°C	35 sec 2 min 8-18 min	32 Every cycle +20 sec
72°C	10 min	1
4°C	hold	1

Ramping up and down: 0.5°C/sec

Capillary electrophoresis

Dilute PCR product
PCR product:H_2O=1:1

PCR product	2.0 µl
Size standard	0.1 µl
Formamide	7.9 µl
Total	10.0 µl

95°C 3min and analyse with genetic analyzer.

Amplicon length analysis

	List	
Primer 4	P14	FAM-CAAGGAGGGAAACAACCGCAGCC
Primer 5	P16	GCAGGCACCGCAACCGCAG
Size standard	S2	GeneScan 500LIZ Size Standard (Applied Biosystems, USA)
Analysis machine	M2	3130*xl* Genetic Analyzer (Applied Biosystems, USA)
Analysis software	A2	GeneMapper Software v4.0 (Applied Biosystems, USA)

PCR reaction (PR11)

Products	Final conc.
KAPA Enhancer	1x
Primer 4	0.2 µM
Primer 5	0.2 µM
dCTP, dATP, dTTP (each)	0.2 mM
7-deaza-2-deoxy GTP	0.2 mM
DMSO	5%
$MgCl_2$	2.0 mM
Taq Robust	0.2 U
DNA	5 ng/µl
Total	20 µl

KAPA Enhancer and *Taq* Robust (Kapa Biosystems, USA)

PCR protocol (PP11)

Temp	Time	Cycle
97°C	5 min	1
97°C 60°C 72°C	30 sec 30 sec 1 min	10
97°C 60°C 72°C	10 sec 15 sec 30 sec	28
72°C	10 min	1

Capillary electrophoresis

Dilute PCR product
PCR product:H_2O=1:39

Diluted PCR product	2.0 µl
Size standard	0.1 µl
Formamide	7.9 µl
Total	10.0 µl

95°C 3min and analyse with genetic analyzer.

Supple table 3D. Methods of each laboratory

Laboratory C

RP-PCR

	List	
Primer 1	P1	FAM-AGTCGCTAGAGGCGAAAGC
Primer 2 (with GGGGCC repeat)	P2	tacgcatcccagtttgagacgGGGGCCGGGGCCGGGGCCGGGG
Primer 3	P3	tacgcatcccagtttgagacg
Size standard	S2	GeneScan 500 LIZ Size Standard (Applied Biosystems, USA)
Analysis machine	M2	3130*xl* genetic analyzer (Applied Biosystems, USA)
Analysis software	A1	Peak Scanner Software v1.0 (Applied Biosystems, USA)

PCR reaction (PR1)

Products	Final conc.
FastStart PCR Master	1x
Primer 1	1.4 µM
Primer 2	0.7 µM
Primer 3	1.4 µM
7-deaza-2-deoxy GTP	0.18 mM
DMSO	7 %
Q solution	1x
$MgCl_2$	0.89 mM
DNA	2.14 ng/µl
Total	28 µl

FastStart PCR Master (Roche, Switzerland)
Q solusion (QIAGEN, Germany)

PCR protocol (PP3)

Temp	Time	Cycle
95°C	8 min	1
95°C 70-56°C 72°C	1 min 1 min 3 min	8 Every cycle -2°C
95°C 56°C 72°C	1 min 1 min 3 min	32
72°C	10 min	1
4°C	hold	1

Capillary electrophoresis

PCR product	2.5 µl
Size standard	0.25 µl
Formamide	7.25 µl
Total	10.0 µl

95°C 2min and 4°C.
Analyse with genetic analyzer.

Amplicon length analysis

	List	
Primer 4	P14	FAM-CAAGGAGGGAAACAACCGCAGCC
Primer 5	P16	GCAGGCACCGCAACCGCAG
Size standard	S2	GeneScan 500LIZ Size Standard (Applied Biosystems, USA)
Analysis machine	M2	3130*xl* genetic analyzer (Applied Biosystems, USA)
Analysis software	A1	Peak Scanner Software v1.0 (Applied Biosystems, USA)

PCR reaction (PR12)

Products	Final conc.
Primer 4	0.4 µM
Primer 5	0.4 µM
7-deaza-2-deoxy GTP	0.2 mM
dNTPs (each)	0.25 mM
DMSO	4 %
Betaine	1.0 M
GeneAmp PCR buffer	1x
AmpliTaq DNA polymerase	1.25 U
DNA	2.4 ng/µl
Total	25.0 µl

AmpliTaq and GeneAmp PCR buffer (Roche, Switzerland)

PCR protocol (PP12)

Temp	Time	Cycle
95°C	5 min	1
95°C 56°C 72°C	1 min 1 min 2 min	30
72°C	10 min	1
4°C	hold	1

Capillary electrophoresis

PCR product	2.5 µl
Size standard	0.25 µl
Formamide	7.25 µl
Total	10.0 µl

95°C 2min and 4°C.
Analyse with genetic analyzer.

Supple table 3D. Methods of each laboratory

Laboratory D

RP-PCR

	List	
Primer 1	P4	FAM-tgtaaaacgacggccagtCAAGGAGGGAAACAACCGCAGCC
Primer 2 (with GGGGCC repeat)	P13	cacgacgttgtaaaacgaCCCCGGCCCCGGCCCCGG
Primer 3	-	none
Size standard	S2	GeneScan 500LIZ Size Standard (Life Technologies, USA)
Analysis machine	M1	3730*xl* DNA Analyzer (Applied Biosystems, USA)
Analysis software	A1	Peak Scanner Software v2.0 (Applied Biosystems, USA)

PCR reaction (PR3)

Products	Final conc.
RG25 buffer	1x
Primer 1	0.2 µM
Primer 2	0.2 µM
dCTP, dATP, dTTP (each)	0.2 mM
7-deaza-2-deoxy GTP	0.2 mM
DMSO	5%
Betaine	1.0 M
Taq DNA polymerase	1.8 U
DNA	2.0 ng/µl
Total	20.0 µl

RG25 buffer (160mM NH_4SO_4, 670mM Tris-HCl (pH 8.8), 1% Tween, 25mM $MgCl_2$)

PCR protocol (PP4)

Temp	Time	Cycle
95°C	5 min	1
95°C 68°C	30 sec 3 min	32
72°C	7 min	1
12°C	hold	1

Capillary electrophoresis

PCR product	2.0 µl
Size standard	0.5 µl
Formamide	7.5 µl
Total	10.0 µl

95°C 5min and analyse with DNA analyzer.

Amplicon length analysis

	List	
Primer 4	P15	CAAGGAGGGAAACAACCGCAGCC
Primer 5	P16	GCAGGCACCGCAACCGCAG
Analysis system	A4	4 % agarose gel

PCR reaction (PR13)

Products	Final conc.
Primer 4	0.2 µM
Primer 5	0.2 µM
AccuPrime buffer A	1x
AccuPrime Taq	0.4 U
DNA	4.2 ng/µl
Total	30.0 µl

AccuPrime GC-Rich DNA Polymerase (Invitrogen, USA)

PCR protocol (PP13)

Temp	Time	Cycle
95°C	3 min	1
95°C 68°C 72°C	30 sec 30 sec 2 min	35
72°C	10 min	1
12°C	hold	1

Supple table 3D. Methods of each laboratory

Laboratory E

RP-PCR

	List	
Primer 1	P4	FAM-tgtaaaacgacggccagtCAAGGAGGGAAACAACCGCAGCC
Primer 2 (with GGGGCC repeat)	P5	caggaaacagctatgaccGGGCCCGCCCCGACCACGCCCCGGCCCCG GCCCCGG
Primer 3	P6	caggaaacagctatgacc
Size standard	S1	GeneScan 500ROX Size Standard (Applied Biosystems, USA)
Analysis machine	M1	3730 DNA Analyzer (Applied Biosystems, USA)
Analysis software	A1	Peak Scanner Software v1.0 (Applied Biosystems, USA)

PCR reaction (PR4)

Products	Final conc.
Qiagen buffer	1x
Primer 1	0.4 µM
Primer 2	0.2 µM
Primer 3	0.4 µM
ATP, CTP, TTP (each)	0.05 mM
GTP	0.0125 mM
7-deaza-2-deoxy GTP	0.0375 mM
Q solution	1x
Taq DNA polymerase	0.75 U
DNA	20 ng/µl
Total	25.0 µl

Taq DNA polymerase (QIAGEN, Germany)

PCR protocol (PP5)

Temp	Time	Cycle
95°C	5 min	1
95°C 70-53.2°C 68°C	30 sec 30 sec 40 sec	49 Every cycle -0.35°C
95°C 58°C 68°C	30 sec 30 sec 40 sec	16
68°C	10 min	1
12°C	hold	1

Capillary electrophoresis

Dilute PCR product
PCR product:H20=1.5:100

Diluted PCR product	5.0 µl
Size standard	0.07 µl
H2O	10.0 µl
Total	15.7 µl

95°C 5min and analyse with DNA analyzer.

Amplicon length analysis

	List	
Primer 4	P14	FAM-CAAGGAGGGAAACAACCGCAGCC
Primer 5	P16	GCAGGCACCGCAACCGCAG
Size standard	S1	GeneScan 500ROX Size Standard (Applied Biosystems, USA)
Analysis machine	M1	3730 DNA Analyzer (Applied Biosystems, USA)
Analysis software	A1	Peak Scanner Software v1.0 (Applied Biosystems, USA)

PCR reaction (PR10)

Products	Final conc.
Primer 4	0.4 µM
Primer 5	0.4 µM
Kit buffer	1x
DNA	2.0 ng/µl
Total	25.0 µl

Kit buffer: One Taq 2x Master Mix with GC Buffer (New England BioLabs, UK)

PCR protocol (PP14)

Temp	Time	Cycle
98°C	5 min	1
97°C 65-55°C 68°C	30 sec 30 sec 1.5 min	11 Every cycle -1°C
97°C 55°C 68°C	30 sec 30 sec 1.5 min	24
68°C	10 min	1
12°C	hold	1

Capillary electrophoresis

Dilute PCR product
PCR product:H20=1.5:100

Diluted PCR product	5.0 µl
Size standard	0.07 µl
H2O	10.0 µl
Total	15.7 µl

95°C 5min and analyse with DNA analyzer.

Supple table 3D. Methods of each laboratory

Laboratory F

RP-PCR

	List	
Primer 1	P10	FAM-AGTACTCGCTGAGGGTGAAC
Primer 2 (with GGGGCC repeat)	P11	cgtacgcatcccagtttgagaGCCCCGGCCCCGGCCCCGG
Primer 3	P12	cgtacgcatcccagtttgaga
Size standard	S2	GeneScan 500LIZ Size Standard (Applied Biosystems, USA)
Analysis machine	M1	3730 DNA Analyzer (Applied Biosystems, USA)
Analysis software	A5	In-house developed Tracl genotyping software

PCR reaction (PR5)

Products	Final conc.
Buffer 2 (kit)	1x
Primer 1	0.33 µM
Primer 2	0.33 µM
Primer 3	0.03 µM
dNTP (each)	0.5 mM
Betaine	2.0 M
Polymeras (TiTaq 50x)	2x
DNA	10 ng/µl
Total	20.0 µl

Expand Long Template PCR system (Roche, Switzerland)

PCR protocol (PP6)

Temp	Time	Cycle
98°C	10 min	1
97°C 53°C 68°C	35 sec 2 min 2 min	10
97°C 53°C 68°C	35 sec 2 min 2-10 min	25 Every cycle +20 sec
68°C	10 min	1
4°C	hold	1

Capillary electrophoresis

PCR product	2.0 µl
Size standard	0.35 µl
Formamide	10.0 µl
Total	12.35µl

95°C 1.5min and analyse with DNA analyzer.

Amplicon length analysis

	List	
Primer 4	P17	FAM-CAGGTGTGGGTTTAGGAGGT
Primer 5	P18	CCAGCTTCGGTCAGAGAAAT
Size standard	S4	GeneScan 600LIZ Size Standard (Applied Biosystems, USA)
Analysis machine	M1	3730 DNA Analyzer (Applied Biosystems, USA)
Analysis software	A5	In-house developed Tracl genotyping software

PCR reaction (PR14)

Product	Final conc.
Primer 4	0.3 µM
Primer 5	0.3 µM
dNTPs (each)	0.3 mM
Betaine	1.0 M
Kapa HiFi GC buffer	1x
Polymerase (kit)	0.3U
DNA	3.3 ng/µl
Total	15.0 µl

Kapa HiFi Hotstart PCR kit (Kapa Biosystems, USA)

PCR protocol (PP15)

Temp	Time	Cycle
95°C	5 min	1
98°C 59°C 72°C	20 sec 15 sec 1 min	33
72°C	5 min	1
4°C	hold	1

Ramping rate: 50%

Capillary electrophoresis

PCR product	2.0 µl
Size standard	0.35 µl
Formamide	10.0 µl
Total	12.35µl

95°C 1.5min and analyse with DNA analyzer.

Supple table 3D. Methods of each laboratory

Laboratory G

RP-PCR

	List	
Primer 1	P4	FAM-tgtaaaacgacggccagtCAAGGAGGGAAACAACCGCAGCC
Primer 2 (with GGGGCC repeat)	P5	caggaaacagctatgaccGGGCCCGCCCCGACCACGCCCCGGCCCCG GCCCCGG
Primer 3	P6	caggaaacagctatgacc
Size standard	S3	GeneScan 400HD Rox Size Standard (Applied Biosystems, USA)
Analysis machine	M1	3730 DNA Analyzer (Applied Biosystems, USA)
Analysis software	A2	GeneMapper v4.0 (Applied Biosystems, USA)

PCR reaction (PR6)

Products	Final conc.
Qiagen buffer	1x
Primer 1	1.0 µM
Primer 2	1.0 µM
Primer 3	1.0 µM
dCTP, dATP, dTTP (each)	0.25 mM
7-deaza-2-deoxy GTP	0.25 mM
DMSO	5%
Betaine	1.0 M
Taq DNA polymerase	2.5 U
DNA *1	12.5 ng/µl
Total	20.0 µl

Taq DNA polymerase, buffer (QIAGEN, Germany)

PCR protocol (PP7)

Temp	Time	Cycle
98°C	10 min	1
97°C 64°C 68°C	35 sec 2 min 8 min	10
97°C 64°C 68°C	35 sec 2 min 8-16 min	25 Every cycle +20 sec

Reduce ramping rate up and down to 0.5°C/sec.

Capillary electrophoresis

Dilute PCR product
PCR product:H20=1:5

Diluted PCR product	2.0 µl
Size standard	0.12 µl
Formamide	7.88 µl
Total	10.0 µl

95°C 2min and analyse with DNA analyzer.

*1: Initial DNA concentration ~250 ng/µl.

Amplicon length analysis

	List	
Primer 4	P14	FAM-CAAGGAGGGAAACAACCGCAGCC
Primer 5	P16	GCAGGCACCGCAACCGCAG
Size standard	S3	GeneScan 400HD Rox Size Standard (Applied Biosystems, USA)
Analysis machine	M1	3730 DNA Analyzer (Applied Biosystems, USA)
Analysis software	A2	GeneMapper v4.0 (Applied Biosystems, USA)

PCR reaction (PR15)

Products	Final conc.
Primer 4	1.0 µM
Primer 5	1.0 µM
dCTP, dATP, dTTP (each)	0.25 mM
7-deaza-2-deoxy GTP	0.25 mM
DMSO	5%
Betaine	1.0 M
Qiagen buffer	1x
Taq DNA polymerase	2.5 U
DNA *2	3.35 ng/µl
Total	20.0 µl

Taq DNA polymerase, buffer (QIAGEN, Germany)

PCR protocol (PP16)

Temp	Time	Cycle
94°C	4 min	1
94°C 62°C 72°C	30 sec 30 sec 1 min	36
72°C	10 min	1

Capillary electrophoresis

Dilute PCR product
PCR product:H20=1:180

Diluted PCR product	3.0 µl
Size standard	0.10 µl
HiDi Formamide	6.90 µl
Total	10.0 µl

95°C 2min and analyse with DNA analyzer.

*2: Initial DNA concentration ~67 ng/µl.

Supple table 3D. Methods of each laboratory

Laboratory H

RP-PCR

List

Primer 1	P1	FAM-AGTCGCTAGAGGCGAAAGC
Primer 2 (with GGGGCC repeat)	P2	tacgcatcccagtttgagacgGGGGCCGGGGCCGGGGCCGGGG
Primer 3	P3	tacgcatcccagtttgagacg
Size standard	S2	GeneScan 500LIZ Size Standard (Applied Biosystems, USA)
Analysis machine	M1	3730 DNA Analyzer (Applied Biosystems, USA)
Analysis software	A2	GeneMapper v4.0 (Applied Biosystems, USA)

PCR reaction (PR1)

Products	Final conc.
FastStart PCR Master	1x
Primer 1	1.4 µM
Primer 2	0.7 µM
Primer 3	1.4 µM
7-deaza-2-deoxy GTP	0.18 mM
DMSO	7 %
Q solution	1x
$MgCl_2$	0.89
DNA	7.1 ng/µl
Total	28.1 µl

FastStart PCR Master (Roche, Switzerland)
Q solusion (QIAGEN, Germany)

PCR protocol (PP8)

Temp	Time	Cycle
95°C	5 min	1
95°C 70-56°C 72°C	30 sec 30 sec 3 min	8 Every cycle -2°C
95°C 56°C 72°C	30 sec 30 sec 3 min	35
72°C	20 min	1
4°C	hold	1

Capillary electrophoresis

Dilute PCR product
PCR product:H_2O=1:19

Diluted PCR product	2.0 µl
Formamide mix	8.6 µl
Total	10.6 µl

Formamide mix=8µl Size standard + 850µl Formamide

No denaturation.
Analyze with DNA analyzer.

Amplicon length analysis

List

Primer 4	P14	FAM-CAAGGAGGGAAACAACCGCAGCC
Primer 5	P16	GCAGGCACCGCAACCGCAG
Size standard	S2	GeneScan 500LIZ Size Standard (Applied Biosystems, USA)
Analysis machine	M1	3730 DNA Analyzer (Applied Biosystems, USA)
Analysis software	A2	GeneMapper v4.0 (Applied Biosystems, USA)

PCR reaction (PR16)

Products	Final conc.
Primer 4	0.5 µM
Primer 5	0.5 µM
7-deaza-2-deoxy GTP	0.18 mM
DMSO	7.1 %
Q solution	1x
$MgCl_2$	0.89 mM
FastStart PCR master	1x
DNA	7.1 ng/µl
Total	28.0 µl

FastStart PCR Master (Roche, Switzerland)
Q solusion (QIAGEN, Germany)

PCR protocol (PP17)

Temp	Time	Cycle
95°C	4 min	1
95°C 70-57°C 72°C	30 sec 30 sec 3 min	14 Every cycle -1°C
95°C 56°C 72°C	30 sec 30 sec 3 min	26
72°C	10 min	1
4°C	hold	1

Capillary electrophoresis

Dilute PCR product
PCR product:H_2O=1:39

Diluted PCR product	2.0 µl
Formamide mix	8.6 µl
Total	10.6 µl

Formamide mix=8µl Size standard + 850µl Formamide

No denaturation.
Analyze with DNA analyzer.

Supple table 3D. Methods of each laboratory

Laboratory I

RP-PCR

	List	
Primer 1	P4	FAM-tgtaaaacgacggccagtCAAGGAGGGAAACAACCGCAGCC
Primer 2 (with GGGGCC repeat)	P5	caggaaacagctatgaccGGGCCCGCCCCGACCACGCCCCGGCCCCG GCCCCGG
Primer 3	P6	caggaaacagctatgacc
Size standard	S1	GeneScan 500ROX Size Standard (Applied Biosystems, USA)
Analysis machine	M3	3500 Genetic Analyzer (Applied Biosystems, USA)
Analysis software	A2	GeneMapper v4.0 (Applied Biosystems, USA)

PCR reaction (PR7)

Products	Final conc.
AccuPrime buffer A	1x
Primer 1	1.4 µM
Primer 2	0.7 µM
Primer 3	1.4 µM
dCTP, dATP, dTTP (each)	0.18 mM
7-deaza-2-deoxy GTP	0.18 mM
Taq DNA polymerase	1.0 U
DNA	12 ng/µl
Total	25.0 µl

AccuPrime GC-rich DNA polymerase, buffer A (Invitrogen, USA)

PCR protocol (PP7)

Temp	Time	Cycle
98°C	10 min	1
97°C 64°C 68°C	35 sec 2 min 8 min	10
97°C 64°C 68°C	35 sec 2 min 8-16 min	25 Every cycle +20 sec
16°C	hold	1

Ramp rate adjusted to 64% for the denaturation and extension steps. Ramp rate adjusted to 25% for the annealing step.

Capillary electrophoresis

PCR product	1.5 µl
Size standard	0.5 µl
Formamide	15.0 µl
Total	17.0 µl

95°C 5min and analyse with genetic analyzer.

Amplicon length analysis

	List	
Primer 4	P15	CAAGGAGGGAAACAACCGCAGCC
Primer 5	P16	GCAGGCACCGCAACCGCAG
Analysis system	A4	3% agarose gel

PCR reaction (PR13)

Products	Final conc.
Primer 4	0.2 µM
Primer 5	0.2 µM
Kit buffer	1x
Kit polymerase	0.4 U
DNA	2.0 ng/µl
Total	25.0 µl

Kit: AccuPrime GC-rich DNA polymerase (Invitrogen, USA)

PCR protocol (PP18)

Temp	Time	Cycle
95°C	3 min	1
95°C 63°C 72°C	30 sec 30 sec 2 min	35
72°C	10 min	1
16°C	hold	1

Supple table 3D. Methods of each laboratory

Laboratory J

RP-PCR

	List	
Primer 1	P4	FAM-tgtaaaacgacggccagtCAAGGAGGGAAACAACCGCAGCC
Primer 2 (with GGGGCC repeat)	P5	caggaaacagctatgaccGGGCCCGCCCCGACCACGCCCCGGCCCCGG CCCCGG
Primer 3	P6	caggaaacagctatgacc
Size standard	S2	GeneScan 500LIZ Size Standard (Applied Biosystems, USA)
Analysis machine	M1	3730*xl* DNA Analyzer (Applied Biosystems, USA)
Analysis software	A2	GeneMapper v4.1 (Applied Biosystems, USA)

PCR reaction (PR8)

Products	Final conc.
Extensor Mastermix	1x
Primer 1	2.5 µM
Primer 2	2.5 µM
Primer 3	0.25 µM
Betaine	1.75 M
DNA	3.3 ng/µl
Total	15 µl

Extensor Mastermix (Thermo Scientific, USA)

PCR protocol (PP9)

Temp	Time	Cycle
95°C	5 min	1
95°C 70-56°C 72°C	30 sec 30 sec 8 min	8 Every cycle -2°C
95°C 56°C 72°C	30 sec 30 sec 8-16 min	26 Every cycle +20 sec
72°C	7 min	1

Capillary electrophoresis

PCR product	1.0 µl
Size standard	0.5 µl
Formamide	8.5 µl
Total	10.0 µl

95°C 2min and on ice. Analyse with DNA analyzer.

Amplicon length analysis

	List	
Primer 4	P14	FAM-CAAGGAGGGAAACAACCGCAGCC
Primer 5	P16	GCAGGCACCGCAACCGCAG
Size standard	S2	GeneScan 500LIZ Size Standard (Applied Biosystems, USA)
Analysis machine	M1	3730*xl* DNA Analyzer (Applied Biosystems, USA)
Analysis software	A2	GeneMapper v4.1 (Applied Biosystems, USA)

PCR reaction (PR17)

Products	Final conc.
Primer 4	0.1 µM
Primer 5	0.1 µM
DMSO	1%
Megamix Blue	1x
DNA	3.3 ng/µl
Total	15 µl

Megamix Blue (Microzone, UK)

PCR protocol (PP19)

Temp	Time	Cycle
95°C	5 min	1
95°C 70-56°C 72°C	30 sec 30 sec 8 min	8 Every cycle -2°C
95°C 56°C 72°C	30 sec 30 sec 8-16 min	26 Every cycle +20 sec
72°C	7 min	1
4°C	hold	1

Capillary electrophoresis

PCR product	1.0 µl
Size standard	0.5 µl
Formamide	8.5 µl
Total	10.0 µl

95°C 2min and on ice. Analyse with DNA analyzer.

Supple table 3D. Methods of each laboratory

Laboratory L

RP-PCR

List

Primer 1	P1	FAM-AGTCGCTAGAGGCGAAAGC
Primer 2 (with GGGGCC repeat)	P2	tacgcatcccagtttgagacgGGGGCCGGGGCCGGGGCCGGGG
Primer 3	P3	tacgcatcccagtttgagacg
Size standard	S1	GeneScan 500XL ROX Size Standard (Applied Biosystems, USA)
Analysis machine	M1	3730*xl* DNA Analyzer (Applied Biosystems, USA)
Analysis software	A1	Peak Scanner Software v1.0 (Applied Biosystems, USA)

PCR reaction (PR1)

Products	Final conc.
FastStart PCR Master	1x
Primer 1	1.4 µM
Primer 2	0.7 µM
Primer 3	1.4 µM
7-deaza-2-deoxy GTP	0.18 mM
DMSO	7 %
Q solution	1x
$MgCl_2$	0.89 mM
DNA	100 ng
Total	28.0 µl

FastStart PCR Master (Roche, Switzerland)
Q solusion (QIAGEN, Germany)

PCR protocol (PP1)

Temp	Time	Cycle
95°C	15 min	1
94°C 70-58°C 72°C	1 min 1 min 3 min	2-8 *
94°C 56°C 72°C	1 min 1 min 3 min	5
72°C	10 min	1

* Every 2°C decrease add 1 cycle.

Capillary electrophoresis

PCR product	2.0 µl
Size standard	0.5 µl
HiDi Formamide	7.0 µl
Total	9.5 µl

95°C 3min and analyse with DNA analyzer.

Amplicon length analysis…not performed.

Supple table 3D. Methods of each laboratory

Laboratory M

RP-PCR

	List	
Primer 1	P4	FAM-tgtaaaacgacggccagtCAAGGAGGGAAACAACCGCAGCC
Primer 2 (with GGGGCC repeat)	P5	caggaaacagctatgaccGGGCCCGCCCCGACCACGCCCCGGCCCCG GCCCCGG
Primer 3	P6	caggaaacagctatgacc
Size standard	S2	GeneScan 500 LIZ Size Standard (Applied Biosystems, USA)
Analysis machine	M1	3730*xl* DNA Analyzer (Applied Biosystems, USA)
Analysis software	A1	Peak Scanner Software v1.0 (Applied Biosystems, USA)

PCR reaction (PR6)

Products	Final conc.
Qiagen buffer	1x
Primer 1	1.0 µM
Primer 2	1.0 µM
Primer 3	1.0 µM
dCTP, dATP, dTTP (each)	0.25 mM
7-deaza-2-deoxy GTP	0.25 mM
DMSO	5%
Betaine	1.0 M
Taq DNA polymerase	2.5 U
DNA	5.0 ng/µl
Total	20.0 µl

Taq DNA polymerase, buffer (QIAGEN, Germany)

PCR protocol (PP7)

Temp	Time	Cycle
98°C	10 min	1
97°C 64°C 68°C	35 sec 2 min 8 min	10
97°C 64°C 68°C	35 sec 2 min 8-16 min	25 Every cycle +20 sec

Ramping up and down: 0.5°C/sec

Capillary electrophoresis

PCR product	1.0 µl
Size standard	0.1 µl
Formamide	19.9 µl
Total	21.0 µl

95°C 5min and analyse with DNA analyzer.

Amplicon length analysis…not performed.

Supple table 3D. Methods of each laboratory

Laboratory N

RP-PCR

	List	
Primer 1	P1	FAM-AGTCGCTAGAGGCGAAAGC
Primer 2 (with GGGGCC repeat)	P2	tacgcatcccagtttgagacgGGGGCCGGGGCCGGGGCCGGGG
Primer 3	P3	tacgcatcccagtttgagacg
Size standard	S1	GeneScan 500ROX Size Standard (Applied Biosystems, USA)
Analysis machine	M2	3130 Genetic Analyzer (Applied Biosystems, USA)
Analysis software	A3	Genotyper v4 (Applied Biosystems, USA)

PCR reaction (PR9)

Products	Final conc.
True Allele PCR premix	1x
Primer 1	1.4 µM
Primer 2	0.7 µM
Primer 3	1.4 µM
7-deaza-2-deoxy GTP	0.16 mM
DMSO	7%
Betaine	0.83 M
$MgCl_2$	0.83 mM
DNA	3.3ng/µl
Total	30.0 µl

True Allele PCR premix (Applied Biosystems, USA)

PCR protocol (PP1)

Temp	Time	Cycle
95°C	15 min	1
94°C 70-58°C 72°C	1 min 1 min 3 min	2-8 *
94°C 56°C 72°C	1 min 1 min 3 min	5
72°C	10 min	1
4°C	hold	1

* Every 2°C decrease add 1 cycle.
Ramp speed: 1°C/sec

Capillary electrophoresis

PCR product	2.0 µl
Size standard	0.5 µl
Formamide	12.5 µl
Total	15.0 µl

95°C 2min and analyse with DNA analyzer.

Amplicon length analysis…not performed.

Supplementary table 4. The definitions of an expansion of the GGGGCC-repeat in *C9orf72* as used in 13 laboratories.

GGGGCC-repeat number (4 laboratories)

> 24 repeats

≥ 30 repeats

> 30 repeats (2 laboratories)

RP-PCR shape (2 laboratories)

Clear saw-tooth sloping pattern

GGGGCC-repeat number + RP-PCR shape (1 laboratory)

>30 repeats + Stutter profile in RP-PCR

GGGGCC-repeat number + RP-PCR shape + Amplicon-length analysis result (1 laboratory)

Saw-tooth pattern over 460 bp (30 repeats) in RP-PCR + One amplicon in amplicon-length analysis

RP-PCR shape + Amplicon-length analysis result (1 laboratory)

Saw-tooth pattern in RP-PCR + One amplicon in amplicon-length analysis

RP-PCR shape + Amplicon-length analysis result + Southern blot result (4 laboratories)

Saw-tooth pattern with concave shape in RP-PCR + One amplicon in amplicon-length analysis + Expanded band in Southern blot

One amplicon in amplicon-length analysis + Saw-tooth profile in RP-PCR + Expanded band in Southern blot

One amplicon in amplicon-length analysis + Long stutter in RP-PCR + Expanded band in Southern blot (2 laboratories)

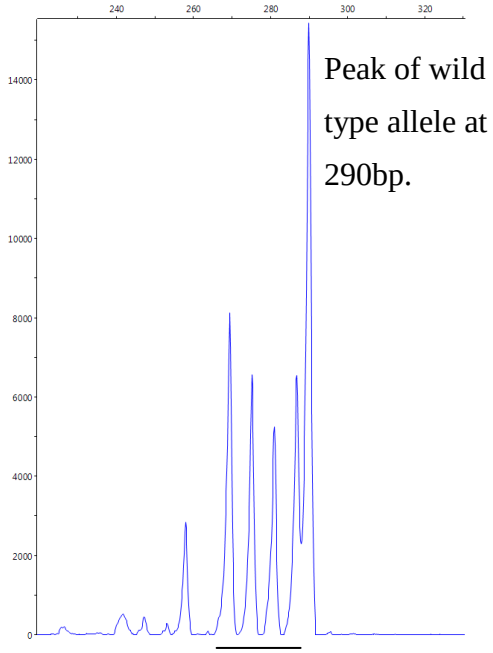
Supplementary table 5. Summary of the Southern blot methods of three laboratories.

	Laboratory I	Laboratory II	Laboratory III
Labelling	Radioisotope	Radioisotope	DIG system
Dose of gDNA	10 µg	10 µg	6-10 µg
Probe primer-F	TGACACACCAAGCGTCATCT	TATTAAGGTTCGCACACGCTA	AGAACAGGACAAGTTGCC
Probe primer-R	CACTGTGGGAGCAGTGTCAT	GACGGCTGACACACCAAG	AACACACACCTCCTAAACC
Probe length	1000bp	210 bp	241 bp
Restriction enzyme	Xba I and Hind III	Xba I and Hind III	Xba I

Supplementary Figure 1a,b,c,d

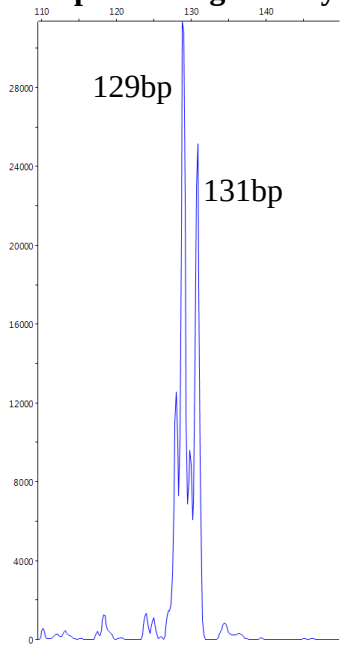
A

RP-PCR



Four peaks of mutant allele.

Amplicon-length analysis

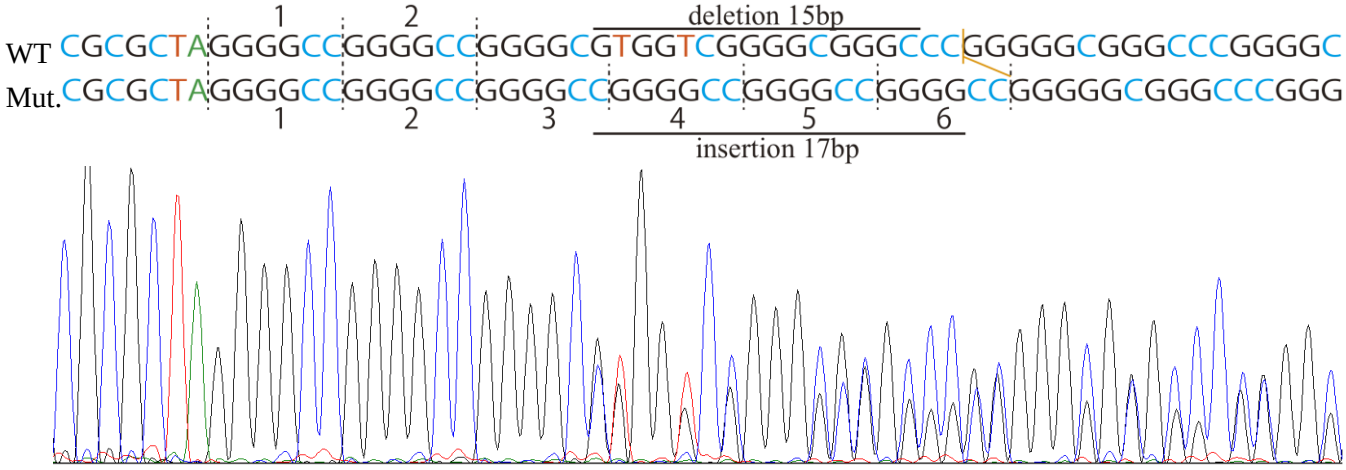


129bp: Peak of wild type allele with 2 repeats.

131bp: Peak of mutant allele with 6 repeats.

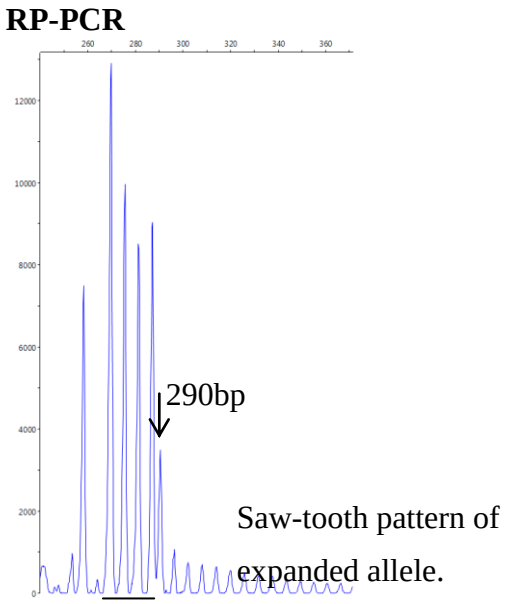
B

Sequence

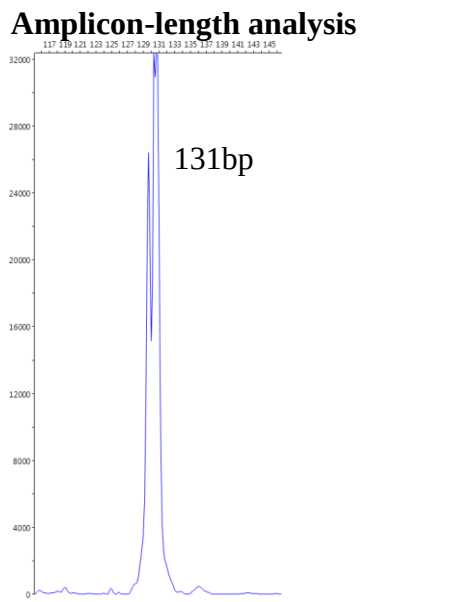


Supplementary figure 1a.b.c.d (cont.)

C

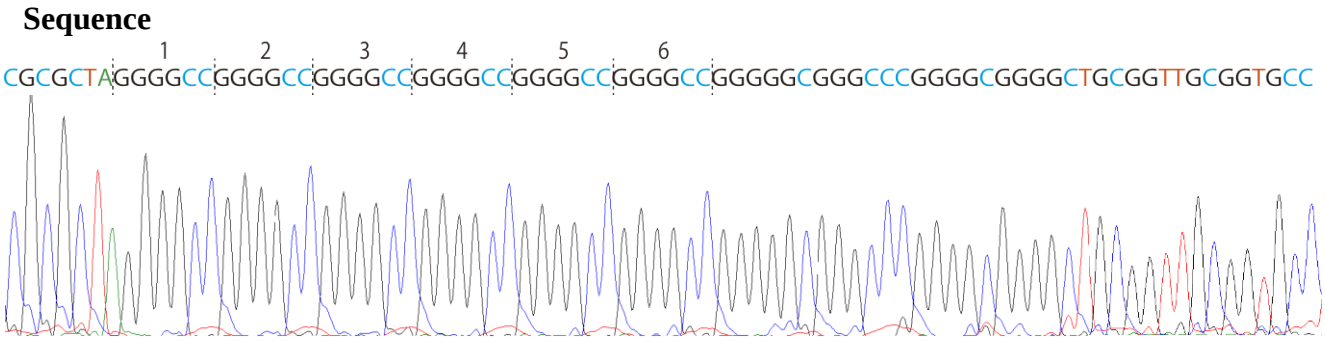


Four peaks of mutant allele.



131bp: Peak of mutant allele with 6 repeats.

D



Supplementary figure 1. Detailed analyses of the results of the three samples with mutations.

(A) The Rp-PCR and amplicon length analysis of sample 8.1. In RP-PCR there are four peaks before the wild-type allele peak at 290 bp. In amplicon-length analysis there are two peaks at 129bp (wild-type allele) and 131bp (the mutant allele), respectively and the two peaks are close together.

As a consequence, four laboratories interpreted this sample as having one amplicon.

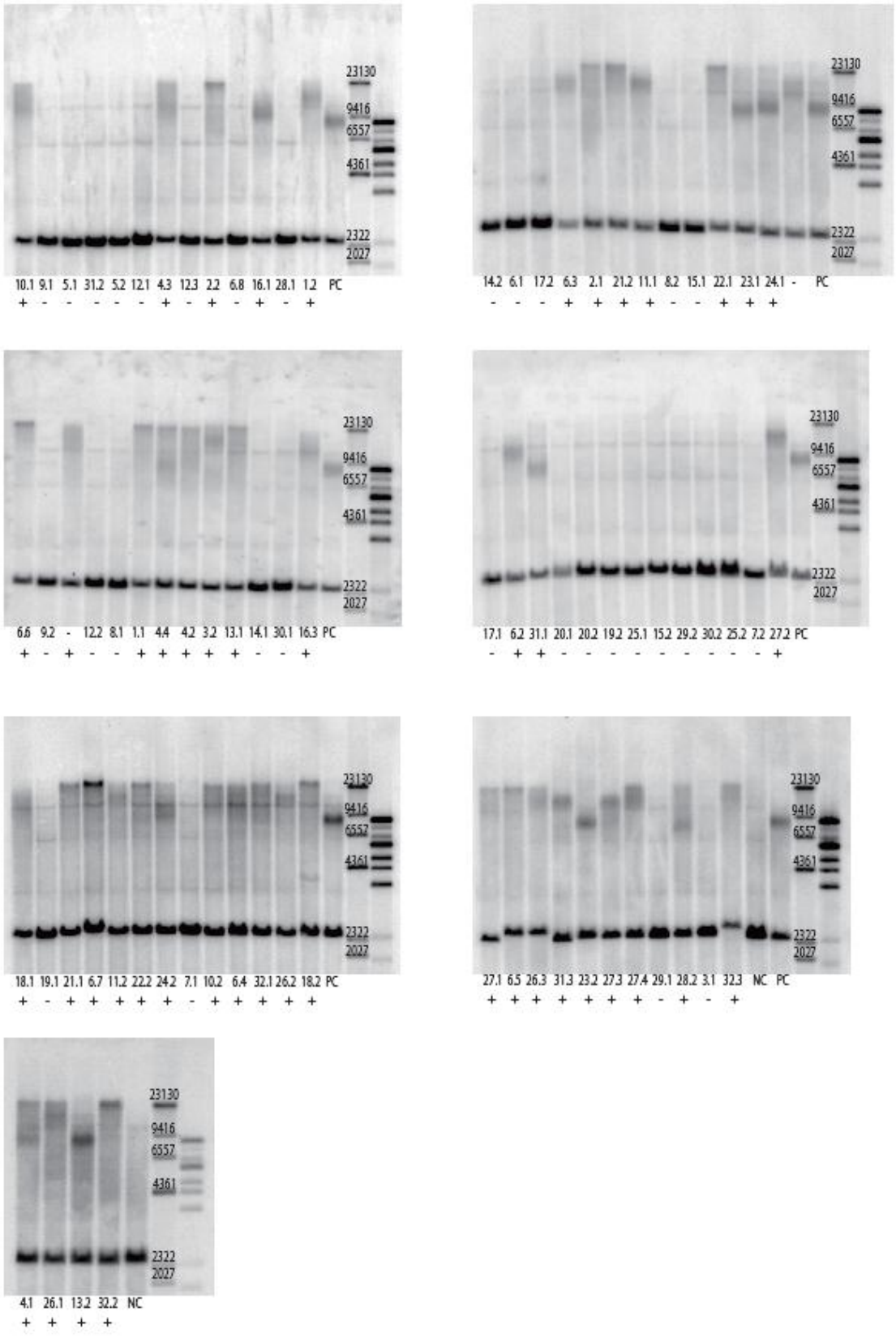
(B) The sequence of sample 8.1. The wild-type allele has two GGGGCC-repeats and the mutant allele has six GGGGCC-repeats with a complex 15 deletion/17bp insertion mutation. Sample 8.1 and Sample 8.2 both carries this mutation.

(C) The RP-PCR and amplicon length analysis results of sample 32.1. In RP-PCR there are four peaks before the 290bp peak (mutant allele) and a saw-tooth pattern peaks (expanded allele). In amplicon-length analysis there is one peak at 131bp (mutant allele) with 6 repeats.

(D) The sequence of 32.1 illustrating the mutant allele which has six repeats with a complex 15bp deletion/17bp insertion mutation.

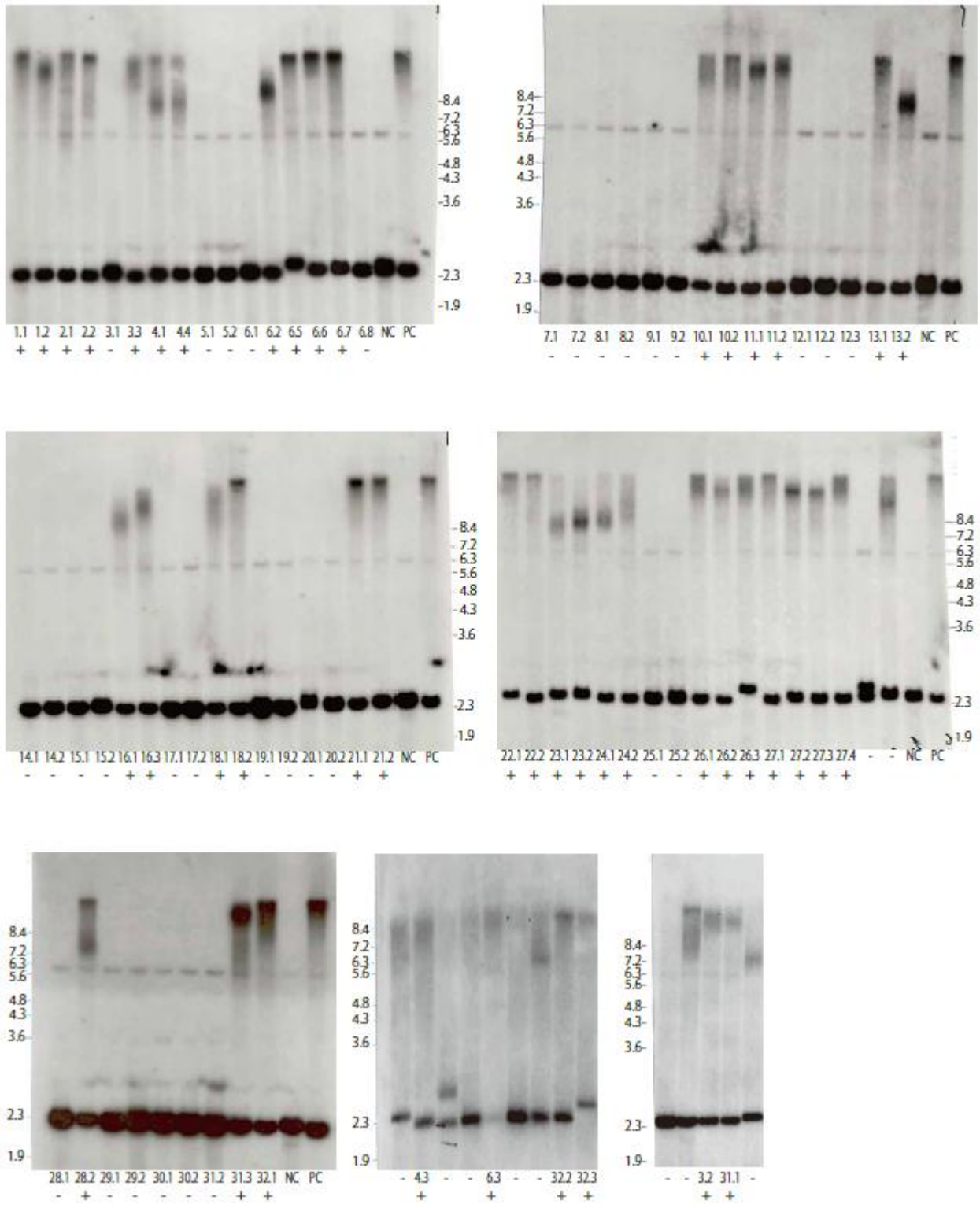
Supplementary Figure 2

Laboratory I



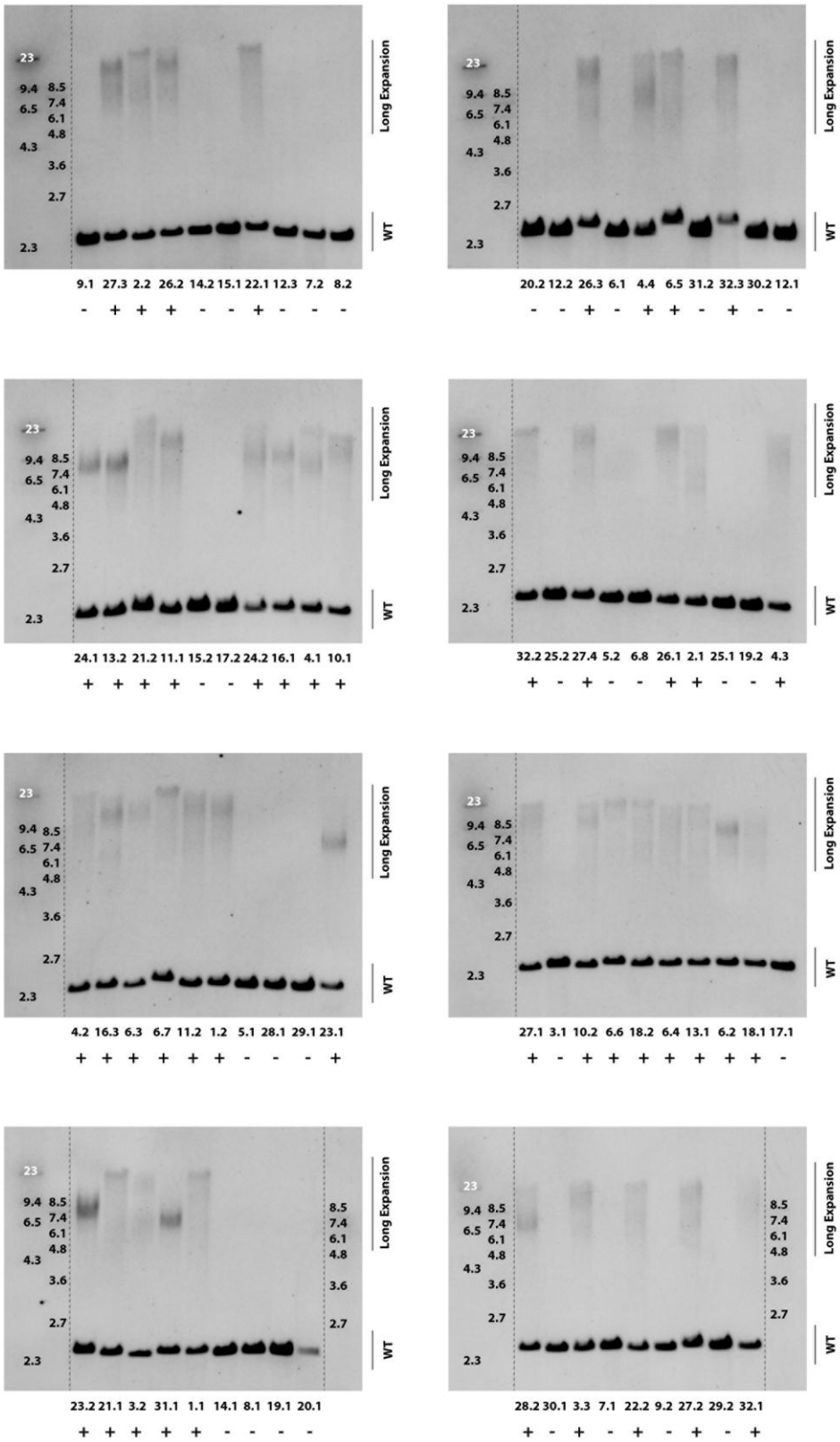
Supplementary Figure 2 (cont.)

Laboratory II



Supplementary Figure II (cont.)

Laboratory III



Supplementary Figure 2. The Southern blot results in three laboratories.

Laboratory III ran out of DNA from one individual (sample 31.1). This laboratory therefore only

performed SB on 77 samples.

PC: Positive control, NC: negative control, +: sample with large repeat expansion, - :sample without large repeat expansion.