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Supplemental Information

Fine and Predictable Tuning of TALEN

Gene Editing Targeting for Improved T Cell

Adoptive Immunotherapy

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Supplementary tables

Sequences of off-site targets predicted in silico

Offtarget name	left_Sequence_With_Mismatches	right_Sequence_With_Mismatches	type
T3v1OS1	tctcttAgatAtgcAA	AGgcTgatcaaagTga	RR
T3v1OS2	tacctctgAgAAAcca	gcTcagatcCCagaga	LR
T3v1OS3	tctcttAAatctgcAc	CcgcaTCtcCaagaga	RR
T3v1OS4	taccCAGgtggggccT	AgTcccTGcagaggta	LL
T3v1OS5	taccGcGAtggggcca	TcTcaTCtcaaagaga	LR
T3v1OS6	taActAtgCAGggcca	ATgcTccacaAaggta	LL
T3v1OS7	tctcttgaGctTcAG	ATgcaTatcaTagaga	RR
T3v1OS8	tGcctcAgtgAggTca	CcAcagatTaaTgaga	LR
T3v1OS9	tacctctgtgTgAAcT	tggcccTAcagagTGA	LL
T3v1OS10	tctcttAaActTcAc	tgTccccTcTgTggta	RL
T3v1OS11	tctctCAGaGctgccc	CggccccacaTaCTta	RL
T3v1OS12	tacctctgCAGgAcCa	gcgcaCTGcaaCgaga	LR
T3v1OS13	tcAcCGtgatctgAgc	tggccAcGcagGTgta	RL
T3v1OS14	tGccGctgGggAgcca	GAgGcccaGagaggta	LL

Offtarget name	left_Sequence_With_Mismatches	right_Sequence_With_Mismatches	type	type
TRAC/T3v2OS1	tGcctctgCggAgcT	TgccccCTagaggta	PD1T3L	PD1T3L
TRAC/T3v2OS2	tcCctGGgatctgcA	TgcagatcaaagaTa	PD1T3R	PD1T3R
TRAC/T3v2OS3	tGcctcATggggcc	atCtctgtgTgTcCa	PD1T3L	TRAC_L
TRAC/T3v2OS4	tGccActgtggggAc	ggcccTGGTgaggCa	PD1T3L	PD1T3L
TRAC/T3v2OS5	tctcaActgAtacaG	TTcaTCtcaGagaga	TRAC_R	PD1T3R
TRAC/T3v2OS6	tctcttAgatAtgcA	GgcTgatcaaagTga	PD1T3R	PD1T3R
TRAC/T3v2OS7	ttgtAccacaAatat	cTTagGtcTCTgaga	TRAC_L	PD1T3R
TRAC/T3v2OS8	tctcAGAGaCctAAg	atafTtgtggTacaa	PD1T3R	TRAC_L
TRAC/T3v2OS9	tctcAGAGaCctAAg	atafTtgtggTacaa	PD1T3R	TRAC_L
TRAC/T3v2OS10	tctcAGAGaCctAAg	atafTtgtggTacaa	PD1T3R	TRAC_L
TRAC/T3v2OS11	ttgtAccacaAatat	cTTagGtcTCTgaga	TRAC_L	PD1T3R
TRAC/T3v2OS12	tctcAGAGaCctAAg	atafTtgtggTacaa	PD1T3R	TRAC_L
TRAC/T3v2OS13	tAtcttgaActgAg	CTccccTcagTggta	PD1T3R	PD1T3L
TRAC/T3v2OS14	ttgtccAaAagaCGt	cAcaTatcaTagaga	TRAC_L	PD1T3R
TRAC/T3v2OS15	tacctctCtAggAcc	TAcTgatTGAagaga	PD1T3L	PD1T3R
TRAC/T3v2OS16	taccAcAgAggggAc	cgTagCtcaaagTTa	PD1T3L	PD1T3R
TRAC/T3v2OS17	tGccActgtggggCAc	TgTagatcaGaTaga	PD1T3L	PD1T3R
TRAC/T3v2OS18	tctAtttgaActgAA	GGatTtgtTggacaa	PD1T3R	TRAC_L
TRAC/T3v2OS19	ttAGTcAacagatat	cgTagTtTaaagaga	TRAC_L	PD1T3R
TRAC/T3v2OS20	tctcttAatcAgAg	TTTagatcaTagaAa	PD1T3R	PD1T3R
TRAC/T3v2OS21	tctcttAAatctgcA	cgcaTCtcCaagaga	PD1T3R	PD1T3R
TRAC/T3v2OS22	tGcctctAAgAgAcc	cAcagatcTaagTga	PD1T3L	PD1T3R
TRAC/T3v2OS23	tGcctctgtgggCcc	cTTAaatcaGagaga	PD1T3L	PD1T3R
TRAC/T3v2OS24	tacctcGAaggAAc	TTcaAatcaaagaga	PD1T3L	PD1T3R
TRAC/T3v2OS25	tGcctcAgtgAggTc	cAcagatTaaTgaga	PD1T3L	PD1T3R

List of oligonucleotides used for NGS sequencing to analyze off-target cleavages

T3v1OS1M1	CCATCTCATCCCTGCGTGTCTCCGACTCAGACGAGTGCGTGCTGAGCAGAACAGTCATGG
T3v1OS1M15	CCATCTCATCCCTGCGTGTCTCCGACTCAGATACGACGTAGCTGAGCAGAACAGTCATGG
T3v1OS2M2	CCATCTCATCCCTGCGTGTCTCCGACTCAGACGCTCGACAACGGCAGGAACCTCCATTAGC
T3v1OS2M16	CCATCTCATCCCTGCGTGTCTCCGACTCAGTCACGTAACGGCAGGAACCTCCATTAGC
T3v1OS3M3	CCATCTCATCCCTGCGTGTCTCCGACTCAGAGACGCACTCAGGATCATACTCTGCCACGC
T3v1OS3M17	CCATCTCATCCCTGCGTGTCTCCGACTCAGCGTCTAGTACAGGATCATACTCTGCCACGC
T3v1OS4M4	CCATCTCATCCCTGCGTGTCTCCGACTCAGAGCACTGTAGTCAGCTTAGGGCCTTTTCTCA
T3v1OS4M18	CCATCTCATCCCTGCGTGTCTCCGACTCAGTCTACGTAGCTCAGCTTAGGGCCTTTTCTCA
T3v1OS5M5	CCATCTCATCCCTGCGTGTCTCCGACTCAGATCAGACACGGCTAGGCAAGGGAGTATGGG
T3v1OS5M19	CCATCTCATCCCTGCGTGTCTCCGACTCAGTGTACTACTCGCTAGGCAAGGGAGTATGGG
T3v1OS6M6	CCATCTCATCCCTGCGTGTCTCCGACTCAGATATCGCGAGAGAACCTCAGAGAGCTACTGC
T3v1OS6M20	CCATCTCATCCCTGCGTGTCTCCGACTCAGACGACTACAGAGAACCTCAGAGAGCTACTGC
T3v1OS7M7	CCATCTCATCCCTGCGTGTCTCCGACTCAGCGTGTCTCTAACAGTGATTGTTTCTAGGTGGC
T3v1OS7M21	CCATCTCATCCCTGCGTGTCTCCGACTCAGCGTAGACTAGACAGTGATTGTTTCTAGGTGGC
T3v1OS8M8	CCATCTCATCCCTGCGTGTCTCCGACTCAGCTCGCGTGTCTCCATCACCTCTTGAGCATTATCA
T3v1OS8M22	CCATCTCATCCCTGCGTGTCTCCGACTCAGTACGAGTATGTCCATCACCTCTTGAGCATTATCA
T3v1OS9M9	CCATCTCATCCCTGCGTGTCTCCGACTCAGTAGTATCAGCACAGGAAAGAAACACACTGTTGG
T3v1OS9M23	CCATCTCATCCCTGCGTGTCTCCGACTCAGTACTCTCGTGACAGGAAAGAAACACACTGTTGG
T3v1OS10M10	CCATCTCATCCCTGCGTGTCTCCGACTCAGTCTCTATGCGAGGCGTGACTTCAATCATCCA
T3v1OS10M24	CCATCTCATCCCTGCGTGTCTCCGACTCAGTAGAGACGAGAGGCGTGACTTCAATCATCCA
T3v1OS11M11	CCATCTCATCCCTGCGTGTCTCCGACTCAGTGATACGTCTCGTATCCCAGCGACTAAGC
T3v1OS11M25	CCATCTCATCCCTGCGTGTCTCCGACTCAGTCGTCGCTCGCGTATCCCAGCGACTAAGC
T3v1OS12M12	CCATCTCATCCCTGCGTGTCTCCGACTCAGTACTGAGCTACGGGAGCTTCACTGAGTCAC
T3v1OS12M26	CCATCTCATCCCTGCGTGTCTCCGACTCAGACATACGCGTCGGGAGCTTCACTGAGTCAC
T3v1OS13M13	CCATCTCATCCCTGCGTGTCTCCGACTCAGCATAGTAGTGCCCTTCTAGCCATGAATGA
T3v1OS13M27	CCATCTCATCCCTGCGTGTCTCCGACTCAGACGCGAGTATCCCCTTCTAGCCATGAATGA
T3v1OS14M14	CCATCTCATCCCTGCGTGTCTCCGACTCAGCGAGAGATACTACGTCATAACACTGCGCGGCC
T3v1OS14M28	CCATCTCATCCCTGCGTGTCTCCGACTCAGACTACTATGTTACGTCATAACACTGCGCGGCC
T3v1OS1R	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGGAGTGTCACTCTGGAGGGCA
T3v1OS2R	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGGCTTGGCTAGATCTGGGA
T3v1OS3R	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGCTGAATTTTCTCGGGCA
T3v1OS4R	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGCAGTCTCTCCCTCACTTGG
T3v1OS5R	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGGACTCTACTCCTGGATCCCCA
T3v1OS6R	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGACCACCTCTGTTTGTCTCAG
T3v1OS7R	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGCAGTCATCTCCTGGAGCC
T3v1OS8R	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGTCATTTGCAGCAACACAGATGG
T3v1OS9R	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGGGCACAAACCTTCTCAAGTAGA
T3v1OS10R	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGCTGAAAGTCACCCCTGCACC
T3v1OS11R	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGCTTATACCCCGAACCTGCA
T3v1OS12R	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGTGGAGCCTCACCATAGGGAC
T3v1OS13R	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGTCTCCATCCTGCCATGAGC
T3v1OS14R	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGGGTTGGAGTTGCTTCTCGGA
T3v1MIDF25	CCATCTCATCCCTGCGTGTCTCCGACTCAGTCGTCGCTCGGACAGAGATGCCGGTCACCATTC
T3v1MIDF26	CCATCTCATCCCTGCGTGTCTCCGACTCAGTCGTCGCTCGGACAGAGATGCCGGTCACCATTC
T3v1MIDR	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGGGACAACGCCACCTTACCTGC

TRAC/T3v2OS1F	AAGACTCGGCAGCATCTCCATTGAGGCATATTCGGGCAGG
TRAC/T3v2OS1-1R	GCGATCGTCACTGTTCTCCAGGCCCTAACTCAAACCCACA
TRAC/T3v2OS2-F	AAGACTCGGCAGCATCTCCATGACTTTGTAGCTCCCTCTGC
TRAC/T3v2OS2-R	GCGATCGTCACTGTTCTCCATAACCTAGGCACATCCTCCC
TRAC/T3v2OS3-1-F	AAGACTCGGCAGCATCTCCATGCACCACAGAACTGGGTGA
TRAC/T3v2OS3-1-R	GCGATCGTCACTGTTCTCCAGGCTTTAATCCAGGATGACTGC
TRAC/T3v2OS4-1-F	AAGACTCGGCAGCATCTCCATTCTTAGCACAGGGCCTTGG
TRAC/T3v2OS4-1-R	GCGATCGTCACTGTTCTCCAGACCTGGCTCGTTTCTGTGA
TRAC/T3v2OS5-1-F	AAGACTCGGCAGCATCTCCAAAACCCCTGGCCACATCTTCA
TRAC/T3v2OS5-1-R	GCGATCGTCACTGTTCTCCAACCTCATGGTCGCAAAGTGG
TRAC/T3v2OS6-1-F	AAGACTCGGCAGCATCTCCAAGTTAGGGGAGGCAGGGAGA
TRAC/T3v2OS6-1-R	GCGATCGTCACTGTTCTCCAACAGCCCCGCTTCTATCCC
TRAC/T3v2OS7-1-F	AAGACTCGGCAGCATCTCCATGAAAAATGAGGAAGGGTGCC
TRAC/T3v2OS7-1-R	GCGATCGTCACTGTTCTCCATCCATGACAACTCTGAGTTAGCA
TRAC/T3v2OS8-2-F	AAGACTCGGCAGCATCTCCATCCTATATAATGCCAGATTAGC
TRAC/T3v2OS8-2-R	GCGATCGTCACTGTTCTCCATGAAAGATGAGGAAGAGTGC
TRAC/T3v2OS13-F	AAGACTCGGCAGCATCTCCAATGGACATAAATTTAAGCTAGCAGC
TRAC/T3v2OS13-R	GCGATCGTCACTGTTCTCCAGCCCTTACACATGTTCTTGCA
TRAC/T3v2OS14-F	AAGACTCGGCAGCATCTCCATGGGGGAGTTACAGAAAAGCC
TRAC/T3v2OS14-R	GCGATCGTCACTGTTCTCCACTGATGGAATTACAGGCATAGCA
TRAC/T3v2OS15-1-F	AAGACTCGGCAGCATCTCCAGCAAGTCAGTCATTTGTCAGGG
TRAC/T3v2OS15-1-R	GCGATCGTCACTGTTCTCCATACTATTCCGGGAGCGCAGGA
TRAC/T3v2OS16-1-F	AAGACTCGGCAGCATCTCCACCCCTTCTCTTCTTCCCTCC
TRAC/T3v2OS16-1-R	GCGATCGTCACTGTTCTCCACATGGTGAGTACTCAGGCCA
TRAC/T3v2OS17-1-F	AAGACTCGGCAGCATCTCCAGCCATAAAATGCCAAAGCC
TRAC/T3v2OS17-1-R	GCGATCGTCACTGTTCTCCAAGAGGTCAGAGTCTGGGCA
TRAC/T3v2OS18-1-F	AAGACTCGGCAGCATCTCCATTGACTCTGTGCCAGGAACC
TRAC/T3v2OS18-1-R	GCGATCGTCACTGTTCTCCAAGGAGAGGAGAGGACCTGGA
TRAC/T3v2OS19-1-F	AAGACTCGGCAGCATCTCCACAGAAAGAGAGAGGAGAAAGAAAGA
TRAC/T3v2OS19-1-R	GCGATCGTCACTGTTCTCCACATGGAACCTTTCTGTTCTCC
TRAC/T3v2OS20-1-F	AAGACTCGGCAGCATCTCCAGCCTTTCTCCTGAGCTGTGA
TRAC/T3v2OS20-1-R	GCGATCGTCACTGTTCTCCAGCTGCAACAATGGTTTACTTTGC
TRAC/T3v2OS21-1-F	AAGACTCGGCAGCATCTCCAAGCACTTCACTTAACTCAGGATCA
TRAC/T3v2OS21-1-R	GCGATCGTCACTGTTCTCCAAAATGAGATGATACCTTTCTGACC
TRAC/T3v2OS22-1-F	AAGACTCGGCAGCATCTCCAGGCATCCTTCCATCAGCACA
TRAC/T3v2OS22-1-R	GCGATCGTCACTGTTCTCCATGCACAATTCTTTTTAACACACGC
TRAC/T3v2OS23-F	AAGACTCGGCAGCATCTCCATGTCTAGATGGGAATGGTTTGC
TRAC/T3v2OS23-R	GCGATCGTCACTGTTCTCCAGCCATGATTTTCTTCCATAGTCA
TRAC/T3v2OS25-2-F	AAGACTCGGCAGCATCTCCATGTATGTTTGTACCTTTAGCCAAC
TRAC/T3v2OS25-2-R	GCGATCGTCACTGTTCTCCAACCTGAAGCACTGTTACCATAG

Total number of reads and total number of events detected, Figure 2C

Non-transfected			
Target	# EvtS	# total reads	estimated indels frequency
PD-1	0	5898	< 0.000170
off-site #1	0	11234	< 0.000089
off-site #2	0	1364	< 0.000733
off-site #3	0	15178	< 0.000066
off-site #4	0	15043	< 0.000066
off-site #5	0	10077	< 0.000099
off-site #6	0	33787	< 0.000030
off-site #7	0	10778	< 0.000093
off-site #8	0	4133	< 0.000242
off-site #9	3	12554	0.000239
off-site #10	0	13595	< 0.000074
off-site #11	0	13689	< 0.000073
off-site #12	21	9066	0.002316
off-site #13	0	5675	< 0.000176
off-site #14	0	2399	< 0.000417
T3v1 PD-1			
Target	# EvtS	# total reads	estimated indels frequency
PD-1	3388	3678	0.921153
off-site #1	0	10714	< 0.000093
off-site #2	0	1990	< 0.000503
off-site #3	0	9304	< 0.000107
off-site #4	0	15664	< 0.000064
off-site #5	0	12330	< 0.000081
off-site #6	2	43075	0.000046
off-site #7	0	12146	< 0.000082
off-site #8	0	3488	< 0.000287
off-site #9	244	20926	0.011660
off-site #10	0	15634	< 0.000064
off-site #11	0	16972	< 0.000059
off-site #12	10	10185	0.000982
off-site #13	0	2126	< 0.000470
off-site #14	3	3889	0.000771

The efficiency of T3v1 PD-1 TALEN-mediated gene processing was analyzed by high-throughput DNA sequencing analysis using 454 sequencing system. The indels frequency is estimated as followed: total number of events/total number of reads.

Total number of reads and total number of events detected, Figure 3C

Non-transfected			
Target	# EvtS	# total reads	estimated indels frequency
PD-1	1	4356	0.00022957
off-site #9	1	14080	7.1023E-05
T3v2 PD-1			
Target	# EvtS	# total reads	estimated indels frequency
PD-1	4141	4559	0.90831323
off-site #9	0	11223	< 0.000089
T3v3 PD-1			
Target	# EvtS	# total reads	estimated indels frequency
PD-1	4010	4780	0.83891213
off-site #9	1	4691	0.00021317

The efficiency of T3v2 and T3v3 PD-1 TALEN-mediated gene processing was analyzed by high-throughput DNA sequencing analysis using 454 sequencing system. The indels frequency is estimated as followed: total number of events/total number of reads.

Total number of reads and total number of events detected, Figure 4A

Non-transfected			
Target	# EvtS	# total reads	estimated indels frequency
PD-1	14	9703	0.001443
TRAC	5	15331	0.000326
off-site #1	1	6727	0.000149
off-site #2	1	9173	0.000109
off-site #3	0	9167	< 0.000109
off-site #4	6	11614	0.000517
off-site #5	0	4584	< 0.000218
off-site #6	0	4157	< 0.000241
off-site #7	4	16037	0.000249
off-site #8	0	16669	< 0.00006
off-site #13	5	15147	0.000330
off-site #14	0	8797	< 0.000114
off-site #15	698	15287	0.045660
off-site #16	1	18109	0.000055
off-site #17	17	9861	0.001724
off-site #18	2	6052	0.000330
off-site #19	3	11415	0.000263
off-site #20	4	8167	0.000490
off-site #21	0	2065	< 0.000484
off-site #22	4	11239	0.000356
off-site #23	8	10088	0.000793
off-site #24	1	5271	0.000190
off-site #25	45	14415	0.003122
T3v2 PD-1/TRAC			
Target	# EvtS	# total reads	estimated indels frequency
PD-1	5539	7059	0.784672
TRAC	9	6190	0.001454
off-site #1	6	12799	0.000469
off-site #2	248	8371	0.029626
off-site #3	1	10716	0.000093
off-site #4	0	4330	< 0.000231
off-site #5	0	4167	< 0.000537
off-site #6	7	13040	0.000537
off-site #7	1	14157	0.000071
off-site #8	7	16926	0.000414
off-site #13	1	12087	0.000083
off-site #14	736	15230	0.048326
off-site #15	1	15038	0.000066
off-site #16	24	12413	0.001933
off-site #17	1	5793	0.000173
off-site #18	1	12288	0.000081
off-site #19	3	8184	0.000367
off-site #20	1	2804	0.000357
off-site #21	2	12478	0.000160
off-site #22	6	8545	0.000702
off-site #23	1	7096	0.000141
off-site #24	18	12156	0.001481
off-site #25	3933	8911	0.441365

In depth characterization of the molecular events generated by T3v2 PD-1 TALEN in combination with TRAC TALEN by high-throughput DNA sequencing analysis using Illumina method. The indels frequency is estimated as followed: total number of events/total number of reads.

Total number of reads and total number of events detected, Figure 4C

Non-transfected			
Target	# EvtS	# total reads	estimated indels frequency
PD-1	265	78440	0.003378378
TRAC	412	184080	0.002238157
v2OS3	6	95819	6.26181E-05
v1OS9	51	135931	0.00037519
T3v1 PD-1/TRAC			
Target	# EvtS	# total reads	estimated indels frequency
PD-1	60009	67419	0.890090331
TRAC	61559	100579	0.612046252
v2OS3	2553	97569	0.026166098
v1OS9	1762	128743	0.013686181
T3v2 PD-1/TRAC			
Target	# EvtS	# total reads	estimated indels frequency
PD-1	65292	71167	0.917447693
TRAC	72287	113150	0.63885992
v2OS3	1520	101328	0.01500079
v1OS9	73	148720	0.000490855
T3v3 PD-1/TRAC			
Target	# EvtS	# total reads	estimated indels frequency
PD-1	80010	92730	0.862827564
TRAC	53601	107114	0.500410777
v2OS3	747	104308	0.007161483
v1OS9	58	129894	0.000446518
T3v4 PD-1/TRAC			
Target	# EvtS	# total reads	estimated indels frequency
PD-1	54533	73740	0.739530784
TRAC	52627	89848	0.585733684
v2OS3	11	133406	8.24551E-05
v1OS9	47	140867	0.000333648

In depth characterization of the molecular events generated by T3v1, T3v2, T3v3 or T3v4 PD-1 TALEN in combination with TRAC TALEN by high-throughput DNA sequencing analysis using Illumina method. The indels frequency is estimated as followed: total number of events/total number of reads.