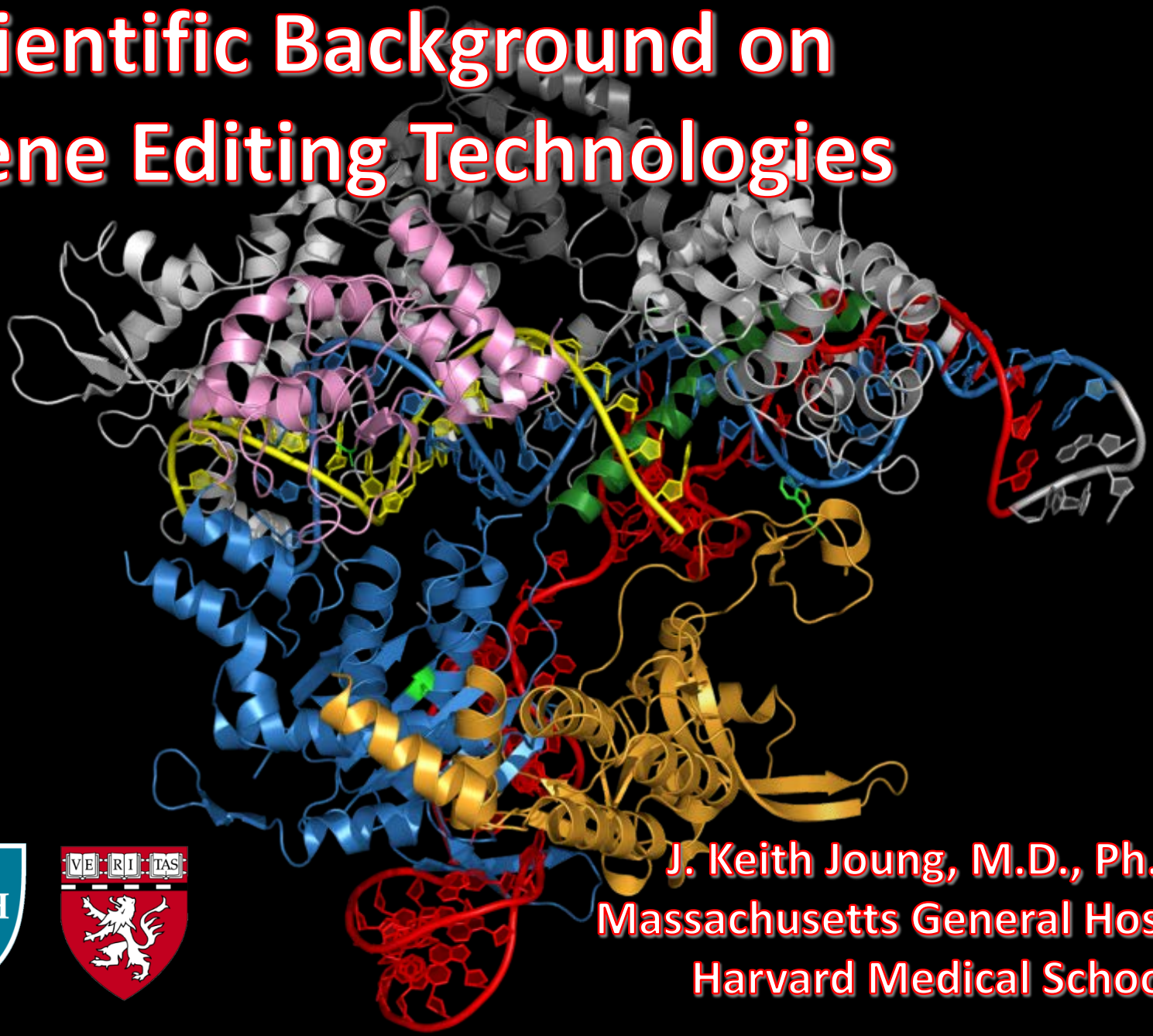
Scientific Background on Gene Editing Technologies



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Harvard Medical School

Conflict of Interest Declaration

- JKJ has financial interests in **Editas Medicine, Hera Testing Laboratories, Poseida Therapeutics, and Transposagen Biopharmaceuticals**
- JKJ is a consultant for **Horizon Discovery**

editas MEDICINE

Transposagen

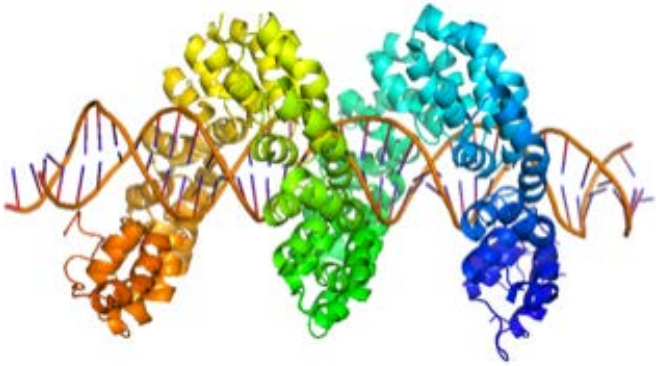
horizon precision genome editing

- JKJ's interests were reviewed and are managed by Massachusetts General Hospital and Partners HealthCare in accordance with their conflict of interest policies

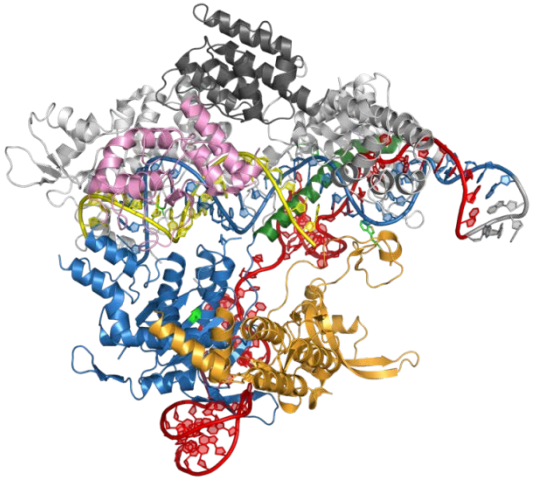
Genome-Editing Nucleases

Zinc Finger Nucleases (ZFNs)

TALE Nucleases (TALENs)

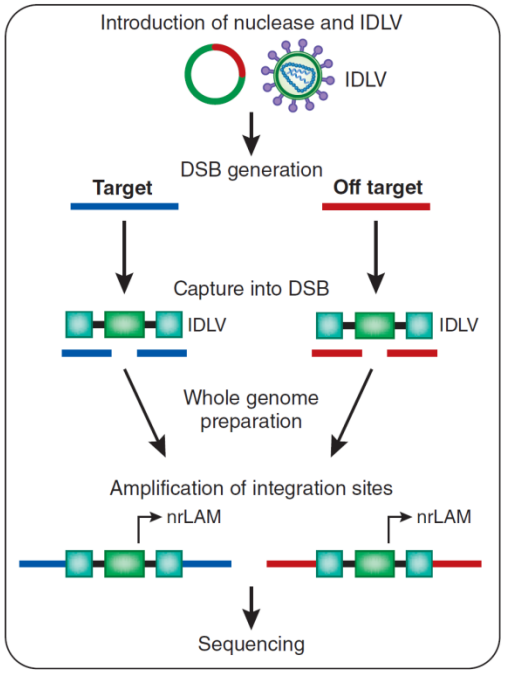


CRISPR RNA-Guided Nucleases

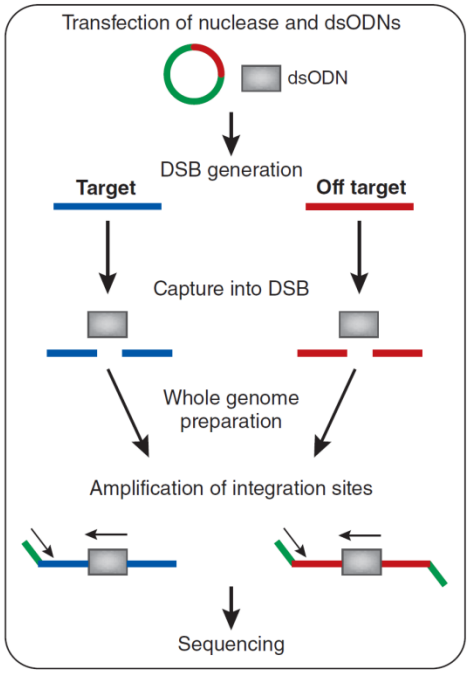


Establish Standards for Genome-wide Off-Target Identification

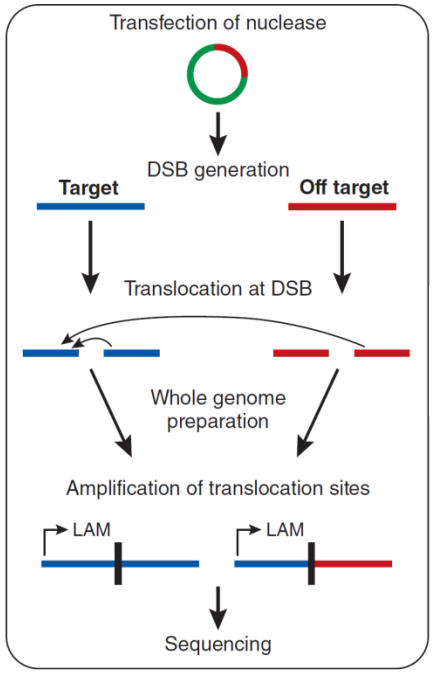
IDLV Capture



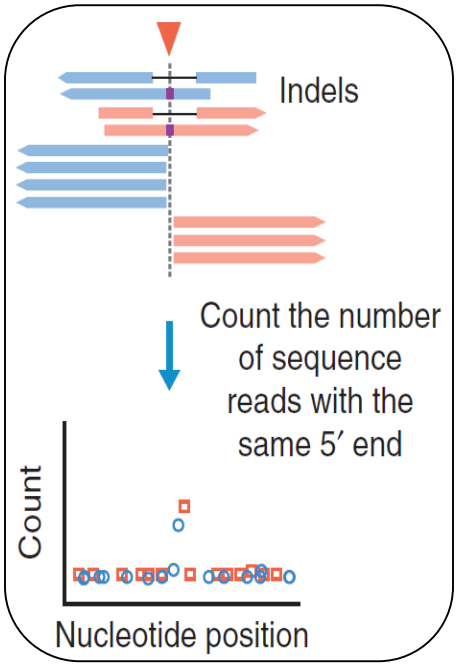
GUIDE-seq



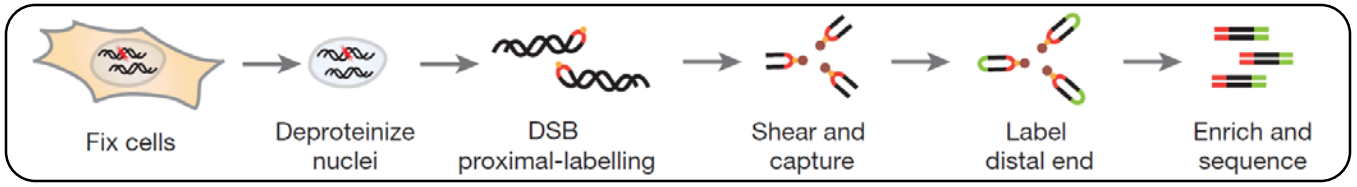
HTGTS



Digenome-seq



BLESS



adapted from Gabriel et al., *Nat Biotechnol.* 2015, Kim et al., *Nat Methods* 2015 & Ran et al., *Cell* 2015

Moving Cas9 Platforms with Reduced Off-Target Effects into the Clinic

CAS9 transcriptional activators for target specificity screening and paired nickases for cooperative genome engineering

Prashant Mali[1,4], John Aach[1,4], P Benjamin Stranges[1], Kevin M Esvelt[2], Mark Moosburner[1], Sriram Kosuri[2], Luhan Yang[3] & George M Church[1,2]

Mali et al., *Nat Biotechnol*. 2013

Fusion of catalytically inactive Cas9 to FokI nuclease improves the specificity of genome modification

John P Guilinger[1–3], David B Thompson[1–3] & David R Liu[1,2]

Guilinger et al., *Nat Biotechnol*. 2014

Double Nicking by RNA-Guided CRISPR Cas9 for Enhanced Genome Editing Specificity

F. Ann Ran,[1,2,3,4,5,11] Patrick D. Hsu,[1,2,3,4,5,11] Chie-Yu Lin,[1,2,3,4,6] Jonathan S. Gootenberg,[1,2,3,4] Silvana Konermann,[1,2,3,4] Alexandro E. Trevino,[1] David A. Scott,[1,2,3,4] Azusa Inoue,[7,8,9,10] Shogo Matoba,[7,8,9,10] Yi Zhang,[7,8,9,10] and Feng Zhang[1,2,3,4,*]

Ran et al., *Cell* 2013

Dimeric CRISPR RNA-guided FokI nucleases for highly specific genome editing

Shengdar Q Tsai[1–4], Nicolas Wyvekens[1–3], Cyd Khayter[1–3], Jennifer A Foden[1–3], Vishal Thapar[1,2], Deepak Reyon[1–4], Mathew J Goodwin[1–3], Martin J Aryee[1,2,4] & J Keith Joung[1–4]

Tsai et al., *Nat Biotechnol*. 2014

Improving CRISPR-Cas nuclease specificity using truncated guide RNAs

Yanfang Fu[1–5], Jeffry D Sander[1–5], Deepak Reyon[1–4], Vincent M Cascio[1–3] & J Keith Joung[1–4]

Fu et al., *Nat Biotechnol*. 2014

Engineered CRISPR–Cas9 nucleases with altered PAM specificities

Benjamin P. Kleinstiver[1,2,3], Michelle S. Prew[1,2], Shengdar Q. Tsai[1,2,3], Ved V. Topkar[1,2], Nhu T. Nguyen[1,2], Zongli Zheng[1,3,4], Andrew P. W. Gonzales[5,6,7], Zhuyun Li[5], Randall T. Peterson[5,6,7], Jing-Ruey Joanna Yeh[5,8], Martin J. Aryee[1,3,9] & J. Keith Joung[1,2,3]

Kleinstiver et al., *Nature* 2015

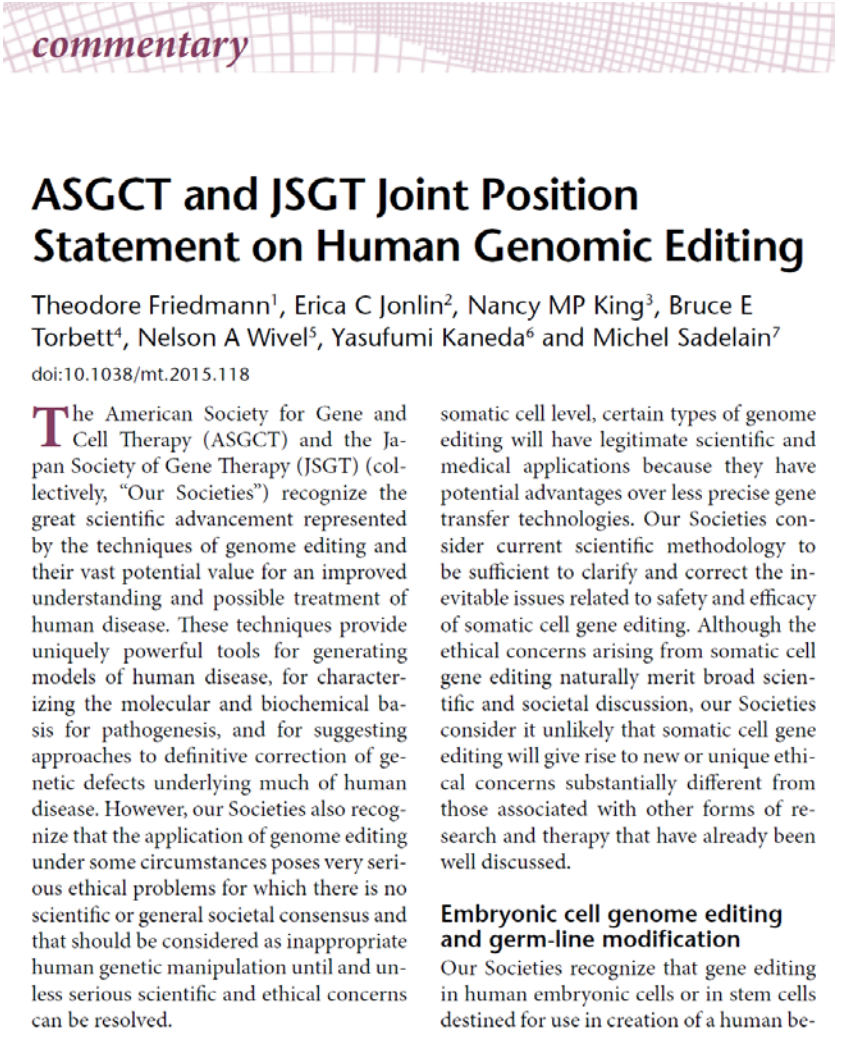
…and additional future improvements to the CRISPR-Cas9 platform

American Society of Gene and Cell Therapy (ASGCT)

- professional society established in 1996 and devoted to application of new genetic and cellular therapies
- has strong and long-standing interest in genome editing technologies for treating human disease
- provides guidance to its members and to the general scientific community through editorials and position statements
- http://www.asgct.org

American Society of Gene and Cell Therapy (ASGCT)

- has already issued (with the Japan Society of Gene Therapy) a position statement on Human Genomic Editing (Aug. 2015)
  - affirms potential benefit and need for research on somatic cell editing
  - discusses potential issues with germline editing and need forfurther scientific progress and broad public consensus



commentary

ASGCT and JSGT Joint Position Statement on Human Genomic Editing

Theodore Friedmann[1], Erica C Jonlin[2], Nancy MP King[3], Bruce E Torbett[4], Nelson A Wivel[5], Yasufumi Kaneda[6] and Michel Sadelain[7]

doi:10.1038/mt.2015.118

The American Society for Gene and Cell Therapy (ASGCT) and the Japan Society of Gene Therapy (JSGT) (collectively, "Our Societies") recognize the great scientific advancement represented by the techniques of genome editing and their vast potential value for an improved understanding and possible treatment of human disease. These techniques provide uniquely powerful tools for generating models of human disease, for characterizing the molecular and biochemical basis for pathogenesis, and for suggesting approaches to definitive correction of genetic defects underlying much of human disease. However, our Societies also recognize that the application of genome editing under some circumstances poses very serious ethical problems for which there is no scientific or general societal consensus and that should be considered as inappropriate human genetic manipulation until and unless serious scientific and ethical concerns can be resolved.

somatic cell level, certain types of genome editing will have legitimate scientific and medical applications because they have potential advantages over less precise gene transfer technologies. Our Societies consider current scientific methodology to be sufficient to clarify and correct the inevitable issues related to safety and efficacy of somatic cell gene editing. Although the ethical concerns arising from somatic cell gene editing naturally merit broad scientific and societal discussion, our Societies consider it unlikely that somatic cell gene editing will give rise to new or unique ethical concerns substantially different from those associated with other forms of research and therapy that have already been well discussed.

Embryonic cell genome editing and germ-line modification

Our Societies recognize that gene editing in human embryonic cells or in stem cells destined for use in creation of a human be-

ASGCT Workshop on Germline Gene Editing
Science, Ethics, Law & Policy