Adding CRISPR to Your Bio-ARROW Protocol

Table of Contents

Work Covered by this Guidance Document:

This guidance document covers how to add the use of CRISPR systems (e.g., CRISPR/Cas9, CRISPR/Cpf1) – whether for genome editing or other purposes (e.g., CRISPR-mediated interference, CRISPRi) – to your Bio-ARROW protocol. This includes genetically modified organisms generated using CRISPR-based genome editing tools, even if the modification was or will be performed by someone else (e.g., a collaborator or company). For additional questions or one-on-one assistance, please contact the Office of Biological Safety at 263-2037 or biosafety@fpm.wisc.edu.

Background:

There are distinct biosafety concerns that arise with the use of CRISPR systems in sexually reproducing organisms, cells, and microbes.

Gene drive: Gene drive becomes a concern when CRISPR/Cas9 or other CRISPR system is used to the edit the genome of a sexually reproducing organism. 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. This can have significant ecological or evolutionary impact if introduced into a wild population. Gene drive occurs when the genome editing machinery incorporates into the genome as a cassette that is then able to copy itself into the other allele, converting a heterozygous state to a homozygous one. 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 subsequently inherited together
- The genome editing components are introduced into embryos or germ line cells

Oncogenic potential: If genome editing leads to the inactivation of tumor suppressors or activation of oncogenes in human cells, then oncogenesis becomes a possibility in the event of exposure. This is a concern when:

- Genome editing components are delivered by a method that can enter human cells (e.g., viral vectors that can infect human cells)
- All components required for genome editing are likely to be introduced in a single exposure event, such as a needle stick or mucosal splash
- Genome editing is designed to target human oncogenes/tumor suppressors or can target human oncogenes/tumor suppressors due to homology

Genome editing in microbes: If CRISPR systems are being used to edit microbial genomes, then it should be considered whether such editing changes the risks associated with the microbe. For example, will the pathogenicity, virulence, transmission, tropism, or antibiotic resistance of the microbe be altered?

If any of these apply to your research, make sure to address how the risks will be mitigated. Because research projects are unique, specific protocols may have additional risks that are not discussed here.

VI. Materials and Activities:

Please check all activities that apply to your research.

VI. Materials and Activities - Recombinant Materials:

Genome editing: Check "Yes" if CRISPR system is being used for genome editing. Check "No" if a nuclease-deficient CRISPR system is being used for non-editing purposes (e.g., CRISPRi).

Gene drive potential and precautions: If you checked "Yes" to Genome editing, be sure to answer all questions in this section, even if gene drive is not a potential risk in your research.

If gene drive is identified as a potential risk, any additional precautions should be described under "Gene drive precautions and containment". These may include, but are not limited to:

- Use of organisms that are incapable of surviving outside of the laboratory environment
- Physical containment measures that prevent escape or release of modified organisms
- Use of laboratory strains that cannot reproduce with wild counterparts

If there is no risk of gene drive in your research, please use this text box to explain why not (e.g., genome editing is being done solely in somatic cells grown in culture).*

**Note if you are using the University of Wisconsin-Madison Biotechnology Center Transgenic Animal Facility (TAF) services:* The standard method used by TAF for generating CRISPR-modified rodents is microinjection of purified Cas9 protein and synthetic gRNA, with or without a homology directed repair template, into one-cell embryos that are then implanted into surrogates. Because the genome editing components cannot be integrated into the genome, there is no risk of gene drive using this method.

Genes and DNA/RNA fragments: Remember to include all genome editing components (e.g., Cas9, sgRNA, and homology directed repair template, if applicable). Under "Use of gene", please list target genes for sgRNAs and homology directed repair templates; a general category with examples is acceptable.

Construct: Include all constructs (e.g., plasmids or viral vectors) used to deliver genome editing components, if applicable.

VI. Materials and Activities - NIH Guidelines:

Please check all appropriate categories that apply to your research.

VI. Materials and Activities - Microbes and Disease-Causing Agents:

Bacteria: Check "Yes" if you are using bacteria to generate or propagate recombinant plasmids containing genome editing components including homology directed repair templates, and fill in the appropriate information.

Viruses: Check "Yes" if you are using viral vectors to deliver genome editing components to cells or animals, and fill in the appropriate information.

VI. Materials and Activities - Cells, Organs, Tissues or Biological Specimens:

Cell culture: List all primary cells or cell lines that will be used for genome editing experiments, or that will be administered recombinant microbes that have undergone genome editing.

VI. Materials and Activities - Vertebrate or Invertebrate Animals:

Add any genetically modified animals created using a CRISPR system, even if not generated in your laboratory. Be sure to specify the gene target(s) and nature of the modification (e.g., knockout, knock-in, point mutation).

Animals genetically modified by your lab: If you are creating genetically modified animals using CRISPR, please describe here.

Animals genetically modified by someone else: If you are receiving animals that have been modified using a CRISPR system from a company or collaborator, please describe here.

VI. Materials and Activities - Plants

Add any genetically modified plants created using a CRISPR system, even if not generated in your laboratory. Be sure to specify the gene target(s) and nature of the modification (e.g., knockout, knock-in, point mutation).

Plants transgenic: Check "Yes" if you are using plants that have been genetically modified using a CRISPR system.

Transgenic plant precautions: If you are using plants that have been genetically modified using a CRISPR system, please answer this question.

Plant containment strategies and Plant experiment restrictions: If you will be working with genetically modified plants that are subject to gene drive, please use these sections to describe measures that will be taken to prevent their release, or why such measures may be unnecessary (e.g., plants cannot survive outside of the laboratory).

VII. Biosafety Precautions - Disinfection/Inactivation:

Please complete this section of the biosafety protocol following the instructions for each question.

Spill/release procedure: If you will be using genome-edited organisms in your research, please upload a document describing what you would do if a genetically modified organism escaped. There is a sample procedure on the OBS website: https://ehs.wiscweb.wisc.edu/wp-content/uploads/sites/25/2017/01/ReleaseOfTransgenicMaterials.pdf. If this meets your needs, you can upload this as your lab's procedure.

VII. Biosafety Precautions - Emergency Response:

Please complete this section of the biosafety protocol following the instructions for each question.

Emergency response special: Please specify information provided to the laboratory worker (and medical professional) in the event of an exposure or potential exposure (e.g., hazard communication regarding the genome editing system, vectors used in association with the system, ability to integrate into human genome, ability to edit the human genome, potential for human epigenetic changes, long/short term effects for a person if known).

Lab specific training: Training should include information about any risks associated with CRISPR use in your research (i.e., gene drive, oncogenic potential, increased pathogenicity of microbes due to genome editing).

VIII. Research Description:

The research description should clearly describe your research from a biosafety perspective.

If you are using genome editing tools such as CRISPR/Cas9, it should be clearly stated whether they will be used in microbes, cells, animals, plants, microbes that will be administered to animals or plants, and/or cells that will be administered to animals or plants. The method by which the genome editing components will be delivered (e.g., viral vectors, transient transfection of plasmids, injection of recombinant Cas9 protein and synthetic sgRNA) should be described, and tropism should be specified (i.e., is it able to enter a human cell). Target genes should be specified; a general category with examples is acceptable (e.g., calcium channel subunit genes such as *Cacna1a* and *Cacna2d1*). If you are targeting human tumor suppressors or oncogenes, this should be explicitly stated and a description of how the risk of oncogenic potential will be mitigated should be provided. Possible off-target effects and/or homology of non-human gene targets to human genes should be addressed. If you are using a CRISPR system to edit a microbial genome, describe the purpose (e.g., investigate gene function, select for mutants). Any expected changes in the pathogenicity, virulence, transmission, tropism, or antibiotic resistance should be clearly stated.

If you are using microbes, animals, or plants that were generated by a collaborator or company using CRISPR-based genome editing tools, this should be clearly stated. Any additional information related to the potential risk of gene drive and how it would be mitigated that is not already included elsewhere in the protocol should be described here. Please clarify if the organism being modified would be at an advantage if inadvertently released into the environment. Describe how risk of release will be mitigated.

If you are using CRISPR systems for purposes other than genome editing (e.g., CRISPRi, CRISPRa, CETCh-seq), the components of the editing system and their intended purpose should be clearly described. Many non-traditional uses of CRISPR are based on mutants that lack nuclease activity. Without this activity, there may be no risk of gene drive. Other risks (e.g., oncogenic potential) may still exist, however, and should be addressed.

Depending on the nature of your specific research, other risks may exist. Please be sure to describe them and how they will be mitigated.

Upload additional documents: Be sure to include all constructs for generation and delivery of genome editing components.