

CRISPR/Cas9 Biosafety Guide

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. Researchers interested in using CRISPR, or other genome editing technologies such as Transcription Activator-Like Effector Nucleases (TALENs) and/or Zinc Finger Nucleases (ZFN), are required to submit their research to the [Temple University Institutional Biosafety Committee](#) (IBC) for review.

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

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:
 - 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. Will unexpected mutations due to off-target are 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.
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.
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!

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

If you have any questions or concerns regarding your research, please contact IBC at ibc@temple.edu.

Adapted from http://rehs.rutgers.edu/pdf_files/crispr.pdf.