In vivo genome engineering with CRISPR-Cas9

These publications demonstrate the application of CRISPR-Cas9 genome engineering techniques for target gene knockout, chromosomal deletions, precise knock-in, or gene correction in a variety of animal models.

Reference	Species/Cells	Target	Type of editing	Cas9 and guide RNA format	Delivery method	Link
Paix et al. Nucl. Acids Res. 2016	*Caenorhabditis elegans*	*dpy-10, gtbp-1, meg-3*	HDR/knock-in of reporter with ssODN or dsDNA, gene replacement	Cas9 protein and synthetic crRNA:tracrRNA (Dharmacon)	Microinjection	http://dx.doi.org/10.1093/nar/gkw502
Friedland *et al. Nat. Methods* 2013	*C. elegans*	*unc-119, dpy-13, klp-12, Y61A9LA.1*	Single gene knockout	Cas9 and sgRNA plasmids	Microinjection	http://dx.doi.org/10.1038/nmeth.2532
Wagner *et al. Nat. Methods* 2014	*Plasmodium falciparum*	*kahrp, eba-175*	HDR/knockin of reporter with plasmid donor	Cas9 and sgRNA plasmids	Electroporation	http://dx.doi.org/10.1038/nmeth.3063
Wang et al. Cell Res. 2013	*Bombyx mori* (silkworm) preblastoderm embryos	*BmBLOS2*	Single gene knockout	Cas9 mRNA and sgRNA (IVT)	Microinjection	http://dx.doi.org/10.1038/cr.2013.146
Ma *et al. Sci. Rep.* 2014	*B. mori* (silkworm) eggs	*BmKu70*	Single gene knockout	Cas9 and sgRNA plasmids	Microinjection	http://dx.doi.org/10.1038/srep04489
Bassett *et al. Cell Rep.* 2013	*Drosophila* preblastoderm embryos	*yellow, white*	Single gene knockout	Cas9 mRNA and sgRNA (IVT)	Microinjection	http://dx.doi.org/10.1016/j.celrep.2013.06.020
Li *et al. Sci. Rep.* 2015	*Papilio xhutus* (butterfly) eggs	*Abd-B, ebony, frizzled*	Single gene knockout	Cas9 mRNA and sgRNA (IVT)	Microinjection	http://dx.doi.org/10.1038/ncomms9212
Kistler *et al. Cell Rep.* 2015	*Aedes aegypti* (mosquito) preblastoderm embryos	*AAEL010779, AAEL004091, AAEL000926, AAEL014228, AAEL002575, AAEL013647, Aaeg-wtrw*	Single gene knockout, HDR/knockout with ssODN, chromosomal deletion with two sgRNAs, HDR/knock-in of reporter with plasmid donor	Cas9 protein or mRNA and sgRNA	Microinjection	http://dx.doi.org/10.1016/j.celrep.2015.03.009
Hammond *et al. Nat. Biotechnol.* 2016	*Anopheles gambiae* (mosquito) zygotes	*AGAP005958, AGAP007280, AGAP011377*	HDR/knock-in/knockout of reporter with plasmid donor	Cas9 and sgRNA plasmids	Microinjection	http://dx.doi.org/10.1038/nbt.3439
Chang *et al. Cell Res.* 2013	*Danio rerio* (zebrafish) zygotes	*etsrp, gata5, gata4*	Single gene knockout and HDR/ knock-in with ssODN	Cas9 mRNA and sgRNA (IVT)	Microinjection	http://dx.doi.org/10.1038/cr.2013.45
Hwang *et al. Nat. Biotechnol.* 2013	*D. rerio* (zebrafish) zygotes	*fh, th1, apoea, rgs4, tph1a, drd3, gria3a, slc6a3, tial1, gsk3b*	Single gene knockout	Cas9 mRNA and sgRNA (IVT)	Microinjection	http://dx.doi.org/10.1038/nbt.2501
Jao *et al. PNAS* 2013	*D. rerio* (zebrafish) zygotes	*egfp, tyr, gol, mitfa, ddx19*	Single and multiple gene(s) knockout	Cas9 mRNA and sgRNA (IVT)	Microinjection	http://dx.doi.org/10.1073/pnas.1308335110
Ansai & Kinoshita. *Biol. Open* 2014	*Oryzias latipes* (Japanese medaka) zygotes	*park7*	Single gene knockout	Cas9 mRNA and sgRNA (IVT)	Microinjection	http://dx.doi.org/10.1242/bio.20148177
Nakayama *et al. Genesis* 2013	*Xenopus* zygotes	*tyr, six3*	Single gene knockout	Cas9 mRNA and sgRNA (IVT)	Microinjection	http://dx.doi.org/10.1002/dvg.22720
Abu-Bonsrah *et al. Sci. Rep.* 2016	*Galus galus* (chicken) embryos	*DGCR8*	Single gene knockout	Cas9 and sgRNA plasmids	Microinjection, electroporation	http://dx.doi.org/10.1038/srep34524
Yang *et al. Cell* 2013	Mouse zygotes	*Tet1-loxP + Tet2-loxP, Sox2-V5, Nanog-mCherry, Oct4-GFP*	HDR/knock-in of reporter or epitope tag with ssODN or dsDNA	Cas9 mRNA and sgRNA (IVT)	Microinjection	http://dx.doi.org/10.1016/j.cell.2013.08.022
Han *et al. RNA Biol.* 2014	Mouse zygotes	*Rian*	Chromosomal deletion with two sgRNAs	Cas9 mRNA and sgRNA (IVT)	Microinjection	http://dx.doi.org/10.4161/rna.29624
Platt *et al. Cell* 2014	Mouse brain and lungs	*Rbfox3, Kras + p53 + Stk11*	Single gene knockout, Kras G12D gene conversion with donor DNA	Cas9 tg-mouse, AAV-sgRNA	Stereotatic injection, intranasal, intratracheal instillation	http://dx.doi.org/10.1016/j.cell.2014.09.014

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Aida *et al.* *Genome Biol.* 2015	Mouse zygotes	*Actb*	HDR/knock-in of reporter with plasmid donor	Cas9 protein and synthetic crRNA:tracrRNA	Microinjection	http://dx.doi.org/10.1186/s13059-015-0653-x
Chen *et al.* *Cell* 2015	Mouse non-small-cell lung cancer cell line	Genes driving tumor growth and metastasis	*In vivo* screening	Cas9 and sgRNA stable integration	Lentiviral particles	http://dx.doi.org/10.1016/j.cell.2015.02.038
Dow *et al. Nat. Biotechnol.* 2015	Mouse ES cells	*Apc, Trp53, chrm. 8* nongenic region	Inducible single and multiple genes knockout	Cas9 and sgRNA tg-mouse	ESC transfection, transgenic mouse produced by blastocyst injection	http://dx.doi.org/10.1038/nbt.3155
Lupiáñez *et al.* *Cell* 2015	Mouse ES cells	*Epha4* TAD	Chromosomal deletion/inversion with two sgRNAs	Cas9 and sgRNA plasmids	ESC transfection, transgenic mouse produced by tetraploid complementation	http://dx.doi.org/10.1016/j.cell.2015.04.004
Wu *et al.* *Cell Res.* 2015	Mouse spermatogonial stem cells (SSC)	*Crygc*	Single gene knockout, HDR/gene correction with ssODN	Cas9 and sgRNA plasmids	SSC electroporation, transgenic mouse produced by SSC transplantation	http://dx.doi.org/10.1038/cr.2014.160
Long *et al.* Science 2016	Mouse cardiac and skeletal muscle	*Dmd*	Exon skipping	Cas9 and sgRNA AAVs	Intramuscular, intraperitoneal and retro-orbital injection	http://dx.doi.org/10.1126/science.aad5725
Nelson *et al.* Science 2016	Mouse cardiac and skeletal muscle	*Dmd*	Exon deletion	Cas9 and sgRNA AAVs	Intramuscular, intraperitoneal and intravenous injection	http://dx.doi.org/10.1126/science.aad5143
Tabebordbar *et al.* Science 2016	Mouse cardiac and skeletal muscle	*Dmd*	Exon deletion	Cas9 and sgRNA AAVs	Intramuscular, intraperitoneal and intravenous injection	http://dx.doi.org/10.1126/science.aad5177
Monteis *et al.* *Mol. Ther.* 2016	Mouse brain	Human *HTT* in tg-mouse	Allele-specific single gene knockout	Cas9 and sgRNA AAVs	Stereotactic injection	http://dx.doi.org/10.1016/j.ymthe.2016.11.010
Mikuni *et al.* *Cell* 2016	Mouse brain	*MeCP2, Actb, Dcx, Rab11a, Fmrp, Arc, Prkca, Cacna1c, Ywhae, Camk2a, Camk2b*	HDR/knock-in of epitope tag with ssODN	Cas9 and sgRNA plasmids	*In utero* electroporation	http://dx.doi.org/10.1016/j.cell.2016.04.044
Hu *et al.* *Cell Res.* 2013	Rat zygotes	*Dusp6, Gata5*	Single gene knockout, chromosomal deletion with 2 sgRNAs	Cas9 mRNA and sgRNA (IVT)	Microinjection	http://dx.doi.org/10.1038/cr.2013.141
Kaneko *et al.* *Sci. Rep.* 2014	Rat zygotes	*Il2rg*	Single gene knockout	Cas9 mRNA and sgRNA (IVT)	Electroporation	http://dx.doi.org/10.1038/srep06382
Wang *et al.* *Sci. Rep.* 2015	Chinese Bama miniature pig zygotes	*Npc1l1*	Single gene knockout	Cas9 mRNA and sgRNA (IVT)	Microinjection	http://dx.doi.org/10.1038/srep08256
Wang *et al. Mol. Ther. Nucleic Acids* 2016	Porcine fetal fibroblasts	*LRRK2*	HDR/knock-in, point mutation with ssODN	Cas9 and sgRNA plasmids	Cell electroporation, transgenic pig produced by somatic cell nuclear transfer	http://dx.doi.org/10.1038/mtna.2016.101
Wang *et al. Sci. Rep.* 2016	Sheep zygotes	*MSTN, ASIP, BCO2*	Single and multiple gene(s) knockout	Cas9 mRNA and sgRNA (IVT)	Microinjection	http://dx.doi.org/10.1038/srep32271
Zou *et al. J. Mol. Cel. Biol.* 2015	Beagle dog zygotes	*MSTN*	Single gene knockout	Cas9 mRNA and sgRNA (IVT)	Microinjection	http://dx.doi.org/10.1093/jmcb/mjv061
Niu *et al. Cell* 2014	Cynomolgus monkey zygotes	*Ppar-g + Rag1*	Multiple genes knockout	Cas9 mRNA and sgRNA (IVT)	Microinjection	http://dx.doi.org/10.1016/j.cell.2014.01.027

Abbreviations: IVT, *in vitro* transcription; HDR, homology-directed repair; ssODN, single-strand oligonucleotide donor; dsDNA, double-strand DNA; tg, transgenic; TAD, topologically associating domain; AAV, adeno-associated virus.

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