Supplementary Tables

Supplementary table 1. The top ten most observed variants of each gRNA and their fraction in each samples time point. The first sequence, in bold, represents the original sequence of each gRNA, dashes represent deleted bases, capital letters represent insertions, underlined letters represent mismatches.

hgRNA	Sequence	% at 0d	% after 1d	% after 3d	% after 5d
A21	**acc ggtcccctccaccccacagtg gggttagagctagaaatagcaagttaacctaa**	61.66	54.24	8.75	1.82
variant 1	acc ggtcccctccaccccacaAgtg gggttagagctagaaatagcaagttaacctaa	1.74	4.55	15.80	10.59
variant 2	acc ggtcccctccaccccacaAAgtg gggttagagctagaaatagcaagttaacctaa	0.00	0.06	4.57	8.54
variant 3	acc ggt-----------------g gggttagagctagaaatagcaagttaacctaa	0.18	0.29	4.28	4.25
variant 4	acc --------------------g gggttagagctagaaatagcaagttaacctaa	0.04	0.25	1.26	6.02
variant 5	acc ggtcccctccacccc------ ----------tagaaatagcaagttaacctaa	0.08	0.10	1.11	1.30
variant 6	acc --------------------- -ggttagagctagaaatagcaagttaacctaa	0.04	0.08	1.14	1.18
variant 7	acc ggtcccctccacccca----- ---------ctagaaatagcaagttaacctaa	0.04	0.16	0.69	0.95
variant 8	acc ggt------------------ ---------ctagaaatagcaagttaacctaa	0.01	0.14	0.60	0.88
variant 9	acc gg------------------- ----tagagctagaaatagcaagttaacctaa	0.02	0.05	0.51	0.37
B21	**acc ggtcggagggaggtgggttag gggttagagctagaaatagcaagttaacctaa**	74.17	73.91	71.25	64.62
variant 1	acc ggtcggagggaggt------- gggttagagctagaaatagcaagttaacctaa	0.00	0.24	1.18	3.55
variant 2	acc ggtcggagggaggtgggtTtag gggttagagctagaaatagcaagttaacctaa	0.00	0.06	0.61	1.29
variant 3	acc ggtcggagggagggggttag gggttagagctagaaatagcaagttaacctaa	0.58	0.61	0.57	0.49
variant 4	acc ggtcggagggaggtgggttag gggttagagctagaaatagcgagttaacctaa	0.48	0.53	0.51	0.44
variant 5	acc ggtcggagggaggtgggttag gggttagagctagagatagcaagttaacctaa	0.51	0.49	0.47	0.46
variant 6	acc ggtcggagggaggtgggttag gggttagagctagaagtagcaagttaacctaa	0.50	0.52	0.44	0.46
variant 7	acc ggtcggaggggggtgggttag gggttagagctagaaatagcaagttaacctaa	0.51	0.50	0.47	0.44
variant 8	gcc ggtcggagggaggtgggttag gggttagagctagaaatagcaagttaacctaa	0.50	0.49	0.45	0.43
variant 9	acc ggtcgggggaggtgggttag gggttagagctagaaatagcaagttaacctaa	0.48	0.44	0.50	0.40
C21	**acc ggtacaccctcaagcagtgtg gggttagagctagaaatagcaagttaacctaa**	62.74	47.14	6.53	1.64
variant 1	acc ggtacaccctcaagcagtTgtg gggttagagctagaaatagcaagttaacctaa	0.98	11.51	28.63	21.92
variant 2	acc ggtacaccctcaagcagtTTgtg gggttagagctagaaatagcaagttaacctaa	0.01	0.12	3.75	7.29
variant 3	acc ggtacaccctcaa-----gtg gggttagagctagaaatagcaagttaacctaa	0.01	0.26	1.37	1.57
variant 4	acc ggtacaccctca--------- -------agctagaaatagcaagttaacctaa	0.02	0.13	1.20	1.92
variant 5	acc g-----------------gtg gggttagagctagaaatagcaagttaacctaa	0.02	0.18	1.14	1.32
variant 6	acc ggtacaccctcaagca----g ---ttagagctagaaatagcaagttaacctaa	0.02	0.13	0.92	1.64
variant 7	acc ggtacaccctcaagcaAgtgtg gggttagagctagaaatagcaagttaacctaa	0.01	0.21	0.93	0.82
variant 8	acc ggtacaccctcaagc------ -------agctagaaatagcaagttaacctaa	0.02	0.13	0.61	0.94
variant 9	acc ggtacaccctcaagca----- -ggttagagctagaaatagcaagttaacctaa	0.03	0.14	0.62	0.71
D21	**acc ggtcacgctacggccaaggtg gggttagagctagaaatagcaagttaacctaa**	64.00	55.82	23.87	14.80
variant 1	acc ggtcacgctacggccaagGgtg gggttagagctagaaatagcaagttaacctaa	0.09	1.19	5.43	8.40
variant 2	acc ggtcacgctacg--------- gggttagagctagaaatagcaagttaacctaa	0.02	0.38	3.97	2.42
variant 3	acc ggtcacgctacg------gtg gggttagagctagaaatagcaagttaacctaa	0.07	0.40	2.14	1.56
variant 4	acc g-----------------gtg gggttagagctagaaatagcaagttaacctaa	0.03	0.34	1.66	2.38
variant 5	acc ggtcac------------gtg gggttagagctagaaatagcaagttaacctaa	0.07	0.38	1.62	1.97
variant 6	acc ggtcac--------------- --------gctagaaatagcaagttaacctaa	0.05	0.32	1.07	1.85
variant 7	acc ggtcacgctac---------- -ggttagagctagaaatagcaagttaacctaa	0.04	0.23	1.12	1.76
variant 8	acc ggtcacgctacggccaa-gtg gggttagagctagaaatagcaagttaacctaa	0.05	0.52	1.11	1.02
variant 9	acc ggtcacgctac---------- gggttagagctagaaatagcaagttaacctaa	0.02	0.11	1.00	1.05

Supplementary table 1 (cont'd).

hgRNA	Sequence	% at 0d	% after 1d	% after 3d	% after 5d
E21	**acc ggttccagagatactgacgtg gggttagagctagaaatagcaagttaacctaa**	64.30	54.57	24.17	9.45
variant 1	acc ggttccagagatactgacCgtg gggttagagctagaaatagcaagttaacctaa	0.23	2.06	8.23	9.88
variant 2	acc ggttccagagatac-----tg gggttagagctagaaatagcaagttaacctaa	0.16	0.49	4.35	6.06
variant 3	acc ggttcc--------------- -----agagctagaaatagcaagttaacctaa	0.16	0.53	3.20	4.90
variant 4	acc g-----------------gtg gggttagagctagaaatagcaagttaacctaa	0.06	0.18	1.28	2.65
variant 5	acc ggttcca-----------gtg gggttagagctagaaatagcaagttaacctaa	0.07	0.17	1.20	1.62
variant 6	acc --------------------- -ggttagagctagaaatagcaagttaacctaa	0.04	0.09	0.97	1.60
variant 7	acc ggttccagagatactgacCCgtg gggttagagctagaaatagcaagttaacctaa	0.00	0.03	0.81	1.86
variant 8	acc ggttccagagatact------ ------gagctagaaatagcaagttaacctaa	0.04	0.10	0.76	1.06
variant 9	acc ggttccagagata-------- ---------ctagaaatagcaagttaacctaa	0.04	0.06	0.57	0.88
F21	**acc ggtgtgtagcgatggctacag gggttagagctagaaatagcaagttaacctaa**	66.81	61.32	30.21	13.94
variant 1	acc ggtgtgtagcgatggctaAcag gggttagagctagaaatagcaagttaacctaa	0.02	0.82	3.30	1.77
variant 2	acc ggtgtgtagcgatggctaCAcag gggttagagctagaaatagcaagttaacctaa	0.02	0.28	1.77	2.23
variant 3	acc ggtgtgtag------------ gggttagagctagaaatagcaagttaacctaa	0.01	0.10	1.32	2.72
variant 4	acc ggtgtgtagcgatg------- --------gctagaaatagcaagttaacctaa	0.01	0.10	1.42	2.43
variant 5	acc ggtg----------------- ----tgtagctagaaatagcaagttaacctaa	0.01	0.11	1.11	2.73
variant 6	acc ggtgtgtag---------cag gggttagagctagaaatagcaagttaacctaa	0.00	0.14	1.05	1.57
variant 7	acc ggtgtgtagcgatggc----- ----tagagctagaaatagcaagttaacctaa	0.00	0.12	1.17	1.32
variant 8	acc ggtgtg--------------- ----tagagctagaaatagcaagttaacctaa	0.01	0.09	0.87	1.72
variant 9	acc ggtgtgtagcgatg------- gggttagagctagaaatagcaagttaacctaa	0.01	0.05	0.80	1.43
G21	**acc ggtatgcggatgcaatctccg gggttagagctagaaatagcaagttaacctaa**	64.07	48.13	16.44	4.98
variant 1	acc ggtatgcggatgcaatctTccg gggttagagctagaaatagcaagttaacctaa	0.43	4.40	10.03	11.26
variant 2	acc ggtatg-------------cg gggttagagctagaaatagcaagttaacctaa	0.04	0.54	2.64	4.36
variant 3	acc g-------------------- gggttagagctagaaatagcaagttaacctaa	0.07	0.73	2.40	3.36
variant 4	acc ggtatgcggatgcaatc--cg gggttagagctagaaatagcaagttaacctaa	0.28	1.46	1.64	0.83
variant 5	acc ggtatgcggat---------- --------gctagaaatagcaagttaacctaa	0.07	0.24	1.53	1.56
variant 6	acc ggtatgcggatgca--TAccg gggttagagctagaaatagcaagttaacctaa	0.06	0.34	1.44	1.28
variant 7	acc ggtatgcggatgcaatctTTccg gggttagagctagaaatagcaagttaacctaa	0.00	0.04	0.58	1.65
variant 8	acc ggtatgcggatgcaat-tccg gggttagagctagaaatagcaagttaacctaa	0.00	0.19	0.82	0.56
variant 9	acc ggtatgcggatg-------cg gggttagagctagaaatagcaagttaacctaa	0.03	0.11	0.46	0.92

Supplementary table 2. Binary distance matrix between hgRNA variants in different subpopulations.

hgRNA A21	**0**	**0,1**	**0,2**	**0,3**	**0,3,1**	**0,3,2**	**0,3,2,1**	**0,3,2,2**	**0,3,2,3**	**0,3,3**	**0,4**	**0,4,1**	**0,4,1,1**	**0,4,1,2**	**0,4,1,3**	**0,4,3**	**0,4,3**	**0,5**	**0,6**
0	0	0.6	0.624	0.592	0.565	0.571	0.657	0.648	0.605	0.568	0.618	0.628	0.685	0.688	0.715	0.612	0.61	0.608	0.617
0,1	0.6	0	0.488	0.434	0.487	0.498	0.606	0.584	0.549	0.501	0.489	0.587	0.714	0.656	0.686	0.589	0.585	0.434	0.468
0,2	0.624	0.488	0	0.46	0.562	0.57	0.656	0.642	0.619	0.578	0.485	0.613	0.708	0.675	0.699	0.608	0.592	0.474	0.488
0,3	0.592	0.434	0.46	0	0.353	0.357	0.473	0.461	0.433	0.362	0.492	0.591	0.693	0.655	0.682	0.595	0.588	0.423	0.419
0,3,1	0.565	0.487	0.562	0.353	0	0.332	0.442	0.445	0.393	0.345	0.538	0.59	0.697	0.64	0.683	0.59	0.584	0.49	0.477
0,3,2	0.571	0.498	0.57	0.357	0.332	0	0.383	0.364	0.345	0.346	0.558	0.6	0.689	0.65	0.684	0.614	0.594	0.524	0.491
0,3,2,1	0.657	0.606	0.656	0.473	0.442	0.383	0	0.325	0.379	0.459	0.665	0.669	0.693	0.662	0.712	0.669	0.651	0.623	0.578
0,3,2,2	0.648	0.584	0.642	0.461	0.445	0.364	0.325	0	0.373	0.412	0.653	0.659	0.671	0.642	0.684	0.644	0.632	0.628	0.575
0,3,2,3	0.605	0.549	0.619	0.433	0.393	0.345	0.379	0.373	0	0.39	0.604	0.628	0.651	0.634	0.676	0.617	0.606	0.585	0.537
0,3,3	0.568	0.501	0.578	0.362	0.345	0.346	0.459	0.412	0.39	0	0.55	0.603	0.693	0.633	0.675	0.594	0.581	0.52	0.502
0,4	0.618	0.489	0.485	0.492	0.538	0.558	0.665	0.653	0.604	0.55	0	0.483	0.68	0.613	0.653	0.506	0.466	0.416	0.515
0,4,1	0.628	0.587	0.613	0.591	0.59	0.6	0.669	0.659	0.628	0.603	0.483	0	0.546	0.456	0.512	0.457	0.442	0.555	0.613
0,4,1,1	0.685	0.714	0.708	0.693	0.697	0.689	0.693	0.671	0.651	0.693	0.68	0.546	0	0.497	0.453	0.561	0.573	0.732	0.72
0,4,1,2	0.688	0.656	0.675	0.655	0.64	0.65	0.662	0.642	0.634	0.633	0.613	0.456	0.497	0	0.472	0.505	0.499	0.662	0.68
0,4,1,3	0.715	0.686	0.699	0.682	0.683	0.684	0.712	0.684	0.676	0.675	0.653	0.512	0.453	0.472	0	0.546	0.543	0.702	0.7
0,4,3	0.612	0.589	0.608	0.595	0.59	0.614	0.669	0.644	0.617	0.594	0.506	0.457	0.561	0.505	0.546	0	0.416	0.602	0.613
0,4,3	0.61	0.585	0.592	0.588	0.584	0.594	0.651	0.632	0.606	0.581	0.466	0.442	0.573	0.499	0.543	0.416	0	0.576	0.591
0,5	0.608	0.434	0.474	0.423	0.49	0.524	0.623	0.628	0.585	0.52	0.416	0.555	0.732	0.662	0.702	0.602	0.576	0	0.458
0,6	0.617	0.468	0.488	0.419	0.477	0.491	0.578	0.575	0.537	0.502	0.515	0.613	0.72	0.68	0.7	0.613	0.591	0.458	0

hgRNA C21	**0**	**0,1**	**0,1,1**	**0,1,1,1**	**0,1,1,2**	**0,1,1,3**	**0,1,2**	**0,1,2,1**	**0,1,2,2**	**0,1,2,3**	**0,1,3**	**0,2**	**0,2,1**	**0,2,2**	**0,2,3**	**0,3**	**0,4**	**0,5**	**0,6**
0	0	0.572	0.503	0.535	0.6	0.553	0.509	0.472	0.506	0.46	0.508	0.557	0.551	0.555	0.546	0.509	0.554	0.552	0.563
0,1	0.572	0	0.311	0.503	0.622	0.557	0.282	0.451	0.396	0.415	0.319	0.434	0.484	0.482	0.492	0.314	0.317	0.287	0.323
0,1,1	0.503	0.311	0	0.438	0.581	0.477	0.328	0.395	0.36	0.385	0.36	0.449	0.493	0.494	0.5	0.335	0.371	0.336	0.365
0,1,1,1	0.535	0.503	0.438	0	0.418	0.385	0.472	0.499	0.477	0.485	0.504	0.561	0.569	0.577	0.589	0.492	0.531	0.509	0.527
0,1,1,2	0.6	0.622	0.581	0.418	0	0.428	0.605	0.609	0.601	0.581	0.608	0.647	0.651	0.659	0.656	0.613	0.645	0.633	0.639
0,1,1,3	0.553	0.557	0.477	0.385	0.428	0	0.543	0.514	0.52	0.525	0.536	0.594	0.615	0.616	0.615	0.55	0.576	0.57	0.579
0,1,2	0.509	0.282	0.328	0.472	0.605	0.543	0	0.388	0.358	0.343	0.311	0.411	0.462	0.463	0.467	0.294	0.366	0.333	0.333
0,1,2,1	0.472	0.451	0.395	0.499	0.609	0.514	0.388	0	0.405	0.392	0.425	0.493	0.513	0.516	0.517	0.415	0.464	0.458	0.469
0,1,2,2	0.506	0.396	0.36	0.477	0.601	0.52	0.358	0.405	0	0.382	0.402	0.493	0.522	0.522	0.532	0.387	0.454	0.403	0.436
0,1,2,3	0.46	0.415	0.385	0.485	0.581	0.525	0.343	0.392	0.382	0	0.405	0.475	0.516	0.519	0.517	0.387	0.463	0.438	0.456
0,1,3	0.508	0.319	0.36	0.504	0.608	0.536	0.311	0.425	0.402	0.405	0	0.426	0.461	0.471	0.466	0.336	0.384	0.348	0.377
0,2	0.557	0.434	0.449	0.561	0.647	0.594	0.411	0.493	0.493	0.475	0.426	0	0.289	0.274	0.259	0.399	0.417	0.416	0.42
0,2,1	0.551	0.484	0.493	0.569	0.651	0.615	0.462	0.513	0.522	0.516	0.461	0.289	0	0.312	0.267	0.456	0.47	0.464	0.486
0,2,2	0.555	0.482	0.494	0.577	0.659	0.616	0.463	0.516	0.522	0.519	0.471	0.274	0.312	0	0.265	0.44	0.474	0.465	0.487
0,2,3	0.546	0.492	0.5	0.589	0.656	0.615	0.467	0.517	0.532	0.517	0.466	0.259	0.267	0.265	0	0.458	0.483	0.48	0.491
0,3	0.509	0.314	0.335	0.492	0.613	0.55	0.294	0.415	0.387	0.387	0.336	0.399	0.456	0.44	0.458	0	0.35	0.303	0.329
0,4	0.554	0.317	0.371	0.531	0.645	0.576	0.366	0.464	0.454	0.463	0.384	0.417	0.47	0.474	0.483	0.35	0	0.328	0.348
0,5	0.552	0.287	0.336	0.509	0.633	0.57	0.333	0.458	0.403	0.438	0.348	0.416	0.464	0.465	0.48	0.303	0.328	0	0.292
0,6	0.563	0.323	0.365	0.527	0.639	0.579	0.333	0.469	0.436	0.456	0.377	0.42	0.486	0.487	0.491	0.329	0.348	0.292	0

Supplementary Note

The sequences of various gRNA constructs used in this study.

>hgRNA-A21
GAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATACAAGGCTGTTAGAGAGATAATTGGA
ATTAATTTGACTGTAAACACAAAGATATTAGTACAAAATACGTGACGTAGAAAGTAATAATTTCTTGGG
TAGTTTGCAGTTTTAAAATTATGTTTTAAAATGGACTATCATATGCTTACCGTAACTTGAAAGTATTTC
GATTTCTTGGCTTTATATATCTTGTGGAAAGGACG AAACACCGGTCCCCTCCACCCCACAGTG GGGTTA
GAGCTAGAAATAGCAAGTTAACCTAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTG
CTTTTTTTT

U6 Promoter Predicted TSS Spacer gRNA Scaffold U6 Terminator

>sgRNA-A21
GAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATACAAGGCTGTTAGAGAGATAATTGGA
ATTAATTTGACTGTAAACACAAAGATATTAGTACAAAATACGTGACGTAGAAAGTAATAATTTCTTGGG
TAGTTTGCAGTTTTAAAATTATGTTTTAAAATGGACTATCATATGCTTACCGTAACTTGAAAGTATTTC
GATTTCTTGGCTTTATATATCTTGTGGAAAGGACG AAACACCGGTCCCCTCCACCCCACAGTG GTTTTA
GAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTG
CTTTTTTTT

U6 Promoter Predicted TSS Spacer gRNA Scaffold U6 Terminator

>A21-Target
AAGAGGATGGTGCAGCAACCAAG AACAAGAAAGTATTTCGATTTCTTGGCTTTATATATCTTGTGGAAA
GGACGAAACACCGGTCCCCTCCACCCCACAGTG **GGG**TTAGAGCTAGAAATAGCAAGTTAA GGTAAGGTG
AGAGGCACCTGTCAGATTGA

Truncated Promoter Spacer Truncated Scaffold with **PAM** Primer-binding Sites

>hgRNA-B21
GAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATACAAGGCTGTTAGAGAGATAATTGGA
ATTAATTTGACTGTAAACACAAAGATATTAGTACAAAATACGTGACGTAGAAAGTAATAATTTCTTGGG
TAGTTTGCAGTTTTAAAATTATGTTTTAAAATGGACTATCATATGCTTACCGTAACTTGAAAGTATTTC

GATTTCTTGGCTTTATATATCTTGTGGAAAGGACG AAACACC GGTCGGAGGGAGGTGGGTTAG GGGTTA
GAGCTAGAAATAGCAAGTTAACCTAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTG
CTTTTTTTT

U6 Promoter   Predicted TSS   Spacer   gRNA Scaffold   U6 Terminator

>hgRNA-C21
GAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATACAAGGCTGTTAGAGAGATAATTGGA
ATTAATTTGACTGTAAACACAAAGATATTAGTACAAAATACGTGACGTAGAAAGTAATAATTTCTTGGG
TAGTTTGCAGTTTTAAAATTATGTTTTAAAATGGACTATCATATGCTTACCGTAACTTGAAAGTATTTC
GATTTCTTGGCTTTATATATCTTGTGGAAAGGACG AAACACC GGTACACCCTCAAGCAGTGTG GGGTTA
GAGCTAGAAATAGCAAGTTAACCTAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTG
CTTTTTTTT

U6 Promoter   Predicted TSS   Spacer   gRNA Scaffold   U6 Terminator

>hgRNA-D21
GAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATACAAGGCTGTTAGAGAGATAATTGGA
ATTAATTTGACTGTAAACACAAAGATATTAGTACAAAATACGTGACGTAGAAAGTAATAATTTCTTGGG
TAGTTTGCAGTTTTAAAATTATGTTTTAAAATGGACTATCATATGCTTACCGTAACTTGAAAGTATTTC
GATTTCTTGGCTTTATATATCTTGTGGAAAGGACG AAACACC GGTCACGCTACGGCCAAGGTG GGGTTA
GAGCTAGAAATAGCAAGTTAACCTAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTG
CTTTTTTTT

U6 Promoter   Predicted TSS   Spacer   gRNA Scaffold   U6 Terminator

>hgRNA-E21
GAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATACAAGGCTGTTAGAGAGATAATTGGA
ATTAATTTGACTGTAAACACAAAGATATTAGTACAAAATACGTGACGTAGAAAGTAATAATTTCTTGGG
TAGTTTGCAGTTTTAAAATTATGTTTTAAAATGGACTATCATATGCTTACCGTAACTTGAAAGTATTTC
GATTTCTTGGCTTTATATATCTTGTGGAAAGGACG AAACACC GGTTCCAGAGATACTGACGTG GGGTTA
GAGCTAGAAATAGCAAGTTAACCTAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTG
CTTTTTTTT

U6 Promoter   Predicted TSS   Spacer   gRNA Scaffold   U6 Terminator

>hgRNA-F21
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ATTAATTTGACTGTAAACACAAAGATATTAGTACAAAATACGTGACGTAGAAAGTAATAATTTCTTGGG
TAGTTTGCAGTTTTAAAATTATGTTTTAAAATGGACTATCATATGCTTACCGTAACTTGAAAGTATTTC
GATTTCTTGGCTTTATATATCTTGTGGAAAGGACG AAACACCGGTGTGTAGCGATGGCTACAG GGGTTA
GAGCTAGAAATAGCAAGTTAACCTAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTG
CTTTTTTTTT

U6 Promoter   Predicted TSS   Spacer   gRNA Scaffold   U6 Terminator

>hgRNA-G21
GAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATACAAGGCTGTTAGAGAGATAATTGGA
ATTAATTTGACTGTAAACACAAAGATATTAGTACAAAATACGTGACGTAGAAAGTAATAATTTCTTGGG
TAGTTTGCAGTTTTAAAATTATGTTTTAAAATGGACTATCATATGCTTACCGTAACTTGAAAGTATTTC
GATTTCTTGGCTTTATATATCTTGTGGAAAGGACG AAACACCGGTATGCGGATGCAATCTCCG GGGTTA
GAGCTAGAAATAGCAAGTTAACCTAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTG
CTTTTTTTTT

U6 Promoter   Predicted TSS   Spacer   gRNA Scaffold   U6 Terminator

>hgRNA-A’26
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TAGTTTGCAGTTTTAAAATTATGTTTTAAAATGGACTATCATATGCTTACCGTAACTTGAAAGTATTTC
GATTTCTTGGCTTTATATATCTTGTGGAAAGGACG AAACACCGGTAGACGCACCTCCACCCCACAGTG G
GGTTAGAGCTAGAAATAGCAAGTTAACCTAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGT
CGGTGCTTTTTTTT

U6 Promoter   Predicted TSS   Spacer   gRNA Scaffold   U6 Terminator

>hgRNA-A’51
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TAGTTTGCAGTTTTAAAATTATGTTTTAAAATGGACTATCATATGCTTACCGTAACTTGAAAGTATTTC

GATTTCTTGGCTTTATATATCTTGTGGAAAGGACG AAACACCGGTAGACGCGGTCACACTGATGCAGCT
AGTATGCACCTCCACCCCACAGTG GGGTTAGAGCTAGAAATAGCAAGTTAACCTAAGGCTAGTCCGTTA
TCAACTTGAAAAAGTGGCACCGAGTCGGTGCT TTTTTTT

U6 Promoter Predicted TSS Spacer gRNA Scaffold U6 Terminator

>hgRNA-A’76
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TAGTTTGCAGTTTTAAAATTATGTTTTAAAATGGACTATCATATGCTTACCGTAACTTGAAAGTATTTC
GATTTCTTGGCTTTATATATCTTGTGGAAAGGACG AAACACCGGTAGACGCTGTGACAGAGCCAACACG
CAGTCTCGGTCACACTGATGCAGCTAGTA TGCACCTCCACCCCACAGTG GGGTTAGAGCTAGAAATAGC
AAGTTAACCTAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCT TTTTTTT

U6 Promoter Predicted TSS Spacer gRNA Scaffold U6 Terminator

>hgRNA-A’101
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ATTAATTTGACTGTAAACACAAAGATATTAGTACAAAATACGTGACGTAGAAAGTAATAATTTCTTGGG
TAGTTTGCAGTTTTAAAATTATGTTTTAAAATGGACTATCATATGCTTACCGTAACTTGAAAGTATTTC
GATTTCTTGGCTTTATATATCTTGTGGAAAGGACG AAACACCGGTAGACGGCTAGATGAAGAGCAAGCG
CATGGACTGTGACAGAGCCAACACGCAGTCTCGGTCACACTGATGCAGCTAGTA TGCACCTCCACCCCA
CAGTGGGGTTAGAGCTAGAAATAGCAAGTTAACCTAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCA
CCGAGTCGGTGCT TTTTTTT

U6 Promoter Predicted TSS Spacer gRNA Scaffold U6 Terminator

>FISSEQ-hgRNA-Design1
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ATTAATTTGACTGTAAACACAAAGATATTAGTACAAAATACGTGACGTAGAAAGTAATAATTTCTTGGG
TAGTTTGCAGTTTTAAAATTATGTTTTAAAATGGACTATCATATGCTTACCGTAACTTGAAAGTATTTC
GATTTCTTGGCTTTATATATCTTGTGGAAAGGACG AAACACCGGTGGAGCAATTCCACAACAC GGGTTA
GAGCTAGAAATAGCAAGTTAACCTAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTG
CCACTGTTGTCTTATACCAACTTTCC TTTTTTT

U6 Promoter Predicted TSS Spacer gRNA Scaffold U6 Terminator
RT Primer Binding Site RCA Primer Binding Site

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>FISSEQ-hgRNA-Design2
GAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATACAAGGCTGTTAGAGAGATAATTGGA
ATTAATTTGACTGTAAACACAAAGATATTAGTACAAAATACGTGACGTAGAAAGTAATAATTTCTTGGG
TAGTTTGCAGTTTTAAAATTATGTTTTAAAATGGACTATCATATGCTTACCGTAACTTGAAAGTATTTC
GATTTCTTGGCTTTATATATCTTGTGGAAAGGACG AAACACCGGTGGAGCAATTCCACAACAC GGGTTA
GAGCTAGAAATAGCAAGTTAACCTAAGGCTAGTCCGTTATCAACTTATTA CACTGTTGTCTTATACCAA
CTTTCCTAATAAGTGGCACCGAGTCGGTGC TTTTTTT
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U6 Promoter   Predicted TSS   Spacer   gRNA Scaffold   U6 Terminator

RT Primer Binding Site   RCA Primer Binding Site