SCIENCE

The New York Times

Scientists Seek Ban on Method of Editing the Human Genome

By NICHOLAS WADE MARCH 19, 2015

policy-ish

Scientists Urge Temporary Moratorium On Human Genome Edits

MARCH 20, 2015 5:12 PM ET

ROB STEIN

npr

All Things Considered

MIT Technology Review

FEATURED STORY

Engineering the Perfect Baby

Scientists are developing ways to edit the DNA of tomorrow's children. Should they stop before it's too late?

By Antonio Regalado on March 5, 2015

Outline

- **Background**
- **Key Issues**
  - **Ethics**
  - **Safety**
  - **Value**

RESEARCH ARTICLE

Protein & Cell

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✉, Junjiu Huang✉

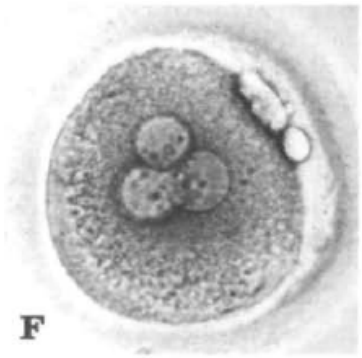
Guangdong Province Key Laboratory of Reproductive Medicine, the First Affiliated Hospital, and Key Laboratory of Gene Engineering of the Ministry of Education, School of Life Sciences, Sun Yat-sen University, Guangzhou 510275, China

✉ Correspondence: hjunjiu@mail.sysu.edu.cn (J. Huang), zhoucanquan@hotmail.com (C. Zhou)

Received March 30, 2015 Accepted April 1, 2015

*ostensible application: beta thalassemia

tri-nuclear zygote -- typically arise due to multiple sperm penetration; generally discarded in IVF



[Balakier, Human Reprod (1999)]

In this report, we used tripronuclear (3PN) zygotes to further investigate CRISPR/Cas9-mediated gene editing in human cells. We found that CRISPR/Cas9 could effectively cleave the endogenous β-globin gene (*HBB*).* However, the efficiency of homologous recombination directed repair (HDR) of *HBB* was low and the edited embryos were mosaic. Off-target cleavage was also apparent in these 3PN zygotes as revealed by the T7E1 assay and whole-exome sequencing. Furthermore, the endogenous delta-globin gene (*HBD*), which is homologous to *HBB*, competed with exogenous donor oligos to act as the repair template, leading to untoward mutations. Our data also

CRISPR-Cas9 genome editing*

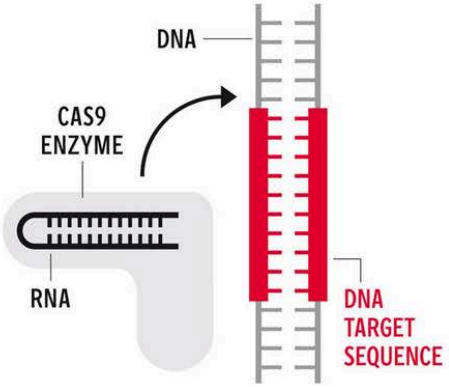
THE INDEPENDENT SATURDAY 25 APRIL 2015

A BRAVE NEW WORLD OF GENOME EDITING

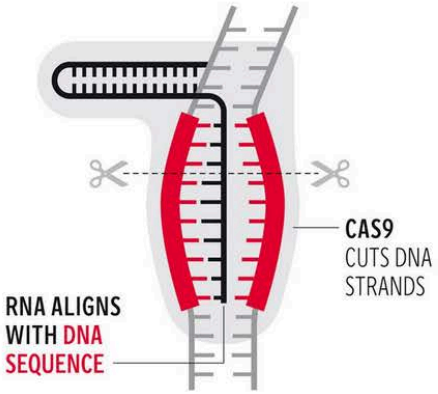
How the Crispr system derived from bacteria works on human cells to correct genetic defects

~20 bases

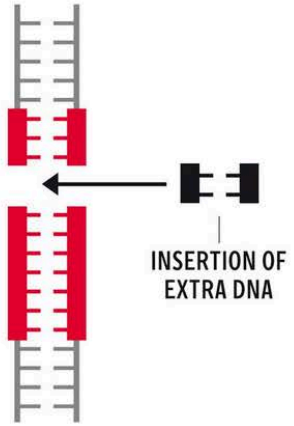
1
An RNA "guide" molecule can be **programmed to match any unique DNA sequence** found in the human genome



2
A special enzyme, called **CAS9, can be attached to the RNA guide**. Its job is to find the target sequence of DNA



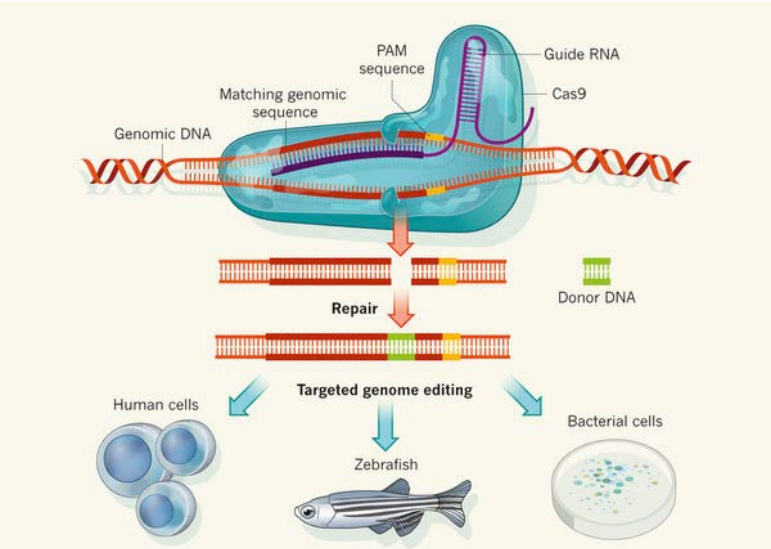
3
The RNA aligns with the target DNA sequence and the **CAS9 attaches and cuts both strands of the DNA double helix**



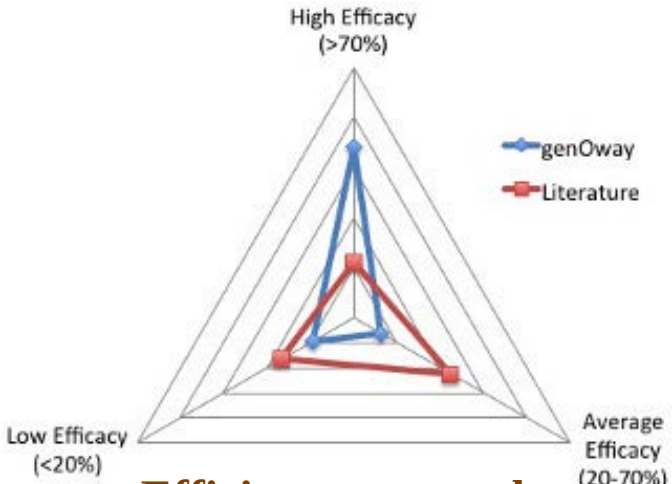
4
The DNA cuts can be **amended with an extra DNA insertion** (above), or a deletion of defective DNA

***'clustered regularly interspaced short palindromic repeats'**

CRISPR-Cas9 genome editing*

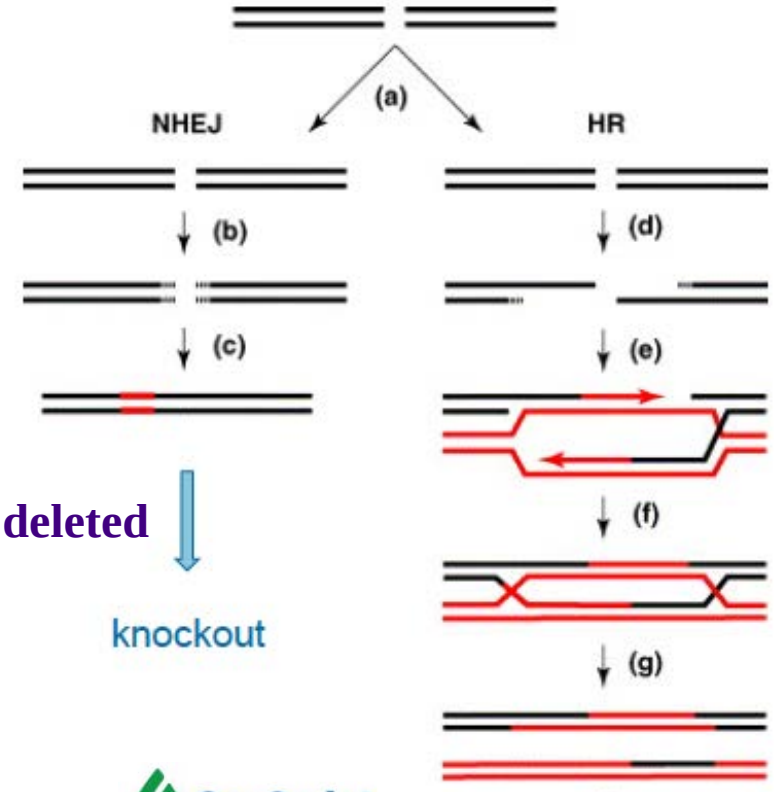


[Charpentier, Nature (2013)]



Efficiency currently generally <100%

Various kinds of potential alterations in gene due to "repaired" DNA



gene deleted

knockout

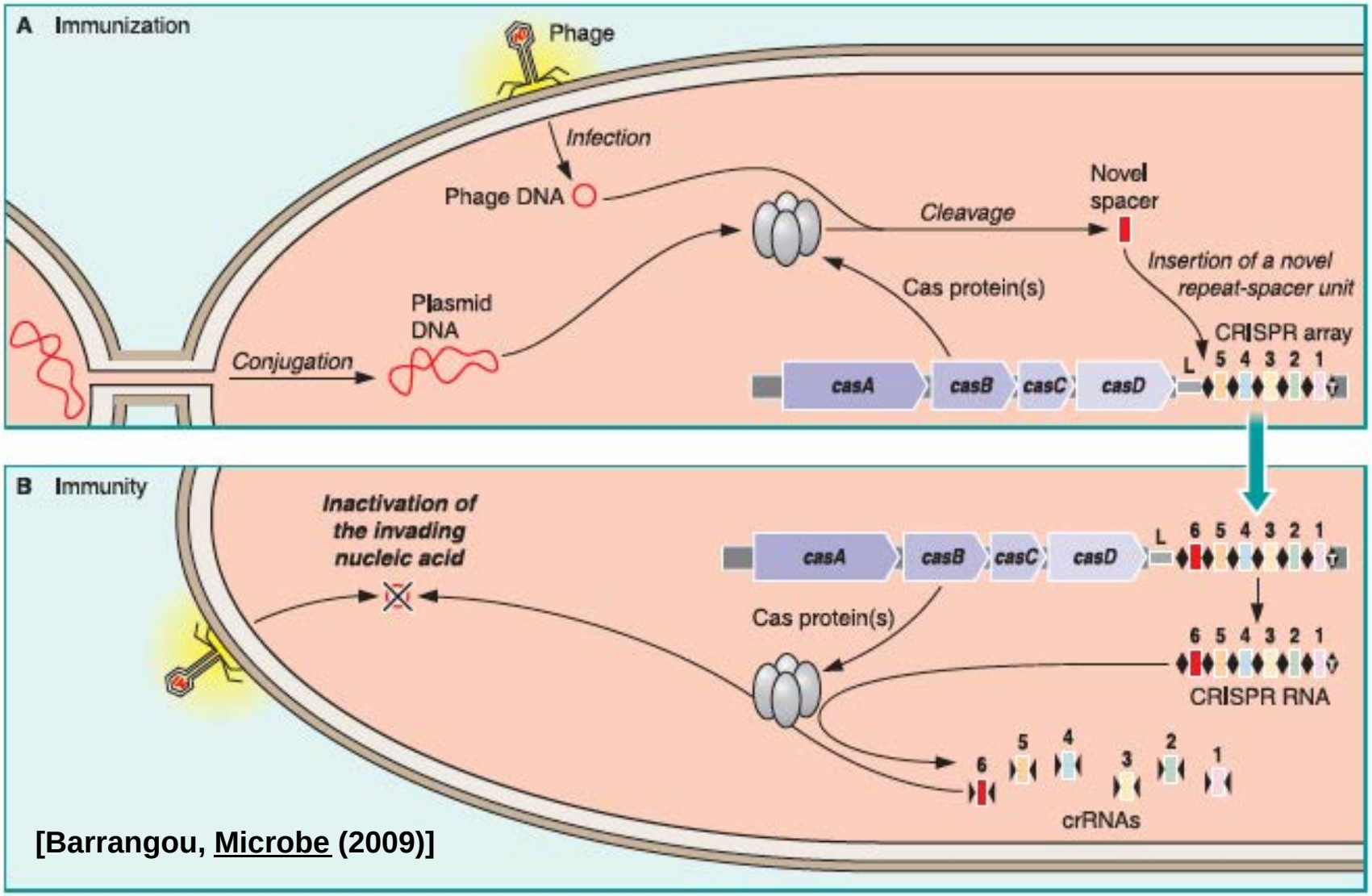
gene inserted

knockin

McGovern Institute video

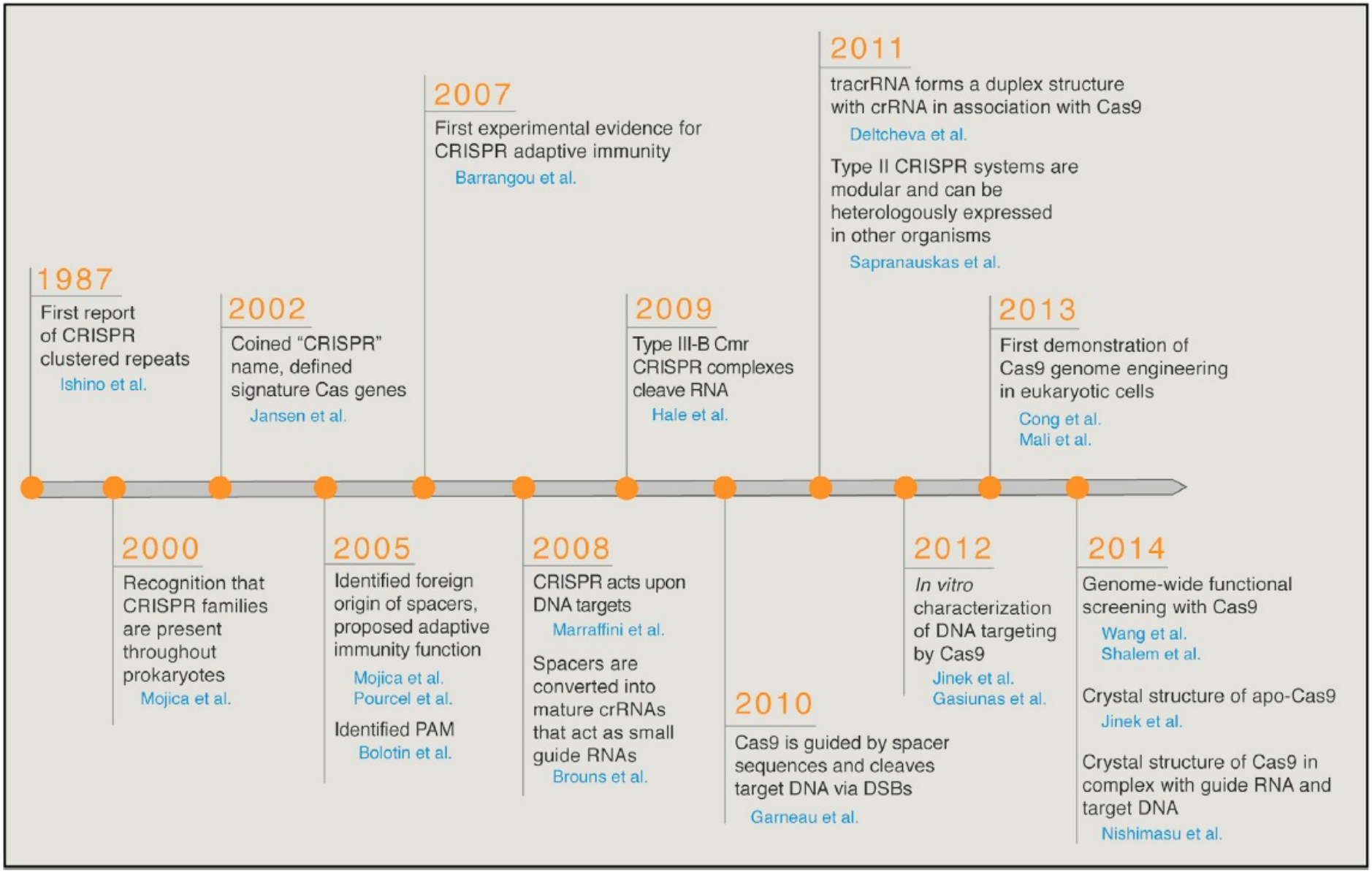
https://www.youtube.com/watch?v=2pp17E4E-O8

CRISPR originally a bacterial "immune response" against foreign DNA (e.g., viruses, other microbes)



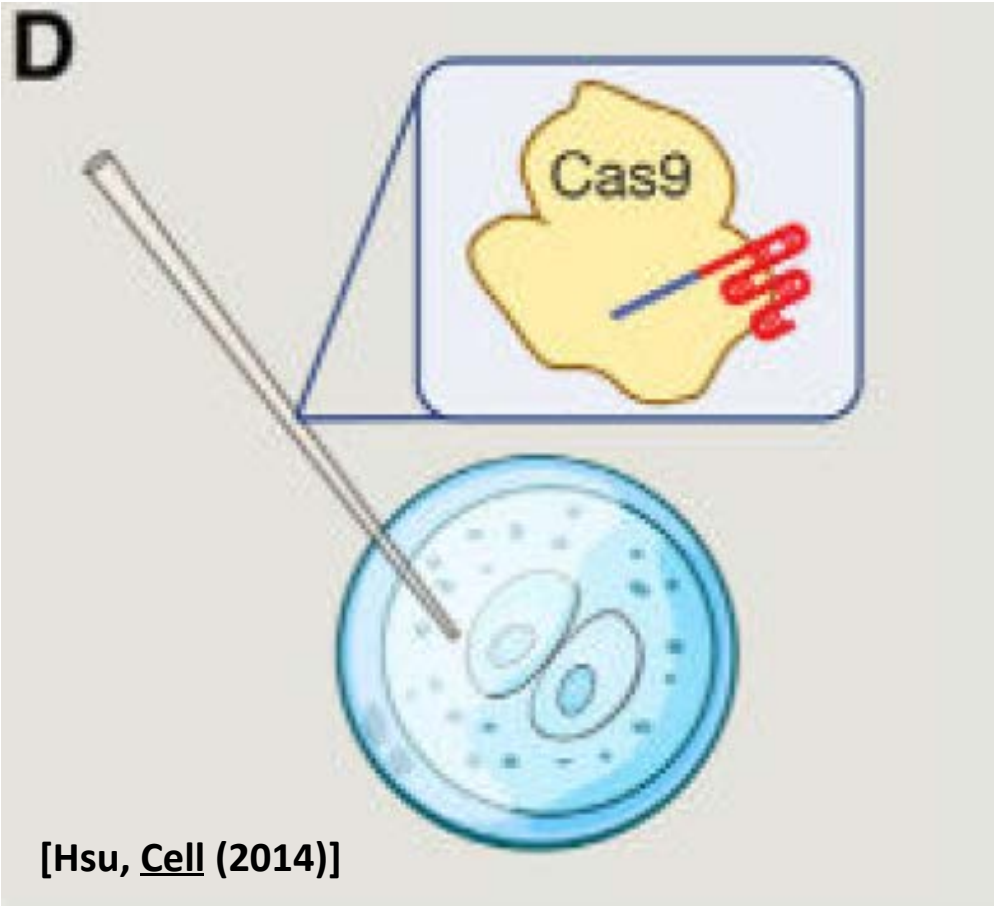
[Barrangou, Microbe (2009)]

CRISPR-Cas9 Historical Timeline



[Hsu, Cell (2014)]

CRISPR-Cas9 genome editing



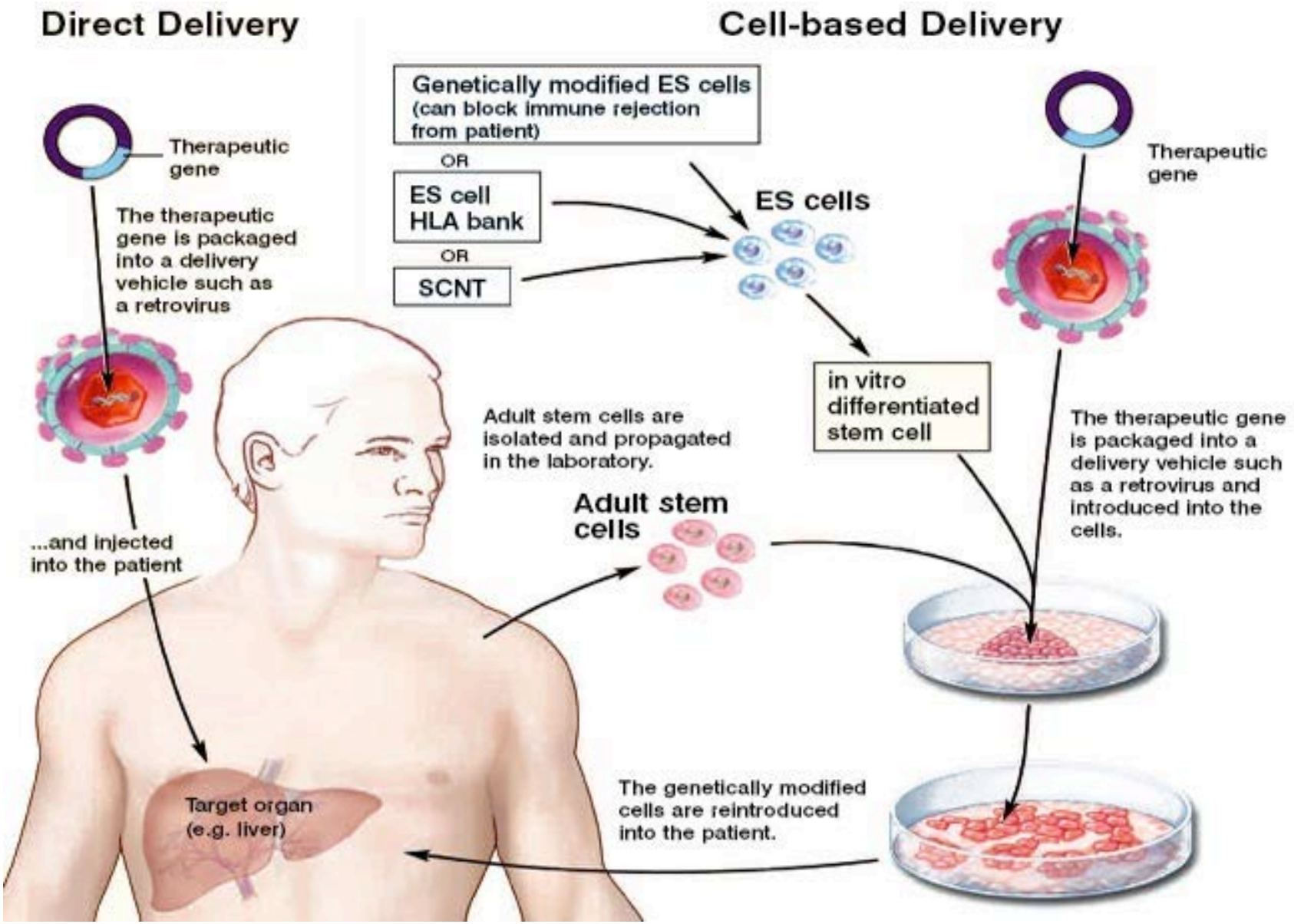
[Hsu, Cell (2014)]

Among myriad kinds of applications in basic science and in medicine, one is correction of genetic diseases

-- examples:

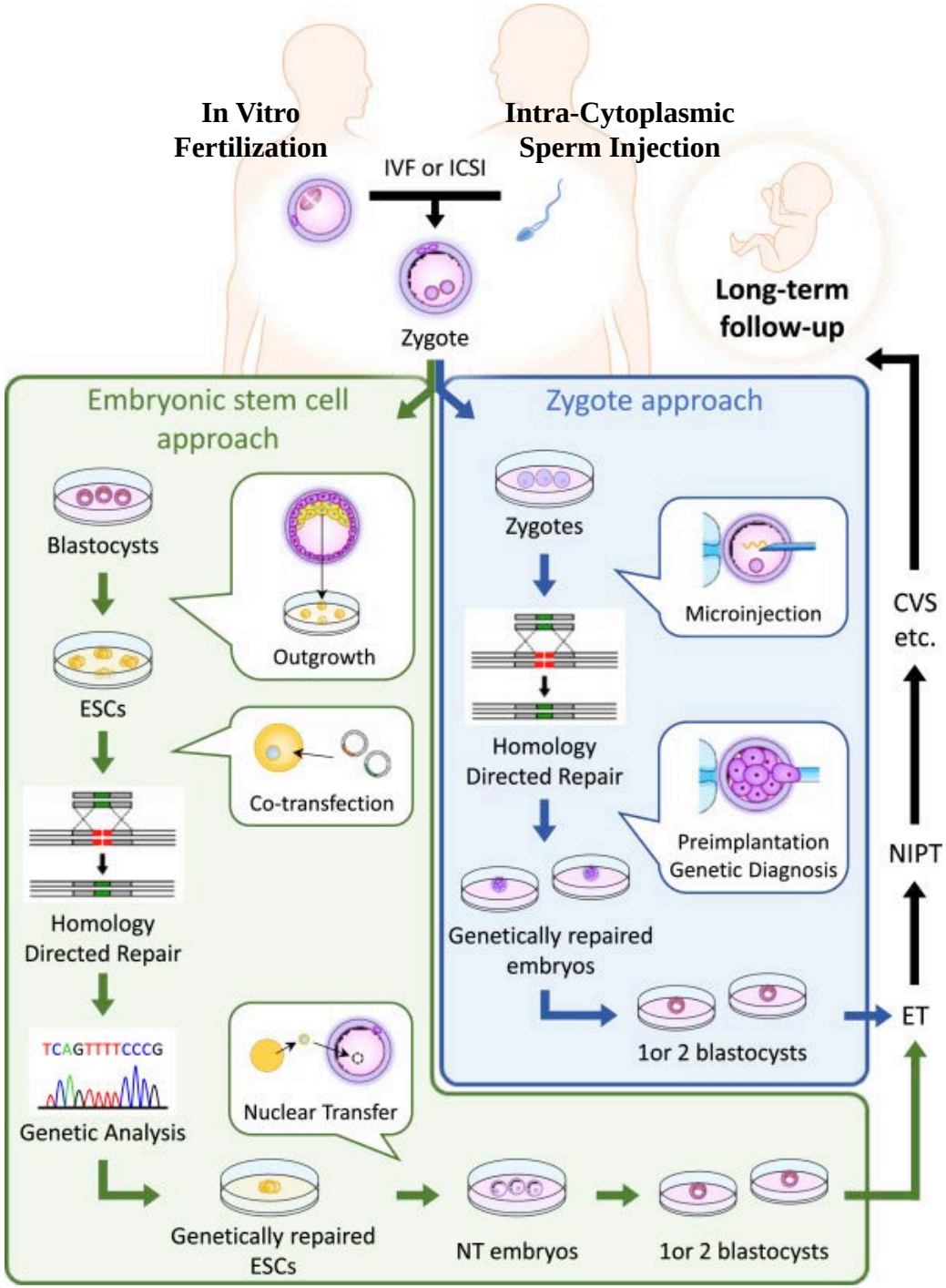
- **Cystic fibrosis**
- **Muscular dystrophy**
- **Huntington's disease**
- **Beta thalassemia**
- **Sickle cell anemia**
- **…**

Overall Context of Gene Therapy



[http://stemcells.nih.gov/info/2001report/pages/chapter11.aspx]

Embryo-Based Gene Therapy



Chorionic Villus Sampling (or Amniocentesis)

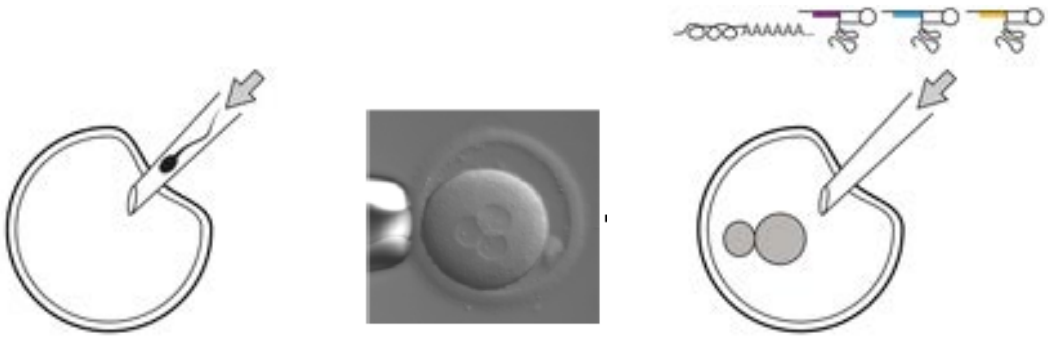
Non-Invasive Parental Genetic Testing

Embryo Transfer

[Araki, Reprod Biol Endocrinol (2014)]

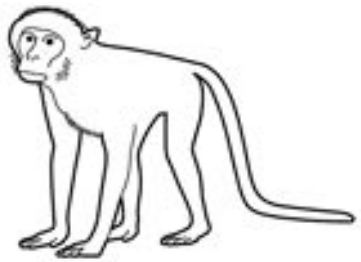
Application to Disease Research – Animal Studies

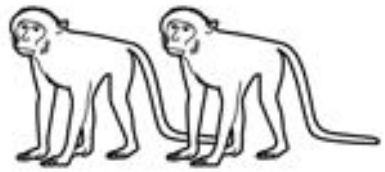
Example: neurological pathologies, in non-human primates



1 Sperm injection Lab technicians inject a single sperm into an unfertilized egg.

2 Genome editing The fertilized egg is injected with "guide" RNAs that target a specific gene, and a template for the DNA-cutting enzyme.





3 Surrogate mother Researchers transfer healthy-looking embryos, now dividing into many cells, into female monkeys. Typically, three embryos are transferred into a surrogate.

4 Primate babies The twins Mingming and Lingling are born with multiple genetic changes, the first live primates created in experiments using CRISPR genome editing.

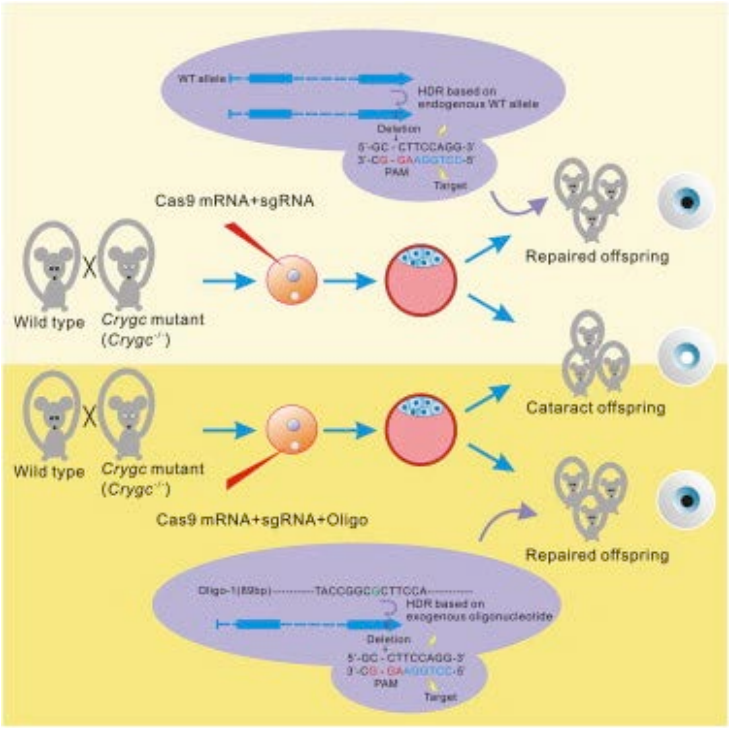
MIT Technology Review

Cell Volume 156, Issue 4, p836–843, 13 February 2014

Generation of Gene-Modified Cynomolgus Monkey via Cas9/RNA-Mediated Gene Targeting in One-Cell Embryos

Yuyu Niu[7], Bin Shen[7], Yiqiang Cui[7], Yongchang Chen[7], Jianying Wang, Lei Wang, Yu Kang, Xiaoyang Zhao, Wei Si, Wei Li, Andy Peng Xiang, Jiankui Zhou, Xuejiang Guo, Ye Bi, Chenyang Si, Bian Hu, Guoying Dong, Hong Wang, Zuomin Zhou, Tianqing Li, Tao Tan, Xiuqiong Pu, Fang Wang, Shaohui Ji, Qi Zhou, Xingxu Huang, Weizhi Ji, Jiahao Sha

Application to Genetic Disease Correction: Animal Studies

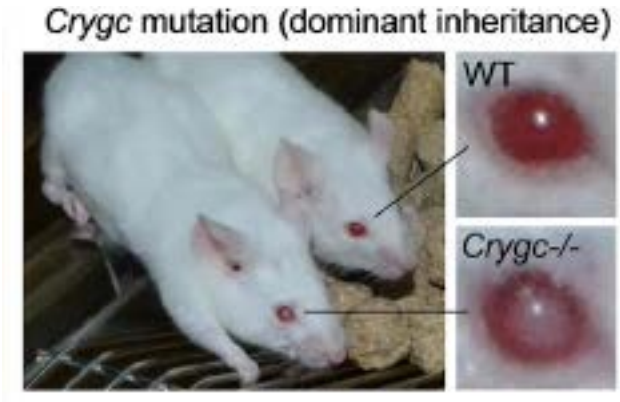


Cell Stem Cell
Brief Report

Cell Stem Cell *13*, 659–662, December 5, 2013

Correction of a Genetic Disease in Mouse via Use of CRISPR-Cas9

Yuxuan Wu,[1,7] Dan Liang,[1,2,7] Yinghua Wang,[1,2] Meizhu Bai,[1,3] Wei Tang,[4] Shiming Bao,[5] Zhiqiang Yan,[5] Dangsheng Li,[6] and Jinsong Li[1,3,*]



Example: cataracts, in mice

Key Issues

- **Ethics I**
  - **germ-line cells vs somatic cells**
    - **alterations enter human heredity**
- **Safety**
  - **unintended consequences**
    - **off-target effects**
    - **gene co-variation effects**
    - **general lack of predictive capability**
- **Value**
  - **actual medical benefit?**
- **Ethics II**
  - **“desirable” traits?**
  - **informed consent?**
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Germ-line vs Somatic Cell Gene Editing

Germ-line =

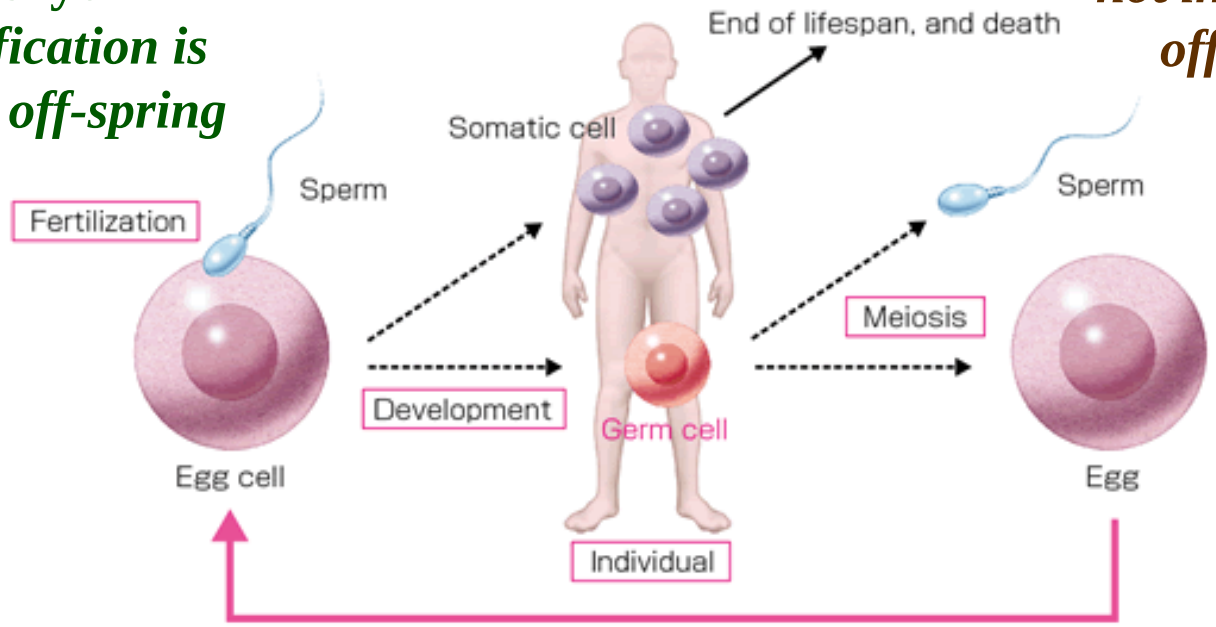
- **Egg**
- **Sperm**
- **Zygote**
- **Embryo**

Gene modification is inherited by off-spring

Somatic =

- **All other tissue/blood cell types**

Gene modification is not inherited by off-spring



[http://csls-text2.c.u-tokyo.ac.jp/inactive/05_06.html]

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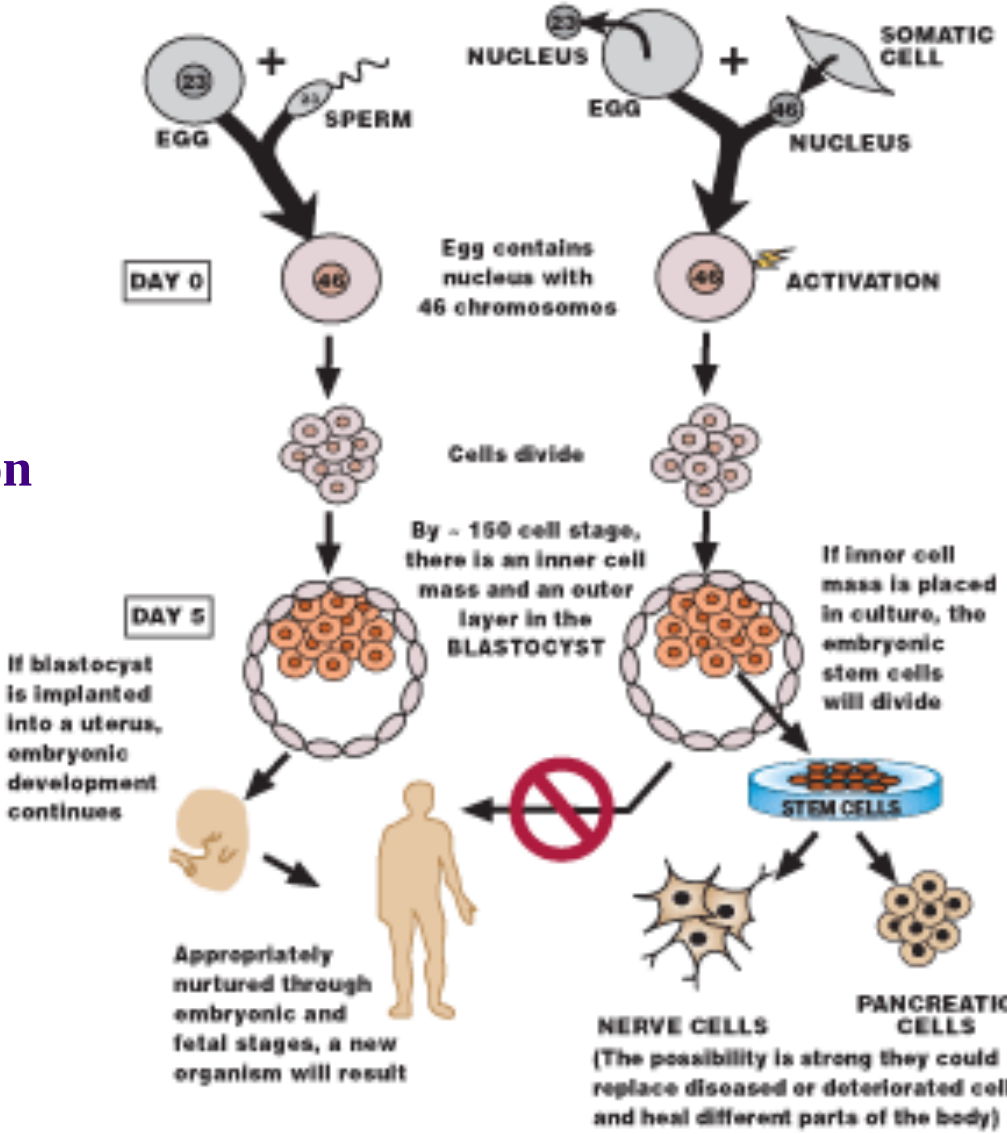

Somatic =

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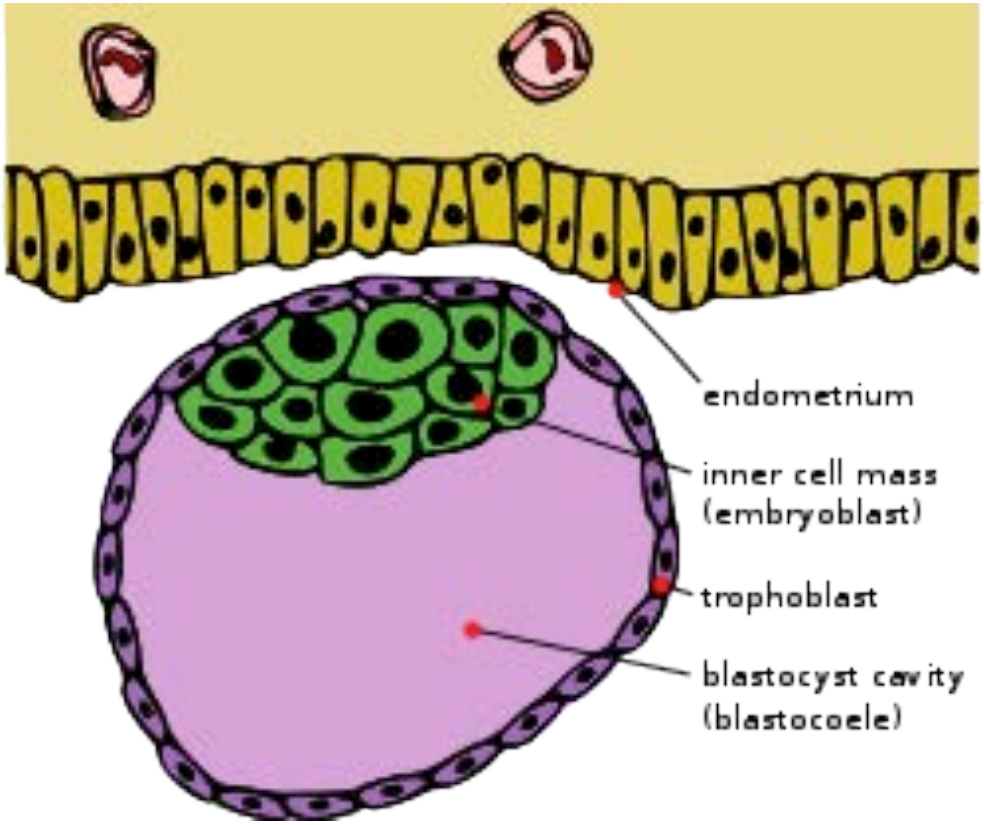
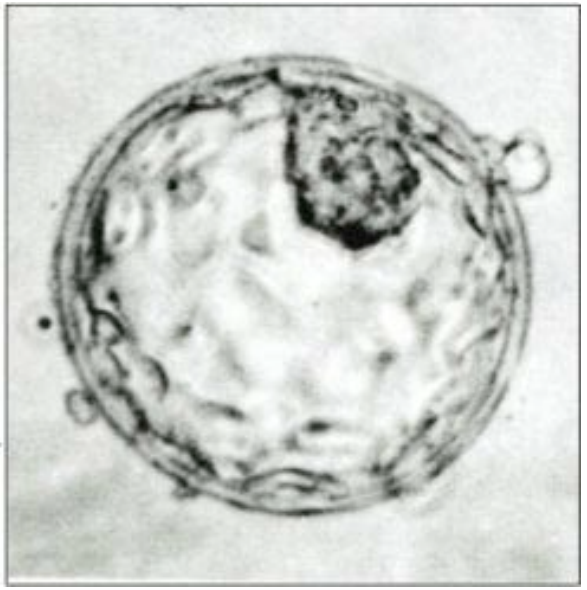
Embryonic Blastocysts

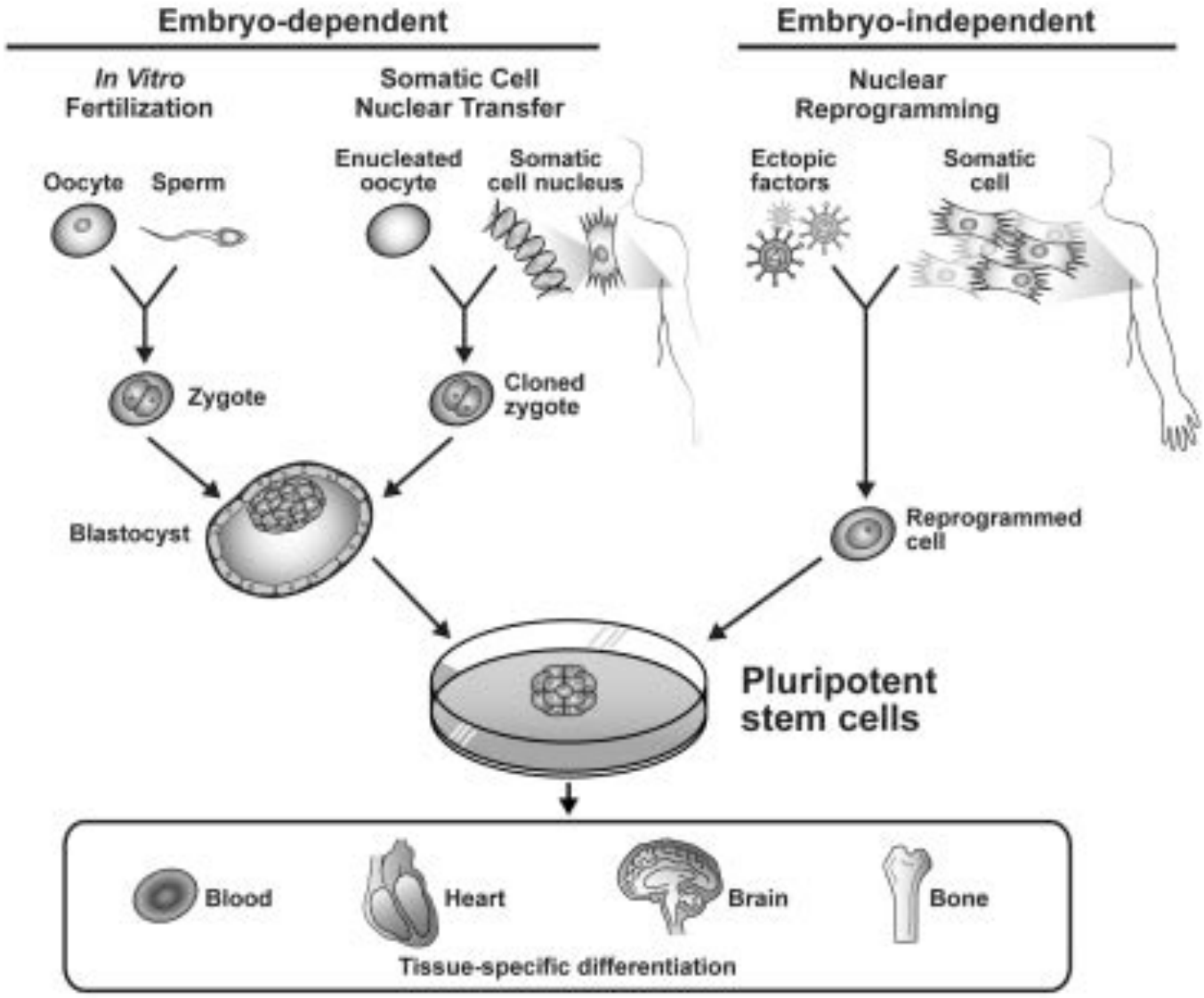




What are sources?

[http://en.wikipedia.org/wiki/Blastocyst]

Embryonic Stem Cell Sources



Mayo Clin Proc. • *July 2011;86(7):634-640*

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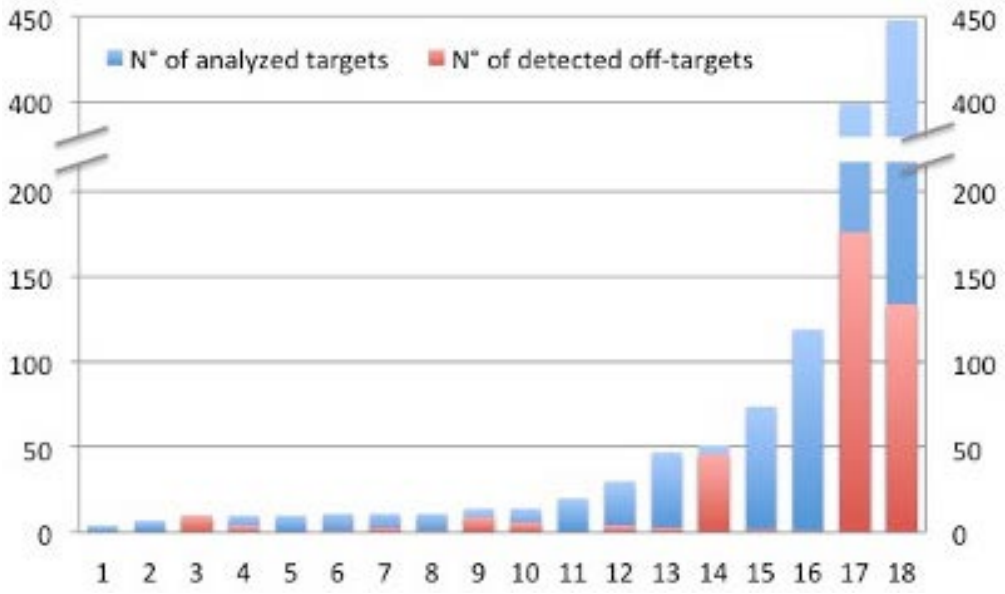
Off-Target Effects

- **Issue of vigorous research, in quantitative analysis and in enhancement of selectivity**
- **Currently, significant probability of off-target gene mutations in cells for which the desired gene is affected**



http://www.genoway.com/technologies/crispr-cas9-technology.htm

PLoS One. 2015 Apr 24;10(4):e0124633. doi: 10.1371/journal.pone.0124633. eCollection 2015.

CCTop: An Intuitive, Flexible and Reliable CRISPR/Cas9 Target Prediction Tool.

Stemmer M[1], Thumberger T[1], Del Sol Keyer M[1], Wittbrodt J[1], Mateo JL[1].

Nat Methods. 2015 Mar;12(3):237-43, 1 p following 243. doi: 10.1038/nmeth.3284. Epub 2015 Feb 9.

Digenome-seq: genome-wide profiling of CRISPR-Cas9 off-target effects in human cells.

Kim D[1], Bae S[1], Park J[2], Kim E[3], Kim S[3], Yu HR[3], Hwang J[4], Kim JI[5], Kim JS[1].

Nat Commun. 2014 Nov 26;5:5507. doi: 10.1038/ncomms6507.

Targeted and genome-wide sequencing reveal single nucleotide variations impacting specificity of Cas9 in human stem cells.

Yang L[1], Grishin D[2], Wang G[3], Aach J[2], Zhang CZ[4], Chari R[2], Homsy J[2], Cai X[5], Zhao Y[5], Fan JB[5], Seidman C[2], Seidman J[2], Pu W[3], Church G[1].

RESEARCH ARTICLE

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CRISPR/Cas9-mediated gene editing in human tripronuclear zygotes

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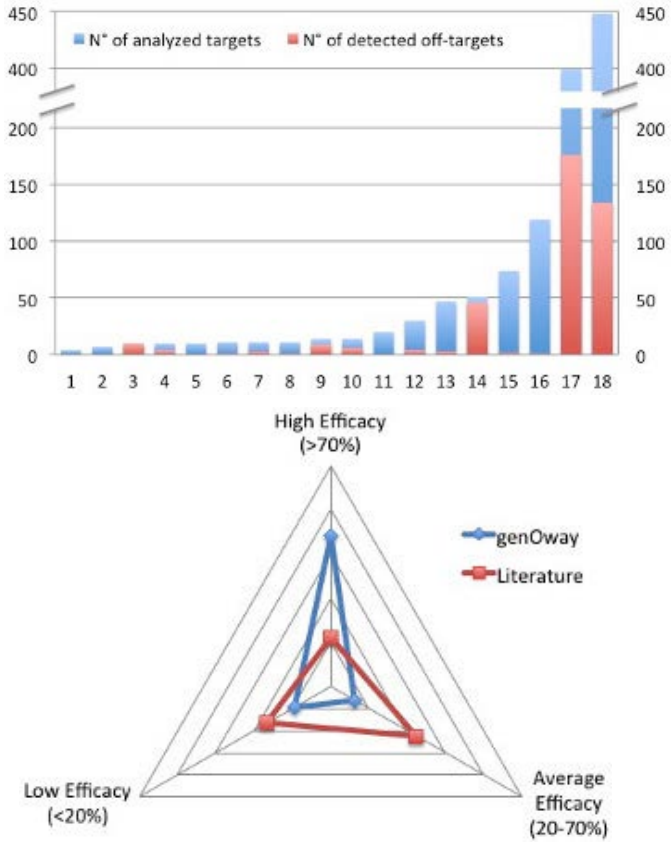
Guangdong Province Key Laboratory of Reproductive Medicine, the First Affiliated Hospital, and Key Laboratory of Gene Engineering of the Ministry of Education, School of Life Sciences, Sun Yat-sen University, Guangzhou 510275, China

✉ Correspondence: hjunjiu@mail.sysu.edu.cn (J. Huang), zhoucanquan@hotmail.com (C. Zhou)

Received March 30, 2015 Accepted April 1, 2015



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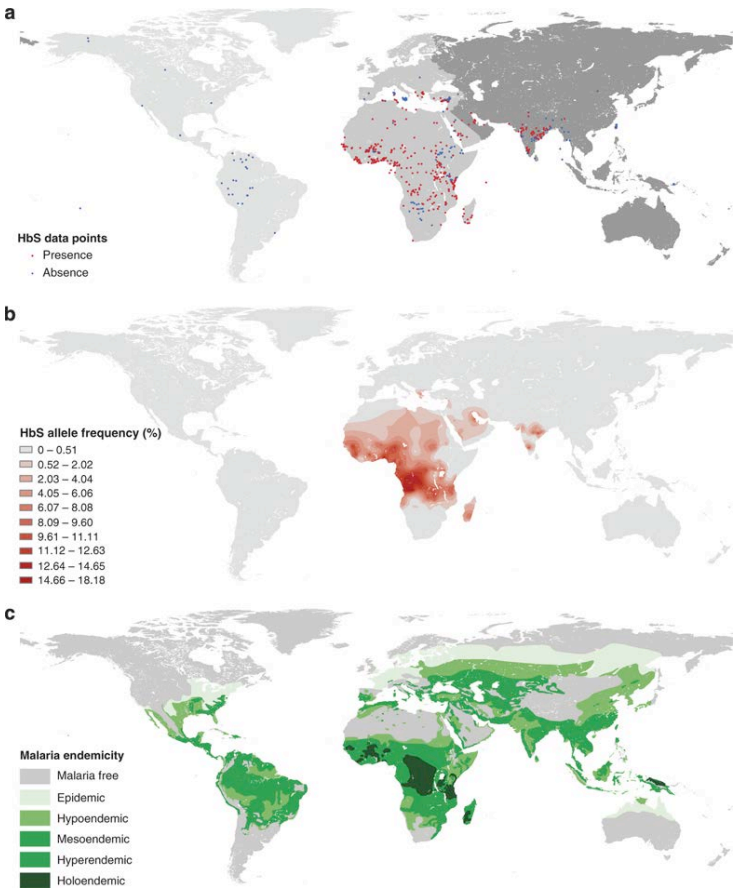
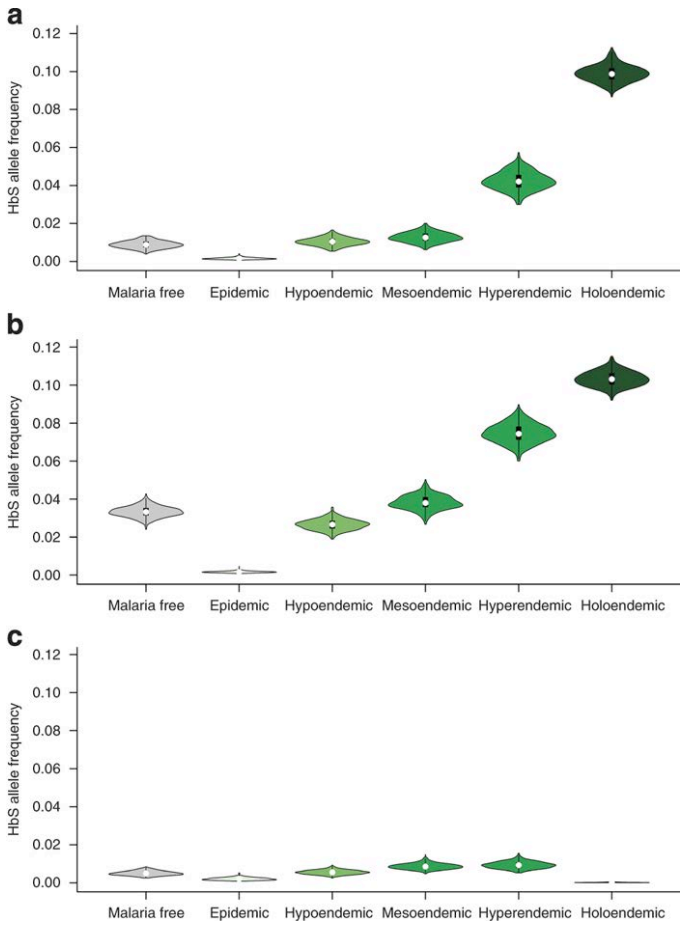
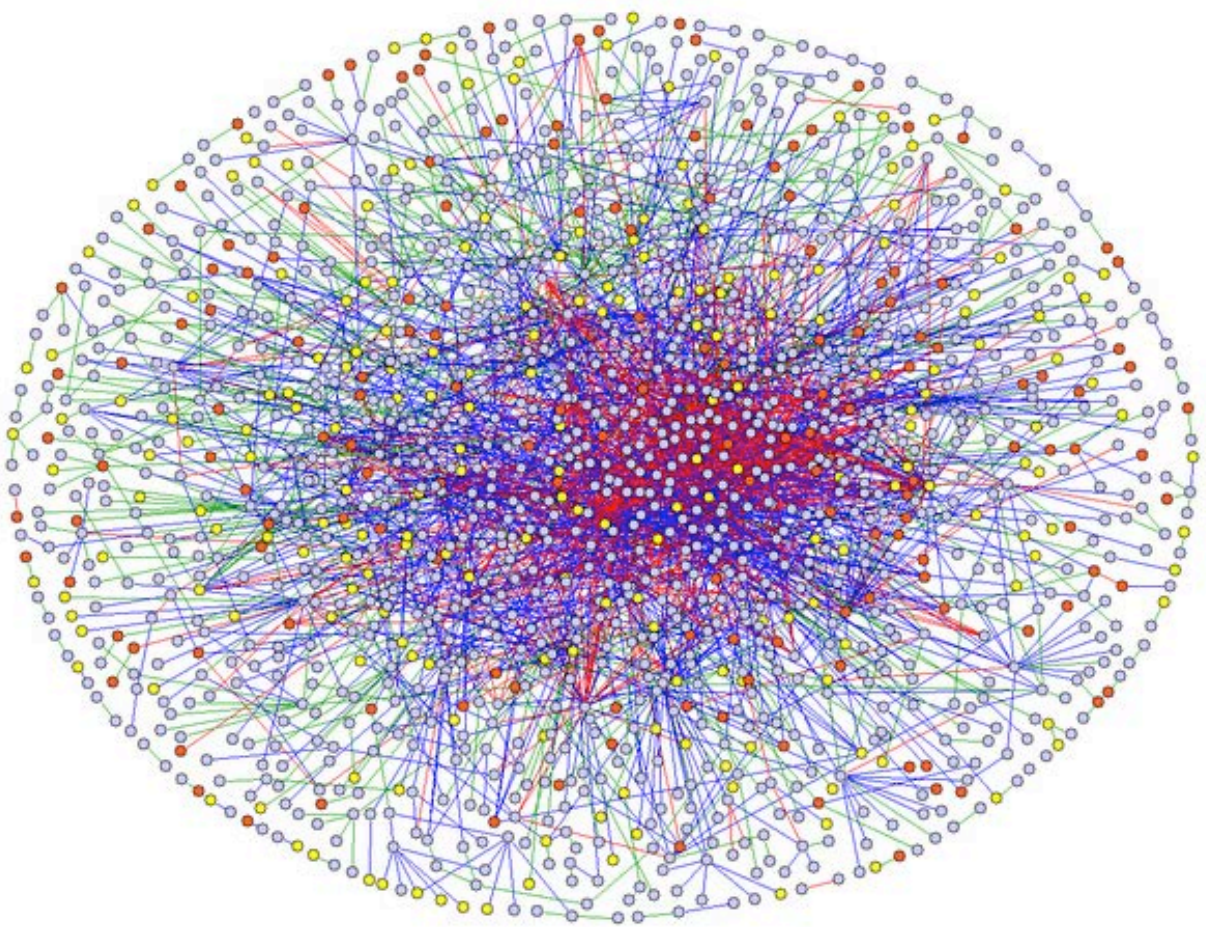
Gene Co-Variation – Sickle Cell Anemia and Malaria



Sickle Cell hemoglobin gene mutation is found more frequently in areas where malaria is prevalent -- it has favorable selection advantage due to protection against malaria parasite survival within red blood cells



[Piel, Nature Comm (2014)]



[Max Planck Inst Molec Genetics, Munich]

General Lack of Predictability

-- dynamic gene network complexity

-- environmental context dependent

Estimated #Protein-Protein Interactions

Fly	**~70,000**
Worm	**~200,000**
Plants	**~300,000**
Human	**~700,000**

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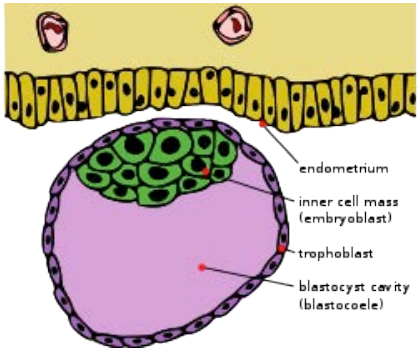
California IVF:
Davis Fertility Center, Inc

Medical Benefit Beyond Current Capabilities?

Preimplantation Genetic Diagnosis (PGD)

Alagille syndrome
Alpers disease
alpha 1 anti-trypsin
alpha-thalassemia
Alport syndrome
anti-Kell antibodies
Becker muscular dystrophy
beta-thalassemia
breast cancer, gene 1 & 2
carbamoyl phosphate synthetase deficiency
central core disease
cerebral arteriopathy (Cadasil)
Charcot-Marie-Tooth syndrome 1A & 1B
chronic granulomatosis disease (CGD)
congenital adrenal hyperplasia
congenital disorder of glycosylation
congenital nephrotic syndrome
connexin 26
Crigler-Najjar syndrome I
Crouzon syndrome
cystic fibrosis
Czech dysplasia
Dejerine-Sottas syndrome
Duchenne muscular dystrophy
early onset Alzheimer disease
early onset torsion dystonia
E-cadherin
ectodermal dysplasia
Emery Dreifuss muscular dystrophy
epidermolysis bullosa, dominant dystrophic
epidermolysis bullosa,
- Herlitz junctional, gene 1 or gene 2
epidermolytic palmoplantar keratosis
facioscapulohumeral muscular dystrophy
familial adenomatous polyposis
familial amytrophic lateral sclerosis
(Lou Gehrig's disease)
Fechtner syndrome
fragile X
fumarase deficiency
galactosemia
Gaucher disease type 2
Goldberg-Shprintzen syndrome
Gorlin syndrome
haemophilia A or B
Hirschsprung's disease
HLA match for Wiskott-Aldrich syndrome
HLA match with beta thalassemia
HLA match with diamond blackfan anemia
HLA match with hyper IgM
HLA match with sickle cell anemia
HLA matching
Holt Oram Syndrome
Hunter syndrome (mucopolysaccharidosis II A)
Huntington disease
Hyper IgM
hypochondroplasia
hypophosphatasia
hypophosphatemic rickets
incontinentia pigmenti
infantile neuroaxonal dystrophy
juvenile neuronal ceroid lipofuscinosis
juvenile retinoschisis
late infantile neuronal ceroid lipofuscinosis
(Batten disease)
Lowe oculocerebrorenal syndrome
medium-chain acyl-CoA dehydrogenase deficiency
medullary thyroid carcinoma (RET)
metachromatic leukodystrophy
mucopolysaccharidosis III B
multiple endocrine neoplasia 2A
multiple hereditary exotoses
myotonic muscular dystrophy
myotubular myopathy
nail-patella syndrome
nemaline myopathy
nephrogenic diabetes insipidus
neurofibromatosis types 1
neurofibromatosis types 2
Norrie disease
oculocutaneous albinism
ornitrine transcarbamylase deficiency
osteogenesis imperfecta type 1
palmoplantar hyperkeratosis
Pendred syndrome
pericentric inversion of X
polycystic kidney disease, autosomal dominant, gene 1
polycystic kidney disease, autosomal dominant, gene 2
polycystic kidney disease, autosomal recessive
proximal myotonic myopathy
psoriasis, susceptibility gene
pulmonary alveolar proteinosis
retinoblastoma
rhesus D disease
Saethre-Chotzen syndrome
Sandhoff disease
sickle-cell anaemia
spinal muscular atrophy 1, 2, or 3
Stickler syndrome
thyroid cancer
transthyretin amyloidosis
Treacher-Collins syndrome
tuberous sclerosis, gene 1
tuberous sclerosis, gene 2
Ullrich congenital muscular dystrophy
vitelliform macular dystrophy
von Hippel-Lindau disease
Wilms tumour
Wiskott-Aldrich syndrome
Wolman disease
X-linked adrenoleukodystrophy
X-linked choroideremia
Zellweger syndrome



While not whole genome sequencing, can examine for particular mutations of concern from parental genetics

[http://www.californiaivf.com/genetic-diagnosis-PGD-CGH.htm]

Medical Benefit Beyond Current Capabilities?

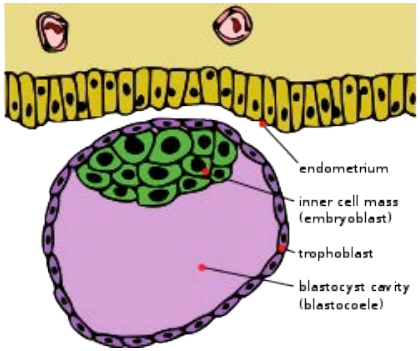
BIOMEDICINE NEWS

MIT Technology Review

DNA Sequencing of IVF Embryos

Researchers are testing whether high-throughput DNA sequencing can help screen out abnormal embryos during in vitro fertilization.

By Susan Young Rojahn on February 14, 2014



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IVF embryos: whole genetic code can be scanned for mutations

Last updated: Thursday 12 February 2015 at 12am PST

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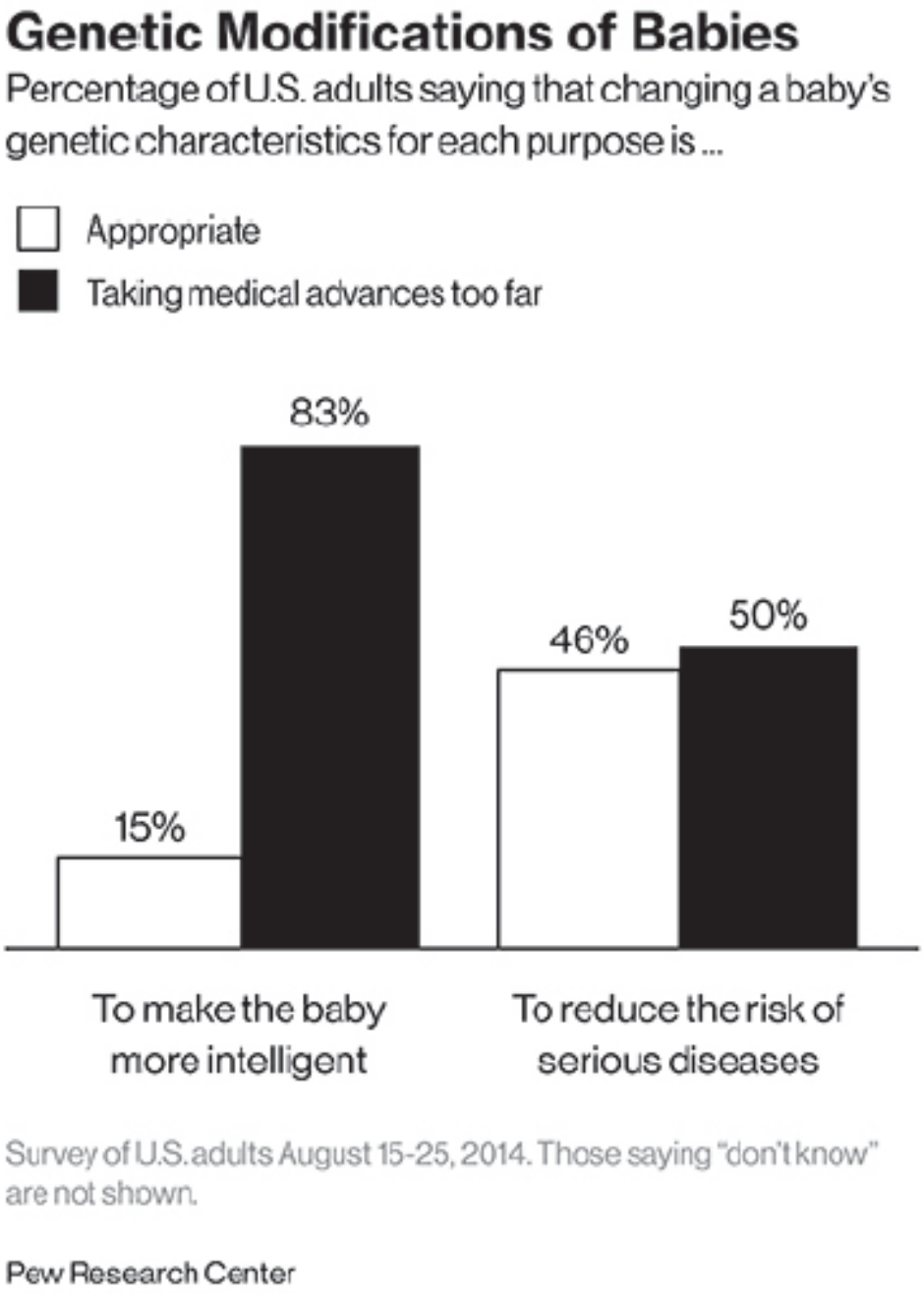
[http://www.medicalnewstoday.com/articles/289279.php]

- **Can do parental genome sequencing in order to ascertain potential risks for which to examine particular embryo genes**
- **Potential for embryo genome sequencing when parental is not available, or for de novo mutations**

Key Issues

- **Ethics I**
  - **germ-line cells vs somatic cells**
    - **alterations enter human heredity**
- **Safety**
  - **unintended consequences**
    - **off-target effects**
    - **gene co-variation effects**
    - **general lack of predictive capability**
- **Value**
  - **actual medical benefit?**
- **Ethics II**
  - **“desirable” traits?**
  - **informed consent? – individual and progeny**
  - **socio-economic equity?**



"Non-Therapeutic" Applications

Imaginable examples?

Scientific Community "Pro-Active" Reaction

Science. 2015 Apr 3;348(6230):36-8. doi: 10.1126/science.aab1028. Epub 2015 Mar 19.

Biotechnology. A prudent path forward for genomic engineering and germline gene modification.

Baltimore D[1], Berg P[2], Botchan M[3], Carroll D[4], Charo RA[5], Church G[6], Corn JE[7], Daley GQ[8], Doudna JA[9], Fenner M[7], Greely HT[10], Jinek M[11], Martin GS[12], Penhoet E[13], Puck J[14], Sternberg SH[15], Weissman JS[16], Yamamoto KR[17].

1. **Strongly discourage clinical application of this technology at this time.**
2. **Create forums for education and discussion**
3. **Encourage open research to evaluate the utility of CRISPR-Cas9 technology for both human and nonhuman model systems.**
4. **Hold an international meeting to consider these issues and possibly make policy recommendation.**

"At present, the potential safety and efficacy issues arising from the use of this technology must be thoroughly investigated and understood before any at-tempts at human engineer-ing are sanctioned, if ever, for clinical testing."

Jennifer Doudna interview

How did the meeting go? Were there some areas of disagreement?

Doudna: It actually went fairly smoothly. There was definitely very animated discussion. This is a topic that people can feel emotion about. It is pretty profound if you talk about clinical applications that could change human evolution. There were different points of view, but not hugely different. I didn't hear anybody at either extreme saying things like "We should edit people tomorrow!" or "We have to get rid of this technology." It was more focused on questions such as "What kind of safety or regulatory matters should be discussed?" It was only a one-day meeting so there wasn't a lot of time to get into other issues such as gene editing triggering a biological chain reaction where a dominant change could spread through a whole population.

Knoepfler Lab Stem Cell Blog **[http://www.ipscell.com/tag/jennifer-doudna/]**

Jennifer Doudna interview

Can you imagine a future point at which you'd support the use of gene editing in humans in a heritable manner? If so, how do we get to that point from where things are today? What do we need to learn first?

Doudna: We need to learn how efficiently it works. What's the best way to deliver it safely and efficiently? Not only efficiency, but also what are the off-target levels? How do we minimize that? What would be a safe level if any of off-targets? I'd like to see basic research like what happens to the DNA in germ cells or pre-germ cells when a double-stranded break occurs? What is the repair process like in those specific cells? Those answers would be interesting from a basic science perspective as well as informing future potential clinical applications.

I feel uncomfortable imagining widespread gene editing use in humans now, but it is possible that there are going to be certain types of very specific applications that could be envisioned as beneficial in the future. I won't be able to make a decision of the wisdom of such an approach until we have more data. What are the real risks? There is always a risk-versus-reward kind of consideration. In which cases is the risk worth the payoff?

Knoepfler Lab Stem Cell Blog **[http://www.ipscell.com/tag/jennifer-doudna/]**

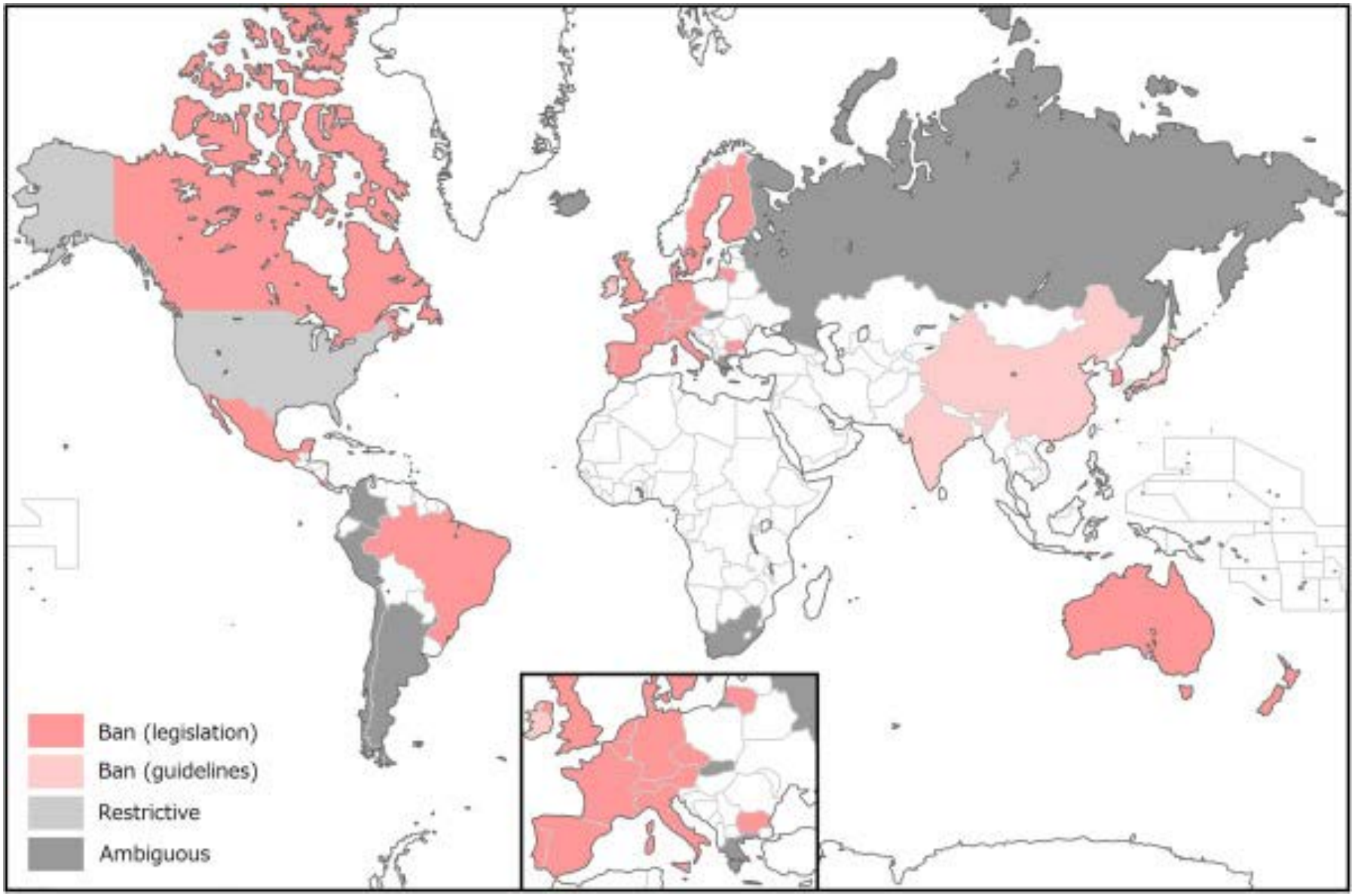
Jennifer Doudna interview

What do we do if someone goes rogue?

Doudna: That's one of the purposes of these meetings: to get out in front of that. I can't guarantee that that might not happen. I can work to form a coalition to say, "here's our considered view of the technology and here's what we see as a prudent way to move forward with this". That's really the best that we can do. There's no way to unlearn what is learned. We can't put this technology to bed. If a person has basic knowledge of molecular biology they can do it. It's not realistic to think we can block it. Same thing with regulations. To imagine that we could have international regulations, it's just not realistic, and in any case, how do you enforce them? I wouldn't feel comfortable hiding away in the lab. The better path is to try to be open and transparent and to educate people who want to understand it. It's such a wonderful technology in many ways. Like any technology it has the potential to be used for good and not so good. We want to put out there the information that people would need to make an informed decision, to encourage appropriate research and discourage forging ahead with clinical applications that could be dangerous or raise ethical issues.

Knoepfler Lab Stem Cell Blog **[http://www.ipscell.com/tag/jennifer-doudna/]**

International Status of Human Germ-line Editing



[Araki, Reprod Biol Endocrinol (2014)]

Status of Human Germ-line Editing in USA

NATURE | BREAKING NEWS

NIH reiterates ban on editing human embryo DNA

Agency issues statement after researchers alter gene in non-viable zygotes.

Sara Reardon

29 April 2015 | Corrected: 29 April 2015

- *"NIH will not fund any use of gene-editing technologies in human embryos."*
- *"The concept of altering the human germ-line in embryos for clinical purposes has been debated over many years… and has been viewed almost universally as a line that should not be crossed."*
- *"…strong arguments against engaging in this activity remain. These include*
  - ✧ *Unquantifiable safety issues*
  - ✧ *Ethical issues presented by altering germ-line in a way that affects the next generation without their consent*
  - ✧ *A current lack of medical applications justifying the use… in embryos."*

[http://www.nih.gov/about/director/04292015_statement_gene_editing_technologies.htm]

Status of Human Germ-line Editing in USA

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NIH reiterates ban on editing human embryo DNA

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29 April 2015 | Corrected: 29 April 2015

- ***"Practically, there are multiple existing legislative and regulatory prohibitions against this kind of work."***
  - ✧ ***"Dickey-Wicker amendment prohibits use of appropriate funds for creation of human embryos for research purposes or for research in which human embryos are destroyed."***
  - ✧ ***"NIH guidelines state that the Recombinant DNA Advisory Committee will not at present entertain proposals for germ-line alteration."***
  - ✧ ***FDA has authority to regulate cell and gene therapy products… which would include human germ-line modification."***

[http://www.nih.gov/about/director/04292015_statement_gene_editing_technologies.htm]