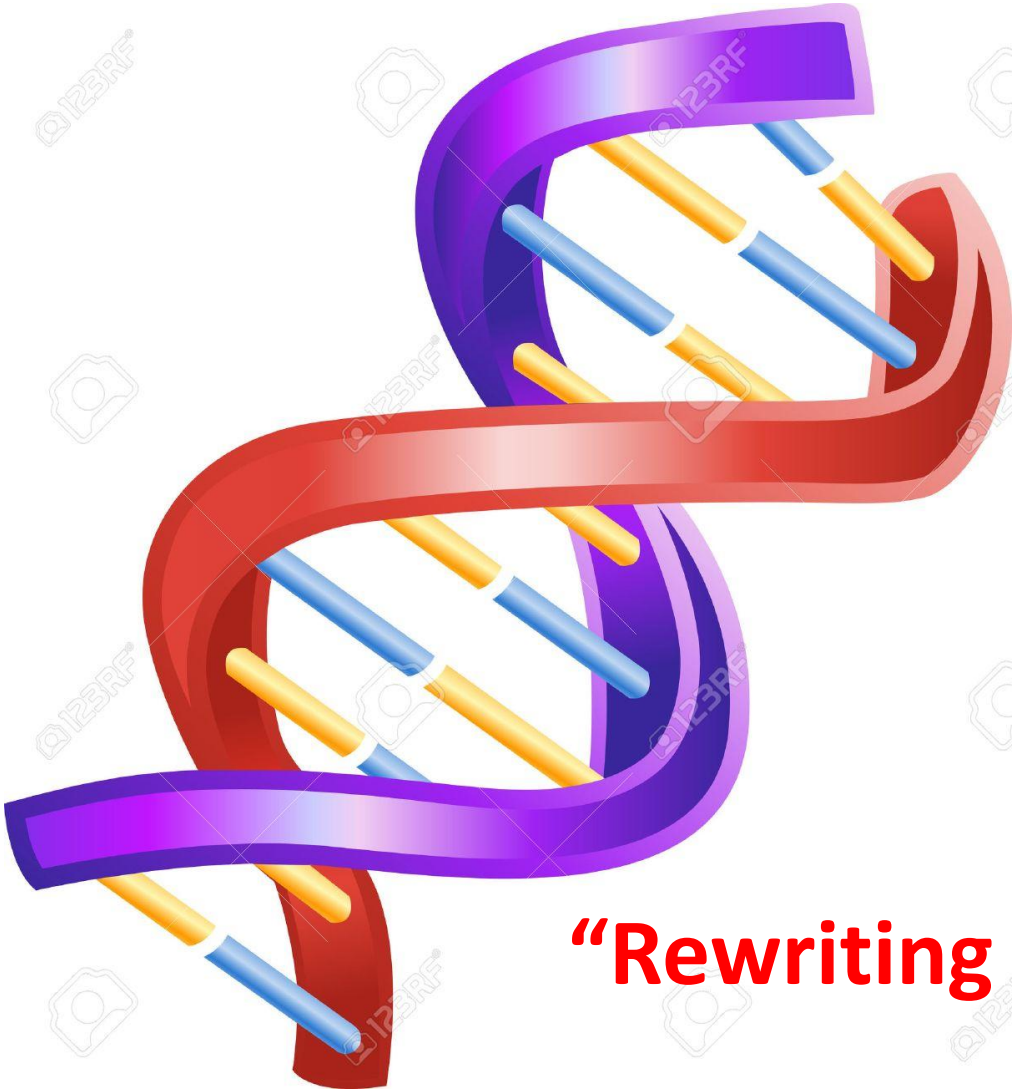


“Rewriting Code of the Life”

G.B.N. Chainy
30/8/19
UU RC

Chinese Scientist Claims to Use Crispr to Make First Genetically Edited Babies

The researcher, He Jiankui, offered no evidence or data to back up his assertions. If true, some fear the feat could open the door to "designer babies." **2018**

Corn and other important crops can now be gene edited by pollen carrying CRISPR

By Jon Cohen Mar. 4, 2019 , 11:00 AM

CRISPR-Cas9 Gene Editing in Lizards through Microinjection of Unfertilized Oocytes

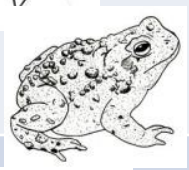
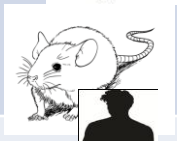
CELL REPORT| VOLUME 28, ISSUE 9, P2288-2292.E3, AUGUST 2019

WHAT IS CRISPR- Cas9 ?

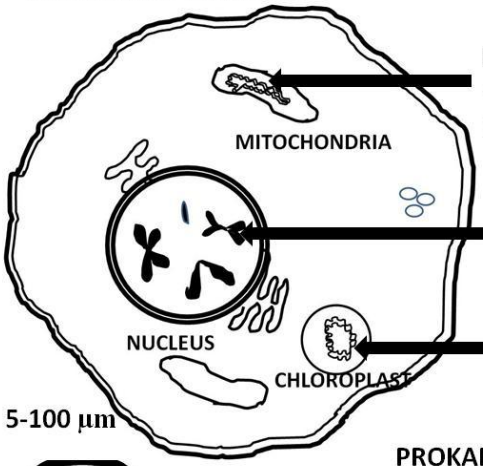
(Clustered Regularly Interspaced Short Palindromic Repeats/CRISPRassociated nuclease 9)

What is a genome?

A genome is an organism's complete set of DNA, including all of its genes. Each genome contains all of the information needed to build and maintain that organism.

SPECIES	CHROMOSOME NUMBER	C VALUE DNA (pg)/COPY GENOME	GENOME SIZE(Mbp)
	1		
	1	0.017	4.24
	14	0.17	12.1
	24	0.87	430
	42	15.46	16000
	22	6	
	40	3.3	2800
	46	3.5	3200

EUKARYOTIC GENOME



PROKARYOTIC GENOME (1 COPY)

0.1-5 μm

VIRAL GENOME (1 COPY)

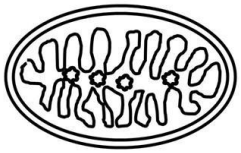
0.02- 0.4 μm

1 base pair(bp) = 660 Dalton

1000 bp = 1 Kilobase (10^6bp)

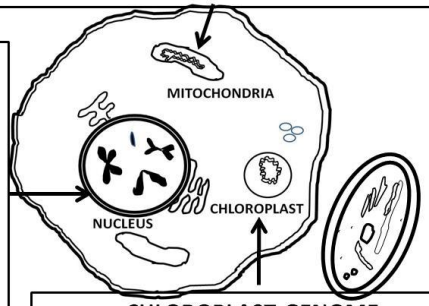
1000 kb = 1 Megabase (10^6bp)

1000 mb = 1 Gigabase (10^9bp)



MITOCHONDRIAL GENOME:
- CIRCULAR, DUPLEX, MULTIPLE COPIES
- > 100 COPIES IN DIFFERENTIATING EMBRYONIC CELLS.
- SIZE 16000-20000 bp IN ANIMALS.
- SIZE 200,000 TO 250,0000 IN PLANTS.

NUCLEAR GENOME:
- LARGE SIZE, DUPLEX, LINEAR, COMPLEX ORGANIZATION.
- MORE THAN ONE DNA MOLECULE.
- MOSTLY DIPLOID STATE
- PRESENCE OF NON CODIND SEQUENCE.
- PRESENCE OF REPETITIVE DNA.
- SIZE 10^6 TO 10^{11} bp

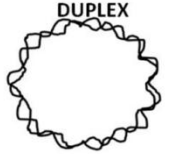


CHLOROPLAST GENOME
- CIRCULAR, DUPLEX, MULTIPLE COPIES.
- > 100 COPIES IN DIFFERENTIATING EMBRYONIC CELLS.
- SIZE 120000-160000 bp.

A TYPICAL PROKARYOTIC GENOME

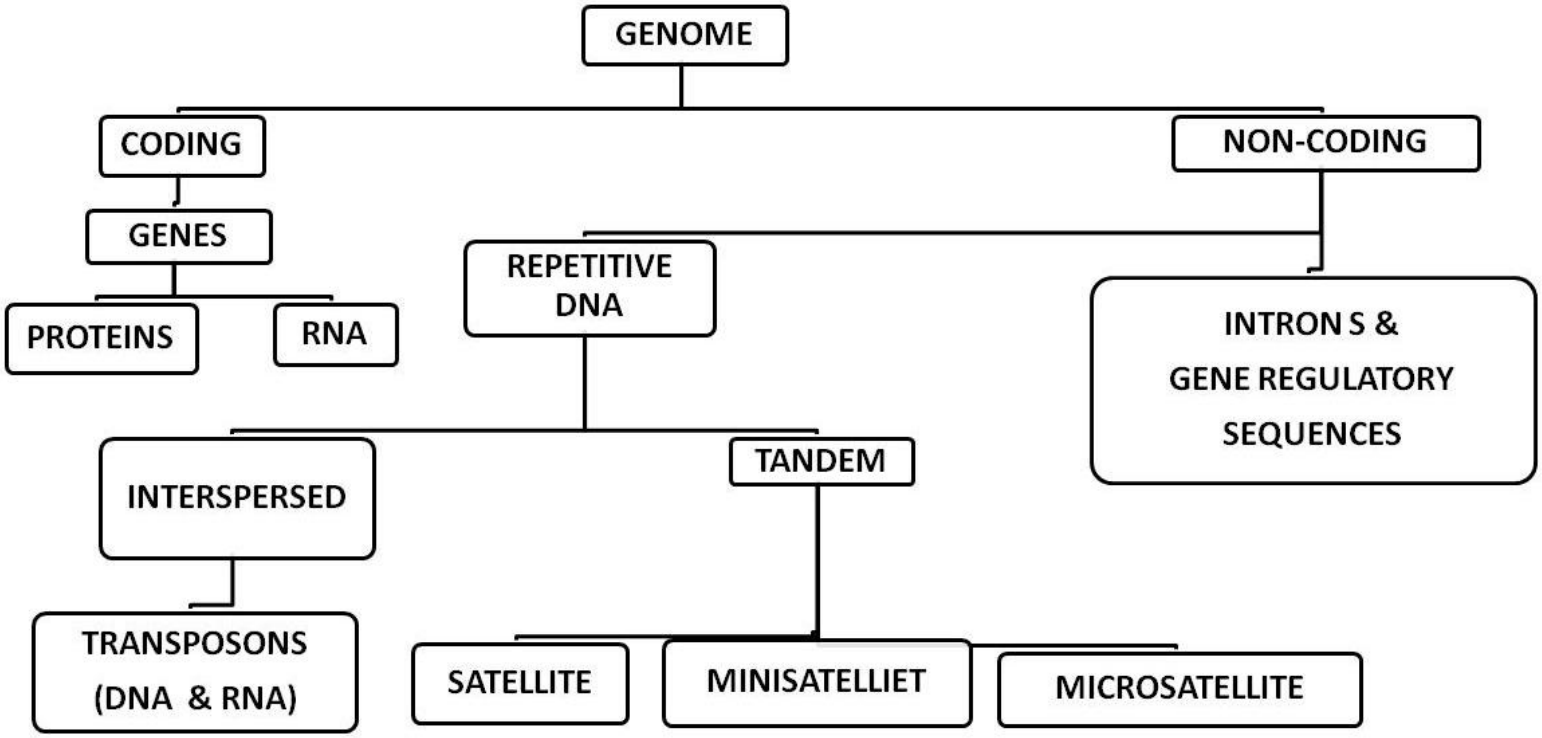
SMALL SIZE (10^5-10^6 bp)

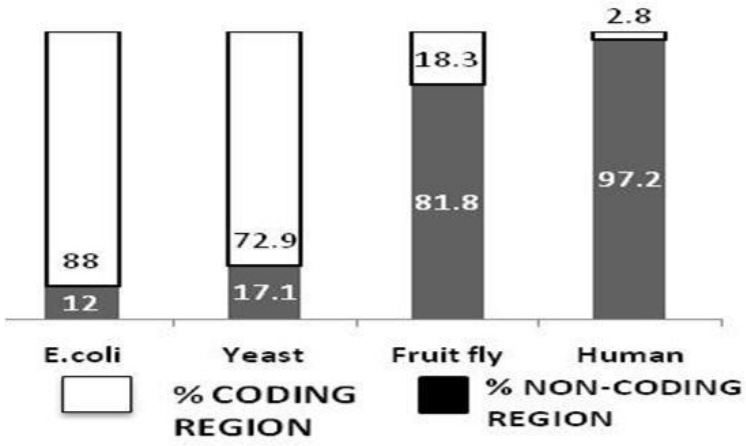
DUPLEX

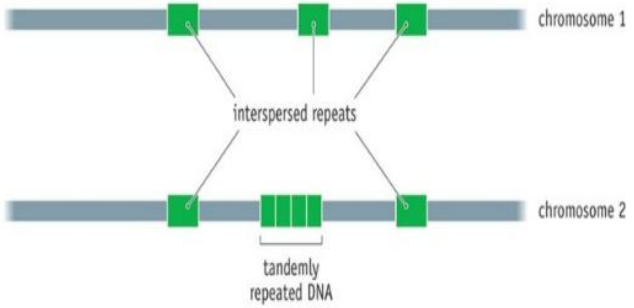


PRESENT FREELY IN CYTOPLASM
SINGLE COPY, COMPACT
NO REPETITIVE SEQUENCE
SINGLE ORIGIN OF REPLICATION

SCHEMATIC PRESENTATION OF GENOME ORGANIZATION







CTAGAGATAGATAGATAGATAGATAGATAGATAGATAGATACTAGAG

DISCOVERY OF CRISPR-Cas9

•The park is about 3,700 hectares..
•The pink lake is 1,400 hectares
• the green one is 700 hectares.
•Both are connected to the sea by canals.

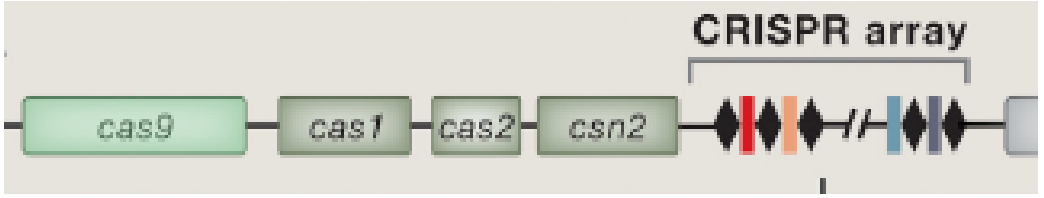
•The strange pink-purple colour of the Torrevieja lagoon is caused by pigments of the **Halobacterium** bacteria and by an alga called ***Dunadiella salina,*** which is responsible for the bright red colour of the lake.
• The Artemia Salina brine shrimp, which lives in the lake, is also red because it feeds on the bacteria.

1993 - 2005 — Francisco Mojica, University of Alicante, Spain

• Characterized CRISPR locus in 1993.
• Coined the term CRISPR through correspondence with Ruud Jansen 2002.
• CRISPER snippets from the genomes of bacteriophage .
• hypothesized, correctly, that CRISPR is an adaptive immune system.

Discovery of Cas9 and PAM

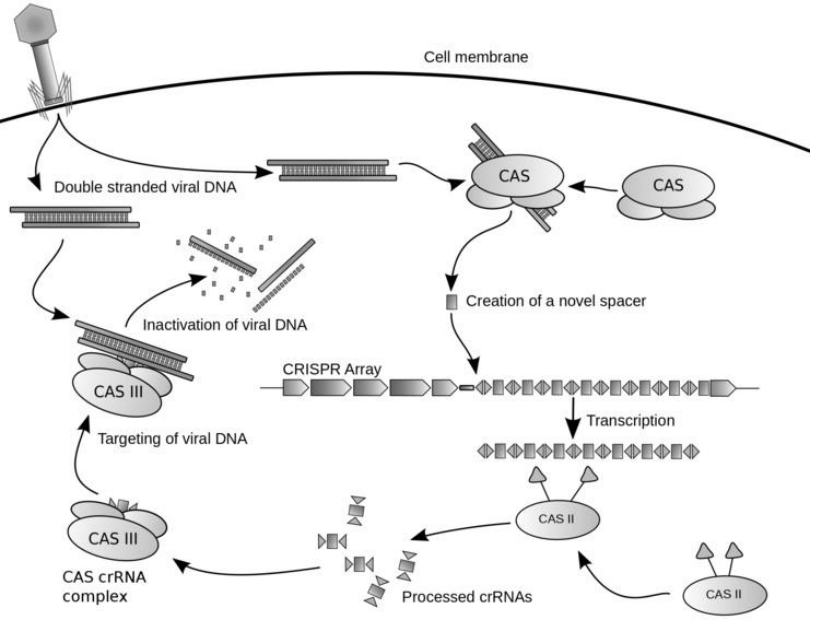
May, 2005 — Alexander Bolotin, French National Institute for Agricultural Research (INRA)



• Noticed unusual CRISPR locus in *Streptococcus thermophilis*.

• Found **Cas 9 gene** encoding large Cas 9 nuclease.

• Furthermore, they noted that the all spacers share a common sequence at one end the **protospacer adjacent motif (PAM)**,



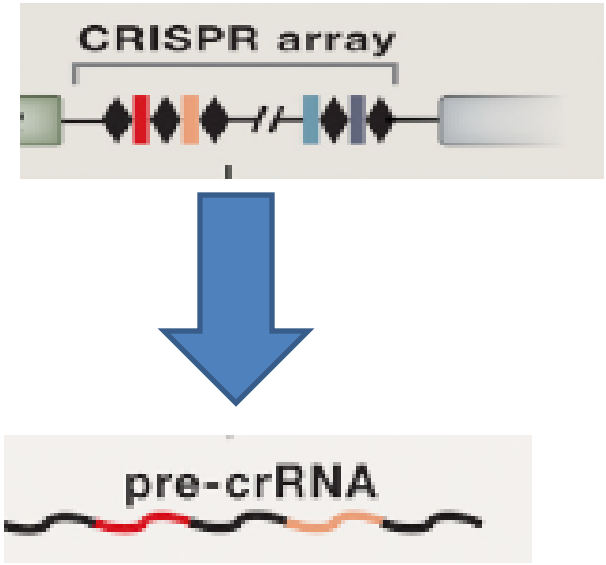
Hypothetical scheme of adaptive immunity

March, 2006 — Eugene Koonin, US National Center for Biotechnology Information, NIH

Koonin was studying clusters of orthologous groups of proteins by computational analysis and proposed a hypothetical scheme for CRISPR cascades as bacterial immune system based on inserts homologous to phage DNA in the natural spacer array, abandoning previous hypothesis that the Cas proteins might comprise a novel DNA repair system.

Experimental demonstration of adaptive immunity. March, 2007 — Philippe Horvath, Danisco France SAS

• S. thermophilus is widely used in the dairy industry to make yogurt and cheese.
• showed experimentally that CRISPR systems are indeed an adaptive immune system
• showed that Cas9 is likely the only protein required for interference, the process by which the CRISPR system inactivates invading phage, details of which were not yet known.



Spacer sequences are transcribed into guide RNAs

August, 2008 — John van der Oost, University of Wageningen, Netherlands

• showed that in *Escherichia coli*, spacer sequences, which are derived from phage, are transcribed into small RNAs, termed CRISPR RNAs (crRNAs), that guide Cas proteins to the target DNA.

CRISPR acts on DNA targets

December, 2008 — Luciano Marraffini and Erik Sontheimer, Northwestern University, Illinois

• Demonstrated that the target molecule is DNA, not RNA. This was somewhat surprising, as many people had considered CRISPR to be a parallel to eukaryotic RNAi silencing mechanisms, which target RNA.

Cas9 cleaves target DNA

December, 2010 — Sylvain Moineau, University of Laval, Quebec City, Canada

• Demonstrated that CRISPR-Cas9 creates double-stranded breaks in target DNA at precise positions, 3 nucleotides upstream of the PAM.

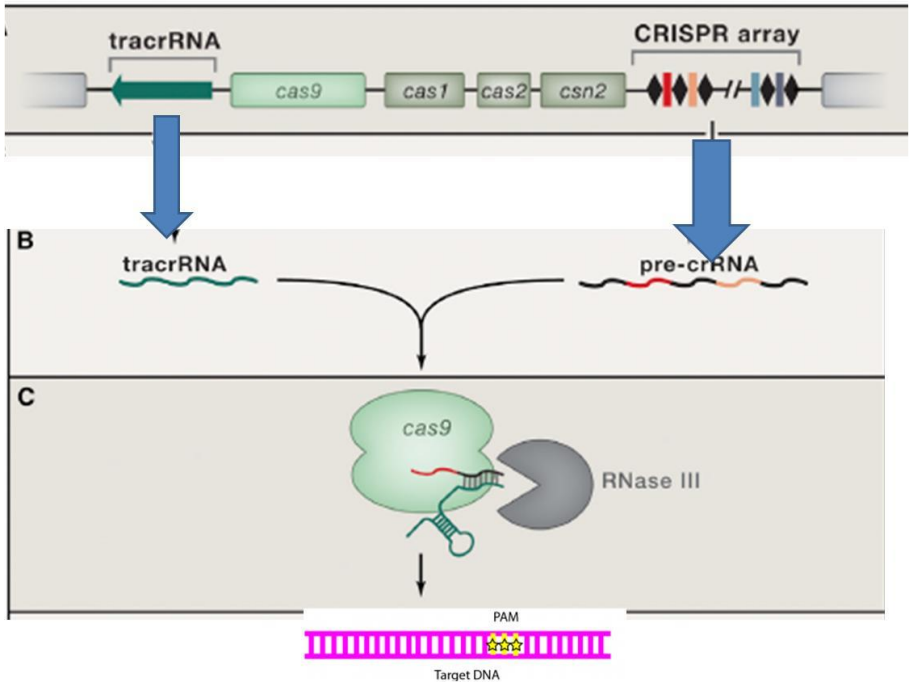
•Confirmed that Cas9 is the only protein required for cleavage in the CRISPR-Cas9 system.

CRISPR is an adaptive immune system.

Discovery of tracrRNA for Cas9 system

March, 2011 — Emmanuelle Charpentier, Umea University, Sweden and University of Vienna, Austria

- Discovered that in addition to the crRNA, a second small RNA exists.
- Named it trans-activating CRISPR RNA (tracrRNA).
- Demonstrated that tracrRNA forms a duplex with crRNA, and that it is this duplex that guides Cas9 to its targets.

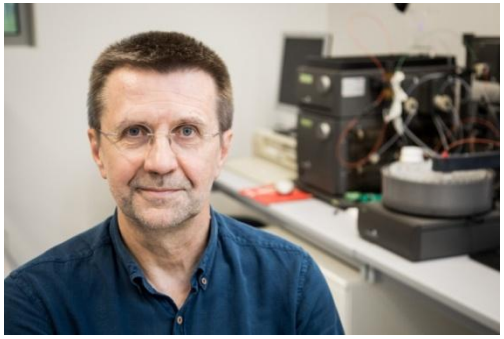


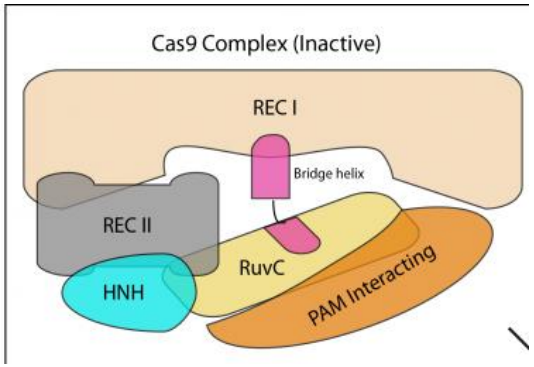
CRISPR-Cas9 :
FROM FUNDAMENTALS TO APPLICATION

CRISPR systems can function heterologously in other species

July, 2011 — Virginijus Siksnys, Vilnius University, Lithuania

•Cloned the entire CRISPR-Cas locus from S. thermophilus (a Type II system) and expressed it in E. coli (which does not contain a Type II system).





Biochemical characterization of Cas9-mediated cleavage

September, 2012 — , Vilnius University, Lithuania

• Purified Cas9 in complex with crRNA from the E. coli strain engineered to carry the *S. thermophilus* CRISPR locus.

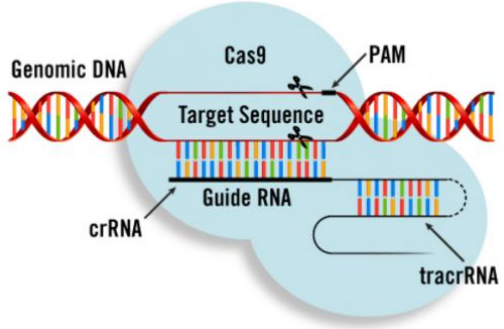
• Mechanistically characterize Cas9's mode of action .

• Verified the cleavage site and the requirement for the PAM.

•RuvC domain cleaves the non-complementary strand while the HNH domain cleaves the complementary site.

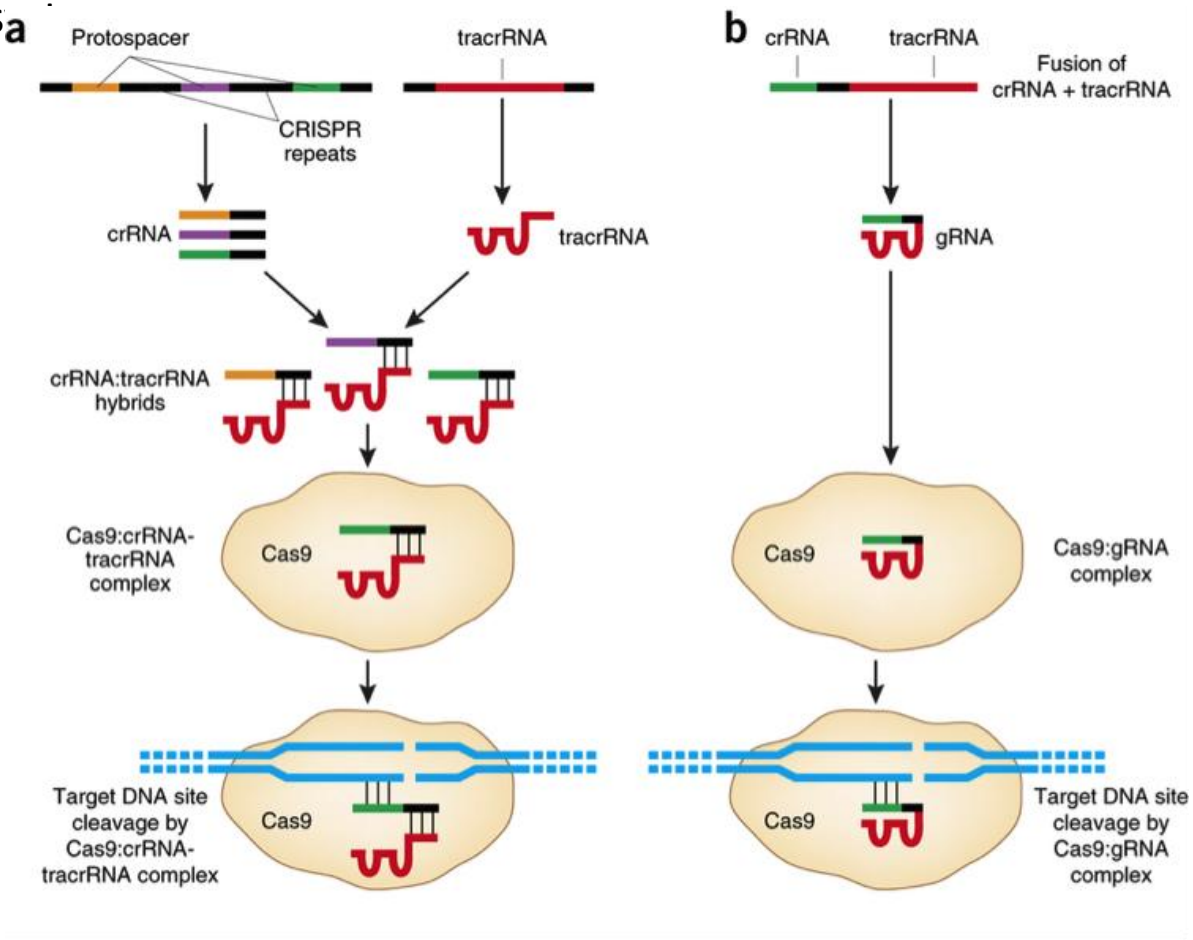
• crRNA could be trimmed down to a 20-nt stretch sufficient for efficient cleavage.

•Reprogramming of Cas9 to target a site by changing the sequence of the crRNA.



June, 2012 — ***Charpentier and Jennifer Doudna,*** *University of California, Berkeley*

- Reported that the crRNA and the tracrRNA could be fused together to create a single, synthetic guide, further simplifying the s

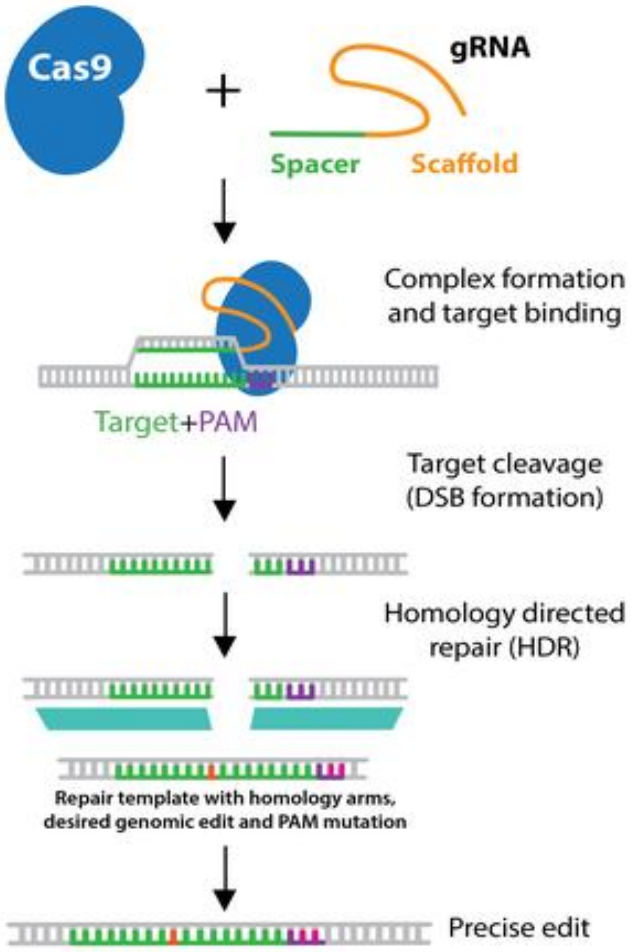


CRISPR-Cas9 harnessed for genome editing

January, 2013 — Feng Zhang, Broad Institute of MIT and Harvard, McGovern Institute for Brain Research at MIT, Massachusetts

•Engineered two different Cas9 orthologs (from *S. thermophilus and S. pyogenes*) and demonstrated targeted genome cleavage in human and mouse cells.

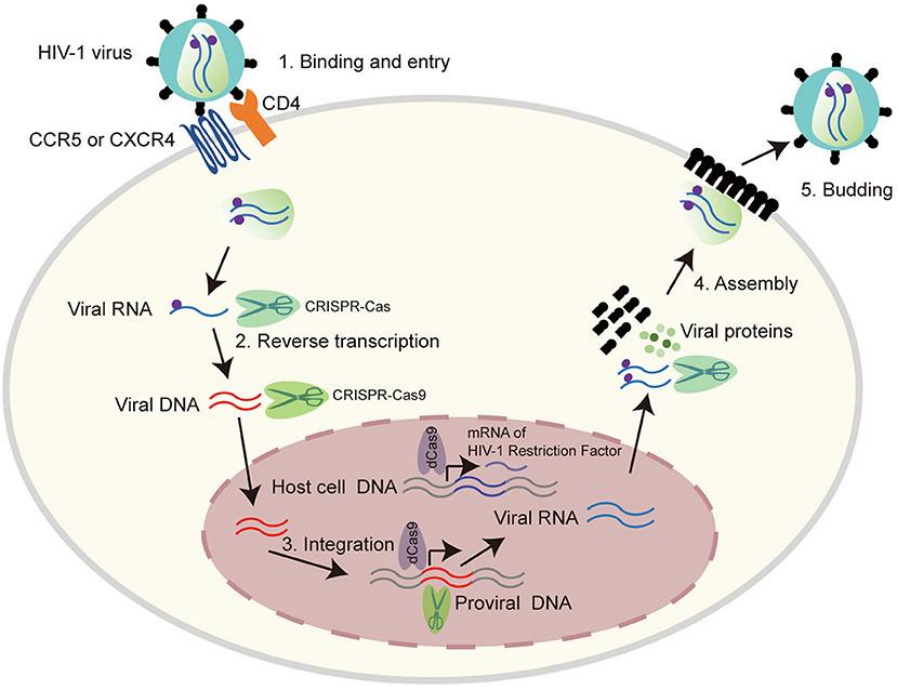
• showed that the system (i) could be programmed to target multiple genomic loci, and (ii) could drive homology-directed repair.

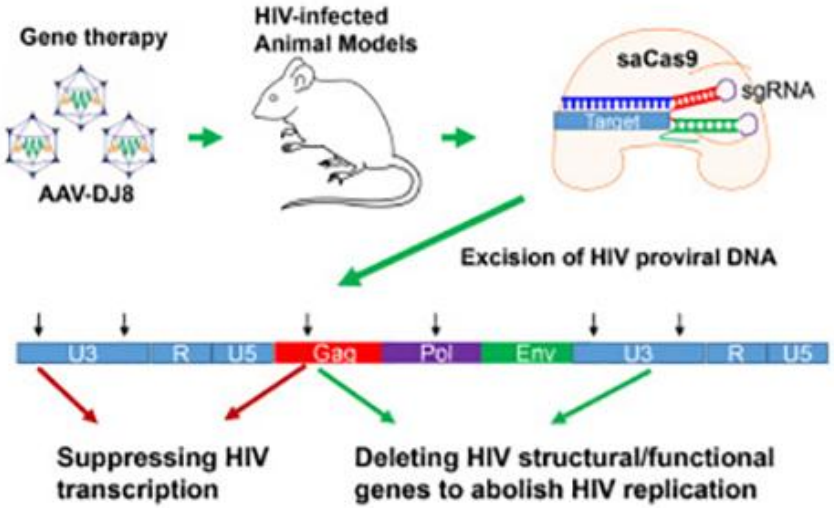


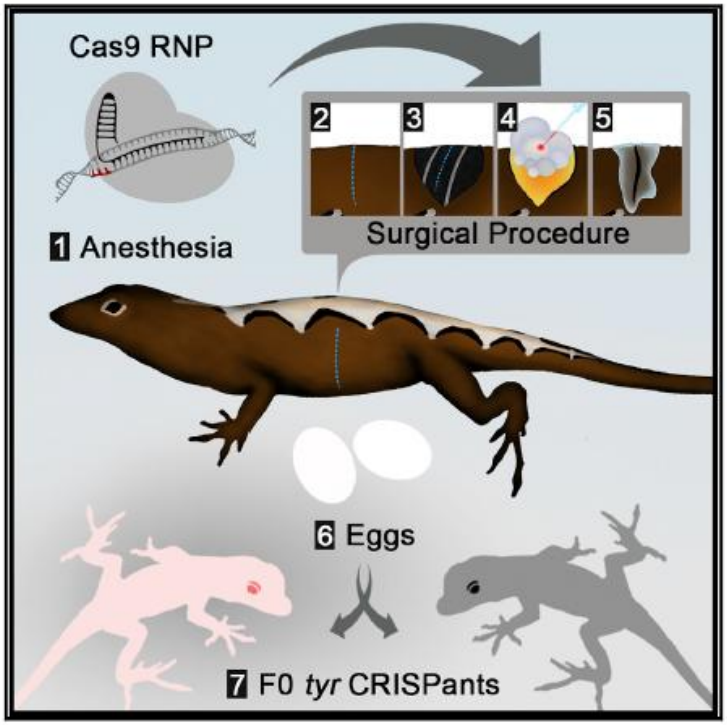
Nature Protocol. 2013 Nov;8(11):2281-2308

Genome engineering using the CRISPR-Cas9 system.

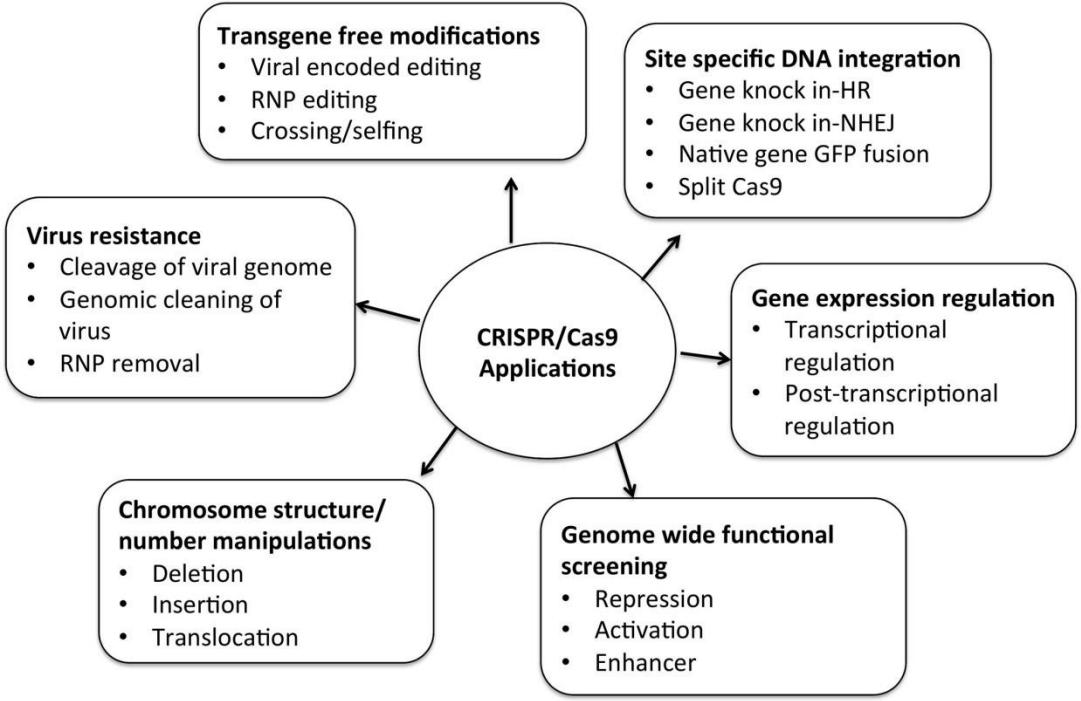
Ran FA, Hsu PD, Wright J, Agarwala V, Scott D, Zhang F.

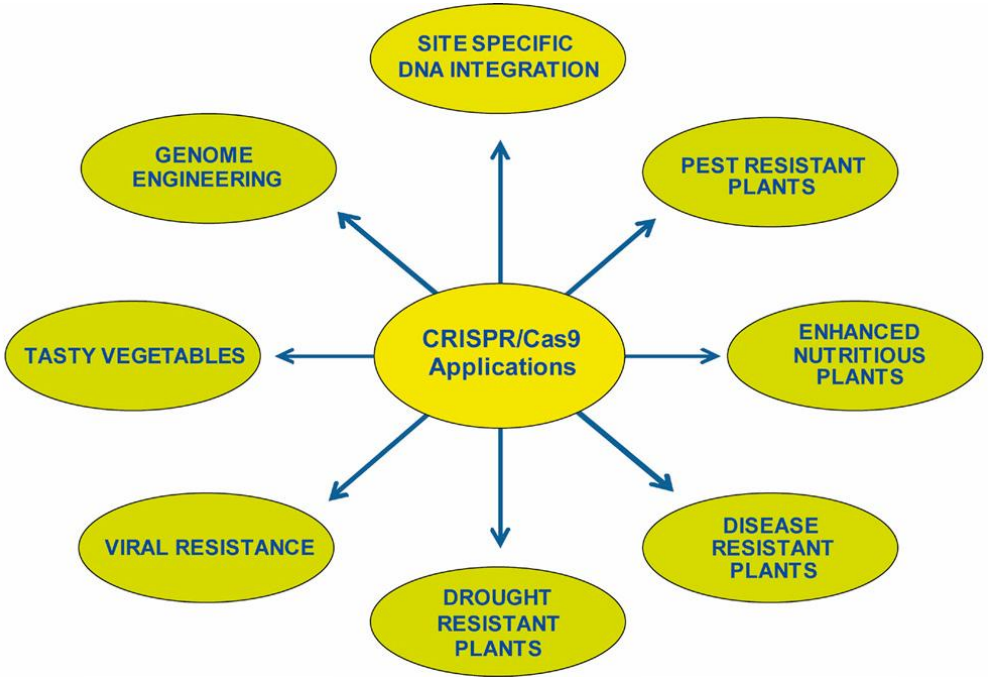

HIV-1 virus
1. Binding and entry
CD4
CCR5 or CXCR4
5. Budding
4. Assembly
Viral proteins
Viral RNA
CRISPR-Cas
2. Reverse transcription
Viral DNA
CRISPR-Cas9
dCas9
mRNA of
HIV-1 Restriction Factor
Host cell DNA
Viral RNA
3. Integration
dCas9
Proviral DNA


Gene therapy
HIV-infected
Animal Models
saCas9
sgRNA
Target
AAV-DJ8
Excision of HIV proviral DNA
U3
R
U5
Gag
Pol
Env
U3
R
U5
Suppressing HIV
transcription
Deleting HIV structural/functional
genes to abolish HIV replication


Cas9 RNP
2
3
4
5
Surgical Procedure
1 Anesthesia
6 Eggs
7 F0 tyr CRISPants

Highlights


Transgene free modifications
• Viral encoded editing
• RNP editing
• Crossing/selfing
Site specific DNA integration
• Gene knock in-HR
• Gene knock in-NHEJ
• Native gene GFP fusion
• Split Cas9
Virus resistance
• Cleavage of viral genome
• Genomic cleaning of virus
• RNP removal
CRISPR/Cas9 Applications
Gene expression regulation
• Transcriptional regulation
• Post-transcriptional regulation
Chromosome structure/ number manipulations
• Deletion
• Insertion
• Translocation
Genome wide functional screening
• Repression
• Activation
• Enhancer


SITE SPECIFIC DNA INTEGRATION
GENOME ENGINEERING
PEST RESISTANT PLANTS
TASTY VEGETABLES
CRISPR/Cas9 Applications
ENHANCED NUTRITIOUS PLANTS
VIRAL RESISTANCE
DISEASE RESISTANT PLANTS
DROUGHT RESISTANT PLANTS

GENOME

READ

COPY

and now u can REWRITE ….


high IQ
no baldness
perfect pitch
rinter
20/20 v
low risk
Alzheimer's
breast canc
strokes

THANK U ALL