## Experiments performed before constructing a competent drive

Evaluate candidate target sequences for cutting efficiency and specificity Evaluate candidate guide RNAs vs equivalent sequences in related species Measure the fitness effects of expressing Cas9 + guide RNAs on the organism Build a 'dependent' cassette with guide RNAs targeting a unique sequence and no Cas9 Calculate homing efficiency in Cas9-expressing organisms with the unique sequence added Determine whether the guide RNAs to be used can cut the relevant loci of related species Generate organisms bearing the proposed genomic alterations without the drive Measure the fitness effects of the genomic alterations in the laboratory Evaluate the stability of non-drive cassettes and genomic alterations in the laboratory Model gene drive spread and population-level effects using laboratory non-drive data Run contained field trials to measure fitness of non-drive alterations in the wild Run contained field trials to measure ecological impact of non-drive alterations in the wild Model gene drive spread and population-level effects using field non-drive data

## Experiments performed after drive construction

Build and test the drive in a laboratory whose location affords ecological containment Measure the rate of drive spread through laboratory populations Test the rate of spread and effectiveness of reversal drives in the laboratory Test the rate of spread and effectiveness of immunizing drives in the laboratory Evaluate drive stability during the spreading process in the laboratory Evaluate drive stability after reaching fixation in the laboratory Determine whether the drive can spread into the loci of related species in the laboratory Model gene drive spread and population-level effects using laboratory drive data Run contained field trials to evaluate all the characteristics listed above Run contained field trials of an autonomously spreading population suppression drive Model gene drive spread and population-level effects using available drive data

## Experiments releasing a "neutral" drive that makes no other changes

Determine whether the drive mechanism itself has any ecological impact Measure gene flow in the target wild population Determine whether two successive drives can recode all members of the population

## **Relative Ecological Risk**

Negligible Negligible Negligible Negligible Negligible Negligible Negligible Negligible None Minor Minor None

Negligible Moderate Moderate Moderate Moderate Moderate None Moderate-High High None

> Moderate Moderate