


Supplementary Information for

CRISPR/Cas9-Mediated Generation of *COL7A1*-Deficient Keratinocyte Model of Recessive Dystrophic Epidermolysis Bullosa

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Table S1: Oligonucleotides and primers

Application	Oligonucleotide/primer	Sequence (5'-3')
Generation of gRNA-coding fragment	ghCOL7A1-S	CACCGACTGCCTAGGATGACGCTG
	ghCOL7A1-A	AAACCAGCGTCATCCTAGGCAGTC
PCR amplification of gRNA target region	COL7A1-F1	TGGATTGGCATAAGGCAGGG
	COL7A1-R2	ATCTGCCCATGCAGAACCTT
Sequencing of the gRNA target region	COL7A1-F2	CACCTCTCTCCC-TGTGCT
	COL7A1-R2	ACGCGCAGGCAAGACCAG
PCR amplification and sequencing of <i>FAT3</i> off-target region	FAT3-F	CTTCCCTGCTCCTTTCCCAG
	FAT3-R	CAAGGACAGAGACACGCTGT
PCR amplification and sequencing of <i>ANKZF1</i> off-target region	ANKZF1-F	GTTGACTGTGGGGACTCTGG
	ANKZF1-R	CCTCCTGCCTTTCCCAACAT
PCR amplification and sequencing of <i>AXIN2</i> off-target region	AXIN2-F	AGGGGTCAGTGCCAAAACAT
	AXIN2-R	ACTACATCCACCACCATGCC
PCR amplification and sequencing of <i>NPHP4</i> off-target region	NPHP4-F	CACCCTTGAAGTCCCTCCAC
	NPHP4-R	GTCCTTCTGAGATCGCGG

PCR; Polymerase chain reaction.

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Table S2: Predicted off-target sites

Off-target sequence	Mismatch position	Mismatch count	MIT off-target score	CDF off-target score	Chromosome position (hg19)	Locus	Genomic context
TAATGCCTAGGATGAGGCTGAAG	..*.....*	2	0.37468	0.034567901	chr9:117192882-117192904	DFNB31	intron
TAATGCCTAGGATGACACTTGGG	..*.....**	3	0.530449741	0.566222222	chr3:113151837-113151859	WDR52-AS1-WDR52/WDR52-AS1	intergenic
TACTGGCTAGGATGAAACTGGGG*.....**	3	0.112770207	0.466666667	chr11:92623965-92623987	FAT3	exon
TCCTGCCAAGGATGACGCTCCGG	.*.....*	3	1.492090395	0.269387755	chr1:1068727-1068749	C1orf159-RP11-465B22.5	intergenic
CACTGCCTGGGCTGACGCTGAGG	*.....*.....*	3	0.846167111	0.15037594	chr20:61012678-61012700	RP5-908M14.5-GATA5	intergenic
TCCTGCCAAGGTTGACGCTGAGA	.*.....*	3	0.276977778	0.014550264	chr18:66654718-66654740	RP11-861L17.3-RP11-861L17.2	intergenic
CACTCCCAGGATGAAGCTGTGG	*.....*.....*	4	0.272333333	0.673469388	chr7:43183211-43183233	HECW1-IT1	intron
TTCTACCCAGGATGAAGCTGTGG	.*.....*	4	0.258544304	0.63030303	chr7:157381590-157381612	PTPRN2	intron
TTCTGCCAAGGATGAAACTGAGG	..*.....**	4	0.104848333	0.543030303	chr22:31646772-31646794	LIMK2	intron
TCATACCTAGGATGGCGCTGGGG	***.....*	4	0.397208228	0.383603175	chr4:28937913-28937935	MESTP3-RP11-292B1.2	intergenic
GACTGTCCACGATGACGCTGGGG	*.....*.....*	4	0.797205949	0.355279503	chr1:5934701-5934723	NPHP4	exon
TACTGCTTTGGAAGAAGCTGAGG*.....*	4	0.039742653	0.3375	chr16:64250429-64250451	RP11-744D14.2-AC012322.1	intergenic
TGCTGCCTTGAAGACACTGAGG	.*.....*.....*	4	0.144140246	0.310153846	chr19:9404893-9404915	CTC-325H20.4-ZNF699/CTC-325H20.4	intergenic
TTCTGCCCACGATGACACTGAGG	.*.....*.....*	4	0.56142625	0.271515151	chr22:20168160-20168182	AC006547.14-XXbac-B444P24.8	intergenic
TACTGACCAGGATGAAGCAGAGG*.....*	4	0.049272081	0.265306123	chr2:109253612-109253634	LIMS1	intron
AAATGCCTAGGAGGAAGCTGGGG	*.....*.....*	4	0.103917498	0.226086956	chr5:68026266-68026288	CTC-537E7.2-CTC-340D7.1	intergenic
AACTCCGAGGATGAAGCTGGGG	*.....*.....*	4	0.272333333	0.22	chr6:100551945-100551967	MCHR2-AS1-PRDX2P4	intergenic
TTCTCCCAGGATGACGCTAAGG	.*.....*	4	0.697447183	0.204545454	chr15:27606535-27606557	GABRG3-AC144833.1	intergenic
AACTGCCTAAAATGTCGCTGAGG	*.....**.....*	4	0.205917821	0.186666667	chr10:101526549-101526571	CUTC-ABCC2	intergenic
TACAGCCCTGGATGGCGCTGGGG	..*.....*	4	0.234278012	0.185714286	chr5:178012701-178012723	COL23A1	intron
AACTCCTTAGGATGACGCAGAGG	*.....*.....*	4	0.359837588	0.182397959	chr12:26013113-26013135	RP11-443N24.3-RP11-443N24.4	intergenic
TACCGCCCAGGCTGAAGCTGGGG	..*.....*.....*	4	0.127203797	0.170278638	chr3:46753357-46753379	TMIE-PRSS50	intergenic
TACTGCCGATGAGGAAGCTGAGG*.....*	4	0.083678408	0.155434782	chr3:612765-612787	AC090044.1	intron
TACTGCCGATGAGGAAGCTGAGG*.....*	4	0.083678408	0.155434782	chr21:9539436-9539458	Gap-CR381670.1	intergenic
AATGCTTAGGATGACTCTGGGG	*.....*.....*	4	0.410516581	0.139648438	chr3:72514258-72514280	RYBP-RP11-654C22.2	intergenic

Table S2: Continued

Off-target sequence	Mismatch position	Mismatch count	MIT off-target score	CDF off-target score	Chromosome position (hg19)	Locus	Genomic context
TTCTGCCGAGAAGGACGCTGAGG	*.....**.....	4	0.307297816	0.139130435	chr7:152536523-152536545	ACTR3B	intron
TTCTGCCGAGAAGGACGCTGAGG	*.....**.....	4	0.307297816	0.139130435	chr7:149967512-149967534	ACTR3C	intron
CCCTGCCTGGGATGATGCTGTGG	**.....*.....	4	0.166395667	0.138147567	chr4:154720444-154720466	SFRP2-AC020703.1	intergenic
CACTGCCTAGGCTGGCACTGAGG	*.....*.....	4	0.08037722	0.136842105	chr13:25476200-25476222	CENPJ	intron
TCCTGCCAGGAAGACCTGAGG	*.....*.....	4	0.23590875	0.127989658	chr19:31454160-31454182	CTC-40019.3-AC020952.1	intergenic
TACTGCCTAGGGTTGAGCTGGGG*.....	4	0.004409678	0.125185185	chr5:172376398-172376420	ERGIC1	intron
TGCTGCCTAAGAAGACCCTGAGG	*.....*.....	4	0.217271959	0.121628959	chr11:10208741-10208763	RP11-748C4.1-SBF2	intergenic
TACTGCCTGGATGGTGTGGGG**.....	4	0.038443137	0.12	chr15:31091096-31091118	GOLGA8UP	intron
TACTGCCTTAGAAGATGCTGAGG**.....	4	0.051127507	0.119289941	chr7:69367849-69367871	AUTS2	intron
CCCTGCCTGGGAGGACGCTGCGG	**.....*.....	4	0.355433782	0.117125111	chr1:2066579-2066601	PRKCZ	intron
TAGTGCCTAAGTTAACGCTGCGG	..*.....**.....	4	0.095245275	0.116666667	chr4:155161554-155161576	DCHS2	intron
CACAGCCTAGGATGCAGCTGGGG	*.....**.....	4	0.072985333	0.111317254	chr18:72955041-72955063	TSHZ1	intron
TACTGGCTAGGATGAACCTAGGG*.....**.....	4	0.025112337	0.110294118	chr6:119236695-119236717	MCM9	intron
TACTGCCTCAGATGGTGTGGGG**.....	4	0.035406129	0.106666667	chr19:1908581-1908603	ADAT3/SCAMP4	intron
CGCTGCCAGGATGAGGCTGGGG	**.....*.....	4	0.272333333	0.105494506	chr20:57195347-57195369	APCDD1L-AS1-MGC4294	intergenic
CACTGCCTGGGACGACGCTGTGG	*.....*.....	4	0.174873421	0.10410642	chr3:129307423-129307445	PLXND1	intron
TCCTGCCAAGGATGGCCCTGGGG	*.....*.....	4	0.163368333	0.096134454	chr10:133008610-133008632	TCERG1L	intron
TACTGCCAGGATGACCCTTGGG**.....**.....	4	0.147449913	0.087843137	chr5:43246184-43246206	NIM1K	intron
TATTACTTAGGATGAGGCTGGGG	..**.....*.....	4	0.174113559	0.074479167	chrX:71562857-71562879	HDAC8	intron
TAAAGCCTAGGAAGACTCTGAGG	..**.....*.....	4	0.220828507	0.071428571	chr6:22433745-22433767	RP3-404K8.2-HDGFL1	intergenic
TACTGCTTGTGATGAGGCTGGGG**.....*.....	4	0.094581352	0.067708333	chr6:150559142-150559164	PPP1R14C	intron
TAATGACTAGGATGAGGATGTGG	..*.....**.....	4	0.031841181	0.066666667	chr16:74008324-74008346	RPSAP56-AC009120.4	intergenic
TACCTCCTAGGACCACGCTGGGG	...**.....**.....	4	0.082499774	0.065678903	chr2:175112699-175112721	OLA1	intron
CACTGTCTAGGATTATGCTGAGG	*.....*.....**.....	4	0.024549488	0.065306123	chr1:65594559-65594581	MRPS21P1-AK4	intergenic

Table S2: Continued

Off-target sequence	Mismatch position	Mismatch count	MIT off-target score	CDF off-target score	Chromosome position (hg19)	Locus	Genomic context
TCCTGCCTAGAATGAGGATGTGG	. * * * ..	4	0.02962442	0.065088757	chr2:163125807-163125829	IFIH1	intron
TACAGCCTAAGATCATGCTGGGG	... * * * ..	4	0.035479776	0.058608059	chr1:186968564-186968586	PLA2G4A-LINC01036	intergenic
TACTGCATAGGATTATACTGGGG * * * ..	4	0.009641657	0.057435898	chr6:44481606-44481628	CDC5L-RP3-449H6.1	intergenic
TTCTGCCTAGGCTCGCGCTGCGG	. * * * ..	4	0.029531989	0.053315106	chr1:2144990-2145012	AL590822.1/RP11-181G12.5/C1orf86/RP11-181G12.4	intergenic
TACTGCTAAGCATGAGGCTGGGG ** * * ..	4	0.093281959	0.042857143	chr9:97724698-97724720	C9orf3	intron
TTCTTCCTAGGATAACTCTGAGG	. * * * ..	4	0.090827917	0.040909091	chr3:18178080-18178102	AC132807.1-SATB1	intergenic
TACTTCCTAGGACGATGATGAGG * * * ..	4	0.019611103	0.039240112	chr11:17629090-17629112	OTOG	intron
TACTGCCTCTGATGCTGCTGAGG ** * * ..	4	0.035406129	0.038961039	chr11:58632351-58632373	GLYATL2-GLYATL1P2	intergenic
TGCTGCCTGGGAGGACTCTGGGG	. * * * ..	4	0.144140246	0.034782609	chr11:2174136-2174158	INS-IGF2/IGF2-INS-IGF2	intergenic
TACTGCCTCTGCTGCCGCTGTGG ** * * ..	4	0.101277997	0.033321941	chr17:63533165-63533187	AXIN2	exon
TTCTGCCTAGGCTGGCGCGGTGG	. * * * ..	4	0.06576318	0.031100478	chr12:43572807-43572829	RP11-118A3.1-AC079603.1	intergenic
TTCTGCCCAGGCTGAGGCTGAGG	. * * * ..	4	0.127203797	0.02944424	chr1:200862759-200862781	C1orf106	intron
TACTGACGAGGCTGAGGCTGAGG * * * ..	4	0.073249464	0.027568922	chr4:170845432-170845454	RP11-205M3.3	intron
AGCTGCCTAGGATGACCGTGTGG	** * * ..	4	0.119478333	0.025098039	chr17:55538633-55538655	MSI2-RP11-118E18.2	intergenic
AACTGCCTGGGATGACTGTGTGG	. * * * ..	4	0.073001262	0.022222222	chr2:220099746-220099768	ANKZF1	exon
TACTGCCTGGGGTGAGTCTGTGG * * * ..	4	0.027171265	0.018518519	chr11:79067067-79067089	TENM4-MIR708	intergenic
TACTTCCAAGGATTACCCTGAGG * * * ..	4	0.086229035	0.015058824	chr9:79421778-79421800	PRUNE2	intron
TACTCCCTAGGAGGACCCAGAGG * * * ..	4	0.070548661	0.013779425	chr3:94216126-94216148	NSUN3-ARMC10P1	intergenic
GACTGCCTGGGATGAGGGTGC GG	. * * * ..	4	0.032613551	0.013080639	chr3:193446631-193446653	OPA1-RN7SL447P	intergenic
TACTGTCTAGGATTAGCCTGGGG * * * ..	4	0.00854056	0.008963585	chr1:217346142-217346164	ESRRG-GPATCH2	intergenic
TTCTGCCTAGGAGGAGGCAGAGG	. * * * ..	4	0.033198795	0.008339487	chr12:40117882-40117904	C12orf40	intron