THREE TECHNOLOGIES THAT CHANGED GENETICS

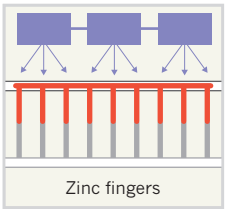
Genome editing uses enzymes that are targeted to sequences of DNA to make cuts. These cuts are then repaired by the cell's machinery. This technology allows scientists to disrupt or modify genes with unprecedented precision. By **Amy Maxmen**, *infographic by* **Denis Mallet**.

FokI

A DNA-cutting enzyme called FokI from the bacterium *Flavobacterium okeanokoites* is fused to proteins that recognize DNA.

~2000

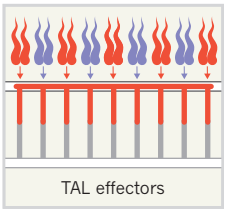
ZFNs Zinc-finger nucleases



One zinc-finger protein (purple) recognizes three DNA bases. Typically, 3 to 6 proteins are linked together to create a DNA-binding domain that is specific for 9 to 18 nucleotides.

2010

TALENs Transcription activator-like effector nucleases



A pair of amino acids (red or purple) in a single TAL effector recognizes one DNA base. Tacking TAL effectors together can generate a recognition domain of up to 40 nucleotides.

ZFNs and TALENs have the same basic form: a string of engineered proteins that recognize a sequence of bases (red) attached to FokI, which cuts the adjacent DNA.

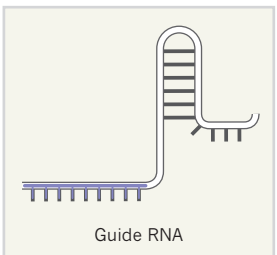
Cas9

A tool developed in 2012 uses Cas9 as its DNA-cutting enzyme. Cas9 is nearly 2.5 times bigger than FokI.

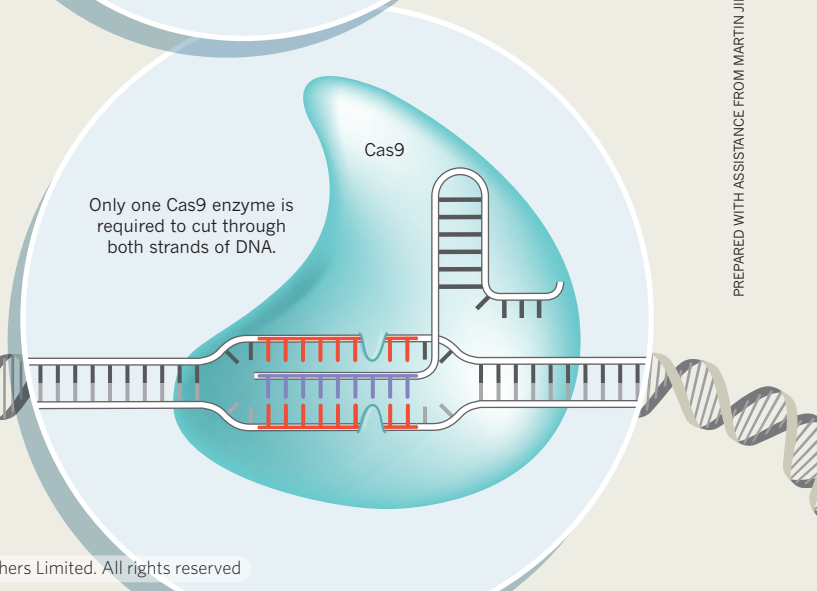
Two FokI enzymes are needed to cut both strands of a double helix. These enzymes are fused together in both ZFNs and TALENs.

2012

CRISPR–CAS9



Cas9 is taken to the correct sequence of DNA bases by a 'guide RNA' with a complementary sequence (purple). This can bind up to 22–23 base pairs.

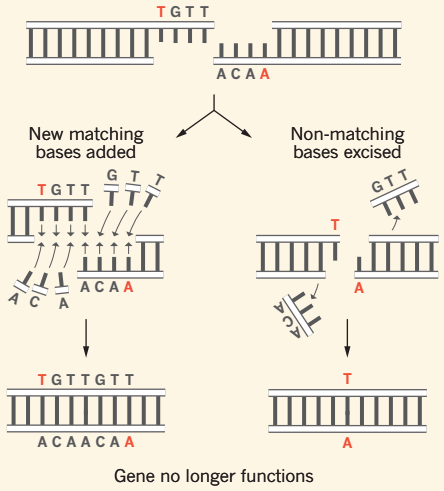


PREPARED WITH ASSISTANCE FROM MARTIN JINEK, UNIVERSITY OF ZURICH

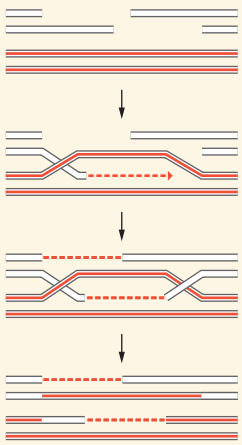
DOUBLE-STRANDED BREAK

All three of the main genome-editing tools (ZFNs, TALENs and CRISPR–Cas9) create a break across both strands of DNA at a specific location, which is repaired in one of two ways to either 'knock out' or 'knock in' a gene.

Non-homologous end joining for gene knock out
DNA is repaired in an error-prone manner — by either adding or removing bases — so that the gene can no longer be translated.



Homology-directed repair for gene knock in
A DNA template, or 'homologous sequence', accompanies the DNA-cutting enzyme so that the repair results in an altered or an inserted gene.



The DNA template (red) lines up next to the double-stranded break.

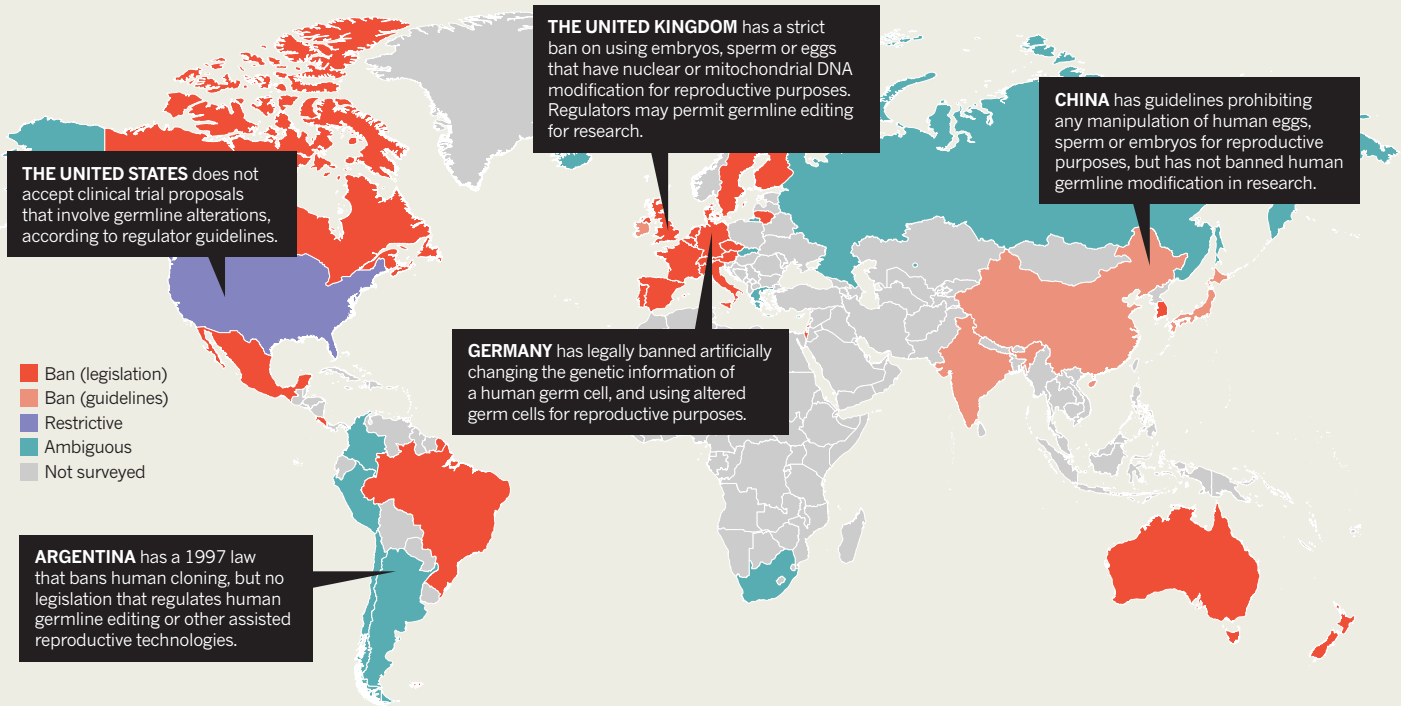
The template then 'invades' the gap, and the cell's DNA synthesis machinery generates complementary code (dashed line).

When the repair process is complete, a segment of DNA from the template may be inserted into the original break.

The template separates, leaving the original break with a new section of code.

LEGAL STATUS

Countries are grappling with how to appropriately regulate human germline editing. As of December, before a Washington DC meeting organized by UK, US and Chinese scientific societies, at least 29 countries have banned germline modification.



THE UNITED KINGDOM has a strict ban on using embryos, sperm or eggs that have nuclear or mitochondrial DNA modification for reproductive purposes. Regulators may permit germline editing for research.

CHINA has guidelines prohibiting any manipulation of human eggs, sperm or embryos for reproductive purposes, but has not banned human germline modification in research.

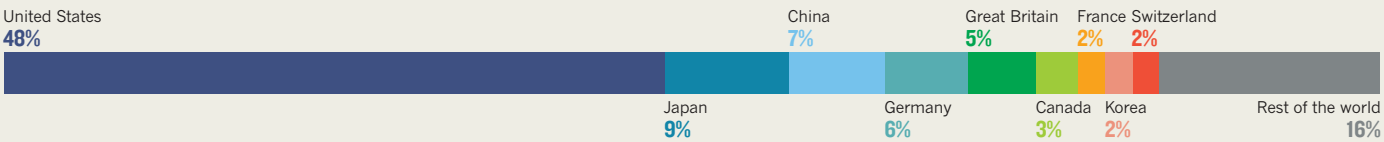
THE UNITED STATES does not accept clinical trial proposals that involve germline alterations, according to regulator guidelines.

GERMANY has legally banned artificially changing the genetic information of a human germ cell, and using altered germ cells for reproductive purposes.

ARGENTINA has a 1997 law that bans human cloning, but no legislation that regulates human germline editing or other assisted reproductive technologies.

POPULARITY

The newest of the three main gene-editing tools, CRISPR–Cas9, has spread far and wide. Massachusetts-based Addgene, a non-profit plasmid repository that distributes CRISPR–Cas9 editing kits to 83 countries, sends the largest proportion of its kits to US researchers.



MAP SOURCE: ARAKI, M & ISHII, T. *REPROD. BIOL. ENDOCRINOL.* **12,** 108 (2014); CHART SOURCE: ADDGENE