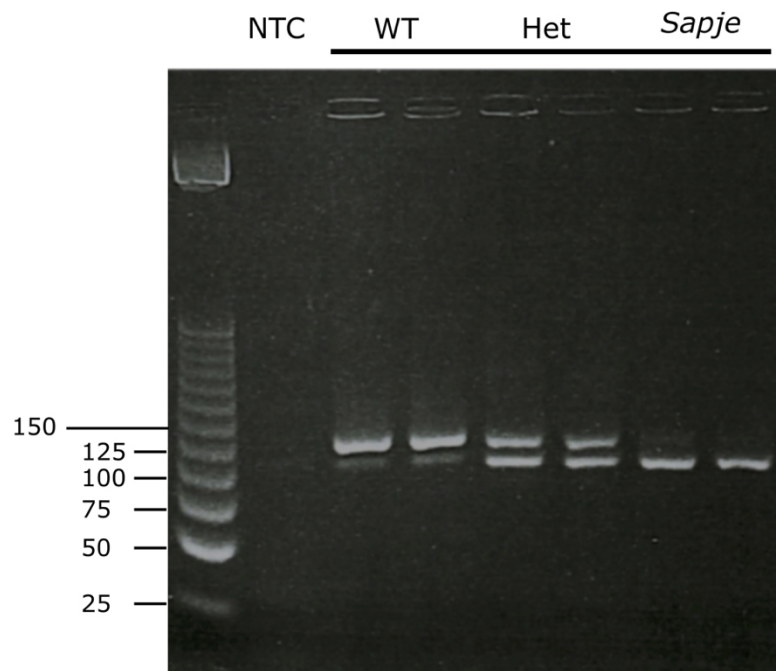


A genotyping method combining primer competition PCR with HRM analysis to identify point mutations in Duchenne animal models

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Supplementary information



Supplementary Figure S1. Optimization of the pcPCR extension and annealing temperature. pcPCR amplification at 60°C results in non-specific additional bands in homozygous samples. These unspecific bands are not observed or greatly diminished at 65°C (Figures 2, 3).

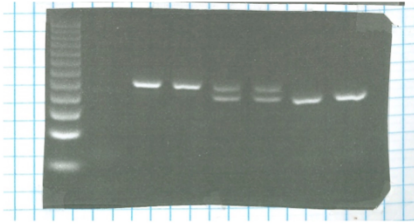


Figure 2

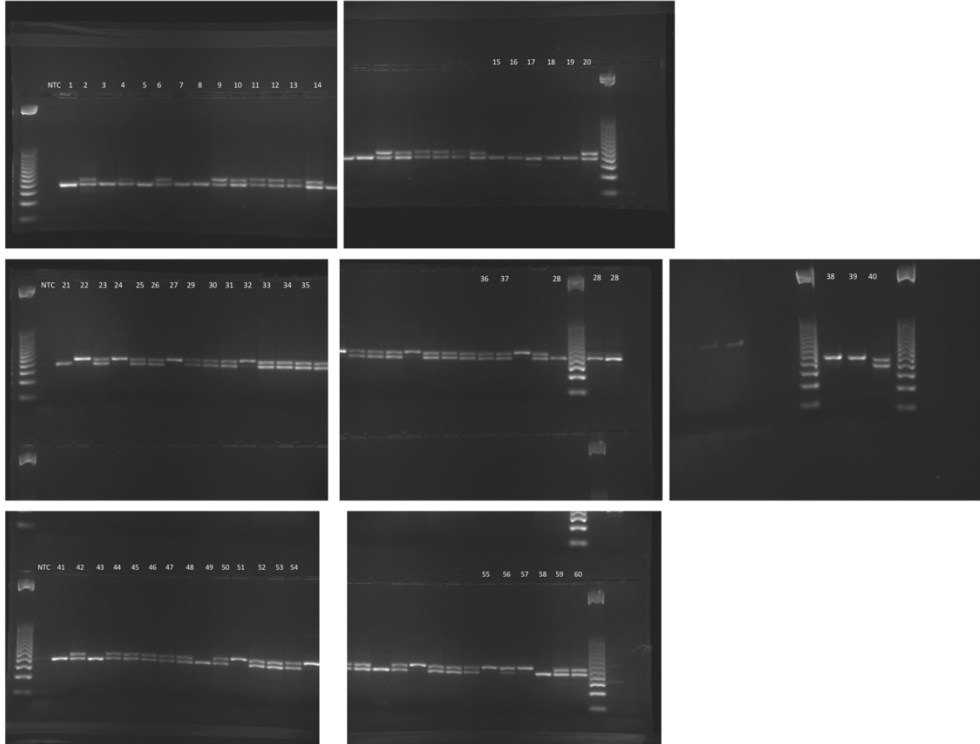


Figure 3

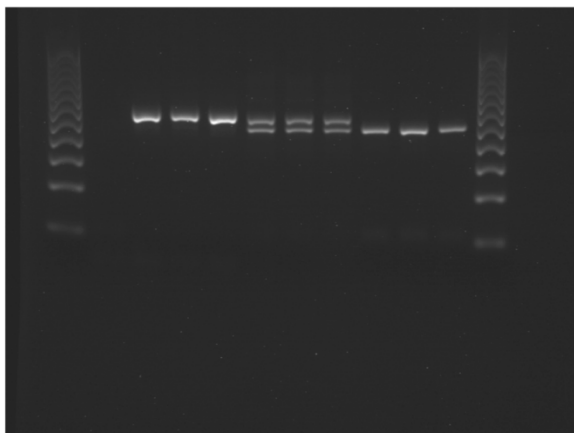
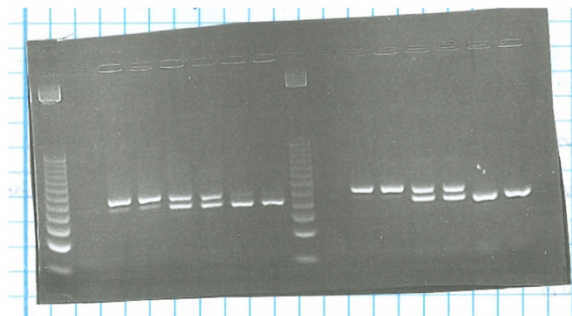


Figure 5 blots



Supplementary Figure 1

Supplementary Figure S2. Uncropped original images from agarose gels

Supplementary Table S1. Genotype and phenotype analyses in 60 zebrafish larvae. *Sapje* phenotype was determined by birefringence analysis. HRM data shows genotypes resolved by HRM analysis of pcPCR products. Cluster, percentage confidence (highest % value), and melting temperature (T_m , mean \pm SD) values are extracted from the HRM analysis. Gel refers to genotypes resolved by 3% agarose gel electrophoresis. Seq refers to genotypes resolved by Sanger sequencing.

Sample	Phenotype	Cluster	Confidence %	T_m	HRM	Gel	Seq
1	<i>Sapje</i>	2	99.8	79.17 \pm 0.06	<i>Sapje</i>	<i>Sapje</i>	<i>Sapje</i>
2	Unaffected	1	98.0	78.77 \pm 0.06	Het	Het	
3	<i>Sapje</i>	2	98.7	79.13 \pm 0.06	<i>Sapje</i>	<i>Sapje</i>	
4	<i>Sapje</i>	2	92.9	78.9 \pm 0.0	<i>Sapje</i>	<i>Sapje</i>	
5	<i>Sapje</i>	2	99.9	79.1 \pm 0.0	<i>Sapje</i>	<i>Sapje</i>	
6	Unaffected	1	99.4	78.8 \pm 0.0	Het	Het	Het
7	<i>Sapje</i>	2	94.2	78.97 \pm 0.06	<i>Sapje</i>	<i>Sapje</i>	
8	<i>Sapje</i>	2	99.3	79.13 \pm 0.06	<i>Sapje</i>	<i>Sapje</i>	<i>Sapje</i>
9	Unaffected	1	94.6	78.77 \pm 0.06	Het	Het	Het
10	Unaffected	1	98.1	78.83 \pm 0.06	Het	Het	
11	Unaffected	1	99.3	78.8 \pm 0.0	Het	Het	
12	Unaffected	1	99.4	78.77 \pm 0.06	Het	Het	
13	Unaffected	1	99.7	78.8 \pm 0.0	Het	Het	
14	Unaffected	1	98.5	78.77 \pm 0.06	Het	Het	
15	<i>Sapje</i>	2	99.5	79.1 \pm 0.0	<i>Sapje</i>	<i>Sapje</i>	
16	<i>Sapje</i>	2	97.9	79 \pm 0.10	<i>Sapje</i>	<i>Sapje</i>	<i>Sapje</i>
17	<i>Sapje</i>	2	98.0	79.1 \pm 0.0	<i>Sapje</i>	<i>Sapje</i>	
18	<i>Sapje</i>	2	99.6	79.1 \pm 0.0	<i>Sapje</i>	<i>Sapje</i>	
19	<i>Sapje</i>	2	99.6	79.1 \pm 0.0	<i>Sapje</i>	<i>Sapje</i>	
20	Unaffected	1	94.5	78.7 \pm 0.0	Het	Het	
21	<i>Sapje</i>	2	99.3	79.1 \pm 0.0	<i>Sapje</i>	<i>Sapje</i>	<i>Sapje</i>
22	Unaffected	3	98.4	78.7 \pm 0.0	WT	WT	WT
23	Unaffected	1	99.6	78.8 \pm 0.0	Het	Het	Het
24	Unaffected	3	98.6	78.7 \pm 0.0	WT	WT	
25	Unaffected	1	99.8	78.7 \pm 0.17	Het	Het	
26	Unaffected	1	99.5	78.8 \pm 0.0	Het	Het	
27	Unaffected	3	98.3	78.7 \pm 0.0	WT	WT	WT
28	<i>Sapje</i>	2	60.4	78.9	<i>Sapje</i>	<i>Sapje</i>	<i>Sapje</i>
		1	97.7	78.9	Het		
		2	98.0	79	<i>Sapje</i>		
29	Unaffected	1	99.4	78.8 \pm 0.0	Het	Het	
30	Unaffected	1	99.8	78.8 \pm 0.0	Het	Het	
31	Unaffected	1	99.4	78.8 \pm 0.0	Het	Het	
32	Unaffected	3	98.7	78.7 \pm 0.0	WT	WT	
33	Unaffected	1	99.2	78.77 \pm 0.06	Het	Het	
34	Unaffected	1	99.7	78.8 \pm 0.0	Het	Het	
35	Unaffected	1	99.5	78.8 \pm 0.0	Het	Het	
36	Unaffected	1	99.6	78.7 \pm 0.0	Het	Het	
37	Unaffected	1	99.3	78.8 \pm 0.0	Het	Het	
38	Unaffected	3	98.2	78.7 \pm 0.0	WT	WT	
39	Unaffected	3	98.2	78.7 \pm 0.0	WT	WT	WT

40	Unaffected	1	71.7	78.6	Het	Het	Het
		1	98.3	78.7	Het		
		3	92.6	78.6	WT		
41	<i>Sapje</i>	2	99.3	79.1 ± 0.0	<i>Sapje</i>	<i>Sapje</i>	<i>Sapje</i>
42	Unaffected	1	99.5	78.8 ± 0.0	Het	Het	
43	<i>Sapje</i>	2	99.6	79 ± 0.0	<i>Sapje</i>	<i>Sapje</i>	<i>Sapje</i>
44	Unaffected	1	99.7	78.7 ± 0.0	Het	Het	
45	Unaffected	1	99.6	78.8 ± 0.0	Het	Het	
46	Unaffected	1	99.7	78.7 ± 0.0	Het	Het	Het
47	Unaffected	1	99.5	78.77 ± 0.06	Het	Het	
48	Unaffected	1	99.1	78.7 ± 0.0	Het	Het	
49	<i>Sapje</i>	2	99.6	79.1 ± 0.0	<i>Sapje</i>	<i>Sapje</i>	
50	Unaffected	1	99.7	78.8 ± 0.0	Het	Het	
51	Unaffected	3	98.7	78.6 ± 0.17	WT	WT	
52	Unaffected	1	99.4	78.77 ± 0.06	Het	Het	
53	Unaffected	1	99.8	78.73 ± 0.06	Het	Het	
54	Unaffected	1	99.7	78.73 ± 0.06	Het	Het	Het
55	Unaffected	3	99.2	78.77 ± 0.06	WT	WT	WT
56	Unaffected	3	99.2	78.73 ± 0.06	WT	WT	WT
57	Unaffected	3	98.7	78.73 ± 0.06	WT	WT	
58	<i>Sapje</i>	2	98.9	79.1 ± 0.0	<i>Sapje</i>	<i>Sapje</i>	
59	Unaffected	1	99.6	78.77 ± 0.06	Het	Het	
60	Unaffected	1	99.7	78.8 ± 0.0	Het	Het	

Supplementary Table S2. Raw dataset and statistical parameters generated from the intra- and inter-assays using 60 zebrafish larvae. Melting temperatures (T_m, Mean, and SD) from 3 pcPCR-HRM independent runs, with their corresponding coefficients of variation (CV, %).

Genotype	Sample	Run 1					Run 2					Run 3					Inter-assay			
		T _m	Mean	SD	CV %	Intra-assay CV%	T _m	Mean	SD	CV %	Intra-assay CV%	T _m	Mean	SD	CV %	Intra-assay CV%	Mean	SD	CV %	Inter-assay CV%
<i>Sapje</i>	1	79.1 79.2 79.2	79.17	0.06	0.07		79.2 79.2 79.2	79.2	0	0		79.1 79.1 79.1	79.1	0	0		79.16	0.05	0.06	
	3	79.2 79.1 79.1	79.13	0.06	0.07		79.2 79.1 79.1	79.13	0.06	0.07		79.1 79.1 79.1	79.1	0	0		79.12	0.02	0.02	
	4	78.9 78.9 78.9	78.9	0	0		79.0 79.0 79.0	79.0	0	0		79.1 79.1 79.1	79.1	0	0		79	0.10	0.13	
	5	79.1 79.1 79.1	79.1	0	0		79.2 79.1 79.1	79.13	0.06	0.07		79.1 79.1 79.0	79.07	0.06	0.07		79.1	0.03	0.04	
	7	79.0 79.0 78.9	78.97	0.06	0.07		79.0 79.0 78.9	78.97	0.06	0.07		78.8 78.8 78.8	78.8	0	0		78.91	0.10	0.12	
	8	79.2 79.1 79.1	79.13	0.06	0.07		79.2 79.2 79.1	79.17	0.06	0.07		79.1 79.1 79.1	79.1	0	0		79.13	0.03	0.04	
	15	79.1 79.1 79.1	79.1	0	0		79.1 79.1 79.1	79.1	0	0		79.1 79.1 79.1	79.1	0	0		79.1	0	0	
	16	78.9 79.0 79.1	79	0.10	0.13		79.0 79.1 79.1	79.07	0.06	0.07		79.2 79.1 79.1	79.13	0.06	0.07		79.07	0.07	0.08	
	17	79.1 79.1 79.1	79.1	0	0		79.1 79.1 79.1	79.1	0	0		79.1 79.1 79.1	79.1	0	0		79.1	0	0	
	18	79.1 79.1 79.1	79.1	0	0		79.1 79.1 79.2	79.13	0.06	0.07		79.1 79.1 79.1	79.1	0	0		79.11	0.02	0.02	
	19	79.1 79.1 79.1	79.1	0	0		79.1 79.2 79.2	79.17	0.06	0.07		79.1 79.1 79.1	79.1	0	0		79.12	0.04	0.05	
	21	79.1 79.1 79.1	79.1	0	0		79.2 79.1 79.1	79.13	0.06	0.07		79.0 79.0 79.0	79.0	0	0		79.08	0.07	0.09	
	28	78.9 78.9 79.0	78.93	0.06	0.07		79.0 79.0 79.1	79.03	0.06	0.07		79.1 79.1 79.1	79.1	0	0		79.02	0.08	0.11	
	41	79.1 79.1 79.1	79.1	0	0		79.1 79.1 79.1	79.1	0	0		79.1 79.1 79.0	79.07	0.06	0.07		79.09	0.02	0.02	
	43	79.0 79.0 79.0	79	0	0		79.1 79.1 79.0	79.07	0.06	0.07		78.8 78.8 78.8	78.8	0	0		78.96	0.14	0.18	
	49	79.1 79.1 79.1	79.1	0	0		79.1 79.1 79.1	79.1	0	0		79.0 79.1 79.0	79.03	0.06	0.07		79.08	0.04	0.05	
	58	79.1 79.1 79.1	79.1	0	0		79.1 79.1 79.1	79.1	0	0		79.0 79.0 79.0	79	0	0		79.07	0.06	0.07	
		Total		79.07	0.08		0.10		79.10	0.06		0.08		79.05	0.10		0.13	79.07	0.03	

WT	22	78.7 78.7 0 0		78.8 78.77 0.06 0.07		78.7 78.7 0 0		78.72 0.04 0.05
		78.7		78.8		78.7		
		78.7		78.7		78.7		
	24	78.7 78.7 0 0		78.7 78.7 0 0		78.7 78.7 0 0		78.7 0 0
		78.7		78.7		78.7		
		78.7		78.7		78.7		
	27	78.7 78.7 0 0		78.8 78.77 0.06 0.07		78.7 78.7 0 0		78.72 0.04 0.05
		78.7		78.8		78.7		
		78.7		78.7		78.7		
	32	78.7 78.7 0 0		78.8 78.8 0 0		78.8 78.77 0.06 0.07		78.76 0.05 0.06
	78.7		78.8		78.7			
	78.7		78.8		78.8			
38	78.7 78.7 0 0		78.8 78.73 0.06 0.07		78.6 78.6 0 0		78.68 0.07 0.09	
	78.7		78.7		78.6			
	78.7		78.7		78.6			
39	78.7 78.7 0 0		78.8 78.73 0.06 0.07		78.7 78.7 0 0		78.71 0.02 0.02	
	78.7		78.7		78.7			
	78.7		78.7		78.7			
51	78.7 78.6 0.17 0.22		78.7 78.63 0.12 0.15		78.6 78.6 0 0		78.61 0.02 0.02	
	78.7		78.7		78.6			
	78.4		78.5		78.6			
55	78.7 78.77 0.06 0.07		78.8 78.8 0 0		78.6 78.6 0 0		78.72 0.11 0.14	
	78.8		78.8		78.6			
	78.8		78.8		78.6			
56	78.7 78.73 0.06 0.07		78.8 78.8 0 0		78.7 78.63 0.06 0.07		78.72 0.08 0.11	
	78.7		78.8		78.6			
	78.8		78.8		78.6			
57	78.7 78.73 0.06 0.07		78.8 78.8 0 0		78.7 78.7 0 0		78.74 0.05 0.06	
	78.7		78.8		78.7			
	78.8		78.8		78.7			
Total	78.70 0.04	0.05	78.75 0.05	0.07	78.67 0.06	0.07	78.71 0.04	0.05
Het	2	78.8 78.77 0.06 0.07		78.8 78.8 0 0		78.6 78.8 0 0		78.72 0.11 0.14
		78.8		78.8		78.6		
		78.7		78.8		78.6		
	6	78.8 78.8 0 0		78.8 78.8 0 0		78.7 78.7 0 0		78.77 0.06 0.07
		78.8		78.8		78.7		
		78.8		78.8		78.7		
	9	78.8 78.77 0.06 0.07		78.8 78.8 0 0		78.7 78.7 0 0		78.76 0.05 0.06
		78.7		78.8		78.7		
		78.8		78.8		78.7		
	10	78.9 78.83 0.06 0.07		78.9 78.87 0.06 0.07		78.6 78.6 0 0		78.77 0.15 0.18
		78.8		78.9		78.6		
		78.8		78.8		78.6		
	11	78.8 78.8 0 0		78.8 78.8 0 0		78.7 78.7 0 0		78.77 0.06 0.07
	78.8		78.8		78.7			
	78.8		78.8		78.7			
12	78.8 78.77 0.06 0.07		78.8 78.8 0 0		78.7 78.7 0 0		78.76 0.05 0.06	
	78.7		78.8		78.7			
	78.8		78.8		78.7			
13	78.8 78.8 0 0		78.8 78.8 0 0		78.7 78.7 0 0		78.77 0.06 0.07	
	78.8		78.8		78.7			
	78.8		78.8		78.7			
14	78.7 78.77 0.06 0.07		78.8 78.8 0 0		78.8 78.77 0.06 0.07		78.78 0.02 0.02	
	78.8		78.8		78.8			
	78.8		78.8		78.7			
20	78.7 78.7 0 0		78.8 78.8 0 0		78.7 78.7 0 0		78.73 0.06 0.07	
	78.7		78.8		78.7			
	78.7		78.8		78.7			
23	78.8 78.8 0 0		78.8 78.8 0 0		78.7 78.7 0 0		78.77 0.06 0.07	
	78.8		78.8		78.7			
	78.8		78.8		78.7			
25	78.8 78.7 0.17 0.22		78.8 78.73 0.12 0.15		78.8 78.77 0.06 0.07		78.73 0.03 0.04	
	78.8		78.8		78.8			
	78.5		78.6		78.7			
26	78.8 78.8 0 0		78.8 78.8 0 0		78.8 78.73 0.06 0.07		78.78 0.04 0.05	
	78.8		78.8		78.7			
	78.8		78.8		78.7			
29	78.8 78.8 0 0		78.8 78.8 0 0		78.7 78.7 0 0		78.77 0.06 0.07	
	78.8		78.8		78.7			

		78.8			78.8			78.7				
	30	78.8 78.8 0 0			78.8 78.8 0 0			78.7 78.7 0 0				78.77 0.06 0.07
		78.8			78.8			78.7				
		78.8			78.8			78.7				
	31	78.8 78.8 0 0			78.8 78.8 0 0			78.7 78.7 0 0				78.77 0.06 0.07
		78.8			78.8			78.7				
		78.8			78.8			78.7				
	33	78.8 78.77 0.06 0.07			78.9 78.87 0.06 0.07			78.7 78.7 0 0				78.78 0.08 0.11
		78.8			78.9			78.7				
		78.7			78.8			78.7				
	34	78.8 78.8 0 0			78.8 78.8 0 0			78.7 78.7 0 0				78.77 0.06 0.07
		78.8			78.8			78.7				
		78.8			78.8			78.7				
	35	78.8 78.8 0 0			78.8 78.8 0 0			78.7 78.7 0 0				78.77 0.06 0.07
		78.8			78.8			78.7				
		78.8			78.8			78.7				
	36	78.7 78.7 0 0			78.8 78.8 0 0			78.7 78.7 0 0				78.73 0.06 0.07
		78.7			78.8			78.7				
		78.7			78.8			78.7				
	37	78.8 78.8 0 0			78.8 78.8 0 0			78.7 78.7 0 0				78.77 0.06 0.07
		78.8			78.8			78.7				
		78.8			78.8			78.7				
	40	78.6 78.63 0.06 0.07			78.7 78.7 0 0			78.6 78.6 0 0				78.64 0.05 0.06
		78.7			78.7			78.6				
		78.6			78.7			78.6				
	42	78.8 78.8 0 0			78.8 78.8 0 0			78.7 78.67 0.06 0.07				78.76 0.08 0.10
		78.8			78.8			78.6				
		78.8			78.8			78.7				
	44	78.7 78.7 0 0			78.8 78.73 0.06 0.07			78.7 78.7 0 0				78.71 0.02 0.02
		78.7			78.7			78.7				
		78.7			78.7			78.7				
	45	78.8 78.8 0 0			78.8 78.8 0 0			78.7 78.7 0 0				78.77 0.06 0.07
		78.8			78.8			78.7				
		78.8			78.8			78.7				
	46	78.7 78.7 0 0			78.8 78.8 0 0			78.7 78.7 0 0				78.73 0.06 0.07
		78.7			78.8			78.7				
		78.7			78.8			78.7				
	47	78.7 78.77 0.06 0.07			78.8 78.8 0 0			78.6 78.6 0 0				78.72 0.11 0.14
		78.8			78.8			78.6				
		78.8			78.8			78.6				
	48	78.7 78.7 0 0			78.7 78.77 0.06 0.07			78.7 78.7 0 0				78.72 0.04 0.05
		78.7			78.8			78.7				
		78.7			78.8			78.7				
	50	78.8 78.8 0 0			78.8 78.8 0 0			78.7 78.7 0 0				78.77 0.06 0.07
		78.8			78.8			78.7				
		78.8			78.8			78.7				
	52	78.7 78.77 0.06 0.07			78.8 78.8 0 0			78.6 78.6 0 0				78.72 0.11 0.14
		78.8			78.8			78.6				
		78.8			78.8			78.6				
	53	78.8 78.73 0.06 0.07			78.8 78.8 0 0			78.7 78.7 0 0				78.74 0.05 0.06
		78.7			78.8			78.7				
		78.7			78.8			78.7				
	54	78.7 78.73 0.06 0.07			78.8 78.8 0 0			78.7 78.7 0 0				78.74 0.05 0.06
		78.7			78.8			78.7				
		78.8			78.8			78.7				
	59	78.7 78.77 0.06 0.07			78.8 78.83 0.06 0.07			78.7 78.7 0 0				78.77 0.07 0.08
		78.8			78.8			78.7				
		78.8			78.9			78.7				
	60	78.8 78.8 0 0			78.8 78.8 0 0			78.6 78.6 0 0				78.73 0.12 0.15
		78.8			78.8			78.6				
		78.8			78.8			78.6				
	Total	78.77 0.05	0.06		78.80 0.03	0.04		78.69 0.04	0.06		78.75 0.06	0.07