

**Table S1 sgRNAs used in this study.** Distances from TSS are based on BDGP Release 6 (August 2014).

Gene targeted	sgRNA sequence (including PAM)	Distance from TSS (from 3' end of PAM)	Strand	Efficiency Score
wg	CCCCGATCCGATCGCATCGTCGG	-78	minus	4.85
	GCAGCTGCAATGCAGGAGTCAGG	-145	plus	4.07
wg	ATGAGGTTGCGCAAATAATCGGG	-363	plus	6.79
	GGAAATGGAAAACTCTGCCCGG	-337	minus	4.26
wg	TATATATTGTATCGTAAATTTGG	125	minus	5.33
	ATTTGTGCGATTAATTCCGCTGG	206	minus	5.79
wg	GCTGCTGACAAACGCAGAGTCGG	22	plus	5.39
	CGTGTGTTTCAGTTAAGCGTTGG	15	plus	7.97
hnt	GCGCAAATAGGATTACACATTGG	-251	minus	4.63
	GGCCGTACTIONCATCTTTATTGG	-304	minus	3.41
hnt	GAGAGAAGAGAGAAGCAGTCTGG	-131	minus	4.55
	ATTTGAAACGAAGAATGAGAAGG	-180	plus	5.30
hnt	AGTTGTATTTATAAATACAACGG	34	minus	4.86
	TGCGTTTGATATTTCTTTGTAGG	173	minus	6.56
hnt	GCCTAAAACAGTGCGAAATCCGG	444	plus	4.81
	AACAGTGCGAAATCCGGAGTTGG	450	plus	5.66
QUAS (#1)	CTCGGGTAATCGCTTATCCTCGG	-103	plus	5.45
QUAS (#2)	CGGATAAACAATTATCCTCACGG	-141	plus	4.69
QUAS (#3)	CCAACGCGTTGGGAGCTCTCCGG	-197	plus	5.35
engrailed (isoform A)	GCGTAACTCTCCCCGACGTCGG	-20	plus	5.72

engrailed (isoform A)	AACTGTCACGGTGAAAGAGAGG	-78	minus	5.09
engrailed (isoform A)	GGCGAGATCCCACAAGTAGCTGG	-121	minus	5.80
engrailed (isoform A)	AGCGAAAATCGATCAGTGTAAAGG	-210	plus	5.65
engrailed (isoform A)	GCTCACTCACTCCTATTAGCTGG	-300	minus	7.33
twist (isoform B)	CAAAATGTCAATTTGAGCAATGG	-14	plus	6.52
twist (isoform B)	GCGGGACGACGATAGAGCGGCGG	-61	plus	6.48
twist (isoform B)	GCCATCCCGCTCCCACTCAATGG	-122	minus	6.56
twist (isoform B)	GCATCGGCAGGTATGACGTCAGG	-156	minus	6.75
twist (isoform B)	ATTTTCTCGAGCGGCAGCGGCGG	-191	minus	6.37
per	GAGTGAGTGTGAGAAAATTCTGG	-50	minus	5.85
per	CCGCCGTCGCTGAGAATCGCTGG	-104	plus	6.38
per	TCGCTCGGGAAAATCGCTGGTCGG	-136	plus	6.03
per	TTCGCCAAGGGTTAATGTTTGG	-151	minus	6.05
y	CATTGGCCTGTCTTCGTCTTCGG	-46	minus	6.83
y	ACGAAGGCGCGGCCAACTTCGG	-101	plus	7.86
y	ATTCGGGTGGTTCAGTGTTCGGG	-135	plus	6.55
y	CGCAAAGTTGGCCGATCTATGGG	-157	minus	4.45
Os	TACCGCTCGTCGGCACTCGGCGG	-39	minus	4.91
Os	ATTCAGATCCGAAGAACCGCAGG	-131	plus	6.27
en	GCGTTAACTCTCCCCGACGTCGG	-19	minus	5.72
en	AACTGTCACGGTGAAAGAGAGG	-77	plus	5.09
en	GGCGAGATCCCACAAGTAGCTGG	-120	plus	5.80
en	AGTGAGTGAGTGACAGCAGTTGG	-164	plus	4.10
en	AGCGAAAATCGATCAGTGTAAAGG	-209	minus	5.65
en	GCTCACTCACTCCTATTAGCTGG	-299	plus	7.33

AttC	TATAGCAATCTATCTCTGAGTGG	-48	plus	6.52
AttC	TATAAATTGGTATTTCATTGTCGG	-63	minus	6.64
AttC	AGCTGAGCAATGTTTCGCACTGG	-138	plus	5.61
AttC	GTGAACCACCTGGTCATTGCGGG	-140	minus	9.39
AttC	ATCCCCTTGAACTACTTGCCGG	-209	plus	6.92
AttC	TAAAATTTGAACTACTCATTGG	-338	minus	6.22
Dro	CGAATCTCTTGTTCATCGATGG	-39	minus	4.31
Dro	AACATGAAAAGTCCCAAGATGG	-101	minus	3.56
Dro	GCCGGTGATTCCCATCTTGGGG	-112	plus	4.30
Dro	ATCAACGAATAGGCGACTGAAGG	-151	plus	5.78
Dro	GCTGCGTAGTTTACATCATTGG	-220	plus	5.30
sna	CCGACGCCGCTGTCGCCATTTGG	-68	plus	7.39
sna	TCCATTTCCACCTCTCTCTCGG	-235	plus	8.15
sna	AAAGTGCTGTTGTTGTTGCTAGG	-117	minus	7.14
sna	GAAATACGCAATAAGGGTATGGG	-141	minus	5.39
sna	GAGAGAGAGAGTGAGAGAGCAGG	-179	minus	5.96