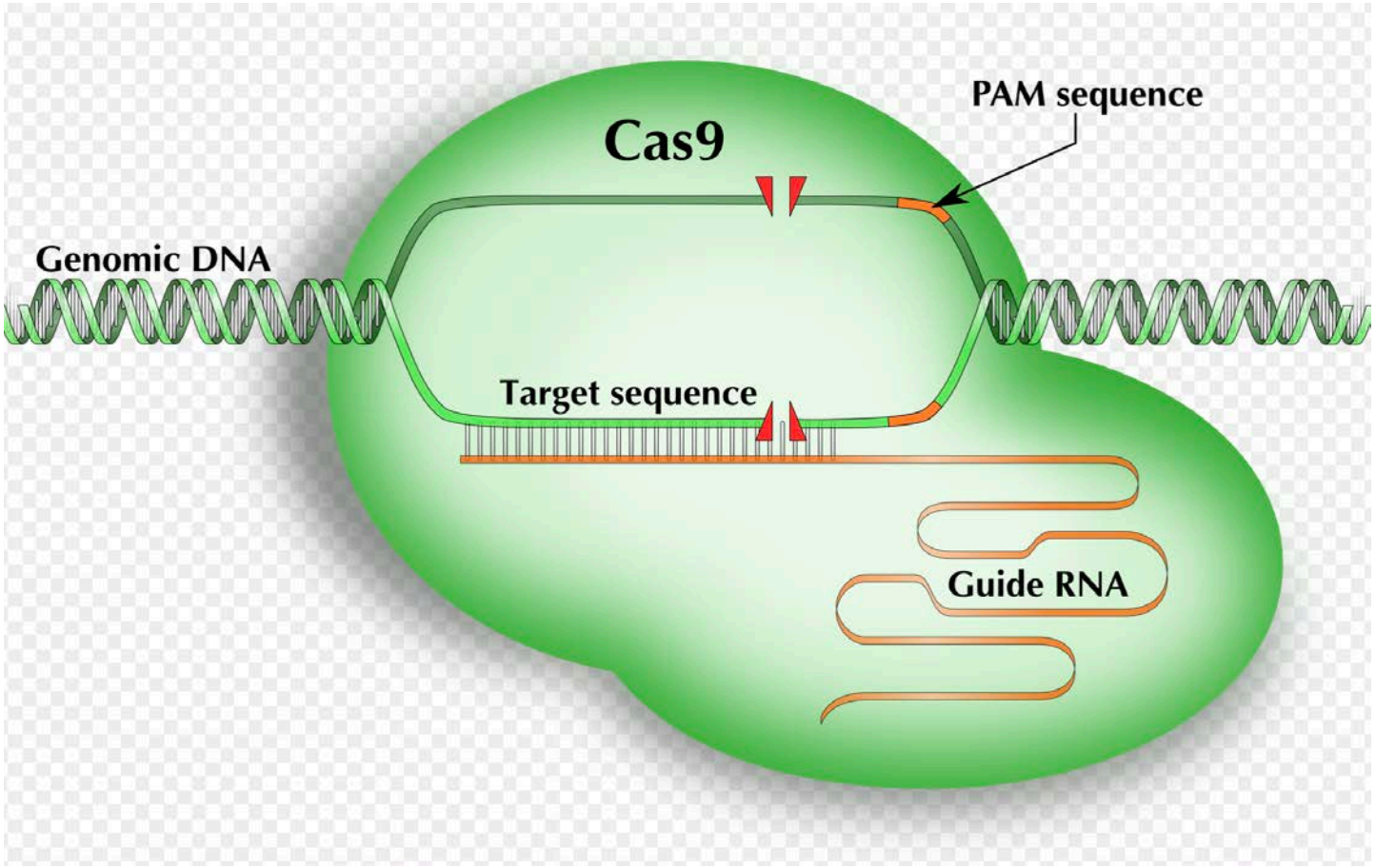
CRISPR-Cas9

RNA-guided DNA endonuclease



- Scans DNA for PAM sequence (NGG)
- Melts DNA and forms RNA::DNA hybrid
- Cleaves both DNA strands

CRISPR discovery

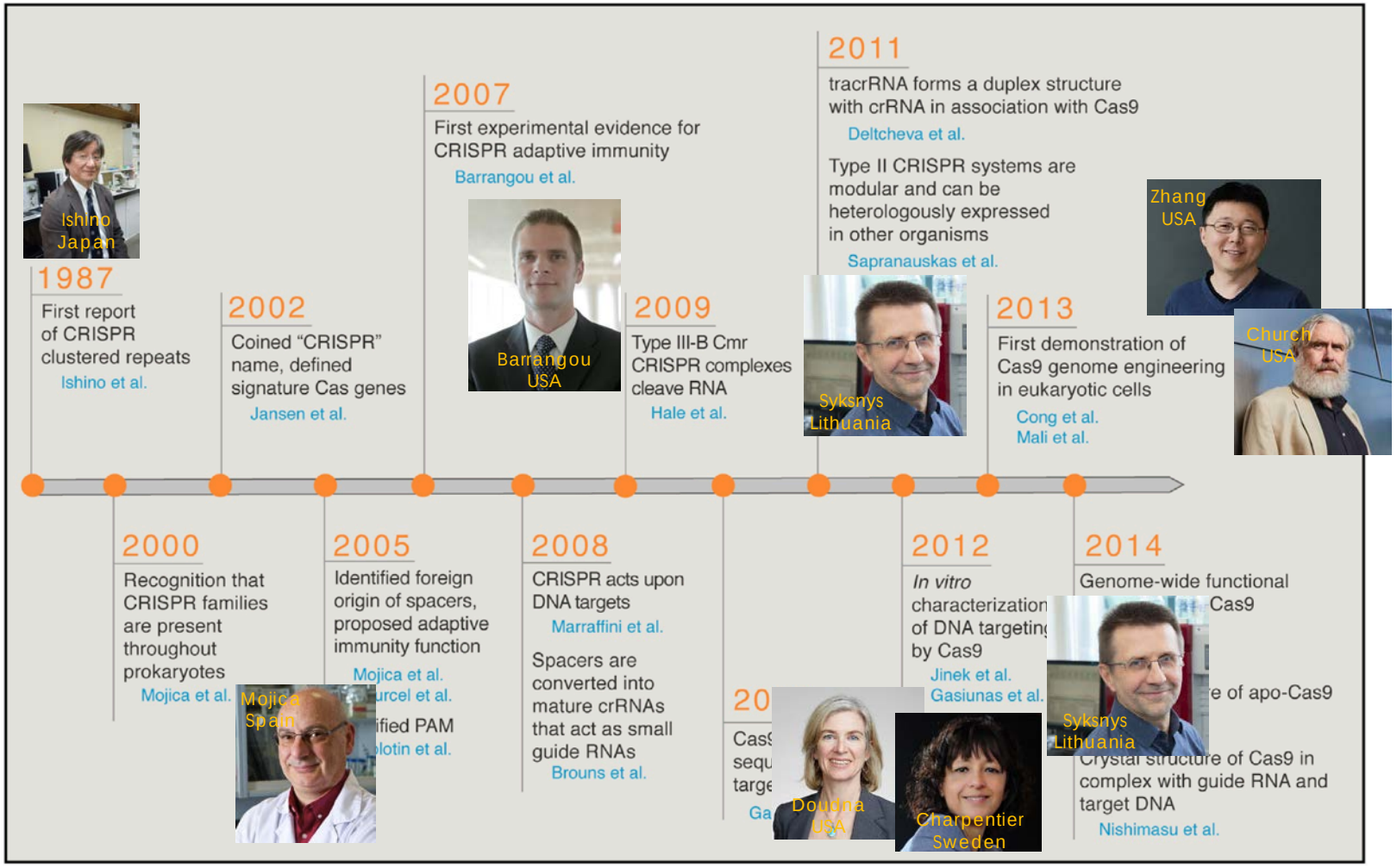
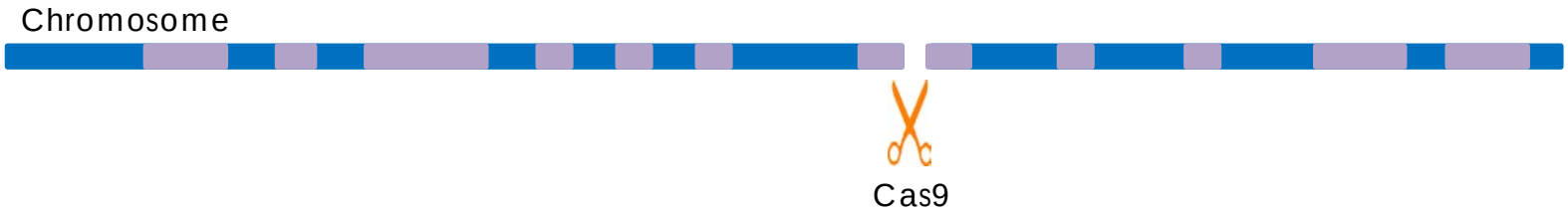
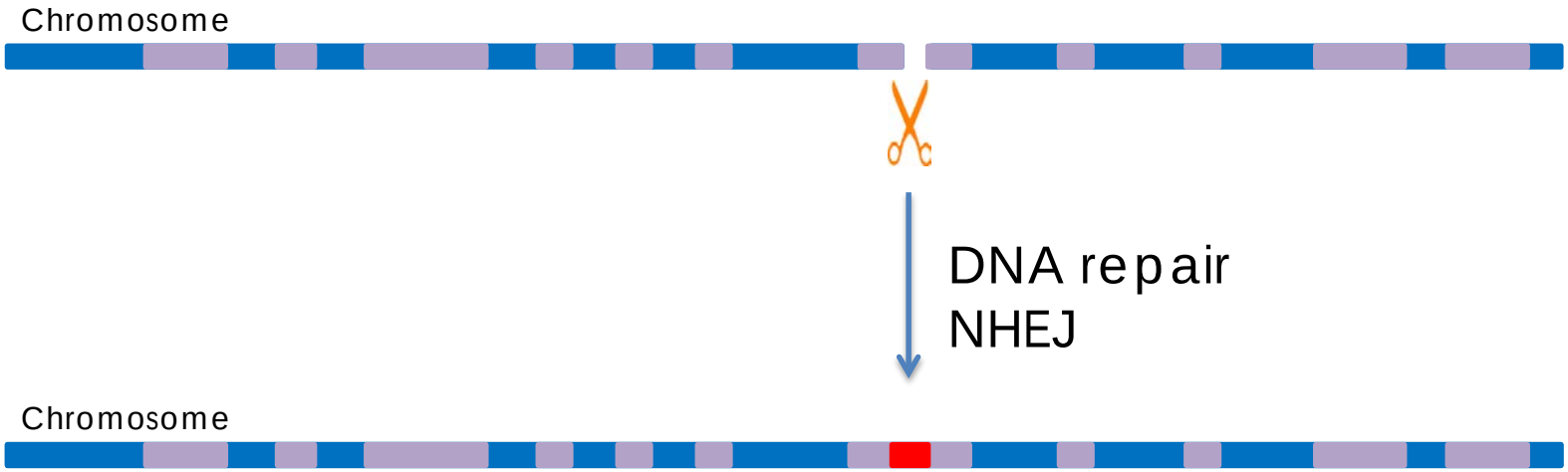


Figure: Hsu, Lander, Zhang: Development and Applications of CRISPR-Cas9 for Genome Engineering; Cell 157, June 5, 2014

Genome editing

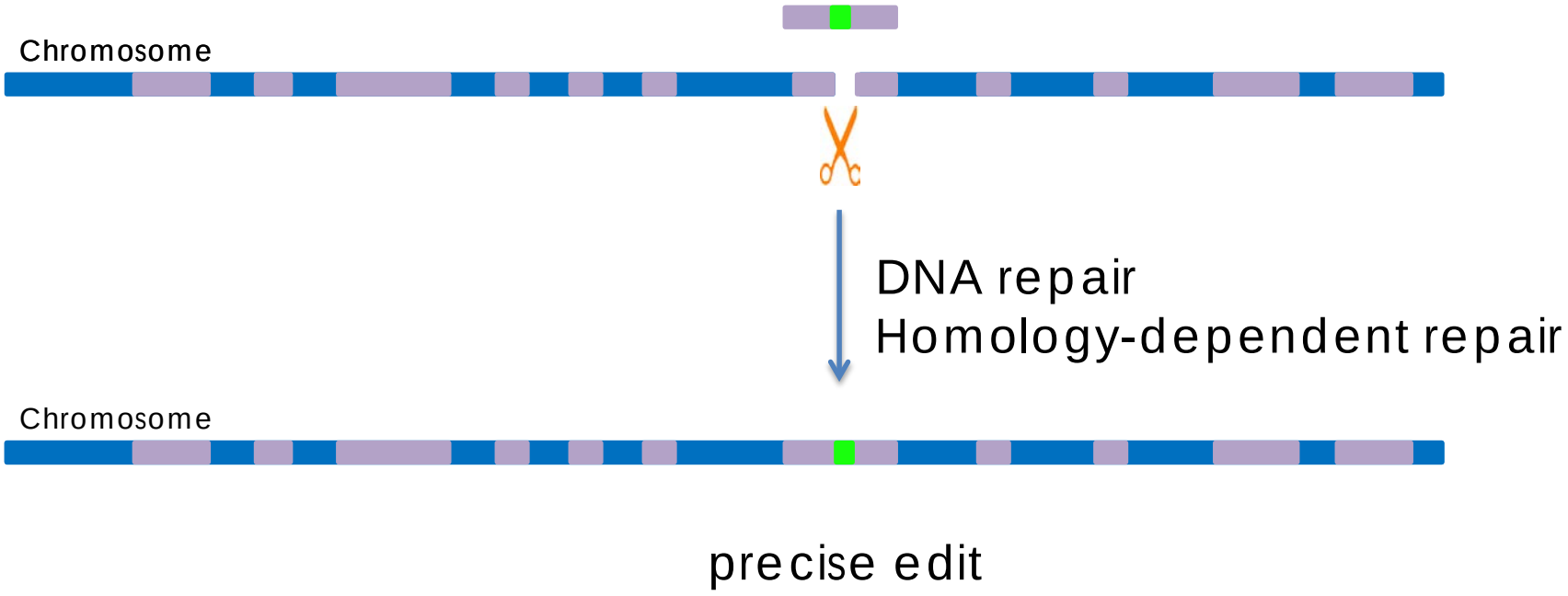


Genome editing

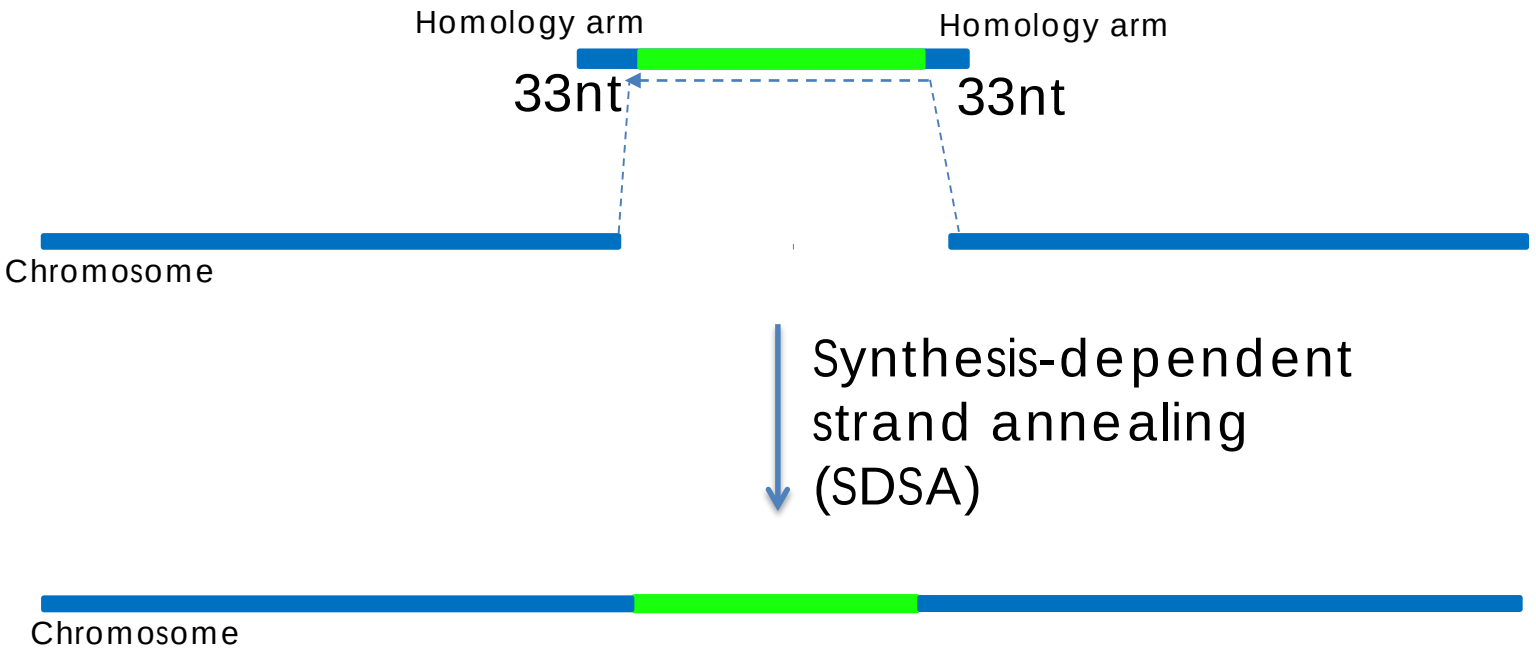


gene knock-out/down

Genome editing

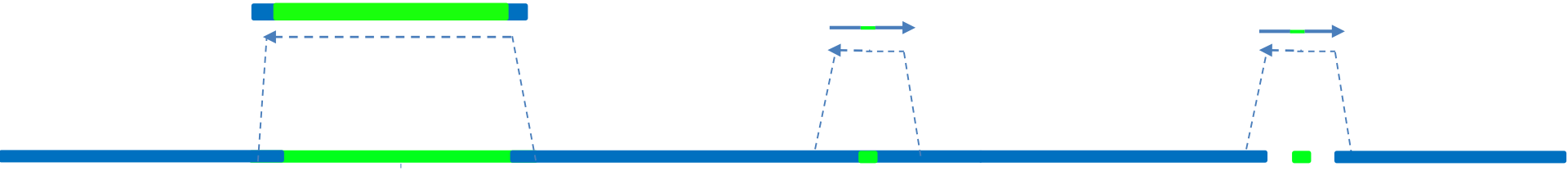


Homology dependent repair
Donor DNA is copied by repair DNA synthesis



"Efficient – requires only 30nt homology arms"

Genome editing

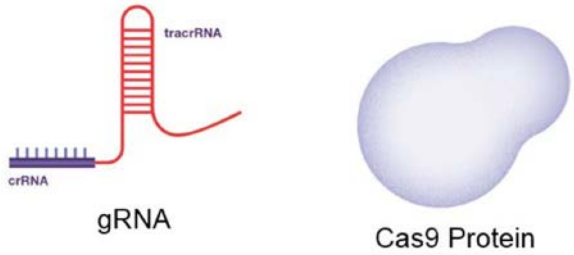


ORF-sized insertions
(GFP tagging)

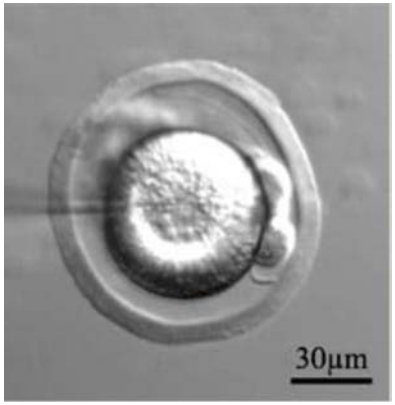
Small insertions/mutations
(Small tags, base changes)

Deletions
(Gene knockouts, replacements)

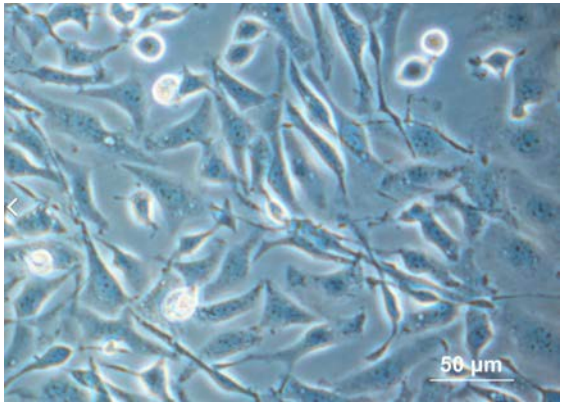
REAGENTS



Donor DNA



Embryo

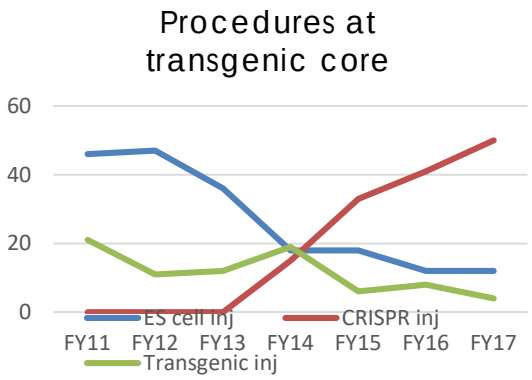


Cells in culture

Applications of genome editing

Research

- Gene discovery – CRISPR screens
- Structure-function studies
- Study human mutations in model systems



Applications of genome editing

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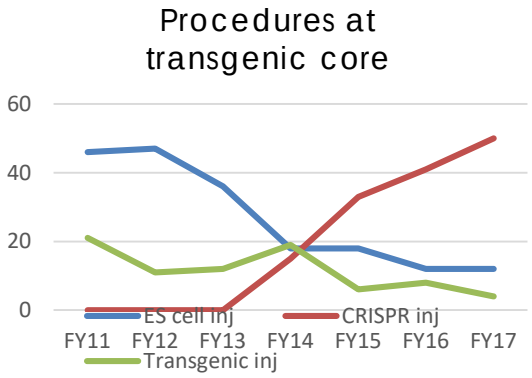


Patient Care

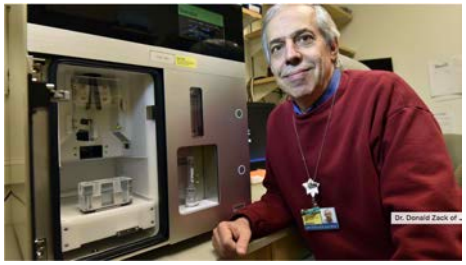
- Gene therapy
  Correct disease-causing mutations

March 8, 2018

Gene Knockout Using New CRISPR Tool Makes Mosquitoes Highly Resistant to Malaria Parasite

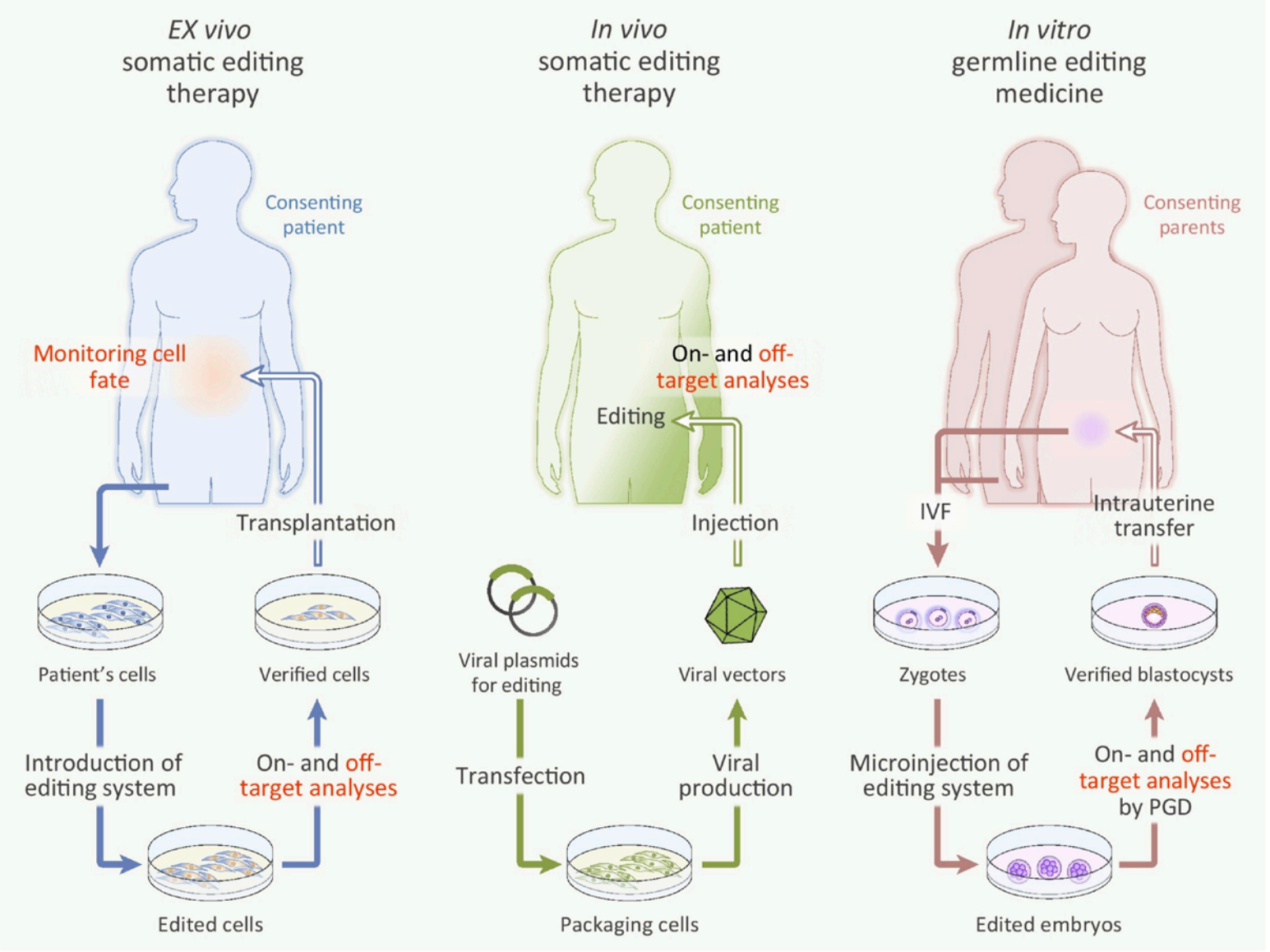
STUDY HIGHLIGHTS THE POTENTIAL OF MOSQUITO GENE-MODIFICATION TO COMBAT MALARIA

Dimopoulos JHU SPH



Eye cells grown at Johns Hopkins may lead to a cure for some forms of blindness

Zack JHU SOM



Providing Appropriate Risk Information on Genome Editing for Patie
MotokoAraki[1]TetsuyaIshii[1]

2017 Recommendations from the National Academy of Sciences and National Academy of Medicine

RECAP OF MAJOR RECOMMENDATIONS

Basic Laboratory Research

- Use existing regulatory processes to oversee human genome editing laboratory research

Somatic Genome Editing

- Use existing regulatory processes for human gene therapy to oversee somatic human genome editing research and uses
- Limit clinical trials or therapies to treatment and prevention of disease or disability at this time
- Evaluate safety and efficacy in the context of risks and benefits of intended use
- Require broad public input prior to extending uses

Germline (Heritable) Genome Editing

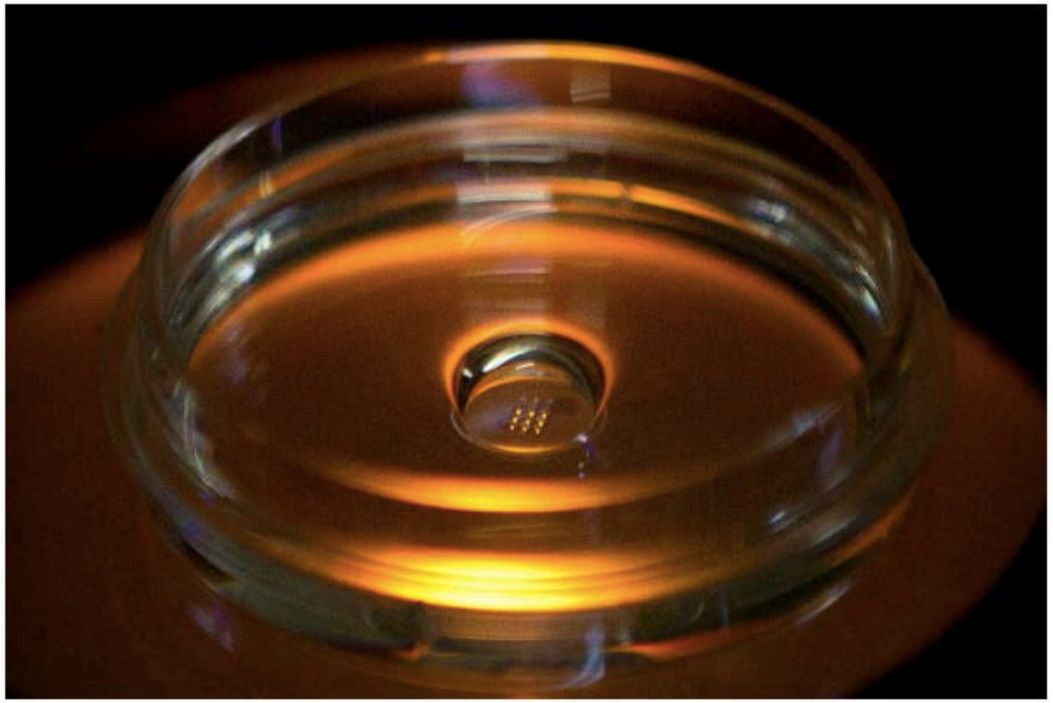
- Permit clinical research trials only for compelling purposes of treating or preventing serious disease or disabilities, and only if there is a stringent oversight system able to limit uses to specified criteria
- Ongoing reassessment and public participation should precede any heritable germline editing

Enhancement

- Do not proceed at this time with human genome editing for purposes other than treatment or prevention of disease and disability
- Encourage public discussion and policy debate with respect to somatic human genome editing for uses other than treatment or prevention of disease and disability

Chinese Scientist Claims to Use Crispr to Make First Genetically Edited Babies

The researcher, He Jiankui, offered no evidence or data to back up his assertions. If true, some fear the feat could open the door to "designer babies."



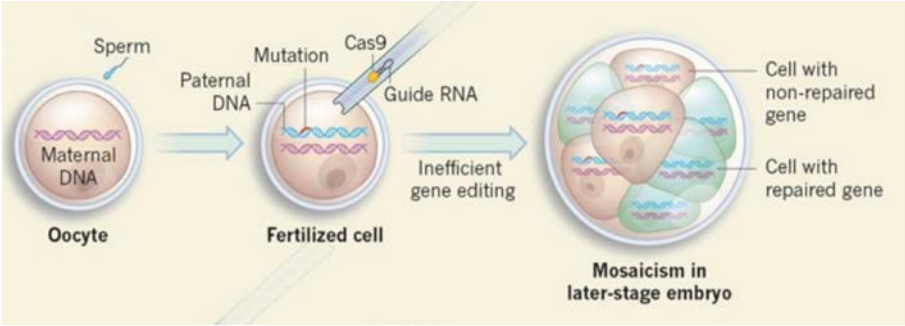
A microplate from the Chinese scientist He Jiankui's lab containing embryos whose genes have been edited. Dr. He's announcement about genetically edited babies prompted a statement from a group of 122 scientists condemning his actions as "crazy." Mark Schiefelbein/Associated Press

November 2018

https://www.bsgct.org/gene-therapy-against-hiv-fighting-the-virus-in-disguise/

Risks:

Mosaicism



Off-target mutations

CRISPR off-target analysis in genetically engineered rats and mice

Keith R. Anderson[1], Maximilian Haeussler[2], Colin Watanabe[3], Vasantharajan Janakiraman[1], Jessica Lund[1], Zora Modrusan[1], Jeremy Stinson[1], Qixin Bei[1], Andrew Buechler[1], Charles Yu[1], Sobha R. Thamminana[1], Lucinda Tam[1], Michael-Anne Sowick[4], Tuija Alcantar[4], Natasha O'Neil[4], Jinjie Li[4], Linda Ta[4], Lisa Lima[4], Merone Roose-Girma[1], Xin Rairdan[4], Steffen Durinck[1] and Søren Warming[1]*

Despite widespread use of CRISPR, comprehensive data on the frequency and impact of Cas9-mediated off-targets in modified rodents are limited. Here we present deep-sequencing data from 81 genome-editing projects on mouse and rat genomes at 1,423 predicted off-target sites, 32 of which were confirmed, and show that high-fidelity Cas9 versions reduced off-target mutation rates in vivo. Using whole-genome sequencing data from ten mouse embryos, treated with a single guide RNA (sgRNA), and from their genetic parents, we found 43 off-targets, 30 of which were predicted by an adapted version of GUIDE-seq.

and 56 potential off-targets are presented, and no off-target mutations were identified. In addition to the identification of lower-frequency off-targets that might otherwise be missed (Supplementary Note 1), deep-sequencing analysis of the mosaic G0 founders rather than their G1 progeny also allows for the selection of founders with high contributions of the desired allele for more efficient breeding.

Twenty-three percent (19/81) of our animal model projects had off-targets (Fig. 1a), defined by at least one animal with at least one allele with Cas9-induced mutations in >3% of the sequence reads in at least one of the analyzed off-target loci. Some animal model projects require the use of two sgRNAs, for example, to generate a

https://www.bsgct.org/gene-therapy-against-hiv-fighting-the-virus-in-disguise/

Regulation of gene editing in human embryos

Banned in more than 40 countries, including
- Canada
- Germany
- South Korea
- France

Allowed under some conditions
- UK: allowed if in the child's interest
- China: requires approval from National Health Commission
- Japan: research allowed – draft guidelines (oct 2018)

USA: - NIH no clinical trials for human germline engineering and no research involving creation or destruction of embryos
- Clinics must get FDA approval to alter genome