

## How to Complete a Risk Assessment for CRISPR Experiments in Animals and Cell Culture

1. **Project Description:** CRISPR specific for [insert species] will be used to inactivate [insert gene] to create a model for [insert disease]. Include how CRISPR will be dosed: viral vector, plasmid, liposome, etc.
2. **Format of Gene Silencing:** Are you attempting random or specific gene silencing? Will silencing be one-step (Crispr/Cas9 and gRNA combined in one vector) or two-step (create cell line containing Crispr/Cas9, then add gRNA) or purchasing pre-transfected cell lines containing CRISPR-Cas9, then transfecting the RNA guide sequences?
3. **Containment Requirements:** Usually **BSL-1** and chemical hygiene practices, containment equipment and facilities for all activities involving non-virus dosing. For virus-vectored CRISPR, **BSL-2** practices including biological safety cabinets are recommended. Centrifuge safety precautions, secondary containers for transport between incubator and BSC. Keep hands away from the eyes, nose and mouth in order to avoid potential exposure of the mucous membranes; eye goggles or face shields may assist in accomplishing this objective.
4. **CRISPR Injection dosing precautions:** The use of sharps should be minimized. Safe-sharp technology is highly recommended during animal dosing.
5. **Spills:** If non-virus vectored, cleanup per the chemical hygiene plan. If virus vectored, the follow BSL-2 spill instructions.
6. **Biohazardous Waste:** Collect in double red bags and transport in a rigid container.
7. **Approved Disinfectants:**
  - i. Non-virus vectored siRNA: soap and water
  - ii. Virus-vectored; disinfectants appropriate for the virus.
8. **Disposal:** Non-virus vectored, as a chemical. Virus-vectored: Decontaminate before disposal; steam sterilization, incineration, chemical disinfection.
9. **Storage:** Store plasmids as per the chemical hygiene plan. Store virus vectors as BSL-2 organisms.
10. **Pathogenicity:** Mucous membranes, ingestion, broken skin and injection. Reasons can be sharps contact, failure to wash hands, skin contamination from dirty gloves or work surfaces.
11. **Modes of Transmission:** Liposomes and plasmids may cross the cell membrane of individual cells. If the gene target is present, it could result in silencing. Liposomes and plasmids are not infectious; once integrated into cells, they do not reproduce. For virus vectored, refer to appropriate virus vector sheet.
12. **Length of gene deletion:** In human and mammalian cells, as well as animals, CRISPR silencing is permanent. It is transmissible to off-spring.
13. **Communicability:** If virus vectored, accidental contact with live virus can result in CRISPR expression.
14. **Medical surveillance and clinical treatment procedure:** Immune suppression is required, as the silencing can affect the immune system. Clinical Operating

Procedure "Virus Vectors" must be listed on risk assessment if used to vector CRISPR.

**15. Stability in Environment:** Refer to appropriate virus vector sheet.


**16. CRISPR concentration, dosage per experiment:** State your stock concentration and the amount used per experiment or kg animal weight.

**17. CRISPR shedding from animals:** Animals will not shed CRISPR if dosed with plasmid formulations. For viral vectors, refer to specific viral vector risk assessment.


**18. CRISPR Information:** Discuss the desired effect of gene editing on the animal or cell line. You must address the potential effects due to accidental worker exposure. If unknown, state that. Points to consider are:

- a. Is the guide sequence specific to animals, humans or could it affect both? Similarity between human and animal guide sequences?
- b. What is known about off-target effects
- c. How much genotype change (dose) is needed for a physical effect?
- d. How does route of exposure affect outcome?

A good source for understanding the transgene being silenced or over-expressed is GENE CARDS (<http://www.genecards.org/>). A snapshot of a sample gene card is shown below:



The Human Gene Compendium



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**LifeMap Sciences**

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**TNFRSF10B Gene**  
protein-coding **GIEx: 73**  
GCID: GC08M022877

**Tumor Necrosis Factor Receptor Superfamily, Member 10b**

**ESCMID** Microbiology & Infectious Diseases Congress

**M** Antibodies / (SH) RNA  
Proteins & Enzymes  
(Access & Kits) Pathways

**NCBI** Molecular Gene Network  
(OMIM) / (OMIM) / (OMIM) / (OMIM)  
PCR Arrays / Pathways / (OMIM) / (OMIM)

**88 related diseases at MoleCards**

**ORIGENE** Proteins  
Antibodies  
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**GenScript** Proteins  
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**Allases for TNFRSF10B gene**

(According to <sup>1</sup>[HGNC](#), <sup>2</sup>[Entrez Gene](#), <sup>3</sup>[UniProt/Swiss-Prot](#), <sup>4</sup>[UniProt/TrEMBL](#), <sup>5</sup>[OMIM](#), <sup>6</sup>[GeneLoc](#), <sup>7</sup>[Ensembl](#), <sup>8</sup>[OMIM](#), <sup>9</sup>[mRNA](#), <sup>10</sup>[tRNAdb](#), <sup>11</sup>[HinvDB](#), <sup>12</sup>[NCBI](#), <sup>13</sup>[NONCODE](#) and/or <sup>14</sup>[RNAdb](#))

[About This Section](#)

This gene clusters with an RNA gene  
Subcategory (RNA class): lncRNA

[Quality score](#) for the **ORGL**, clustered with this gene is 3

**Allases**  
Tumor Necrosis Factor Receptor Superfamily, Member 10b<sup>1,4</sup>

DR5 <sup>1,2</sup>	TRICK2B <sup>4</sup>
TRAILR2 <sup>1,1,1</sup>	TRICK2 <sup>4</sup>
Death Receptor 5 <sup>1,1</sup>	Apoptosis Inducing Protein TRICK2A/2B <sup>4</sup>
KILLER <sup>1,1</sup>	Apoptosis Inducing Receptor TRAIL-R3 <sup>4</sup>
TRAIL-R2 <sup>1,1</sup>	Cytotoxic TRAIL Receptor-2 <sup>4</sup>
TRICK2 <sup>1,1</sup>	Death Domain Containing Receptor For TRAIL/Apo-2L <sup>4</sup>
ZTNFR9 <sup>1,1</sup>	Fas-Like Protein <sup>4</sup>
TNF-Related Apoptosis-Inducing Ligand Receptor 2 <sup>1,1</sup>	FS3-Regulated DNA Damage-Inducible Cell Death Receptor(Killer) <sup>4</sup>
CD262 <sup>2</sup>	Tumor Necrosis Factor Receptor Superfamily Member 10B <sup>4</sup>
KILLER/DR5 <sup>4</sup>	Tumor Necrosis Factor Receptor-Like Protein ZTNFR9 <sup>4</sup>
TRICK2A <sup>4</sup>	CD262 Antigen <sup>4</sup>
	TRAIL Receptor 3 <sup>4</sup>

External Ids: [HGNC: 11905<sup>1</sup>](#) [Entrez Gene: 8759<sup>2</sup>](#) [Ensembl: ENSG00000120889<sup>3</sup>](#) [OMIM: 603613<sup>5</sup>](#) [UniProtKB: O14763<sup>3</sup>](#)

**ORGL**, members:

[NONCODE<sup>13</sup>](#) [n407670](#) [express<sup>4</sup>](#)

[Export aliases for TNFRSF10B gene to outside databases](#)

Previous GC Identifiers: GC08M022647 GC08M023231 GC08M022899 GC08M022933 GC08M021422

To better understand potential human outcomes from accidental silencing, you can see if information exists in the JAX Mouse Genome Informatics: (<http://www.informatics.jax.org/batch>). Enter the gene designation, and then look to see if a mouse knockout phenotype exists. If so, add that information to the risk assessment.

**MGI** Celebrate 25 years with us Keywords, Symbols, or IDs **Quick Search**

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## MGI Batch Query

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Input	Output
<p>Type: Search all input types</p> <p>Source:</p> <p>Enter Text: <input type="text"/> Upload File: <input type="button" value="Upload File"/></p> <p>IDSymbols List:</p> <p>*tab, space, and newline separated ids.</p> <p><b>Search</b> <b>Reset</b></p>	<p><b>Gene Attributes:</b></p> <p><input checked="" type="checkbox"/> Nomenclature <input type="checkbox"/> Genome Location <input type="checkbox"/> Ensembl ID</p> <p><input type="checkbox"/> Entrez Gene ID <input type="checkbox"/> VEGA ID</p> <p><b>Additional Information:</b></p> <p><input type="checkbox"/> Gene Ontology (GO) <input type="checkbox"/> Mammalian Phenotype (MP) <input type="checkbox"/> Human Disease (OMIM)</p> <p><input type="checkbox"/> Alleles <input type="checkbox"/> Gene Expression <input type="checkbox"/> RefSNP ID</p> <p><input type="checkbox"/> GenBank/RefSeq ID <input type="checkbox"/> UniProt ID <input checked="" type="radio"/> None</p>

Contributing Projects: Mouse Genome Database (MGD), Gene Expression Database (GXD), Mouse Tumor Biology (MTB), Gene Ontology (GO), MouseCyc

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Last database update: 09/09/2014 09:02:19

To gain more information on gene mutations related to cancer, the database Atlas of Genetics Oncology (<http://atlasgeneticsoncology.org/index.html>) can be consulted:

Atlas of Genetics and Cytogenetics in Oncology and Haematology

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Leukemia predisposing genes in Perugia, Italy, on October 24-25, 2015

1st International Workshop on Cancer Genetic & Cytogenetic Diagnostics in Nijmegen from April 6 to April 8, 2016