Gene drive reversibility introduces new layer of biosafety

November 16 2015

In parallel with their development of the first synthetic gene drives - which greatly increase the chance a specific gene will be passed on to all offspring - George Church, Ph.D., and Kevin Esvelt, Ph.D., helped pioneer proactive biosafety measures to ensure that gene drives are investigated effectively and safely in confined laboratory experiments. They envision that synthetic gene drives designed using an RNA-guided gene editing system known as CRISPR-Cas9 - which works like a pair of molecular scissors to precisely cut or edit DNA - could one day be used outside of the lab to prevent transmission of deadly insect and animal-borne diseases and eradicate invasive species that threaten the ecosystem and agriculture.

Now, in a new study published in *Nature Biotechnology* on November 16, a team led by Church and Esvelt at the Wyss Institute for Biologically Inspired Engineering at Harvard University and Harvard Medical School (HMS) demonstrates effective safeguarding mechanisms for working with gene drives and unveils a first-of-its-kind method for reversing the changes they spread.

"Any claim of reversibility of modern technology requires strong evidence," said Church, who is a Wyss Core Faculty member, the Robert Winthrop Professor of Genetics at HMS, and Professor of Health Sciences and Technology at Harvard and MIT. "This is a major step in that direction for the field of synthetic biology."

Alongside researchers on the Wyss Institute's Synthetic Biology

platform, Church and Esvelt, who is a Wyss Technology Development Fellow, have led the gene drive research community in discussions about responsible laboratory conduct and proactive confinement guidelines for the safeguarding of gene drive research. Their latest study verifies the efficacy of safeguarding protocols developed by their team, such as increased and improved physical biocontainment barriers and the introduction of so-called "molecular confinement" mechanisms which use genetic engineering to block laboratory organisms from surviving and reproducing in the highly unlikely event they ever escaped into the ecosystem.

"The gene drive research community has been actively discussing what should be done to safeguard shared ecosystems, and now we have demonstrated that the proposed safeguards work extremely well and should therefore be used by every gene drive researcher in every relevant lab organism," said Esvelt.

CRISPR gene drives work by using sequences of RNA to guide the gene-cutting Cas9 protein to a specific target gene for editing. The molecular confinement mechanisms developed by the team prevent gene drives from functioning in the wild by manipulating these biological components. By separating the guide RNA and the Cas9 protein so that they are not encoded together in the same organism, or by inserting an artificial sequence into the targeted gene, gene drives can only be activated in lab organisms and are therefore not able to function in the wild.

"Using yeast in the lab, we also showed that a trait imposed on a population using a gene drive could be reversed," said the paper's first co-first author James Dicarlo, a graduate research assistant at the Wyss Institute and HMS. The team notes that using this safeguard, essentially any population-level change mediated by a gene drive could be subsequently overwritten if the need ever arose. In such a case, the

originally imposed trait would be reversed and the biological "machinery" of the CRISPR gene drive system - the guide RNAs and the Cas9 protein - would remain present, albeit rendered inactive, in the DNA of organisms.

The reversibility mechanism isn't just a useful backup to have on hand in case a gene drive ever had an unexpected side-effect; the ability to impose or reverse gene drive effects could also one day prove useful for the management of disease transmitting organisms such as mosquitoes, invasive species, and crop-destroying insects.

Although more research needs to be done in the lab before gene drives will ever potentially be ready for use outside of confined laboratory experiments, researchers now have the tools to perform those experiments safely. And in the meantime, gene drives themselves are useful lab tools for perturbing the genomes of lab organisms and unlocking new insights into the complex interplay of genes.

"Gene drive technology has great potential to solve global problems, such as malaria, for which we have no solutions today," said Wyss Institute Founding Director Donald Ingber, M.D., Ph.D, who is also the Judah Folkman Professor of Vascular Biology at HMS and Boston Children's Hospital and Professor of Bioengineering at the Harvard John A. Paulson School of Engineering and Applied Sciences. "But the field needs to proactively develop safeguard mechanisms and reversibility capabilities to ensure the safety of this new technology and enable its enormous potential for doing good. I am proud that our team - led by George Church and Kevin Esvelt - is championing this charge."

More information: Safeguarding CRISPR-Cas9 gene drives in yeast, *Nature Biotechnology* , DOI: 10.1038/nbt.3412