Guidance on Safe Use of CRISPR/Cas9 Genome Editing Technology

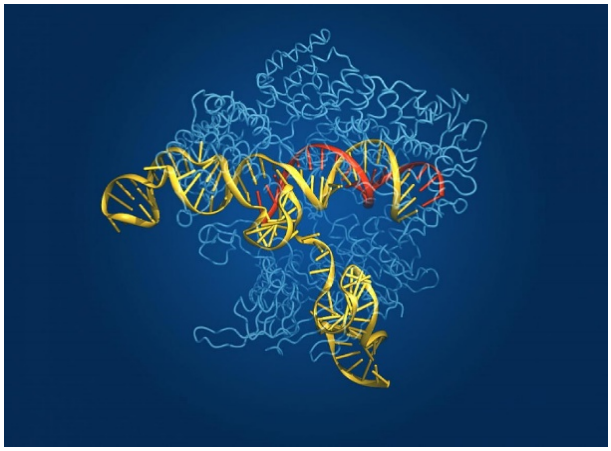
1. BACKGROUND

The process for editing genomic sequences within cells has been greatly simplified due to recent technological discoveries. These new procedures are rapidly becoming used for a broad variety of strategies, from inactivating a selected gene to insertion of engineered, foreign sequences. Furthermore, the ability to introduce site-specific double-stranded DNA breaks vastly enhances the repair processes that are required for genome editing and also allows for introduction of various desired permanent genetic changes including insertions, deletions and substitutions - in the genomes of organisms.

Zinc Finger Nucleases (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 in genome engineering. The newest of these methods is CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats). As a very powerful genome editing technology and due to its adaptability, simplicity and low cost, it has become widely used.

There are three major types of CRISPR systems (CRISPR - I-III). The type II CRISPR/Cas9 system takes advantage of the bacterial immune system. It is derived from *Streptococcus pyogenes* and consists of 2 components: 1) a "guide" RNA (gRNA) and 2) a non-specific CRISPR-associated endonuclease (Cas9).

The gRNA is a synthetic RNA composed of a "scaffold" sequence necessary for Cas9-binding and a user selected of approximatively 20 nucleotide "targeting" sequence, which defines the genomic target to be modified. By changing the targeting sequence present in the gRNA, the genomic target of Cas9 changes. As a result, an organism's genome can be cut at any desired location by having the Cas9 protein and the appropriate gRNA delivered into a cell.



Caption: Crystal structure of the Cas9 gene-editing enzyme (light blue) in complex with an RNA guide (red) and its target DNA (yellow).
Credit: Bang Wong, Broad Institute of Harvard and MIT, Cambridge, MA

2. Challenges with CRISPR/Cas9 Use in Research

CRISPR/Cas9 system use does not present, in itself, a risk to the researchers or community. The result of its use has generated, however, various concerns such as:

1. ***Potential for off-target genome editing effects or on-target events with unintended consequences***

CRISPR/Cas9 technology can induce site-specific DNA mutations in human DNA and off-target effects of the CRISPR/Cas9 system have been observed. They are a partial result of incomplete homologies between the gRNA and other regions of the genome. The implications of these off-target effects are not currently known. However, exposures of laboratory workers to CRISPR/Cas9 materials could potentially generate inactivation of tumor suppressor genes, activation of oncogenes, etc. Of high importance is also the fact that while usually biosafety is concerned with individuals at risk from lab-acquired infections, in this case populations unaware of the experiments are at risk from an accident.

Post exposure prophylaxis for researchers working with the CRISPR/Cas9 tools and inadvertently becoming exposed to the materials used is not yet known to be available.

2. ***Creation of synthetic gene drives***

Gene drives creation could be a result of the CRISPR/Cas9 technology use; in these instances, a targeted modification of an organism's genome generates a more efficient spread of a trait through the intended population of sexually reproducing organisms, as compared to Mendelian inheritance. In time, the targeted genetic alteration would be expected to spread to all members of the population.

Considerations regarding unknown risks to both target and non-target species and overall ecosystem must be made during the initial phase of a research proposal development, since creation of gene drives can have important evolutionary and ecological impacts. In the laboratory, creation of gene drives and inadvertent release of the organisms kept under containment is an additional concern.

Some recent studies, however, suggest that factors such as natural genetic variation and inbreeding may represent substantial impediments to the propagation of CRISPR drives.

3. ***Creation of high-risk biological agents*** (dual use research of concern)

Biosecurity concerns have been highlighted with regards to the possibility of generating biological agents of high risk by means of CRISPR/Cas9 technology: e.g. increasing pathogenicity, host-range, transmissibility; introduction of antibiotic resistance and increasing toxin production in various crops, etc. by organizations or persons with malicious intent.

3. Research Proposals involving CRISPR/Cas9

Use of CRISPR/Cas9 tools require prior KAUST Institutional Biosafety and Bioethics Committee (IBEC) approval since they fall under the r/s N/A research. KAUST researchers are advised to consider the following questions (see ***Table 1.***) when developing their research protocols involving CRISPR/Cas9 or other genome editing technologies. Their consideration will allow KAUST Institutional Biosafety and Bioethics Committee to more easily assess the biosafety concerns associated with proposed protocols.

Table 1. Risk Assessment and Risk Management - genome editing tools

Risk Assessment Questions (Technology used: CRISPR/Cas9, Zinc Finger Nucleases, TALENs, etc.).	**Possible mitigation/ Risk management**
1. Will the research involving the technology be done in bacteria, cell cultures, invertebrates, vertebrates, plants?	Technology use in: a) Animals – might require also IACUC approval b) Insects and plants - require specialized containment c) Human cell lines - require **BSL-2** practices and procedures d) Human clinical studies – see *Note1* below table*
2. If animal work is involved, will syringes be used for injections?	If YES, 'no recapping' should be strictly enforced; syringes with integral safety features must be used.
3. How will the technology be delivered to the host? (by means of viral vectors, plasmids, liposomes, nanoparticles, etc.?) 3.1 Will the gRNA and Cas9 be delivered on a single transfer plasmid/ transfer vector/ delivery vehicle together, or on separate plasmids/ transfer vectors/ delivery vehicles (greater safety achieved)?	a) Level of containment is determined by the gene type and viral vector used. Retro/ Lenti viruses can stably integrate into host cell chromosome; can inactivate tumor suppressor genes or activate proto-oncogenes. If using a human target gene sequence, it will include Adeno Associated Viral vectors (AAV). b) When both Cas9 and gRNA are delivered together on the same viral vector additional risks for laboratory workers might be present (e.g. possibility of inactivating one or more human tumor suppressor genes due to possible autoinoculation, ingestion, inhalation, etc.) Any potential risks from accidental exposures should be considered and experimental design justified. c) Examples of Research activities and recommended biocontainment levels: *BSL-2/ ABSL-2*: - genes associated with toxicity or allergenicity - gRNA and Cas9 on the same vector or plasmid - replication defective viruses, ecotropic retroviral vectors, other defective vectors in the same risk group - inserted nucleic acid targeting cell cycle, cell division, cell activation, cell growth, etc. *BSL-2 + (enhanced)*: - gRNA and Cas9 on the same plasmid or vector - retroviral vectors with amphotropic packaging cell lines - lentiviral vectors - tumor suppressor gene knock-out

	- human cellular or viral oncogene knock in - large libraries targeting the human genome
4. Will you be constructing a gene drive (viral or non-viral delivery) in invertebrates, vertebrates or plants?	Describe experiment's safety aspects of the delivery mechanism and address the containment guidelines identified below (use at least 2 strategies to ensure containment): I. **Molecular containment** – 1) will you use gRNA and nuclease in separate loci ('split drive')? 2) Will a synthetic target be used that is absent from the wild type target organism? Did you engineer a 'reversal drive' to be able to alter the drive created in the population? II. **Ecological containment** – Conduct experiments in areas with no wild populations in which modifications could take place. III. **Reproductive containment** – Use Laboratory organism/ isolate that cannot reproduce with wild type organisms; use synthetic sequences not found in the wild. IV. **Barrier containment** – Describe containment barriers utilized to ensure modified organism will not be released within the environment (e.g. physical, chemical, temperature control, etc.)
5. Will the technology target embryos or germ line cells? (beyond standard transgenic animal protocols)	a) Requires IACUC approval b) See also *Note 2**
6. How many genes do you plan on targeting? - Single? - Multiple (how many)? - Libraries (hundreds/thousands/more?)	The risk of the off-target effects are possible if multiple genes are targeted; risk further increases if tumor suppressor genes are targeted. Higher containment might be needed.
7. Do you know or anticipate potential off target effects or mutations?	a) Consider Cas9 variants/ mutants for increased specificity and safety during gene editing (reduce off-target effects) More information about Cas9 variants: http://www.genecopoeia.com/resource/crispr-cas9-specificity-taming-off-target-mutagenesis/ b) In case of use of systems targeting non-human genes, a genome target scan of the gRNA sequence highly recommended to identify potential off-target effects on

	the human genome in order to assess the risks. To conduct the genome target scan please see: www.rgenome.net/cas-offinder If high homology between the animal and human gRNA sequences, higher containment measures might be required.

*Note 1** Human clinical studies involving CRISPR/Cas9 are not currently pursued at KAUST. Please contact IBEC at ibec@kaust.edu.sa to obtain the most current KAUST position related to the use of genome editing technologies in humans.

*Note 2** The United States National Institutes of Health's (NIH) Policy on genome editing technologies prohibits genome-editing experiments in human embryos. Genome-editing experiments in human somatic cells are allowed. Please contact IBEC at ibec@kaust.edu.sa to obtain most current KAUST position related to the use of genome editing technologies in humans.

4. References

1. Carroll D*. Mol. Therapy* 24(3) 412-413. 2. Akbur et. al. *Science* 349(6251): 927-929. https://www.nih.gov/about-nih/who-we-are/nih-director/statements/statement-nih-funding-research-using-gene-editing-technologies-human-embryos
2. A good source from GENE CARDS for understanding the transgene(s) being silenced, restricted, activated or over-expressed: http://www.genecards.org/
3. Gene drive information
   https://wyss.harvard.edu/gene-drive-reversibility-introduces-new-layer-of-biosafety/

   https://wyss.harvard.edu/staticfiles/newsroom/pressreleases/Gene%20drives%20FAQ%20FINAL.pdf
4. Akbari, Omar S., et al. "Safeguarding Gene Drive Experiments in the Laboratory." *Science* 349.6251 (2015): 927-9. Web.
5. Bae S., Park J., & Kim J.-S. Cas-OFFinder: A fast and versatile algorithm that searches for potential off-target sites of Cas9 RNA-guided endonucleases. Bioinformatics 30, 1473-1475 (2014).

5. CONTACT

Please contact IBEC at ibec@kaust.edu.sa or Research Safety (ResearchSafety@kaust.edu.sa) for questions.