

Primers for Analysis of ROSA26 Gene Editing			
ID	Primer Binding Target	Sequence	PCR Amplicon
1	EF1 α promoter (5' end, Reverse Orientation)	TTCTCTAGGCACCGGTTCAATTGC	5' Junction
2	<i>EGFP</i> (3' end, Forward Orientation)	GTCCTGCTGGAGTTCGTGACC	3' Junction
3	<i>ROSA26</i> locus (5' end, Forward orientation, outside of homology arms)	CTGATTGGCTTCTTTCTCCC GCC	5' Junction
4	Ad5 genomic location 18452-18472	CGTACGAGGAGGCACTAAAGC	Internal Ad5 Hexon gene
5	Ad5 genomic location in Hexon gene	CATCATCGAAGGGGTAGCCAT	Internal Ad5 Hexon gene
6	<i>ROSA26</i> locus (3' end, Reverse orientation, past homology arms)	GAGTCAAGCCAGTCCAAGAGAAAGC	3' Junction
7	<i>hAAT</i> (3' end, Forward orientation)	CTCCAAGGCCGTGAAGG	3' Junction
8	<i>ROSA26</i> locus (3' end, Reverse orientation, past homology arms)	AAGCTCACAAGACCTTAGGTCAGGAAAGAC	3' Junction
9	<i>EGFP</i> (Forward orientation)	GAAGCAGCACGACTTCTTCAAG	<i>EGFP</i> qPCR
10	<i>EGFP</i> (Reverse orientation)	AAGTCGATGCCCTT CAGCTC	<i>EGFP</i> qPCR
11	<i>EGFP</i> (Taqman Probe)	[6FAM]CAAGGACGACGGCAACTACAAGACCCGC[BHQ1]	<i>EGFP</i> qPCR
12	<i>Hexon</i> (Forward orientation)	TACGCACGACGTGACCACA	<i>Hexon</i> qPCR
13	<i>Hexon</i> (Reverse orientation)	ATCCTCACGGTCCACAGGG	<i>Hexon</i> qPCR
14	<i>Hexon</i> (Taqman Probe)	[6FAM]ACCGGTCCCAGCGTTTGACGC[BHQ1]	<i>Hexon</i> qPCR
15	<i>mActin</i> (Forward orientation)	AGCTGGAGGACTCCGAGACT	<i>mActin</i> qPCR
16	<i>mActin</i> (Reverse orientation)	TGGCACTTCTCCTGCACCTT	<i>mActin</i> qPCR
17	<i>mActin</i> (Taqman Probe)	[HEX]TAGACGCCTGCACAAGCCGCC[BHQ1]	<i>mActin</i> qPCR
18	<i>ROSA26</i> (3' end, reverse orientation)	[Btn]GAGTCAAGCCAGTCCAAGAGAAAGC	LAM-PCR linear amplification
19	Plus Orientation Linker (5' to 3'), anneals	5'-3' CTGAAGGGCTCAGGTTACACA GGCACGCTCGTAGGAGGTGTTCCAGTTCACCACG	LAM-PCR, Linker

	negative orientation linker		
20	Negative Orientation Linker (3' to 5'), anneals positive orientation linker	3'-5' [3d_C]CGAGCCTCCACAAGGTCAAGTGGTGC GTAC	LAM-PCR, Linker
21	LAM-PCR Linker, Forward orientation	CTGAAGGCTCAGGTTACACAGGCAC	LAM-PCR, 1st nested PCR
22	<i>ROSA26</i> , 3' end reverse orientation (outside of right homology arm)	AAGCTCACAAGACCTTAGGTCAGGAAAGAC	LAM-PCR, 1st nested PCR
23	LAM-PCR Linker, Forward orientation	GCTCGTAGGAGGTGTTCCAGTTCACC	LAM-PCR, 2nd nested PCR
24	<i>ROSA26</i> , 3' end reverse orientation (inside right homology arm)	GAAGGAGCGGGAGAAATGGATATGAAG	LAM-PCR, 2nd nested PCR
25	<i>ROSA26</i> , 5' end, forward orientation (inside left homology arm)	GGAGGGTCAGCGAAAGTAGCTC	LAM-PCR, Wild-type qPCR
26	<i>ROSA26</i> , 5' end, reverse orientation (inside left homology arm)	ATGGACTCAACTTGCACGAACAC	LAM-PCR, Wild-type qPCR
27	<i>ROSA26</i> , 5' end (inside left homology arm)	[6FAM]CTTCCTCTGGGGGAGTCGTTTTACCCGCC[BHQ1]	LAM-PCR, Wild-type qPCR