The University of Tulsa Institutional Biosafety Committee –Standard Operation Procedures (SOPs)

SOP Title: **Use of Gene Editing Technologies**		
SOP Number: **IBC-SOP-004**	Revision Number: 2	Effective Date: 6/15/2020

1. PURPOSE

1.1 The purpose of this document is to assist investigators and reviewers in evaluating specific biosafety issues related to gene-editing (CRISPR/Cas9 or similar protocols) and the information needed when describing research activities using gene-editing technologies (such as CRISPR/Cas9) to make risk assessment determinations.

2. KEY WORD DEFINITIONS:

2.1 **IBC**: Institutional Biosafety Committee
2.2 **PI**: Principal investigator
2.3 **CRISPR**: Clustered Regularly Interspaced Short Palindromic Repeat
2.4 **TALENS**: Transcription Activator-Like Effector Nucleases
2.5 **ZFN**: Zinc Finger Nucleases
2.6 **NIH OBA**: National Institutes of Health Office of Biotechnology Activities
2.7 **NIH Guidelines**: *NIH Guidelines for Research Involving Recombinant and Synthetic Nucleic Acid Molecules*
2.8 **Enhanced Review**: Protocol submissions that may not be subject to IBC review and oversight currently under Section III-F of the *NIH Guidelines* but where the TU IBC at its discretion has opted to review certain types of research even if it currently falls in the List of Exempt Experiments, require increased 'Enhanced' review by TU IBC. Gene editing technologies, such as CRISPR/Cas9, fall into this category of **Enhanced** review and must be submitted and approved by the TU IBC before research can commence. Factors that can lead to increased, ***Enhanced*** review may include new procedures, equipment or technologies not yet addressed in the *NIH Guidelines*, etc.

3. CRISPER AND OTHER GENOME EDITING TECHNOLOGY OVERVIEW

3.1 CRISPR/Cas9 is a very powerful genome editing technology that is currently being used in many research projects. This system is revolutionizing the life sciences field by making genome modification easier and faster than ever before. This technique uses a system of adaptive immunity that bacteria use, and has been expanded to disrupt or alter genes in all types of cells. Expression of CRISPR/Cas9 (or other Cas proteins) and guide RNAs allows for integration into the host genome, which is stable and permanent for the life of the cell. The TU IBC considers the potential for disruption of genes not targeted by guide RNA sequences, which are known as "off-target" effects, to also be a safety issue that should be addressed when using gene-editing technology.

3.2 CRISPR-Cas systems are able to target DNA sequences that are complementary to a ~20 nucleotides (nt) CRISPR single guide (sg)RNA. The region of the sgRNA sequence that is complementary to the DNA target sequence in the 'edited' organism(s) is known as the protospacer. Prior to DNA cleavage, the Cas proteins bind to the target DNA, by recognizing a specific 3 nt sequence known as the Protospacer Adjacent Motif (PAM). For Cas9, the PAM sequence reads 'NGG', where N can be any nucleotide base. Depending on the Cas system, the PAM sequence may vary, though it is necessary for successful gene editing.

3.3 The NIH makes clear that the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules* (*NIH Guidelines*), Section IV-A (Policy), is a living document and as such cannot be complete, nor able to foresee the utilization of new genetic manipulation that could enable work to be accomplished faster, more efficiently or at larger scale. Thus, the responsibility is on the institution and those associated with it to **adhere to the intent as well as the specifics of the NIH Guidelines**.

3.4 The University of Tulsa IBC discussed safety concerns based on the current state of knowledge with respect to this fast-evolving technology and in keeping with all appropriate institutionally recognized safety guidelines and regulations.

3.4.1 If new knowledge is discovered that substantiates changes, the Committee will convene discussion to provide the most appropriate safety guidance to the TU research community.

3.5 Researchers interested in using CRISPR, or other genome editing technologies such as Transcription Activator-Like Effector Nucleases (TALENS) and/or Zinc Finger Nucleases (ZFN), **<u>are required</u>** to submit their research to The University of Tulsa Institutional Biosafety Committee (IBC) for review.

3.5.1 The TU IBC will review all gene-editing technology (such as CRISPR/Cas9) protocols. The ***Enhanced*** review process will be used by the TU IBC (if a protocol falls in the current List of Exempt Experiments but it using gene-editing technology. The TU IBC, through the IBC Chair or chair designate, will determine what level of increased 'Enhanced' review procedures will be used.

- Enhanced review can take the form of:
    1. Review by the TU IBC Chair, Chair designate or TU IBC Member with subject matter expertise;
    2. Review by a sub-committee of TU IBC Member with subject matter expertise; ***or***
    3. Review at a convened meeting of the TU IBC with a quorum of members present (similar to Non-Exempt review procedures).
    4. Forms of review 1 & 2 above, may approve a protocol, conditionally approve a protocol, ask for additional information/clarification ('table'), or request that the protocol be reviewed at a convened meeting of the TU IBC with a quorum of members present, but <u>cannot</u> disapprove/reject a protocol.

5. If the form of review is review at a convened meeting with a quorum of members present, the TU IBC may approve a protocol, conditionally approve a protocol, ask for additional information/clarification ('table') or not approve/reject a protocol.

- Once approved, TU IBC protocols using gene-editing are subject to Annual Administrative Reviews , similar to Non-Exempt protocol requirements

4. IBC GUIDANCE ON CRISPR-Cas9 AND SIMILAR TECHNOLOGY

4.1 Below is a list of questions that may help investigators and reviewers to evaluate the biosafety issues related to specific CRISPR/Cas9 protocols. Other genome editing technologies include Zinc Fingers and TALENS. Biosafety concerns for these technologies would be similar to those described here for CRISPR-based systems.

4.2 **Principal Investigators (PIs):**

In order to properly assess the research, the Principal Investigator should address the following questions when submitting an IBC protocol for review:

4.2.1 Is gene editing, genome modification, or similar technology (CRISPR, TALENs, zinc fingers, etc.) being used as the part of the protocol? If yes, describe the experimental design in the IBC form, including:

- How will the gRNA and Cas9 be delivered to the cells or tissues?
- How was/were the targeting sequence(s) designed? Specify the gRNA nucleotide sequence and the PAM sequence in the target genome.
- How was/were off-target site/s evaluated?

4.2.2 Which organism(s) is (are) being modified? Targeting of human cells presents additional risk to laboratory workers due to the potential for accidental ingestion, inhalation, injection or other routes of administration. Describe how these risks are reduced in your experiment. Remember that highly homologous genes in non-human species may target human genes as well and consider this in your design.

4.2.3 Will CRISPR work be done in cell culture, in whole organisms, or both? If human cell cultures are used, BSL2 procedures are required. If animal cell cultures are used, recombinant DNA or viral vector procedures will apply, depending on your design. In whole organisms, IACUC or IRB approval will be required.

4.2.4 Will unexpected mutations due to off-target binding be expected? The mutant forms of Cas9 can help significantly decrease off-targeting effects during gene editing (when using CRISPR/Cas9). Researchers should consider using the mutant Cas9 forms to increase the specificity and decrease off-target effects.

4.2.5 How will CRISPR-Cas9 be delivered (e.g., viral vector, plasmid, liposome, nanoparticles, etc.)? If it is a viral delivery, will the Cas9 and gRNA be delivered together on a single transfer vector/plasmid or on separate transfer vectors/plasmids (since it imparts greater safety)? Cases where both Cas9 and gRNA are delivered using the same viral vector may present additional risks for laboratory workers if there is the possibility of inactivating one or more human tumor suppressor genes, as one example. Please consider any potential risks to humans from accidental exposure and justify your experimental design in light of this risk.

4.2.6 If animal work is involved, will syringes be used for injections? If so, syringes with integral safety features must be used and 'no recapping' strictly enforced!

4.2.7 Will the research involve the creation of a gene drive experiment (i.e., a system that greatly increases the probably that a trait will be passed on to offspring)?

4.2.8 All gene drive experiments must include full descriptions of biological and engineering containment protocols that are customized for the organism and the gene editing strategy. Please be aware that these will be scrutinized in detail due to the danger of releasing a gene-drive organism into the environment.

4.2.9 Will the gene editing technology be used to target embryos/germ line cells? If so, the biosafety protocol must include an approved or submitted IACUC number.

4.2.10 Will the gene editing technology be used for human gene therapy research? If so, the biosafety protocol must include IRB submission information.

4.3 TU IBC Member Review: Risk Assessment Questions and Risk Mitigation

4.3.1 Will the research be done in bacteria, cell cultures, plants, insects, or whole animals?
Answer: Using human-derived cell lines will require BSL-2 practices. Use in animals will require IACUC approval. Use in plants or insects will require specific containment.

4.3.2 Will you perform gene editing in human embryos or germ-line cells?
Answer: Applications of such experiments for human use are not permitted.

4.3.3. How will this technology be delivered to the host: using plasmids, viral vectors, nanoparticles, other delivery methods?
Answer: The risks of using viral vectors must be considered e.g. Retro/Lentiviruses can stably integrate into the host cell chromosome and activate proto-oncogenes or suppress tumor suppressor genes at the site of insertion. Containment levels for the mode of delivery will be assessed based on the vector and gene hazards.

4.3.4. Are the guide RNA (gRNA) sequences specific to humans/animals or can it affect both?
Answer: What is the homology between the human and the animal gRNA sequences? A higher containment measures may be required if high homology.

4.3.5. How many genes are being targeted: Single? Multiple? Libraries: hundreds/thousands/more?
Answer: Off-target effects are possible if multiple genes are targeted and tumor suppressor genes may be among those targeted. Higher containment practices maybe required if off-target effects are anticipated.

4.3.6 Do the experiments involve an environmental release of the modified organisms? What are the possible effects on the population due to the changes created in the organism? Can this lead to 'gene drive' i.e. a 100% chance that certain traits may spread through an entire population of sexually reproducing organisms (vs. normal gene inheritance, in which there is a 50% chance)?
Answer: Gene drive containment can be obtained as follows: (labs should use at least two of these strategies)

Molecular: 'Split drive': Separate the components required for the gene drive i.e.: add gRNA to a cell line/animal already expressing CRISPR/cas9. Higher containment may be recommended if cas9 and gRNA are delivered on the same plasmid/vector.
Have a 'reversal drive' in place to alter the drive created in the population
Ecological: Perform experiments in areas lacking the wild populations in which changes can occur.
Reproductive: Use laboratory strains that cannot reproduce in the wild and synthetic sequences that are not found in the wild
Barrier: Use strict containment measures to prevent the release/escape of the modified organisms e.g. physical barriers, temperature control, air blast fans etc.

4.3.7 Do you anticipate any known potential off-target effects or mutations (i.e. disruption of genes not directly targeted by the gRNA sequences)?
- How will you determine the unknown off-target effects/ mutations?
- What are the human/animal exposure concerns with the off-target effects?
- How much genotype change is needed to cause an apparent physical effect on the organism? How does the route of exposure affect the outcome?

Answer: -Using a variant form of Cas9 can increase the target specificity and reduce the off-target effects.

5 REQUIREMENT FOR BIOSAFETY LEVEL 2 LABORATORY

5.1 The University of Tulsa Institutional Biosafety Committee (IBC) requires Biosafety Level 2 measures when nuclease-based genome editing technologies (e.g., CRISPR/Cas, CRISPR/Cpf1, TALENs, ZFNs) are used with a target DNA sequence that shows 90% or greater similarity to human genomic sequence (based on BLAST and BLAT searches to

the human genome). This requirement is to be followed whether or not the model organisms themselves require BSL-2 containment. The TU IBC has established this requirement for safety reasons. Specifically, to prevent a lab mishap that could result in the accidental exposure (injection or other introduction) of nuclease-based DNA-editing to the investigators.

5.2 **The measures listed below are to be followed:**

5.2.1 The principal investigator ensures that laboratory personnel receive appropriate training about the potential hazards associated with the work involved.

5.2.2 A biohazard sign must be posted on the entrance to the laboratory.

5.2.3 Access to the lab is restricted when work is being conducted.

5.2.4 Only personnel with appropriate authorization may enter a BSL-2 facility while BSL-2 research activity is in progress.

5.2.5 Laboratory must have self-closing doors or be locked when work is being conducted.

5.2.6 Sink and eyewash must be available.

5.2.7 A policy for handling sharps must be in place.

5.2.8 Investigators must use standard PPE consisting of a lab coat, gloves, and eye protection.

5.2.9 The committee recommends that this work be conducted in a BSL-2 biosafety cabinet if available. This is absolutely required for organisms considered Risk Group 2 agents.

6 STANDARD OPERATION PROCEDURES APPROVAL

6.1 Version 2 approved 6/15/2020

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Approved by: ____ _____ Date Approved: 6/15/2020

TU IBC Chair

https://osp.od.nih.gov/wp-content/uploads/NIH_Guidelines.pdf