UH IBC: Guided Gene Drive Technology

Guideline 1.0, Version 1.o
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PURPOSE

The purpose of this guideline is to describe the Institutional Biosafety Committee (IBC) review of gene drive technology.

INTRODUCTION

RNA-Guided Gene Drives risk is not defined by the capability to infect and cause disease in a susceptible human or animal host, but instead, the main point of risk management is to consider effects to the natural ecosystem. Depending on the aim of the particular RNA-Guided Gene Drive, there is potential to alter populations of organisms in manners which could have positive effects on human health, but both direct and indirect effects on the environment and the living organisms that inhabit it. (Wyss Institute Harvard University)

The **C**lustered **R**egularly **I**nterspaced **S**hort **P**alindromic **R**epeats (CRISPR)/Cas9) CRISPR-**a**ssociated protein **S**ystem is an incredibly powerful genome editing technology. CRISPR other genome editing technologies, such as Transcription Activator-Like Effector Nucleases (TALENS), Meganucleases (MGN) and Zinc Finger Nucleases (ZFN), require to submit their research disclosures with the IBC registration.

Prior to CRISPR/Cas9, genome engineering approaches relied upon the use of customizable DNA-binding protein nucleases that required scientists to design and generate a new nuclease-pair for every genomic target. Largely due to its simplicity and adaptability, CRISPR has rapidly become one of the most popular approaches for genome engineering selectively activate or repress target genes, purify specific regions of DNA, and even image DNA in live cells using fluorescence microscopy (S. Moisyadi Jun 2016)

The greatest laboratory risks posed by CRISPR, other gene editing technologies, and also existing conventional rDNA research remain:

- Presence of replication competent viruses
- Insertion activation of oncogenes (more likely with MLV than lentiviruses)
- Risk of tumor suppressor gene inactivation
- Other (site-specific risk assessment)

Viral vectors, plasmids, and nanoparticles are being used to deliver CRISPR systems. CRISPR research is being performed in many organisms, from *E. coli* to human cells lines to animals.

Viral vectors, plasmids, and nanoparticles are being used to deliver CRISPR systems.
Mouse and human guided RNA will be used.

Off-target mutations may occur, or be unknown.
There may be unwanted immune system reactions.
Infection may be possible from viral vectors.

There may be challenges associated with CRISPR activity over time.

Some experiments have the Cas9 protein and gRNA being transiently expressed; the CRISPR DNA is not being stably integrated into the cell's genome.

In some experiments, targeted genes lead to reduced host fitness (e.g., slower growth rate); these strains would be less competitive if inadvertently released to natural environment.
OSHA Bloodborne Pathogens Standard is followed for work with human materials (e.g., human cell lines). There is potential for needle sticks or other exposures. Eliminating needles and other sharps when doing the work is recommended when possible.

BIOSAFETY REQUIREMENTS (University of Pittsburg Feb 2016)

- **BSL/ABSL-1: Recommended for non-viral, non-human cell use**

  The IBC will consider the use of gene drives in cell culture work viruses for use at BSL/ABSL-1:

  Transfection of cells in culture, except for human-derived cells

- **BSL/ABSL-2: Recommended for viruses or use in human cells***

  The IBC will consider the use of gene drives in cell culture work viruses for use at BSL/ABSL-2:

  Transfection in human-derived cells
  Transduction of cells in culture

*Viral vector must be at 3rd generation or later. 1st and 2nd generation may require containment BSL/ABSL 2+.

1) All work involving potential gene drive systems should be preceded by a thorough assessment by the relevant biosafety authorities of the risk of unwanted release from the laboratory. Seek guidance from external experts and make their evaluation available to others.

2) All laboratory gene drive experiments should employ at least two stringent confinement strategies (see the table) whenever possible to minimize the risk of altering wild populations. Using one form of confinement may be justified only if relevant biosafety authorities determine that it will reduce the probability of release to a level that is acceptably low. This probability must be defined on a case-by-case basis. The analyses necessary to confidently predict the efficacy of confinement strategies for gene drive systems are in a nascent form. Therefore, any proposal to use one rather than multiple forms of confinement requires even greater scrutiny and extensive deliberation between regulatory authorities and scientists.

3) Organisms carrying gene drive constructs that could spread if the reproductively capable life stages were to escape in transit should not be distributed to other institutions until formal biosafety

guidelines are established. Whenever possible, laboratories should instead send DNA constructs or information sufficient to reconstruct the gene drive. Protocols for distributing materials should be established in discussion with the wider research community and other relevant stakeholders.

BSL-1 or ABSL-1	Cas9 and gRNA on separate plasmid
	Plasmid or vector no capable of infecting human cells
	Standard cloning vector (<50% of Risk Group 2 pathogen)
	Research in non-pathogenic *E. coli* (K-12, other*) Saccharomyces cerevisiae, B. subtilis* , other Risk Group 1 cell lines)
	Replication defective Adeno Associated Virus Vector (AAV)
BSL-2 or ABSL-2	Cas9 and gRNA on same plasmid or vector
	Replication defective Adenovirus, Herpesvirus, ecotropic Retroviralvectors, other Risk Group defective vectors
	Research in human or non-human primate cell line (COS-7)
	Inserted nucleic acid targeting cell cycle or cell division, transcription,cell activators, cell growth
	Genes associated with toxicity or allergenicity
BSL-2+ or ABSL2+	Cas9 and gRNA on same plasmid or vector
	Lentiviral vectors
	Retroviral vectors with amphotropic packaging cell lines
	Vaccinia virus and VSV (lab strain) vector
	Large libraries targeting the human genome
	Human cellular or viral oncogene knock-in
	Tumor suppressor gene knock-out
BSL-3 or ABSL-3	Any research with CRSPR/Cas9 involving Risk Group 3 materials.

RISK ASSESSMENT

In order to perform a proper risk assessment, the researcher will provide the following:

1 Does your research involve CRISPR or another gene editing technology? If yes, you will need to describe the technology (e.g., CRISPR/Cas9, ZNF, TALENS, Meganucleases) that is being proposed.

2 For CRISPR systems, are the guide RNA (gRNA) and nuclease on the same plasmid, vector, or delivery vehicle?

If so, can this plasmid, vector, or delivery vehicle transfect or infect a human cell and can the gRNA or CRISPR nuclease be expressed in human cells?

3 For CRISPR research involving viral vectors, a Genome Target Scan (GT-Scan) for off target affects by your gRNA must be completed. This is necessary to determine if there is homology to human DNA and for assessing the risk of potential exposure in the event of an unanticipated incident. (**References:** Bae et al., 2014; O'Brien and Bailey, 2014)

4 Will the genome editing technology be used in prokaryotes, eukaryotes, or mammalian cells? If so, please specify which.

5 How is the gene editing technology being delivered (e.g., nanoparticles, plasmid, lentivirus, adeno-associated virus, etc.)?

6 Will the gene editing technology target embryos or germ line cells? **

7 Will the gene editing technology be used for human gene transfer research? **

8 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) (**Reference:** Akbar et al., 2015).

**No gene editing of the germ line, human embryos, or germ cells for clinical application is allowed. Gene editing of human embryos and germ cells for scientific purpose may be allowed, but must be evaluated on a case- by-case basis by the appropriate federal and local scientific review committees.

Attach the following to your IBC Registration

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. **Containment Requirements:**

   Usually **BSL-1** and biological 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.

3. **CRISPR Injection dosing precautions:** The use of sharps should be minimized.

   Safe-sharp technology is highly recommended during animal dosing.

4. **Spills:**

   If non-virus vectored, cleanup per the biological spill plan.

   If virus vectored, follow BSL-2 spill instructions.

5. **Biohazardous Waste:** Collect in double red bags and transport in a rigid container. Autoclave with appropriate time, pressure and temperature (with quality control)

6. **Approved Disinfectants:** Non-virus vectored siRNA: soap and water
   Virus-vectored; disinfectants appropriate for the virus.

7. **Disposal:** Non-virus vectored, as a biological spill plan

   Virus-vectored: Decontaminate before disposal; steam sterilization, incineration, chemical disinfection.

8. **Storage:** Store plasmids per chemical hygiene plan.
   Store virus vectors as BSL-2 organisms.

9. **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.

10. **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.

11. **Length of gene deletion:** In human and mammalian cells, as well as animals, CRISPR silencing is permanent. It is transmissible to off-spring.

12. **Communicability:** If virus vectored, accidental contact with live virus can result in CRISPR expression.

13. **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.

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

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

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

17. **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

Potentially stringent confinement strategies for gene drive research

Multiple stringent confinement strategies should be used whenever possible.

TYPE	STRINGENT CONFINEMENT STRATEGY	EXAMPLES
Molecular	Separate components required for genetic drive	sgRNA and Cas9 in separate loci (*8*)
	Target synthetic sequences absent from wild organisms	Drive targets a sequence unique to laboratory organisms (*3*,*4*,*8*)
Ecological	Perform experiments outside the habitable range of the organism	*Anopheles* mosquitoes in Boston
	Perform experiments in areas without potential wild mates	*Anopheles* mosquitoes in Los Angeles
Reproductive	Use a laboratory strain that cannot reproduce with wild organisms	*Drosophila* with compound autosomes*
Barrier	Physical barriers between organisms and the environment	Triply nested containers, >3 doors (*6*)
	•Remove barriers only when organisms are inactive	Anesthetize before opening (*6*)
	•Impose environmental constraints	Low-temperature room, air-blast fans
	•Take precautions to minimize breaches due to human error	Keep careful records of organisms, one investigator performs all experiments (*6*)

*An example of reproductive confinement would be *Drosophila* laboratory strains with a compound autosome, where both copies of a large autosome are conjoined at a single centromere. These strains are fertile when crossed inter se but are sterile when outcrossed to any normal or wild-type strain because all progeny are monosomic or trisomic and die early in development.

Akbari et al. (2015). Safeguarding gene drive experiments in the laboratory. Science; 349(6251): 927-8.
http://www.sciencemag.org.ezproxy1.lib.asu.edu/content/349/6251/927.full.pdf

What safeguards and confinement strategies are available?

Molecular confinement involves building gene drives that can spread through populations of transgenic laboratory organisms but not wild organisms. For example, an sgRNA-only drive will spread exclusively through populations that already express Cas9 from an unlinked locus, while a Cas9+sgRNA drive targeting a synthetic sequence will only spread in transgenic laboratory populations with that sequence. Both methods are easy to implement and have been tested in yeast5.

Ecological confinement involves performing experiments in a geographic area where escaped organisms won't be able to find mates. For example, ongoing experiments attempting to build gene drives in tropical mosquito vectors of diseases such as malaria and dengue are currently being performed in regions that don't have resident

populations of the relevant mosquito species.

Reproductive confinement involves working with laboratory organisms that can't reproduce with wild ones. For example, Drosophila lines with compound autosomes are completely infertile when mated to wild fruit flies. It's also worth noting that gene drive experiments are less hazardous in organisms that seldom reproduce sexually because the drive must be much more efficient and minimally harmful in order to spread.

Barrier confinement seeks to keep the organisms in the laboratory. It varies by organism, but your local biosafety officer should be familiar with appropriate measures. Barriers should be a component of all gene drive confinement strategies, but they should not be relied on exclusively because historical studies of pathogen research have conclusively shown that barrier protocols are vulnerable to human error. And with gene drives, one mistake can be enough.

Reversal drives are designed to overwrite a previous gene drive and thereby undo the genetic changes driven by the earlier intervention. While an initial reversal drive cannot restore the exact original sequence, it can restore the original protein-coding sequence using a recoding strategy; a subsequent drive can restore the wild-type sequence (save for the residual sgRNAs and possibly cas9 gene). An immunizing reversal drive is a variant that also spreads through the wild population and immunizes it against the first drive. Laboratories interested in building candidate gene drives intended for eventual release should consider building an appropriate immunizing reversal drive at the same time to mitigate the potential effects of an accidental release.