

**Table e-1. Primers for initial RT amplification**

<b>Patient Mutation</b>	<b>Primer Name</b>	<b>RT-primer Sequence (5'-3')</b>	<b>Size (bp)</b>	<b>Tm (°C)</b>
Deletion exon 45–50	Exon 55-56R1	ACCTTGGAGGTCTTGCCATTG	21	55.4
Deletion exon 48–50	Exon 55-56R1	ACCTTGGAGGTCTTGCCATTG	21	55.4
Deletion exon 49–50	Exon 55-56R1	ACCTTGGAGGTCTTGCCATTG	21	55.4
Deletion exon 50	Exon 55-56R1	ACCTTGGAGGTCTTGCCATTG	21	55.4
Deletion exon 52	Exon 55-56R1	ACCTTGGAGGTCTTGCCATTG	21	55.4

Abbreviations: RT = reverse transcription; Tm = temperature.

**Table e-2. Primers for first-round PCR**

<b>Patient Mutation</b>	<b>Primer</b>	<b>Primer Name</b>	<b>Primary Primer Sequence (5'-3')</b>	<b>Size (bp)</b>	<b>Tm (°C)</b>
Deletion exon 45–50	Forward	h42F1	ACTGTCCGTGAAGAAACGATGATG	24	56.9
	Reverse	h53R2	TGCATCTACTGTATAGGGACCCTCCTT	27	58.6
Deletion exon 48–50	Forward	h45F1	GGATGGCATTGGGCAGCG	18	59.4
	Reverse	h53R2	TGCATCTACTGTATAGGGACCCTCCTT	27	58.6
Deletion exon 49–50	Forward	h46F1	ATGAATTTGTTTTATGGTTGGAGGA	25	54.9
	Reverse	h53R2	TGCATCTACTGTATAGGGACCCTCCTT	27	58.6
Deletion exon 50	Forward	h47F1	AACTGGAGGACCCGTGCTTGTA	22	57.3
	Reverse	h53R2	TGCATCTACTGTATAGGGACCCTCCTT	27	58.6
Deletion exon 52	Forward	h49F	ACCGGATGTGGAAGAGATTTTG	22	54.6
	Reverse	h54R	TTATCATGTGGACTTTTCTGGTATCA	26	53.8

Abbreviations: PCR = polymerase chain reaction; Tm = temperature.

**Table e-3. Primers for second-round nested PCR**

<b>Patient Mutation</b>	<b>Primer</b>	<b>Primer Name</b>	<b>Primary Primer Sequence (5'-3')</b>	<b>Size (bp)</b>	<b>T<sub>m</sub> (°C)</b>
Deletion exon 45–50	Forward	h43Fi	GCCTGTGGAAAGGGTGAAGC	20	55.9
	Reverse	h52Ri	TTTTGGGCAGCGGTAATGAG	20	55.2
Deletion exon 48–50	Forward	h46F2	CACTTGAACCTGGAAAAGAGCAG	23	54.8
	Reverse	h52Ri	TTTTGGGCAGCGGTAATGAG	20	55.2
Deletion exon 49–50	Forward	h47F1	AAGTGGAGGACCCGTGCTTGTA	22	57.3
	Reverse	h52Ri	TTTTGGGCAGCGGTAATGAG	20	55.2
Deletion exon 50	Forward	h48Fo	GTTTCCAGAGCTTTACCTGAGAA	23	51.5
	Reverse	h52Ro	TCTTGATTGCTGGTCTTGTTTTT	23	52.6
Deletion exon 52	Forward	h50Fo	GTTAGAAGATCTGAGCTCTGAGTGG	25	52.6
	Reverse	h53R2	TGCATCTACTGTATAGGGACCCTCCTT	27	58.6

Abbreviations: PCR = polymerase chain reaction; T<sub>m</sub> = temperature.

**Table e-4. Flagship pathologist individual patients, percent dystrophin-positive fibers**

Subject Number <sup>a</sup>	Study Visit	Percent Dystrophin-Positive Fibers			
		Rater 1	Rater 2	Rater 3	Average
<b>Treated Patients</b>					
A	Week 180	3.1624	6.6736	3.7948	4.5436
B	Week 180	1.1795	1.8113	1.2602	1.4170
C	Week 180	26.2412	33.1382	25.2288	28.2027
E	Week 180	19.7439	25.2252	17.1994	20.7228
F	Week 180	7.3295	8.9733	4.9432	7.0820
G	Week 180	9.9343	16.5651	11.7427	12.7474
H	Week 180	15.9671	26.9596	21.5111	21.4793
I	Week 180	21.5631	29.3457	20.9599	23.9562
J	Week 180	33.9856	42.9014	23.6238	33.5036
K	Week 180	16.6747	23.0722	17.6678	19.1382
L	Week 180	16.6044	19.5841	19.2652	18.4846
				<b>Mean</b>	<b>17.39</b>
<b>Untreated Samples</b>					
G		0.9926	1.6409	0.6410	1.0915
K		2.5283	2.6859	2.5323	2.5822
L		0.0000	0.3854	0.1866	0.1907
DMD 1		0.0000	0.3232	0.1404	0.1546
DMD 2		0.1096	0.6339	0.1126	0.2854
DMD 3		3.7158	4.0333	4.1064	3.9518

DMD 4	0.3682	0.3997	0.4036	0.3905
DMD 5	0.3205	0.3846	0.3731	0.3594
DMD 6	1.1105	1.2699	0.9373	1.1059
			<b>Mean</b>	<b>1.12</b>

<sup>a</sup>Patient D declined to participate in the optional muscle biopsy at study week 180.

The interrater reliability (IRR) analysis of the 3 raters using their average measurements showed an excellent level of concordance based on the commonly cited cutoffs for qualitative ratings of agreement based on the intraclass correlation coefficient (ICC) values.<sup>16</sup> Table e-5 shows the IRR calculated values for treated patients, control samples, and the combination of treated patients and control samples. ICC values ranged from 0.839 to 0.994 across all comparisons, with the 95% confidence interval lower bound value ranging from 0.597 to 0.991. Pairwise (3 possible combinations of 2 raters each) and an all-rater ICC evaluation were performed.<sup>17-19</sup>

**Table e-5. Concordance, Flagship Pathologist**

<b>Treated Patients, Study Week 180</b>			
<b>Raters</b>	<b>ICC</b>	<b>95% Confidence Interval Lower Bound</b>	<b>Concordance Established (95% LB &gt;0.40)</b>
Rater 1, Rater 2, Rater 3	0.917	0.849	Yes
Rater 1, Rater 2	0.911	0.625	Yes
Rater 1, Rater 3	0.889	0.851	Yes
Rater 2, Rater 3	0.839	0.597	Yes
<b>Control Samples, Study Week 180</b>			
<b>Raters</b>	<b>ICC</b>	<b>95% Confidence Interval Lower Bound</b>	<b>Concordance Established (95% LB &gt;0.40)</b>
Rater 1, Rater 2, Rater 3	0.986	0.979	Yes
Rater 1, Rater 2	0.971	0.953	Yes
Rater 1, Rater 3	0.994	0.991	Yes
Rater 2, Rater 3	0.970	0.952	Yes
<b>Treated Patients and Control Samples, Study Week 180</b>			
<b>Raters</b>	<b>ICC</b>	<b>95% Confidence Interval Lower Bound</b>	<b>Concordance Established (95% LB &gt;0.40)</b>
Rater 1, Rater 2, Rater 3	0.943	0.911	Yes
Rater 1, Rater 2	0.937	0.824	Yes
Rater 1, Rater 3	0.924	0.903	Yes
Rater 2, Rater 3	0.891	0.780	Yes

Abbreviations: ICC = intraclass correlation coefficient; LB, lower bound.

**Table e-6. Summary of Becker muscular dystrophy and normal sample dystrophin expression**

Patient ID	Assay			
	Western Blot	Bioquant	PDPF Flagship	PDPF NCH
Untreated BMD and Normal Control Samples				
BMD 1	32.19	87.3	98.1	97.8
BMD 2	18.43	57.7	94.0	95.5
BMD 3	2.31	29.0	44.2	70.7
Control 1	85.36	145.1	99.2	98.8
Control 2	95.84	106.5	98.6	98.3
Control 3	51.97	87.3	98.5	97.9

Abbreviations: BMD = Becker muscular dystrophy; NCH = Nationwide Children's Hospital; PDPF = percent dystrophin-positive fibers.