

## CRISPR/Cas9 Rutgers Biosafety Guide Sheet

### A. Overview and background of CRISPR/Cas9:

Recent discoveries have simplified the process for editing genomic sequences within cells. These new procedures are rapidly becoming used for a broad variety of strategies, from inactivating a selected gene to insertion of engineered, foreign sequences. While genomic methods for homologous recombination have been used for many years, the ability to introduce a site-specific break in DNA vastly enhances the repair processes that are required for genome editing. Zinc Fingers (class of engineered DNA-binding protein that facilitate targeted editing of genome) and TALENs (Transcription activator-like effector proteins bind to specific DNA-binding domain and make targeting editing of the genome) are among the commonly used techniques used for genome editing. The newest and simplest of these methods is known as CRISPR. It is an incredibly powerful genome editing technology and due to its simplicity and adaptability, it has rapidly become one of the most popular approaches for genome engineering. (<https://www.addgene.org/crispr/guide/>).

The Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) Type II system is a bacterial immune system that has been modified for genome engineering. CRISPR normally consists of two components: a “guide” RNA (gRNA) and a non-specific CRISPR-associated endonuclease (Cas9). The gRNA is a short synthetic RNA composed of a “scaffold” sequence necessary for Cas9-binding and a user-defined ~20 nucleotide “spacer” or “targeting” sequence which defines the genomic target to be modified. Thus, one can change the genomic target of Cas9 by simply changing the targeting sequence present in the gRNA.

#### Using Cas9 variants for increased specificity and safety during gene editing

Recent research shows that mutant forms of Cas9 can help significantly decrease off-targeting effects during gene editing (when using CRISPR/Cas9). Researchers should consider using these mutant Cas9 forms to increase the specificity and decrease off-target effects. Please refer to this link for more information about Cas9 variants (<http://www.genecopoeia.com/resource/crispr-cas9-specificity-taming-off-target-mutagenesis/> )

#### Gene drive research using CRISPR/Cas9

Gene drives are genetic systems that transfers targeted genetic alternation made in one generation to be passed on to offspring, which allows them to spread to all members of the population eventually.

Gene drive research and its implementation can have major ecological and/ or evolutionary impacts. Since risk depends on the alteration of species rather than on the drive itself, proposed gene drive experiments must be evaluated on a case-by-case basis. Some considerations for safe gene drive research are as follows:

- Build and optimize gene drives in a species without risking release into wild populations (by separating required components (so they can't be copied together) and having them cut sequences that aren't present in wild populations.
- Build and test a reversal drive for every primary drive that could spread a trait through a wild population as driven alterations can be reversed with another drive.
- Immunize populations against gene drives (as necessary).
- Use safeguards and containment barriers:
  - *Ecological* (Prevent organisms from escaping)
  - *Reproductive*: Use organisms that cannot reproduce with the wild counterpart

- *Molecular confinement*: Split drive mechanisms

## **B. Questions/Issues to consider (risk-assessment) for reviewing or submitting CRISPR-Cas9 protocols**

Below is a list of questions that may help reviewers and investigators 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.

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, including:
  - a. How will the gRNA and Cas9 be delivered to the cells or tissues?
  - b. How was/were the targeting sequence(s) designed?
  - c. How was/were off-target site/s evaluated?
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.
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. 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.
5. 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!
6. Will the research involve the creation of a gene drive experiment (i.e., a system that greatly increases the probability that a trait will be passed on to offspring)? See previous section ([under Gene drive research using CRISPR/Cas9](#)) for biosafety guidance on gene drive experiments.

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.
7. 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.
8. Will the gene editing technology be used for human gene therapy research? If so, the biosafety protocol must include IRB submission information.

**Resources:**

- A good source from GENE CARDS for understanding the transgene(s) being silenced, restricted, activated or over-expressed:  
<http://www.genecards.org/>
- A good resource from Addgene for working with CRISPR-Cas9:  
<https://www.addgene.org/CRISPR/>
- Harvard-Yale CRISPR-Cas9 symposium presentations  
<http://www.harvardyalesymposium.com/#!presentations/wiq15>
- Gene drive information
  - a) <http://wyss.harvard.edu/viewpressrelease/227/gene-drive-reversibility-introduces-new-layer-of-biosafety>
  - b) <http://wyss.harvard.edu/staticfiles/newsroom/pressreleases/Gene%20drives%20FAQ%20FINAL.pdf>
  - c) Akbari, Omar S., et al. "Safeguarding Gene Drive Experiments in the Laboratory." *Science* 349.6251 (2015): 927-9. Web.
- Enhancing CRISPR targeting specificity and decreasing off-target effects  
<http://blog.addgene.org/enhancing-crispr-targeting-specificity-with-espcas9-and-spcas9-hf1>  
<http://www.genecopoeia.com/resource/crispr-cas9-specificity-taming-off-target-mutagenesis/>