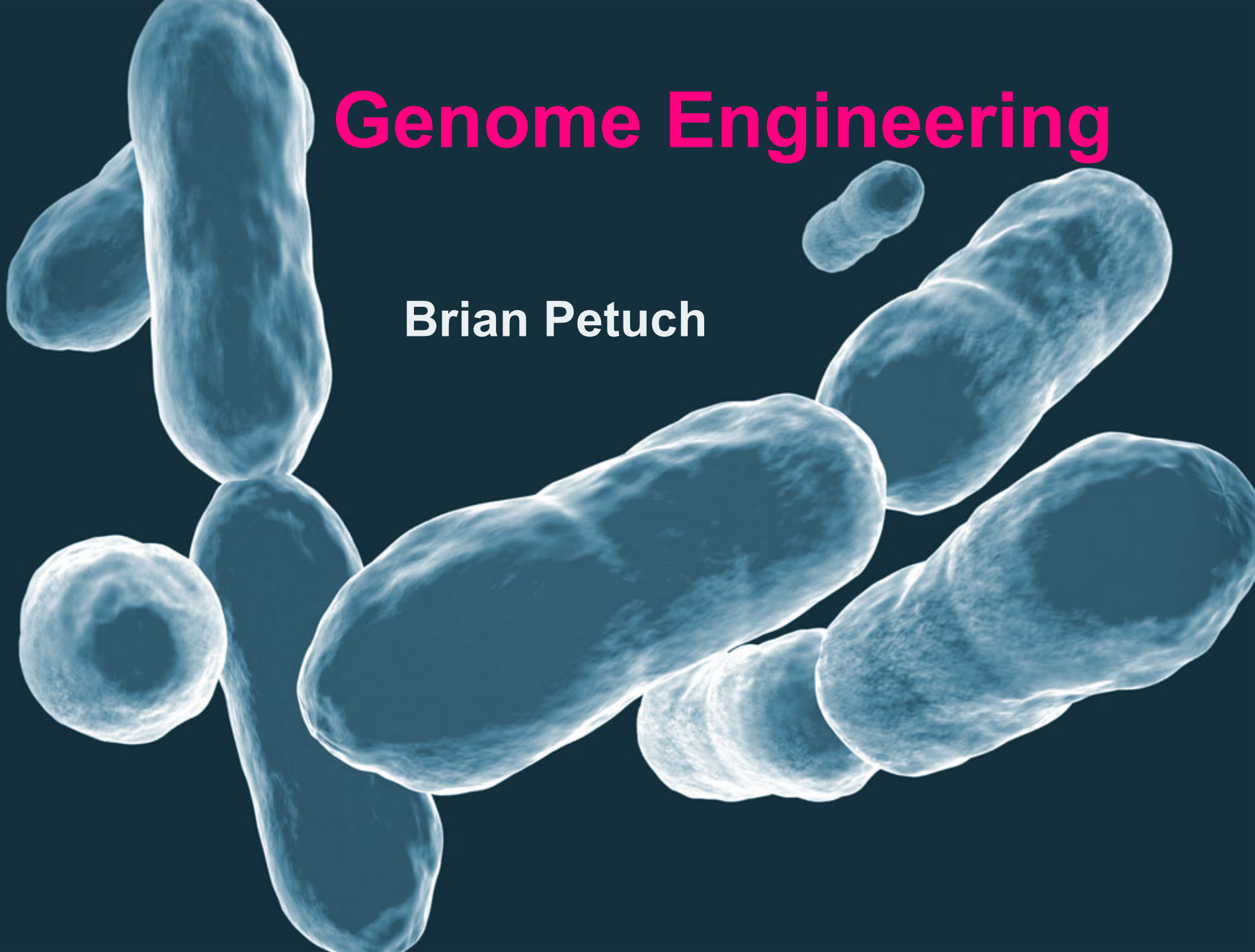


# Genome Engineering

Brian Petuch

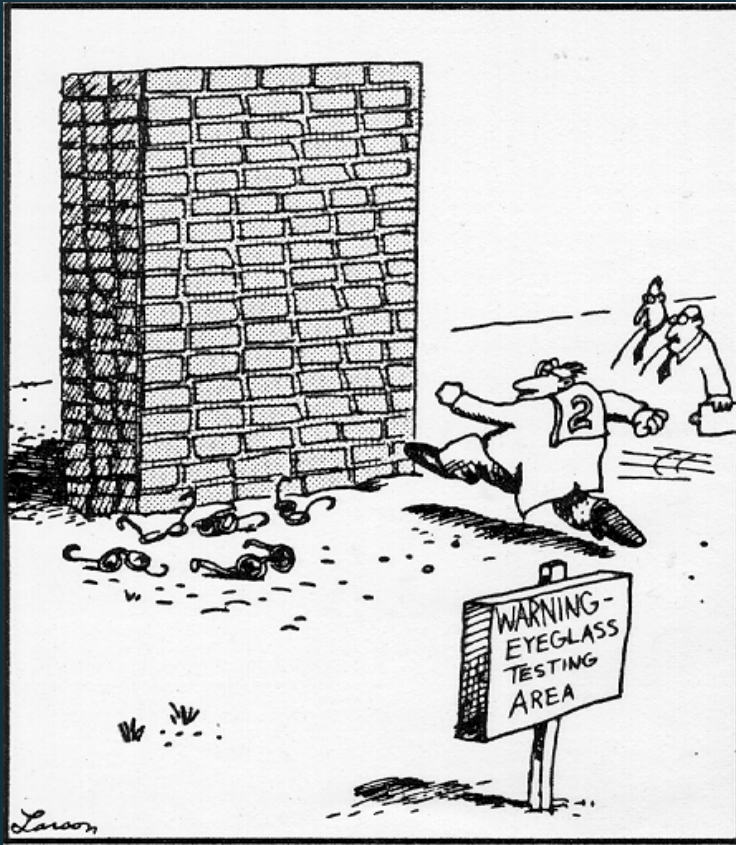


# Guiding Principles

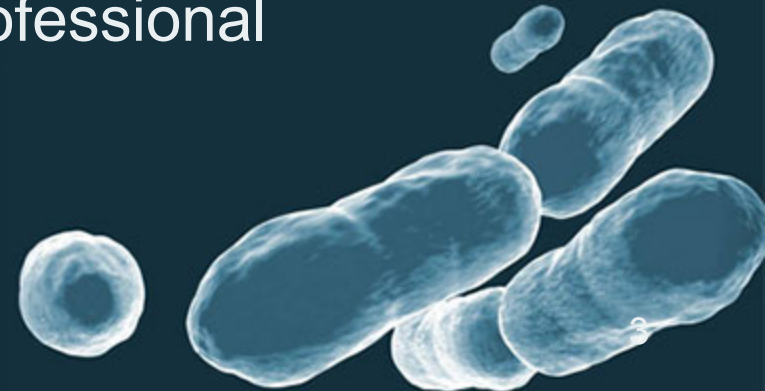
- An understanding of the details of gene engineering is essential for understanding protocols when making recommendations on:
  - Risk assessment
  - Containment levels
  - Safe work practices
  - Training



# Risk Assessment: It's a Matter of Perspective...



- Investigators who submit rDNA protocols want to perform their experiments safely
- However, their perception of the risks involved will not necessarily be the same as those of a biosafety professional



# Challenges of Genome Engineering Risk Assessment

- A= transformation methodology
- B= expression construct
- Quite often...  $A + B = A^*$
- But sometimes...  $A + B = C$
- Worst case...  $A \times B = Z^2$
- Need realistic risk assessment for
  - Protection of personnel
  - Guidance for containment & work practices





# CRISPR-CAS9 GENOME ENGINEERING



# CRISPR-Cas9

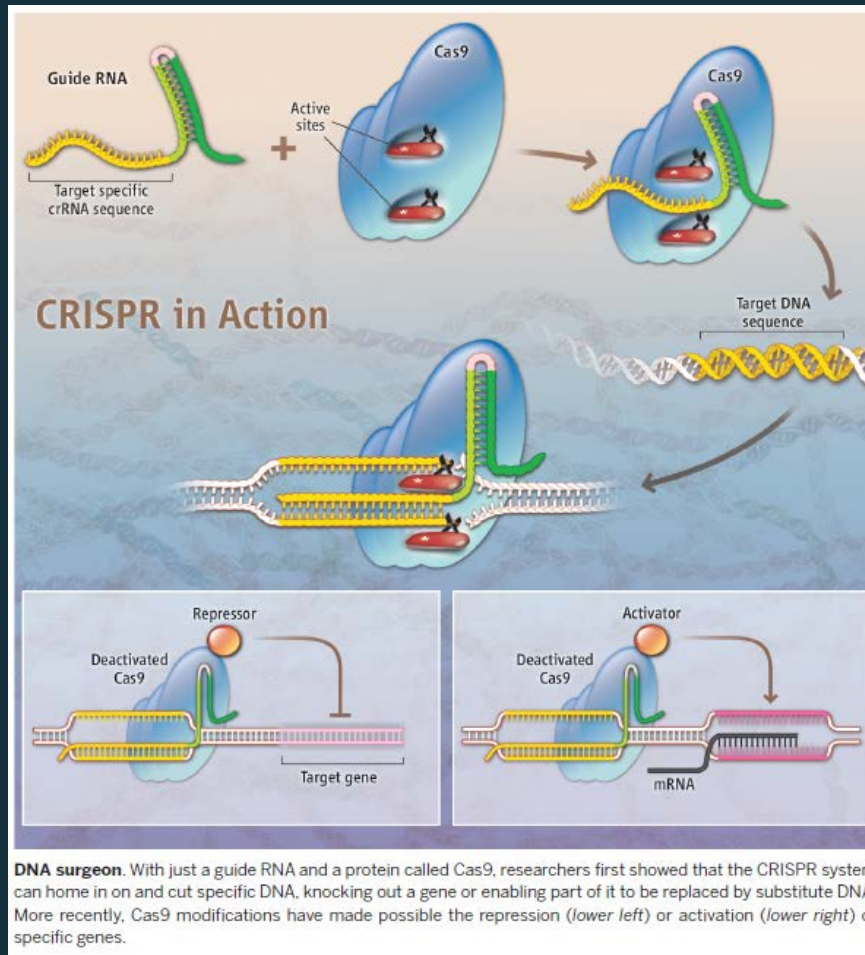
## Genome Engineering



- CRISPR/Cas9 Genome Engineering
  - CRISPR-associated protein (Cas) system provides prokaryotes with adaptive immunity to viruses and plasmids.
  - When Cas9, a protein, is complexed with two RNAs called CRISPR RNA (crRNA) and trans-activating crRNA (tracrRNA), it forms a sequence-specific endonuclease that cleaves foreign genetic sequences to protect host cells.



# CRISPR-Cas9 Genome Engineering




# CRISPR-Cas9 Genome Engineering



**Table 1.** Comparison of three types of engineered nucleases

	<b>ZFN</b>	<b>TALEN</b>	<b>RGEN</b>
DNA-binding determinant	Zinc finger proteins	TAL effectors	crRNA or sgRNA
Nuclease	FokI	FokI	Cas9
Length of target site	18 to 36 bp	30 to 40 bp	23 bp
Targetable sequences	Guanine-rich	No limitations	End with GG (PAM)
Off-target effects	High	Low	Variable
Cytotoxicity	Variable to high	Low	Low
Size	~1 kbp × 2	~3 kbp × 2	4.2 kbp (Cas9) + 0.1 kbp (sgRNA)
Public resources	Zinc finger consortium Addgene	Seoul National University ( <a href="http://www.talenlibrary.net">www.talenlibrary.net</a> )	Addgene
Commercial resources	Sigma-Aldrich	Life Technologies Collectis	ToolGen



# CRISPR-Cas9 Genome Engineering

**Table 1.** *Organisms that have been modified using the CRISPR-Cas9 system*

Organism	Mutations created in		Alleles generated by		References
	Cultured cells	Organism (heritable?)	NHEJ	HDR	
<b>Vertebrates</b>					
Axolotl		✓	✓		Flowers et al. 2014
Frog		✓ (Yes)	✓		Blitz et al. 2013; Nakayama et al. 2013; Guo et al. 2014
Human	✓		✓	✓	For review, see Sander and Joung 2014
Medaka		✓ (Yes)	✓		Ansai and Kinoshita 2014
Mouse	✓	✓ (Yes)	✓	✓	For review, see Sander and Joung 2014
Monkey		✓	✓		Niu et al. 2014b
Pig	✓	✓ (Yes)	✓		Hai et al. 2014; Sato et al. 2014
Rabbit		✓	✓		Yang et al. 2014
Rat	✓	✓ (Yes)	✓	✓	Li et al. 2013a,b, 2014b; Ma et al. 2014b,c,d
Tilapia		✓ (Yes)	✓		Li et al. 2014a
Zebrafish		✓ (Yes)	✓	✓	For review, see Auer et al. 2014
<b>Invertebrates</b>					
Freshwater flea		✓ (Yes)	✓		Nakanishi et al. 2014
Fruit fly	✓	✓ (Yes)	✓	✓	For review, see Gratz et al. 2013; Bassett and Liu 2014
Roundworm		✓ (Yes)	✓	✓	For review, see Waaijers and Boxem 2014
Silkworm	✓	✓ (Yes)	✓	✓	Wang et al. 2013b; Daimon et al. 2014; Liu et al. 2014b; Ma et al. 2014a; Wei et al. 2014
<b>Plants</b>					
Corn		✓	✓		Liang et al. 2014
Liverwort		✓ (Yes)	✓		Sugano et al. 2014
Rice		✓ (Yes)	✓		For review, see Belhaj et al. 2013
Sorghum		✓	✓		Jiang et al. 2013b
Sweet orange		✓	✓		Jia and Wang 2014
Thale cress		✓ (Yes)	✓	✓	For review, see Belhaj et al. 2013
Tobacco		✓ (Yes)	✓	✓	For review, see Belhaj et al. 2013
Wheat	✓		✓		Upadhyay et al. 2013

We limited our list to those organisms that provide platforms for the study of development (as indicated for some organisms, only cells derived from the organism have been modified to date). Blanks indicate "not tested." For the organisms in which the CRISPR-Cas9 system has been used extensively, see recent reviews. Given the rapid advances in the field, we apologize for any organisms or references that were inadvertently not included.

# CRISPR-Cas9 Genome Engineering The Good



One-step generation of different immunodeficient mice with multiple gene modifications by CRISPR/Cas9 mediated genome engineering

Jiankui Zhou<sup>a,1</sup>, Bin Shen<sup>a,1</sup>, Wensheng Zhang<sup>b,1</sup>, Jianying Wang<sup>a</sup>, Jing Yang<sup>a</sup>, Li Chen<sup>a</sup>, Na Zhang<sup>c</sup>, Kai Zhu<sup>c</sup>, Juan Xu<sup>a</sup>, Bian Hu<sup>a</sup>, Qibin Leng<sup>c,\*\*</sup>, Xingxu Huang<sup>a,\*</sup>

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<sup>b</sup> Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge CB10 1SA, UK

<sup>c</sup> Key Laboratory of Molecular Virology and Immunology, Institute Pasteur of Shanghai, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, 225 South Chongqing Road, Shanghai, China



# CRISPR-Cas9 Genome Engineering The Bad

## RESEARCH ARTICLE

### CRISPR/Cas9-mediated gene editing in human tripronuclear zygotes

Puping Liang, Yanwen Xu, Xiya Zhang, Chenhui Ding, Rui Huang, Zhen Zhang, Jie Lv, Xiaowei Xie, Yuxi Chen, Yujing Li, Ying Sun, Yaofu Bai, Zhou Songyang, Wenbin Ma, Canquan Zhou<sup>✉</sup>, Junjiu Huang<sup>✉</sup>

Guangdong Province Key Laboratory of Reproductive Medicine, the First Affiliated Hospital, and Key Laboratory of Gene Engineering of the Ministry of Education, School of Life Sciences, Sun Yat-sen University, Guangzhou 510275, China

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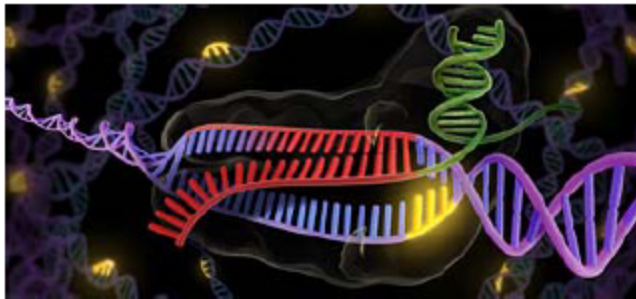
# CRISPR-Cas9 Genome Engineering The Bad

## Scientists urge caution in using new CRISPR technology to treat human genetic disease

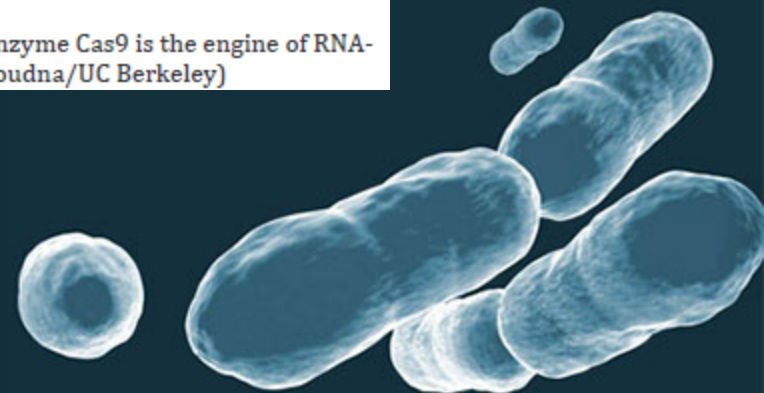
*By Robert Sanders, Media Relations | March 19, 2015*

BERKELEY —

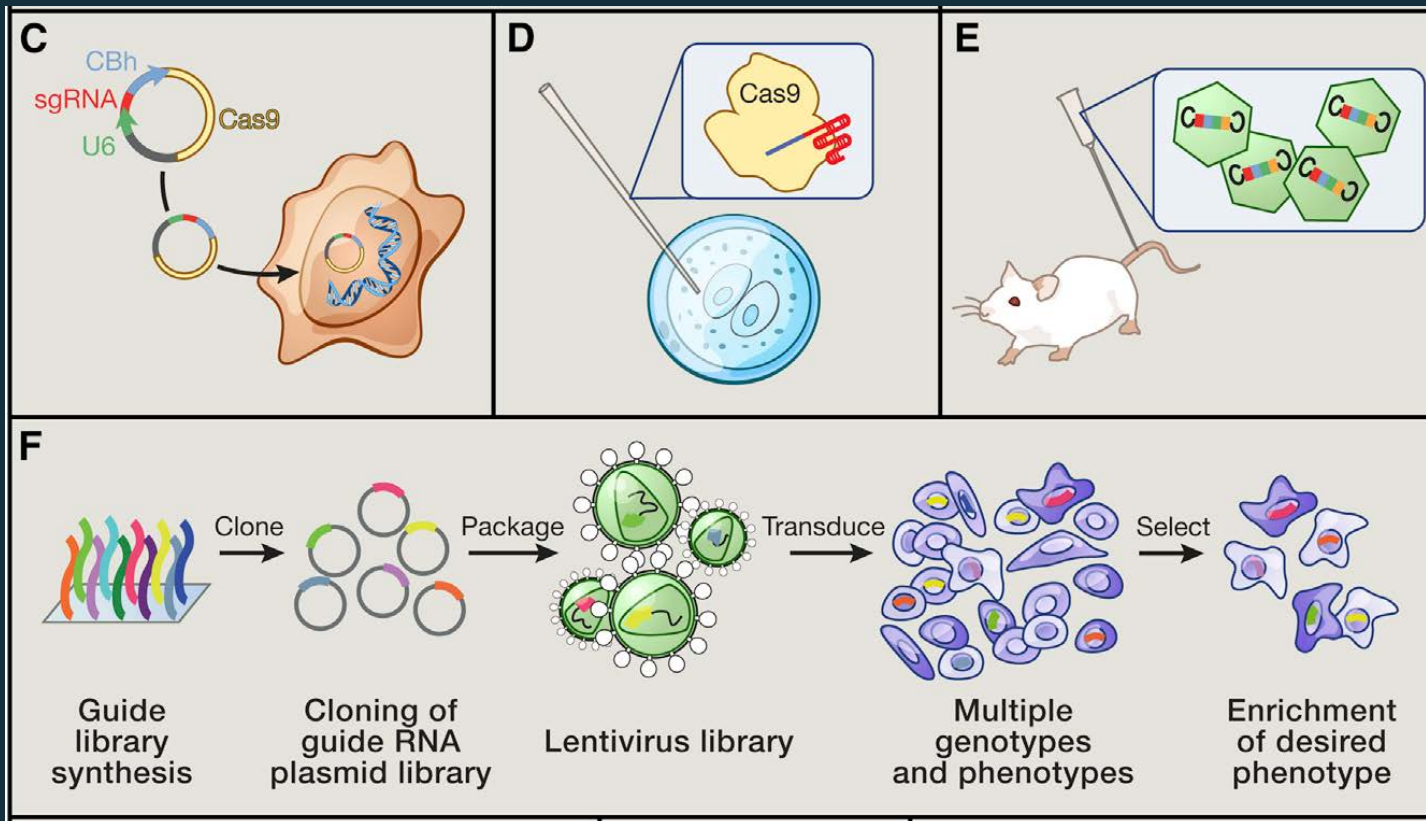
A group of 18 scientists and ethicists today warned that a revolutionary new tool to cut and splice DNA should be used cautiously when attempting to fix human genetic disease, and strongly discouraged any attempts at making changes to the human genome that could be passed on to offspring.



The bacterial enzyme Cas9 is the engine of RNA-programmed genome engineering in human cells. (Graphic by Jennifer Doudna/UC Berkeley)



# CRISPR-Cas9 Delivery



Hsu, et al., Cell 157, 2014, 1262-1278

# Lentivirus Vectors



- Based on HIV
  - 3<sup>rd</sup> + generation vectors only have 3 HIV genes
  - Replication incompetent
  - Genes needed for replication on multiple plasmids in host cells
  - Efficiently transduce both dividing and non-dividing cells
  - Long term gene expression
  - Chromosome integration
- Reproducibly transduce cells and animals
  - Gene knockdown
  - Gene overexpression



# Adenovirus Vectors



- 49 immunologically distinct types; AdV5 most common
  - Replication incompetent
  - Efficiently transduce both dividing and non-dividing cells
  - Multiplicity of infection >25 copies per cell possible
  - Expression can be 10–20% of total cell protein
  - Does not integrate into cell genome (cytoplasmic expression)
  - Specificity:
    - AAV1: CNS, Eye, Heart, Lung, Skeletal muscle
    - AAV2: CNS, Eye
    - AAV5: CNS, Eye, Lung
    - AAV6: Adipose, Heart, Liver, Lung, Skeletal muscle
    - AAV8: Adipose, CNS, Eye, Liver, Skeletal muscle;
    - AAV9: Adipose, CNS, Eye, Heart, Liver, Lung, Skeletal muscle



# Adeno-Associated Virus Vectors



- Large percentage of population is seropositive for exposure to various strains of adeno-associated virus (AAV).
- AAV never identified as an etiologic agent of human disease.
- AAV is a naturally 'defective' virus requiring a helper virus for productive replication
- AAV establishes a latent infection by genome integration into a specific locus of human chromosome 19.



# Virus Vector Risk Assessment

- Risk of replication competent virus production
- Risk of mutagenic insertion (Lenti, AAV)
- Transgene effects
  - Growth-regulating products
  - Products released into circulation
  - Products with a general effect on the host-immune system
  - Oncogenes and tumor suppressors
  - RNA interference that inhibit (knock down) the above genes
- Use in animal models
- Indicator cell lines for bioassay
  - Lower biosafety level?

# Viral Vector Risk Assessment

- Specific Biosafety Guidance Available
  - Lentivirus
    - Use Guideline
    - Medical protocol
    - Derogation to RG-1 Formula
  - Adenovirus
    - Use guideline
    - No medical treatment guideline
  - AAV
    - Use guideline
    - No medical treatment guideline



# Virus Vector Risk Assessment

- Medical Exposure Protocol (lenti vectors)
  - Risk of transgene
  - Immunosuppression
  - HIV-infected employees
  - Medical Exposure Protocol
    - Baseline HIV test
    - Oral HIV Integrase Inhibitor
    - Monitoring test-COBAS® AmpliPrep/COBAS® TaqMan® HIV-1

# Virus Vector Risk Assessment



- Lentivirus Transfected Cell Line Derogation
  - Dutch COGEM advisory CGM/090331-03 “Grading of Laboratory Work with Lentiviral Vectors.”

$$\text{Reduction ratio} = (20W \times 200I \times 2^{2.4T})/Ci$$

- W: Washes (20x per wash)
- I: Trypsin washing step (200x per treatment)
- T: Days incubated at 37° C



# RNAi Risk Assessment Resources

- Understand the Gene Target
  - <http://www.genecards.org/>



The screenshot shows the GeneCards website interface for the TNFRSF10B gene. The page includes a navigation bar with links for Home, GeneCards Guide, Suite, Terms and Conditions, About Us, User Feedback, and Mirror sites. A search bar is present with a 'Search' button and an 'Advanced Search' link. The main content area features the GeneCards logo, the gene name 'TNFRSF10B Gene', and its classification as a protein-coding gene with 73 GIFts. It also mentions 'Tumor Necrosis Factor Receptor Superfamily, Member 10b' and 'ESCMIID Microbiology & Infectious Diseases Congress'. Below this, there are several partner logos including M (Antibodies/cDNA/RNAi), SAbion Sciences Gene Network, ORIGENE, and GenScript. The main content area is divided into sections: 'Aliases for TNFRSF10B gene' (listing various database identifiers like HGNC, Entrez, UniProt, etc.), 'This gene clusters with an RNA gene' (Subcategory: lncRNA), 'Quality score for the ORGUL clustered with this gene is 3', 'Aliases' (listing various protein names like TRICK2B, DR5, TRAILR, etc.), 'External ids' (HGNC: 11905, Entrez Gene: 8795, etc.), 'ORGUL members' (NONCODE4: n407670), and 'Export aliases for TNFRSF10B gene to outside databases'. At the bottom, it lists 'Previous GC identifiers: GC08M022647 GC08M023231 GC08M022899 GC08M022933 GC08M021422'.



# RNAi Risk Assessment Resources



- **Entrez Gene summary for [APOL1 Gene](#):**  
This gene encodes a secreted high density lipoprotein which binds to apolipoprotein A-I. Apolipoprotein A-I is a relatively abundant plasma protein and is the major apoprotein of HDL. It is involved in the formation of most cholesteryl esters in plasma and also promotes efflux of cholesterol from cells. This apolipoprotein L family member may play a role in lipid exchange and transport throughout the body, as well as in reverse cholesterol transport from peripheral cells to the liver. Several different transcript variants encoding different isoforms have been found for this gene. (provided by RefSeq, Nov 2008)
- **GeneCards Summary for APOL1 Gene:**  
APOL1 (apolipoprotein L, 1) is a protein-coding gene. Diseases associated with APOL1 include [phencyclidine abuse](#), and [trypanosomiasis](#), and among its [related super-pathways](#) are *Scavenging of Heme from Plasma*. GO annotations related to this gene include *lipid binding* and *chloride channel activity*. An important paralog of this gene is [APOL4](#).
- **UniProtKB/Swiss-Prot: [APOL1\\_HUMAN, O14791](#) Function:** May play a role in lipid exchange and transport throughout the body. May participate in reverse cholesterol transport from peripheral cells to the liver



# RNAi Risk Assessment Resources

- Understand the Effects of Gene Knock-out
  - JAX Mice Database (<http://jaxmice.jax.org/query/f?p=205:1:0>)

Home > JAX® Mice & Services > Find JAX® Mice > Search JAX® Mice database

We are developing a new and improved search interface.  
Try the beta version of the [JAX® Mice database search](#)

JAX® Mice Database Search


**Basic Search**


Search by Gene / Allele / Strain / Common Name


Enter Stock Number    
Direct link to strain information

**Most popular JAX® Mice strains**  
Quick links to strain, physiological and phenotypic data for our most commonly referenced strains.  
[Learn more](#)

**Donate a strain**  
Submit your novel mouse strain to The Jackson Laboratory.  
[Learn more](#)

 Find Mice by  
**Therapeutic Area**

 **Cre Mice**  
by Site of Expression

 **Reporter Mice**  
by Site of Expression

# Risk Assessment

- Effect of Silencing on:
  - Growth-regulating genes
  - Genes with a general effect on the host-immune system
  - Knockout of genes involved in tumor suppression
  - Off-target effects
- Persistence of viral delivery vector
  - Integration
  - Cytoplasmic
- Exposure Protocol (Lenti only)





# SUMMARY AND QUESTIONS

