CRISPR 유전자가위 기술과 생명과학 혁명

김진수
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Genome Editing Highlighted by Science and Nature

MIT Technology Review

10 Breakthrough Technologies 2016

Precise Gene Editing in Plants

CRISPR offers an easy, exact way to alter genes to create traits such as disease resistance and drought tolerance.

Immune Engineering

Genetically engineered immune cells are saving the lives of cancer patients. That may be just the start.

Key Players in Engineering Crops

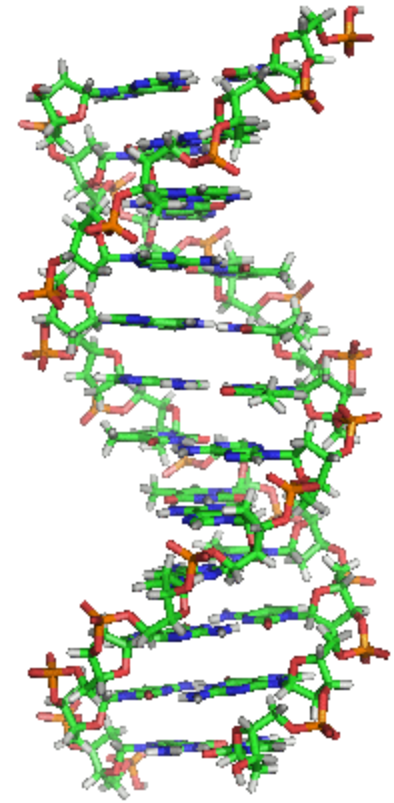
- The Sainsbury Laboratory and John Innes Centre, Norwich, U.K.
- Seoul National University
- University of Minnesota
- Institute of Genetics and Developmental Biology, Beijing

유전적 다양성

- 유전자의 차이가 개체의 차이를 대부분 설명함
- 유전자에 따라 질병에 대한 감수성이 달라짐

유전질환: Genetic Disorders

- 혈우병, 겸상 적혈구증 등 10,000 종이 있음
- 전체 신생아의 1%가 유전질환자임
- 소아과 진료의 40%에 해당
- 대부분 완치 불가능
- 돌연변이 유전자를 다음 세대에 물려 줌



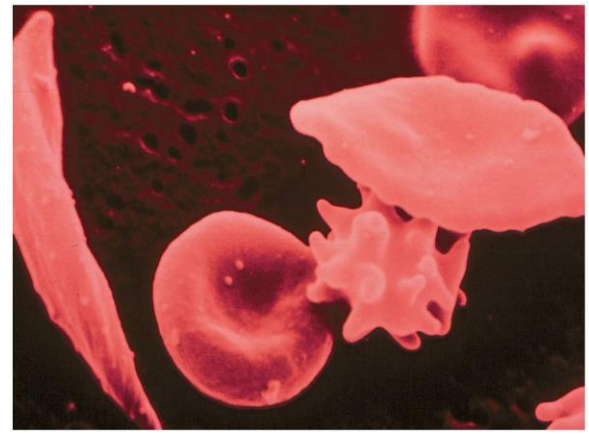
정상 적혈구와 낫모양 적혈구

HBB Sequence in Normal Adult Hemoglobin (Hb A):

Nucleotide	CTG	ACT	CCT	GAG	GAG	AAG	TCT
Amino Acid	Leu	Thr	Pro	Glu	Glu	Lys	Ser
	3			6			9

HBB Sequence in Mutant Adult Hemoglobin (Hb S):

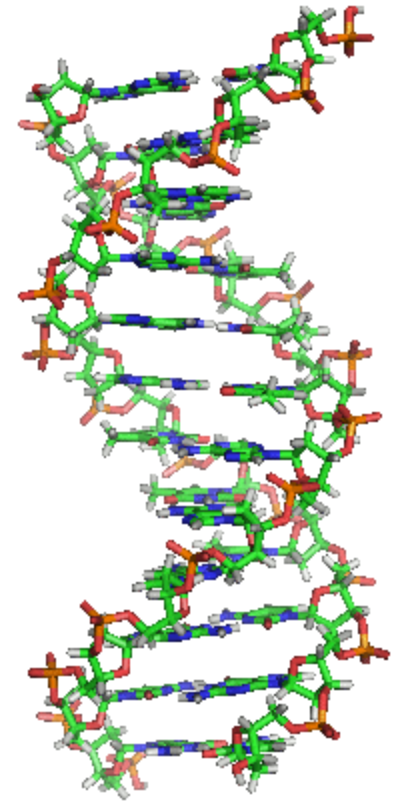
Nucleotide	CTG	ACT	CCT	GTG	GAG	AAG	TCT
Amino Acid	Leu	Thr	Pro	Val	Glu	Lys	Ser
	3			6			9



- 흑인 500명 중 한 명 비율로 발생하는 빈혈증
- 단일염기 변이로 인해 헤모글로빈 아미노산 한 개가 바뀜
- 낫모양 적혈구 형성, 기능 상실

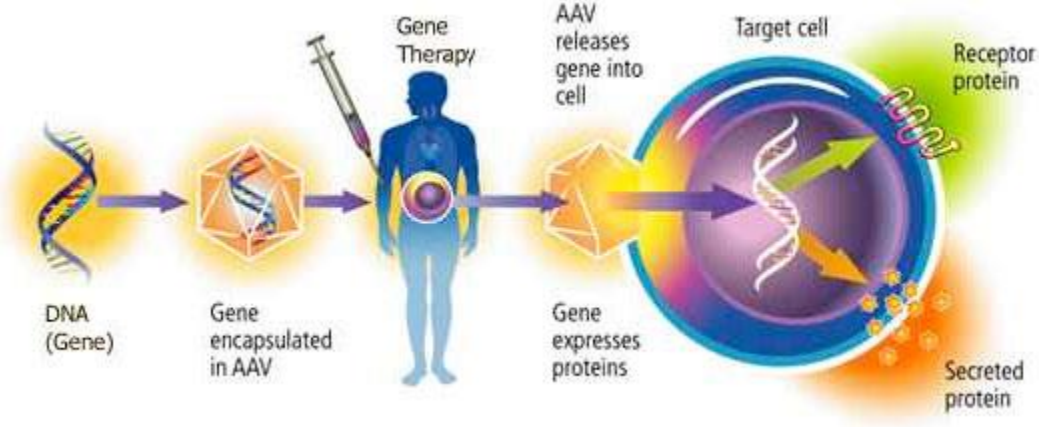
유전자 변이의 종류와 원인

- 단일염기 변이: Single Nucleotide Polymorphism
- 구조 변이: 결실, 역위, 전좌 등
- 체세포 변이와 생식세포 변이
- 변이의 원인
  - DNA 복제 효소 오작동: 10억분의 1
  - 화합물: 일부 약제 포함
  - 활성 산소: 대사 부산물
  - 방사능, 자외선
  - 이중나선 절단: Double Strand Break



유전자 치료의 전망과 한계

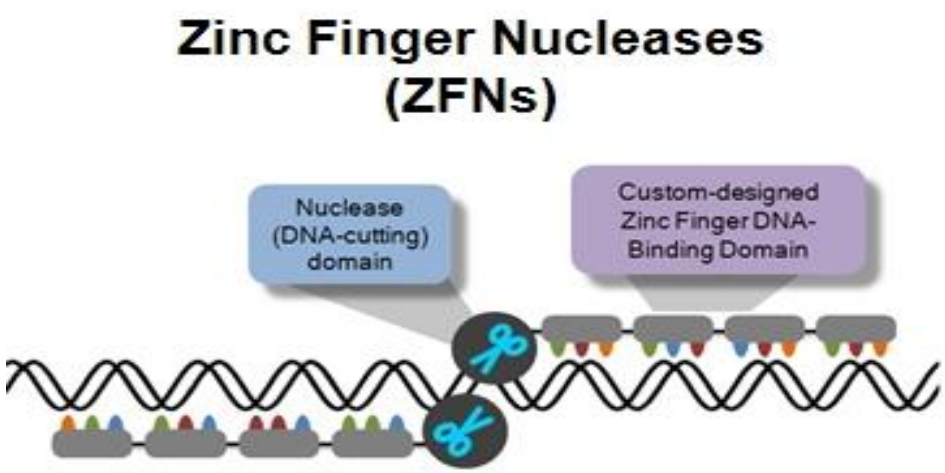
- 치료 효과가 기대되는 유전자를 전달체에 실어 환자 체내에 투여함
- 바이러스, 플라즈미드 DNA 등을 전달체로 사용
- 치료용 유전자, 전달체 DNA가 환자 세포에 무작위 삽입될 수 있음: 암 발생 가능
- 8번 인자와 같이 큰 유전자는 전달체 수용 불가

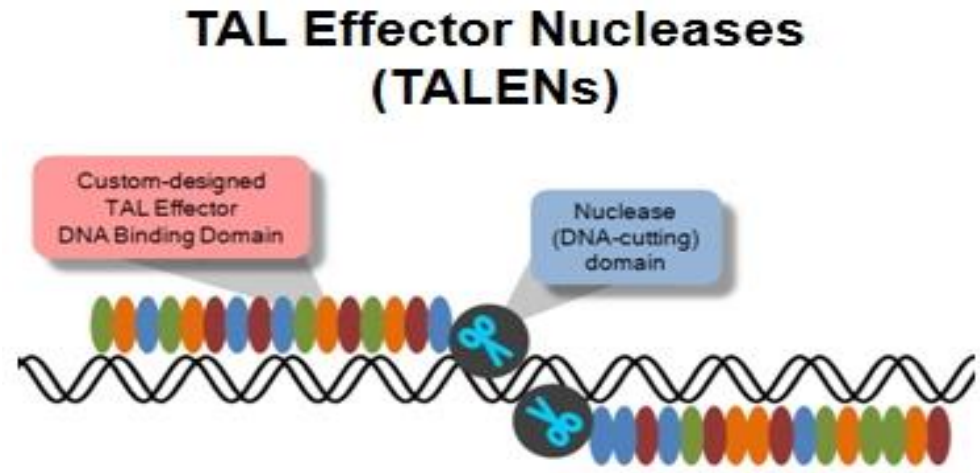


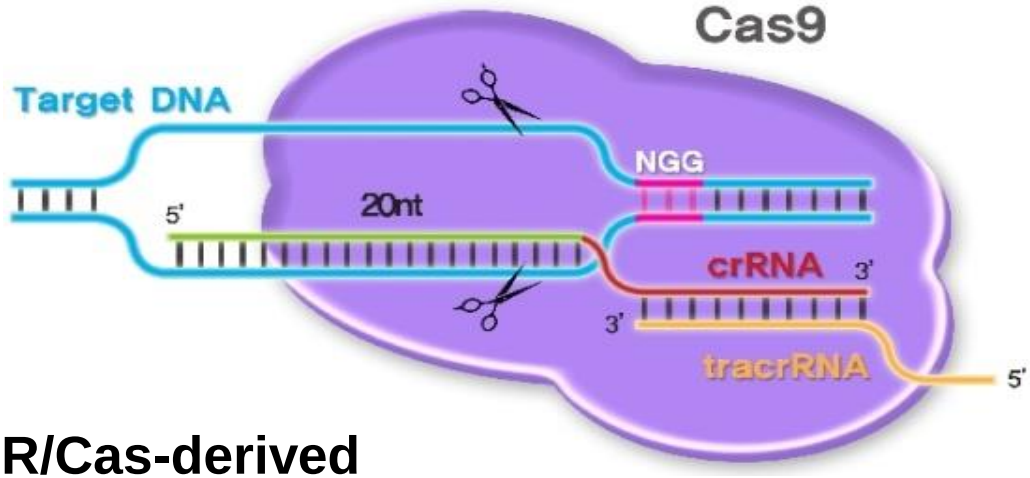
유전체 교정: Genome Editing

- 유전자가위를 사용해 세포 내 유전자 DNA를 절단함
- 절단된 DNA가 수선되는 과정에서 변이가 발생함
- 치료용 유전자를 안전한 위치에 삽입할 수 있음
- 유전질환, 암, 감염성 질환의 치료법으로 개발되고 있음
- 가축, 농작물, 어류, 곤충 등에 널리 활용됨

유전자가위: Programmable Nuclease







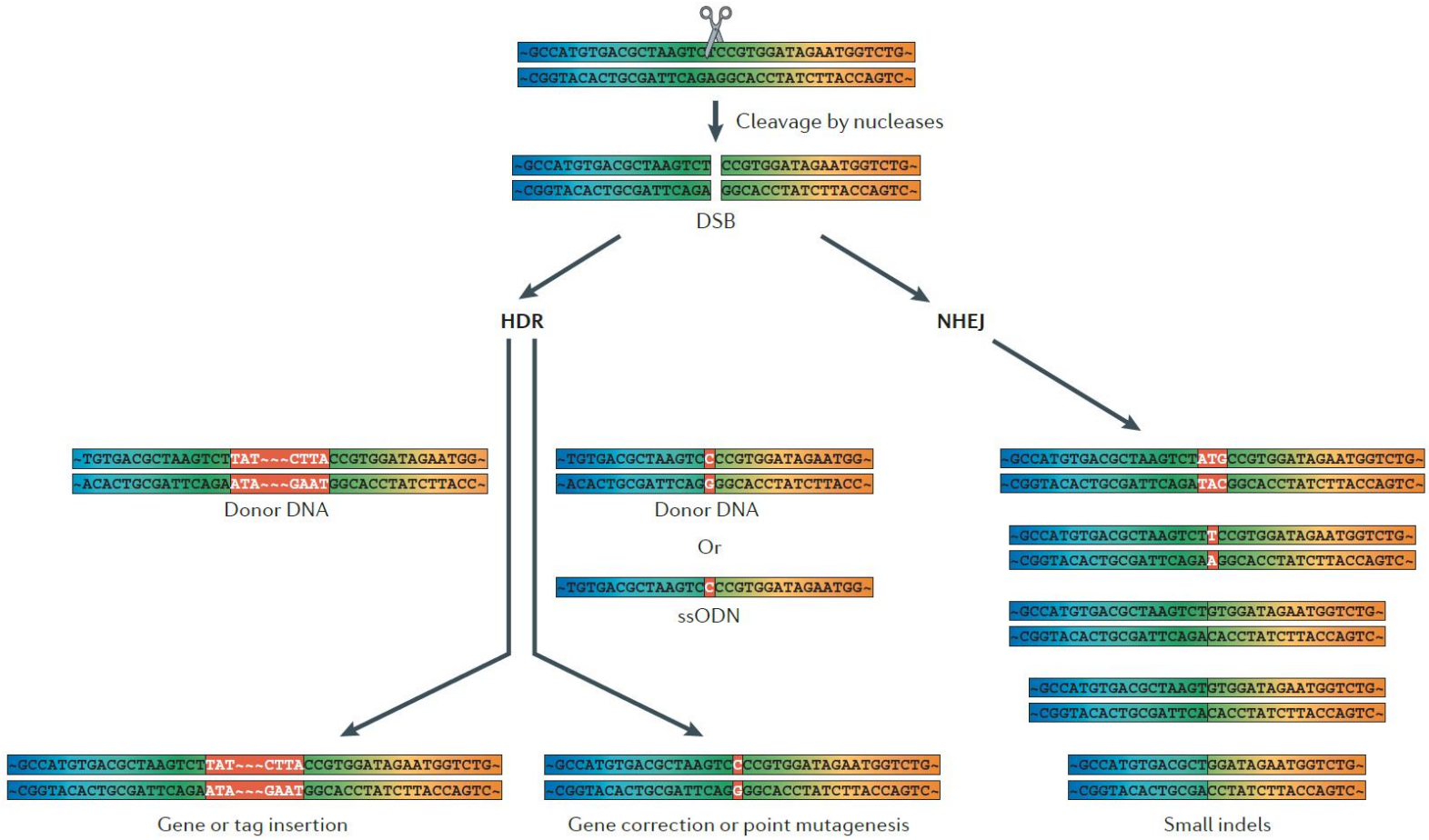
CRISPR/Cas-derived
RNA-guided endonuclease
(RGEN)

Comparison of Programmable Nucleases

	ZFN	TALEN	CRISPR RGEN
Success rate	~24%	>99%	~90%
Average mutation rate	<10%	~20%	~20%
Length of target site	20 to 36 bp	30 to 40 bp	23 bp
Restriction in target site	Guanine-rich	Start with T and end with A	End with GG (PAM)
Design density	One per ~100 bp	One per every bp	One per 8 bp
Off-target effects	High	Low	Variable
Size	2 x ~2 kbp	2 x ~3 kbp	4.2 kbp + gRNA

Kim H & Kim JS, Nat. Rev. Genet. (2014)

Double-Strand Break Repair



Kim H & Kim JS, Nat. Rev. Genet. (2014)

- Gene-targeting efficiency is boosted drastically by DSBs.
- DSBs also induce NHEJ, an error-prone DSB repair system.

CRISPR

RNA-Guided ENdonuclease

NEWS & VIEWS

BIOTECHNOLOGY

Rewriting a genome

A bacterial enzyme that uses guide RNA molecules to target DNA for cleavage has been adopted as a programmable tool to site-specifically modify genomes of cells and organisms, from bacteria and human cells to whole zebrafish.

50 | NATURE | VOL 495 | 7 MARCH 2013

NEWS AND VIEWS

RNA guides genome engineering

Claudio Mussolino & Toni Cathomen

The Cas9 endonuclease is reprogrammed by RNAs for site-specific modification of eukaryotic and microbial genomes.

VOLUME 31 NUMBER 3 MARCH 2013 NATURE BIOTECHNOLOGY

RESEARCH HIGHLIGHTS

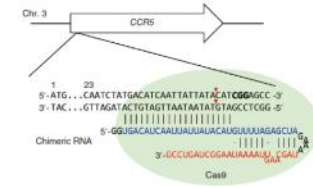
GENETICS

RNA-guided gene editing

Targeted gene modification can be guided by programmable RNA in bacteria, zebrafish and mammalian cells.

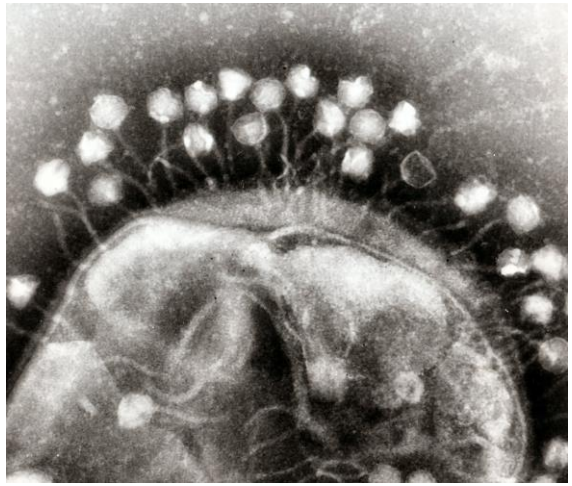
Humble creatures, prokaryotes and viruses, have an illustrious history of providing biologists with molecular tools. Where, after all, would molecular biology—or for that matter, all of genomics—be without restriction endonucleases or adjacent motif (PAM), which takes the form NGG, immediately following the 20-base-pair target sequence.

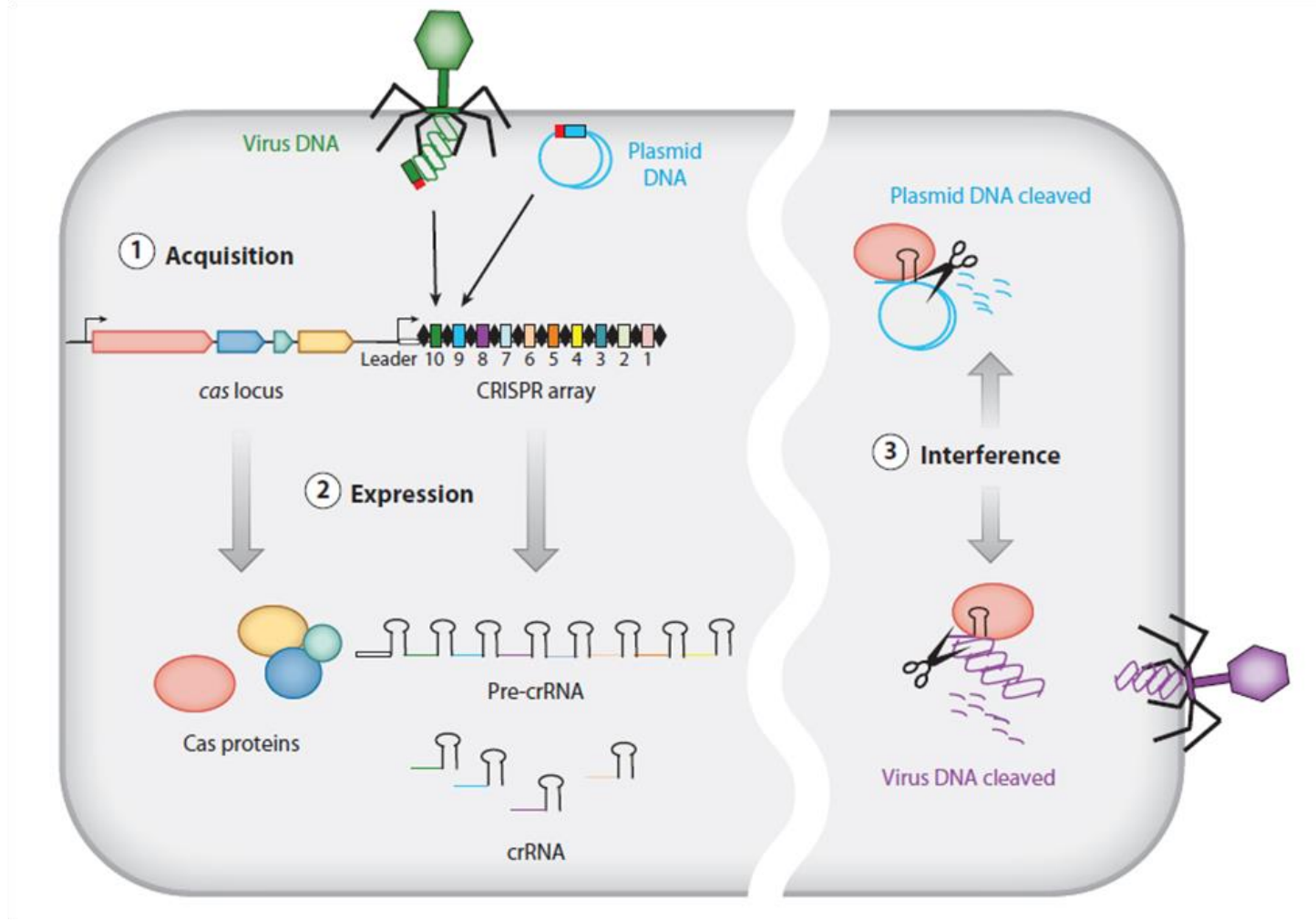
In contrast to current tools, zinc-finger nucleases and transcription activator–like effector nucleases (TALENs), targeting of a new sequence with CRISPR-Cas requires the design of only a new RNA guide and not a new pair of enzymes. The efficiency of



NATURE METHODS | VOL.10 NO.3 | MARCH 2013 | 189

CRISPR: Adaptive Immune System in Bacteria

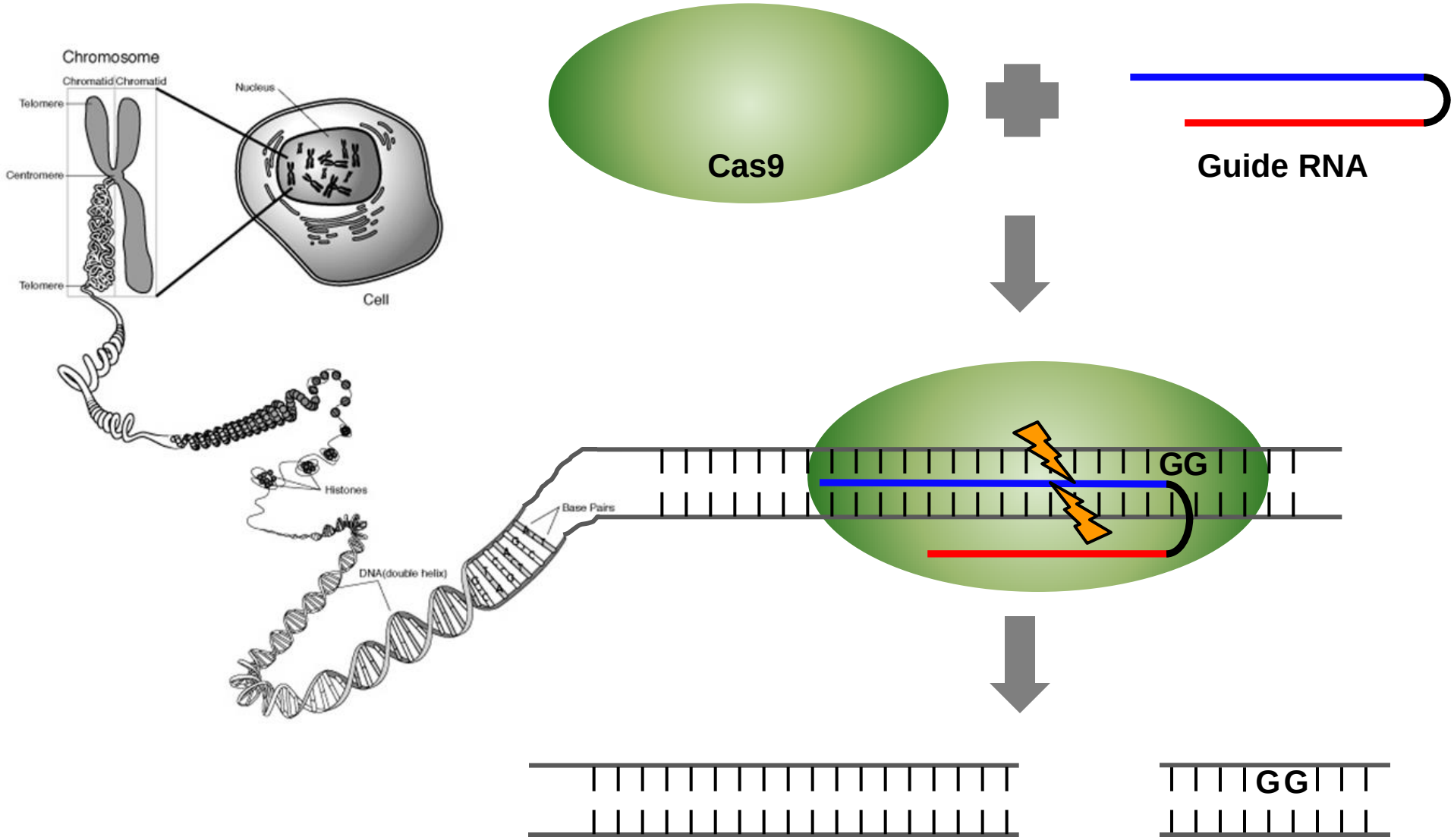




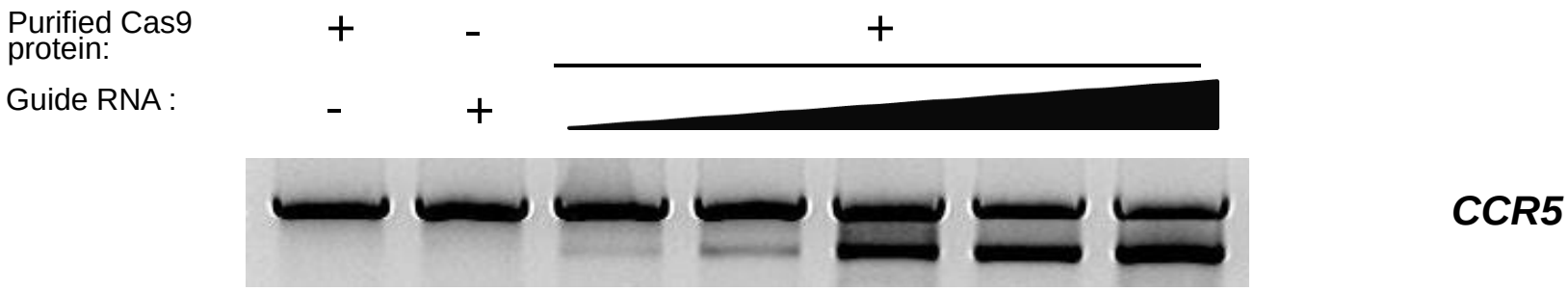
CRISPR–Cas system

- Clusters of Regularly Interspaced Palindromic Repeats
- CRISPR-Cas9: RNA-guided programmable nucleases

RNA-Guided ENdonuclease (RGEN)



Plasmid-free Gene Knockout in Human Cells

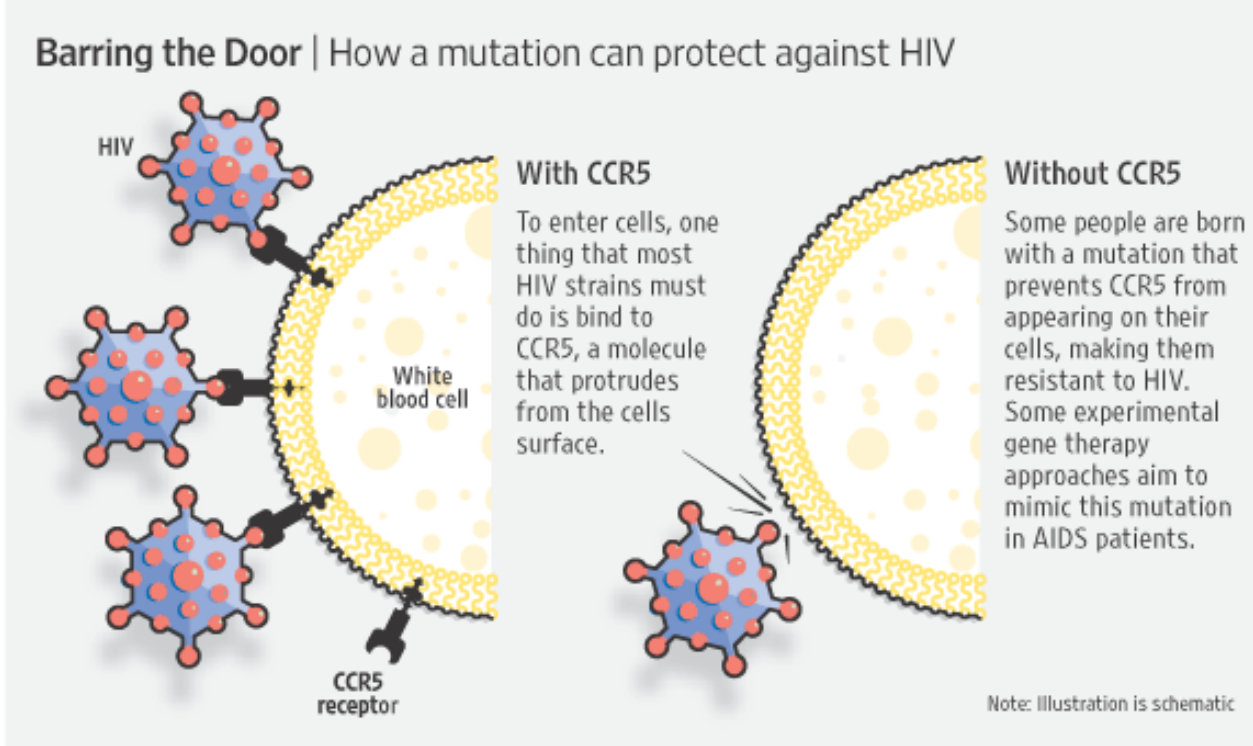



```
CAATCTATGACATCAATTATTATA-CATCGGAGCCCTGCCAAAAAATCAA  WT
CAATCTATGACATCAATTATTATAACATCGGAGCCCTGCCAAAAAATCAA  +1
CAATCTATGACATCAATTATTAT--------------GCCAAAAAATCAA -13
CAATCTATGACATC---------------GGAGCCCTGCCAAAAAATCAA -14
CAATCTATGACAT-------------------GCCCTGCCAAAAAATCAA -18
CAATCTATGACATCAATTATTAT--------------------AAATCAA -19
CAATCTATGACATC-------------------------CAAAAAATCAA -24
CAATCTATGACA-------------------------------AAATCAA -30
```

Cho & Kim et al. Nature Biotechnol (2013)

- Purified Cas9 protein and in vitro transcribed sgRNA are used.
- Mutation frequencies range from 1% to 98%.

Gene/cell therapy targeting *CCR5*



- CCR5 is an essential co-receptor of HIV infection.
- *CCR5* Δ32 deletion leads to resistance to HIV infection.
- Homozygote *CCR5* Δ32 carriers are healthy (1% of Caucasian)

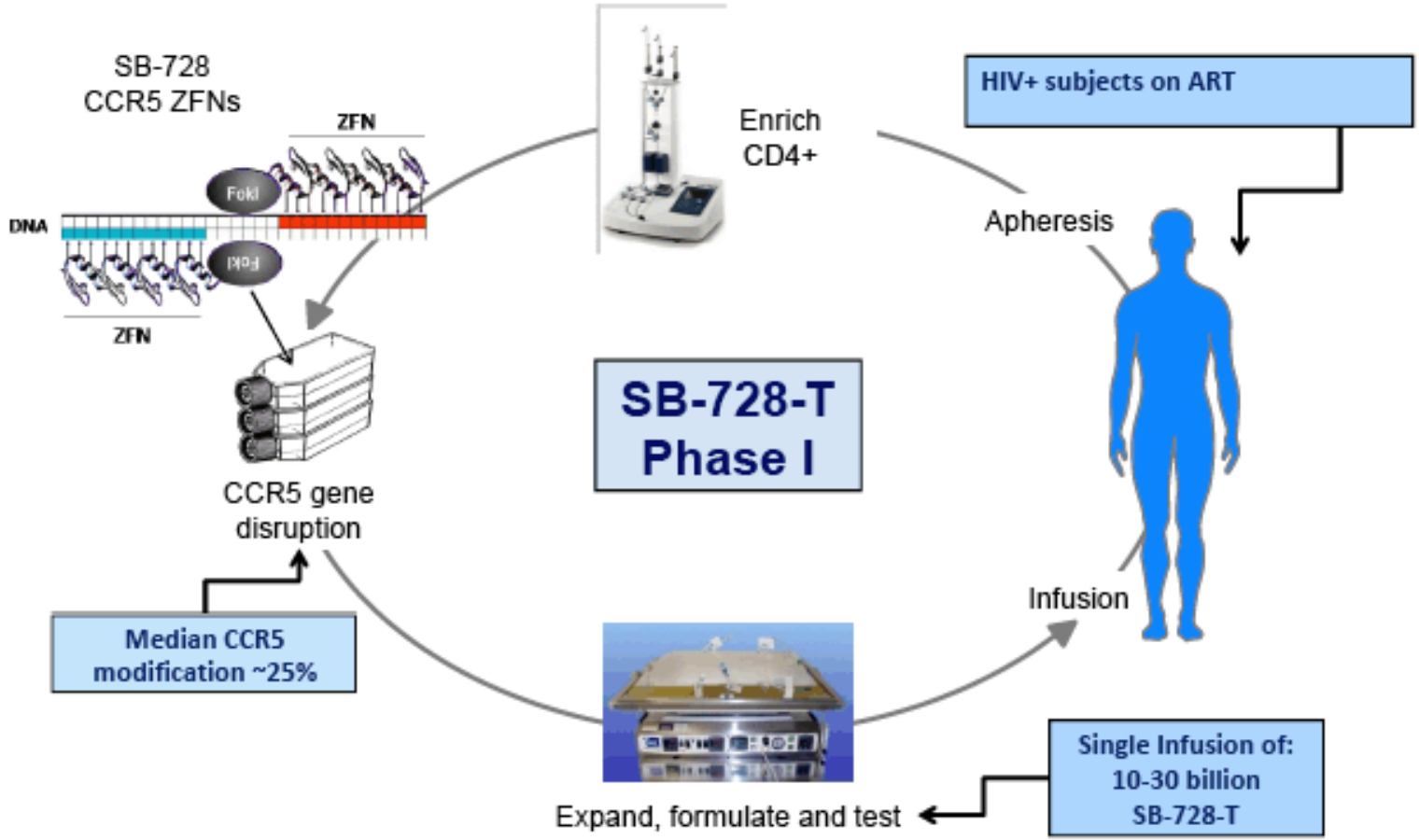
Proof negative. The apparent cure of Timothy Ray Brown (*left*) has given momentum to novel interventions like the gene therapy that Matt Sharp (*right*) received.

The Emerging Race To Cure HIV Infections

Timothy Ray Brown's startling fate has pushed to the front a daunting research challenge that long seemed a fool's errand

13 MAY 2011 VOL 332 SCIENCE www.sciencemag.org

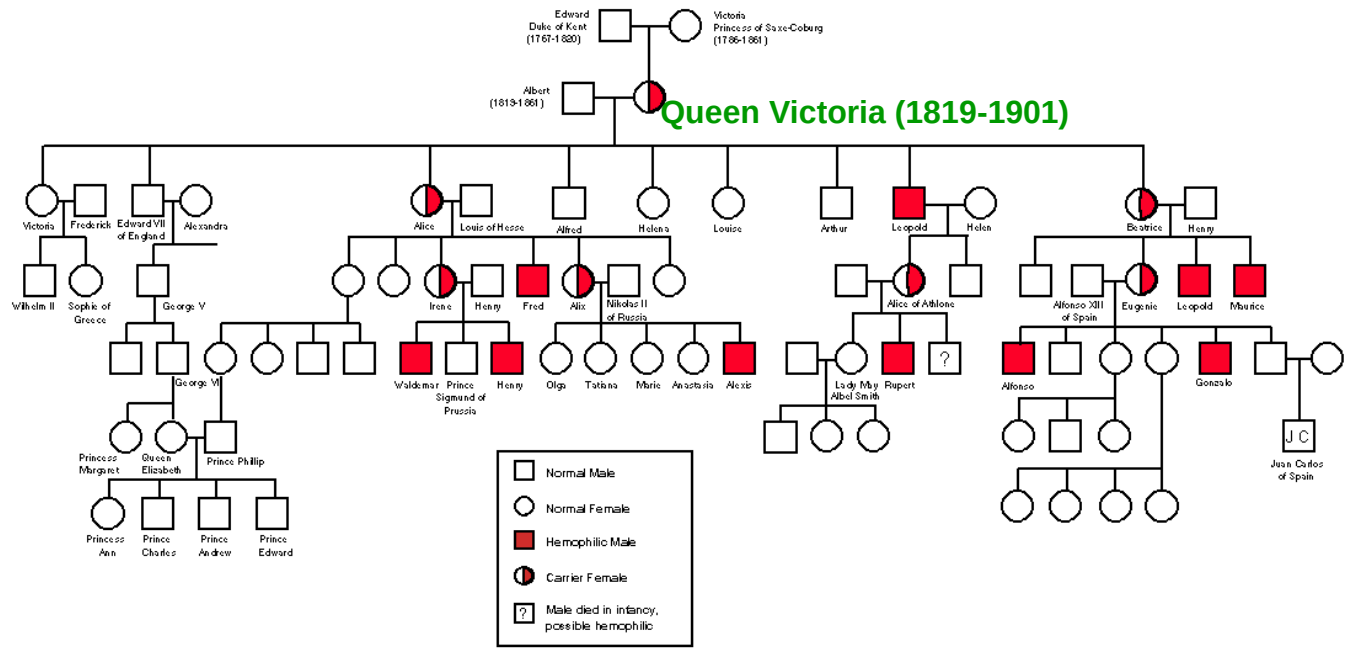
SB-728-T: Zinc Finger Nuclease Driven CCR5 Modified Autologous CD4+ T-cells



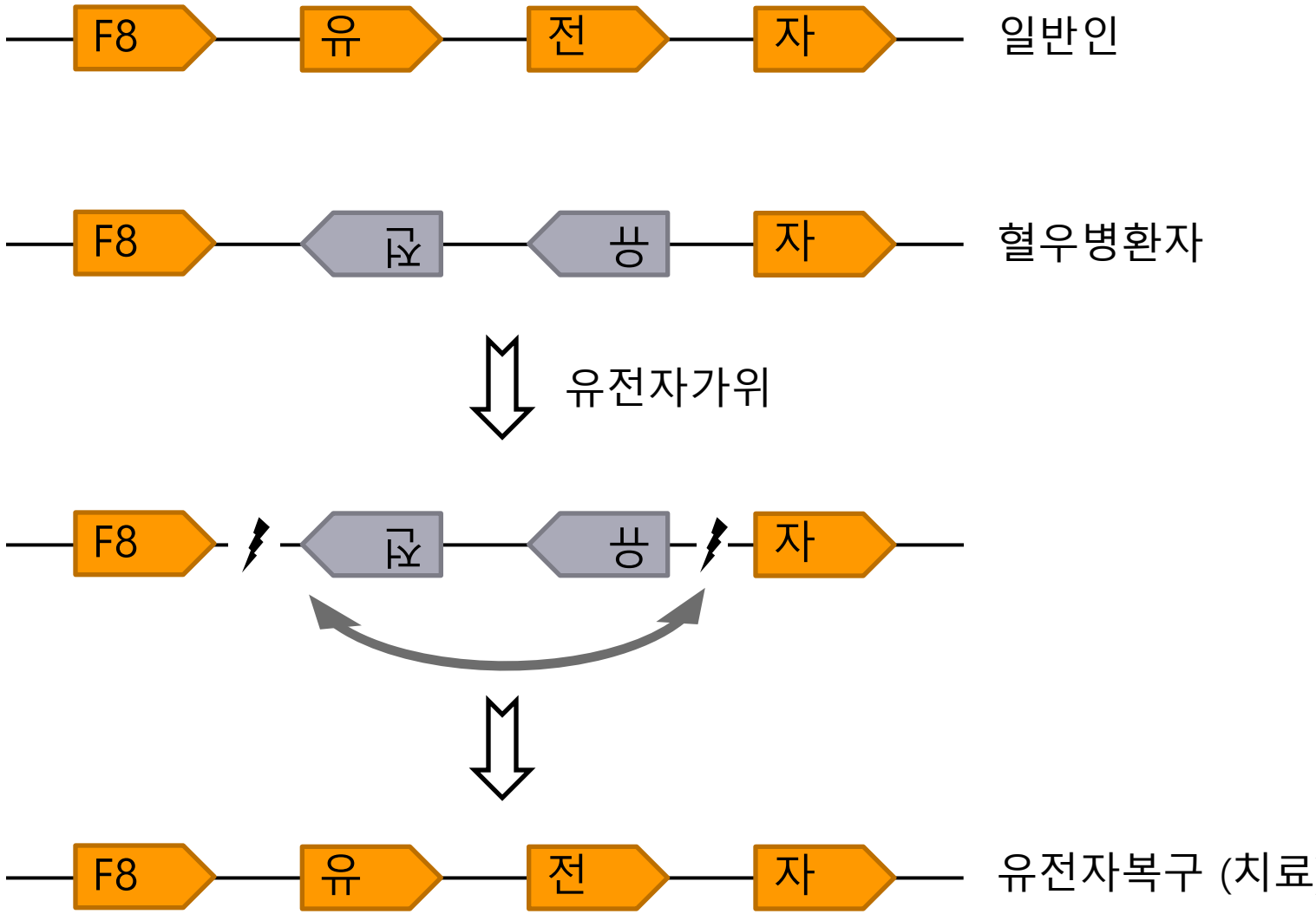
- T cells from HIV+ patients are treated with a programmable nuclease.
- CCR5-inactive T cells are delivered back to patients

혈우병: The Royal Disease

Queen Victoria and her royal family



- British Queen Victoria was a carrier of the hemophilia gene.
- Almost half of the severe form of hemophilia A is caused by DNA inversion.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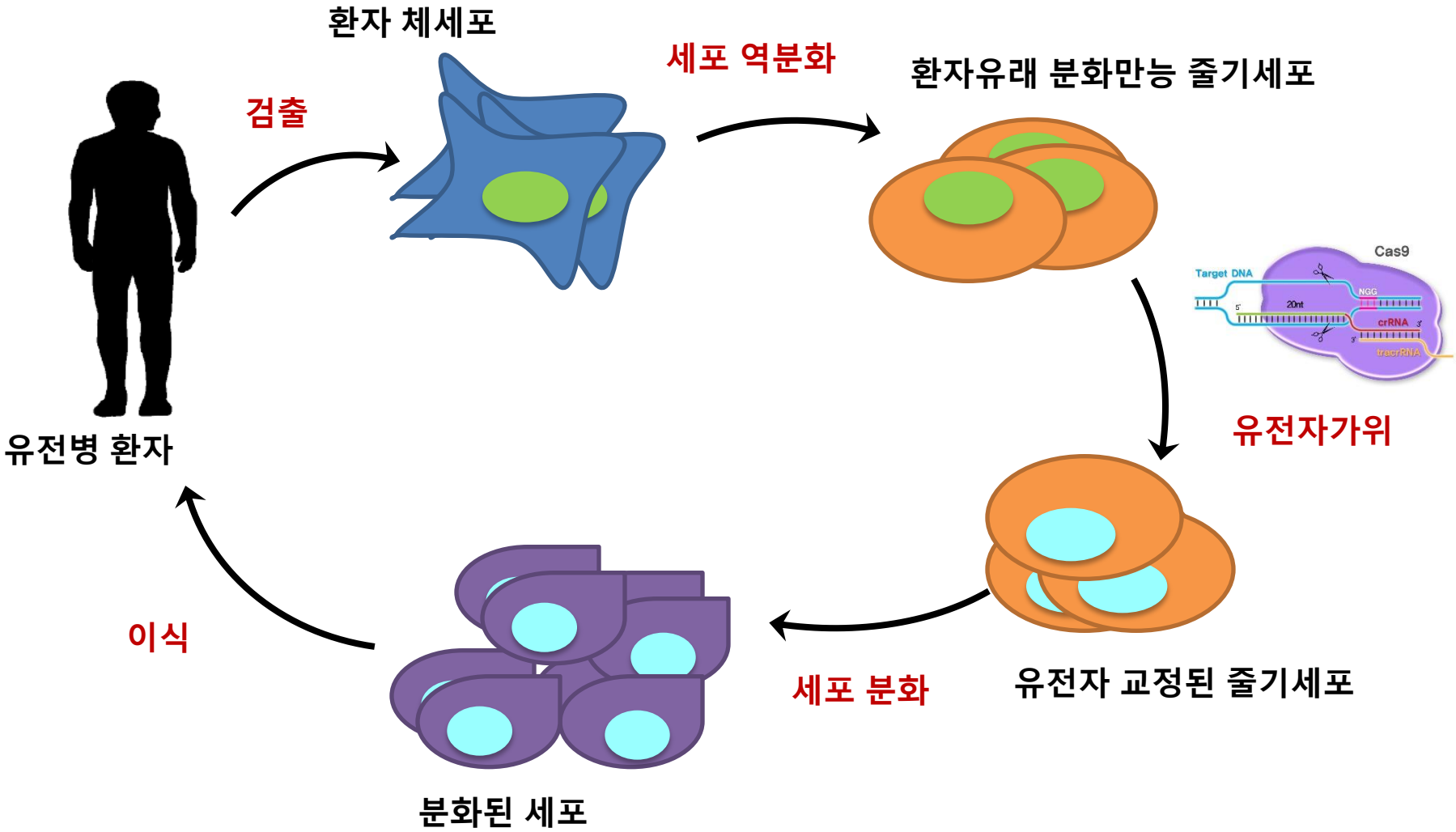
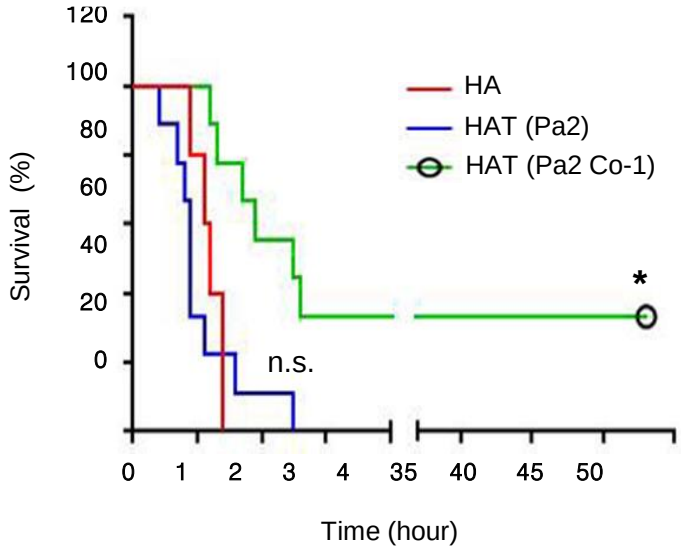
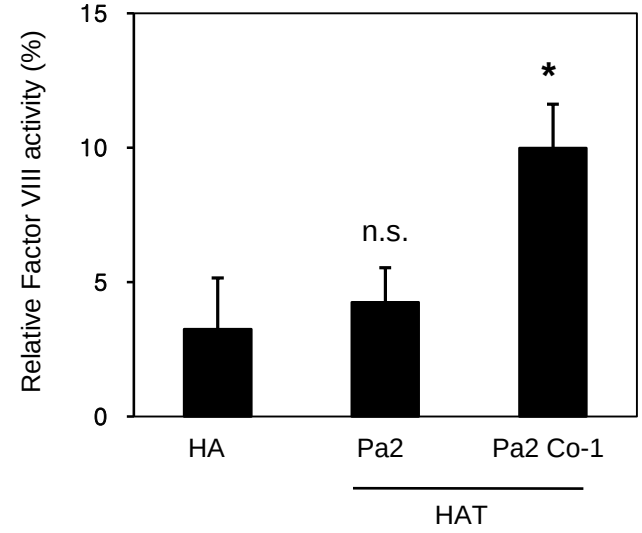
유전자복구 (치료)

줄기세포와 유전자 교정


환자 체세포
세포 역분화
환자유래 분화만능 줄기세포
검출
Cas9
Target DNA
NGG
20nt
crRNA
tracrRNA
유전병 환자
유전자가위
이식
세포 분화
유전자 교정된 줄기세포
분화된 세포

혈우병 생쥐의 세포치료 성공

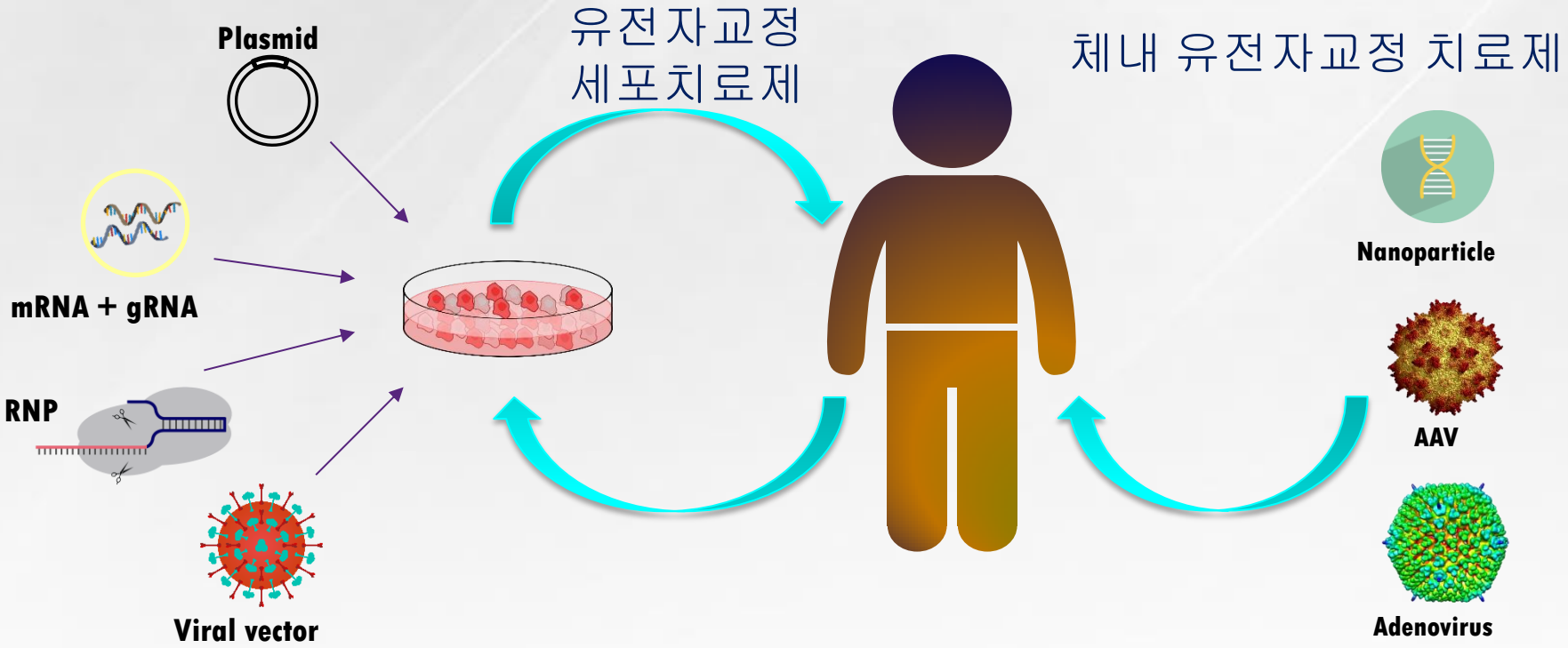




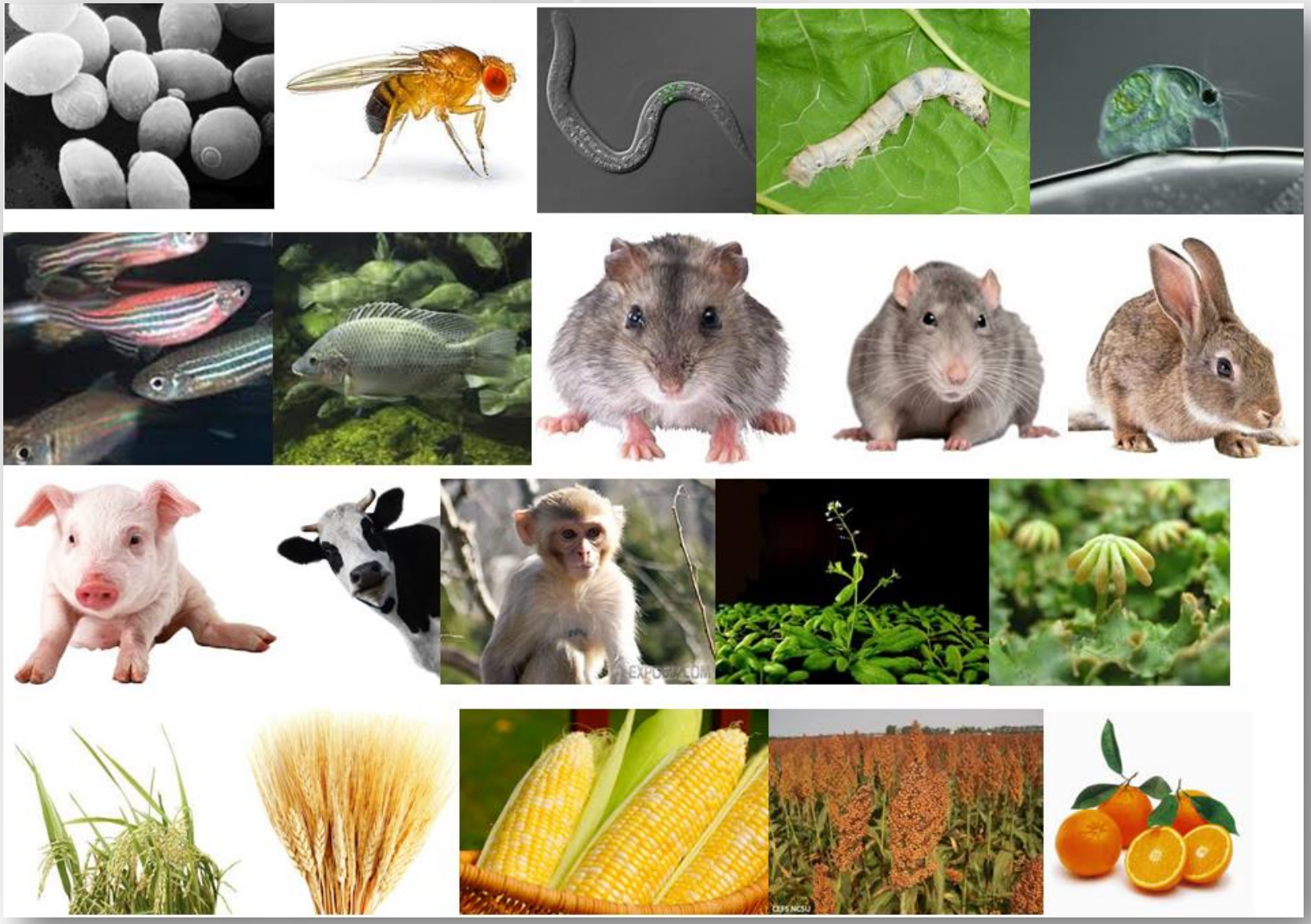
Park et al. Cell Stem Cell (2015)

- 환자 유래 역분화 줄기세포를 교정한 후 내피세포로 분화시킴
- 이를 혈우병 생쥐에 주입하니 출혈 증상이 현저히 개선됨

유전자 수술: Genome Surgery



유전자교정 동식물



Naturally-occurring *MSTN* KO animals

- Myostatin inhibits muscle differentiation and growth.
- Animals lacking myostatin have extensive muscles.

NATURE | NEWS

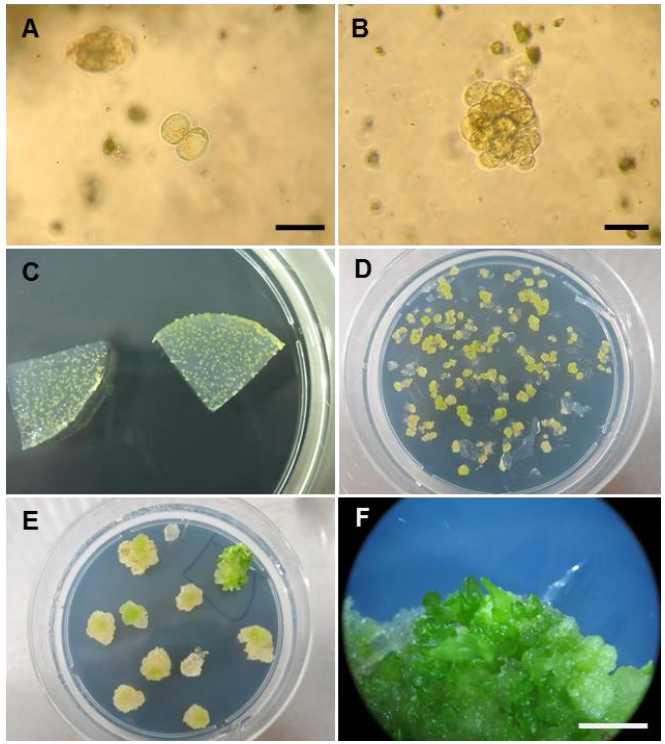
Super-muscly pigs created by small genetic tweak

Researchers hope the genetically engineered animals will speed past regulators.

David Cyranoski

30 June 2015

DNA-free RNA-guided Genome Editing in Lettuce



Woo & Kim et al. Nature Biotechnology, in press

- 크리스퍼 유전자가위 사용해 상추 원형질체 유전자 교정
- 외부 DNA 도입 없이 상추 자체 유전자만 교정됨
- 자연적 변이와 구별할 수 없어 GMO로 규제하는 것이 불합리함

NATURE | NEWS

CRISPR tweak may help gene-edited crops bypass biosafety regulation

Technique deletes plant genes without adding foreign DNA.

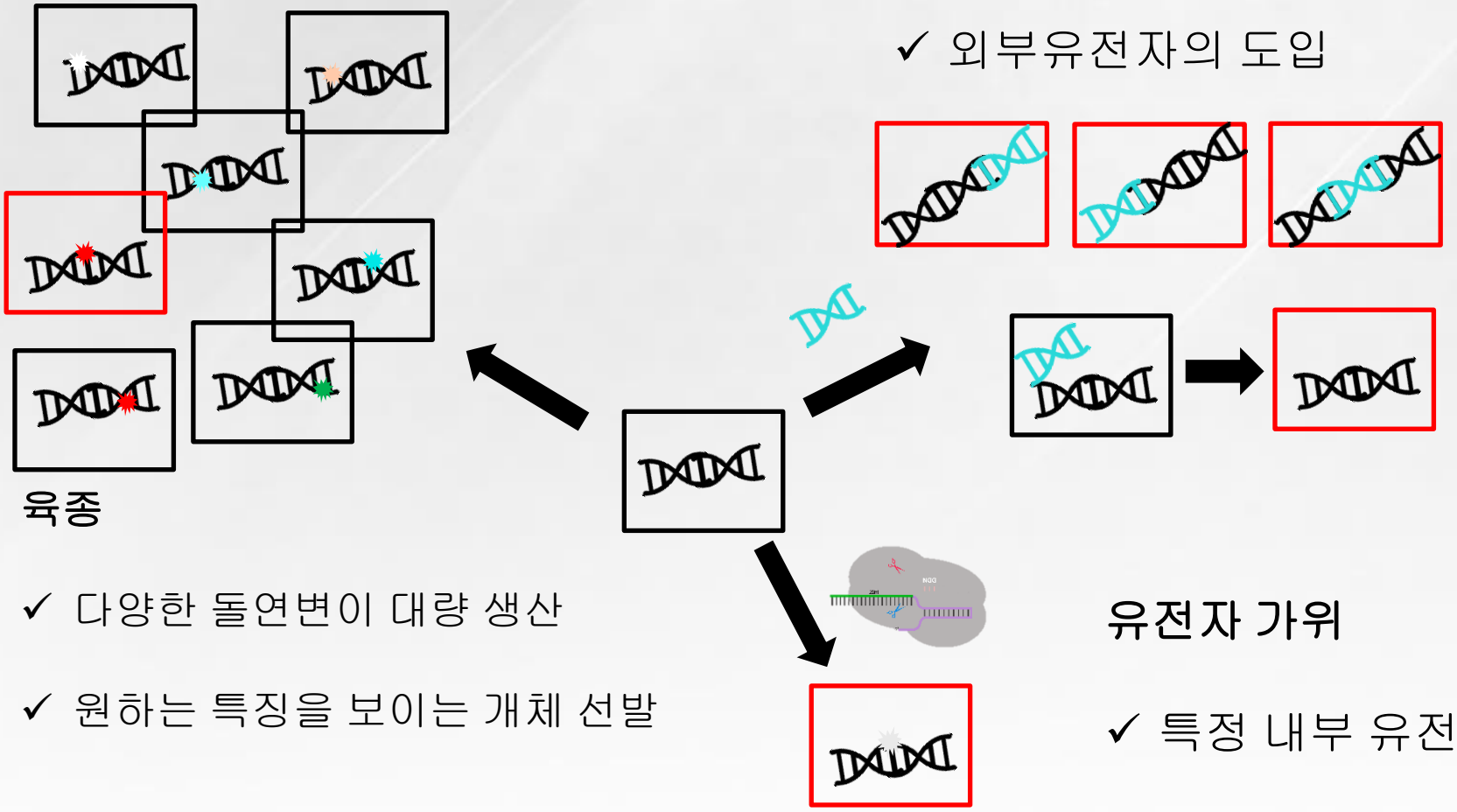
David Cyranoski

19 October 2015

Bananageddon and Banana Save Project

- Banana is infected by a fungus.
- Gros Michel is an old banana variety extinct in 1950’s.
- Cavendish banana will be extinct in 10~20 years.
- Genome editing can be used to make banana resistant to the fungus.

육종 vs GMO vs 유전자 가위



육종

✓ 다양한 돌연변이 대량 생산

✓ 원하는 특징을 보이는 개체 선발

GMO

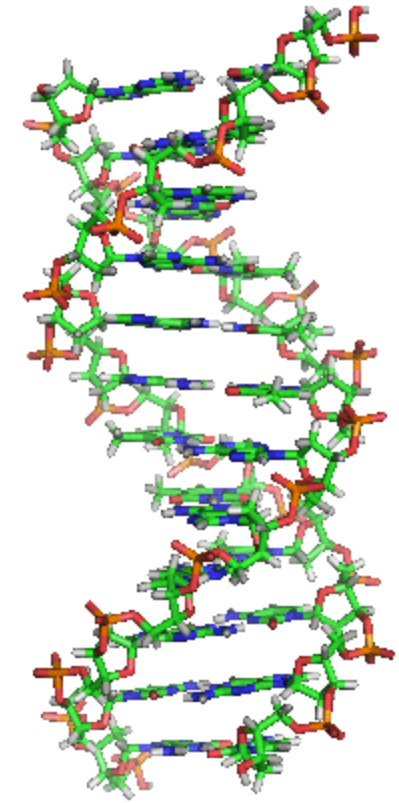
✓ 외부유전자의 도입

유전자 가위

✓ 특정 내부 유전자 변이

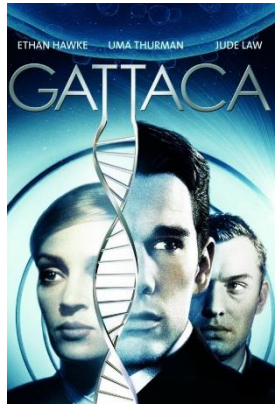
CRISPR Revolution

- Biomedical research
- Drug target discovery
- Gene and cell therapy
- Plant biotechnology
- Animal biotechnology



유전자가위 기술을 둘러싼 논란

- 인간 생식세포와 체세포 치료의 구별 필요
- 생식세포 유전자 교정: 임상과 연구 구별
- 유전자 교정과 강화: 예) 혈우병 치료와 IQ 향상
- 농작물, 가축 대상: GMO와 같이 규제할 것인가?
- 환경에 대한 영향: 예) 유전자 드라이브로 모기 박멸



유전자가위 기술 개선 과제

- 효율적 세포 내 전달 방법
- 상동재조합(HDR) vs. 비상동재접합(NHEJ)
- 오프타겟 돌연변이 측정 및 제어

생명윤리법 제47조

- 유전자치료에 관한 연구는 다음 각 호의 모두에 해당하는 경우에만 할 수 있다.

주요 국가별 유전자치료 허용 기준

한국	①유전질환, 암, 후천성면역결핍증, 그 밖에 생명을 위협하거나 심각한 장애를 일으키는 질병 ②현재 이용 가능한 치료법이 없거나 다른 치료와 비교할 때 현저히 우수할 것으로 예측되는 경우 ①과 ②를 모두 충족해야 허용
미국	약사법상 유전자치료 질환 연구제한 없음 단 임상시험 참여자 안전 가이드 라인과 유전자치료제 신약허가 심의 강화
유럽	유전자치료제 등 첨단치료제의 연구개발 범위 제한 없음 유럽의약품청의 첨단치료제 위원회 심의통해 임상시험 및 신약허가

자료:보건복지부

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