

Table 1. Phenotype and viability of *Tyr* edited zygotes and mice by CRISPR-EZ

Strain	Genome Editing Method	Zygotes Electroporated	Zygotes Recovered After Electroporation	Zygotes Transferred ^a	Mice Born	Viability	Genotyping ^b		
							No Mutation	HDR	Indel
B6N	2 pulses	30	30	10	5	50	0	0	5 (100%)
B6N	6 pulses	40	22	22	6	27.2727	0	1 (17%)	6 (100%)
B6J	4 pulses	35	29	29	15	51.7241	5 (33%)	1 (7%)	10 (67%)
B6J	6 pulses	35	34	34	16	47.0588	0	10 (62.5%)	16 (100%)
B6J	8 pulses	35	30	29	12	41.3793	0	6 (50%)	12 (100%)
B6J	4 pulses	100 ^c	80	48	26	54.1667	3 (12%)	14 (62%)	23 (88%)

a) Number of Zygotes transferred to pseudopregnant females after electroporation.

b) HDR and an Indel events are not mutually exclusive.

c) 100 Zygotes were electroporated in a single cuvette

Table 2. Results of CRISPR-EZ and Microinjection Deletion Experiments.

Gene ^a	Method	Date of Electroporation or Microinjection	Number of sgRNAs Delivered	Zygotes Injected ^b or Loaded into Single Cuvette ^c	Zygotes Recovered ^d	Zygotes Transferred ^e	Pups Born	Viability (%)	Edited Pups ^f	Edited Pups (%)
Cfap57	CRISPR-EZ	3/22/17	4	50	49	40	4	10.0	4	100.0
Clcnkb	CRISPR-EZ	1/18/17	4	60	58	40	14	35.0	8	57.1
Cndp2	CRISPR-EZ	1/23/17	4	62	50	40	6	15.0	4	66.7
Ddx6	CRISPR-EZ	12/14/16	4	70	64	40	20	50.0	5	25.0
Fubp1	CRISPR-EZ	1/23/17	4	60	48	35	6	17.1	3	50.0
Gsg1l	CRISPR-EZ	12/5/16	4	70	45	44	7	15.9	3	42.9
Sfmbt2	CRISPR-EZ	12/5/16	4	70	55	44	29	65.9	3	10.3
Sh3rf2	CRISPR-EZ	1/11/17	2	60	51	40	17	42.5	4	23.5
Tuba1a	CRISPR-EZ	1/11/17	4	60	52	40	14	35.0	0	0.0
Unc5cl	CRISPR-EZ	9/28/16	4	60	49	40	8	20.0	6	75.0
Copb1	CRISPR-EZ	9/28/16	4	43	41	30	6	20.0	1	16.7
Kpna2	CRISPR-EZ	9/29/16	4	89	62	40	11	27.5	2	18.2
Ocel1	CRISPR-EZ	10/7/16	4	45	35	36	24	66.7	0	0.0
Slc25a18	CRISPR-EZ	10/7/16	4	44	33	33	16	48.5	1	6.3
Ipo7	CRISPR-EZ	10/7/16	4	50	36	36	13	36.1	8	61.5
Xpo5	CRISPR-EZ	10/10/16	4	56	50	40	12	30.0	8	66.7
Mcts1	CRISPR-EZ	10/10/16	4	53	50	40	12	30.0	3	25.0
Wdr82	CRISPR-EZ	10/11/16	4	66	52	40	11	27.5	1	9.1
Cyp4v3	CRISPR-EZ	10/11/16	4	63	41	40	8	20.0	6	75.0
Cdhr2	CRISPR-EZ	12/14/16	4	72	65	40	8	20.0	0	0.0
Eif3i	CRISPR-EZ	12/14/16	4	72	63	40	3	7.5	0	0.0
Cfap57	Microinjection	1/5/17	4	50	36	36	6	16.7	0	0.0
Clcnkb	Microinjection	8/15/16	3	65	50	50*	9	18.0	0	0.0
Cndp2	Microinjection	8/22/16	4	48	36	34	3	8.8	0	0.0
Ddx6	Microinjection	7/11/16	4	50	37	37	6	16.2	0	0.0
Fubp1	Microinjection	8/22/16	4	42	35	34	4	11.8	0	0.0
Gsg1l	Microinjection	10/17/16	4	65	40	40	22	55.0	0	0.0
Sfmbt2	Microinjection	10/24/16	4	45	35	35	9	25.7	0	0.0
Sh3rf2	Microinjection	5/16/16	4	55	40	40	29	72.5	1	3.4
Tuba1a	Microinjection	4/18/16	4	76	60	60*	5	8.3	1	20.0
Cand1	Microinjection	10/12/16	4	58	40	40	9	22.5	1	11.1
Slc22a16	Microinjection	10/12/16	4	63	51	40	21	52.5	0	0.0
Ubac2	Microinjection	10/17/16	4	70	40	40	13	32.5	0	0.0
Map3k13	Microinjection	10/18/16	4	50	35	35	18	51.4	5	27.8
Sliitrk2	Microinjection	10/18/16	4	50	40	40	18	45.0	1	5.6
Csmc3	Microinjection	10/19/16	4	100	92	80**	22	27.5	4	18.2
Fam132a	Microinjection	10/19/16	4	40	35	35	11	31.4	0	0.0
Maged2	Microinjection	10/19/16	4	35	30	30	8	26.7	0	0.0
Marveld3	Microinjection	10/20/16	4	59	48	40	14	35.0	0	0.0
Naa10	Microinjection	10/20/16	4	58	50	40	10	25.0	1	10.0
Serhl	Microinjection	10/20/16	4	49	40	40	8	20.0	0	0.0
Tlk2	Microinjection	10/24/16	4	70	41	40	13	32.5	1	7.7
Cdc42ep3	Microinjection	10/25/16	4	55	45	40	17	42.5	4	23.5
Magohb	Microinjection	10/25/16	4	70	44	40	10	25.0	0	0.0
1700034E13Rik	Microinjection	12/8/16	4	60	42	40	14	35.0	3	21.4
2810474O19Rik	Microinjection	12/8/16	4	90	56	55*	11	20.0	3	27.3
4833420G17Rik	Microinjection	12/9/16	4	50	45	40	12	30.0	1	8.3
8030462N17Rik	Microinjection	12/9/16	4	80	68	60*	5	8.3	0	0.0

a) Gene names highlighted in grey were involved in direct comparisons between CRISPR-EZ and Microinjection.

b) All zygotes from each electroporation experiment were loaded into the same cuvette. Conditions across all procedures were 8uM Cas9 Protein, 6 pulses at 30V with 3ms pulse intervals.

c) All zygotes from each experiment were individually injected with a cocktail of up to 4 sgRNAs and injection buffer. 100ng/μL Cas9, 20ng/μL Each sgRNA.

d) Zygote recovery and survival was based on visual cues. Those that appeared lysed were discarded.

e) For the majority of experiments, embryos were transferred to two recipient females each, with approximately 20 embryos per female.

*) Instead of 2 recipient females, 3 were used.

**) Instead of 2 recipient females, 4 were used.

f) "Edited" is defined by presence of desired PCR amplicon. Animals may be Heterozygous or Mosaic.

Table 3. Sequences and expected editing results of sgRNAs used in Electroporation Experiments.

Gene ^a	Fwd Primer	Rev Primer	5'gRNA-1	5'gRNA-2	3'gRNA-1	3'gRNA-2	Targeted exon for deletion	Unedited amplicon Size (bp)	Predicted deletion size (bp) ^b
Unc5cl	cttcagacagcaggttaggtacc	cacctcaagaacctcaattgag	gggtcatcgctactcgtttg	aggccaccgagagctaaactg	agcatgctaaatgaatgaag	agaacagtggtcctcagagag	3	1214	580-684
Copb1	ccagctccctctagaattttg	ctgaatccatgctcacactctagg	tggaaltccaactgttcaga	ggcagctlagcccccgtcc	gaacttagacgacacaccac	gtagaaaagggttggggatg	3,4	3323	2,508-2,672
Kpna2	ctgacttgagaatccaatgtcatgg	gctgtttctctagaaaagcaatttc	tgactgtgaaaacagtgtag	ggtcagacaactcataatg	ggatgttttaaaggggaa	tgtcacttaaaagcagcct	4	1127	697-780
Oce1	ctctgggacaggtgacaccgag	gtgagatccatgctattgagcc	cgcgcgctcatgctcagagag	gatccgcaactctgggtgctg	fgcttggggcctgctcag	gctcaggttagtttcaactg	1,2,3,4	2203	1,714-1,837
Slc25a18	gttctcgaggcatctttgaagg	gtcatcatgtgggctgagctc	agagaggttctclagcctgtg	gctctcagggaggggtgctg	tgctacaggtcacaagaatg	tggaagaatlaattcctcgg	3,4	945	510-592
Ipo7	gttgatccccctggatttag	gatgctcagcagtaagacc	agaggatcacaacctcttc	gtagggatcaaaactagagc	agccctactccaataatca	gtgcatgtgtgtccattg	2	940	395-627
Xpo5	gggttgacattcaactgtatggg	cagcacagaaacctttaatcttc	agccctggagatgqcatag	gctgaggactggaatccagg	gcacctgtgtgctcatgaa	gcacaggtgctgctaact	2	1569	547-1,164
Mcts1	gcagtgtatctccattcctagattc	ctccttgggtgtgttgaatgac	agcttagcaataggcctgac	gtaaggaagaccaattcct	gccaccactcatgcttta	agtttggattgttccaag	2,3	2551	1,427-2,239
Wdr82	cctctgttgattctgtttgtg	ccaaccctgcttttagaag	caaccacataatgacttagc	gggggagaggatctcgaggg	tgcaaatcaaacacaacg	gctggaggagtgaggtagc	2	1172	659-871
Cyp4v3	gcatgtagcagacattttactgac	gaagacattgcacaacagcttc	gaatcaacgcccaacacat	gggtgtgagctcagagg	tgcaacaataaacaccgag	ggctatatgcatgtctgtt	2	934	406-623
Cdhr2	cagggacaagcctttcaaacgc	agcagaaccgtgggttcctgg	gactcttgcctcagctgtg	tgctcgaaggaaacatggg	gcalgtatgccctacactg	gtgggactaatgactgatg	4,5	1191	1,030-1,133
Eif3i	ccatgtgtgtttgtatgtgtgtgc	ctggcctgaaactcagaaaacc	cgggagggcatgagctgtag	agcctcattagcaggactcg	agtggtcctgtcacaagaag	agaagtgagtagactctccg	5,6	1272	722-811
Cfap57	ccaataaaccagccctggg	tgtaaggtttgtgcttgatgc	atagacagggccaccatacac	agtatagctctactacatg	ctcctgaagcctccaccg	catctcctggaatgctgtg	4	1198	656-846
Clnkb	gacaggttttctgggctggag	gctgggaagctggaccctacc	gccaagctacaagacatcc	gaaataagccggccggtgg	none	gacagtgaggggtaggaca	5	943	435-548
Cndp2	gctctgaaggaaagagatgaccc	aatagacataaccagttctgtagg	gctgtccctacaagaactg	gtggttgagatgttaccct	gggatattactgtgaccg	ggaagctgcaagcctcagg	4	1516	955-1051
Ddx6	attatcgtgctgctcattggg	caactattcaatacacagagcctcagc	gctaagaactacagatactct	gttactglaagttacagcct	tgaaatgacgctgcatgtgt	gtgctacaggtgtagcat	3	1657	932-1099
Fubp1	gcatgtgagaagttaggattagactagc	aaagggccacaatacagtcagtc	gttacgtacaattgaacgag	tggtgggtgttttaaatg	gtgattctgactgaatgaa	ggtaaggttgggtgtgct	2	1343	635-823
Gsg1l	cagggacaagccttcaaacgc	agcagaaccgtgggttctgg	gtctctctgcttgaagtg	tgccctctccactcccgcg	gctgcgagttccagtgctg	gaaatgactcaggccgaag	1	1191	799-885
Sfmbt2	gaactcattgctcgcagagcagc	ctlaaggaggctgagttgctgc	gacatttccagctaaagtg	gaaagctgcataaaagggg	gagccacaataagagtttgt	tgtggctggggtaaatctt	4	1782	1039-1436
Sh3rf2 ^c	gtlgagggagcctaagttctgagtc	caggttgacatgctagtagtcc	agtagtgcaggctgcaacg	tggtttgggtagagtgagg	ggctgggcatggagactagg	gatctctattgtctcactc	5	836	383(EZ) ,292-532
Tuba1a	caagtgccatgatgaaataccaggtc	gtaacctgtcaactcacaatgtgc	gcagtattaaggtgacatct	ggagtcacatcctaataac	gtgtgccagcttctatgctt	tggaaggttgggtcccag	2,3,4	2623	2037-2286

a) Gene names highlighted in grey were involved in direct comparisons between CRISPR-EZ and Microinjection.

b) For each deletion, up to 4 sgRNAs were combined during the electroporation. Two were designed upstream of the targeted exon and two were designed downstream. Shortest and Longest predicted deletion sizes are noted.

c) Electroporation was performed with 5'gRNA-1 and 3'gRNA-1. Microinjection was performed with all four sgRNA.