а







Detected Cas9: MYC locus1 off-targets







Figure legend

Fig. 1 PEM-seq Detects Off-target Hotspots of CRISPR/Cas9

(a) Overview of PEM-seq. To prepare PEM-seq libraries, primer extension generated a copy of template with biotinylated primer followed by bridge adapter ligation and DNA amplification. Gray bars represented bait region while orange ones for captured prey region;
"N" indicated RMB in the bridge adapter. Arrows indicated positions and orientations of primers. See supplemental protocols for details.

(b) Circos plots of Cas9:RAG1A libraries. Three biological replicates were showed from outside to inside, and the displayed translocation junctions were 19,494, 16,005 and 18,078. Genome-wide translocation junctions binned into 5-Mb regions (blue lines) were plotted on a log scale. Chromosomes were showed with centromere to telomere in clockwise direction. Red arrow indicated the Cas9:RAG1A cleavage site. Colored lines connected the on-target site to the off-target hotspots.

(c) Zoomed-in view of Cas9:RAG1A translocation junctions (binned into 2Mb) on chromosome 7. Red arrows indicated identified off-target hotspots. Cen represented centromere and p/q indicated the chromosome arms. The Pearson correlation coefficient was 0.99 between replicates 1 and 2, 0.99 between replicates 1 and 3, 0.98 between replicates 2 and 3.

(d) Scatter plot of Cas9:RAG1A off-target hotspots in 293T, HCT116, K562 and U2OS cells.*y* axis showed frequency of each hotspot per 100,000 editing events (indels plus translocation). Red asterisks indicated the off-target hotspots detected by PEM-seq but not by LAM-HTGTS.

(e) Venn Diagram showing the overlap for RAG1A off-target hotspots among indicated cell lines. Legend is the same as showed in panel (d).

Fig. 2 Assessing Editing Efficiencies of CRISPR/Cas9 by PEM-seq

(a) Outcomes of Cas9-induced DSBs. Germline represents un-cut or perfect rejoining; indels is derived from errored rejoining; translocation involves a second DSB.

(b) Percentages of germline, indels and translocation showed by pie graph for Cas9:RAG1A. Mean ± SD. (c) Frequency of indels for Cas9:RAG1A detected by PEM-seq, RFLP, T7EI assay and single cell RFLP. Averages were indicated by black lines. DNA from different batches were marked in different colors.

(d) Composition of Cas9:RAG1A translocation either joined with off-target DSBs or genomewide low-level DSBs. Mean \pm SD.

(e) Composition of indels of Cas9:RAG1A libraries within ± 20 bp around cleavage-site. Note that part of deletions also involved insertion, indicated in gray. Mean \pm SD.

(f) Frequency of junctions (binned into 50 bp) within \pm 5 kb (\pm 20 bp excluded) around Cas9:RAG1A cleavage-site. Upper schematic showed different DNA repair products detected by PEM-seq, including inversion, deletion, and excision. Yellow box indicates the Cas9 target site. Blue and red arrows indicate the translocation orientations. Purple arrow indicates the position and orientation of primer used for PEM-seq. In the bottom panel, black dotted line indicates the Cas9 cleavage-site, while yellow dotted line indicates the primer locus. Junction numbers for each region were indicated.

(g) Frequency of junctions (binned into 1kb) for 5-50 kb downstream of Cas9:RAG1A cleavage-site. Black dotted lines indicate the boundaries of the 5-50 kb region. Purple arrow indicates the primer used for PEM-seq. Junction numbers for each region were indicated.

Fig. 3 Cas9 Nickase Shows Lower Off-target Activity with Loss of Target Editing Efficiency Schematic of RAG1A, RAG1B, RAG1C and RAG1G target-site positions. Purple arrows indicated the biotinylated primers used for PEM-seq. Yellow boxes represented gRNA target sites and red bars indicated Cas9 cleavage site. In the lower panels, pie graph showed the compositions of germline, indels, and translocation for indicated Cas9 treatment in HEK293T cells. Mean \pm SD.

Fig. 4 Editing Efficiencies and Specificity of Cas9 Variants

(a) Schematic of Cas9 domains and corresponding point mutations for Cas9 variants. Red letters indicated mutated amino acids of indicated Cas9 variants.

(b) Editing efficiencies detected by PEM-seq for Cas9 variants targeting RAG1A in HEK293T cells. Target site sequence was listed above and the red letters indicated the PAM sequence. Error bars, mean \pm SD. Two-tailed t-test, *, p < 0.05.

(c) Frequencies of total translocation junctions in 1-kb regions around off-target hotspots for indicated variants targeting RAG1A in HEK293T cells. Total numbers of identified off-target hotspots for each Cas9 variant were showed above the bar. Error bars, mean \pm SD. Two-tailed t-test, *, p < 0.05; ** p < 0.01.

(d) Scatter plot of RAG1A off-target hotspots for indicated variants. *y* axis showed frequency of each hotspot per 100,000 editing events (indels plus translocation). Note that the WT libraries presented in (b-d) were independently prepared from the ones in Fig. 1 and 2. Sequencing reads for the libraries in this figure was less (~60%) and the identified hotspots were only 38, but still more than LAM-HTGTS.

(e-g) Editing efficiencies and off-target hotspots for Cas9 variants targeting *EMX1* site in HEK293T cells, depicted as described in the legend to panels b-d.

Fig. 5 AcrIIA4 Blocks Cas9 Off-target Activity Less Effectively

(a) Editing efficiencies detected by PEM-seq for Cas9:RAG1A in HEK293T cells with indicated mass ratios of Cas9 over AcrIIA4. Error bars, mean \pm SD. Two-tailed t-test, **, p < 0.01.

(b) Frequencies of translocation junctions in 1-kb regions around off-target hotspots for Cas9:RAG1A in HEK293T cells with indicated ratios of Cas9 over AcrIIA4. Total numbers of identified off-target hotspots for indicated samples were showed above the bars. Error bars, mean \pm SD. Two-tailed t-test, *, p < 0.05; ** p < 0.01.

(c) Composition of indels and off-target junctions for Cas9:RAG1A libraries with indicated ratios of Cas9 over AcrIIA4. Total junction numbers from pooled three libraries were indicated above the bars. Note that the total bar length of each library was normalized to the same though inhibitor-treated samples always contained less junctions than un-treated ones. (d-f) Editing efficiencies and off-target hotspots in other Cas9-targeting loci with 1:1 Cas9 over AcrIIA4, depicted as described in the legend to panels (a-c). Note that for 1:1 ratio described in panels (a-c), the amount of plasmid DNA used for transfection for Cas9:RAG1A, AcrIIA4 and blank were 2 μg, 2 μg and 4 μg respectively; while for 1:1 in panels (d-f), the amounts were 2 μg, 2 μg and 0, which led to a higher transfection efficiency

of Cas9:RAG1A.

Supplementary Fig. S1 Genomic translocation features revealed by PEM-seq. (a) Workflow of PEM-seq pipeline. Raw data was subjected to data preprocess, reads alignment and de-duplicates with RMB. Then unique reads were categorized into germline, indels and translocation according to the sequence features. See online methods for details. (b) *In vitro* Cas9 digestion on RAG1A off-target hotspots. Indicated amplified fragments were incubated with purified Cas9 for 20 hrs. "On" was the RAG1A on-target site. "NC", negative control with no Cas9:RAG1A target sites but can be targeted by Cas9:*MYC1*. Red triangles indicated the un-cleaved fragment, while blue triangles indicated the larger cleaved fragments. The detailed information of these off-targets were showed in Supplementary Table S2.

(c) Circos plots of Cas9:RAG1A libraries with RAG1A OT6 and OT8 loci as bait in 293T cells. Red triangles indicated the bait sites and red asterisks indicated the detected off-target, and blue triangles indicated the position of RAG1A on-target sites. The detailed information of these off-targets are showed in Supplementary Table S2.

(d) Frequencies of translocation junctions in genetic and intergenic regions for Cas9:RAG1A in indicated cell lines. Error bars, mean \pm SD.

(e) Distribution profiles of off-target hotspots-excluded translocation junctions in each chromosome for Cas9:RAG1A in indicated cell lines. Red arrow indicated the target site-containing chromosome. Error bars, mean \pm SD.

(f) Circos plots of Cas9:RAG1A libraries in HCT116, K562 and U2OS cell lines, depicted as described in the legend to Fig.1b.

Supplementary Fig. S2 Detecting indels generated by CRISPR/Cas9.

(a) Frequencies of indels detected by PEM-seq from titrated amounts of raw reads extracted from Cas9:RAG1A library with 14-bp or 7-bp RMB. The original read size for the Cas9:RAG1A library was 7.85 million. We randomly took 1 M to 7M (one sample per 0.5M) raw reads to go through the PEM-seq "SuperQ" pipeline individually. The 7-bp RMB was truncated from 14-bp RMB for simulation analysis. If the RMB got saturated, the frequencies of indels would increase due to the miss-assignment of germline as showed by the red line. (b) Frequencies of indels for libraries with indicated ratios of Cas9:RAG1A-treated and untreated DNA. The frequency of indels consistently increased about 7% per 25% more Cas9-treated DNA.

(c) Frequencies of indels detected by T7EI for Cas9:RAG1A in HEK293T cells.

Cas9:RAG1A cleavge site was PCR amplified and either mock cleaved (–) or T7EI cleaved (+). Length of the uncut and cleaved DNA, and frequencies of indels for each lane was indicated. See online methods for details.

(d) Frequencies of indels detected by RFLP for Cas9:RAG1A in HEK293T cells.

Cas9:RAG1A targeting sequence was underlined by blue and PAM sequence by yellow. Purple arrow indicated the Cas9 cutting site. Red sequence indicated the endonuclease *Sty*I recognition site and red line showed the cutting site. Length of the uncut and *Sty*I-cleaved DNA, and frequencies of indels for each lane were indicated. See online methods for details. (e) Table for single cell RFLP in HEK293T cells statistics. Three biological repeats were showed. See online methods for details.

(f) Frequencies of indels for Cas9:RAG1A detected by PEM-seq and TIDE. Note that DNA used here was different batch as that used in Fig. 2. Red box indicates the Cas9:RAG1A target site. DNA from the same batch were marked in the same color. n.s. not significant. See online methods for details.

Supplementary Fig. S3 Editing efficiency and off-target hotspots detected by PEM-seq for Cas9 variants.

(a) Circos plots of Cas9 variants for *EMX1* locus libraries, depicted as described in the legend to Fig. 1b.

(b-d) Editing efficiencies and off-target hotspots for Cas9 variants targeting *MYC* locus1 in HEK293T cells, depicted as described in the legend to panels Fig.4b-d.

Supplementary Fig. S4 AcrIIA4 suppresses Cas9 activity.

(a) Scatter plot of Cas9:RAG1A off-target hotspots in HEK293T cells with indicated ratios of Cas9 over AcrIIA4. *y* axis showed frequency of each hotspot per 500 editing events (indels + translocation). There low-frequency off-target hotspots detected in 3:1 libraries but not in no-inhibitor libraries were on the list of 53 total Cas9:RAG1A off-target sites. Only 19 off-target hotspots were detected in the no-inhibitor control libraries, because blank plasmid mock added into the transfection mixture reduced the delivery efficiency of Cas9:RAG1A plasmid. (b) Editing efficiencies detected by PEM-seq for *Sa*Cas9:*MYC* locus1 with or without AcrIIA4 inhibitor. Error bars, mean \pm SD.

(c) Translocation junctions captured for Cas9:RAG1A on- and off-target sites with or without AcrIIA4 inhibitor identified by *Sa*Cas9:*MYC* locus1 bait. Total numbers of Cas9:RAG1A on- and off-target junctions for each treatment were showed above the bar. Fold changes of junctions between different treatments were indicated.

Supplementary Table S1: Information of PEM-seq libraries

RAG1A locus in different cell lines										
Nuclease	Cell line	Locus	GFP (%)	DNA	Reads	Trans- location	Indels	Germline	Editing efficiency	Pearson correlation coefficient
	293T	RAG1A	95%	20ug	7,851,886	19,494	290,742	460,327	39.7%	1.00
Cas9	293T	RAG1A	90%	20ug	6,632,310	16,005	236,954	459,240	36.5%	1.00
	293T	RAG1A	93%	20ug	6,939,348	18,078	237,726	406,670	39.0%	1.00
Total				60ug	21,423,544	53,577	765,422	1,326,237		
	HCT116	RAG1A	84%	20ug	2,594,049	1,002	34,315	453,894	6.1%	1.00
Cas9	HCT116	RAG1A	81%	20ug	3,093,171	1,161	34,702	466,884	6.2%	1.00
	HCT116	RAG1A	79%	20ug	2,687,388	647	30,707	465,548	5.3%	1.00
Total				60ug	8,374,608	2,810	99,724	1,386,326		
	K562	RAG1A	97%	20ug	5,714,664	5,564	177,063	998,723	13.8%	1.00
Cas9	K562	RAG1A	96%	20ug	5,171,097	4,977	129,098	869,775	11.8%	1.00
	K562	RAG1A	97%	20ug	5,088,244	4,586	139,803	899,627	12.2%	1.00
Total				60ug	15,974,005	15,127	445,964	2,768,125		
	U2OS	RAG1A	80%	20ug	4,069,162	2,496	60,684	746,677	7.2%	1.00
Cas9	U2OS	RAG1A	80%	20ug	3,837,414	2,122	56,258	693,266	7.2%	1.00
	U2OS	RAG1A	80%	20ug	3,652,282	2,019	52,057	657,544	7.0%	1.00
Total				60ug	11,558,858	6,637	168,999	2,097,487		
				Other lo	oci around RAC	G1A				
	293T	RAG1B	90%	20ug	3,080,750	1,654	90,793	435,451	18.8%	1.00
Cas9	293T	RAG1B	88%	20ug	3,212,193	1,295	79,374	376,688	19.4%	1.00
	293T	RAG1B	88%	20ug	2,126,589	927	48,035	200,496	21.7%	1.00
Total				60ug	8,419,532	3,876	218,202	1,012,635		
	293T	RAG1C	90%	20ug	1,201,887	988	73,467	200,535	28.2%	1.00
Cas9	293T	RAG1C	88%	20ug	1,997,275	1,552	89,084	271,415	26.6%	1.00
	293T	RAG1C	82%	20ug	3,017,360	1,784	137,037	401,451	29.3%	1.00
Total				60ug	6,216,522	4,324	299,588	873,401		
	293T	RAG1A&G	92%	20ug	5,329,428	2,071	128,249	360,296	21.6%	1.00
Cas9n	293T	RAG1A&G	86%	20ug	2,612,348	1,785	89,569	324,397	17.8%	1.00
	293T	RAG1A&G	83%	20ug	2,616,692	1,589	88,624	318,448	18.4%	1.00
Total				60ug	10,558,468	5,445	306,442	1,003,141		
RAG1A and DNA titration libraries										
4:0	293T	RAG1A	-	20ug	2,765,758	10,562	146,721	328,693	32.4%	-
3:1	293T	RAG1A	-	20ug	2,244,669	6,617	98,938	334,469	24.0%	-

2:2	293T	RAG1A	-	20ug	2,447,355	4,827	77,638	456,722	15.3%	-
1:3	293T	RAG1A	-	20ug	1,814,991	2,322	40,017	497,239	7.8%	-
0:4	293T	RAG1A	-	20ug	1,718,514	45	8,995	542,549	1.6%	-
				Cas9	:RAG1A varian	ts				
	293T	RAG1A	87%	20ug	3,842,485	11,159	156,800	339,285	36.2%	1.00
Cas9	293T	RAG1A	89%	20ug	4,726,942	18,801	227,188	416,858	40.0%	1.00
	293T	RAG1A	80%	20ug	4,147,416	8,087	137,379	388,652	31.8%	1.00
Total				60ug	12,716,843	38,047	521,367	1,144,795		
	293T	RAG1A	98%	20ug	4,752,710	10,281	196,184	327,216	36.9%	1.00
D1135E	293T	RAG1A	96%	20ug	3,500,646	10,085	193,552	310,619	38.7%	1.00
	293T	RAG1A	96%	20ug	4,186,260	10,531	192,087	281,209	41.2%	1.00
Total				60ug	12,439,616	30,897	581,823	919,044		
	293T	RAG1A	92%	20ug	3,225,144	5,381	137,897	249,179	37.0%	1.00
eCas9	293T	RAG1A	90%	20ug	3,084,029	5,052	133,061	327,003	30.4%	1.00
	293T	RAG1A	88%	20ug	1,846,304	3,105	88,385	211,194	31.6%	1.00
Total				60ug	8,155,477	13,538	359,343	787,376		
	293T	RAG1A	87%	20ug	2,227,781	13,222	59,680	162,041	46.0%	1.00
FeCas9	293T	RAG1A	82%	20ug	2,476,621	17,906	86,764	188,102	59.8%	1.00
	293T	RAG1A	81%	20ug	2,181,993	11,605	47,357	139,839	45.9%	1.00
Total				60ug	6,886,395	42,733	193,801	489,982		
				Cas	EMX1 variant	s				
	293T	EMX1	83%	20ug	2,259,843	3,561	182,614	202,850	57.9%	1.00
SpCas9	293T	EMX1	96%	20ug	1,728,790	13,255	253,009	138,626	68.9%	1.00
	293T	EMX1	97%	20ug	1,617,812	6,148	196,836	80,635	73.6%	1.00
Total				60ug	5,606,445	22,964	632,459	422,111		
	293T	EMX1	86%	20ug	2,245,786	4,408	281,487	234,844	63.8%	1.00
D1135E	293T	EMX1	93%	20ug	1,442,261	5,278	219,216	128,526	68.2%	1.00
	293T	EMX1	98%	20ug	1,430,186	4,982	206,844	84,695	72.9%	1.00
Total				60ug	5,118,233	14,668	707,547	448,065		
	293T	EMX1	90%	20ug	2,825,769	3,978	252,905	168,454	67.4%	1.00
eCas9	293T	EMX1	94%	20ug	1,349,284	5,640	238,614	145,073	66.7%	1.00
	293T	EMX1	90%	20ug	1,577,042	4,374	189,306	93,930	75.2%	1.00
Total				60ug	5,752,095	13,992	680,825	407,457		
	293T	EMX1	86%	20ug	2,015,130	4,328	213,106	257,933	53.4%	1.00
FeCas9	293T	EMX1	96%	20ug	1,751,609	5,358	268,147	156,880	66.0%	1.00
	293T	EMX1	96%	20ug	1,530,437	2,797	170,044	146,198	56.7%	1.00
Total				60ug	5,297,176	12,483	651,297	561,011		
				Cas9:M	YC-locus1 vari	ants				
	203T	MYC-	97%	2000	3 881 155	0 377	1/10 327	207 022	13 0%	1.00
	2001	locus1	5170	2009	0,004,400	3,511	170,021	201,022	-0.070	1.00
Cas9	203T	MYC-	87%	2000	5 007 042	5 956	134 327	323 853	33.1%	1 00
	2001	locus1	0170	Loug	0,007,042	0,000	107,021	020,000	00.170	1.00
	293T	MYC-	85%	20ug	3,040,133	8,301	178,424	297,689	43.4%	1.00

		locus1								
Total				60ug	11,931,630	23,634	462,078	828,564		
	293T	MYC- locus1	98%	20ug	3,759,229	11,409	215,672	204,814	52.1%	1.00
D1135E	293T	MYC- locus1	91%	20ug	1,410,295	4,519	113,828	175,876	42.4%	1.00
	293T	MYC- locus1	73%	20ug	3,409,507	6,840	188,745	509,110	35.9%	1.00
Total				60ug	8,579,031	22,768	518,245	889,800		
	293T	MYC- locus1	98%	20ug	4,222,772	9,585	172,112	195,976	47.4%	1.00
eCas9	293T	MYC- locus1	88%	20ug	3,047,053	6,952	141,436	302,895	35.6%	1.00
	293T	MYC- locus1	98%	20ug	4,155,923	7,005	141,635	174,922	45.2%	1.00
Total				60ug	11,425,748	23,542	455,183	673,793		
	293T	MYC- locus1	98%	20ug	3,738,130	5,910	137,411	199,781	41.0%	1.00
FeCas9	293T	MYC- locus1	89%	20ug	3,481,196	4,462	101,718	186,367	38.9%	1.00
	293T	MYC-	98%	20ug	2,374,662	4,426	114,930	170,927	40.4%	1.00
		100031								
Total		100031		60ug	9,593,988	14,798	354,059	557,075		
Total		100031		60ug AcrIIA4	9,593,988 I inhibitor librar	14,798 ies	354,059	557,075		
Total	293T	RAG1A	72%	60ug AcrIIA 20ug	9,593,988 i inhibitor librar 1,513,624	14,798 ies 2,713	354,059 37,828	557,075 207,508	20.3%	1.00
Total	293T 293T	RAG1A RAG1A	72%	60ug AcrIIA 20ug 20ug	9,593,988 i inhibitor librar 1,513,624 1,259,867	14,798 ies 2,713 2,357	354,059 37,828 33,008	557,075 207,508 199,679	20.3% 19.6%	1.00
Total	293T 293T 293T	RAG1A RAG1A RAG1A	72% 68% 54%	60ug AcrIIA 20ug 20ug 20ug	9,593,988 I inhibitor librar 1,513,624 1,259,867 1,081,871	14,798 ies 2,713 2,357 825	354,059 37,828 33,008 17,400	557,075 207,508 199,679 167,655	20.3% 19.6% 15.0%	1.00 1.00 1.00
Total 1:0 Total	293T 293T 293T	RAG1A RAG1A RAG1A	72% 68% 54%	60ug AcrIIA2 20ug 20ug 20ug 60ug	9,593,988 i inhibitor librar 1,513,624 1,259,867 1,081,871 3,855,362	14,798 ies 2,713 2,357 825 5,895	354,059 37,828 33,008 17,400 88,236	557,075 207,508 199,679 167,655 574,842	20.3% 19.6% 15.0%	1.00 1.00 1.00
Total 1:0 Total	293T 293T 293T 293T	RAG1A RAG1A RAG1A RAG1A	72% 68% 54% 76%	60ug AcrIIA4 20ug 20ug 20ug 60ug 20ug	9,593,988 inhibitor librar 1,513,624 1,259,867 1,081,871 3,855,362 1,055,671	14,798 ies 2,713 2,357 825 5,895 614	354,059 37,828 33,008 17,400 88,236 6,602	557,075 207,508 199,679 167,655 574,842 225,486	20.3% 19.6% 15.0% 1.8%	1.00 1.00 1.00 0.98
Total 1:0 Total 3:1	293T 293T 293T 293T 293T 293T	RAG1A RAG1A RAG1A RAG1A RAG1A	72% 68% 54% 76% 76%	60ug AcrIIA2 20ug 20ug 20ug 60ug 20ug 20ug	9,593,988 inhibitor librar 1,513,624 1,259,867 1,081,871 3,855,362 1,055,671 1,276,393	14,798 ies 2,713 2,357 825 5,895 614 806	354,059 37,828 33,008 17,400 88,236 6,602 7,878	557,075 207,508 199,679 167,655 574,842 225,486 273,475	20.3% 19.6% 15.0% 1.8% 1.8%	1.00 1.00 1.00 0.98 0.98
Total 1:0 Total 3:1	293T 293T 293T 293T 293T 293T 293T	RAG1A RAG1A RAG1A RAG1A RAG1A RAG1A	72% 68% 54% 76% 76% 54%	60ug AcrIIA2 20ug 20ug 20ug 60ug 20ug 20ug 20ug	9,593,988 inhibitor librar 1,513,624 1,259,867 1,081,871 3,855,362 1,055,671 1,276,393 1,064,759	14,798 ies 2,713 2,357 825 5,895 614 806 350	354,059 37,828 33,008 17,400 88,236 6,602 7,878 4,820	557,075 207,508 199,679 167,655 574,842 225,486 273,475 209,925	20.3% 19.6% 15.0% 1.8% 1.8% 1.3%	1.00 1.00 1.00 0.98 0.98 0.98
Total 1:0 Total 3:1 Total	293T 293T 293T 293T 293T 293T 293T	RAG1A RAG1A RAG1A RAG1A RAG1A RAG1A	72% 68% 54% 76% 54%	60ug AcrIIA4 20ug 20ug 20ug 20ug 20ug 20ug 20ug 60ug	9,593,988 inhibitor librar 1,513,624 1,259,867 1,081,871 3,855,362 1,055,671 1,276,393 1,064,759 3,396,823	14,798 ies 2,713 2,357 825 5,895 614 806 350 1,770	354,059 37,828 33,008 17,400 88,236 6,602 7,878 4,820 19,300	557,075 207,508 199,679 167,655 574,842 225,486 273,475 209,925 708,886	20.3% 19.6% 15.0% 1.8% 1.8% 1.3%	1.00 1.00 1.00 0.98 0.98 0.98
Total 1:0 Total 3:1 Total	293T 293T 293T 293T 293T 293T 293T 293T	RAG1A RAG1A RAG1A RAG1A RAG1A RAG1A RAG1A	72% 68% 54% 76% 76% 54% 68%	60ug AcrIIA2 20ug 20ug 20ug 20ug 20ug 20ug 60ug 20ug	9,593,988 inhibitor librar 1,513,624 1,259,867 1,081,871 3,855,362 1,055,671 1,276,393 1,064,759 3,396,823 1,253,450	14,798 ies 2,713 2,357 825 5,895 614 806 350 1,770 377	354,059 37,828 33,008 17,400 88,236 6,602 7,878 4,820 19,300 5,776	557,075 207,508 199,679 167,655 574,842 225,486 273,475 209,925 708,886 278,637	20.3% 19.6% 15.0% 1.8% 1.8% 1.3% 0.7%	1.00 1.00 1.00 0.98 0.98 0.98 0.98
Total 1:0 Total 3:1 Total 1:1	293T 293T 293T 293T 293T 293T 293T 293T	RAG1A RAG1A RAG1A RAG1A RAG1A RAG1A RAG1A RAG1A	72% 68% 54% 76% 54% 54% 68% 68%	60ug AcrIIA2 20ug 20ug 20ug 20ug 20ug 20ug 20ug 20u	9,593,988 inhibitor librar 1,513,624 1,259,867 1,081,871 3,855,362 1,055,671 1,276,393 1,064,759 3,396,823 1,253,450 1,038,947	14,798 ies 2,713 2,357 825 5,895 614 806 350 1,770 377 293	354,059 37,828 33,008 17,400 88,236 6,602 7,878 4,820 19,300 5,776 5,237	557,075 207,508 199,679 167,655 574,842 225,486 273,475 209,925 708,886 278,637 239,284	20.3% 19.6% 15.0% 1.8% 1.8% 1.3% 0.7% 0.9%	1.00 1.00 1.00 0.98 0.98 0.98 0.98 0.98 0.98
Total 1:0 Total 3:1 Total 1:1	293T 293T 293T 293T 293T 293T 293T 293T	RAG1A RAG1A RAG1A RAG1A RAG1A RAG1A RAG1A RAG1A RAG1A	72% 68% 54% 76% 76% 54% 68% 64% 48%	60ug AcrIIA2 20ug 20ug 20ug 20ug 20ug 20ug 20ug 20u	9,593,988 inhibitor librar 1,513,624 1,259,867 1,081,871 3,855,362 1,055,671 1,276,393 1,064,759 3,396,823 1,253,450 1,038,947 1,214,018	14,798 ies 2,713 2,357 825 5,895 614 806 350 1,770 377 293 100	354,059 37,828 33,008 17,400 88,236 6,602 7,878 4,820 19,300 5,776 5,237 4,703	557,075 207,508 199,679 167,655 574,842 225,486 273,475 209,925 708,886 278,637 239,284 252,257	20.3% 19.6% 15.0% 1.8% 1.8% 1.3% 0.7% 0.9% 0.4%	1.00 1.00 1.00 0.98 0.98 0.98 0.98 0.98 0.97 0.98
Total 1:0 Total 3:1 Total 1:1	293T 293T 293T 293T 293T 293T 293T 293T	RAG1A RAG1A RAG1A RAG1A RAG1A RAG1A RAG1A RAG1A RAG1A	72% 68% 54% 76% 76% 54% 68% 68% 64% 48%	60ug AcrIIA2 20ug 20ug 20ug 20ug 20ug 20ug 20ug 20u	9,593,988 inhibitor librar 1,513,624 1,259,867 1,081,871 3,855,362 1,055,671 1,276,393 1,064,759 3,396,823 1,253,450 1,038,947 1,214,018 3,506,415	14,798 ies 2,713 2,357 825 5,895 614 806 350 1,770 377 293 100 770	354,059 37,828 33,008 17,400 88,236 6,602 7,878 4,820 19,300 5,776 5,237 4,703 15,716	557,075 207,508 199,679 167,655 574,842 225,486 273,475 209,925 708,886 278,637 239,284 252,257 770,178	20.3% 19.6% 15.0% 1.8% 1.8% 1.3% 0.7% 0.9% 0.4%	1.00 1.00 1.00 0.98 0.98 0.98 0.98 0.98 0.98 0.97 0.98
Total 1:0 Total 3:1 Total 1:1 1:3	293T 293T 293T 293T 293T 293T 293T 293T	RAG1A RAG1A RAG1A RAG1A RAG1A RAG1A RAG1A RAG1A RAG1A RAG1A	72% 68% 54% 76% 54% 54% 68% 68%	60ug AcrIIA4 20ug 20ug 20ug 20ug 20ug 20ug 20ug 20ug	9,593,988 inhibitor librar 1,513,624 1,259,867 1,081,871 3,855,362 1,055,671 1,276,393 1,064,759 3,396,823 1,253,450 1,038,947 1,214,018 3,506,415 1,210,093	14,798 ies 2,713 2,357 825 5,895 614 806 350 1,770 377 293 100 770 199	354,059 37,828 33,008 17,400 88,236 6,602 7,878 4,820 19,300 5,776 5,237 4,703 15,716 4,770	557,075 207,508 199,679 167,655 574,842 225,486 273,475 209,925 708,886 278,637 239,284 252,257 770,178 229,174	20.3% 19.6% 15.0% 1.8% 1.8% 1.3% 0.7% 0.9% 0.4% 0.6%	1.00 1.00 1.00 0.98 0.98 0.98 0.98 0.98 0.97 0.98
Total 1:0 Total 3:1 Total 1:1 Total 1:3	293T 293T 293T 293T 293T 293T 293T 293T	RAG1A RAG1A RAG1A RAG1A RAG1A RAG1A RAG1A RAG1A RAG1A RAG1A RAG1A	72% 68% 54% 76% 76% 54% 68% 68% 68% 68% 68%	60ug AcrIIA 20ug 20ug 20ug 20ug 20ug 20ug 20ug 20ug	9,593,988 inhibitor librar 1,513,624 1,259,867 1,081,871 3,855,362 1,055,671 1,276,393 1,064,759 3,396,823 1,253,450 1,038,947 1,214,018 3,506,415 1,210,093 1,442,245	14,798 ies 2,713 2,357 825 5,895 614 806 350 1,770 377 293 100 770 199 291	354,059 37,828 33,008 17,400 88,236 6,602 7,878 4,820 19,300 5,776 5,237 4,703 15,716 4,770 6,618	557,075 207,508 199,679 167,655 574,842 225,486 273,475 209,925 708,886 278,637 239,284 252,257 770,178 229,174 330,241	20.3% 19.6% 15.0% 1.8% 1.8% 1.3% 0.7% 0.9% 0.4% 0.6% 0.5%	1.00 1.00 1.00 0.98 0.98 0.98 0.98 0.98 0.97 0.98 0.97 0.98
Total 1:0 Total 3:1 Total 1:1 Total 1:3	293T 293T 293T 293T 293T 293T 293T 293T	RAG1A RAG1A RAG1A RAG1A RAG1A RAG1A RAG1A RAG1A RAG1A RAG1A RAG1A RAG1A	72% 68% 54% 76% 54% 54% 68% 68% 64% 48% 68% 69% 62%	60ug AcrIIA2 20ug 20ug 20ug 20ug 20ug 20ug 20ug 20u	9,593,988 inhibitor librar 1,513,624 1,259,867 1,081,871 3,855,362 1,055,671 1,276,393 1,064,759 3,396,823 1,253,450 1,038,947 1,214,018 3,506,415 1,210,093 1,442,245 891,225	14,798 ies 2,713 2,357 825 5,895 614 806 350 1,770 377 293 100 770 199 291 102	354,059 37,828 33,008 17,400 88,236 6,602 7,878 4,820 19,300 5,776 5,237 4,703 15,716 4,770 6,618 3,555	557,075 207,508 199,679 167,655 574,842 225,486 273,475 209,925 708,886 278,637 239,284 252,257 770,178 229,174 330,241 148,599	20.3% 19.6% 15.0% 1.8% 1.8% 1.3% 0.7% 0.9% 0.4% 0.6% 0.5% 1.1%	1.00 1.00 1.00 0.98 0.98 0.98 0.98 0.98 0.97 0.97 0.97 0.97 0.97
Total 1:0 Total 3:1 Total 1:1 Total 1:3 Total	293T 293T 293T 293T 293T 293T 293T 293T	RAG1A RAG1A RAG1A RAG1A RAG1A RAG1A RAG1A RAG1A RAG1A RAG1A RAG1A RAG1A	72% 68% 54% 76% 76% 54% 68% 68% 64% 48% 68% 68% 69% 62%	60ug AcrIIA 20ug 20ug 20ug 20ug 20ug 20ug 20ug 20ug	9,593,988 inhibitor librar 1,513,624 1,259,867 1,081,871 3,855,362 1,055,671 1,276,393 1,064,759 3,396,823 1,253,450 1,038,947 1,214,018 3,506,415 1,210,093 1,442,245 891,225 3,543,563	14,798 ies 2,713 2,357 825 5,895 614 806 350 1,770 377 293 100 770 199 291 102 592	354,059 37,828 33,008 17,400 88,236 6,602 7,878 4,820 19,300 5,776 5,237 4,703 15,716 4,770 6,618 3,555 14,943	557,075 207,508 199,679 167,655 574,842 225,486 273,475 209,925 708,886 278,637 239,284 252,257 770,178 229,174 330,241 148,599 708,014	20.3% 19.6% 15.0% 1.8% 1.8% 1.3% 0.7% 0.9% 0.4% 0.6% 0.5% 1.1%	1.00 1.00 1.00 0.98 0.98 0.98 0.98 0.98 0.97 0.97 0.97 0.97 0.97
Total 1:0 Total 3:1 Total 1:1 Total 1:3 Total	293T 293T 293T 293T 293T 293T 293T 293T	RAG1A RAG1A RAG1A RAG1A RAG1A RAG1A RAG1A RAG1A RAG1A RAG1A RAG1A RAG1A RAG1A	72% 68% 54% 76% 76% 54% 68% 68% 64% 48% 68% 69% 62% 61%	60ug AcrIIA2 20ug 20ug 20ug 20ug 20ug 20ug 20ug 20u	9,593,988 inhibitor librar 1,513,624 1,259,867 1,081,871 3,855,362 1,055,671 1,276,393 1,064,759 3,396,823 1,253,450 1,038,947 1,214,018 3,506,415 1,210,093 1,442,245 891,225 3,543,563 2,958,042 2,545,677	14,798 ies 2,713 2,357 825 5,895 614 806 350 1,770 377 293 100 770 199 291 102 592 4,174	354,059 37,828 33,008 17,400 88,236 6,602 7,878 4,820 19,300 5,776 5,237 4,703 15,716 4,770 6,618 3,555 14,943 69,064 70 250	557,075 207,508 199,679 167,655 574,842 225,486 273,475 209,925 708,886 278,637 239,284 252,257 770,178 229,174 330,241 148,599 708,014 423,828	20.3% 19.6% 15.0% 1.8% 1.8% 1.3% 0.7% 0.9% 0.4% 0.6% 0.5% 1.1% 22.3%	1.00 1.00 1.00 0.98 0.98 0.98 0.98 0.98 0.97 0.98 0.97 0.97 0.97 0.97 0.97 0.97
Total 1:0 Total 3:1 Total 1:1 Total 1:3 Total 1:3 Total 1:0	293T 293T 293T 293T 293T 293T 293T 293T	RAG1A RAG1A RAG1A RAG1A RAG1A RAG1A RAG1A RAG1A RAG1A RAG1A RAG1A RAG1A RAG1A RAG1A	72% 68% 54% 76% 54% 68% 64% 48% 68% 68% 69% 62% 61% 60%	60ug AcrIIA4 20ug 20ug 20ug 20ug 20ug 20ug 20ug 20ug	9,593,988 inhibitor librar 1,513,624 1,259,867 1,081,871 3,855,362 1,055,671 1,276,393 1,064,759 3,396,823 1,253,450 1,038,947 1,214,018 3,506,415 1,210,093 1,442,245 891,225 3,543,563 2,958,042 3,545,677 2,552,555	14,798 ies 2,713 2,357 825 5,895 614 806 350 1,770 377 293 100 770 199 291 102 592 4,174 3,962	354,059 37,828 33,008 17,400 88,236 6,602 7,878 4,820 19,300 5,776 5,237 4,703 15,716 4,770 6,618 3,555 14,943 69,064 72,852	557,075 207,508 199,679 167,655 574,842 225,486 273,475 209,925 708,886 278,637 239,284 252,257 770,178 229,174 330,241 148,599 708,014 423,828 430,571	20.3% 19.6% 15.0% 1.8% 1.8% 1.3% 0.7% 0.9% 0.4% 0.6% 0.5% 1.1% 22.3% 23.3%	1.00 1.00 1.00 0.98 0.98 0.98 0.98 0.98 0.98 0.97 0.97 0.97 0.97 0.97 0.97 0.97 0.97

10371RAG1A58%20.902.83.90938.001.0.82431.6.911.0.8040.0.911112037RAG2A65%2.0.903.6.003.6.014.0.3244.0.9.010.0.9111111165%2.0.907.35.3831.0.904.0.9.141.0.910.0.911121111111111111111111111111111111111237RAG1B65%2.001.0.8.9.1274.0.91.0.9.131.0.9.11.0.9.11111237RAG1B65%2.001.0.9.1276.0.93.0.9.123.0.9.123.0.9.121.0.9.11.0.9.11112.0.37RAG1B7.5%2.0.91.0.9.1276.0.93.0.9.123.0.9.121.0.9.11.0.9.11112.0.37RAG1B7.5%2.0.91.0.9.23.0.9.23.0.9.123.0.9.123.0.9.123.0.9.121.0.9.11.0.9.11112.0.37RAG1B7.5%2.0.91.0.9.23.0.9.123.0.9.123.0.9.123.0.9.123.0.9.123.0.9.121.0.9.1 <th>Total</th> <th></th> <th></th> <th></th> <th>60ug</th> <th>9,042,047</th> <th>11,265</th> <th>198,554</th> <th>1,198,868</th> <th></th> <th></th>	Total				60ug	9,042,047	11,265	198,554	1,198,868		
11111237RAG1A5%20.93.746.393.047.8324.05.354.03.650.09%0.09%1237RAG1B6%20.97.335.381002.6074.09.11.01.801.01.911237RAG1B6%20.91.363.3810810.2011.02.911.03.931.02.911.02.911.03.931.02.911.02.911.03.931.02.911.02.9		293T	RAG1A	56%	20ug	2,638,509	380	10,624	515,716	1.6%	0.97
9337RAG1A58%20092,620,333067,8154,33,161,1%0,99Total164%20091,33,33,6838016,56112,2811,0%1,0%2937RAG1B64%20091,630,327638016,28121,2831,1%1,0%2037RAG1B65%20091,603,92763835,28222,3931,1%1,0%1002037RAG1B67%20091,01,2776,3736,846336,5421,7%1,0%2037RAG1B67%20092,057,9576,376,364336,5421,6%0,0%1012037RAG1B67%20092,077,976,376,364336,5421,6%0,0%1012037RAG1B67%20092,37,7972,6748,61634,61631,6714,00%1,00%1012037RAG1B67%20092,37,7972,6748,61834,61631,67033,6801,00%1012037RAYC157%20092,37,5782,48390,61833,61633,61831,67033,68031,	1:1	293T	RAG1A	55%	20ug	2,476,639	364	7,632	460,536	0.9%	0.96
TotalImage in the image in the image. The image is the image in the image. The image in the image. The image is the image in the image. The image is the image in the image in the image in the image. The image is there in the image in there		293T	RAG1A	58%	20ug	2,620,035	306	7,815	433,166	1.1%	0.96
2837RAG1B64%6101.333.3938016.0119.21310.01%1.10.01100RAG1B65%1001.685.3910.0217.28010.01%11.00101RAG1B65%1001.685.3910.0210.0210.0110.0110.01101RAG1B65%1001.01010.0110.0110.0110.0110.0110.01101RAG1B65%1001.01010.0110.0110.0110.0110.0110.01101RAG1B67%1001.01010.0110.0110.0110.0110.0110.01101RAG1B67%10010.0110.0110.0110.0110.0110.0110.0110110110110110010.0110.0110.0110.0110.0110.0110110110110010.0110.0110.0110.0110.0110.0110.0110110110110010.0110.0110.0110.0110.0110.0110.0110110110110010.0110.0110.0110.0110.0110.0110.0110110110110110110.0110.0110.0110.0110.0110.0110110110110110110110110110.0110.0110.0110110110110110	Total				60ug	7,735,183	1,050	26,071	1,409,418		
<table-container> 1110 2931 RAG1B 6% 200 1.069.27 4.03 2.03 2.17.28 1.0.1% 1.0.5% Total 1 100 100 1.0.01.27 1.0.01 21.507 249.305 1.1.5% 1.0.01 Total 2937 RAG1B 688 2009 1.0.02.27 68 5.0.2 252.28 1.0.% 0.0.9 1010 2937 RAG1B 7.0% 2002 2.0.7.97 2.0.8 0.0.6 33.6.42 1.0.9 0.0.9 1011 2937 RAG1B 5.00 2.0.05.905 2.0.1 8.0.05 34.0.90 3.5.6% 1.0.0 1011 2937 RAG1B 5.00 2.0.1.0 2.0.1 3.4.0.90 3.5.6% 1.0.0 1023 MYC1 5.8% 2.00 3.5.6.10 2.0.1 3.4.0.90 3.5.6% 1.0.0 1034 MYC1 5.8% 2.00 3.5.6.10 2.0.1.0 2.0.1.0 2.0.1.0 2.0.1.0 2.0.1.0 2.0.1.</table-container>		293T	RAG1B	64%	20ug	1,336,396	380	16,561	192,913	10.8%	1.00
<table-container> 2931 RAG1B 6% 200 1,809,12 4,304,12 6,107 69,404 669,607 1 101 RAG1B 6% 200 4,304,21 1,179 69,404 669,607 1 0 102 RAG1B 6% 200 1,003,237 650 5,252 2,35,602 1,604 0,091 102 RAG1B 6% 200 2,016,00 9 3,63,62 1,604 0,091 103 RAG1B 6% 200 2,016,00 3,63,62 1,604 0,091 104 RAC1B 6% 200 2,63,739 2,631 8,016 3,61,712 3,63,64 1,001 104 MC1 5% 200 2,73,789 2,643 92,177 3,163,64 3,161 3,161 3,161,64 3,161,64 3,161,64 3,161,64 3,161,64 3,161,64 3,161,64 3,161,64 3,161,64 3,161,64 3,161,64 3,161,64 3,161,64 3,161,64 3,161,64 <t< td=""><td>1:0</td><td>293T</td><td>RAG1B</td><td>65%</td><td>20ug</td><td>1,668,598</td><td>369</td><td>19,288</td><td>217,289</td><td>10.9%</td><td>1.00</td></t<></table-container>	1:0	293T	RAG1B	65%	20ug	1,668,598	369	19,288	217,289	10.9%	1.00
IndiaIndiaIndiaIndiaIndiaIndiaIndiaIndiaIndia1000RAG106002001.00.2006003.003.05.201.01.000.00.002017RAG107002000.00.000.003.03.003.05.001.00.000.00.002017RAG107002000.00.000.00.003.05.003.00.001.00.002018MC107002000.00.000.00.003.00.003.00.003.00.001.00.002019MC107002.00.002.00.000.00.003.00.003.00.003.00.003.00.003.00.002010MC10700700700.00700.00700.00700.00700.00700.00700.002011MC10700700700.00700.00700.00700.00700.00700.00700.002011MC10700700700.00700.00700.00700.00700.00700.00700.002011MC10700700.00700.00700.00700.00700.00700.00700.00700.002011MC10700700.00700.00700.00700.00700.00700.00700.00700.002011MC10700700.00700.00700.00700.00700.00700.00700.00700.002011MC10700.00700.00700.00700.00700.00700.00700.00<		293T	RAG1B	66%	20ug	1,809,127	430	23,597	249,305	11.5%	1.00
2937RAG1B68%20u1,00,23768%5,32252,2891,7%1,002937RAG1B7%20u1,791,627587,872325,6621,6%0.992031RAG1B7%20u2,050,90908,36914,693772937MYC15%20u2,377,972,63780,618316,71234,0%1,002937MYC15%20u2,375,8922,94494,12434,0903,5%1,002937MYC15%20u2,358,5822,94494,12434,0903,3%1,001012937MYC15%20u3,785,56426444,5083,3%1,002937MYC15%20u2,374,6081378,35125,17012,9%1,002937MYC15%20u2,687,18030015,15747,4812,8%1,002937MYC15%20u2,687,1902,4805,20130,64016,5%1,002937MYC37%20u2,897,1902,4806,20130,64016,5%1,002937MYC37%20u2,897,1902,4806,20130,64016,5%1,002937MYC37%20u2,897,1902,4806,20130,64016,5%1,002937MYC37%20u2,897,1902,4806,20130,6401,3%1,002937 <td>Total</td> <td></td> <td></td> <td></td> <td>60ug</td> <td>4,814,121</td> <td>1,179</td> <td>59,446</td> <td>659,507</td> <td></td> <td></td>	Total				60ug	4,814,121	1,179	59,446	659,507		
111 2937 RAG1B 75% 20u 1.791.627 58 7.872 325.82 1.6% 0.991 2837 RAG1B 70% 20u 2.005.905 90 8.364 336.54 1.8% 0.991 101 2937 MYC1 57% 20u 2.735.892 2.16 80.618 316.712 34.0% 1.000 2937 MYC1 58% 20u 2.735.892 2.944 94.124 34.1969 33.6.% 1.000 2937 MYC1 58% 20u 2.736.892 2.944 94.124 34.1969 3.36.% 1.000 1001 1001 55% 20u 2.736.892 1.92 4.172 2.937 0.001 3.315.40 1.910 1.00 1.000 1.001 1.001 1.001 1.001 1.001 1.001 1.001 1.001 1.001 1.001 1.001 1.001 1.001 1.001 1.001 1.001 1.001 1.001 1.001 1.00		293T	RAG1B	68%	20ug	1,603,237	68	5,932	252,289	1.7%	1.00
2931RAG1B70%2002005, 908,364336,5241,8%0,99Total76006400,76921622,16891,6937772937MYC157%2002,37,8922,83780,61831,61034,00934,0051,002937MYC157%2002,73,8922,84880,41231,61035,00935,	1:1	293T	RAG1B	75%	20ug	1,791,627	58	7,872	325,862	1.6%	0.99
Indep int		293T	RAG1B	70%	20ug	2,005,905	90	8,364	336,542	1.8%	0.99
2931MYC157%20ug2,377,7972,63780,618316,71234,0%1,002937MYC154%20ug2,468,9672,58180,095334,09033.6%1,002937MYC158%20ug3,515,540254,37992,771U12937MYC155%20ug3,515,5403,515427,55440,5083,3%1,002937MYC155%20ug3,515,403,515427,55440,5083,3%1,002937MYC155%20ug2,515,403,635440,5083,3%1,002937MYC155%20ug2,563,7937,3338,2341,169,6901,002937MYC380%20ug2,680,7102,45062,019327,0661,002937MYC372%20ug2,520,6993,603406,85016,6%1,002937MYC368%20ug2,826,8913,6161,617,491,414,7801,002937MYC368%20ug2,826,5816,5121,414,3851,4040,992937MYC368%20ug2,826,5815,13351,4721,443,591,4040,992937MYC368%20ug2,826,5815,13351,4721,443,591,4041,002937MYC368%20ug2,867,4351,9341,4746,38,441,001,002937MYC368%<	Total				60ug	5,400,769	216	22,168	914,693		
1.102931MYC154%20ug2.468,9672.58180.095334,09033.6%1.002937MYC158%20ug7.758,8922.94494,124341,96935.6%1.00Total2937MYC152%20ug7.582,6568.162254,37992,7712.9712.9712937MYC152%20ug2.374,6081.3001.517477,4812.8%1.002937MYC155%20ug2.663,79130.0115,157477,4812.8%1.0070102937MYC35%20ug2.663,79130.0115,157477,4812.8%1.0070312937MYC37%20ug3.263,93973338,2341,169,6901.6%1.002937MYC37%20ug2.871,1092.4506.019380,84016.6%1.0070312937MYC37%20ug3.28,6803.08466.093406,8501.6%1.00714127%20ug3.28,5803.08466.093406,8501.6%1.0071512937MYC368%20ug2.983,856499918,06551,79491.4%0.9971512937MYC368%20ug2.985,56027815,325444,3351.3%0.9971612937MYC368%20ug2.865,5615.07123,3351.4726.3%1.00 <th< td=""><td></td><td>293T</td><td>MYC1</td><td>57%</td><td>20ug</td><td>2,377,797</td><td>2,637</td><td>80,618</td><td>316,712</td><td>34.0%</td><td>1.00</td></th<>		293T	MYC1	57%	20ug	2,377,797	2,637	80,618	316,712	34.0%	1.00
10001001100010001000100010001000Total100100010001000100010001000100010102937MYC152%20003,51,5024,32014,72044,05003,33%1,00010102937MYC152%20302,374,60014,72044,05003,33%1,00010102937MYC155%20002,663,73035,3343,3211,168,6001,00010101010100066002,663,73073338,2321,169,6001,0001,0001010101010102,937MYC380%20002,260,903,0866,03330,80416,6701,00010102937MYC380%20002,260,903,0866,033406,8501,0001,0001,00010102037MYC380%20002,260,903,0866,033406,8501,0001,0001,00010101010101010101,0001,0001,0001,0001,0001,0001,0001,0001011101110101,0001,0001,0001,0001,0001,0001,0001,0001011101110101,0001,0001,0001,0001,0001,0001,0001,0001011101110101,0001,0001,0001,0001,0001,0001,000<	1:0	293T	MYC1	54%	20ug	2,468,967	2,581	80,095	334,090	33.6%	1.00
<table-container>IndiaIndiaIndiaState<th< td=""><td></td><td>293T</td><td>MYC1</td><td>58%</td><td>20ug</td><td>2,735,892</td><td>2,944</td><td>94,124</td><td>341,969</td><td>35.6%</td><td>1.00</td></th<></table-container>		293T	MYC1	58%	20ug	2,735,892	2,944	94,124	341,969	35.6%	1.00
1937 1MYC1 5% 20up 3,51,54u 14,72u 14,05u 3,33u 1,01u 1211 2937 MYC1 5% 20up 2,374,08u 137u 8,351 251,70u 2,93u 1,00u 7014 MYC1 5% 20up 2,633,73u 30u 1,5157 477,48u 2,89u 1,00u 7014 Term Term 500g 2,633,39u 733 58,23u 1,669eu 1,00u 7014 Term Term 500g 2,631,30u 58,23u 320.5u 32,0u	Total				60ug	7,582,656	8,162	254,837	992,771		
111 2937 MYC1 58% 20up 2,374.608 137 8,351 251.701 2.9% 1.00 70tal MYC1 55% 20up 2,683.791 300 15,157 477,481 2.8% 1.00 70tal MYC3 80% 20up 2,680,416 2,977 58,929 327.080 16.7% 1.00 100 2937 MYC3 80% 20up 2,871,109 2,450 62,019 38,040 16.6% 1.00 2937 MYC3 7% 20up 3,276,018 66,059 36,050 17.6% 1.00 1.00 2037 MYC3 6% 20up 2,871,610 2,450 66,059 16,059 1.4% 0.99 111 MYC3 6% 20up 2,805,650 27.68 16,323 144,754 1.4% 0.99 111 MYC3 6% 20up 2,805,650 16,98 1,914 1.4% 0.99 111 MYC3		293T	MYC1	52%	20ug	3,515,540	296	14,726	440,508	3.3%	1.00
2931 MYC1 55% 20ug 2,663,791 300 15,177 477,481 2.8% 1.00 Total Image: Constraint of the	1:1	293T	MYC1	58%	20ug	2,374,608	137	8,351	251,701	2.9%	1.00
Total (i) 60ug 8,553,93 733 8,234 1,169,690 (i) 293T MYC3 80% 20ug 2,690,416 2,977 58,929 327,096 16.7% 1.00 293T MYC3 72% 20ug 2,871,109 2,450 62,019 380,840 16.6% 1.00 293T MYC3 77% 20ug 3,226,089 3,086 66,093 406,850 15.6% 1.00 Total I 60ug 8,787,614 8,513 187,04 144,86 0.09 11:0 MYC3 68% 20ug 2,83,856 499 18,066 517,949 1.4% 0.99 11:1 MYC3 68% 20ug 2,83,856 499 15,322 444,355 1.3% 1.00 11:1 MYC3 68% 20ug 3,65,559 1,088 51,335 1.472,40 1.00 1.00 11:10 293T EMX1 84% 20ug 3,267,308		293T	MYC1	55%	20ug	2,663,791	300	15,157	477,481	2.8%	1.00
2937 MYC3 80% 20ug 2,690,416 2,977 58,929 327,096 16.7% 1.00 2937 MYC3 72% 20ug 2,871,109 2,450 62,019 380,840 16.6% 1.00 2937 MYC3 77% 20ug 3,226,089 3,086 66,093 406,850 15.6% 1.00 Total 60ug 8,787,614 8,513 187,041 1,114,786 60.993 0.993 1.4% 0.993 1:10 68% 20ug 2,983,856 499 18,066 517,949 1.4% 0.993 1:11 2937 MYC3 68% 20ug 2,835,559 1,988 51,325 1,44,355 1.36% 1.00 1:10 2937 EMX1 84% 20ug 3,267,308 61,303 184757 63.8% 1.00 1:10 2937 EMX1 84% 20ug 3,267,308 61,203 186,125 1.00 1.00 <td>Total</td> <td></td> <td></td> <td></td> <td>60ug</td> <td>8,553,939</td> <td>733</td> <td>38,234</td> <td>1,169,690</td> <td></td> <td></td>	Total				60ug	8,553,939	733	38,234	1,169,690		
1:0 2937 MYC3 72% 20ug 2,871,109 2,450 62,019 380,840 16.6% 1.00 2937 MYC3 77% 20ug 3,226,089 3,086 66,093 406,850 15.6% 1.00 Total 60ug 8,787,614 8,513 187,041 1,114,786 0.99 1:1 2937 MYC3 68% 20ug 2,983,856 499 18,096 517,949 1.4% 0.99 1:1 2937 MYC3 68% 20ug 2,885,560 278 15,322 444,385 1.3% 0.99 2937 MYC3 69% 20ug 2,855,531 5,071 234,331 184757 63.8% 1.00 100 2937 EMX1 84% 20ug 2,857,331 5,071 234,331 184757 63.8% 1.00 110 2937 EMX1 84% 20ug 3,267,308 6,209 246,013 180120		293T	MYC3	80%	20ug	2,690,416	2,977	58,929	327,096	16.7%	1.00
2937 MYC3 77% 20ug 3,226,089 3,086 66,093 406,850 15.6% 1.00 Total · 60ug 8,787,614 8,513 187,041 1,114,786 · 2937 MYC3 68% 20ug 2,983,856 499 18,096 517,949 1.4% 0.99 1:10 2937 MYC3 68% 20ug 2,805,560 278 15,322 444,385 1.3% 0.99 2937 MYC3 69% 20ug 2,964,143 321 17,917 510,070 1.4% 1.00 70tal · · 60ug 8,753,559 1,098 51,335 1,472,404 · · · 2037 EMX1 84% 20ug 3,267,308 6,209 246,013 180120 65,556 1.00 2037 EMX1 84% 20ug 3,384,228 651 30,153 432677 6.66% 1.00 1:11 2937 EMX1	1:0	293T	MYC3	72%	20ug	2,871,109	2,450	62,019	380,840	16.6%	1.00
Total···60ug8,78,76,148,513187,0411,114,786·293TMYC368%20ug2,98,38649918.096517,9491.4%0.991:1293TMYC368%20ug2,805,66027815,322444,3851.3%0.99293TMYC369%20ug2,964,14332117,917510,0701.4%1.0070tal··60ug8,753,5591.09851,3351,472,4041:0·EMX184%20ug2,862,5315,071234,33118475763.8%1.001:0·EMX184%20ug2,634,4046,145243,54418704966.1%1.00293TEMX184%20ug2,634,4046,145243,54418704966.6%1.00293TEMX184%20ug3,637,85117,42672,888551,9266.6%1.001:1····60ug3,537,8511,12139,5525046907.3%1.001:11············293TEMX178%20ug3,537,8511,12139,5525046907.3%1.001:11············293TEMX178%20ug3,537,8511,121 <td< td=""><td></td><td>293T</td><td>MYC3</td><td>77%</td><td>20ug</td><td>3,226,089</td><td>3,086</td><td>66,093</td><td>406,850</td><td>15.6%</td><td>1.00</td></td<>		293T	MYC3	77%	20ug	3,226,089	3,086	66,093	406,850	15.6%	1.00
2931MYC368%20ug2,983,85649918,096517,9491.4%0.9911:12937MYC368%20ug2,805,56027815,322444,3851.3%0.992937MYC369%20ug2,964,14332117,917510,0701.4%1.00TotalImage: Constraint of the constr	Total				60ug	8,787,614	8,513	187,041	1,114,786		
111 293T MYC3 68% 20u 2,805,560 278 15,322 444,385 1.3% 0.99 293T MYC3 69% 20u 2,964,143 321 17,917 510,070 1.4% 1.00 Total Image: Constraint of the state		293T	MYC3	68%	20ug	2,983,856	499	18,096	517,949	1.4%	0.99
293TMYC369%20ug2,964,14332117,917510,0701.4%1.00Total $(1,1,1)$ $(1,1,1)$ $(1,1,1)$ $(1,1,1)$ $(1,1,1)$ $(1,1,1)$ $(1,1,1)$ $(1,1,1)$ 203TEMX184%20ug $(2,852,531)$ $(5,071)$ $(23,331)$ $(1,8175)$ $(63,8\%)$ $(1,00)$ $(1,0,1)$ $(293T)$ EMX184% $(20ug)$ $(2,852,531)$ $(5,071)$ $(24,613)$ (180120) $(65,5\%)$ $(1,00)$ $(293T)$ EMX184% $(20ug)$ $(2,634,404)$ $(6,145)$ $(24,544)$ (180120) $(66,55)$ $(1,00)$ Total $(293T)$ EMX1 84% $(20ug)$ $(2,634,404)$ $(6,145)$ $(24,544)$ (180120) $(64,15)$ $(1,00)$ Total $(293T)$ EMX1 74% $(20ug)$ $(3,54,243)$ $(7,425)$ $(23,546)$ $(51,592)$ $(6,6\%)$ $(1,00)$ $(1,1)$ $(293T)$ EMX1 74% $(20ug)$ $(3,537,851)$ $(1,121)$ $(39,552)$ (504690) $(7,3\%)$ $(1,00)$ $(1,1)$ $(293T)$ $(EMX1)$ 78% $(20ug)$ $(3,537,851)$ $(1,121)$ $(39,552)$ (504690) $(7,3\%)$ $(1,00)$ $(1,1)$ $(293T)$ $(EMX1)$ 78% $(20ug)$ $(3,537,851)$ $(1,121)$ $(39,552)$ (504690) $(7,3\%)$ $(1,00)$ $(1,1)$ $(293T)$ $(EMX1)$ $(7,5\%)$ $(1,04)$ $(1,04)$ $(1,04)$ $(1,22)$ $(1,121)$ $(1,121)$ <	1:1	293T	MYC3	68%	20ug	2,805,560	278	15,322	444,385	1.3%	0.99
Total!!60u87535591,09851,3351,472,404!293TEMX184%20u2,852,5315,071234,33118475763.8%1.00293TEMX184%20u3,267,30862.09246.01318010966.1%1.00203TEMX184%20u2,634,40461.45243,54418704964.1%1.007040EMX184%20u2,634,40461.4573.888551,92664.1%1.007041EMX184%20u3,84,22865130,1534326776.6%1.00203TEMX174%20u3,537,8511,12139,5525046907.3%1.00111293TEMX178%20u3,508,24086130,153520,9596.6%1.00203TEMX178%20u3,508,24086138,750520,9596.6%1.00111293TEMX178%60u10,403,3192.633184,551.454,531.00111293TFEMX178%60u10,403,3192.633184,551.454,531.0011111111111131,5111,52351,6881,176,151.1641.10111293TFRAG1A- (7321112.243,177,790367,4811.1111293TFRAG1A- (7321112.1<31,189		293T	MYC3	69%	20ug	2,964,143	321	17,917	510,070	1.4%	1.00
2937EMX184%20ug2,852,5315,071234,33118475763.8%1.002937EMX184%20ug3,267,3086,209246,01318012065.5%1.002937EMX184%20ug2,634,4046,145243,54418704964.1%1.00TotalImage: Constraint of the state	Total				60ug	8,753,559	1,098	51,335	1,472,404		
1:0293TEMX184%20ug3,267,3086,209246,01318012066.5%1.00293TEMX184%20ug2,634,4046,145243,54418704966.1%1.00TotalII60ug8,754,24317,425723,888551,926II293TEMX174%20ug3,384,22865130,1534326776.6%1.001:1293TEMX178%20ug3,537,8511,12139,5525046907.3%1.00293TEMX178%20ug3,508,24086138,7505209596.8%1.007otalII60ug10,430,3192,633108,4551,458,326IITotalII60ug8,551,29111,523518,6881,176,615IICas9:RAIIIIIIIIIIICas9:RA293TRAG1A- OT32IIIIIIIICas9:RA293TRAG1A- OT32IIIIIIIICas9:RA293TRAG1A- OT32IIIIIIIICas9:RA293TRAG1A- OT32IIIIIIIICas9:RA293TRAG1A- OT32IIIIIIII<		293T	EMX1	84%	20ug	2,852,531	5,071	234,331	184757	63.8%	1.00
293TEMX184%20ug2,634,4046,145243,54418704964.1%1.00Total $$	1:0	293T	EMX1	84%	20ug	3,267,308	6,209	246,013	180120	65.5%	1.00
TotalImage: sector		293T	EMX1	84%	20ug	2,634,404	6,145	243,544	187049	64.1%	1.00
293TEMX174%20ug3,384,22865130,1534326776.6%1.001:1293TEMX178%20ug3,537,8511,12139,5525046907.3%1.00293TEMX178%20ug3,508,24086138,7505209596.8%1.00TotalII60ug10,430,3192,633108,4551,458,326IITotalII60ug8,551,29111,523518,6881,176,615IICas9:RA293TRAG1A- OT3210ug2,849,1172777,790464,411G1A293TRAG1A- OT3210ug1,851,1892255,299357,882Cas9:RA293TRAG1A- OT32-10ug4,722,8038021,076338,448Cas9:RA293TRAG1A10ug4,722,8038021,076338,448	Total				60ug	8,754,243	17,425	723,888	551,926		
1:1293TEMX178%20ug3,537,8511,12139,5525046907.3%1.00293TEMX178%20ug3,508,24086138,7505209596.8%1.00Total $-$ 60ug10,430,3192,633108,4551,458,326 $ -$ Total $-$ 60ug8,551,29111,523518,6881,176,615 $ -$ Total $ -$ Cas9:RA $293T$ RAG1A- OT32 $ -$ Cas9:RA293TRAG1A- OT32 $ -$ Cas9:RA293TRAG1A- OT32 $ -$ Cas9:RA293TRAG1A- OT32 $ -$ Cas9:RA293TRAG1A- OT32 $ -$ Cas9:RA293TRAG1A- OT32 $ -$ Cas9:RA293TRAG1A- OT32 $ -$ <th< td=""><td></td><td>293T</td><td>EMX1</td><td>74%</td><td>20ug</td><td>3,384,228</td><td>651</td><td>30,153</td><td>432677</td><td>6.6%</td><td>1.00</td></th<>		293T	EMX1	74%	20ug	3,384,228	651	30,153	432677	6.6%	1.00
293TEMX178%20ug3,508,24086138,7505209596.8%1.00Total-60ug10,430,3192,633108,4551,458,326Total-60ug8,551,29111,523518,6881,176,615Cas9:RA G1A293TRAG1A- OT32-10ug2,849,1172777,790 $A64,411$ Cas9:RA G1A293TRAG1A- OT32-10ug1,851,1892255,299357,882Cas9:RA293TRAG1A- OT32-10ug4,722,8038021,076338,448	1:1	293T	EMX1	78%	20ug	3,537,851	1,121	39,552	504690	7.3%	1.00
Total Image: Marking the system of the system		293T	EMX1	78%	20ug	3,508,240	861	38,750	520959	6.8%	1.00
Total Image: Marriad matrix mat	Total				60ug	10,430,319	2,633	108,455	1,458,326		
Cas9:RA 293T RAG1A- 0T32 - 10ug 2,849,117 277 7,790 464,411 - - - G1A 293T RAG1A- 0T32 - 10ug 1,851,189 225 5,299 357,882 - - Cas9:RA 293T RAG1A- 0T32 - 10ug 1,851,189 225 5,299 357,882 - - Cas9:RA 293T RAG1A- - 10ug 4,722,803 80 21,076 338,448 - -	Total				60ug	8,551,291	11,523	518,688	1,176,615		
Cas9:RA 2331 OT32 10ug 2,040,111 211 1,000 404,411 404,411 G1A 293T RAG1A- OT32 - 10ug 1,851,189 225 5,299 357,882 - - Cas9:RA 293T RAG1A- OT32 - 10ug 4,722,803 80 21,076 338,448 - -		293T	RAG1A-	_	10ua	2 849 117	277	7 790	464 411	_	_
G1A 293T RAG1A- 0T32 - 10ug 1,851,189 225 5,299 357,882 - - Cas9:RA 293T RAG1A- - 10ug 4,722,803 80 21,076 338,448 - -	Cas9:RA	2001	OT32		loug	2,010,117	<i>ב</i> וו	1,100	101,711		-
OT32 OT32 OUg 1,001,100 220 0,200 001,002 I <thi< td=""><td>G1A</td><td>293T</td><td>RAG1A-</td><td>_</td><td>1000</td><td>1 851 189</td><td>225</td><td>5 299</td><td>357 882</td><td>_</td><td>_</td></thi<>	G1A	293T	RAG1A-	_	1000	1 851 189	225	5 299	357 882	_	_
Cas9:RA 293T RAG1A- - 10ug 4,722,803 80 21,076 338,448 - -		2001	OT32		loug	1,001,100	220	0,200	007,002	_	_
	Cas9:RA	293T	RAG1A-	-	10ug	4,722,803	80	21,076	338,448	-	-

G1A		OT29								
	293T	RAG1A- OT29	-	10ug	2,936,487	116	13,809	227,911	-	-
					PEM-seq contr	ol libraries		1		
			Lo	cus	DNA	Total	reads	Translocat ion	Indels	Germline
Cas9 va	riants	293T	RA	G1A	20ug	3,964	1,448	50	45,241	605,764
Cas	9	293T	RA	G1B	20ug	769	966	14	4,617	698,247
Cas	9	293T	RA	G1C	20ug	1,18 ⁻	1,902	11	4,473	272,936
Cas9 inl	nibitor	293T	RA	G1A	20ug	1,695	5,139	7	6,102	363,426
Cas	9	HCT116	RA	G1A	20ug	3,040),041	13	11,711	538,051
Cas	9	K562	RA	G1A	20ug	6,657	7,170	67	27,757	1,299,770
Cas	9	U2OS	RA	G1A	20ug	4,197	7,923	42	17,885	838,120
Cas9 va	riants	293T	EN	/IX1	20ug	2,18	5,150	9	10,682	477,511
Cas9 va	riants	293T	M`	YC1	20ug	2,347	7,509	58	7,534	463,882
1:1 Acı	·IIA4	293T	RA	G1A	20ug	1,239	9,225	7	3,273	279,986
1:1 Acı	·IIA4	293T	RA	G1B	20ug	1,239	9,225	7	3,273	279,986
1:1 Acı	·IIA4	293T	EN	/IX1	20ug	3,532	2,528	6	10704	654373
1:1 Acı	·IIA4	293T	M`	YC1	20ug	2,346	6,147	58	7,534	463,882
1:1 Acı	·IIA4	293T	M`	YC2	20ug	1,332	2,715	25	7,521	298,450
SaCas9 /	AcrIIA4	293T	M`	YC1	20ug	2,062	2,054	23	5,812	451,326
Cas	9	293T	RA O	G1A- T32	10ug	2,366	6,218	26	5,293	381,173
Cas	9	293T	RA O	G1A- T29	10ug	1,864	1,579	8	12,214	176,109

(The Pearson correlation coefficient is for replicates 1 and 2, 2 and 3, 1 and 3 from top to bottom for each group.)

Supplementary Table S2: Cas9:RAG1A off-targets in different cell lines, *in vitro* digestion for Cas9:RAG1A off-targets and identification for Cas9:RAG1A off-targets with baits from Cas9: RAG1A off-targets

Cas9:RAG1A off-targets in different cell lines										
Chromosome	Off-targets	Sequence	293T	HCT116	K562	U2OS				
chr11	RAG1A	GCCTCTTTCCCACCCACCTT GGG	-	-	-	-				
chr12	1	TCCTCCTCCCCACCCACCTT CAG	724.9	87.6	88.5	139.0				
chr15	2	TCCTCTCTCCCACCCACCTC CGG	481.7	104.5	34.3	58.6				
chr19	3	ACCCCTTCCCCACCTACCTT GGG	277.9	83.4	32.0	82.0				
chr7	4	CCCTCCTTCCCACCCACTTT GGG	277.9	26.8	6.6	9.4				
chr11	5	ACGTCTTCCCCACCCACCTG GGG	226.7	22.6	4.6	23.4				
chr14	6	ACTTCCTTCCCACCCACCTT CAG	222.5	25.4	10.7	12.5				
chr7	7	GCCTCTTCCACACCCACCCT GGG	164.5	17.0	10.2	12.5				
chr1	8	TCCTCTTCTCCACCCACCTC TGG	127.4	15.5	-	-				
chr17	9	GTCTCTTTCTCACCCACTTT GGG	76.3	26.8	4.1	6.3				
chr7	10	CACCCTTTCCCATCCACCTT TGG	69.1	7.1	20.1	7.8				
chr8	11	ACCTCCTTCCCGCCCACCTG GGG	68.1	2.8	2.3	7.0				
chr11	12	TCCTCTTCTCCACCCACCAT AGG	63.3	15.5	3.1	2.3				
chr20	13	GTCTCTTTCCCATCCACCTT TGA	56.4	-	3.1	-				
chr13	14	GCCTCTTTACCACCTCACCT TGG	47.3	15.5	-	-				
chr4	15	TTCTCTTCCCCACCCACCTT TGA	44.8	4.2	3.1	-				
chr11	16	GCCCCTCCCCCACCCACCCT GGG	35.1	11.3	-	2.3				
chrX	17	ACCACTTCCCCACCCACCTC AGG	18.5	-	-	-				
chr17	18	GACTCTTTTCCACCCACCCT AGG	16.0	-	-	-				
chr12	19	GCTTCTTCCCCACCCACACT TGG	12.1	11.3	-	-				
chr3	20	ACCTCTTCCCTCCCCACCTT TGG	10.3	-	-	-				
chr7	21	TGCCTCTCCCCACCCACCCT TGG	9.6	-	-	-				
chr1	22	CACCCTTCCCCACCCACCCT GGG	9.3	-	1.02	-				
chr17	23	ACCTCTTATCCACCCACCTT GGC	9.1	-	-	-				
chr7	24	GCCCCTGCCCACCCACCTG TGG	6.1	-	-	-				
chr11	25	GCCCCTACCCACCCACCTG GGT	5.8	-	1.27	-				
chr5	26	TTTTCTTCCCCACCCACCTC GGG	5.8	-	-	3.12				
chr22	27	TGCTCTTCCCCACCCACCCA AGG	4.9	-	-	-				
chr16	28	GCCCCTTCCCCACCCACCCT GTG	3.3	-	-	-				
chr14	29	GGCTCCTCCTCACCCACCTC TGG	2.5	-	-	-				
chr11	30	TACCCTTCCCCATCCACCCT GGG	2.4	-	-	-				
chr11	31	TCCCCTTCACCACCCACCTG AGG	2.4	-	-	-				
chr3	32	GTCTCTTTCCCACCCACCTT GTT	2.4	-	-	-				
chr11	33	TTATCTTTCCCAACCACCTT TGG	2.2	-	-	-				
chr4	34	ACCTCTTTCCATCCCACCTT GGG	2.2	-	-	-				
chr10	35	TCATCTTCCCCACCCACCGT GGG	2.1	-	-	-				
chr15	36	CCCTCCTTCCCACCCACCTC CGA	1.9	-	-	-				

chr11	37	ACCTCATCCCCACTCACCTG GGG	1.8	-	-	-
chr12	38	GCCTCTTTTCCACCCACCTA ATT	1.5	-	-	-
chr6	39	GTGTATTCCCCACCCACCTT GGG	1.5	-	-	-
chr8	40	ACCTCGTCCCCACCCACCCT GGA	1.5	-	-	-
chr3	41	GCCTCATCCACATCCACCTT CGG	1.3	-	-	-
chr11	42	GCCTCTCCGCCACCACCAG AGG	1.2	-	-	-
chr7	43	GCCTCCTCCCCACCCACTCT GAG	1.2	-	-	-
chr16	44	CGCTCTGTCCCACCCACCTC CGG	1.1	-	-	-
chr11	45	ACATCAACCCCACCCACCGT AGG	0.9	-	-	-
chr12	46	GCCTCCTTCCCCTCCACCCC AGG	0.9	-	-	-
chr2	47	CTCCCCTTCCCACCCACCTC TGG	0.8	-	-	-
chr8	48	GCCTCATCCCCACCCACCCA AGG	0.8	-	-	-
chr11	49	GTCTCAAACCCACCCACCTT GGC	0.5	-	-	-
chr14	50	CCCCCTTTCCCACCCACCTT CTG	0.5	-	-	-
chr16	51	GCCTCAGTCCCACCCACCCC AGG	0.5	-	-	-
chr7	52	TCCTCTTCCTCTCCCACCTC CGG	0.5	-	-	-
chr16	53	TCCTCCTCTCCACCCACCTC TGG	0.2	-	-	-
		In vitro digestion for Cas9:RAG	IA off-targe	ts		
Chromosome	Off-targets	Sequence				
chr12	OT1	TCCTCCTCCCCACCCACCTT CAG	Re	lated to off-tar	get #1 in Tab	le S4
chr14	OT2	ACTTCCTTCCCACCCACCTT CAG	Re	lated to off-tar	get #6 in Tab	le S4
chr8	OT3	ACCTCCTTCCCGCCCACCTG GGG	Rel	ated to off-targ	jet #12 in Tal	ble S4
chr17	OT4	GACTCTTTTCCACCCACCCT AGG	Rel	ated to off-targ	jet #18 in Tal	ble S4
chr10	OT5	AGCTCCTCCTCACCCACCTC GGG	Rel	ated to off-targ	jet #30 in Tal	ble S4
chr16	OT6	GCCTCAGTCCCACCCACCCC AGG	Rel	ated to off-targ	jet #32 in Tal	ble S4
chr17	OT7	CGCCTCTTACCATCCACCTT GGG	Rel	ated to off-targ	jet #38 in Tal	ble S4
chr8	OT8	ACCTCGTCCCCACCCACCCT GGA	Rel	ated to off-targ	jet #29 in Tal	ble S4
	Cas9:RAG	1A off-targets using baits from C	as9: RAG1	A OT6 and	OT8	
Chromosome	Off-targets	Sequence				
chr16	OT6	GCCTCAGTCCCACCCACCCC AGG	Rel	ated to off-targ	jet #32 in Tal	ble S4
chr19	1	ACCCCTTCCCCACCTACCTT GGG	Re	lated to off-tar	get #2 in Tab	le S4
chr7	2	CCCTCCTTCCCACCCACTTT GGG	Re	lated to off-tar	get #4 in Tab	le S4
chr17	3	GTCTCTTTCTCACCCACTTT GGG	Re	lated to off-tar	get #9 in Tab	le S4
chr20	4	GTCTCTTTCCCATCCACCTT TGA	Rel	ated to off-targ	get #11 in Tab	ble S4
chr8	OT8	ACCTCGTCCCCACCCACCCT GGA	Rel	ated to off-targ	jet #29 in Tal	ble S4
chr1	1	TCCTCTTCTCCACCCACCTC TGG	Re	lated to off-tar	get #7 in Tab	le S4
chr17	2	GTCTCTTTCTCACCCACTTT GGG	Re	lated to off-tar	get #9 in Tab	le S4

Supplementary Table S3: Off-targets for other Cas9:RAG1 sites

Chromosome	Off-targets	Sequence	Frequency
chr11	RAG1B	GACTTGTTTTCATTGTTCTC AGG	-
chr14	1	TCCTTGTTTTCATTGTTCTC TGG	581.9
chr4	2	CATTTGTTTTCATTGTTCTC TGG	69.2
chr11	RAG1A/G	GCCTCTTTCCCACCCACCTT GGG	-
chr12	1	TCCTCCTCCCCACCCACCTT CAG	4.1
chr11	2	ACGTCTTCCCCACCCACCTG GGG	3.2
chr7	3	CCCTCCTTCCCACCCACTTT GGG	1.8
chr19	4	ACCCCTTCCCCACCTACCTT GGG	1.8
chr13	5	GCCTCTTTACCACCTCACCT TGG	1.4

Supplementary Table S4: Off-targets for Cas9:RAG1A variants

Chromosome	Off-targets	Sequence	WT	D1135E	eCas9	FeCas9
chr11	RAG1A	GCCTCTTTCCCACCCACCTT GGG	-	-	-	-
chr12	1	TCCTCCTCCCCACCCACCTT CAG	713.8	497.3	3.8	4.4
chr19	2	ACCCCTTCCCCACCTACCTT GGG	348.1	179.8	13.0	6.2
chr11	3	ACGTCTTCCCCACCCACCTG GGG	336.5	137.7	8.2	-
chr7	4	CCCTCCTTCCCACCCACTTT GGG	221.3	75.5	1.0	-
chr15	5	TCCTCTCTCCCACCCACCTC CGG	212.6	65.8	1.0	0.9
chr14	6	ACTTCCTTCCCACCCACCTT CAG	188.0	99.0	1.4	-
chr1	7	TCCTCTTCTCCACCCACCTC TGG	123.0	31.8	-	-
chr7	8	GCCTCTTCCACACCCACCCT GGG	119.0	52.4	-	-
chr17	9	GTCTCTTTCTCACCCACTTT GGG	77.3	24.9	0.7	-
chr7	10	CACCCTTTCCCATCCACCTT TGG	65.4	31.8	-	-
chr20	11	GTCTCTTTCCCATCCACCTT TGA	45.5	17.8	-	-
chr8	12	ACCTCCTTCCCGCCCACCTG GGG	40.1	32.6		
chr13	13	GCCTCTTTACCACCTCACCT TGG	38.8	17.2	-	-
chr11	14	TCCTCTTCTCCACCCACCAT AGG	36.5	31.6	-	-
chr4	15	TTCTCTTCCCCACCCACCTT TGA	29.6	15.6	-	-
chr11	16	GCCCCTCCCCACCCACCCT GGG	25.8	4.7	-	-
chr7	17	GCCTCTCCCCACCCACCCTT GGC	11.0	5.7	-	-
chr17	18	GACTCTTTTCCACCCACCCT AGG	10.3	4.7	-	-
chr3	19	ACCTCTTCCCTCCCCACCTT TGG	8.9	0.8	-	-
chr12	20	GCTTCTTCCCCACCCACACT TGG	8.7	1.8	-	-
chr1	21	CACCCTTCCCCACCCACCCT GGG	7.6	9.1	-	-
chr11	22	GCCCCTACCCACCCACCTG GGT	6.5	4.5	-	-
chr16	23	GCCCCTTCCCCACCCACCCT GTG	6.1	2.4	-	-
chr7	24	GCCCCTGCCCCACCCACCTG TGG	5.8	1.6	-	-
chrX	25	ACCACTTCCCCACCCACCTC AGG	4.9	2.8	-	-
chr14	26	GGCTCCTCCTCACCCACCTC TGG	4.9	-	-	-
chr17	27	ACCTCTTATCCACCCACCTT GGC	4.3	0.8	-	-
chr22	28	GCCCCTTACCCATCCACCCT GGG	4.0	-	-	-
chr8	29	ACCTCGTCCCCACCCACCCT GGA	2.5	-	-	-
chr10	30	AGCTCCTCCTCACCCACCTC GGG	2.4	-	-	-
chr11	31	TACCCTTCCCCATCCACCCT GGG	1.8	-	-	-
chr16	32	GCCTCAGTCCCACCCACCCC AGG	1.6	-	-	-
chr22	33	TGCTCTTCCCCACCCACCCA AGG	1.5	2.0	-	-
chrX	34	CCTTCTTTCCCACCCACATG GGG	1.5	-	-	-
chr5	35	TTTTCTTCCCCACCCACCTC GGG	0.9	5.5	-	-
chr10	36	TCATCTTCCCCACCCACCGT GGG	0.7	-	-	-
chr11	37	TTATCTTTCCCAACCACCTT TGG	0.5	-	-	-
chr17	38	CGCCTCTTACCATCCACCTT GGG	0.5	-	-	-
chr4	39	GCCTCCCTCCCAACCAGGAT GAT	-	-	2.7	-

Chromosome	Off-targets	Sequence	WT	D1135E	eSpCas9	Fe <i>Sp</i> Cas9
chr2	EMX1	GAGTCCGAGCAGAAGAAGAA GGG	-	-	-	-
chr15	1	GAGTCTAAGCAGAAGAAGAA GAG	129.3	75.1	0.8	-
chr5	2	GAGTTAGAGCAGAAGAAGAA AGG	100.0	59.3	-	-
chr2	3	GAGGCCGAGCAGAAGAAGA CGG	64.9	8.9	0.5	-
chr5	4	AAGTCTGAGCACAAGAAGAA TGG	25.8	3.3	-	-
chr8	5	GAGTCCTAGCAGGAGAAGAA GAG	26.4	5.0	-	-
chr1	6	AAGTCCGAGGAGAGGAAGAA AGG	3.8	0.7	-	-
chr11	7	AAGCCCGAGCAAAGGAAGAA AGG	2.5	-	-	-
chrX	8	GAGTCCGGGAAGGAGAAGAA AGG	2.5	0.9	-	-
chr5	9	GAGCCGGAGCAGAAGAAGGA GGG	2.5	-	-	-
chr1	10	GAGCCTGAGCAGAAGGAGAA GGG	1.7	-	-	-
chr15	11	AAGTCCCGGCAGAGGAAGAA GGG	1.1	-	-	-
chr2	12	AAGGCAGAGCAGAGGAAGAG AGG	0.9	-	-	-
chr2	13	GAGTCTAAGCAGGAGAATAA AGG	1.1	-	-	-
chr6	14	ACGTCTGAGCAGAAGAAGAA TGG	0.8	-	-	-
chr15	15	GAGCCTGAGGAACAGGAGGA GGA	0.5	-	-	-
chr3	16	GAATCCAAGCAGGAGAAGAA GGA	0.6	-	-	-
chr1	17	GATTCCTACCAGAAGAAGAA TGG	0.5	-	-	-
chr13	18	GAGTAGGAGCAGGAGAAGAA GGA	0.5	-	-	-

Supplementary Table S5: Off-targets for Cas9:EMX1 variants

Supplementary Table S6: Off-targets for Cas9:MYC	21 variants
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Chromosome	Off-targets	Sequence	WT	D1135E	eSpCas9	FeSpCas9
chr8	MYC-1	GGGATGTGGAGCTTGGCTAT GGG	-	-	-	-
chr8	1	AGGAAGTGGAGCTTGGCCTT GGG	68.2	21.0	-	-
chr8	2	AGGAAGTGGAGCTTGGCCTT GGG	67.8	21.0	-	-
chr9	3	GGGGTGTGGAGCTTGACTAT GAG	54.7	14.3	27.8	2.3
chr4	4	AGGATGAAGAGATTGGCTAT GGG	43.3	3.9	-	-
chr3	5	GGGAAGTGGAACCTGGCTCT GGG	35.7	12.7	-	-
chr12	6	TGGATGTGCAGCCTGGCTAT TGG	31.6	6.4	-	-
chr12	7	GGTATGCAGAGCTTGGCTTT CGG	25.8	5.4	-	-
chr18	8	GAGGTGTGGAGCTTGGTTAC TGG	8.4	1.5	-	-
chr1	9	GGGTTGCAGAGCTTGGCTAC GGG	6.7	-	-	-
chr2	10	GGGGTGTGGAGCATGGCTTT GGG	4.7	1.2	-	-
chr14	11	GGTGTGTGGAGCATGGCTAT TGG	4.3	-	-	-
chr15	12	TGAATGTGGAACTTGGCTAT TAG	3.9	-	-	-
chr20	13	AATATGTGGAGCTTGGCTAT AGC	3.4	0.8	-	-
chr10	14	GGGAAGAGGAGCCTGGCTTT GGG	3.0	-	-	-
chr2	15	GGGACTTGGAGCTTGGCTTT AGG	2.8	-	-	-
chr12	16	AGGGTCTGGAGCTTGGCTAA GGG	2.4	1.7	-	-
chr17	17	AGGATGTGGAGCTTGGATAT CAG	2.2	-	-	-
chr1	18	AAGATGTGGAACTTGGTTAC GGG	1.9	-	-	-
chr8	19	GAGATAAGGCCCTTGGCTAT GGG	1.5	-	-	-
chr2	20	GG-ATGAGGAG[G]CTTGGCTA-	0.9	0.6	-	-
		GGG				
chr8	21	AGGAGATGGAGCCTGGCTTA GGG	-	1.2	-	_
chr20	22	GAGATGTGGAGCCTGACTAT GGA	-	-	1.1	-
chr1	23	AAGACATGGAGCCTGGCTAT GGT	-	-	_	_
chr20	24	AAGACTAGGATCTTGGATAA GGG	-	-	-	-

Chromosome	Off-targets	Sequence	1:0	3:1	1:1	1:3
chr11	RAG1A	GCCTCTTTCCCACCCACCTT GGG	-	-	-	-
chr12	1	TCCTCCTCCCCACCCACCTT CAG	3.4	13.6	11.6	10.2
chr14	2	ACTTCCTTCCCACCCACCTT CAG	1.1	6.4	9.6	8.4
chr11	3	ACGTCTTCCCCACCCACCTG GGG	1.0	13.3	13.8	7.8
chr7	4	CCCTCCTTCCCACCCACTTT GGG	0.9	5.5	3.2	3.5
chr19	5	CCCCTTCCCC-ACCTACCTT GGG	0.8	1.8	3.9	-
chr15	6	TCCTCTCTCCCACCCACCTC CGG	0.6	2.2	4.2	2.7
chr7	7	GCCTCTTCCACACCCACCCT GGG	0.5	3.8	7.0	5.3
chr1	8	TCCTCTTCTCCACCCACCTC TGG	0.5	2.3	2.9	3.8
chr17	9	GTCTCTTTCTCACCCACTTT GGG	0.4	1.4	1.9	1.3
chr7	10	CACCCTTTCCCATCCACCTT TGG	0.3	1.7	0.9	0.8
chr13	11	GCCTCTTTACCACC[T]CACCTT GGG	0.2	1.2	-	2.7
chr11	12	TCCTCTTCTCCACCCACCAT AGG	0.2	0.7	1.2	-
chr8	13	ACCTCCTTCCCGCCCACCTG GGG	0.1	0.8	2.7	3.1
chr12	14	GCTTCTTCCCCACCCACACT TGG	0.1	0.3	-	0.8
chr20	15	GTCTCTTTCCCATCCACCTT TGA	0.1	0.9	-	0.6
chr11	16	CGCCCTCCCC[C]ACCCACCCT GGG	0.1	0.7	-	1.1
chr4	17	TTCTCTTCCCCACCCACCTT TGA	0.1	0.7	-	-
chr11	18	GCCCCTACCCACCCACCTG GGT	0.1	-	-	-
chr17	19	GACTCTTTTCCACCCACCCT AGG	0.1	-	-	-
chr11	20	CCTCGTGATCCACCCACCTC GGG	-	0.4	-	-
chr1	21	CACCCTTCCCCACCCACCCT GGG	-	0.6	-	-
chr17	22	ACCTCTTATCCACCCACCTT GGC	-	0.6	-	_

Supplementary Table S7: Off-targets for Cas9:RAG1A locus with AcrIIA4 inhibitor

Supplementary Table S8: Off-targets for Cas9:RAG1A locus with AcrIIA4 inhibitor, and the bait DSBs were generated by *Sa*Cas9:*MYC1*

Chromosome	Off-targets	Sequence	1:0	1:1
chr11	RAG1A	GCCTCTTTCCCACCCACCTT GGG	616.0	33.8
chr8	1	TCCTCCTCCCCACCCACCTT CAG	1367.9	295.0
chr12	2	ACTTCCTTCCCACCCACCTT CAG	468.8	55.5
chr7	3	ACGTCTTCCCCACCCACCTG GGG	269.3	38.3
chr14	4	CCCTCCTTCCCACCCACTTT GGG	255.0	33.8
chr15	5	CCCCTTCCCC-ACCTACCTT GGG	220.9	33.8
chr7	6	TCCTCTCTCCCACCCACCTC CGG	181.7	19.8
chr1	7	GCCTCTTCCACACCCACCCT GGG	179.2	29.4
chr19	8	TCCTCTTCTCCACCCACCTC TGG	172.6	24.9
chr17	9	GTCTCTTTCTCACCCACTTT GGG	123.2	56.8
chr7	10	CACCCTTTCCCATCCACCTT TGG	103.8	20.4
chr13	11	GCCTCTTTACCACC[T]CACCTT GGG	75.9	7.7
chr20	12	TCCTCTTCTCCACCCACCAT AGG	66.7	3.8
chr11	13	ACCTCCTTCCCGCCCACCTG GGG	46.8	10.9
chr4	14	GCTTCTTCCCCACCACACT TGG	36.1	8.9
chr12	15	GTCTCTTTCCCATCCACCTT TGA	34.6	7.0
chr7	16	CGCCCTCCCC[C]ACCCACCCT GGG	31.1	17.9
chr11	17	TTCTCTTCCCCACCCACCTT TGA	28.5	7.7
chr17	18	GCCCCTACCCACCCACCTG GGT	23.9	3.2
chr11	19	GACTCTTTTCCACCCACCCT AGG	21.4	3.8
chr17	20	CCTCGTGATCCACCCACCTC GGG	19.9	-
chr1	21	CACCCTTCCCCACCCACCCT GGG	14.3	-
chr22	22	ACCTCTTATCCACCCACCTT GGC	12.2	-
chrX	23	ACCACTTCCCCACCCACCTC AGG	12.2	4.5
chr2	24	CTCCCCTTCCCACCCACCTC TGG	9.7	-
chr8	25	GCCTCATCCCCACCCACCCA AGG	8.1	-
chr7	26	GCCCCTGCCCACCCACCTG TGG	7.6	-
chr7	27	ACCTCCTAACCCCCACCTT GGG	6.6	-
chr8	28	ACCTCGTCCCCACCCACCCT GGA	6.6	-
chr16	29	TCCTCCTCTCCACCCACCTC TGG	6.1	-
chr22	30	GCCCCTTACCCATCCACCCT GGG	6.1	-
chr10	31	TCATCTTCCCCACCCACCGT GGG	5.6	-
chr16	32	GCCTCAGTCCCACCCACCCC AGG	5.1	-
chr3	33	ACCTCTTCCCTCCCCACCTT TGG	4.1	-
chr1	34	TCTTCTCCCCCACCCACCCT GGG	3.6	-
chr6	35	ACCCCCTTCCCACCCACCCT GAG	3.6	-
chr8	36	TCCTCCCTCCCACCCACCTG AGG	3.6	-
chr8	37	GCCTCTTTCCTGCCCACCTG TGG	1.5	-

Supplementary Table S9: Off-targets for Cas9 with 1:1 AcrIIA4

Chromosome	Off-targets	Sequence	1:0	1:1
chr11	RAG1A	GCCTCTTTCCCACCCACCTT GGG		
chr12	1	TCCTCCTCCCCACCCACCTT CAG	1.9	5.8
chr11	2	ACGTCTTCCCCACCCACCTG GGG	0.9	4.4
chr19	3	ACCCCTTCCCCACCTACCTT GGG	0.8	1.4
chr7	4	CCCTCCTTCCCACCCACTTT GGG	0.7	2.0
chr14	5	ACTTCCTTCCCACCCACCTT CAG	0.6	3.6
chr15	6	TCCTCTCTCCCACCCACCTC CGG	0.5	1.8
chr17	7	GTCTCTTTCTCACCCACTTT GGG	0.2	1.4
chr7	8	GCCTCTTCCACACCCACCCT GGG	0.2	0.9
chr1	9	TCCTCTTCTCCACCCACCTC TGG	0.2	1.1
chr11	10	TCCTCTTCTCCACCCACCAT AGG	0.2	-
chr7	11	CACCCTTTCCCATCCACCTT TGG	0.2	1.2
chr13	12	GCCTCTTTACCACC[T]CACCTT GGG	0.1	1.3
chr20	13	GTCTCTTTCCCATCCACCTT TGA	0.1	0.5
chr8	14	ACCTCCTTCCCGCCCACCTG GGG	0.1	0.9
chr11	15	GCCCCTCCCCACCCACCCT GGG	0.1	0.6
chr4	16	TTCTCTTCCCCACCCACCTT TGA	0.1	0.4
chr17	17	ACCTCTTATCCACCCACCTT GGC	0.1	0.1
chrX	18	ACCACTTCCCCACCCACCTC AGG	0.1	-
chr11	19	GCCCCTACCCACCCACCTG GGT	0.1	-
chr17	20	GACTCTTTTCCACCCACCCT AGG	0.1	-
Chromosome	Locus	Sequence	1:0	1:1
chr11	RAG1B	GACTTGTTTTCATTGTTCTC AGG		-
chr14	1	TCCTTGTTTTCATTGTTCTC TGG	2.5	1.1
chr4	2	CATTTGTTTTCATTGTTCTC TGG	0.2	0.4
Chromosome	Off-targets	Sequence	1:0	1:1
chr8	MYC-locus1	GGGATGTGGAGCTTGGCTAT GGG	-	-
chr9	1	GGGGTGTGGAGCTTGACTAT GAG	0.2	0.4
chr8	2	AGGAAGTGGAGCTTGGCCTT GGG	0.1	0.6
chr8	3	AGGAAGTGGAGCTTGGCCTT GGG	0.1	0.6
chr4	4	AGGATGAAGAGATTGGCTAT GGG	0.1	0.2
chr12	5	GGTATGCAGAGCTTGGCTTT CGG	0.1	0.4
chr12	6	TGGATGTGCAGCCTGGCTAT TGG	0.1	0.4
chr3	7	GGGAAGTGGAACCTGGCTCT GGG	0.1	0.6
chr18	8	GAGGTGTGGAGCTTGGTTAC TGG	0.1	-
chr20	9	AAGACTAGGATCTTGGATAA GGG	0.1	-
chr2	10	GGGGTGTGGAGCATGGCTTT GGG	0.1	-
chr14	11	GGTGTGTGGAGCATGGCTAT TGG	0.1	-
chr15	12	TGAATGTGGAACTTGGCTAT TAG	0.1	-
chr17	13	AGGATGTGGAGCTTGGATAT CAG	0.1	-

Chromosome	Locus	Sequence	1:0	1:1
chr8	MYC-locus2	GTACATGCAGTTCTGCATCT TGG	-	-
chr6	1	ATAAATACAGTTCTGCATCT GGG	1.0	3.6
chr2	2	GTAAATGCAGTTCTGCATTT AGG	0.1	0.9
chr5	3	GCACATGCAGCCTTGCATCT GGG	0.1	0.4

Supplementary Table S10: gRNA sequences for PEM-seq

Locus	Sequence
RAG1A	GCCTCTTTCCCACCCACCTT (GGG)
RAG1B	GACTTGTTTTCATTGTTCTC (AGG)
RAG1C	GCACCTAACATGATATATTA (AGG)
RAG1G	GAAAGAGGCTGCCATGCTGGCTG (AGG)
EMX1	GAGTCCGAGCAGAAGAAGAA (GGG)
MYC-locus1	(TTTA) GGGATGTGGAGCTTGGCTAT (GGGAAT)
MYC-locus2	(TTTA) GTACATGCAGTTCTGCATCT (TGGGGT)

Supplementary Table S11: Oligos used for PEM-seq

Name	Sequence5'→3'	Purpose
Bridge adapters oligos (PEM-seq)		
BA-up	/5phos/CCA CGC GTG CTC TAC ANN NNT NNN ANN	link ssDNA with RMB
	NTN NNN AGA TCG GAA GAG CAC ACG TCT GAA CTC	
	CAG T-NH2(C7)	
BA-lower	TGT AGA GCA CGC GTG GNN NNN N-NH2(C7)	common bridge oligo
Oligos for tagging PCR		
P5-I5	AAT GAT ACG GCG ACC ACC GAG ATC TAC ACA CAC	Hiseq tail
	TCT TTC CCT ACA CGA CGC	
P7	CAA GCA GAA GAC GGC ATA CGA GAT	Hiseq tail
Oligos for nested PCR		
I7-B01	CAG AAG ACG GCA TAC GAG ATC GTG ATG TGA CTG	Hiseq tail mutiplex
	GAG TTC AGA CGT GTG C	
I7-B02	CAG AAG ACG GCA TAC GAG ATA CAT CGG TGA CTG	Hiseq tail mutiplex
	GAG TTC AGA CGT GTG C	
I7-B03	CAG AAG ACG GCA TAC GAG ATG CCT AAG TGA CTG	Hiseq tail mutiplex
	GAG TTC AGA CGT GTG C	
I7-B04	CAG AAG ACG GCA TAC GAG ATT GGT CAG TGA CTG	Hiseq tail mutiplex
	GAG TTC AGA CGT GTG C	
I7-B05	CAG AAG ACG GCA TAC GAG ATC ACT GTG TGA CTG	Hiseq tail mutiplex
	GAG TTC AGA CGT GTG C	
I7-B06	CAG AAG ACG GCA TAC GAG ATA TTG GCG TGA CTG	Hiseq tail mutiplex
	GAG TTC AGA CGT GTG C	
I7-B07	CAG AAG ACG GCA TAC GAG ATG ATC TGG TGA CTG	Hiseq tail mutiplex
	GAG TTC AGA CGT GTG C	
I7-B08	CAG AAG ACG GCA TAC GAG ATT CAA GTG TGA CTG	Hiseq tail mutiplex
	GAG TTC AGA CGT GTG C	
I7-B09	CAG AAG ACG GCA TAC GAG ATC TGA TCG TGA CTG	Hiseq tail mutiplex
	GAG TTC AGA CGT GTG C	
I7-B10	CAG AAG ACG GCA TAC GAG ATA AGC TAG TGA CTG	Hiseq tail mutiplex
	GAG TTC AGA CGT GTG C	
I7-B11	CAG AAG ACG GCA TAC GAG ATG TAG CCG TGA CTG	Hiseq tail mutiplex
	GAG TTC AGA CGT GTG C	
I7-B12	CAG AAG ACG GCA TAC GAG ATT ACA AGG TGA CTG	Hiseq tail mutiplex
	GAG TTC AGA CGT GTG C	
I7-R01	CAG AAG ACG GCA TAC GAG ATT AGT GCG TGA CTG	Hiseq tail mutiplex
	GAG TTC AGA CGT GTG C	
I7-R02	CAG AAG ACG GCA TAC GAG ATG CTA CAG TGA CTG	Hiseq tail mutiplex
	GAG TTC AGA CGT GTG C	
I7-R03	CAG AAG ACG GCA TAC GAG ATA ATC CGG TGA CTG	Hiseq tail mutiplex
	GAG TTC AGA CGT GTG C	

I7-R04	CAG AAG ACG GCA TAC GAG ATA CTG GTG TGA CTG	Hiseq tail mutiplex
	GAG TTC AGA CGT GTG C	
I7-R05	CAG AAG ACG GCA TAC GAG ATT GTC ACG TGA CTG	Hiseq tail mutiplex
	GAG TTC AGA CGT GTG C	
I7-R06	CAG AAG ACG GCA TAC GAG ATC GGT TAG TGA CTG	Hiseq tail mutiplex
	GAG TTC AGA CGT GTG C	
I7-R07	CAG AAG ACG GCA TAC GAG ATG TCT AGG TGA CTG	Hiseq tail mutiplex
	GAG TTC AGA CGT GTG C	
I7-R08	CAG AAG ACG GCA TAC GAG ATT GAA CTG TGA CTG	Hiseq tail mutiplex
	GAG TTC AGA CGT GTG C	
I7-R09	CAG AAG ACG GCA TAC GAG ATC TAG TCG TGA CTG	Hiseq tail mutiplex
	GAG TTC AGA CGT GTG C	
I7-R10	CAG AAG ACG GCA TAC GAG ATA TCG AAG TGA CTG	Hiseq tail mutiplex
	GAG TTC AGA CGT GTG C	
I7-R11	CAG AAG ACG GCA TAC GAG ATC CGA TGG TGA CTG	Hiseq tail mutiplex
	GAG TTC AGA CGT GTG C	
I7-R12	CAG AAG ACG GCA TAC GAG ATG AAC ATG TGA CTG	Hiseq tail mutiplex
	GAG TTC AGA CGT GTG C	
oligos for RAG1A/B locus		
bioRAG14/B	Biotin/GGA CTG CTG GAG ATT GCT CCA GAG AGG	primer extension
	GTT TCC CCT CAA AGG AAT CCT TC	
	ACT CTT TCC CTA CAC GAC GCT CTT CCG ATC TAG	Hiseq tail mutiplex
	CGA GGA TCT CAC CCG GAA CAG C	
15-R2-RAG1A/B	ACT CTT TCC CTA CAC GAC GCT CTT CCG ATC TGC	Hiseq tail mutiplex
	CTA GGA TCT CAC CCG GAA CAG C	
15-R3-RAG1A/B	ACT TTT CCC TAC ACG ACG CTC TTC CGA TCT AGG	Hiseq tail mutiplex
	AAG GAT CTC ACC CGG AAC AGC	
15-R4-RAG1A/B	ACT CTT TCC CTA CAC GAC GCT CTT CCG ATC TTC	Hiseq tail mutiplex
	AGA GGA TCT CAC CCG GAA CAG C	
RAG1A-RFLP-F	GGG AGG CAA AGA TGA ATC AAA G	RAG1A-RFLP
RAG1A-T7EI-F	CCC CCT GGA AGA CTG CTT TA	RAG1A-RFLP
RAG1A-R/T-R	AGG ACT GCT GGA GAT TGC TC	RAG1A-RFLP/T7EI
oligos for RAG1C locus		
bioRAG1C	Biotin/CCT GAG AAC AAT GAA AAC AAG TC	primer extension
	ACT CTT TCC CTA CAC GAC GCT CTT CCG ATC TAG	Hiseq tail mutiplex
IS-R1-RAG1C	CGT ATC AAT ATC CCA CTG ATG TAT C	
	ACT CTT TCC CTA CAC GAC GCT CTT CCG ATC TGC	Hiseq tail mutiplex
	CTT ATC AAT ATC CCA CTG ATG TAT C	
	ACT CTT TCC CTA CAC GAC GCT CTT CCG ATC TAG	Hiseq tail mutiplex
10-R3-RAG IC	GAT ATC AAT ATC CCA CTG ATG TAT C	
	ACT CTT TCC CTA CAC GAC GCT CTT CCG ATC TTC	Hiseq tail mutiplex
	AGT ATC AAT ATC CCA CTG ATG TAT C	

oligos for MYC locus1		
bioMYC1	Biotin/CGG GAA GGA AGT TGG CAT CTG TCC TG	primer extension
I5-R1-MYC1	ACT CTT TCC CTA CAC GAC GCT CTT CCG ATC TAG	Hiseq tail mutiplex
	CGA GAG CCA TTC TCT GGC TCA G	
I5-R2-MYC1	ACT CTT TCC CTA CAC GAC GCT CTT CCG ATC TGC	Hiseq tail mutiplex
	CTA GAG CCA TTC TCT GGC TCA G	Hissa tail mutiplay
I5-R3-MYC1	GAA GAG CCA TTC TCT GGC TCA G	
	ACT CTT TCC CTA CAC GAC GCT CTT CCG ATC TTC	Hiseq tail mutiplex
I5-R4-MYC1	AGA GAG CCA TTC TCT GGC TCA G	
oligos for MYC locus2		
	Biotin/TTG CGA CTC TCA GCT GAA TCC ACT GCT	primer extension
	G	
15-R1-MYC2	ACT CTT TCC CTA CAC GAC GCT CTT CCG ATC TAG	Hiseq tail mutiplex
	CGT GCT CCC ATG GCA TCA TGA C	
I5-R2-MYC2	ACT CTT TCC CTA CAC GAC GCT CTT CCG ATC TGC	Hiseq tail mutiplex
	CTT GCT CCC ATG GCA TCA TGA C	
I5-R3-MYC2	ACT CTT TCC CTA CAC GAC GCT CTT CCG ATC TAG	Hiseq tail mutiplex
	ACT CTT TCC CTA CAC GAC GCT CTT CCG ATC TTC	Hisea tail mutinley
I5-R4-MYC2	AGT GCT CCC ATG GCA TCA TGA C	
oligos for EMX1 locus		
	Biotin/CCC ATC AGG CTC TCA GCT CAG CCT GAG	primer extension
DIOTINEMX1	TGT TGA G	
15-R1-EMX1	ACT CTT TCC CTA CAC GAC GCT CTT CCG ATC TAG	Hiseq tail mutiplex
	CGC CCA GGT GAA GGT GTG GTT C	
I5-R2-EMX1	ACT CTT TCC CTA CAC GAC GCT CTT CCG ATC TGC	Hiseq tail mutiplex
	CTC CCA GGT GAA GGT GTG GTT C	
I5-R3-EMX1	ACT CTT TCC CTA CAC GAC GCT CTT CCG ATC TAG	Hiseq tail mutiplex
	GAC CCA GGT GAA GGT GTG GTT C	
I5-R4-EMX1	ACT CTT TEE CTA CAE GAE GET CTT CEG ATE TTE	Hiseq tail muliplex
oligos for RAG1A off-targets		
	GGG TGT CAC GGG CCG TGG GAC AGG TC	primer extension
I5-R1-SpCAS9-RAG1A-OT6	CGC ATC GGT TGC CAT GGC TAC TG	
	ACT CTT TCC CTA CAC GAC GCT CTT CCG ATC TGC	Hiseq tail mutiplex
I5-R2-SpCAS9-RAG1A-OT6	CTC ATC GGT TGC CAT GGC TAC TG	
	ACT CTT TCC CTA CAC GAC GCT CTT CCG ATC TAG	Hiseq tail mutiplex
10-R0-000809-RAG1A-U10	GAC ATC GGT TGC CAT GGC TAC TG	
bioSpCAS9-RAG1A-OT8	CCT CCC TGA CTA GTG GTT AAC GGA CTT G	primer extension

	ACT CTT TCC CTA CAC GAC GCT CTT CCG ATC TAG	Hiseq tail mutiplex
15-R1-3pCA39-RAG1A-018	CGC GCT TTC CTG GCG GTT ACA G	
15-R2-SpCAS9-RAG1A-OT8	ACT CTT TCC CTA CAC GAC GCT CTT CCG ATC TGC	Hiseq tail mutiplex
13-112-300-33-114-010	CTC GCT TTC CTG GCG GTT ACA G	
15-R3-SpCAS9-RAG1A-OT8	ACT CTT TCC CTA CAC GAC GCT CTT CCG ATC TAG	Hiseq tail mutiplex
	GAC GCT TTC CTG GCG GTT ACA G	
RAG1A-OT1-F	GGC TGT CCA TGT TGT CCT TC	In vitro digestion
RAG1A-OT1-R	ACA CCT CTG GTT AGA GAT AAA GTG	In vitro digestion
RAG1A-OT2-F	CTT GCG ATC TGT CTG TCT CC	In vitro digestion
RAG1A-OT2-R	GAT GTG GAG ACT GAT TAA GCC	In vitro digestion
RAG1A-OT3-F	AGG CCA GTG GTC ACG TGG CTC	In vitro digestion
RAG1A-OT3-R	GGC CGA GGC CTG GAG ACA GG	In vitro digestion
RAG1A-OT4-F	ACA GAC ATA CAC GTG AGG TC	In vitro digestion
RAG1A-OT4-R	AAG TAC AGT CGG ACT TGG CAG	In vitro digestion
RAG1A-OT5-F	GCT GCC TCA GCG GCC AGG GCC	In vitro digestion
RAG1A-OT5-R	TCC TCC CAT CCC AAG GTG CC	In vitro digestion
RAG1A-OT6-F	GGC CTG ATG GCC AGA GAG GG	In vitro digestion
RAG1A-OT6-R	GAA TAA TTA CAG GGG AGT CAG CC	In vitro digestion
RAG1A-OT7-F	CAT GAG CCT CTG GAA TCT GTC	In vitro digestion
RAG1A-OT7-R	GTC ACA TTC ACA GGT CCG AG	In vitro digestion
RAG1A-OT8-F	TCT GGC TAA GCC GTT AGG CGC	In vitro digestion
RAG1A-OT8-R	CCT GTG TTT TCT GGA CAC AGA AC	In vitro digestion
RAG1A-on-target-F	GGG AGG CAA AGA TGA ATC AAA G	In vitro digestion
RAG1A-on-target-R	GGA TCG AGT CAA CAT CTG CC	In vitro digestion
Negative-control-F	GCC TCT GAA AAC CTA TGG CC	In vitro digestion
Negative-control-R	GTT GCA CAA TGG CAG AGA CC	In vitro digestion