Supplementary Information - Improving laboratory animal genetic reporting: LAG-R guidelines

Supplementary Tables

Supplementary Table 1: Examples of the use of LAG-R recommendations for a rodent model and a zebrafish model

Two examples are provided. The first one, in mouse, is based on Mianne *et al*¹. The second one, in zebrafish, is based on Hayot *et al*².

CRITERIA TO REPORT ON FOR ALL RESEARCH ANIMALS ¹			EXAMPLE IN MOUSE	EXAMPLE IN ZEBRAFISH	
		Official name of species, strain, and sub-strain, as applicable,			
		of the animal. Alternatively, for farm animals, indicate breed			
		https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwta			
		x.cgi.		Danio rerio, AB strain	
Genetic background		Describe type of breeding for strain/stock/breed using	Mus musculus, C57BL/6NTac substrain	temperature dark-light cycle water chemistry and fish	
description including	1	species standard wording.	Mutagenized embryos are described in Materials and	feed and feeding frequency are described in Materials	
Strain/breed/stock type		For example, in rodent: outbred, inbred, hybrid, congenic ² ,	Methods. Stock used for germ line transmission are	and Methods. Mutant zebrafish line and origin of	
		documented mixed or non-documented genetic background.		wildtype line utilized to maintain genetic quality of the	
		For example, in zebrafish: specify which wildtype		colony are described in Materials and Methods.	
		background (AB, TU, TL); if mixed background, provide a			
		clear explanation of the breeding scheme and estimation of			
		Specify breeding schemes used to maintain stock and			
		generate experimental animals. Include the genotype of the			
Breeding scheme and		parents when possible. This is particularly important to trace	Heterozygous <i>Cdh23^{em1H}</i> mice were backcrossed onto	Heterozygous mutant zebrafish were backcrossed onto	
stability program (only	2	the origin of sex chromosomes in congenic strains ² .	C57BL/6NTac.	AB strain obtained from the European Zebrafish	
		Specify breeding strategy to maintain genetic quality of the		Resource Center (EZRC, Stock number #24433).	
		colony; indicate known family tree.			
Source of animals	3	Name origin of strain(s). Name supplier or repository or	The Mary I yon Centre at MRC Harwell	F7RC	
	J	other origin of animals used in the experiment.			
International nomenclature		Name strain according to internationally agreed standard			
	4	when available. ³	C57BL/6NTac-Cdh23 ^{em1H} /H	<i>chd8</i> ^{sa19827} (TL)	
		Use research resource identifier (RRID) when applicable.			
Strain or stock identifier	5	Show unique identifier of strain or stock used by the supplier	EM:11508	EZRC#24433	
······································	•	or the repository.			

Genetic background validation	6	Indicate if, when (at what breeding generation) and how the genetic background was verified (i.e., sequencing, SNP (single- nucleotide polymorphism) panel, STR panel, genetic testing chip panel).	F1 animals were verified using the SNP panel described in Supplementary Table 7 (in this document, not Supplemental from initial paper)	N/A
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ADDITIONAL CRITERIA TO REPORT ON FOR ANIMALS WITH GENETIC				ΕΧΔΜΡΙ Ε IN 7EBRAFISH	
ALTERATION ¹					
Name of mutant allele	7	Detail the shorthand used in article, and official nomenclature. ⁴ Use unique identifier (e.g. MGI ID) when applicable.	Shorthand in the publication is <i>Cdh23</i> ^{753A>G} ; MGI: 5661070; official nomenclature is <i>Cdh23</i> ^{ahl+em1H}	Shorthand in the publication is <i>chd8^{c667T}</i> ; EZRC: #24433; ID: ZDB-ALT-160601-32; official nomenclature is <i>chd8^{sa19827}</i>	
Allele type	8	Specify the method of model creation: naturally occurring allele/gene targeting/genome editing/additive transgenesis/chemical or physical mutagenesis/viral insertion/site-specific recombination/transposition.	Genome editing in one cell stage embryo. Materials & Methods describes in detail how the genome editing experiments were performed.	Adult males treated with ENU	
Intended and observed consequence of mutagenesis	9	Detail whether allele is a frameshift, deletion, coding or non- coding variant, overexpression, conditional allele, humanization, reporter, structural variation. Detail new gene product if known.	This mutation reinforces the splice donor sequence mutated in C57BL/6N mice.	The mutation leads to a premature stop codon.	
Model summary description	10	Provide a short summary of genetic modification and background used for establishing genetic alteration.	A short summary as provided by MGI. The CRISPR/Cas9 system was used to correct the <u>Cdh23^{ahl}</u> allele (c.753A>G). This engineered point mutation mimics the C variant of SNP rs257098870 (G on negative gene strand) that is found in the 129S1/SvImJ, C3H/HeSnJ, I/LnJ, YBR/Ei, and MRL/MpJ strains. It restores the G-GT splice donor site that has changed to A-GT in C57BL/6 (and most other strains), where it causes skipping of in- frame exon 7. (<i>J:90559, J:236297</i>). Mutation was introduced in C57BL/6NTac mice.	Adult males from TL background were treated with ENU in the Stemple Lab. The mutant line carries the point mutation c.C667T (p.Glu223*) at position: Chr 2: 38115067 (GRC211). The mutation leads to a premature stop. Information on the mutant line can be found on ZFIN at <u>http://zfin.org/ZDB-ALT-160601-32#summary</u> .	
DNA sequence	11	Provide access to the sequence of the genetic modification: targeting vector, donor template or vector for transgenesis. If employed in the mutagenesis process, provide the sequence of donor (e.g., targeting vector, oligodeoxynucleotide, transgene or template sequence used for mutagenesis; DNA or prime editing guide). ⁵ Annotate genomic sequences with corresponding genome assembly version and coordinates. Use universal format; i.e., .fasta or .gb. Annotate features.	The sequence of the genetic modification corresponds to the sequence of the donor template. Sequence of one representative F1 animal is presented in Figure 2. Supplementary Table S1 contains the sequences of the donor.	Position of the mutation and annotation can be found on ZFIN at <u>http://zfin.org/ZDB-ALT-160601- 32#summary</u> .	
Allele schematic	12	Consider presenting a map of the genetic modification.	Described in Figure 1.	Available on ZFIN. <u>http://zfin.org/ZDB-ALT-160601-</u> <u>32#summary</u> .	
Material availability/source of materials	13	Describe how to access available materials (plasmids, mutant cells, animals and/or germplasm). RRID and/or repository identifier.	Animals and/or germplasm is accessible through the Infrafrontier repository; repository identifier: EM:11508. Plasmids are available through contacting MRC Harwell.	The mutant line is available at EZRC (#24433) and ZIRC (Catalog ID. ZL12033.03)	
Obvious phenotype and welfare concern	14	Specify salient phenotypes, such as issues with viability and/or fertility, or immunodeficiency. Describe the severity	Age-related hearing loss is corrected by the mutations. No welfare issues are associated with this mutation.	All fish lines reproduce normally, andchd8 homozygote mutants were recovered in the expected Mendelian	

		of the associated phenotype. If necessary, include any		ratio. A skewed sex ratio was observed with almost only
		requirement to mitigate welfare concerns. Include		males.
		publication, archive or database reference if available.		
		For mice, consider SHIRPA (SmithKline Beecham/Harwell/Imperial College/Royal London		
		Hospital/phenotype assessment) description (supp ref 3).		
Initial reference	15	Detail whether report is the initial description of mutant and/or mention initial publication of materials.	This paper is the initial reference. PMID: 26876963	This paper is the initial reference. PMID: 36375841
Genotyping assay	16	Describe assay and sequence of primers used for genotyping of established colony.	The animals from the established colony are genotyped using an allelic discrimination assay run according to Kamau et al., 2012) with the following primers: Cdh23 F Primer: GACATGGATCCTATCTTCATCAACC Cdh23 R Primer: CCAGCACGGGCTAGAGAAC Wild-type Allele probe (FAM-Labelled): CACTCTCCTCCC <u>A</u> GTGAG Mutant Allele probe (TET-Labelled): CTCCTCC <u>G</u> GTGAGC	The animals were genotyped as described in Materials and Methods and by utilizing the following primers: Chd8 F primer GTCAGACTCAAGTGCTGCAG Chd8 R primer GACACTTTGGTCGGAT The PCR product was digested by the <i>Rsal</i> enzyme, a restriction enzyme whose restriction site is disrupted by the sa19827 mutation.
Enzyme and other reagents used for genome engineering	17	Describe enzymes (nuclease, recombinase) if used to generate mutation including number and sequence of guide(s) for ribonucleoproteins if relevant. Detail reagents. ⁶	Materials & Methods describes the genome editing reagents used to generate the model including the origin of Cas9 sequence used. Supplementary Table S1 contains the sequences of the guides.	N/A
Validation of allele sequence	18	If done, describe how the region of interest was validated. ⁷	See Figure 2 and Results.	N/A
Validation of allele structure	19	If done, describe the precise method used for validation of chromosomal or allele structure, and the outcome. ⁷	This experiment was not performed. The depth of whole genome sequencing performed did not allow estimation of the structural variation.	N/A
Validation to exclude additional integration of mobilized sequence	20	If done, describe the method and outcome of analyzing the material for additional integrations of donor templates ⁵ or reintegration of deleted segments. ⁷	Copy counting of ssODN is described in Materials & Methods. Whole genome sequencing is described in the Results section.	N/A
Evaluation of potential off-target activity	21	Genome editing off-target is defined as a genomic position and/or nucleic acid sequence distinct from the target. If done, describe the method, selection criteria and outcome of off- target analysis.	Off-target analysis is described in the Results and Supplemental sections.	N/A

Note 1. Essential criteria are indicated in bold; recommended criteria are indicated in italic. The information itself, or a reference to a source, should be detailed.

Note 2. Congenic strains are examples of the importance of correct breeding scheme, as their genetic composition varies according to the parental origin of the sex chromosomes and the mitochondrial genome.

In addition, the identity of the region around a transmitted allele remains that of the original strain.

Note 3. Definitions and guidelines for nomenclature of mouse and rat strains are described at https://www.informatics.jax.org/mgihome/nomen/strains.shtml

Note 4. Guidelines for Nomenclature of Genes, Genetic Markers, Alleles, and Mutations in Mouse and Rat are described at https://www.informatics.jax.org/mgihome/nomen/gene.shtml.

Note 5. Note that donor sequence can differ from mutagenesis outcome.

Note 6. Some recommendations for genome-editing formulations are reported in supp ref 4.

Note 7. If not done, indicate that this assay was not performed.

Supplementary Table 2: LAG-R checklist for reviewers

CRITERIA TO REPORT ON FOR ALL RESEARCH ANIMALS ¹ check				
Genetic background description including Strain/breed/stock type	1	Official name of species, strain, and sub-strain, as applicable, of the animal. Alternatively,forfarmanimals,indicatehttps://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi.Describe type of breeding for strain/stock/breed using species standard wording.For example, in rodent: outbred, inbred, hybrid, congenic², documented mixed or non-documented genetic background. For example, in zebrafish: specify which wildtypebackground (AB, TU, TL); if mixed background, provide a clear explanation of thebreeding scheme and estimation of percentage of each background.		
Breeding scheme and stability program (only relevant for rodents) ³	2	Specify breeding schemes used to maintain stock and generate experimental animals. Include the genotype of the parents when possible. This is particularly important to trace the origin of sex chromosomes in congenic strains ² . Specify breeding strategy to maintain genetic quality of the colony; indicate known family tree.		
Source of animals	3	Name origin of strain(s). Name supplier or repository or other origin of animals used in the experiment.		
International nomenclature	4	Name strain according to internationally agreed standard when available. ³ Use research resource identifier (RRID) when applicable.		
Strain or stock identifier	5	Show unique identifier of strain or stock used by the supplier or the repository.		
Genetic background validation	6	Indicate if, when (at what breeding generation) and how the genetic background was verified (i.e., sequencing, SNP (single-nucleotide polymorphism) panel, STR panel, genetic testing chip panel).		
ADDITIONAL CRITERIA TO I	REPC	ORT ON FOR ANIMALS WITH GENETIC ALTERATION ¹		
Name of mutant allele		Detail the shorthand used in article, and official nomenclature. ⁴ Use unique identifier (e.g. MGI ID) when applicable.		
Allele type	8	Specify the method of model creation: naturally occurring allele/gene targeting/genome editing/additive transgenesis/chemical or physical mutagenesis/viral insertion/site-specific recombination/transposition.		
Intended and observed consequence of mutagenesis	9	Detail whether allele is a frameshift, deletion, coding or non-coding variant, overexpression, conditional allele, humanization, reporter, structural variation. Detail new gene product if known.		
Model summary description	10	Provide a short summary of genetic modification and background used for establishing genetic alteration.		
DNA sequence	11	Provide access to the sequence of the genetic modification: targeting vector, donor template or vector for transgenesis. If employed in the mutagenesis process, provide the sequence of donor (e.g., targeting vector, oligodeoxynucleotide, transgene or template sequence used for mutagenesis; DNA or prime editing guide). ⁵ Annotate genomic sequences with corresponding genome assembly version and coordinates. Use universal format; i.e., .fasta or .gb. Annotate features.		
Allele schematic	12	Consider presenting a map of the genetic modification.		
Material availability/source of materials	13	Describe how to access available materials (plasmids, mutant cells, animals and/or germplasm). RRID and/or repository identifier.		
Obvious phenotype and welfare concern	14	Specify salient phenotypes, such as issues with viability and/or fertility, orimmunodeficiency. Describe the severity of the associated phenotype. If necessary, includeany requirement to mitigate welfare concerns. Include publication, archive or databasereference if available.For mice, consider SHIRPA (SmithKline Beecham/Harwell/Imperial College/Royal LondonHospital/phenotype assessment) description (supp ref 3).Detail whether report is the initial description of mutant and/or mention initial		
initial reference	15	publication of materials.		

Genotyping assay	16	Describe assay and sequence of primers used for genotyping of established colony.	
Enzyme and other reagents used for genome engineering	17	Describe enzymes (nuclease, recombinase) if used to generate mutation including number and sequence of guide(s) for ribonucleoproteins if relevant. Detail reagents. ⁶	
Validation of allele sequence	18	If done, describe how the region of interest was validated. ⁷	
Validation of allele structure		If done, describe the precise method used for validation of chromosomal or allele structure, and the outcome. ⁷	
Validation to exclude additional integration of mobilized sequence	20	If done, describe the method and outcome of analyzing the material for additional integrations of donor templates ⁴ or reintegration of deleted segments. ⁷	
Evaluation of potential off- target activity	21	Genome editing off-target is defined as a genomic position and/or nucleic acid sequence distinct from the target. If done, describe the method, selection criteria and outcome of off-target analysis.	

Note 1. Essential criteria are indicated in bold; recommended criteria are indicated in italic. The information itself, or a reference to a source, should be detailed.

Note 2. Congenic strains are examples of the importance of correct breeding scheme, as their genetic composition varies according to the parental origin of the sex chromosomes and the mitochondrial genome. In addition, the identity of the region around a transmitted allele remains that of the original strain.

Note 3. Definitions and guidelines for nomenclature of mouse and rat strains are described at

https://www.informatics.jax.org/mgihome/nomen/strains.shtml

Note 4. Guidelines for Nomenclature of Genes, Genetic Markers, Alleles, and Mutations in Mouse and Rat are described at https://www.informatics.jax.org/mgihome/nomen/gene.shtml.

Note 5. Note that donor sequence can differ from mutagenesis outcome.

Note 6. Some recommendations for genome editing formulations are reported in supp ref 4.

Note 7. If not done, indicate that this assay was not performed.

Supplementary Table 3: Examples of techniques for the analysis of genetic background

Techniques	Limitations	Reference	
SND DCD/gDCD papel	Availability of a panel for		
Sive PCR/YPCR parler	the species/strain	5,0	
Short tandem repeat (STR),			
simple sequence length	Availability of a panel for	7_11	
polymorphism (SSLP),	the species/strain	7-11	
microsatellites			
Chip panel		5,12	
		Obvious traits relevant to the	
Overt phonetype		species/strain/sub-	
Overt prienotype		strain/breed/stock (e.g. coat	
		color) 13	
Short and long read WGS	May be complex in outbred	14	
(high depth)	context	14	

Supplementary Table 4: Examples of methods for evaluation of targeted allele quality (sequence and structure)

Methods for allele sequence

PCR followed by Sanger sequencing

PCR; subcloning followed by sequencing

PCR followed by NGS¹

PCR for long amplicons and targeted NGS sequencing

DNA enrichment (e.g., Samplix capture, Cas9 capture, adaptive sampling) with deep short- or long-read NGS sequencing²

Methods for allele structure

Southern blot

qPCR, dPCR

Microarray

Targeted NGS sequencing of long amplicons¹

DNA enrichment (e.g., inverse PCR-based techniques such as Targeted Locus

Amplification capture, Samplix, Cas9 capture, adaptive sampling) with long-read sequencing

FISH

Fiber-FISH

Note 1. Limited by PCR amplicon size.

Note 2. Depends on experimental design and read depth.

Supplementary Table 5: Examples of methods for evaluation of offtarget genetic changes

Additional insertion of	Structural alterations	Discrete sequence
donors ¹		alterations
Southern blot	Southern blot	PCR & Sanger sequencing ²
dPCR and qPCR	Optical genome mapping	High-throughput NGS
		analysis of PCR amplicons
		from algorithm-predicted
		off-target loci
Plasmid backbone PCR	G-banding	NGS WGS (high depth)
TLA and other inverse	FISH	
PCR based methods		
DNA capture (e.g.	Fiber-FISH	
Samplix capture, nCATS)		
followed by deep short		
or long read NGS		
sequencing ³		
Chip panel	Short- and long-read WGS	
	(high depth)	

Note 1. and other mobilized elements (e.g., deleted fragments)

Note 2. List of sites at higher risk for unintended edits can be bioinformatically estimated or experimentally estimated by a capture method (e.g., GUIDE-seq, CIRCLE-seq, LAM–HTGTS, UDiTaS[™], Digenome-seq CHANGE-seq, DISCOVER-seq.)

Note 3. Depends on experimental design and read depth.

Supplementary Table 6: Examples of support for nomenclature relating to laboratory animals (Metazoa clade)

Resource	Acronym	Species	Relevant website and resource	
International Commission on		Animals	https://www.iczn.org/	
Zoological Nomenclature		Animais		
Anolis Gene Nomenclature		Anolis	15	
Committee	AGINC	carolinensis		
National Association of Animal	ΝΑΑΡ	Pos tourus	https://www.naab-css.org/uniform-	
breeders	NAAD	BOS LUUIUS	breed-codes	
Official Cattle Breeds and codes	DCMS	Postaurus	https://www.gov.uk/guidance/official-	
(British Cattle Movement Services)	DCIVIS	BOS LUUIUS	cattle-breeds-and-codes	
WormBasa	N/A	Caenorhabditis	https://wiki.wormbase.org/index.php/No	
Wonnbase	N/A	elegans	<u>menclature</u>	
Zohrafish Nomonclature Committee	ZNC	Dania raria	https://zfin.org/zf info/news/committee	
	ZINC	Dunio reno	<u>s.html</u>	
FlyBase	N/A	Drosophila	https://wiki.flybase.org/wiki/FlyBase:No	
Tybase		melanogaster	<u>menclature</u>	
Chicken Gene Nomenclature	CGNC	Gallus gallus	16	
Committee	CONC	domesticus		
International Committee on			https://www.informatics.jay.org/mgihom	
Standardized Genetic Nomenclature	ICSGNM	Mus musculus	e/nomen/	
for Mice				
Rat Genome Nomenclature	RGNC	Rattus	https://rgd.mcw.edu/nomen/nomen.sht	
Committee		norvegicus	<u>ml</u>	
Xenopus Nomenclature Committee	XNC	Xenopus sp.	https://www.xenbase.org	
Breeds of Livestock	N/A	N/A	https://breeds.okstate.edu/	

Supplementary Table 7: Genetic background quality control for line C57BL/6NTac-Cdh23^{em1H}/H

Eight allelic discrimination assays that discriminate the C57BL/6NTac from the C57BL/6J backgrounds were run according to Kamau *et al* ¹⁷. All probes contained a 3' BHQ-1 plus modification.

			C57BL/6NTac		C57BL/6J	
Assay name	Forward sequence	Reverse primer sequence	Probe 1 5' modification	Probe 1 sequence	Probe 2 5' modification	Probe 2 sequence
4_28.49	CTGAAGGTCTCCATTGCCACAT	GGCCATGCCAAACATTATCTGA	FAM	AGGGCTAGTGTGTCTTCTTG	TET	AGGGCTAGTGTGTTTTCTTG
6_67.21	ACTCTTAAAGCAGTCCTGTCACTT	GACAAAGAAAGACAGTAACACACAC	FAM	AAAGTGAGATGTCTATCAGGTA	TET	AAAGTGAGATGTCTTTCAGGT
10_57.79	TCATGTGCGCATTGTATGTGTG	GGTAGGTTGAGCGCCTATCTC	TET	CAATTACACATGCACATCTCAATTACAC ATGTACATCTG	FAM	CAATTACACATGCACATCT
13_27.04	GCTCAGAGCAAAGGCAAACACT	TGCCACCCACCCAAGTAG	FAM	CCCATCTATGCAGTTC	TET	ATCCCATCTGTGCAGTT
9_21.20	GGAGAAGACTTCCTCCATACAGA	CCCAGCAGGAGAGGCTAA	FAMC	AGGGCGCCAGGCC	TET	ACATACTGGAGGGTGCCAG
10_80.19	GGCAGCAACAATGTGTGAGA	ATGCAGCATCGCTCTGTCTTG	FAM	CAATGCACCCATTAAGTAC	TET	AACAATGCACCCTTTAAGTA
11_04.40	CAACCCTGGTTGCACAAATGAG	CCTTAGGAAGGGCATGGTTCTA	TETC	CAGTGGGTTTGCTTAGTAAGCTCAGTG GGTTTGTTTAGTAA	FAM	CTCAGTGGGTTTGTTTAGTAACAGTGG GTTTGCTTAGTAAG
16_17.32	GGACTCCTACAAACACCCTGA	CTTTGTCCTGTCTGTGCCTTA	FAM	ATGCTCATGTCCCTTAAA	TET	TGCTCATGTTCCTTAAA

Supplementary References

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