XXXVIII
SABATO DELL'ANDROLOGIA

COLLOQUI IN PMA
TRA GINECOLOGI, BIOLOGI E ANDROLOGI

17 FEBBRAIO 2018

PADERNO DUGNANO

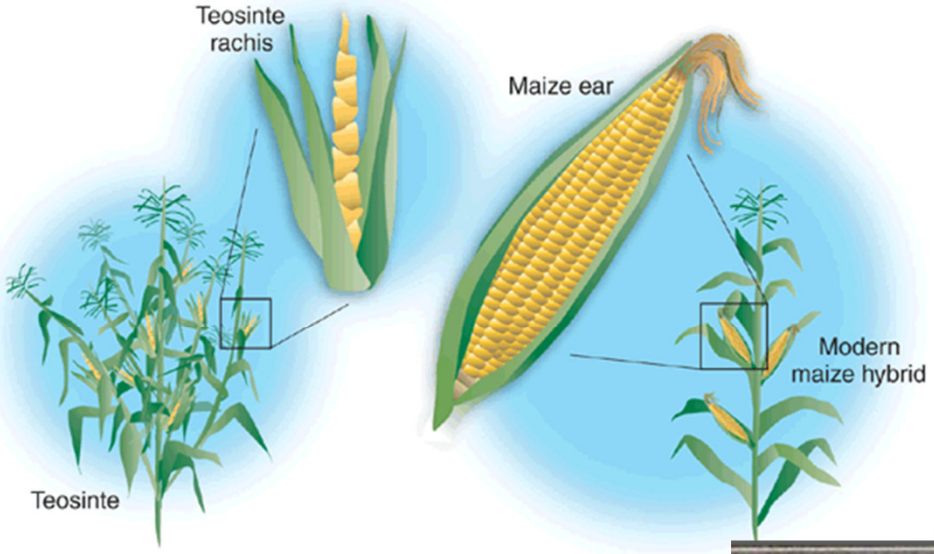
Clinica San Carlo - Via Ospedale, 21
(Auditorium del Nuovo Ospedale)

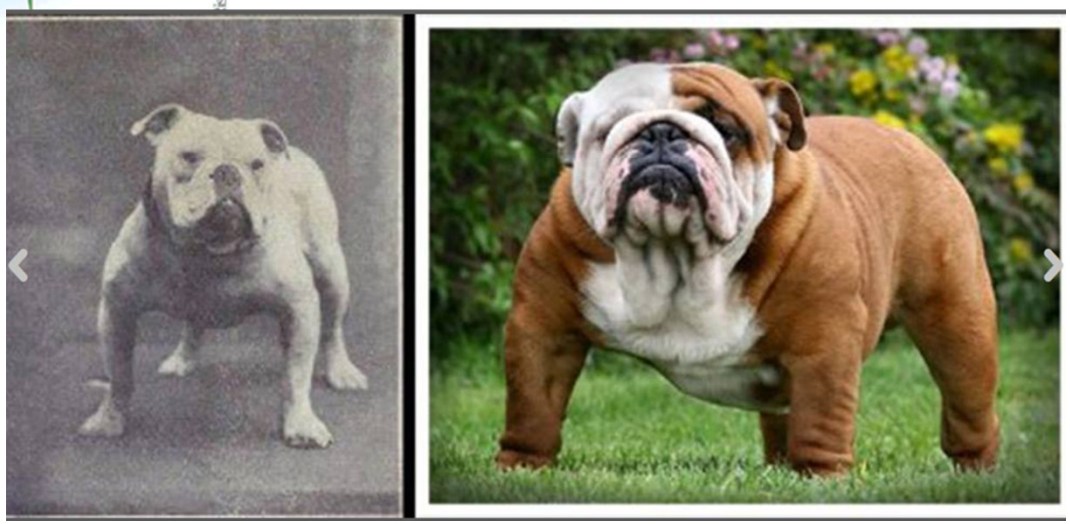
CON IL PATROCINIO DI S.I.R.U.

Lettura: Le nuove frontiere di ingegneria genetica in embriologia

A. Paffoni

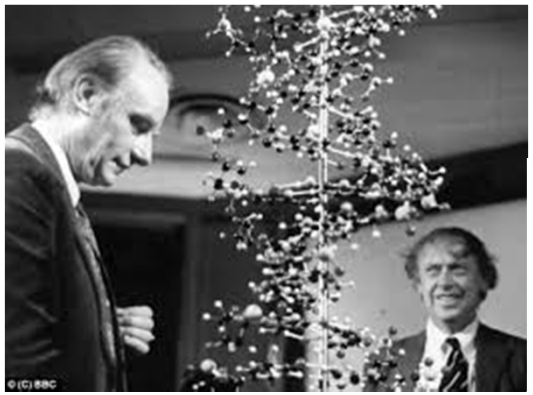
Ingegneria Genetica





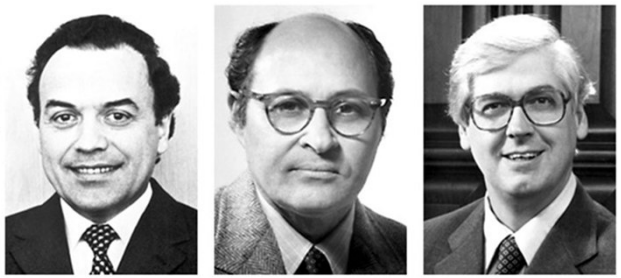
Ingegneria Genetica
Tecnologia del DNA ricombinante

DNA



1953

ENZIMI RESTRIZIONE

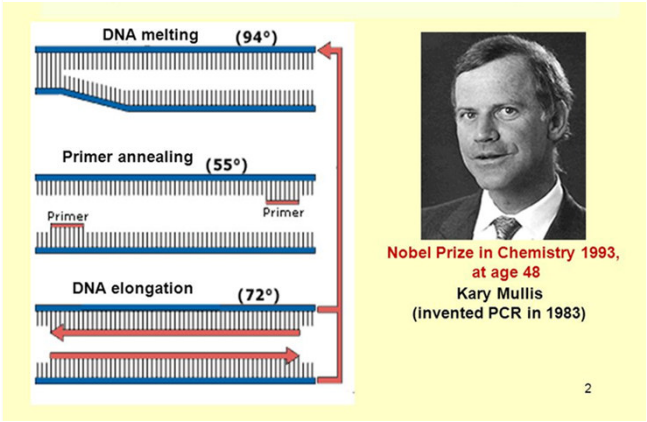


Werner Arber | Daniel Nathans | Hamilton O. Smith

The Nobel Prize in Physiology or Medicine 1978 was awarded jointly to Werner Arber, Daniel Nathans and Hamilton O. Smith *"for the discovery of restriction enzymes and their application to problems of molecular genetics"*.

1973

PCR



1983

Ingegneria Genetica
Tecnologia del DNA ricombinante

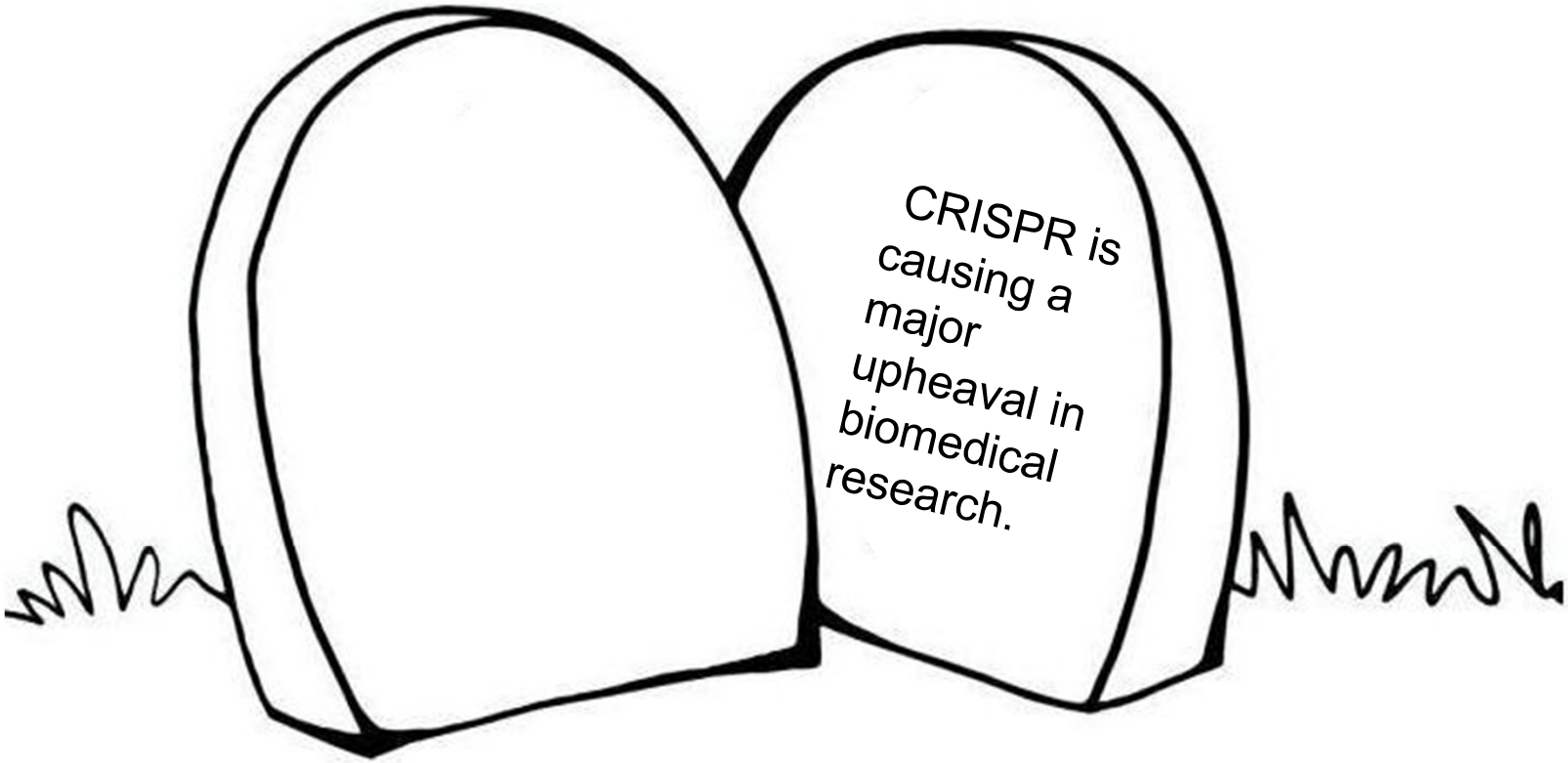
APPLICAZIONI PER LA SALUTE:

. Produzione di farmaci, ormoni, antibiotici, vaccini

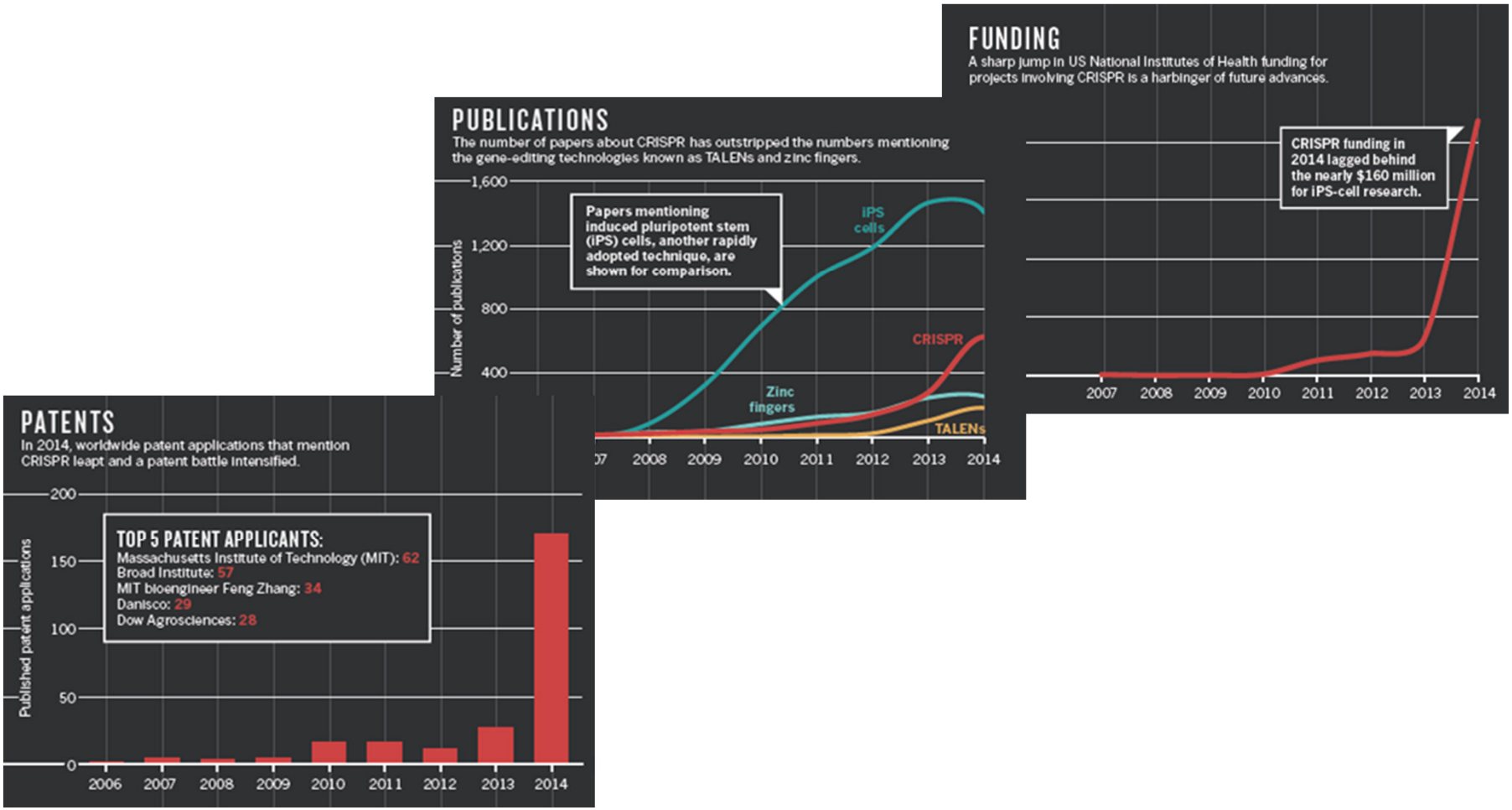
. Correzione di patologie genetiche

CRISPR, THE DISRUPTOR

CRISPR-Cas system allows researchers to quickly change the DNA of nearly any organism — including humans

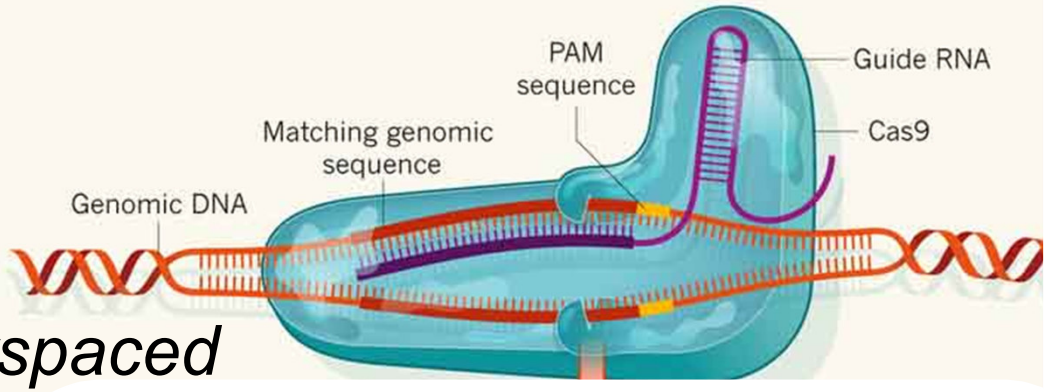


THE RISE OF CRISPR



NATURE | VOL 522 | 4 JUNE 2015

CRISPR- Cas, what?



CRISPR

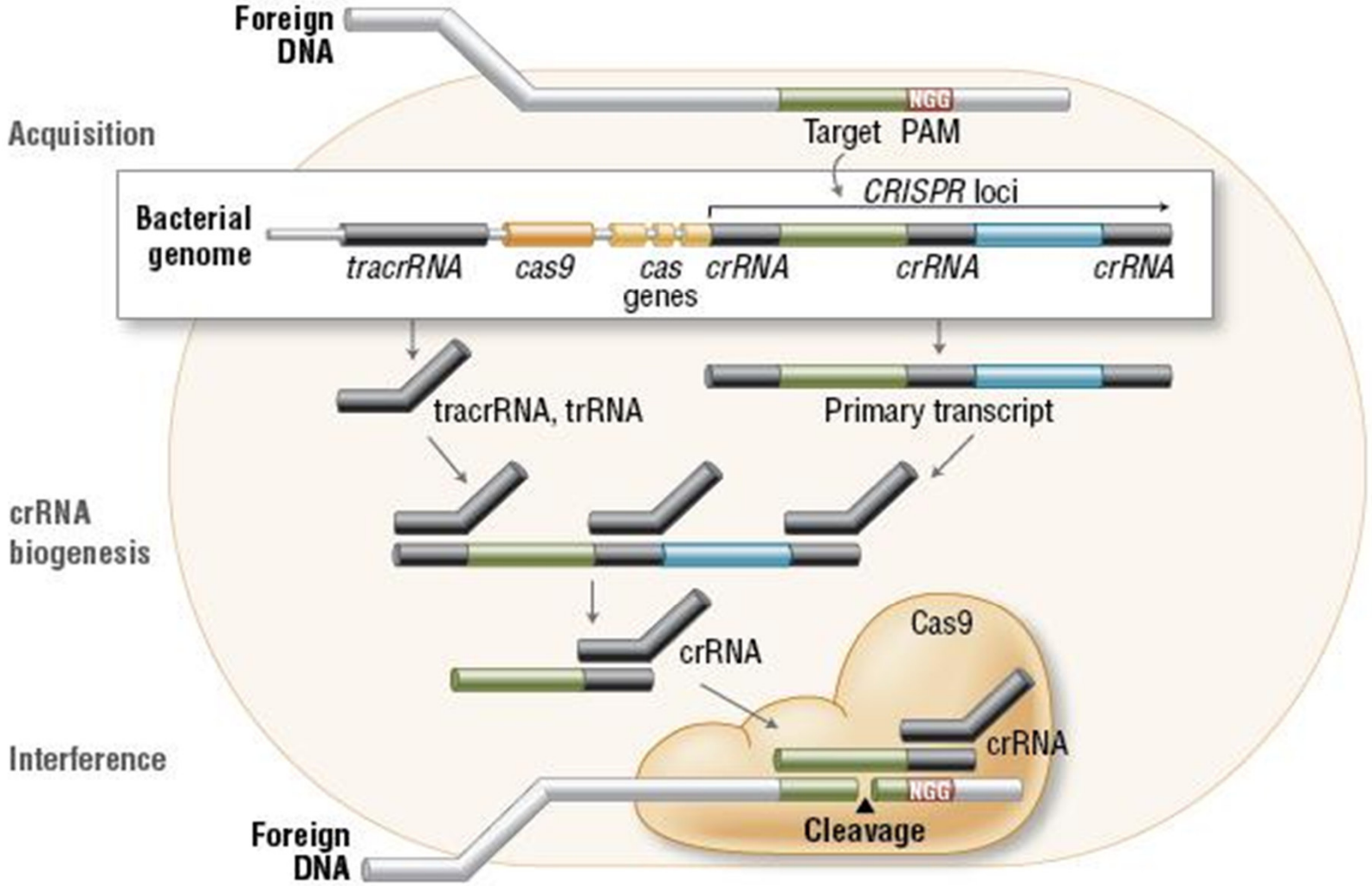
Clustered Regularly Interspaced

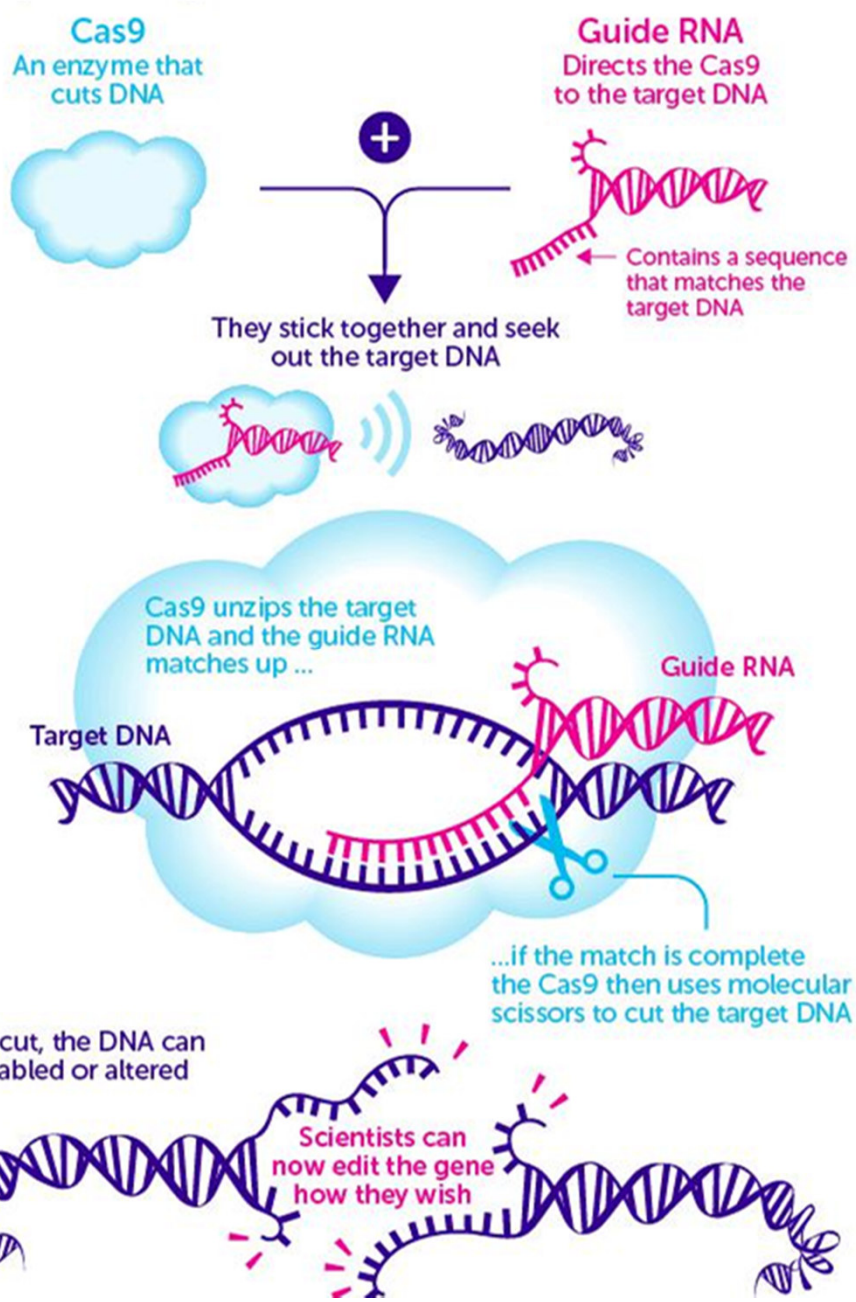
Short Palindromic Repea

Cas

CRISPR-associated syst

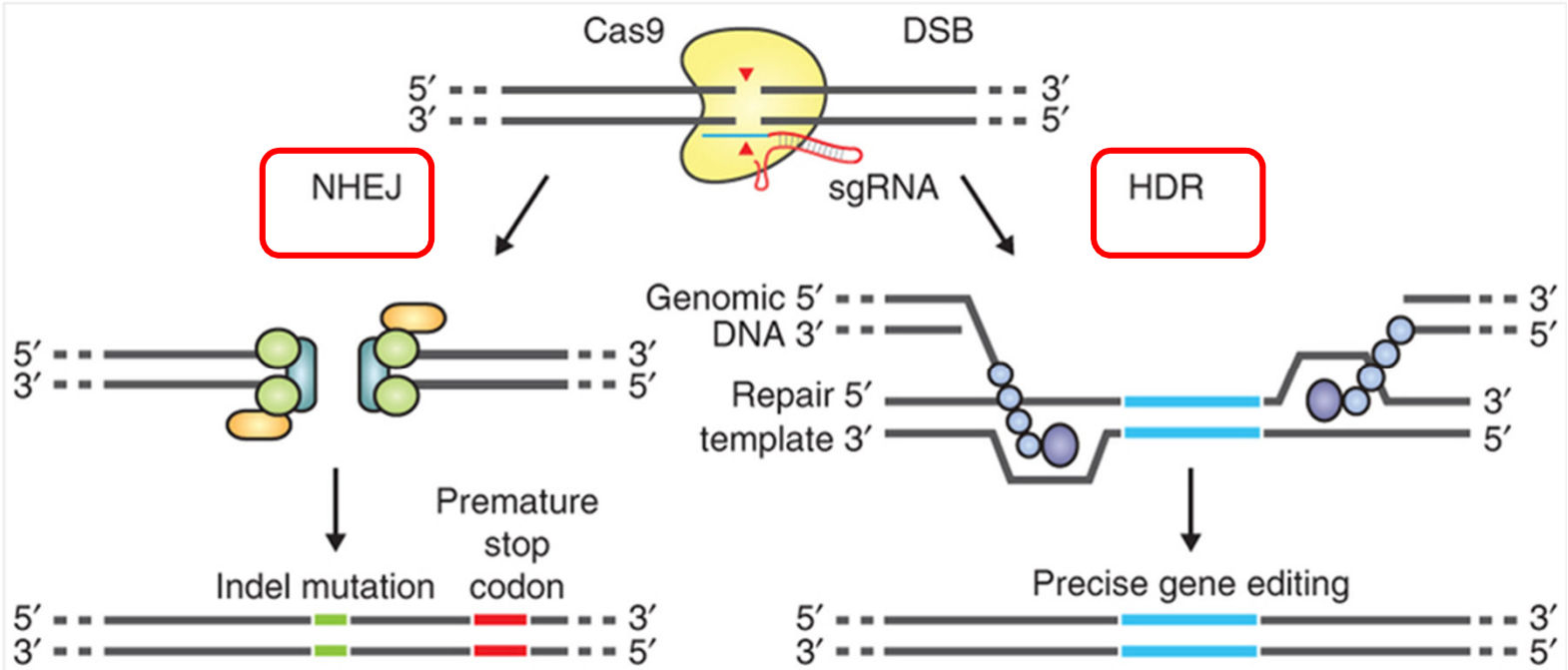
CRISPR-Cas: adaptive immune system




Cas9
An enzyme that cuts DNA
Guide RNA
Directs the Cas9 to the target DNA
Contains a sequence that matches the target DNA
They stick together and seek out the target DNA
Cas9 unzips the target DNA and the guide RNA matches up ...
Guide RNA
Target DNA
...if the match is complete the Cas9 then uses molecular scissors to cut the target DNA
Once cut, the DNA can be disabled or altered
Scientists can now edit the gene how they wish

DSB repair promotes genome editing

F Ann Ran, Patrick D Hsu, Jason Wright, Vineeta Agarwala, David A Scott & Feng Zhang
Nature Protocols **8**, 2281–2308 (2013) | doi:10.1038/nprot.2013.143
Published online 24 October 2013



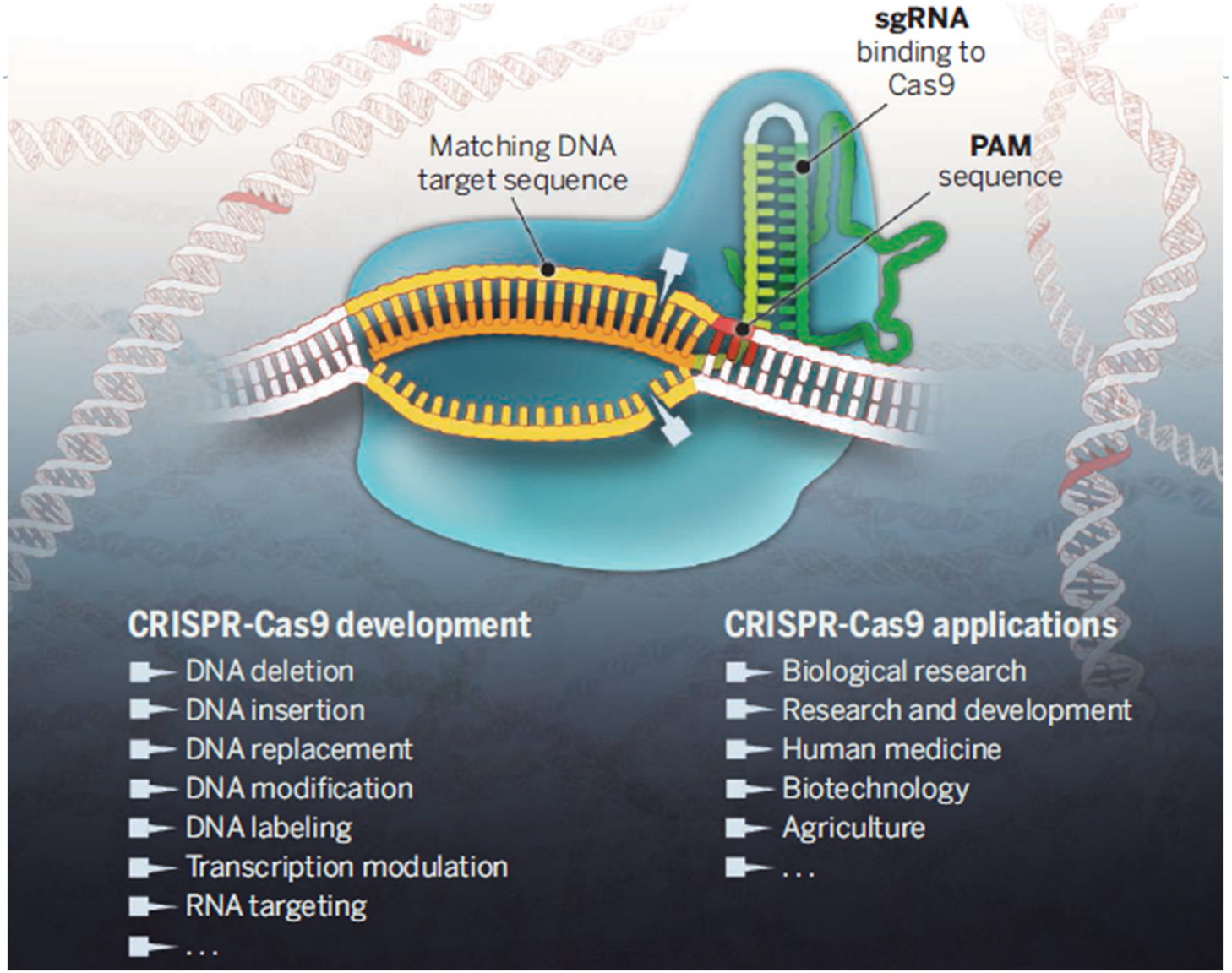
DSBs induced by Cas9 (yello be repaired in one of two ways. In the error-prone NHEJ pathway, the ends of a e processed by endogenous DNA repair machinery and re indel mutations at the site of junction. Indel mutation of a gene can result in frameshifts an odon, resulting in gene knockout. Alternatively, a rep d or ssODN can be supplied to leve vs high fidelity and precise editing. Single-stranded n DR.

A CASO !!!
IL GENE VIENE INTERROTTO

IL GENE VIENE CORRETTO IN MODO SPECIFICO

CRISPR-Cas9

Unlike other gene-editing methods, it is **cheap, quick and easy to use**, and it has swept through labs around the world as a result. Researchers hope to use it to adjust human genes to eliminate diseases, create hardier plants, wipe out pathogens and much more besides


sgRNA
binding to
Cas9
Matching DNA
target sequence
PAM
sequence
CRISPR-Cas9 development
DNA deletion
DNA insertion
DNA replacement
DNA modification
DNA labeling
Transcription modulation
RNA targeting
...
CRISPR-Cas9 applications
Biological research
Research and development
Human medicine
Biotechnology
Agriculture
...

The power of the technology

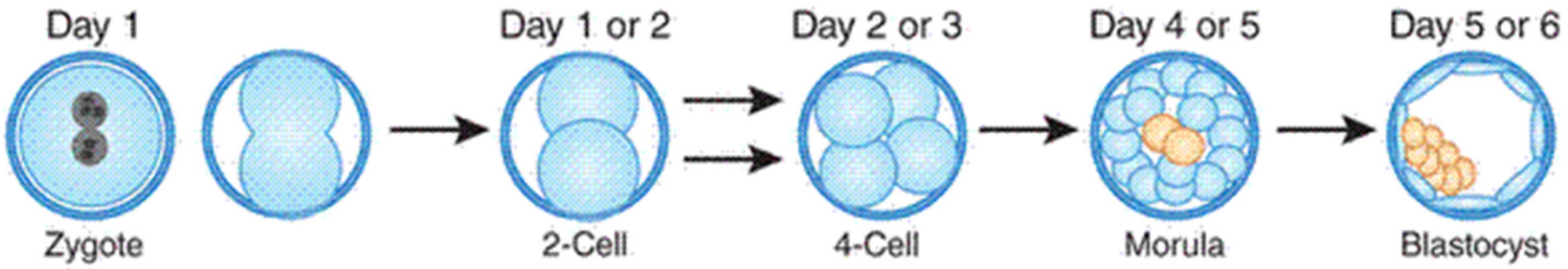
- Precise reproduction of tumor-associated mutations (translocations)
- Analysis of gene function (sgRNA library)
- Protect plants from disease
- Correct genetic mutations in inherited disorders
- Correct genetic mutations responsible for infertility
- Prevent transmission of genetic mutations

IVF

Germline genome editing

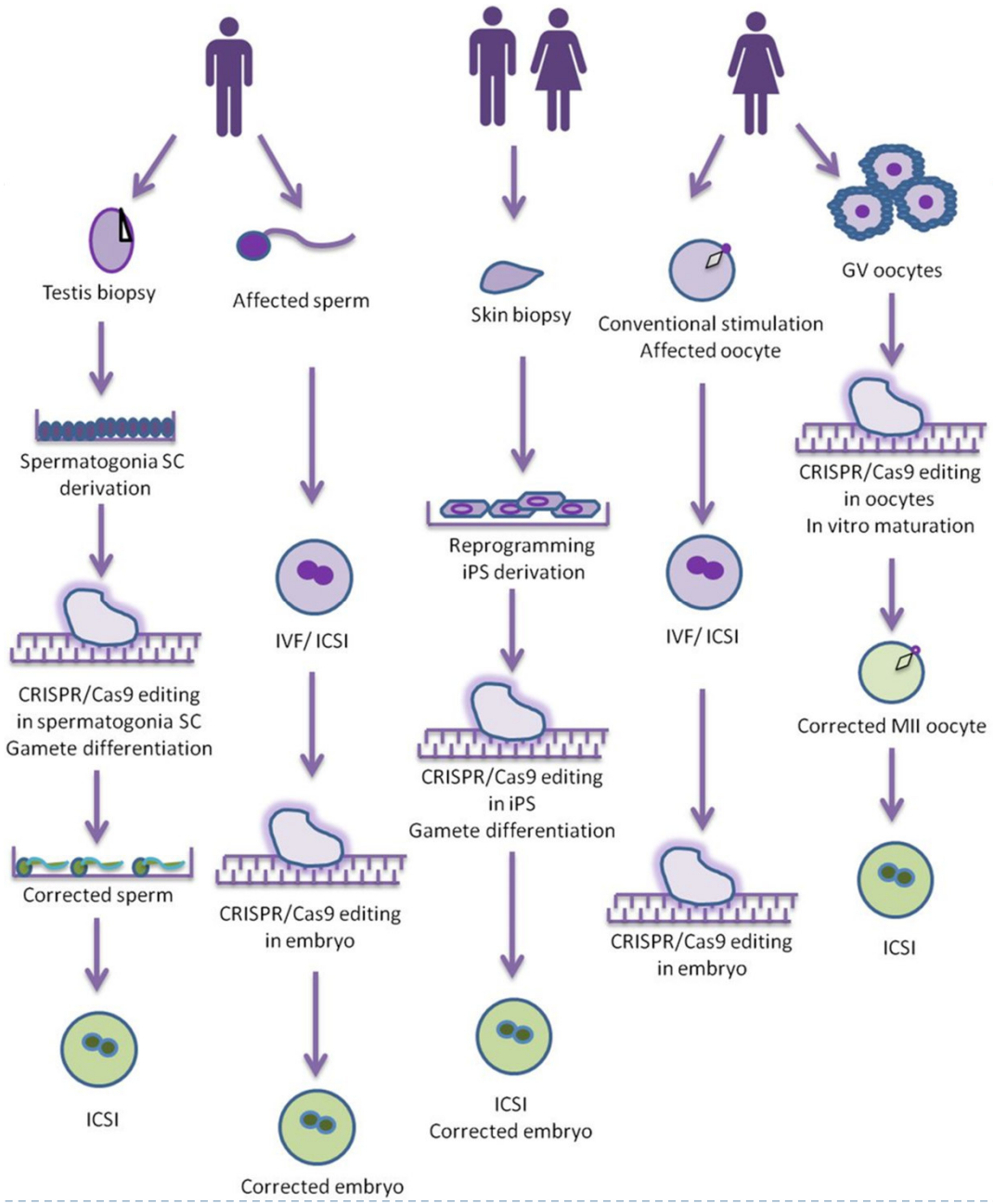
Possible targets

- Gametes
- Zygotes-embryos
- Stem cells (spermatogonial, iPS)



n° cellule

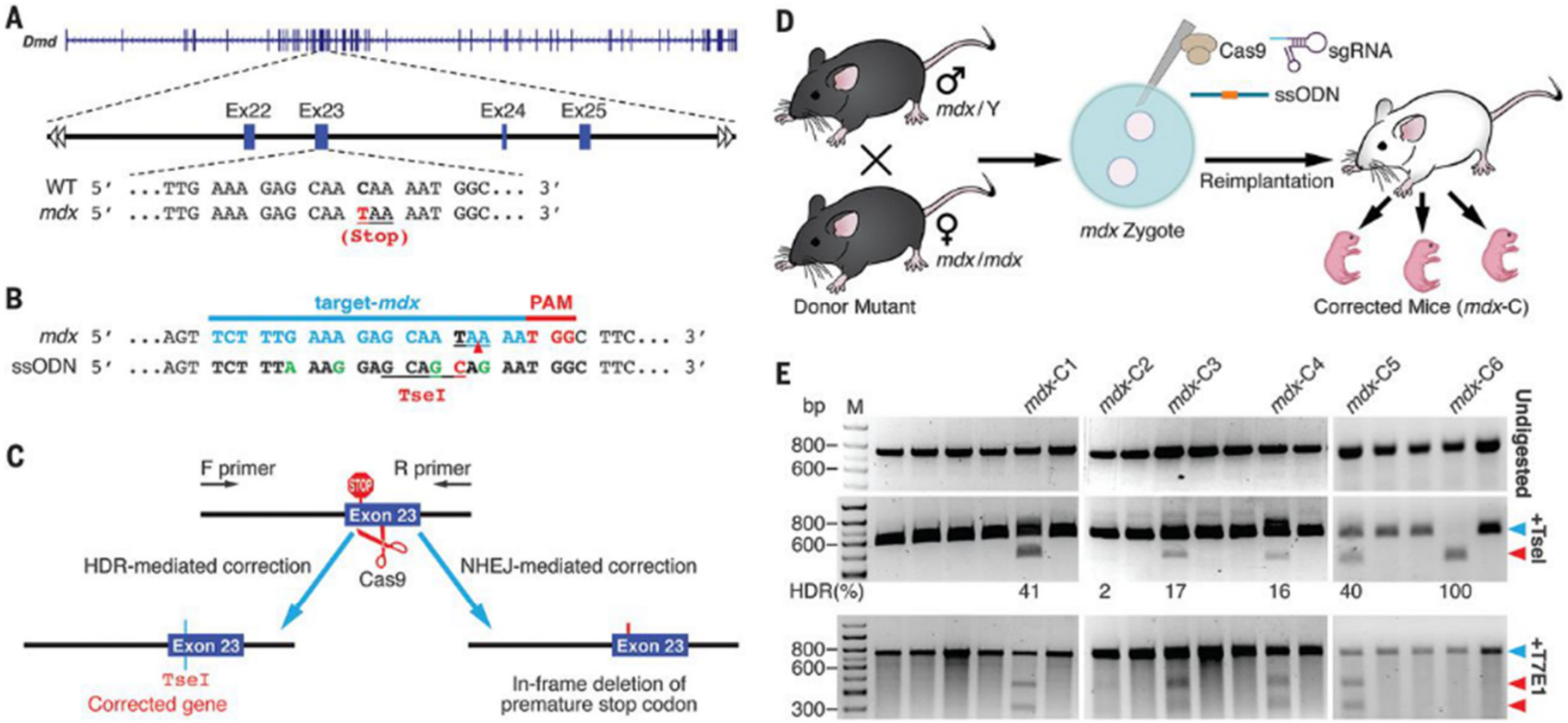
efficacia correzione



Genome engineering through CRISPR/Cas9 technology in the human germline and pluripotent stem cells

Hum Reprod Update. 2016;22(4):411-419. doi:10.1093/humupd/dmw005

Correzione patologie genetiche (DMD)



Chengzu Long et al. Science 2014;345:1184-1188

Protein Cell 2015, 6(5):363–372
DOI 10.1007/s13238-015-0153-5

Protein & Cell

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✉, 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
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Received March 30, 2015 Accepted April 1, 2015

EDITING – Efficacy in 3PN human zygotes

C

A

Targeted editing of the *HBB* gene in human 3PN zygotes by intra-cytoplasmic injection

Group No.	Cas9/gRNA/ssDNA (ng/μL)	Survived /injected	GFP+	PCR-amplified	Cas9-cleaved	Edited with ssDNA	Recombined with HBD
1	100/20/2	10/11	6	6	4	0	1
2	100/20/20	22/29	17	17	7	1	0
3	200/40/200	12/14	12	10*	6	2	2
4	200/40/200	27/32	24	21*	11	1	4
Total	-	71/86 (82.6%)	59	54	28 (51.9%)	4 (14.3%)	7 (25.0%)

Protein Cell 2015, 6(5):363–372

CRISPR/Cas9-mediated gene editing in human tripronuclear zygotes

- In this study, 3PN zygotes were used to investigate the specificity and fidelity of the CRISPR/Cas9 system.
- Most of the DSBs generated by Cas9 in 3PN zygotes were also repaired through NHEJ.
- ssDNA-mediated editing occurred only in 4 embryos (14.3%) and the edited embryos were mosaic, similar to findings in other model systems
- Endogenous homologous sequences were also used as HDR templates, with an estimated editing efficiency of 25%

Protein Cell 2015, 6(5):363–372

Correction of a pathogenic gene mutation in human embryos

Hong Ma[1]*, Nuria Marti-Gutierrez[1]*, Sang-Wook Park[2]*, Jun Wu[3]*, Yeonmi Lee[1], Keiichiro Suzuki[3], Amy Koski[1], Dongmei Ji[1], Tomonari Hayama[1], Riffat Ahmed[1], Hayley Darby[1], Crystal Van Dyken[1], Ying Li[1], Eunju Kang[1], A.-Reum Park[2], Daesik Kim[4], Sang-Tae Kim[2], Jianhui Gong[5,6,7,8], Ying Gu[5,6,7], Xun Xu[5,6,7], David Battaglia[1,9], Sacha A. Krieg[9], David M. Lee[9], Diana H. Wu[9], Don P. Wolf[1], Stephen B. Heitner[10], Juan Carlos Izpisua Belmonte[3]§, Paula Amato[1,9]§, Jin-Soo Kim[2,4]§, Sanjiv Kaul[10]§ & Shoukhrat Mitalipov[1,10]§

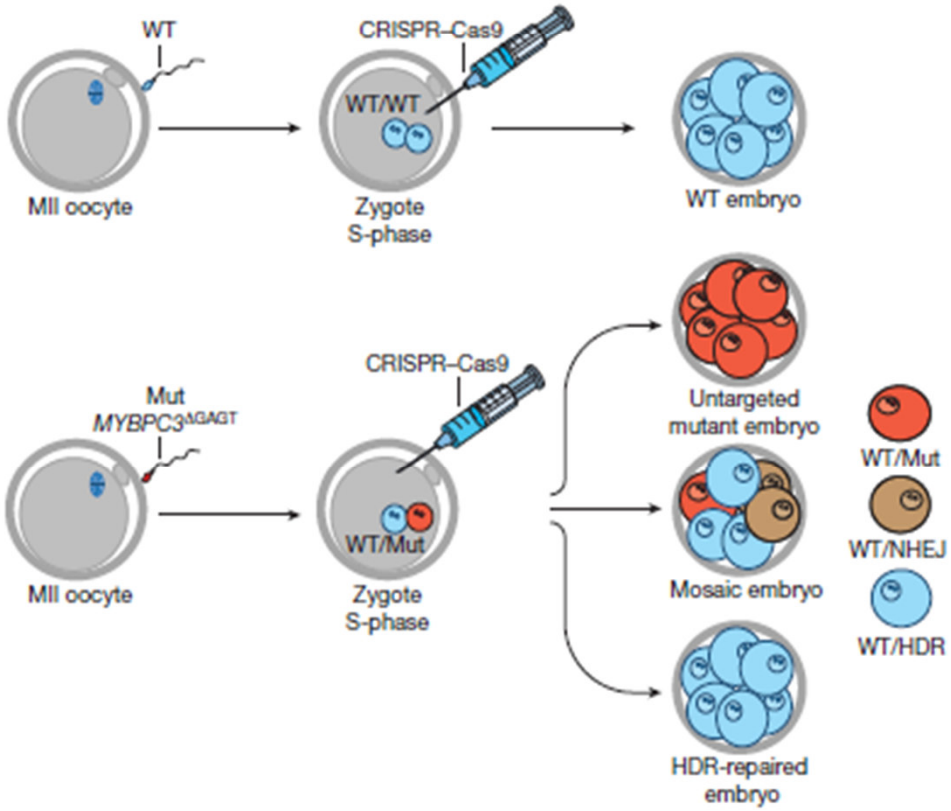
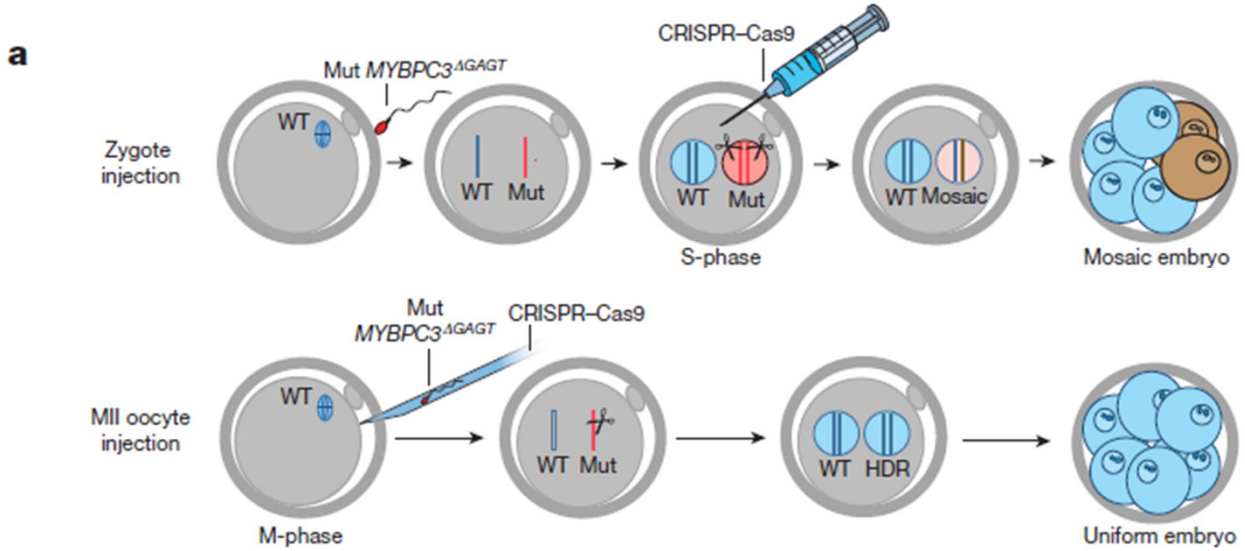


Figure 1 | Gene correction in S-phase-injected human embryos.
Schematic of *MYBPC3*$^{\Delta GAGT}$ gene targeting by injection of CRISPR–Cas9

Ma, Nature, 2017

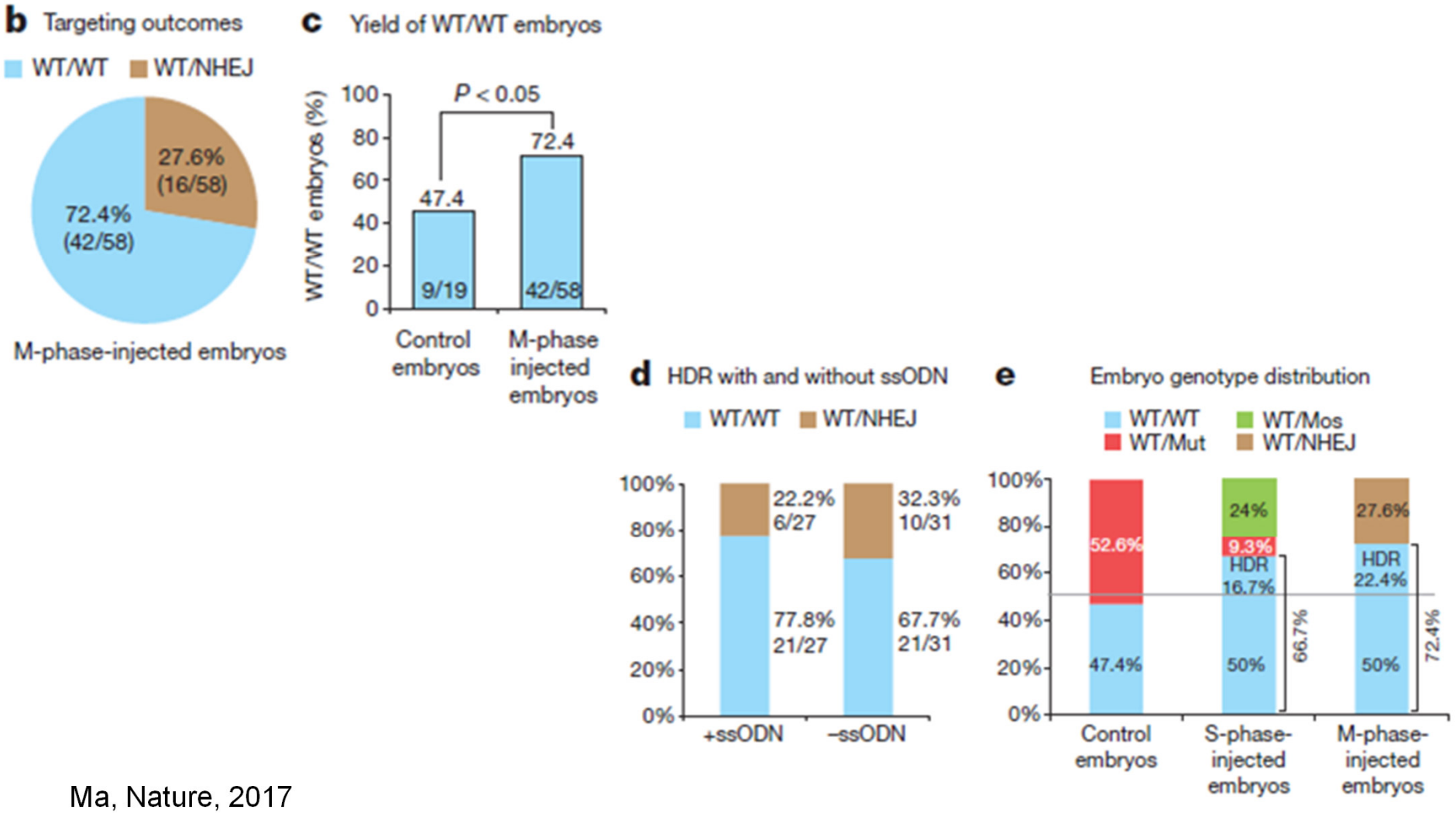
Correction of a pathogenic gene mutation in human embryos

Hong Ma[1]*, Nuria Marti-Gutierrez[1]*, Sang-Wook Park[2]*, Jun Wu[3]*, Yeonmi Lee[1], Keiichiro Suzuki[3], Amy Koski[1], Tomonari Hayama[1], Riffat Ahmed[1], Hayley Darby[1], Crystal Van Dyken[1], Ying Li[1], Eunju Kang[1], A.-Reum Park[2], [1], Sang-Tae Kim[2], Jianhui Gong[5,6,7,8], Ying Gu[5,6,7], Xun Xu[5,6,7], David Battaglia[1,9], Sacha A. Krieg[9], David M. Lee[9], [1], Don P. Wolf[1], Stephen B. Heitner[10], Juan Carlos Izpisua Belmonte[3]§, Paula Amato[1,9]§, Jin-Soo Kim[2,4]§, Sanjiv Kau[1], Shoukhrat Mitalipov[1,10]§



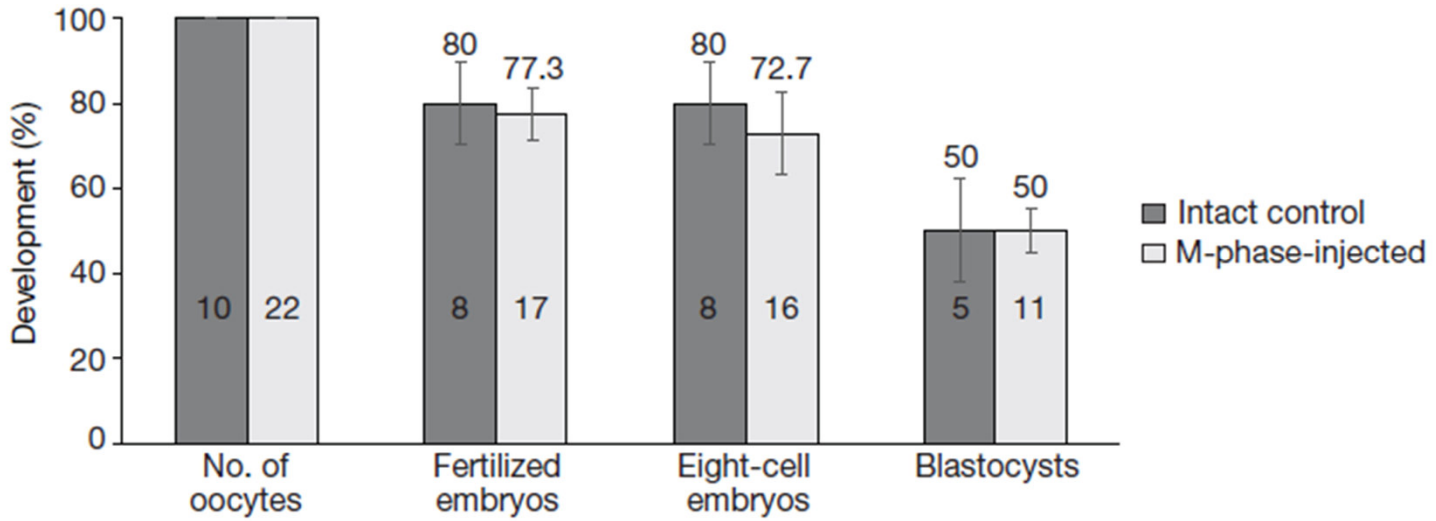
Ma, Nature, 2017

Correction of a pathogenic gene mutation in human embryos

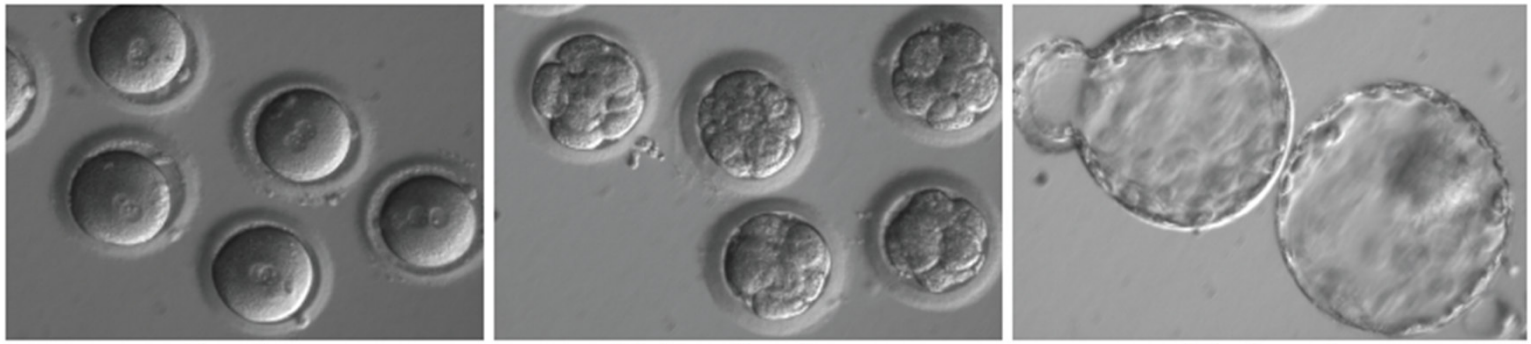


Ma, Nature, 2017

a Fertilization and preimplantation development of CRISPR–Cas9-injected oocytes



b



Ma, Nature, 2017

Gene Correction, Human 2PN model

- CRISPR/CAS9 is effective as a gene-editing tool in human zygotes.
- Homology-directed repair is frequent, mainly due to the use of homologous wild type maternal sequences.
- Mosaicism can be reduced modulating the cycle cell stage at which DNA repair was induced

Ma, Nature, 2017

Protein Cell 2017, 8(11):811–822
DOI 10.1007/s13238-017-0475-6

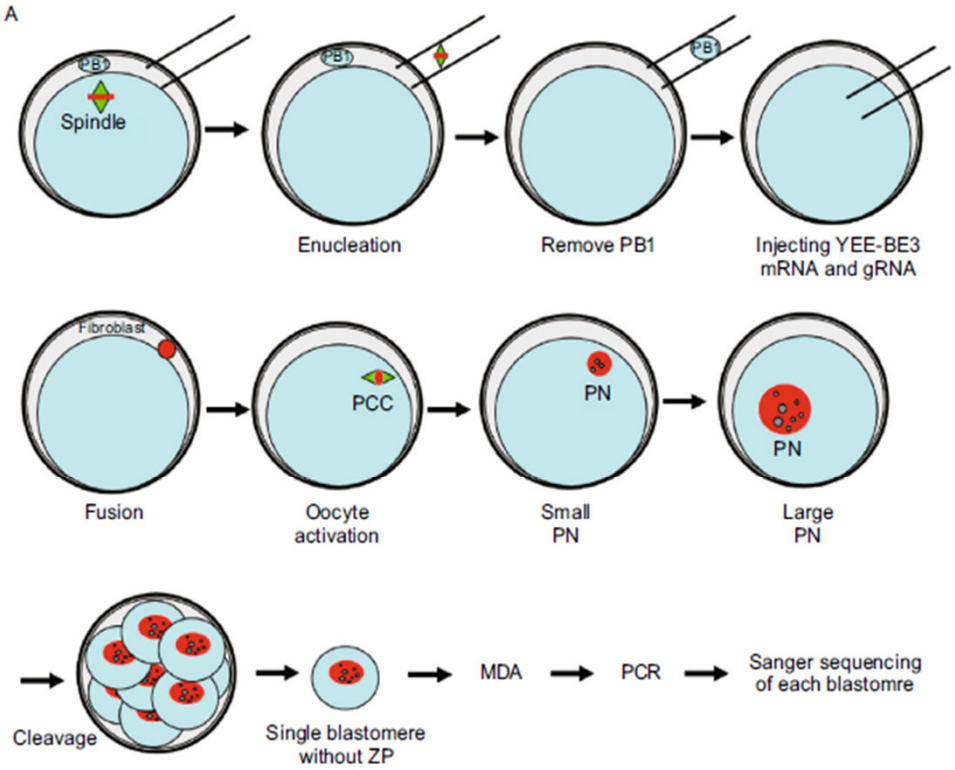
Protein & Cell

SHORT ARTICLE

Correction of β-thalassemia mutant by base editor in human embryos

Puping Liang[1,2], Chenhui Ding[2], Hongwei Sun[1], Xiaowei Xie[1], Yanwen Xu[2], Xiya Zhang[1], Ying Sun[1], Yuanyan Xiong[1], Wenbin Ma[1], Yongxiang Liu[2], Yali Wang[2], Jianpei Fang[3], Dan Liu[4], Zhou Songyang[1,2,4]✉, Canquan Zhou[2]✉, Junjiu Huang[1,2]✉

HBB - Gene correction



B

Survived embryo No. (Injected embryo No.)	Activated embryo No.	Harvested embryo No.	Total blastomere No.	MDA-amplified blastomere No.	PCR-amplified blastomere No.	<Homozygous> $G_{-2}G_{-2}/G_{-2}G_{-2}$ blastomere No. (%)	<Heterozygous> $A_{-2}G_{-2}/G_{-2}G_{-2}$ blastomere No. (%)	<Wild-type> $A_{-2}G_{-2}/A_{-2}G_{-2}$ blastomere No. (%)
28 (35)	24	20#	73	73	48*	37 (77.1)	3 (6.3)	8 (16.7)

- Homozygous mutation disease embryos obtained with nuclear transfer
- Gene correction efficiency over 23%
- In mosaic embryos the % of repaired blastomeres was over 20%

Liang, Prot Cell, 2017

Possible uses of genome editing in embryology

- Both parents carriers of an Autosomal Recessive Disease (CF, b-tal…)
- One member affected of an Autosomal [illegible] Disease or a chromosomal structural [illegible]
- Correction of the affected gene in the g[illegible]line of the affected member or in embryos

PGD !

- Generation of disease models
- Study of genes involved in pre and post implantation development

Safety-Ethical issues

- how to study safety?
- do we need to move to clinical practice? How?
- use of **PGD** to identify modified embryos and **off-target effects**

Off-target effects

The possibility that CRISPR-Cas RGNs might cause additional, unwanted genetic changes has been largely unexplored, so Joung's team set out to investigate the occurrence of "off-target" mutations in human cells expressing CRISPR-Cas RGNs. Since the interaction between the guiding RNA segment and the target DNA relies on only 20 nucleotides, they hypothesized that the RNA might also recognize DNA segments that differed from the target by a few nucleotides

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

A highly specific SpCas9 variant is identified by in vivo screening in yeast.

Casini A[1], Olivieri M[1], Petris G[1], Montagna C[1], Reginato G[1], Maule G[1], Lorenzin F[2], Prandi D[2], Romanel A[2], Demichelis F[2], Inga A[3], Cereseto A[1].

Casini et al., *Nat biotech* 2018

…and additional future improvements to the CRISPR-Cas9 platform

SHUTTERSTOCK

Don’t edit the human germ line

"There is a mentality that as long as it works, we don't have to understand how or why it works."


GENOME
EDITOR
BIO
ETHICS
REVIEW