Biosafety Protocol Registration Form F – CRISPR/Cas9 (Gene Drives)

Protocol Title:

Principal Investigator (PI)/ Teaching Lab Instructor:

Section 1 - Description of Biological Materials

1. Provide the species and common name for all organisms that will be used in "gene drive" experiments:

2. Describe the function or intended function of the "gene drive" transgene construct (include any designed or engineered assembly of sequences):

3. Source of the genetic material that will be used:

4. Select the category of "gene drive" that will be used:

☐ Homing – defining features (includes CRISPR/Cas9 type systems) *(skip Section 3 – Selective Survival of Gene Drives)*

- at least one encoded nuclease
- a target site for that nuclease in the host genome
- reliance on host cell DNA repair machinery to fix the induced break using a homology-based process

☐ Selective survival – defining features *(skip Section 2 – Homing Gene Drives)*

- at least one encoded molecule ("toxin") that reduces an activity essential to the host cell/organism
- this "toxin" must threaten the health of cells/gametes/embryos even if they do not inherit the encoding gene
- (optional) at least one encoded molecule that restores the essential activity ("antidote")

☐ Combination of homing and selective survival features

Idaho State University

Section 2 - Homing Gene Drive Risk Assessment

5. What is the source of the nuclease?

6. Is the nuclease an integrated gene? ☐ Yes ☐ No

7. If integrated, explain the relationship between the integration site and the target site of the nuclease. Otherwise type N/A:

8. What is the nature of the target site? ☐ Engineered ☐ Present in wild type genome

9. If present in the wild type genome, is the target site(s) conserved or polymorphic in wild populations?
☐ Yes ☐ No

10. If conserved, is the target site present in other species where fertile hybridization is possible?
☐ Yes ☐ No

11. Explain the potential for resistance to develop and be selected for at the target site for the gene drive transgene?

12. For the host organism, are there anticipated fitness costs to individuals heterozygous or homozygous for the transgene? ☐ Yes ☐ No

13. Are anticipated fitness cost limited to a specific sex? ☐ Yes ☐ No

Section 3 - Selective Survival Gene Drive Risk Assessment

14. Will an essential function be reduced in one sex only for the host organism? ☐ Yes ☐ No

15. Non sex-distorter questions:

- Will the essential function be reduced in cells/embryos that do not inherit the transgene?
☐ Yes ☐ No

- Is a separate gene provided to restore the activity of the essential function?
  ☐ Yes ☐ No

- Explain the relationship between the gene responsible for reducing an essential function (toxin) and the corresponding rescue gene (antidote)?

16. Sex-distorter - Explain the relationship between the gene responsible for reducing an essential function and the chromosome region that determines the sex that is unaffected by the transgene:

Section 4 – Facilities and Containment

17. Can the transgene (or combination of transgenes) be reasonably expected to persist or spread through a natural population if introduced? ☐ Yes ☐ No

18. Select the most appropriate risk level associated with the transgene (or combination of transgenes) to reasonably persist or spread through a natural population if introduced:

☐ Very low – transgene that cannot persist/spread in the wild (gene drive examples include homing-drives absence of target sequence or underdominance)

☐ Low – transgene that can persist in the wild, but cannot spread (gene drive examples include homing-drives where species does not exit in area or target site is limited)

☐ High – transgene that may spread/persist in the wild, but cannot transfer to new species (gene drive examples include homing-drives possessing resistant alleles that cannot be selected or the target site is absent in related species)

☐ Very high – transgene that is likely to spread/persist in the wild and presents a significant risk of horizontal transfer to new species (gene drive examples include homing-drives with resistance alleles that cannot be selected or target site is conserved in related species)

19. Provide the following information for biosafety cabinets that will be used:

Make/model/serial number:

Location:

Date last certified:

20. Describe standard operating procedures for preventing release into the environment (include access restrictions, record keeping, and disposal):

20. Describe standard operating procedures for training and reporting in the event of a minor or major breach that may lead to inadvertent release into the environment:

Section 5 - Risk Assessment Acknowledgement by PI/Instructor - *Please initial next to each requirement*

PI/instructor will maintain a laboratory-specific biosafety manual

PI/instructor will maintain a laboratory-specific Standard Operating Procedures to minimize risk of release of transgenes and gene drive modified organisms into the environment

PI/instructor is responsible for conducting risk assessment training for all personnel working under this protocol and maintain record that all personnel trained understand risks associated

PI/Instructor assures that the use of the "gene drive" will be conducted in accordance with the BMBL (Biosafety in Microbiological and Biomedical Laboratories) published by the CDC and NIH