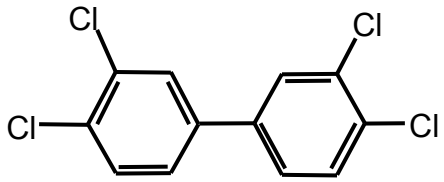
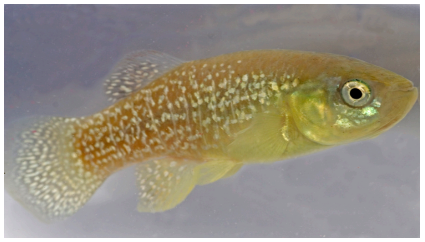
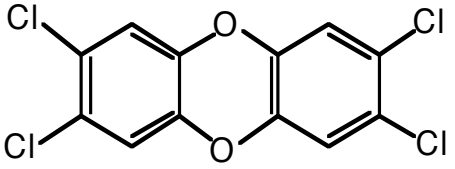
CHE Partnership call - April 2017

Gene Editing: Where Genomic Technologies Meet Environmental Health

CRISPR ways to understand gene-environment interactions







Mark Hahn

with Neel Aluru, Sibel Karchner,
and many other colleagues and collaborators

Woods Hole Oceanographic Institution, Woods Hole, MA

Boston University Superfund Basic Research Program

(http://www.busrp.org)

Boston University
Superfund Research Program

Outline

- Gene-Environment Interactions
- Chemical-adapted populations of fish
- Population genomics reveals critical role of aryl hydrocarbon receptor (AHR) signaling pathway
- CRISPR-Cas9 gene targeting
- Targeting AHR pathway genes in a non-model organism
- Significance

Gene-Environment Interactions

- Genes control response to chemicals
  - pollutants, drugs, nutrients
  - how sensitive?
  - metabolism and excretion

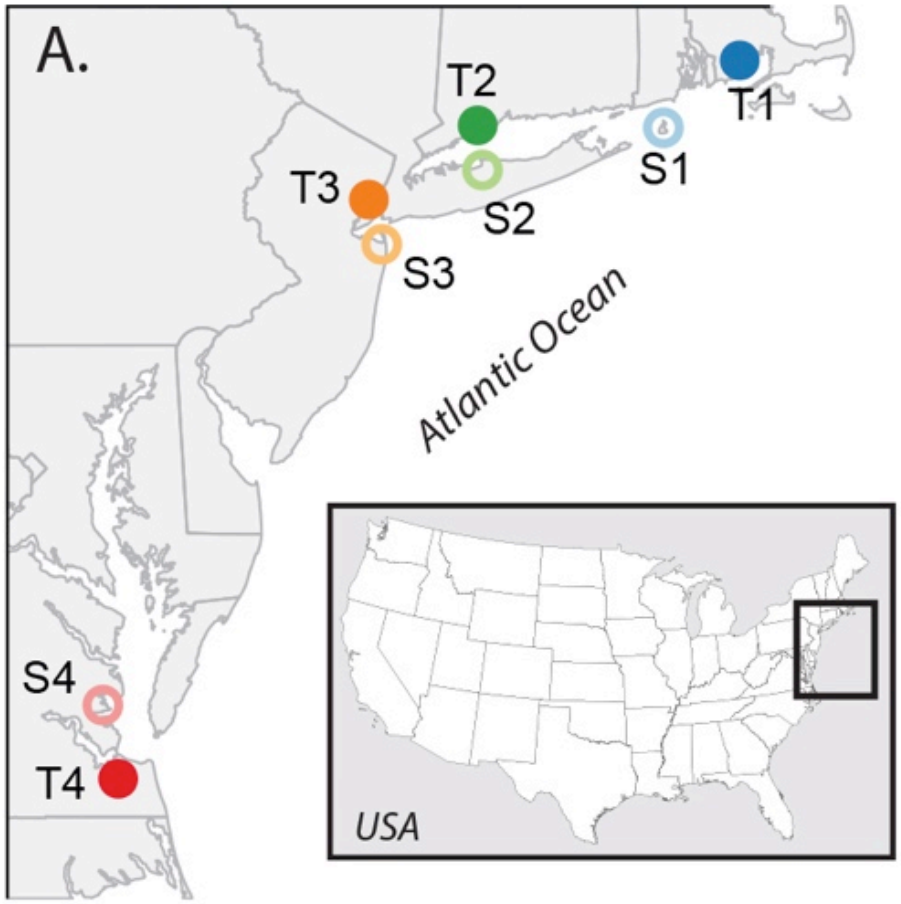
- Chemicals affect genes
  - altered gene expression
  - genetic damage
  - selection for genetic variants

Ecological Impacts at Superfund Sites

What is the impact of
long-term (multi-generational) exposure to
contaminants at Superfund sites?

- Effects on populations; multiple generations.
- Adaptation to rapidly changing environment?
- Mechanisms of adaptation?
  - Molecular basis?
  - Independent or shared among sites?
- Costs / Trade-offs

Tolerance (resistance) to aromatic hydrocarbons in four populations of Atlantic killifish *Fundulus heteroclitus*

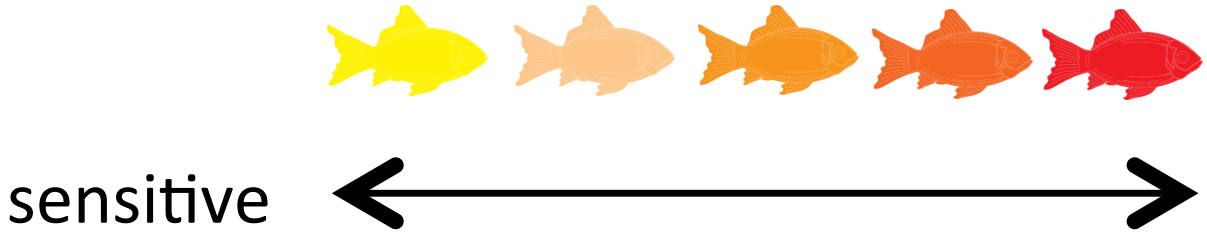


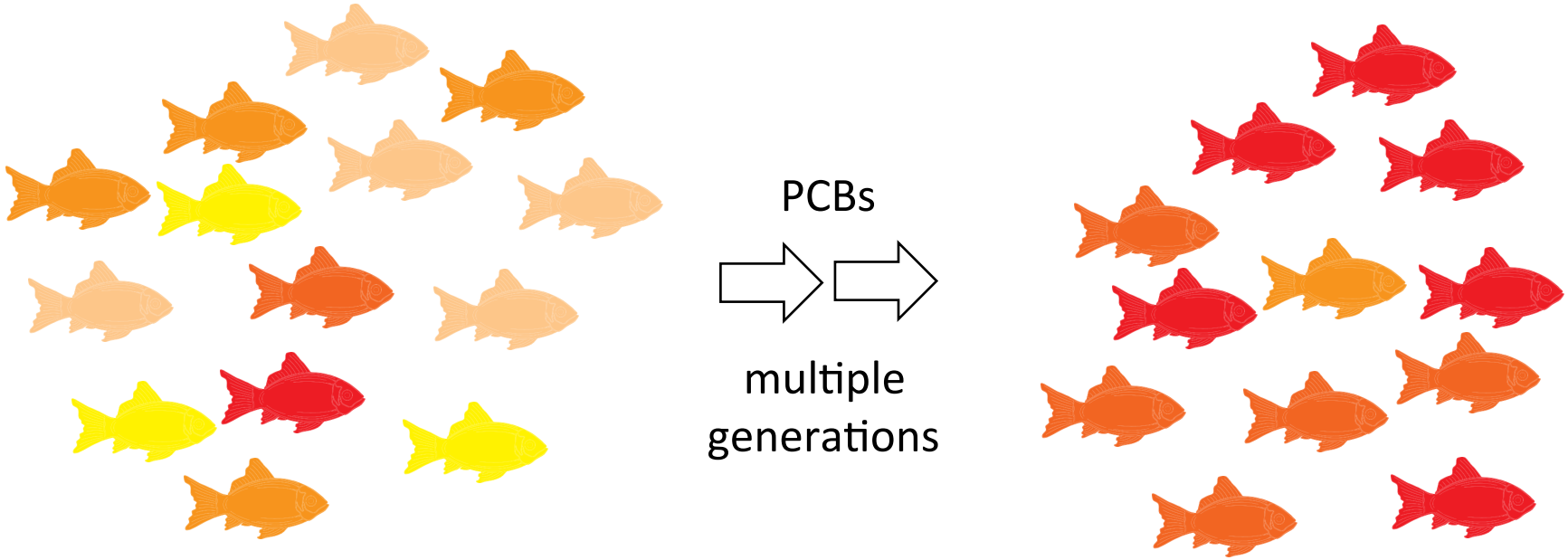
T=Tolerant S=Sensitive

- New Bedford Harbor, MA — PCBs
- Bridgeport, CT — PCBs, PAHs
- Newark, NJ — Chlorinated dioxins
- Elizabeth River, VA — PAHs

Reid *et al.* 2016 *Science* 354: 1305

Evolution of Resistance by Natural Selection





original population
(1940)

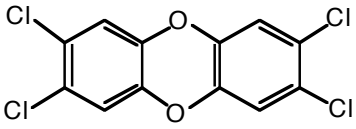
current population
(2017)

Population genomic study

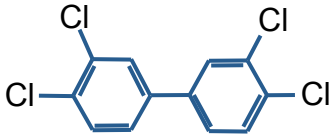
- Eight populations
- Four T-S pairs
- 48 fish per population
- Transcriptomics (gene expression)
- Genome sequencing
- Identification of genes under selection

Reid *et al.* 2016 *Science* 354: 1305

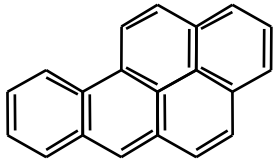
Aryl Hydrocarbon Receptor (AHR) Pathway



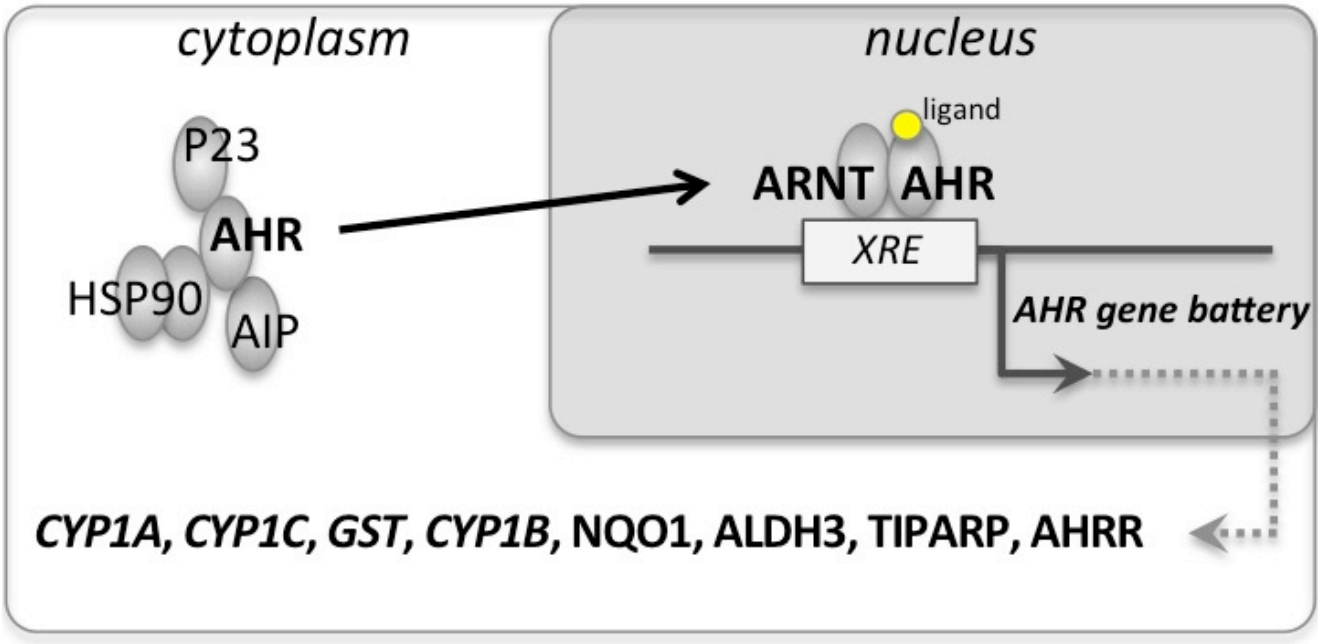
TCDD (dioxin)



PCB

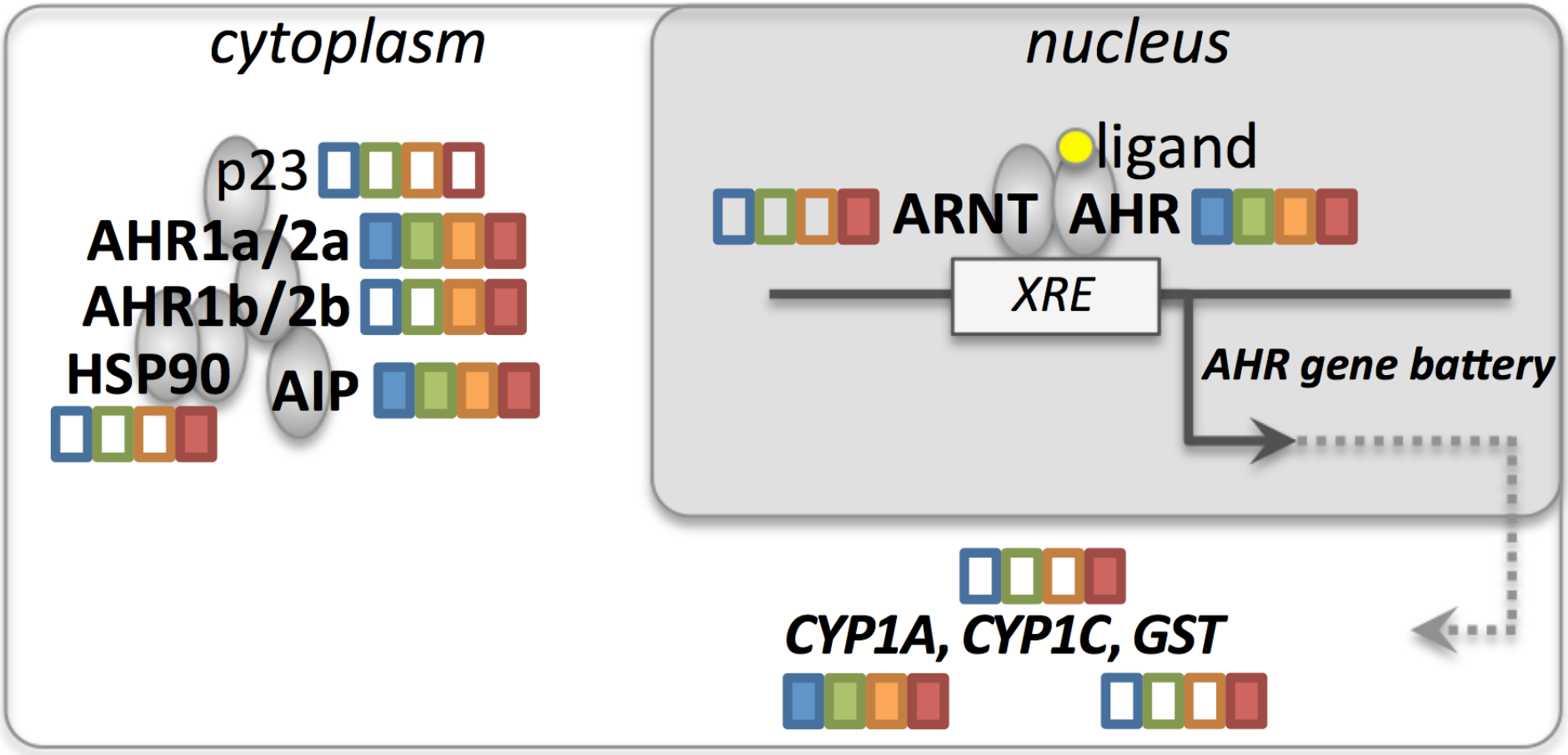


BaP (PAH)



- AHR is a transcription factor (regulates gene expression)
- Activated by TCDD, PCBs, PAHs (and others)
- Involved in mechanism of toxicity in vertebrate animals
- Physiological roles in development and immune system

AHR Pathway as a shared target of selection in multiple populations of resistant killifish



Colors = populations
Filled boxes = under selection

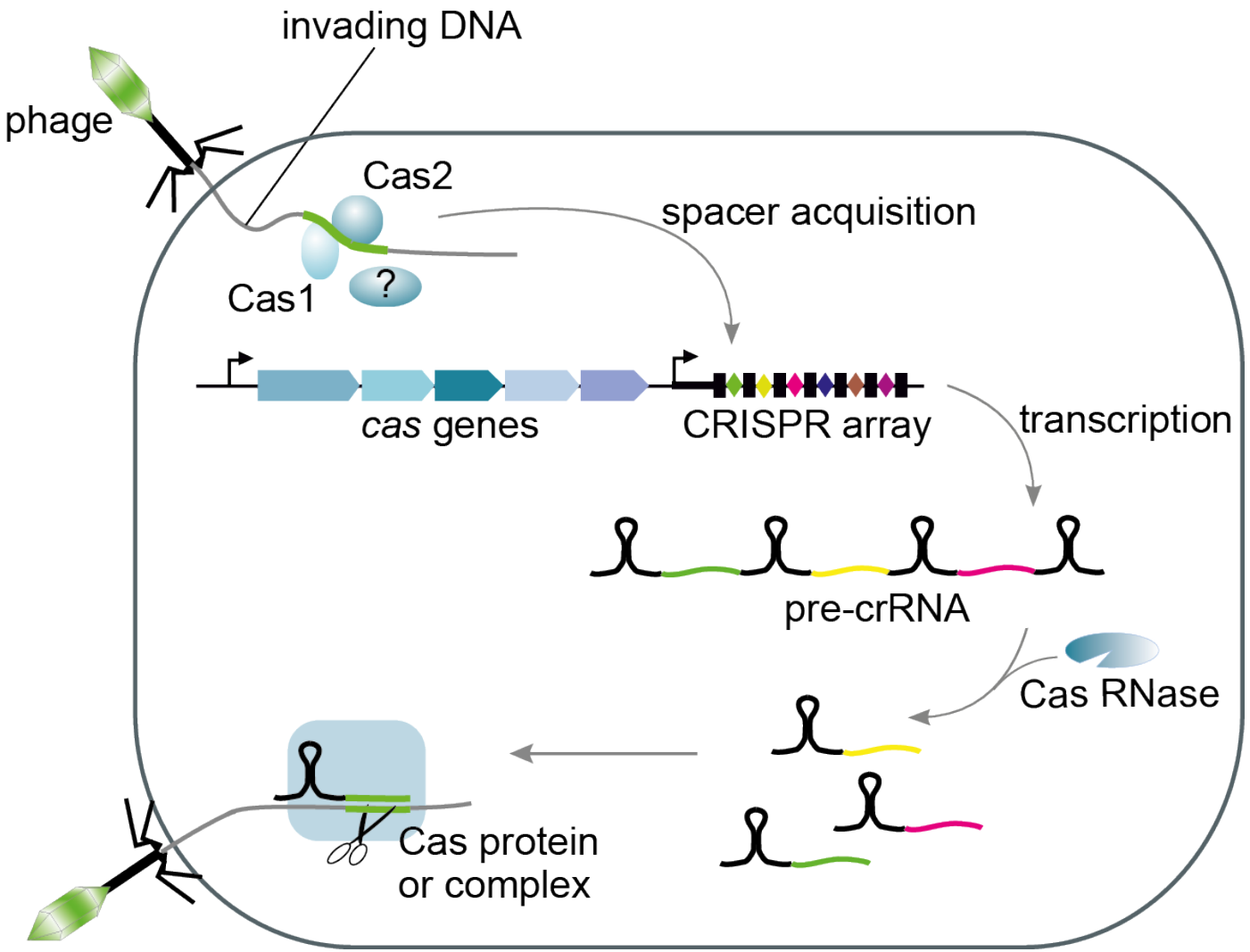
Reid *et al.* 2016 *Science* 354: 1305

Loss-of-function approaches

- Widely used way to understand gene function
- Untargeted mutagenesis
  - radiation, chemicals, retroviral
- Knock-down (reduce) expression
  - RNA interference, anti-sense RNA
- Gene knock-out (KO) (homologous recombination)
  - mice (1989)
- Targeted genome editing
  - Zinc finger nuclease (ZFN) (2005)
  - Transcription activator-like effector nucleases (TALENs)
  - Clustered regularly interspaced short palindromic repeats (CRISPR) (2013)

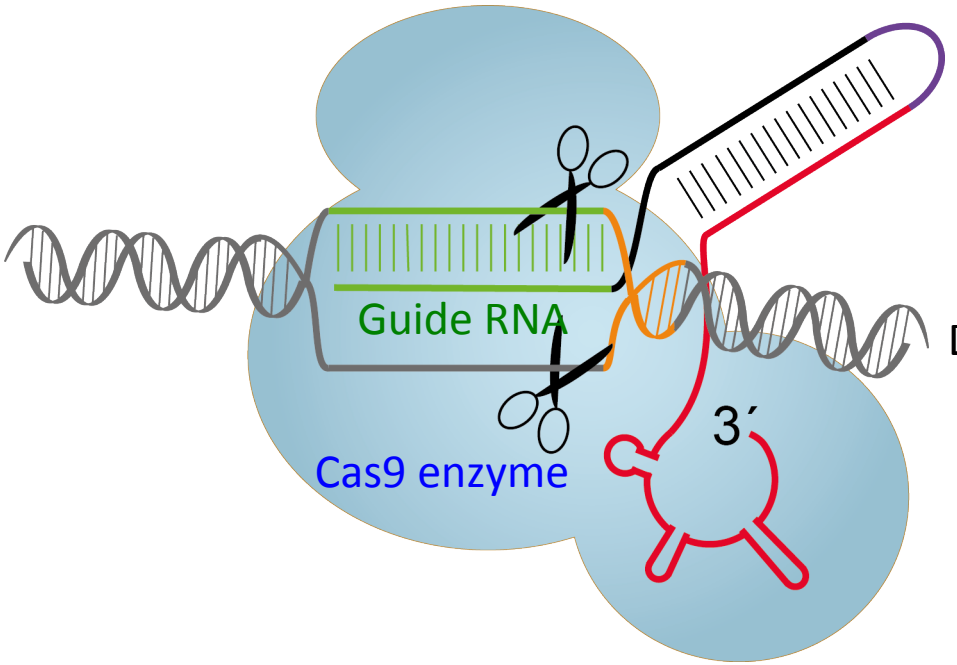
The CRISPR-Cas* adaptive immune system

**Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated (Cas)*

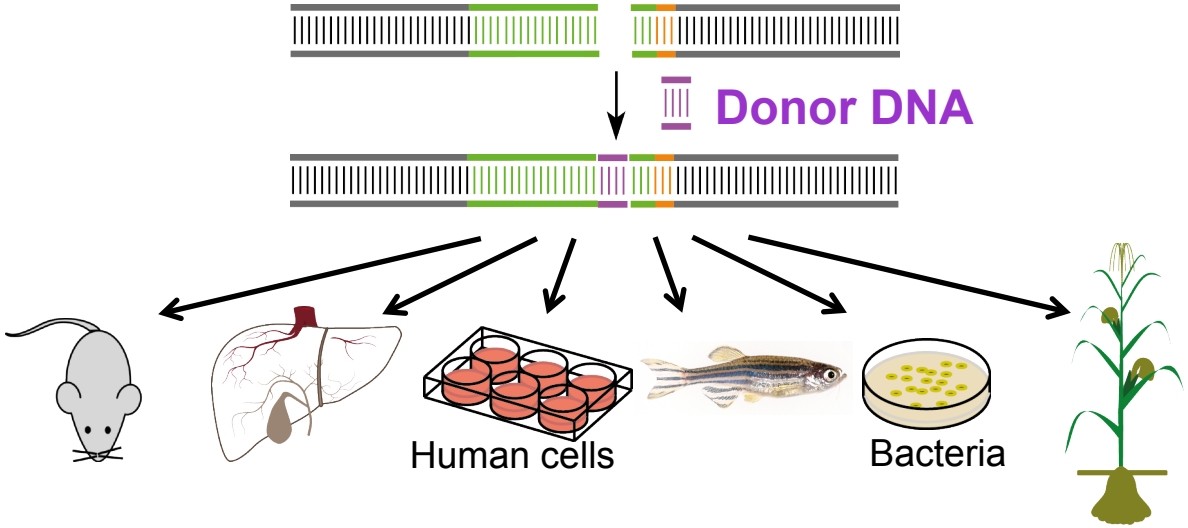


E. Charpentier (http://www.nationalacademies.org/gene-editing/Gene-Edit-Summit/index.htm)

RNA-programmable CRISPR-Cas9



- Guide RNA directs Cas9 to target
- Cas9 endonuclease cuts DNA
- Can be used in most organisms



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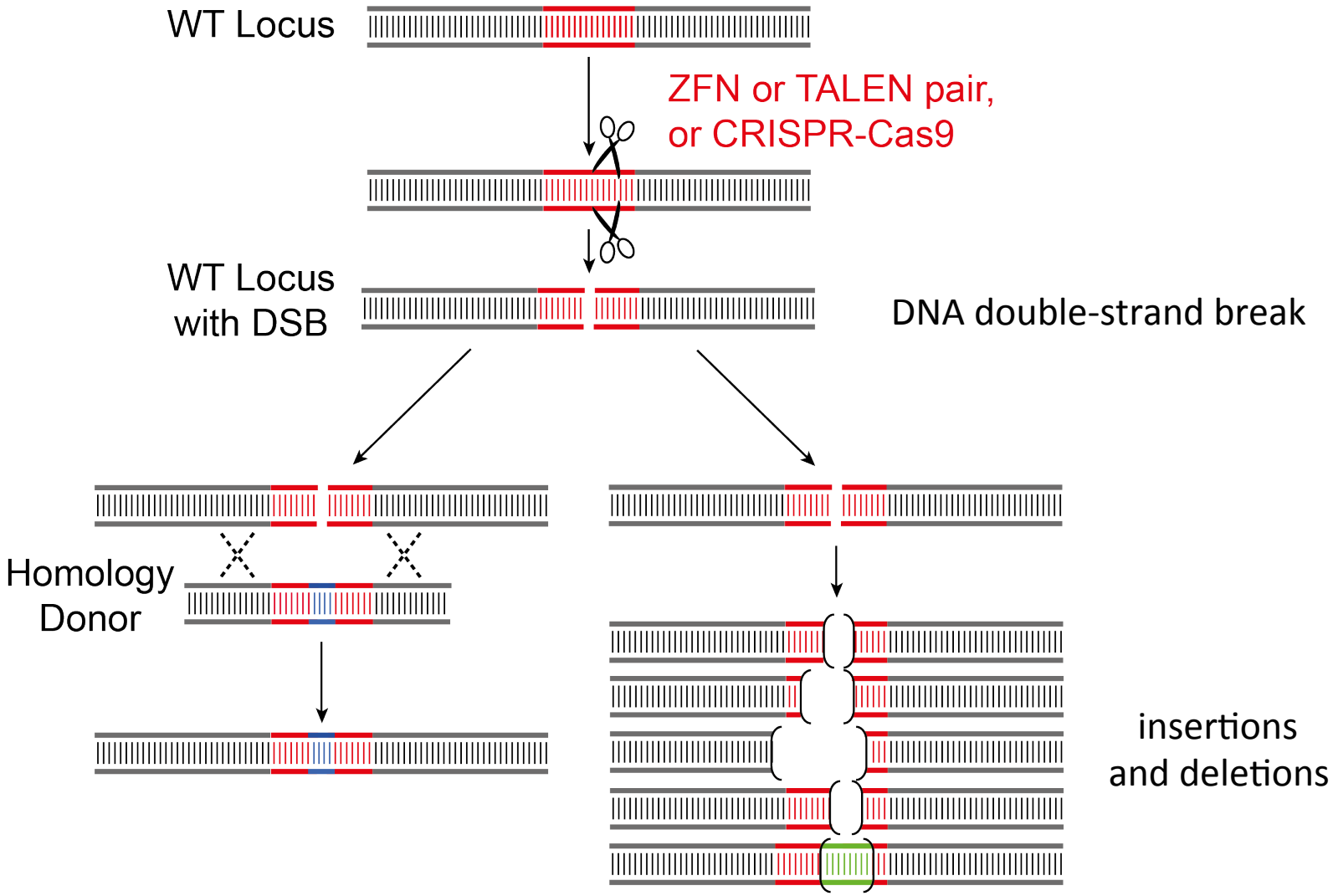
Two types of genome editing with CRISPR-Cas9



Knock-in

HDR:
Targeted Modification

NHEJ:
Error Prone Repair

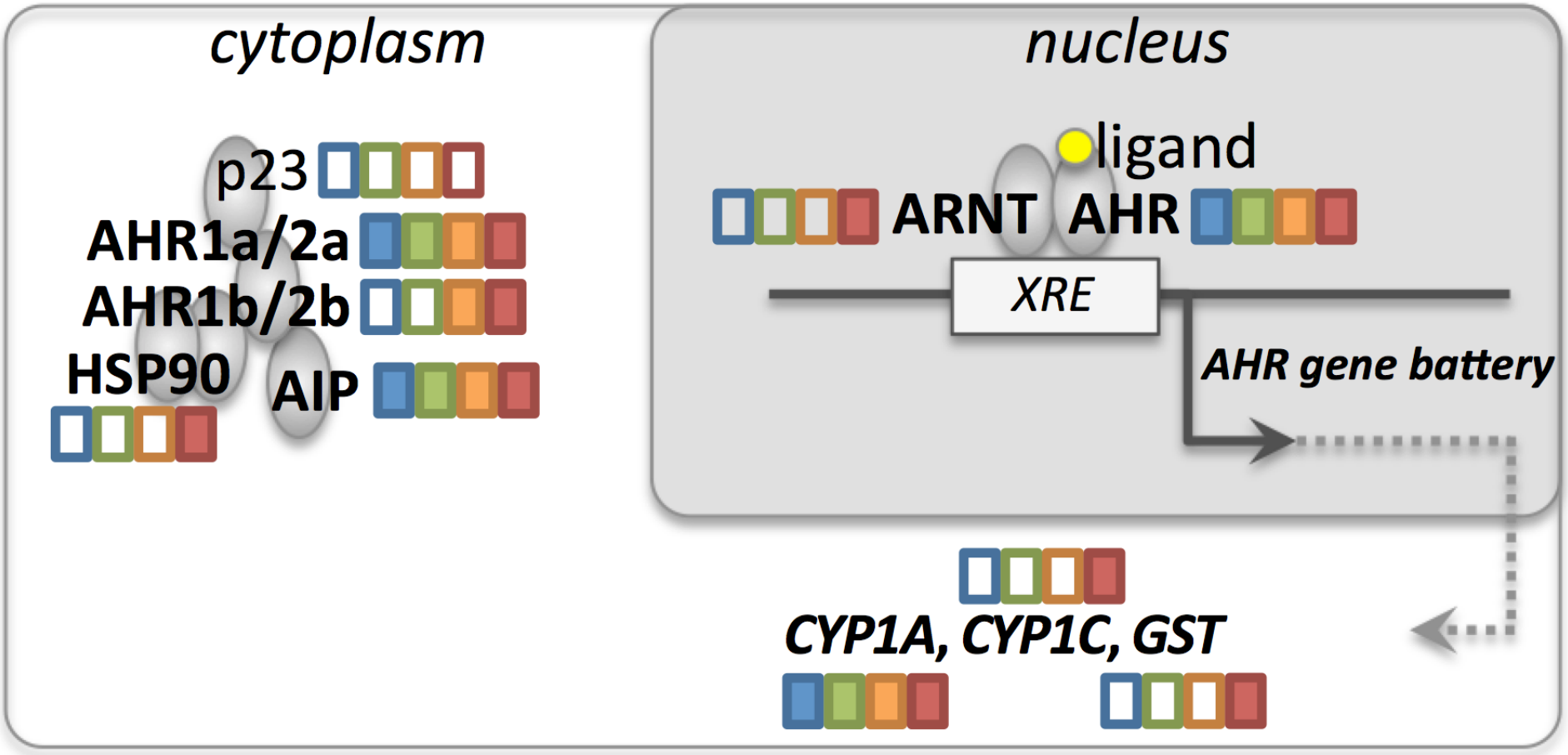
Knock-out

E. Charpentier (http://www.nationalacademies.org/gene-editing/Gene-Edit-Summit/index.htm)

Uses of CRISPR-Cas9 (selected examples)

- Targeted gene inactivation (knock-out)
- Precision gene editing, e.g. introduction of SNP
- Genome-wide gene targeting (screening) (Chris Vulpe)
- Tissue-specific gene inactivation (conditional KO)
- Targeted insertion of exogenous gene
- Targeted insertion of fluorescent protein tag (e.g. GFP)
- Targeted tagging of DNA
- Targeted transcriptional regulation (activation or inhibition)
- Targeted epigenetic modification (e.g. methylation)

Using CRISPR to target genes in the AHR Pathway: *AHR1, AHR2, AIP*

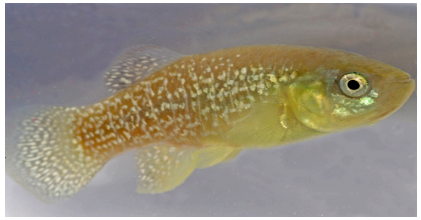


Reid *et al.* 2016 *Science* 354: 1305

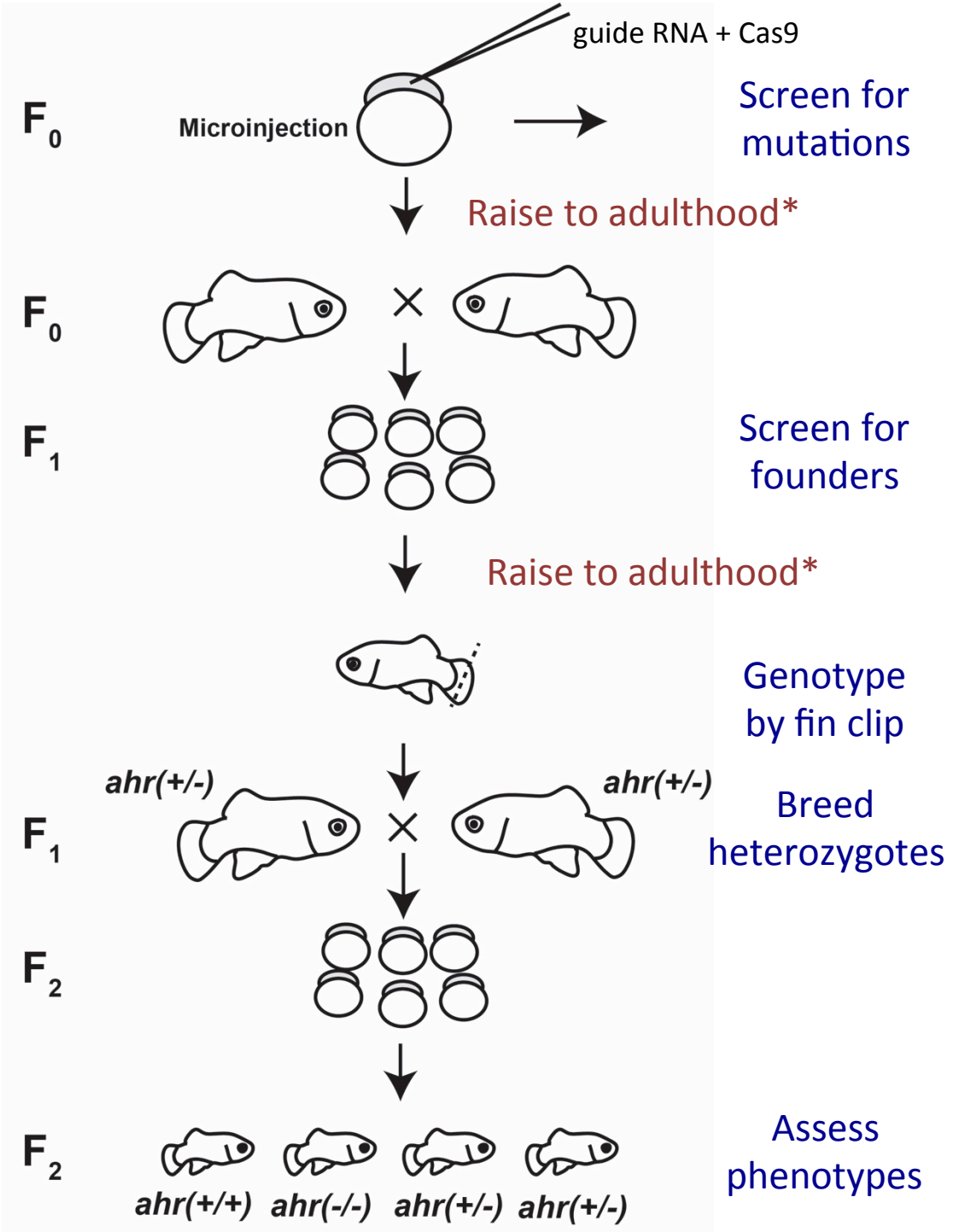
Targeting AHR and AIP genes in zebrafish and killifish

*Raising to adulthood

Zebrafish: 3 months

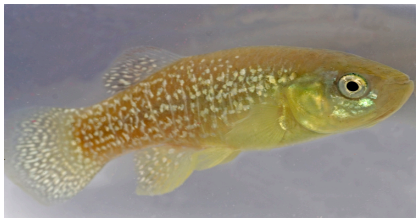


Killifish: 2 years (!)



Aluru *et al.* 2015 Aquat Toxicol 158: 192

Progress in generating knock-out and knock-in killifish and zebrafish



Zebrafish:

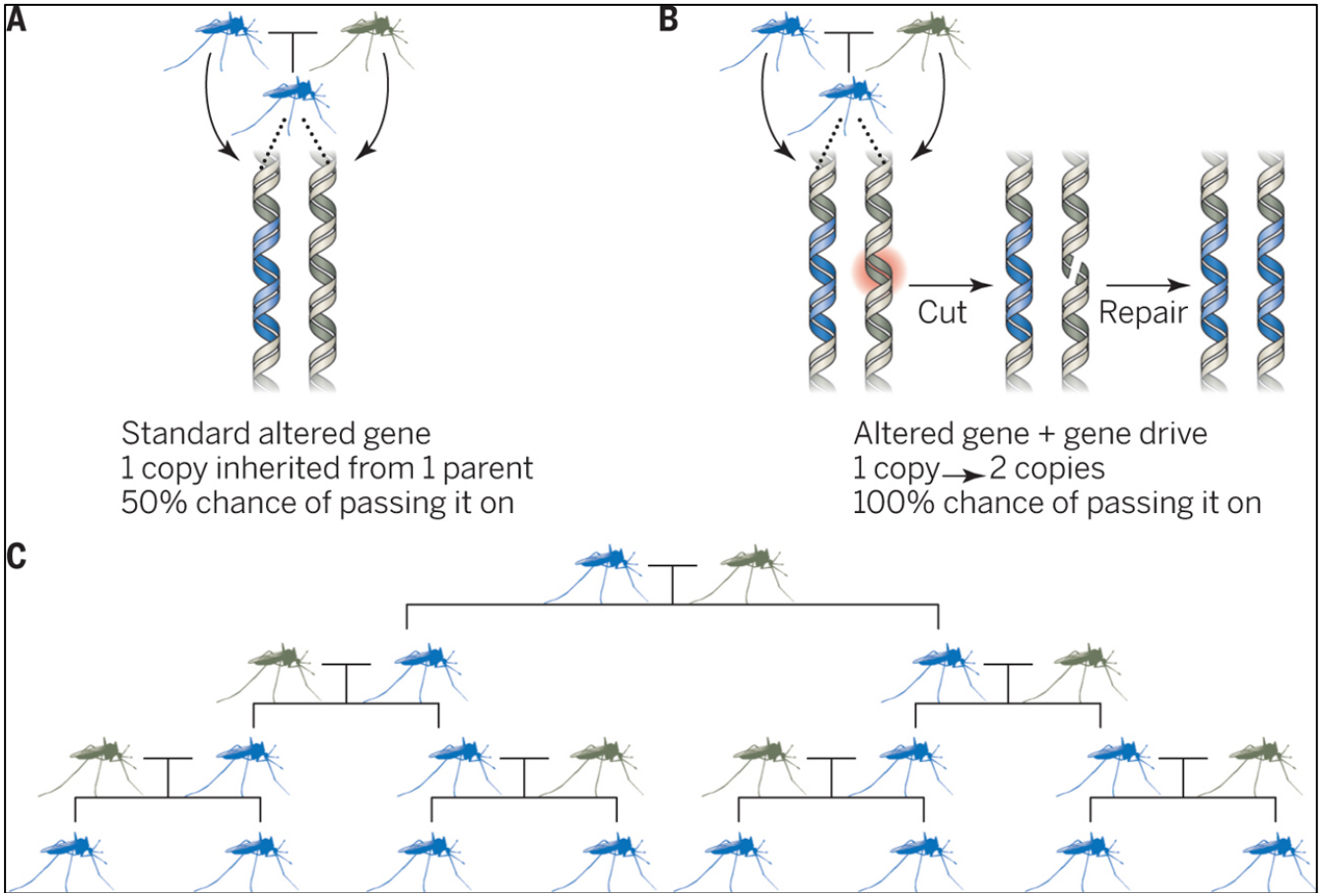
- **AHR1b:** mutant line established
- **AHRRa:** mutant line established
- **AIP mutants:** potential founders being raised and screened
- **AIP knock-ins:** in progress

Killifish:

- **AHR2a:** founders identified
- **AHR2b:** founders identified
- **AIP mutants:** potential founders being raised

We are NOT:

- Releasing genetically engineered fish into the environment.
- Using *Gene Drives* (self-propagating genome editing cassette causes mutagenic chain reaction)



Oye *et al.* (2014) *Science* 345: 626

Implications / Significance

- Which genes (AHRs, AIP) control sensitivity to PCBs.
- How long-term exposure leads to functional genetic changes in AHR pathway genes in a population.
- How knock-out (complete loss of function) might differ from knock-down (reduced function), e.g. genetic compensation (basic research).
- Lessons for human health: Humans have homologous genes that control sensitivity to these chemicals.
- What are the costs (trade-offs) of altering the function of these genes?

Thank you!!

Woods Hole Oceanographic Institution

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Noah Reid

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Andrew Whitehead
Noah Reid

Boston University Superfund Research Program

NIH National Institute of Environmental Health Sciences

American taxpayers

Additional Information

http://www.busrp.org

http://www.epa.gov/nbh/

http://www.whoi.edu/science/B/people/mhahn/hahn.html

Aluru N, Karchner SI, Franks DG, Nacci D, Champlin D, Hahn ME (2015) Targeted mutagenesis of aryl hydrocarbon receptor 2a and 2b genes in Atlantic killifish (*Fundulus heteroclitus*). *Aquatic Toxicology* 158: 192-201.

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