

Materials List for

Zygote Microinjection for Creating Gene Cassette Knock-in and Flox Alleles in Mice

Yoko Tanimoto^{*1}, Natsuki Mikami^{*1,2}, Miyuki Ishida¹, Natsumi Iki¹, Kanako Kato¹, Fumihiro Sugiyama¹, Satoru Takahashi¹, Seiya Mizuno¹

¹Laboratory Animal Resource Center and Trans-Border Medical Research Center, University of Tsukuba

²Ph.D. Program in Human Biology, School of Integrative and Global Majors, University of Tsukuba

*These authors contributed equally

Correspondence to: Seiya Mizuno at konezumi@md.tsukuba.ac.jp

URL: <https://www.jove.com/video/64161>

DOI: [doi:10.3791/64161](https://doi.org/10.3791/64161)

Materials

Name	Company	Catalog Number	Comments
Atipamezole Hydrochloride	ZENOAG	-	
Autoclip Wound Clip	BD	427631	
Autoclip Wound Clip Applier	BD	427630	
Butorphanol	Meiji Seika Pharma	-	
C57BL/6J mice	Jackson Laboratory Japan	-	older than 10 weeks old
Calibrated Pipet, 100ul	Drummond Scientific Company	2-000-100	for collecting zygote and embryo transfer
Cas9	Thermo Fisher Scientific	A36499	
Cas9 Nuclease Reaction Buffer	NEB	B7203	
CellTram 4r oil	Eppendorf	5196000030	
CRISPOR		http://crispor.tefor.net/	web tool for genome editing experiments with the CRISPR-Cas9 system
crRNA	IDT	-	
Gel/PCR Extraction Kit	FastGene	FG-91302	
hCG	ASKA Animal Health	-	
Hyaluronidase	Merck Sigma-Aldrich	H3884	
ICR mice	Jackson Laboratory Japan	-	older than 10 weeks old, weight 28 G or more
Inverted microscope	Leica		
KOnezumi		https://www.md.tsukuba.ac.jp/LabAnimalResCNT/KOanimals/konezumi.html	a web application for automating gene disruption strategies to generate knockout mice
M16 medium	Merck Sigma-Aldrich	M7292	
M2 medium	Merck Sigma-Aldrich	M7167	
Medetomidine	ZENOAG	-	
Micro shears	Natsume Seisakusho	MB-54-1	
Microforge	Narishige	MF-900	for fire polishing of holding pipette and bending the microinjection needle
Micromanipulator units	Narishige		
Micropipette puller	Sutter Instrument	P-1000	programmable pipette puller
Midazolam	Maruishi Pharmaceutical	-	
MILLEX-GV 0.22 µm filter	Merck Millipore	SLGV033R	

Mineral oil	Nacalai	26114-75	for zygote culture and injection chamber
Petri dish (35mm, untreated)	Iwaki	1000-035	for zygote culture
PIEZO micromanipulator	PRIME TECH	PMM-150	
Plasmid Mini Kit	FastGene	FG-90502	mini prep spin column kit
PMM Operation Liquid	PRIME TECH	KIT-A	operation liquid for microinjection
PMSG	ASKA Animal Health	-	
Polyvinylpyrrolidone	Merck Sigma-Aldrich	P5288	
RNase	QIAGEN	19101	
RNase Free Water	IDT	-	tracrRNA Accessory Reagents
Serrefine clamp	Natsume Seisakusho	C-18	
Sodium dodecyl sulfate	SIGMA	151-21-3	
Suture needle with thread	Natsume Seisakusho	C11-60B2	
Thin wall borosilicate glass without filament	Sutter Instrument	B100-75-10	for microinjection needle and holding pipette
tracrRNA	IDT	-	