Case Western Reserve University
Institutional Biosafety Committee

Guidance regarding the use of Gene Editing Techniques

Gene editing technology allows scientists to modify the DNA of organisms, including plants, animals, and humans. This includes the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) Type II system, which is a bacterial immune system that has been adapted for genome engineering. This field has revolutionized research by enabling precise alterations to genetic material, which can lead to advancements in understanding diseases, developing therapies, and improving agricultural practices. In accordance with the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines)*, experiments using nucleic acids to introduce genetic modifications need to be submitted to the Case Western Reserve University's Institutional Biosafety Committee (IBC) for review.

Researchers need to conduct a risk assessment when designing CRISPR or gene editing experiments and implement risk mitigation strategies based on the identified risks. The justification for the design considering the risks should be communicated in the IBC protocol.

Risk Assessment

Delivery mechanism: The mechanism by which the gRNA and Cas9 will be delivered can have an impact on the risks to laboratory workers in the event of an exposure.

- Delivery of the gRNA and Cas9 on separate transfer vectors or plasmids imparts greater safety than delivering them together on one vector/plasmids.
- Targeting human cells or use of a viral vector with broad tropism increases the risk to workers.
- Delivery by lentivirus presents additional risks:
    - Oncogenic potential due to insertion into the host cell chromosomes – potential for inactivation of a tumor suppressor or activation of an oncogene.
    - Risk of replication competent lentivirus (RCL): viral vectors can recombine with endogenous viruses and regain virulence factors. Three plasmid systems present a higher risk compared to four or five plasmid systems which have further separated the viral packaging genes and have removed the Tat gene.

Genetic targets:

- Highly homologous genes in non-human species may target human genes as well.
- Editing a human oncogene, tumor suppressor gene, or immunomodulatory gene can carry a high risk, particularly when all components required for genome editing are likely to be introduced in a single exposure event, such as a needlestick or splash.
- Pooled CRISPR libraries may contain gRNAs targeting thousands of genes, some of which may be of higher risk (oncogenic or immunomodulatory).

- Editing a microbial genome could change the risks associated with the microbe, if the pathogenicity, virulence, transmission, tropism, or antibiotic resistance is altered.

Gene Drive: Promotes the spread of a genetic trait or modification through a population because it is inherited more often than Mendelian segregation would predict.

- A concern when a CRISPR system is used to edit the genome of a sexually reproducing organism.
- CRISPR can lead to gene drive if both of the following conditions are met:
    - All components required for genome editing can be incorporated into the genome and are subsequently inherited together.
    - The genome editing components are introduced into embryos or germ line cells.

Risk Mitigation Considerations:

- The targeting sequences should be designed and evaluated for off-target sites in humans.
- Mutant forms of Cas9 can help decrease off-targeting effects during gene editing.
- For experiments using lentivirus, use of a four or five plasmid system will reduce risk of RCLs.
- Administering Cas9 separately or using cells/ animals that already contain Cas9 decreases risk to the researcher.
- Containment practices must be based on the type of vector, vector design/components, mode of delivery, and potential off-target effects and hazards associated with the gene(s) of interest. Experiments that carry higher risk should implement enhanced BSL2 (BSL2+) containment (BSL3 work practices in a BSL2 facility), which include:
    - All work conducted in a biosafety cabinet (BSC) and biohazardous waste collected within the BSC
    - Increased PPE: wraparound fluid-resistant disposable gown, two pairs of gloves, safety glasses; outer gloves removed before removing hands from BSC.
    - In-line HEPA filter for vacuum line
    - Needle protection devices
    - Use of sealed rotors and/or safety cups for centrifugation – rotors/safety cups loaded and unloaded in BSC
- If a gene drive is anticipated or possible, consideration should be given to physical containment that prevents the escape or release of modified organisms, or use of organisms that cannot survive outside the laboratory environment.