

IMMUNE CELLS HAVING CO-EXPRESSED TGFBR SHRNAS**CROSS REFERENCE TO RELATED APPLICATIONS**

[0001] This application claims the benefit of and priority to U.S. Provisional Application No. 63/375,519, filed September 13, 2022, U.S. Provisional Application No. 63/489,840, filed March 13, 2023, U.S. Provisional Application No. 63/495,867, filed April 13, 2023, and U.S. Provisional Application No. 63/516,484, filed July 28, 2023, the disclosures of each of which are hereby incorporated by reference in their entireties for all purposes.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted electronically and is hereby incorporated by reference in its entirety. Said XML copy, created on October 12, 2023, is named ANB-215WO_SL.xml, and is 298,845 bytes in size.

BACKGROUND

[0003] Cancer is a disease characterized by uncontrollable growth of cells. Many approaches to treating cancer have been tried, including drugs and radiation therapies. Recent cancer treatments have sought to use the body's own immune cells to attack cancer cells. One promising approach uses T cells that are taken from a patient and genetically engineered to produce chimeric antigen receptors, or CARs, receptor proteins that give the T cells a new ability to target a specific protein. The receptors are chimeric because they combine antigen-binding and T-cell activating functions into a single receptor.

[0004] Immunotherapy using CAR-T cells is promising because the modified T cells have the potential to recognize cancer cells in order to more effectively target and destroy them. After the T cells are engineered with the CARs, the resulting CAR-T cells are introduced into patients to attack tumor cells. Once CAR-T cells are infused into a patient, they come in contact with their targeted antigen on a cell. The CAR-T cells bind to the antigen and become activated. Upon antigen engagement, CAR T cells can proliferate exponentially, initiate antitumor cytokine production, and target tumor cell killing.

[0005] However, there remain some limitations to CAR T cell-based immunotherapy. In particular, CAR-T cells can lack peripheral survival, can have reduced expansion and effector function, are susceptible to suppression and exhaustion, and may not result in memory T cell persistence. Thus, additional therapies targeting T cell intrinsic pathways are needed to address these roadblocks for CAR-T therapy.

SUMMARY

[0005a] In one aspect, provided herein is one or more recombinant nucleic acids comprising:

- a. a first nucleic acid sequence at least 15 nucleotides in length complementary to an mRNA encoding human TGF- β Receptor 2 (TGFB2) comprising the sequence set forth in SEQ ID NO: 2, and
- b. a second nucleic acid sequence at least 15 nucleotides in length complementary to an mRNA encoding human TGF- β Receptor 2 (TGFB2) comprising the sequence set forth in SEQ ID NO: 2, wherein the first nucleic acid sequence and the second nucleic acid sequence are distinct.

[0005b] In another aspect, provided herein is an expression vector comprising the recombinant nucleic acids described herein.

[0005c] In another aspect, provided herein is an immune cell comprising one or more recombinant nucleic acids comprising:

- a. a first nucleic acid sequence at least 15 nucleotides in length complementary to an mRNA encoding human TGF- β Receptor 2 (TGFB2) comprising the sequence set forth in SEQ ID NO: 2, and
- b. a second nucleic acid sequence at least 15 nucleotides in length complementary to an mRNA encoding human TGF- β Receptor 2 (TGFB2) comprising the sequence set forth in SEQ ID NO: 2, wherein the first nucleic acid sequence and the second nucleic acid sequence are distinct.

[0005d] In another aspect, provided herein is a population of cells comprising the immune cell described herein.

[0005e] In another aspect, provided herein is a use of the immune cell described herein in the manufacture of a medicament for treating a disease in a subject, wherein the immune cell is to be administered to the subject.

[0005f] In another aspect, provided herein is a use of the one or more recombinant nucleic acids described herein in the manufacture of a medicament for treating a disease in a subject, wherein the one or more recombinant nucleic acids is to be administered to the subject.

[0005g] In another aspect, provided herein is a method comprising contacting a cell obtained from a subject with one or more recombinant nucleic acids, wherein the one or more recombinant nucleic acids comprise:

- a. a first nucleic acid sequence at least 15 nucleotides in length complementary to an mRNA encoding human TGF- β Receptor 2 (TGFB2) comprising the sequence set forth in SEQ ID NO: 2, and
- b. a second nucleic acid sequence at least 15 nucleotides in length complementary to an mRNA encoding human TGF- β Receptor 2 (TGFB2) comprising the sequence set forth in SEQ ID NO: 2, wherein the first nucleic acid sequence and the second nucleic acid sequence are distinct.

[0005h] In another aspect, provided herein is the one or more recombinant nucleic acids described herein or the immune cell described herein, for use in therapy.

[0006] In one aspect, provided herein are one or more recombinant nucleic acids comprising a nucleic acid sequence at least 15 nucleotides in length complementary to an mRNA encoding human TGF- β Receptor 2 (TGFB2) comprising the sequence set forth in SEQ ID NO: 2.

[0007] In one aspect, provided herein are one or more recombinant nucleic acids comprising a nucleic acid sequence at least 15 nucleotides in length complementary to an mRNA encoding human TGF- β Receptor 1 (TGFB1) comprising the sequence set forth in SEQ ID NO: 1.

[0008] In one aspect, provided herein are one or more recombinant nucleic acids comprising: a first nucleic acid sequence at least 15 nucleotides in length complementary to an mRNA encoding human TGF- β Receptor 2 (TGFB2) comprising the sequence set forth in SEQ ID NO: 2, and a second nucleic acid sequence at least 15 nucleotides in length complementary an mRNA encoding human TGF- β Receptor 2 (TGFB2) comprising the sequence set forth in SEQ ID NO: 2.

[0009] In one aspect, provided herein are one or more recombinant nucleic acids comprising: a first nucleic acid sequence at least 15 nucleotides in length complementary an mRNA encoding human TGF- β Receptor 2 (TGFB2) comprising the sequence set forth in SEQ ID NO: 2, and a second nucleic acid sequence at least 15 nucleotides in length complementary to an mRNA encoding human TGF- β Receptor 1 (TGFB1) comprising the sequence set forth in SEQ ID NO: 1.

[0010] In some embodiments, the nucleic acid sequence is at least 16, 17, 18, 19, 20, 21, or 22 nucleotides in length.

[0011] In some embodiments, the nucleic acid is a short hairpin RNA (shRNA), a small interfering RNA (siRNA), a double stranded RNA (dsRNA), or an antisense oligonucleotide.

[0012] In some embodiments, the nucleic acid is an shRNA.

[0013] In some embodiments, the nucleic acid or first nucleic acid comprises a sequence selected from the group consisting of the sequences set forth in SEQ ID NOs: 6-84.

[0014] In some embodiments, the first and second nucleic acids each comprises a sequence selected from the group consisting of the sequences set forth in SEQ ID NOs: 36-84.

[0015] In some embodiments, the nucleic acid or first nucleic acid comprises the sequence set forth in SEQ ID NO: 81.

[0016] In some embodiments, the nucleic acid or first nucleic acid comprises the sequence set forth in SEQ ID NO: 51 or 53.

[00180] FIG. 20A shows the tumor volume post tumor implant in mice treated with ICTs expressing Logic Gates 1-5, RNP or PBS generated from donor 1. FIG. 20B shows the total T cells and expansion of the ICTs on day 12 post inoculation followed by contraction by day 21. FIG. 20C shows total T cells expressing the priming receptor on days 12 and 21. FIG. 20D show the tumor volume post tumor implant in mice treated with ICTs expressing Logic Gates 1-5, RNP or PBS generated from donor 2. FIG. 20E shows the total T cells and expansion of the ICTs on day 12 post inoculation followed by contraction by day 21. FIG. 20F shows total T cells expressing the priming receptor on days 12 and 21.

[00181] FIG. 21A shows tumor growth inhibition (TGI) in the single positive CAR antigen-only flank. FIG. 21B shows tumor growth inhibition (TGI) in the dual positive primeR antigen/CAR antigen flank.

[00182] FIG. 22 shows that TGFBR knockdown protects ICT cells against TGF β - mediated inhibition.

[00183] FIG. 23 shows that the ICT+shRNA was more potent than a conventional CAR-T benchmark.

[00184] FIG. 24 shows *in vivo* tumor volume in the 786-O xenograft model after injection of the indicated ICTs expressing quad shRNAs.

[00185] FIG. 25 shows *in vivo* tumor volume in the A498 xenograft model after injection of the ICTs expressing quad shRNAs as compared to FAS-PTPN2 shRNA.

[00186] FIG. 26 shows a schematic of an exemplary dual shRNA expression cassette for expression of shRNAs against *FAS* and *TGFBR2* in the 3G-E format (SEQ ID NO: 264).

[00187] FIG. 27 shows a schematic of an exemplary triple shRNA expression cassette for expression of shRNAs against *FAS* and *TGFBR2* in the 3G-E-3G format (SEQ ID NO: 265).

[00188] FIG. 28 shows a schematic of an exemplary triple shRNA expression cassette for expression of shRNAs against *TGFBR2* and *FAS* in the 3G-3G-3G format (SEQ ID NO: 266).

[00189] FIG. 29 shows a schematic of an exemplary triple shRNA expression cassette for expression of shRNAs against *FAS* and *TGFBR2* in the 3G-3G-3G format (SEQ ID NO: 267).

[00190] FIG. 30 shows a schematic of an exemplary quadruple shRNA expression cassette for expression of shRNAs against *FAS*, *PTPN2*, and *TGFBR2* in the 3G-E-3G-3G format (SEQ ID NO: 268).

DETAILED DESCRIPTION

Definitions

[00191] Terms used in the claims and specification are defined as set forth below unless otherwise specified.

20 kB of adjacent genes), *etc.* These challenges can be addressed, in part, through the identification and use of safe harbor loci or safe harbor sites (SHS), which are sites in which genes or genetic elements can be incorporated without disruption to expression or regulation of adjacent genes.

[00384] The most widely used of the putative human safe harbor sites is the AAVS1 site on chromosome 19q, which was initially identified as a site for recurrent adeno-associated virus insertion. Other potential SHS have been identified on the basis of homology, with sites first identified in other species (e.g., the human homolog of the permissive murine Rosa26 locus) or among the growing number of human genes that appear non-essential under some circumstances. One putative SHS of this type is the CCR5 chemokine receptor gene, which, when disrupted, confers resistance to human immunodeficiency virus infection. Additional potential genomic SHS have been identified in human and other cell types on the basis of viral integration site mapping or gene-trap analyses, as was the original murine Rosa26 locus. The three top SHS, AAVS1, CCR5, and Rosa26, are in close proximity to many protein coding genes and regulatory elements. (See Sadelain, M., et al. (2012). Safe harbours for the integration of new DNA in the human genome. *Nature reviews Cancer*, 12(1), 51-58, the relevant disclosures of which are herein incorporated by reference in their entirety).

[00385] The AAVS1 (also known as the PPP1R12C locus) on human chromosome 19 is a known SHS for hosting transgenes (e.g. DNA transgenes) with expected function. It is at position 19q13.42. It has an open chromatin structure and is transcription-competent. The canonical SHS locus for AAVS1 is chr19: 55,625,241–55,629,351. See Pellenz *et al.* “New Human Chromosomal Sites with "Safe Harbor" Potential for Targeted Transgene Insertion.” *Human gene therapy* vol. 30,7 (2019): 814-828, the relevant disclosures of which are herein incorporated by reference. An exemplary AAVS1 target gRNA and target sequence are provided below:

- AAVS1-gRNA sequence:
 ggggccactagggacaggatGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCC
 GTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTT (SEQ ID NO: 142)
- AAVS1 target sequence: ggggccactagggacaggat (SEQ ID NO: 143)

[00386] CCR5, which is located on chromosome 3 at position 3p21.31, encodes the major co-receptor for HIV-1. Disruption at this site in the CCR5 gene has been beneficial in HIV/AIDS therapy and prompted the development of zinc-finger nucleases that target its third exon. The canonical SHS locus for CCR5 is chr3: 46,414,443–46,414,942. See Pellenz *et al.* “New Human Chromosomal Sites with "Safe Harbor" Potential for Targeted Transgene

specific integration techniques, as described herein or known to those of ordinary skill in the art. Examples of site-specific techniques for integration into the safe harbor loci include, without limitation, homology-dependent engineering using nucleases and homology independent targeted insertion using Cas9 or other CRISPR endonucleases.

[00396] In some embodiments, the insert is integrated at a safe harbor site by introducing into the engineered cell, (a) a targeted nuclease that cleaves a target region in the safe harbor site to create the insertion site; and (b) the nucleic acid sequence (insert), wherein the insert is incorporated at the insertion site by, *e.g.*, HDR. Examples of non-viral delivery techniques that can be used in the methods of the present disclosure are provided in US Application Nos. 16/568,116 and 16/622,843, the relevant disclosures of which are herein incorporated by reference in their entirety.

[00397] Examples of integration sites contemplated are provided in **Table D**.

Table D: sgRNA sequences

sgRNA ID	SEQ ID NO:	sgRNA Sequence	sgRNA start coor GRCH38	sgRNA Target Loci	Integration Site	Median (% Modified), summarized from 2 donors, 2 primersets
sgRNA_1	144	GCACCTGAATACCACG CCTG	chr16:88811818	APRT	APRT	79.28
sgRNA_2	145	CGCCTGCGATGTAGTC GATG	chr16:88811551	APRT	APRT	78.60
sgRNA_3	146	CAGGACGGGCGAGAT GTCCC	chr16:88811640	APRT	APRT	85.25
sgRNA_4	147	CTGAATCTTTGGAGTA CCTG	chr15:44715425	B2M	B2M	78.51
sgRNA_5	148	GGCCACGGAGCGAGA CATCT	chr15:44711550	B2M	B2M	94.75
sgRNA_6	149	AAGTCAACTTCAATGT CGGA	chr15:44715515	B2M	B2M	70.97
sgRNA_7	150	GCTTGGAGGCCTGATC AGCG	chr19:36141111	CAPNS1	CAPNS1	89.34
sgRNA_8	151	CTTATCTCTTCGCAGC GAGG	chr19:36142301	CAPNS1	CAPNS1	91.09
sgRNA_9	152	CACACATTACTCCAAC ATTG	chr19:36142676	CAPNS1	CAPNS1	71.98
sgRNA_10	153	TTCCGCAAAATAGAGC CCCA	chr3:105746019	CBLB	CBLB	91.55
sgRNA_11	154	TGCACAGAACTATCGT ACCA	chr3:105751622	CBLB	CBLB	91.43
sgRNA_12	155	GCAATAAGACTCTTTA AAGA	chr3:105853470	CBLB	CBLB	76.18
sgRNA_13	156	CAAAGAGATTACGAAT GCCT	chr1:116754658	CD2	CD2	89.80
sgRNA_14	157	CAAGGCACCCCAGGTT TCCA	chr1:116754663	CD2	CD2	92.70
sgRNA_15	158	TTACGAATGCCTTGGA AACC	chr1:116754666	CD2	CD2	92.82

sgRNA_16	159	CAGAGACGCATCTGAC CCTC	chr11:11831554 0	CD3E	CD3E	90.96
sgRNA_17	160	CATGCAGTTCTCACAC ACTG	chr11:11831371 5	CD3E	CD3E	87.47
sgRNA_18	161	GTGTGAGAACTGCATG GAGA	chr11:11831371 5	CD3E	CD3E	86.65
sgRNA_19	162	TCTCATTTTCAGGAAAC CACT	chr11:11834974 8	CD3G	CD3G	87.24
sgRNA_20	163	AGTCATACACCTTAAC CAAG	chr11:11834975 4	CD3G	CD3G	87.99
sgRNA_21	164	TTC AAGGAAACCAGTT GAGG	chr11:11835245 8	CD3G	CD3G	86.55
sgRNA_22	165	GAGCCTTGCCTGGAAA TCTG	chr11:61118177	CD5	CD5	84.03
sgRNA_23	166	AAGCGTCAAAAGTCTG CCAG	chr11:61118324	CD5	CD5	89.19
sgRNA_24	167	CGTTCCAACTCGAAGT GCCA	chr11:61118121	CD5	CD5	83.11
sgRNA_25	168	GAGCGACTGGGACAC GGTGA	chr9:136866246	EDF1	EDF1	88.84
sgRNA_26	169	GCTGCGCAAGAAGGG CCCTA	chr9:136866211	EDF1	EDF1	91.04
sgRNA_27	170	TTGTTCTGGCCAGCAG CCCC	chr9:136863433	EDF1	EDF1	85.98
sgRNA_28	171	CTTCCAGAGCCACATC ATCG	chr19:48965791	FTL	FTL	93.10
sgRNA_29	172	GGGACTCACCAGAGA GAGGT	chr19:48965601	FTL	FTL	88.86
sgRNA_30	173	CGGTCGAAATAGAAG CCCTA	chr19:48965770	FTL	FTL	93.14
sgRNA_31	174	AAAAGGATATTGTGCA ACTG	chr10:87933015	PTEN	PTEN	92.37
sgRNA_32	175	TGTGCATATTTATTAC ATCG	chr10:87933183	PTEN	PTEN	90.64
sgRNA_33	176	TTTGTGAAGATCTTGA CCAA	chr10:87933087	PTEN	PTEN	85.36
sgRNA_34	177	TGTCATGCTGAACCGC ATTG	chr18:12830972	PTPN2	PTPN2	87.94
sgRNA_35	178	CCACTCTATGAGGATA GTCA	chr18:12859219	PTPN2	PTPN2	92.45
sgRNA_36	179	TTGACATAGAAGAGGC ACAA	chr18:12836828	PTPN2	PTPN2	93.96
sgRNA_37	180	GAGTACTACACTCAGC AGCA	chr12:6952098	PTPN6	PTPN6	89.61
sgRNA_38	181	TCACGCACAAGAAAC GTCCA	chr12:6954872	PTPN6	PTPN6	82.74
sgRNA_39	182	AGGTCTCGGTGAAACC ACCT	chr12:6951610	PTPN6	PTPN6	91.27
sgRNA_40	183	AGCATTATCCAAAGAG TCCG	chr1:198696873	PTPRC	PTPRC	88.88
sgRNA_41	184	ATATTAATTCTTACCA GTGG	chr1:198692370	PTPRC	PTPRC	88.95
sgRNA_42	185	AGCTTTAAATCAAGGT TCAT	chr1:198756176	PTPRC	PTPRC	96.89
sgRNA_43	186	ATCCCGAGCCCTAAGG TGCA	chr11:67436325	PTPRCA P	PTPRCAP	84.08
sgRNA_44	187	GGCAGCGCGGAGGAC AGCGT	chr11:67436285	PTPRCA P	PTPRCAP	97.74
sgRNA_45	188	CTCAGGGGGGCTACTAC	chr11:67436170	PTPRCA	PTPRCAP	91.50

		CACC		P		
sgRNA_46	189	GTCACCGACGAGACC AGAAG	chr5:82277810	RPS23	RPS23	79.40
sgRNA_47	190	GTCGTGGACTTCGTAC TGCT	chr5:82277843	RPS23	RPS23	83.07
sgRNA_48	191	TAATTTTTAGGCAAGT GTCG	chr5:82277860	RPS23	RPS23	61.94
sgRNA_49	192	TTAGCTGTTAGACTTG AATA	chr14:51993810	RTRAF	RTRAF	85.50
sgRNA_50	193	CGAGAGCCGTCAACTT GCGT	chr14:51989652	RTRAF	RTRAF	85.64
sgRNA_51	194	CGGCTTCAACTGCAAA GGTG	chr14:51989700	RTRAF	RTRAF	88.77
sgRNA_52	195	TATGAAAAAGCAGAG CGACT	chr15:43793025	SERF2	SERF2	89.61
sgRNA_53	196	TCTGGCGGGCGAGCTC ACGC	chr15:43792989	SERF2	SERF2	86.73
sgRNA_54	197	CTCACGCTGGTTACCG CCTA	chr15:43792977	SERF2	SERF2	80.57
sgRNA_55	198	AAAGATTACGAAC TTC CCTG	chr12:46207559	SLC38A1	SLC38A1	92.24
sgRNA_56	199	GTAAAAACAGACATG CCTA	chr12:46229232	SLC38A1	SLC38A1	91.51
sgRNA_57	200	ATGCCTAAGGAGGTTG TACC	chr12:46229246	SLC38A1	SLC38A1	79.48
sgRNA_58	201	CTCCAGGTATCCCATC GAAA	chr18:47869418	SMAD2	SMAD2	79.53
sgRNA_59	202	CACCAAATACGATAGA TCAG	chr18:47870532	SMAD2	SMAD2	86.61
sgRNA_60	203	TGGCGGCGTGAATGGC AAGA	chr18:47896729	SMAD2	SMAD2	82.91
sgRNA_61	204	TAGGATGGTAGCACAC AACC	chr16:11255478	SOCS1	SOCS1	92.25
sgRNA_62	205	CAGCAGCAGAGCCCC GACGG	chr16:11255432	SOCS1	SOCS1	83.79
sgRNA_63	206	CGGCGTGCGAACGGA ATGTG	chr16:11255296	SOCS1	SOCS1	84.24
sgRNA_64	207	TATAGACGCTGCCCCGA CGTC	chr15:40038895	SRP14	SRP14	95.12
sgRNA_65	208	TCCAAAGAAGGGTACT GTGG	chr15:40038368	SRP14	SRP14	92.14
sgRNA_66	209	ACAGTACCCTTCTTTG GAAT	chr15:40038358	SRP14	SRP14	65.82
sgRNA_67	210	GCGACGGGCGCATCTA CGTG	chr12:12046957 2	SRSF9	SRSF9	83.68
sgRNA_68	211	CCCGACCTCCATAAGT CCTG	chr12:12046570 0	SRSF9	SRSF9	92.56
sgRNA_69	212	GGGGTCCTCGAAGCGC ACGA	chr12:12046942 6	SRSF9	SRSF9	89.94
sgRNA_70	213	TGCTCTGTTTAGAAGA TGAC	chr5:32591641	SUB1	SUB1	79.36
sgRNA_71	214	ATATTCTTTTCTAGTTA AAG	chr5:32591566	SUB1	SUB1	70.93
sgRNA_72	215	CCTGTAAAGAAACAA AAGAC	chr5:32591614	SUB1	SUB1	93.66
sgRNA_73	216	TGGAGAAAGACGTAA CTTCG	chr4:105234315	TET2	TET2	83.53
sgRNA_74	217	TCTGCCCTGAGGTATG CGAT	chr4:105234747	TET2	TET2	90.97

sgRNA_75	218	ATTCCGCTTGGTGAAA ACGA	chr4:105235656	TET2	TET2	89.62
sgRNA_76	219	CAGGCACAATAGAAA CAACG	chr3:114295571	TIGIT	TIGIT	92.65
sgRNA_77	220	CCATTTGTAATGCTGA CTTG	chr3:114295700	TIGIT	TIGIT	60.75
sgRNA_78	221	CTGGGTCACCTGTGCC GTGG	chr3:114295634	TIGIT	TIGIT	87.99
sgRNA_79	222	GTCAGGGTTCTGGATA TCTG	chr14:22547508	TRAC	TRAC	98.20
sgRNA_80	223	TGGATTTAGAGTCTCT CAGC	chr14:22547541	TRAC	TRAC	88.15
sgRNA_81	224	CTGCGGCTGTGGTCCA GCTG	chr14:22550661	TRAC	TRAC	94.77
sgRNA_82	225	ACAAAAGTGTGCTAGA CATG	chr14:22547658	TRAC	TRAC	87.86
sgRNA_83	226	TTCTTCCCCAGCCCAG GTAA	chr14:22547778	TRAC	TRAC	89.85
sgRNA_84	227	CGTCATGAGCAGATTA AACC	chr14:22550625	TRAC	TRAC	95.81
sgRNA_85	228	GAGAGCGCCTGCGAC CCGAG	chr19:58544980	TRIM28	TRIM28	89.44
sgRNA_86	229	CCAGCGGGTGAAGTAC ACCA	chr19:58544869	TRIM28	TRIM28	94.79
sgRNA_87	230	GGAGCGCTTTTCGCCG CCAG	chr19:58544839	TRIM28	TRIM28	91.81
sgRNA_88	231	TGAGGCCTGGACCTTA TGCA	chr10:33134193	chr10:331 30000- 33140000	desert_1 (GS88)	69.44
sgRNA_89	232	CCTGGTGGAGTGAACC ATGA	chr10:33132917	chr10:331 30000- 33140000	desert_1 (GS89)	95.25
sgRNA_90	233	CAAGCACTTAGGTTCC CCTG	chr10:33134633	chr10:331 30000- 33140000	desert_1 (GS90)	91.13
sgRNA_91	234	GGTCTCCCTACAATTC AGCG	chr10:72294568	chr10:722 90000- 72300000	desert_2 (GS91)	92.02
sgRNA_92	235	CACAGCGCGTGACTGC AATG	chr10:72298268	chr10:722 90000- 72300000	desert_2 (GS92)	90.22
sgRNA_93	236	TCTGGGGCACCAATTC TAGG	chr10:72292786	chr10:722 90000- 72300000	desert_2 (GS93)	86.35
sgRNA_94	237	GAGCCATGCTTGGCTT ACGA	chr11:12834257 6	chr11:128 340000- 12835000 0	desert_3 (GS94)	91.24
sgRNA_95	238	GTACAAGTACTTATCT CATG	chr11:12834359 2	chr11:128 340000- 12835000 0	desert_3 (GS95)	89.02
sgRNA_96	239	GAGATAACAACATAA CAACA	chr11:12834717 0	chr11:128 340000- 12835000 0	desert_3 (GS96)	96.47
sgRNA_97	240	CATATTCCATAGTCTT TGGG	chr11:65425000	chr11:654 25000- 65427000	desert_4 (GS97)	88.54

				(NEAT1)		
sgRNA_98	241	CTGCCCTTAGCAACT TAGG	chr11:65425507	chr11:654 25000- 65427000 (NEAT1)	desert_4 (GS98)	92.76
sgRNA_99	242	TGTTTAAAAATATGTT GACA	chr11:65426264	chr11:654 25000- 65427000 (NEAT1)	desert_4 (GS99)	90.76
sgRNA_100	243	CCAGGAATGGAACTC ACGC	chr15:92830315	chr15:928 30000- 92840000	desert_5 (GS100)	87.84
sgRNA_101	244	GAGGCCGCTGAATTAA CCCG	chr15:92831850	chr15:928 30000- 92840000	desert_5 (GS101)	85.32
sgRNA_102	245	ATACACGCACACTTGC AGAA	chr15:92831131	chr15:928 30000- 92840000	desert_5 (GS102)	99.92
sgRNA_103	246	GAGCAGACAGAAACC CAGGG	chr16:11225670	chr16:112 20000- 11230000	desert_6 (GS103)	87.92
sgRNA_104	247	TGAGTCTCCAAACAGA ACAG	chr16:11226284	chr16:112 20000- 11230000	desert_6 (GS104)	88.53
sgRNA_105	248	TAATATCACTGACTTC ACGG	chr16:11225029	chr16:112 20000- 11230000	desert_6 (GS105)	87.65
sgRNA_106	249	TACACACAATGTAAGC AGCA	chr2:87467461	chr2:8746 0000- 87470000	desert_7 (GS106)	71.79
sgRNA_107	250	GGGAGCTCAATTTCGAA ACCA	chr2:87468809	chr2:8746 0000- 87470000	desert_7 (GS107)	65.89
sgRNA_108	251	TTGGACAGGTGAGACA GTCG	chr2:87467001	chr2:8746 0000- 87470000	desert_7 (GS108)	72.64
sgRNA_109	252	AAGCTCACTCAGATAG TGTG	chr3:186511316	chr3:1865 10000- 18652000 0	desert_8 (GS109)	76.89
sgRNA_110	253	CAGGAGAACCACCTTA CACG	chr3:186515260	chr3:1865 10000- 18652000 0	desert_8 (GS110)	86.31
sgRNA_111	254	GGACAGACCCTGATTC ACAA	chr3:186519655	chr3:1865 10000- 18652000 0	desert_8 (GS111)	85.47
sgRNA_112	255	ACATGGCAGTCTATGA ACAG	chr3:59451154	chr3:5945 0000- 59460000	desert_9 (GS112)	87.77
sgRNA_113	256	CCTATAGAGAGTACTA CTTG	chr3:59456416	chr3:5945 0000- 59460000	desert_9 (GS113)	79.33
sgRNA_114	257	CCAACCGGGTCTTCAT TACG	chr3:59457029	chr3:5945 0000- 59460000	desert_9 (GS114)	92.21
sgRNA_115	258	TCAAGCGTAGAGTTCC GAGT	chr8:127993006	chr8:1279 80000-	desert_10 (GS115)	93.07

				12800000 0		
sgRNA_116	259	TCATGCAATTATGGAC CCAG	chr8:127994663	chr8:1279 80000- 12800000 0	desert_10 (GS116)	89.40
sgRNA_117	260	CGGGAAAGTGACTGG CCATG	chr8:127996766	chr8:1279 80000- 12800000 0	desert_10 (GS117)	87.45
sgRNA_118	261	TGAGATTGAAATCAAA TCGG	chr9:7974159	chr9:7970 000- 7980000	desert_11 (GS118)	84.84
sgRNA_119	262	TATGCAATATTCATCA CGCG	chr9:7977914	chr9:7970 000- 7980000	desert_11 (GS119)	85.44
sgRNA_120	263	AATGTGTAAATCAAA TGCA	chr9:7976895	chr9:7970 000- 7980000	desert_11 (GS120)	83.48

CRISPR-Cas Editing

[00398] One effective example of gene editing is the CRISPR-Cas approach (e.g. CRISPR-Cas9). This approach incorporates the use of a guide polynucleotide (e.g. guide ribonucleic acid or gRNA) and a Cas endonuclease (e.g. Cas9 endonuclease).

[00399] As used herein, a polypeptide referred to as a “Cas endonuclease” or having “Cas endonuclease activity” refers to a CRISPR-related (Cas) polypeptide encoded by a Cas gene, wherein a Cas polypeptide is a target DNA sequence that can be cleaved when operably linked to one or more guide polynucleotides (see, e.g., US Pat. No. 8,697,359). Also included in this definition are variants of Cas endonuclease that retain guide polynucleotide-dependent endonuclease activity. The Cas endonuclease used in the donor DNA insertion method detailed herein is an endonuclease that introduces double-strand breaks into DNA at the target site (e.g., within the target locus or at the safe harbor site).

[00400] As used herein, the term “guide polynucleotide” relates to a polynucleotide sequence capable of complexing with a Cas endonuclease and allowing the Cas endonuclease to recognize and cleave a DNA target site. The guide polynucleotide can be a single molecule or a double molecule. The guide polynucleotide sequence can be an RNA sequence, a DNA sequence, or a combination thereof (RNA-DNA combination sequence). A guide polynucleotide comprising only ribonucleic acid is also referred to as “guide RNA”. In some embodiments, a polynucleotide donor construct is inserted at a safe harbor locus using a guide RNA (gRNA) in combination with a Cas endonuclease (e.g. Cas9 endonuclease).

[illegible]