RECOMMENDED READING

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INSPIRED CELL SOLUTIONS

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.

Species	Stage/tissue	Reference	Target	Type of editing	Cas9 and guide RNA source	Delivery method	Link
Mouse	Neonatal pups inner ear hair cells	Gal *et al. Nature* 2018	*Tmc1*	Single gene knockout	Cas9 protein and sgRNA (IVT)	Intracochlear injection of cationic lipid complex	http://dx.doi.org/10.1038/nature25164
Mouse	Pre-implantation embryos	Yoon *et al. Nat. Commun.* 2018	*Tyr*	Single gene knockout	Cas9 and sgRNA in rAAV vector	Oviduct injection of rAAV particles	http://dx.doi.org/10.1038/s41467-017-02706-7
Mouse	Brain	Zhou *et al. Nat. Neurosci.* 2018	*Ascl1, Neurog2* and *Neurod1; Dkk1* and *Hbb*	Multiplex transcriptional activation	dCas9 tg-mouse, tandem sgRNAs in rAAV	Stereotactic injection of rAAV particles	http://dx.doi.org/10.1038/s41593-017-0060-6
Mouse	Liver	Jiang *et al. Cell Res.* 2017	*Pcsk9*	Single gene knockout	Cas9 mRNA and sgRNA (IVT)	Intravenous injection of lipid-like nanoparticles (LLNs)	http://dx.doi.org/10.1038/cr.2017.16
Mouse	Zygotes	Lei *et al. Nat. Communic.* 2017	*Igf2/H19* imprinting region	CpG methylation	dCas9-MQ1 and sgRNA plasmids	Microinjection	http://dx.doi.org/10.1038/ncomms16026
Mouse	Muscle, kidney and liver	Liao *et al. Cell* 2017	*Fst, Kl, Il10,* Pdx1, Utrn	Transcriptional activation	Cas9 tg-mouse, truncated gRNA in rAAV vector	Intramuscular, intravenous or intracerebral injection	http://doi.org/10.1016/j.cell.2017.10.025
Mouse	Retina	Latella *et al. Mol. Ther. Nucl Acids* 2016	Human RHO in tg-mouse	Single gene knockout	Cas9 and sgRNA plasmids	*In vivo* electroporation	http://dx.doi.org/10.1038/mtna.2016.92
Mouse	Cardiac and skeletal muscle	Long *et al. Science* 2016	*Dmd*	Exon skipping	Cas9 and sgRNA AAVs	Intramuscular, intraperitoneal and retro-orbital injection	http://dx.doi.org/10.1126/science.aad5725
Mouse	Brain	Mikuni *et al. Cell* 2016	*Mecp2, Actb, Dcx, Rab11a, Fmr1, Arc, Pkca, Cacna1c, Ywhae, Camk2a, Camk2b*	Epitope tag with ssODN	Cas9 and sgRNA plasmids	*In utero* electroporation	http://dx.doi.org/10.1016/j.cell.2016.04.044
Mouse	Brain	Monteis *et al. Mol. Ther.* 2016	*Human HTT in tg-mouse*	Allele-specific single gene knockout	Cas9 and sgRNA plasmids	Stereotactic injection	http://dx.doi.org/10.1016/j.ymthe.2016.11.010

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Mouse	Cardiac and skeletal muscle	Nelson *et al. Science* 2016	*Dmd*	Exon deletion	Cas9 and sgRNA AAVs	Intramuscular, intraperitoneal and intravenous injection	http://dx.doi.org/10.1126/science.aad5143
Mouse	Cardiac and skeletal muscle	Tabebordbar *et al. Science* 2016	*Dmd*	Exon deletion	Cas9 and sgRNA AAVs	Intramuscular, intraperitoneal and intravenous injection	http://dx.doi.org/10.1126/science.aad5177
Mouse	Liver	Yin *et al. Nat. Biotechnol.* 2016	*Fah*	Gene correction with rAAV vector	Cas9 mRNA/lipid nanoparticles, sgRNA + HDR template in rAAV vector	Intravenous injection	http://dx.doi.org/10.1038/nbt.3471
Mouse	Zygotes	Aida *et al. Genome Biol.* 2015	*Actb*	Reporter knock-in with plasmi donor	Cas9 protein, synthetic crRNA: tracrRNA	Microinjection	http://dx.doi.org/10.1186/s13059-015-0653-x
Mouse	Non-small-cell lung cancer cell line	Chen *et al. Cell* 2015	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
Mouse	Zygotes	Han *et al. RNA Biol.* 2014	*Rian*	Chromosomal deletion with two sgRNAs	Cas9 mRNA and sgRNA (IVT)	Microinjection	http://dx.doi.org/10.4161/rna.29624
Mouse	Lungs	Maddalo *et al. Nature* 2014	*Eml4-Alk*	Chromosomal rearrangement	Cas9 and two sgRNAs in adenoviral vector	Intratracheal instillation	http://dx.doi.org/10.1038/nature13902
Mouse	Zygotes	Yang *et al. Cell* 2013	*Tet1-loxP* + *Tet2-loxP, Sox2-V5,* Nanog-mCherry, *Oct4-GFP*	Reporter knock-in, epitope tag with ssODN or dsDNA	Cas9 mRNA and sgRNA (IVT)	Microinjection	http://dx.doi.org/10.1016/j.cell.2013.08.022
Rat	Retina	Suzuki *et al. Nature* 2016	*Mertk*	Gene correction by NHEJ-mediated targeted integration	Cas9, sgRNA and donor template in rAAV vector	Subretinal injection	http://dx.doi.org/10.1038/nature20565
Rat	Zygotes	Kaneko *et al. Sci. Rep.* 2014	*Il2rg*	Single gene knockout	Cas9 mRNA and sgRNA (IVT)	Electroporation	http://dx.doi.org/10.1038/srep06382
Rat	Zygotes	Yoshimi *et al. Nat. Commun.* 2014	*Tyr, Asip, Kit*	Gene correction with ssODN	Cas9 mRNA and sgRNA (IVT)	Microinjection	http://dx.doi.org/10.1038/ncomms5240
Rat	Zygotes	Hu *et al. Cell Res.* 2013	*Dusp6, Gata5*	Single gene knock-out, chromosomal deletion with 2 sgRNAs	Cas9 mRNA and sgRNA (IVT)	Microinjection	http://dx.doi.org/10.1038/cr.2013.141
Cynomolgus monkey	Zygotes	Niu *et al. Cell* 2014	*PPARG* + *RAG1*	Multiple genes knockout	Cas9 mRNA and sgRNA (IVT)	Microinjection	http://dx.doi.org/10.1016/j.cell.2014.01.027
Dog (beagle)	Zygotes	Zou *et al. J. Mol. Cel. Biol.* 2015	*MSTN*	Single gene knockout	Cas9 mRNA and sgRNA (IVT)	Microinjection	http://dx.doi.org/10.1093/jmcb/mjv061
Cattle	Fetal fibroblasts	Ikeda *et al. Sci. Rep.* 2017	*IARS*	Gene correction with dsDNA fragment	Cas9 and sgRNA plasmids	Cell electroporation, transgenic embryos produced by SCNT	http://dx.doi.org/10.1038/s41598-017-17968-w
Cattle	Fetal fibroblasts	Gao *et al. Gen. Biol.* 2017	*SLC11A1*	Gene insertion with Cas9 nickase and plasmid donor	Cas9n and sgRNA plasmid	Cell electroporation, transgenic embryos produced by SCNT	http://dx.doi.org/10.1186/s13059-016-1144-4
Pig	Fetal fibroblasts	Wang *et al. Mol. Ther. Nucl. Acids* 2016	*APP, LRRK2*	Gene mutation with ssODN	Cas9 and sgRNA plasmids	Cell electroporation, transgenic pig produced by SCNT	http://dx.doi.org/10.1038/mtna.2016.101
Chinese Bama miniature pig	Zygotes	Wang *et al. Sci. Rep.* 2015	*NPC1L1*	Single gene knockout	Cas9 mRNA and sgRNA (IVT)	Microinjection	http://dx.doi.org/10.1038/srep08256
Goat	Zygote	Wang *et al. Sci. Rep.* 2015	*MSTN* and *FGF5*	Multiple genes knockout	Cas9 mRNA and sgRNA (IVT)	Microinjection	http://dx.doi.org/10.1038/srep13878
Sheep	Zygotes	Wang *et al. Sci. Rep.* 2016	*MSTN, ASIP, BCO2*	Single and multiple genes knockout	Cas9 mRNA and sgRNA (IVT)	Microinjection	http://dx.doi.org/10.1038/srep32271
Rabbit	Zygotes	Song *et al. Cell. Mol. Life Sci.* 2016	*TYR*	Gene deletion with two gRNAs	Cas9 mRNA and sgRNA (IVT)	Microinjection	http://dx.doi.org/10.1007/s00018-016-2143-z

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Rabbit	Zygotes	Jiang *et al. Sci. Rep.* 2018	*ATP7B*	Gene mutation with ssODN	Cas9 mRNA and sgRNA (IVT)	Microinjection	http://dx.doi.org/10.1038/s41598-018-19774-4
Ferret *(Mustela putorius furo)*	Zygotes	Kou *et al. Cell Res.* 2015	*DCX, ASPM* and *DISC1*	Single gene knockout	Cas9 mRNA and sgRNA (IVT)	Microinjection	http://dx.doi.org/10.1038/cr.2015.130
Chicken *(Galus galus)*	Embryos	Abu-Bonsrah *et al. Sci. Rep.* 2016	*DGCR8*	Single gene knockout	Cas9 and s gRNA plasmid	Microinjection, electroporation	http://dx.doi.org/10.1038/srep34524
Japanese medaka *(Oryzias latipes)*	Zygotes	Ansai & Kinoshita. *Biol.* Open 2014	*park7*	Single gene knockout	Cas9 mRNA and sgRNA (IVT)	Microinjection	http://dx.doi.org/10.1242/bio.20148177
Zebrafish *(Danio rerio)*	Zygotes	Chang *et al. Cell Res.* 2013	*etsrp, gata5, gata4*	Single gene knockout, sequence knock-in with ssODN	Cas9 mRNA and sgRNA (IVT)	Microinjection	http://dx.doi.org/10.1038/cr.2013.45
Zebrafish *(D. rerio)*	Zygotes	Hwang *et al. Nat. Biotechnol.* 2013	*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
Zebrafish *(D. rerio)*	Zygotes	Jao *et al. PNAS* 2013	*egfp, tyr, gol, mitfa, ddx19*	Single and multiple genes knockout	Cas9 mRNA and sgRNA (IVT)	Microinjection	http://dx.doi.org/10.1073/pnas.1308335110
Frog *(Xenopus)*	Zygotes	Nakayama *et al. Genesis* 2013	*tyr, six3*	Single gene knockout	Cas9 mRNA and sgRNA (IVT)	Microinjection	http://dx.doi.org/10.1002/dvg.22720
Silkworm *(Bombyx mori)*	Preblastoderm embryos	Wang *et al. Cell Res* 2013	*BLOS2*	Single gene knockout	Cas9 mRNA and sgRNA (IVT)	Microinjection	http://dx.doi.org/10.1038/cr.2013.146
Silkworm *(B. mori)*	Eggs	Ma *et al. Sci Rep* 2014	*BmKu70*	Single gene knockout	Cas9 and sgRNA plasmids	Microinjection	http://dx.doi.org/10.1038/srep04489
Butterfly *(Papilio xhutus)*	Eggs	Li *et al. Sci Rep* 2015	*Abd-B, ebony, frizzled*	Single gene knockout	Cas9 mRNA and sgRNA (IVT)	Microinjection	http://dx.doi.org/10.1038/ncomms9212
Drosophila	Lethal at pupal stage	Lin *et al. Genetics* 2015	*Wg*	Transcriptional activation	dCas9-VPR and sgRNA tg-flies	Cross of transgenics flies	http://dx.doi.org/10.1534/genetics.115.181065
Drosophila	Preblastoderm embryos	Bassett *et al. Cell Rep.* 2013	*yellow, white*	Single gene knockout	Cas9 mRNA and sgRNA (IVT)	Microinjection	http://dx.doi.org/10.1016/j.celrep.2013.06.020
Mosquito *(Anopheles stephensi)*	Zygotes	Gantz *et al. PNAS* 2015	*kh*	Gene drive	Cas9 protein + plasmid expressing Cas9, sgRNA and gene drive	Microinjection	http://doi.org/10.1073/pnas.1521077112
Mosquito *(A. gambiae)*	Zygotes	Hammond *et al. Nat Biotechnol* 2016	*AGAP005958, AGAP007280, AGAP011377*	Gene drive	Cas9, sgRNA and gene drive plasmids	Microinjection	http://dx.doi.org/10.1038/nbt.3439
Mosquito *(Aedes aegypti)*	Preblastoderm embryos	Kistler *et al. Cell Rep.* 2015	*AAEL010779, AAEL004091, AAEL000926, AAEL014228, AAEL002575, AAEL013647, Aaeg-wtrw*	Gene knockout with ssODN, chromosomal deletion with two sgRNAs, reporter knock-in with plasmid donor	Cas9 protein or mRNA and sgRNA	Microinjection	http://dx.doi.org/10.1016/j.celrep.2015.03.009
Caenorhabditis elegans	Gonads	Paix *et al. Nucl. Acids Res.* 2016	*dpy-10, gtbp-1, meg-3*	Reporter knock-in with ssODN or dsDNA, gene replacement	Cas9 protein, synthetic crRNA:tracrRNA (Dharmacon)	Microinjection	http://dx.doi.org/10.1093/nar/gkw502
C. elegans	Gonads	Friedland *et al. Nat.* Methods 2013	*unc-119, dpy-13, klp-12, Y61A9LA.1*	Single gene knockout	Cas9 and sgRNA plasmids	Microinjection	http://dx.doi.org/10.1038/nmeth.2532

Abbreviations: IVT, *in vitro* transcription; HDR, homology-directed repair; ssODN, single-strand oligonucleotide donor; dsDNA, double-strand DNA; tg, transgenic; rAAV, recombinant adeno-associated virus; SCNT, somatic cell nuclear transfer.

If you have any questions

t +44 (0) 1223 976 000 (UK) or +1 800 235 9880 (USA); +1 303 604 9499 (USA)
f + 44 (0)1223 655 581
w horizondiscovery.com/contact-us **or** dharmacon.horizondiscovery.com/service-and-support
Horizon Discovery, 8100 Cambridge Research Park, Waterbeach, Cambridge, CB25 9TL, United Kingdom

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