



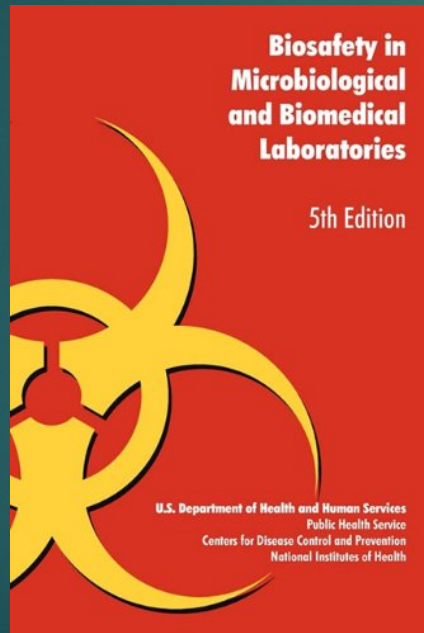
Gene Editing: Biosafety Risk Assessment Becomes **CRISPR**

Insights from the Harvard-Yale Symposium , May 3 and 4 2016, Boston, MA

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Traditional Risk Assessment

- ▶ Biosafety guidance documents available to find risk group information and other pertinent biosafety information




An illustration on a red background. A white DNA double helix is shown. A black hand is holding a lit dynamite stick with a red fuse and a small starburst at the tip, positioned as if about to drop it onto the DNA. The overall theme is genetic editing with potential risks.


CRISPR, THE DISRUPTOR

BY HEIDI LEDFORD

A powerful gene-editing technology is the biggest game changer to hit biology since PCR. But with its huge potential come pressing concerns.



'The agent risk of an RG2/RG3 agent is well defined. For emerging technologies like CRISPR, we have to wrap our brains around the outcomes'



Harvard Yale Symposium 2016 to
discuss risks posed by CRISPR/Cas9
research - May 3 and 4 2016, Boston,
MA

Main Concerns

- ▶ Oversight for CRISPR is difficult because the technology is evolving so quickly.
- ▶ There is no global (or national) consensus on what should /should not be done with CRISPR.
- ▶ Decisions about CRISPR are being made by experts, often with little input from non-experts (e.g., general public, religious community, social scientists, biosafety professionals, and so on).
- ▶ There is great potential for good, but also fear that CRISPR will be misused, on purpose or by accident.
- ▶ Who is responsible for what is created using CRISPR?
- ▶ Who is responsible if something goes awry?

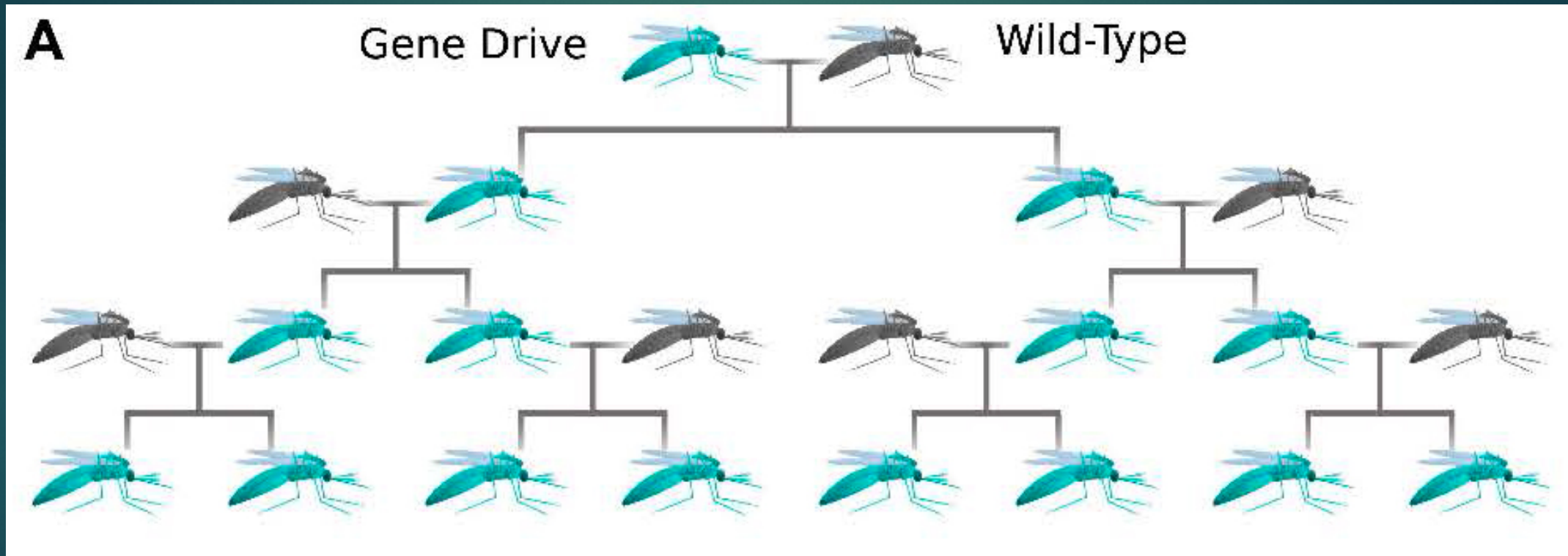
Power of collective wisdom

- ▶ Biosafety professionals must work collaboratively with investigators
- ▶ Investigators carry the onus of responsibility for the research
- ▶ BSO's understanding of the technology may never be as comprehensive as the investigators
- ▶ Utilize willing investigators as teachers to ensure this active form of collaboration is harmonized on behalf of the institution

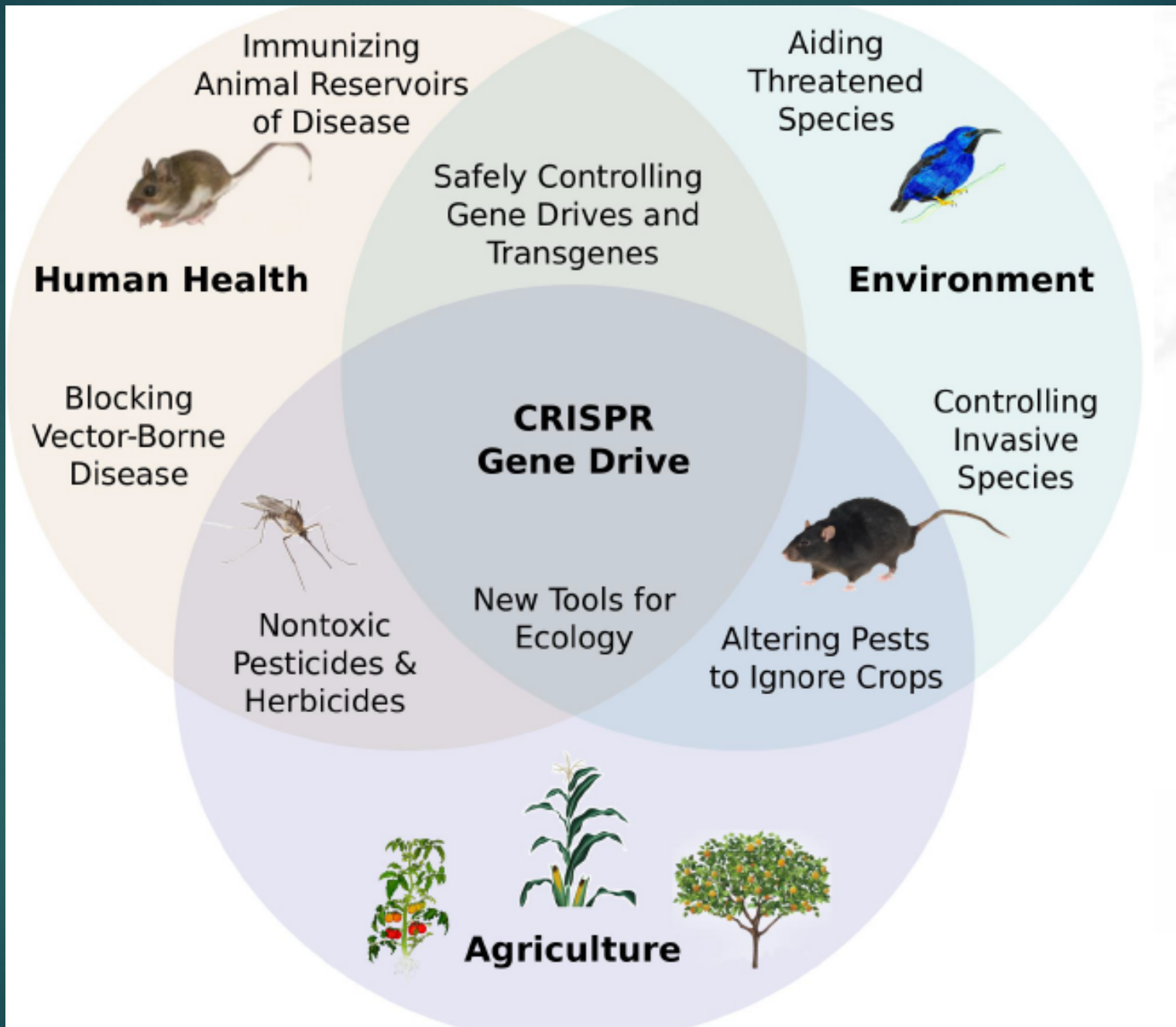
Overview of Biosafety Risks

- To all personnel in the lab
- Environmental?
- Ethical?
- Public Health?
- Public perception?

Regulating Gene Drive Experiments



Gene drive systems are capable of altering the traits of wild populations and associated ecosystems.



Extrinsic Confinement

Barriers

Prevent organisms from escaping the laboratory



Ecological

Escaped organisms cannot find mates or persist in the environment outside the lab



Can fail due to human error or deliberate action

Intrinsic Confinement

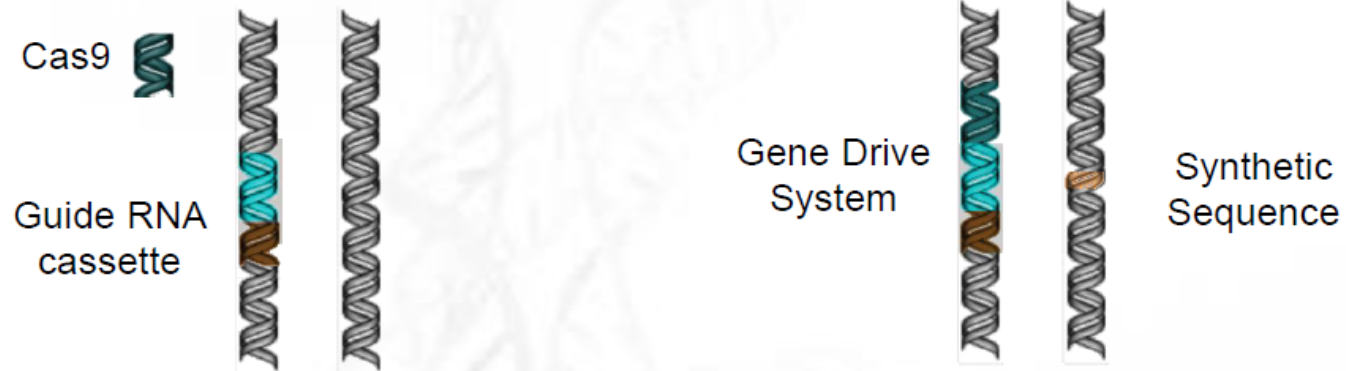
Reproductive Use organisms that cannot reproduce with wild counterparts



Molecular Build drive systems that only function in laboratory populations

Split drive: only acts in transgenic Cas9-expressing organisms

Target synthetic site: only acts in lab organisms with the target sequence



Probability of failure is unaffected by human actions

Esvelt KM, Smidler AL, Catteruccia F, Church GM (2014) *eLife*
DiCarlo J*, Chavez A*, Dietz S, Esvelt KM^, Church GM^ (2015) *Nature Biotechnology*

Potentially stringent confinement strategies for gene drive research.

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

Omar S. Akbari et al. *Science* 2015;349:927-929

Science

Are you creating a gene drive? Flowchart

- ▶ Will the experiment make transgenic, sexually reproducing organisms?
- ▶ Is an entire CRISPR system encoded in a single DNA construct?
- ▶ Could it self-insert into the genome?
- ▶ It's likely a gene drive

Safeguards

Use intrinsic confinement whenever possible (*always* for basic science)

Use extrinsic confinement even more cautiously when it is not

Always use *at least* two confinement strategies

Akbari et al (2015) *Science*

CRISPR library biosafety concerns

Framework for Risk Assessment and Risk Management

Risk Assessment	Risk Management
Pathogen	Practices (good work practices)
Procedures	Protective equipment (clothing and equipment)
Personnel	Place (facility design)

GENETIC SCREENING OF HUMAN CELLS WITH CRISPR/CAS9 LIBRARIES

- ▶ Gene editing technique (CRISPR) capable of causing permanent deletion of genes
- ▶ Human genome targeted in this experiment
- ▶ Lentiviral vector with VSV-G envelope protein (expanded tropism)
- ▶ 50,000 different Lentiviral vectors ordered
- ▶ Each vector with 3 Guide RNA sequences against human genes
- ▶ Tumor suppressor genes among those targeted
- ▶ Off target effects possible

Workspace for Conversion to BSL-2 enhanced



Dedicated or temporary designation for BSL-2 enhanced
Primary or secondary cell culture room for the lab
Scale of lentivirus production

Genetic screening of Human Cells with CRISPR/Cas9 Libraries

- This protocol could not be performed by the lab in their current location, but was critical to the lab
- Partners were selected who:
 - Provided training in advanced biosafety and additional precautions
 - Intensive internship that culminated with a live Biosafety work practice observation exam
 - Helped write detailed high-risk biosafety protocols for this work
 - Sharps eliminated, all work in a biosafety cabinet, sealed centrifuge buckets

Genetic screening of Human Cells with CRISPR/Cas9 Libraries

- Provided secure biohazard research lab space to conduct the research
 - Much less foot traffic, can reserve for dedicated time periods
 - All containment equipment and protective clothing available
- Protocol initiated safely without issues noted

CRISPR Risk Questions

- Will your research involve gene editing technologies (i.e. CRISPR/Cas9, TALEN, Zinc Finger Nucleases, Meganucleases)
- If CRISPR is involved, are the guide RNA sequence and the Cas endonuclease on the same plasmid or delivery vehicle?
- If yes, can the plasmid, vector or delivery vehicle infect a human cell?
- Does the project involve a viral vector?
- Is this a gene drive experiment?
- Will the research involve embryos or germ line cells (outside of standard transgenic animal protocols)?

Additional Questions for Consideration

○ Discuss the potential for off-target effects?

How many genes have been targeted?

○ Single?

○ Multiple? (how many? _____)

○ Library? (List #, i.e. hundreds, thousands, more? _____)

○ # of unique vectors associated with gene editing library _____?

○ # of gene editing sequences targeting each gene in the library
(per vector) _____?

CRISPR Biocontainment

BSL-2 or ABSL-2

- Cas9 and gRNA on same plasmid or vector
- Replication defective Adenovirus, Herpesvirus, ecotropic Retroviral vectors, other Risk Group defective vectors
- Research in human or non-human primate cells
- Inserted nucleic acid targeting cell cycle or cell division, transcription, cell activators, cell growth
- Genes associated with toxicity or allergenicity

CRISPR Biocontainment

BSL-2 + (BSL-2 enhanced) or ABL2+

- Cas9 and gRNA on same plasmid or vector
- Lentiviral vectors
- Retroviral vectors with amphotropic packaging cell lines
- Vaccinia virus and VSV (lab strain) vectors
- Large libraries targeting the human genome
- Human cellular or viral oncogene knock-in
- Tumor suppressor gene knock-out

CRISPR Biocontainment

BSL-3 or ABSL3

- **Any research with CRISPR/Cas9 involving Risk Group 3 Pathogens (i.e. Chikungunya virus, Mycobacterium tuberculosis, West Nile Virus, Coxiella burnetii, Yersinia pestis, Rift Valley Fever virus, Yellow Fever virus)**



Ethics of CRISPR/Cas9 research

Talking Points

- ▶ How can we move forward towards new solutions for major problems in biomedicine and the environment without causing more harm than good?
- ▶ Risk is never zero and outcomes are uncertain – until tried.
- ▶ What trade-off between the chance of benefit and risk of harm is justifiable?
- ▶ What can be done to manage and mitigate risks to biological safety?
- ▶ In how far can scientists/developers take responsibility for future applications? Should they?

What makes this research special?

- ▶ Biosafety usually concerned with individuals at risk from lab-acquired infections
- ▶ Here populations who are likely unaware of the experiments are at risk from an accident
- ▶ Rates of accident occurrence that we may accept as the price of progress when they affect 1-10 people probably not acceptable when they affect millions

Closing

- ▶ Commit to transparency from the proposal stage onwards
- ▶ Collective scrutiny is better than any regulation for ensuring safety
- ▶ Closed-door science + increasingly powerful technology = ?

Questions?

