Μεθοδολογία της Μοριακής Γενετικής Φυτών

Μεθοδολογία στη μελέτη ανάπτυξης των φυτών

1. Πρότυπα μοντέλα στη μελέτη ανάπτυξης των φυτών
2. Μέθοδοι μετασχηματισμού και μεταλλαξιγένεσης
3. Απόκτηση μεταλλαγμένων σειρών Arabidopsis
4. Γενετική ανάλυση μεταλλαγμένων σειρών
5. Φαινοτυπική ανάλυση μεταλλαγμένων σειρών
6. Απομόνωση του γονιδίου που σχετίζεται με μία μετάλλαξη
7. Τρόποι μελέτης της έκφρασης και λειτουργίας ενός γονιδίου
8. Παραδείγματα μελετών μοριακής γενετικής

Απόκτηση μεταλλαγμένων σειρών *Arabidopsis thaliana*

1. Λήψη ήδη δημιουργηθέντων μεταλλαγμένων σειρών που είναι καταχωρημένες σε τράπεζες γενετικού υλικού και σπερμάτων

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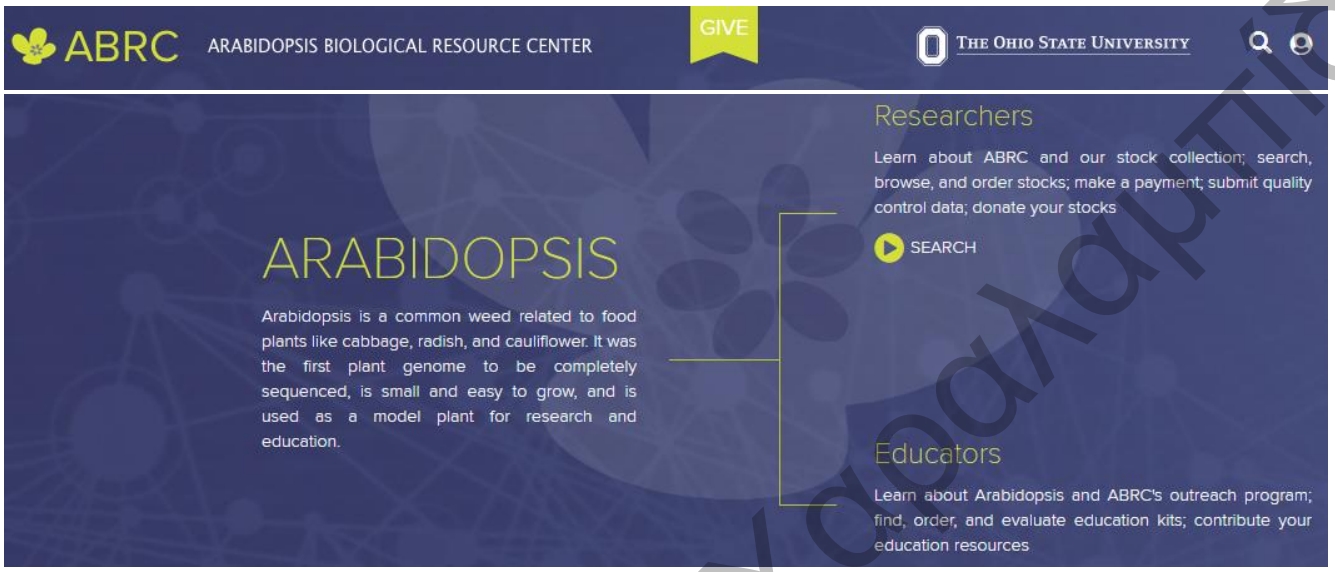
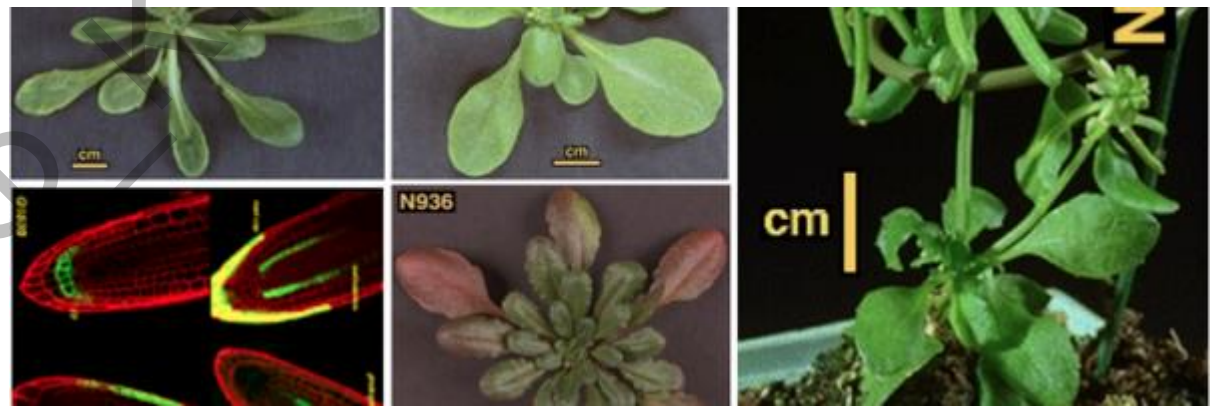
Λήψη αναγνωρισμένων και «χαρακτηρισμένων» μεταλλαγμάτων



The Nottingham Arabidopsis Stock Centre

UK Research and Innovation



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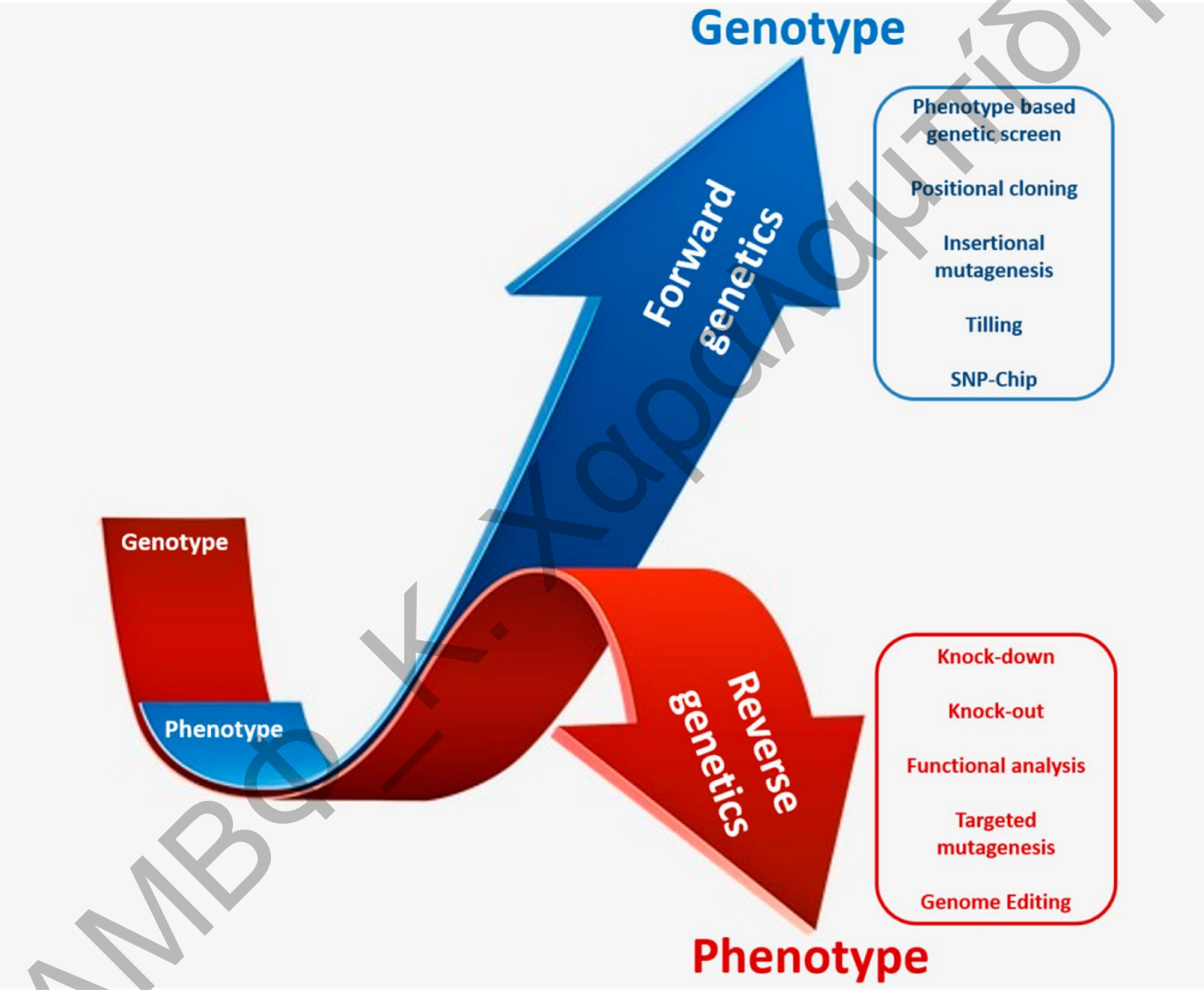
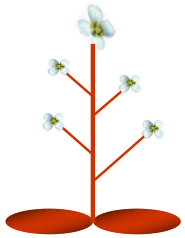
Forward vs. Reverse Genetics



Forward vs. Reverse Genetics

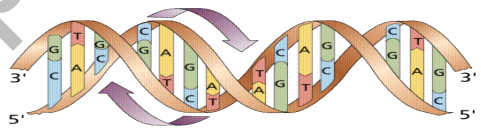
μεταλλαξιγένεση



Φαινότυπος

FORWARD GENETICS

Γονίδιο



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ATCGATTATATGCACGGCAGCAGA
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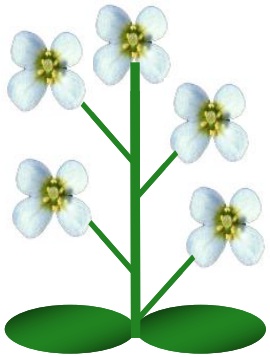
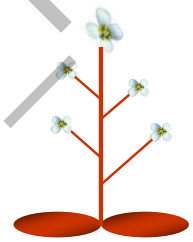


Προσεγγίσεις Μοριακή Γενετική Φυτών

συμπληρωματικότητα



Φαινότυπος

REVERSE GENETICS

«Προωθητική γενετική» (forward genetics)…

…from mutant phenotype to gene, from gene to protein function

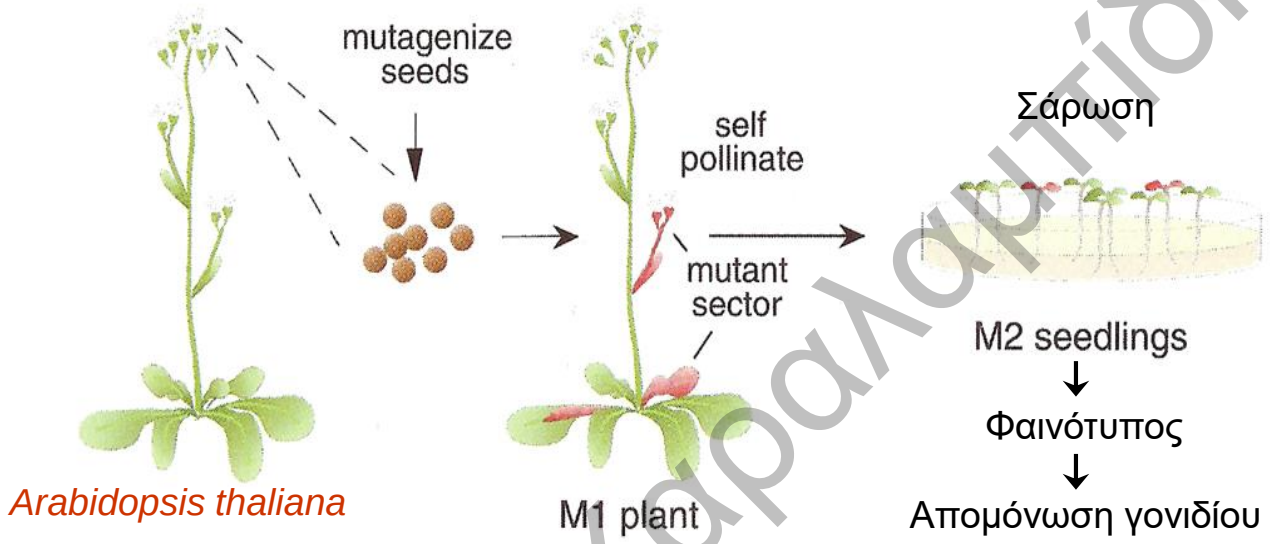
Περιλαμβάνει τη διαδικασία:

- ✓ Λήψη ή δημιουργία τυχαίων μεταλλάξεων με μεταλαξιγόνα
- ✓ Σάρωση για την εύρεση του επιθυμητού φαινότυπου
- ✓ Χαρακτηρισμό του φαινότυπου των μεταλλαγμένων σειρών
- ✓ Απομόνωση του γονιδίου που ευθύνεται για τον φαινότυπο
- ✓ Λεπτομερείς μελέτη της λειτουργίας γονιδίου/πρωτεΐνης

π.χ.

- ➢ Γονίδια που ελέγχουν την άνθηση των φυτών
- ➢ Γονίδια που εμπλέκονται στην άμυνα των φυτών έναντι παθογόνων
- ➢ Γονίδια που εμπλέκονται στη βιοσύνθεση δευτερογενών μεταβολιτών

Forward Genetics - Η διαδικασία περιορίζεται από...



1. Ο αριθμός των σπερμάτων που είναι εφικτός να σαρωθεί
2. Ο πιθανός αριθμός των γονιδίων που ελέγχουν ένα χαρακτηριστικό

- Γονίδια που ελέγχουν την άνθηση των φυτών
- Γονίδια που εμπλέκονται στην άμυνα των φυτών έναντι παθογόνων
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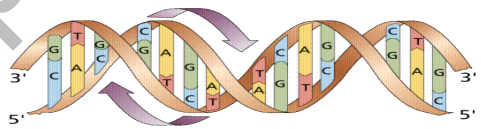
Forward vs. Reverse Genetics

μεταλλαξιγένεση

Φαινότυπος

FORWARD GENETICS

Γονίδιο



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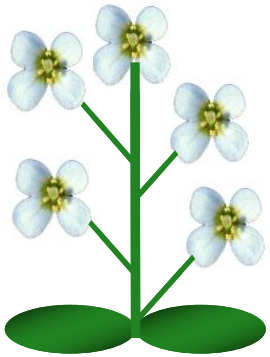


Προσεγγίσεις Μοριακή Γενετική Φυτών

συμπληρωματικότητα

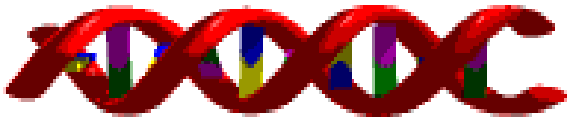
Φαινότυπος

REVERSE GENETICS

«Αντίστροφη γενετική» (reverse genetics)...

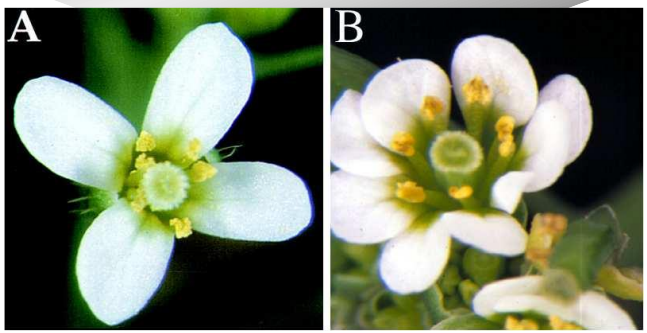
...from gene to mutant phenotype, to function

gene



find or generate a knockout mutant in YFG

analyze the mutant to see if there is any defect



correlate the defect with biological processes

further characterization of gene function

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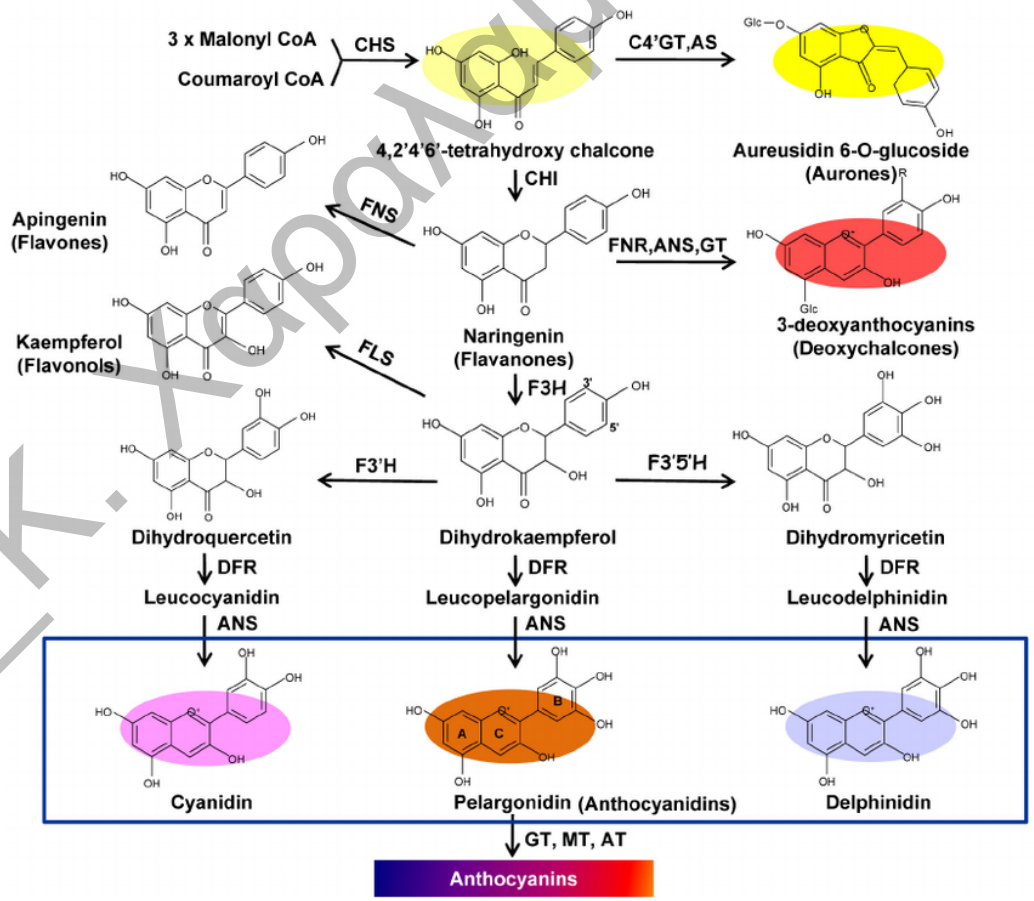
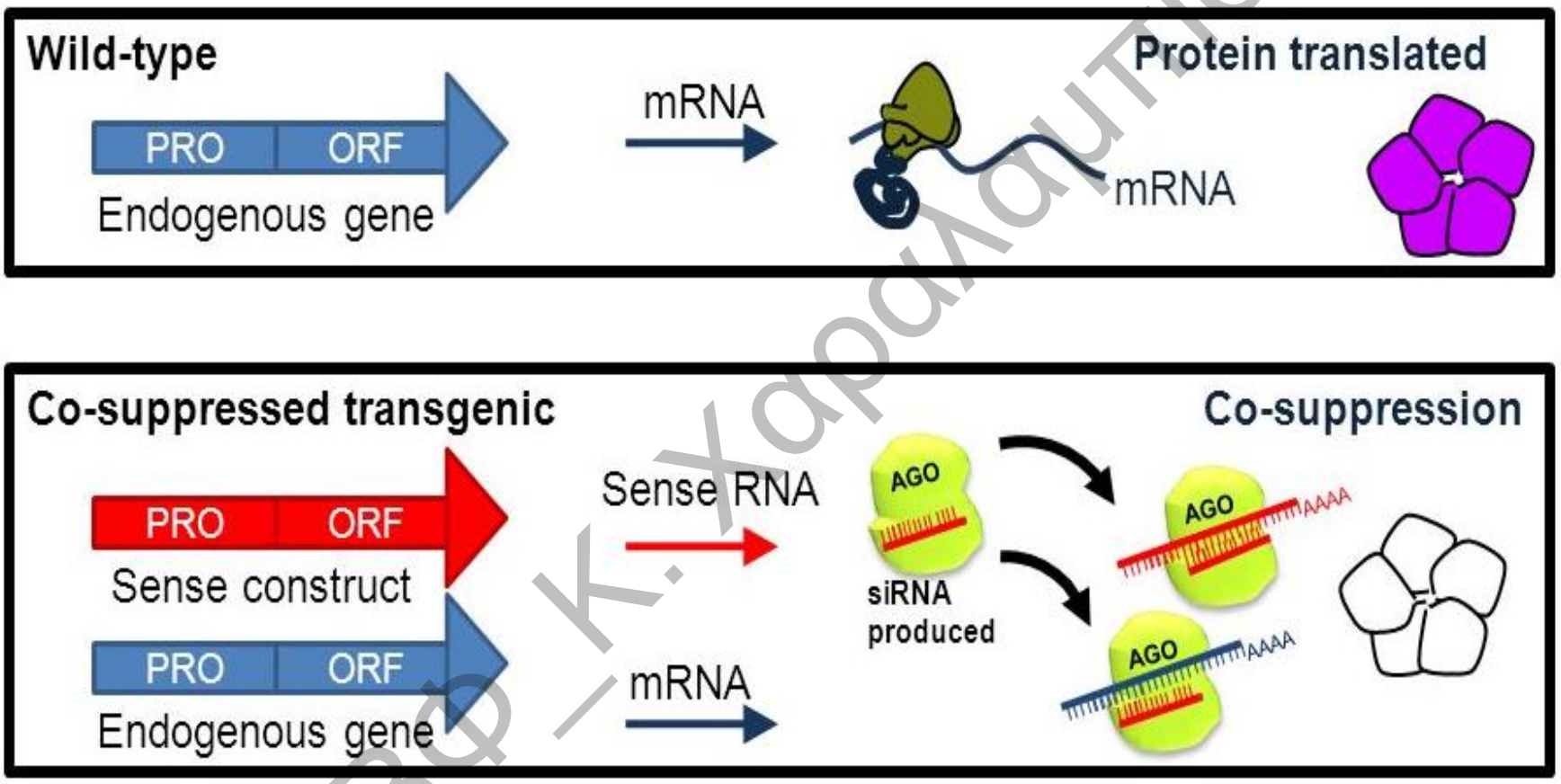
Gene silencing – PTGS in Petunia στις αρχές τις δεκαετίας του 1990

Υπερέκφραση του γονιδίου CHS που συμμετέχει στην βιοσύνθεση της χρωστικής των πετάλων οδηγεί στην ανάπτυξη λευκών ανθέων.



Co-suppression is a consequence of siRNA production



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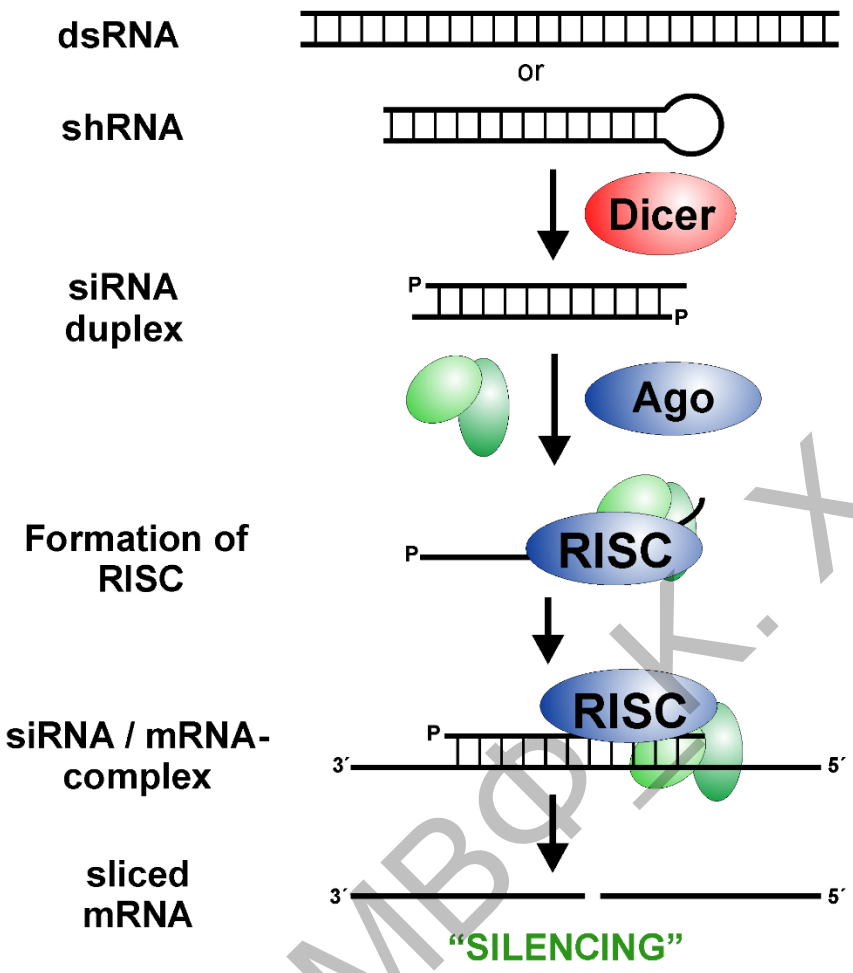
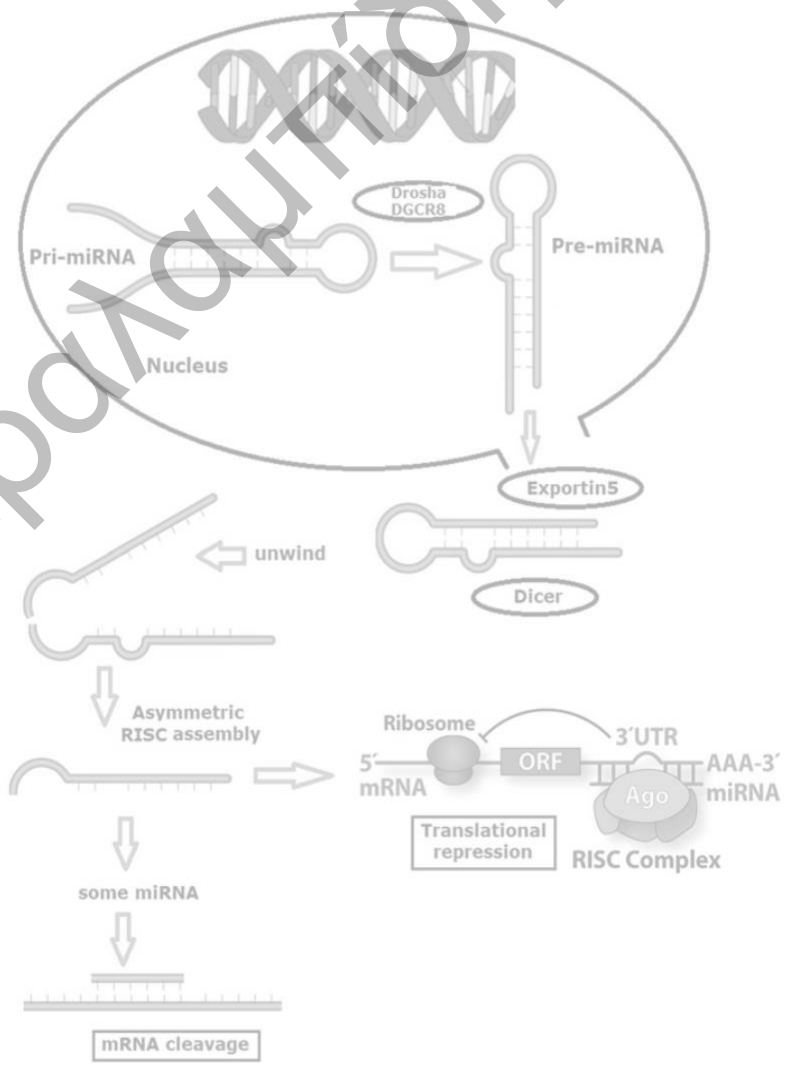
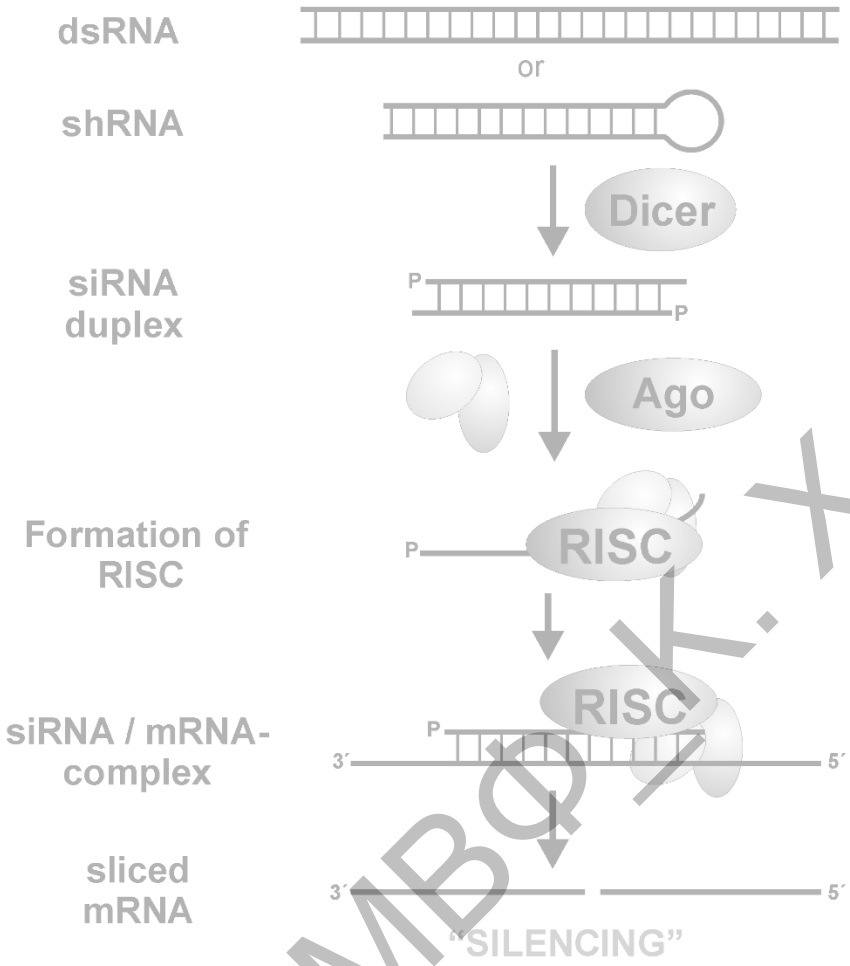


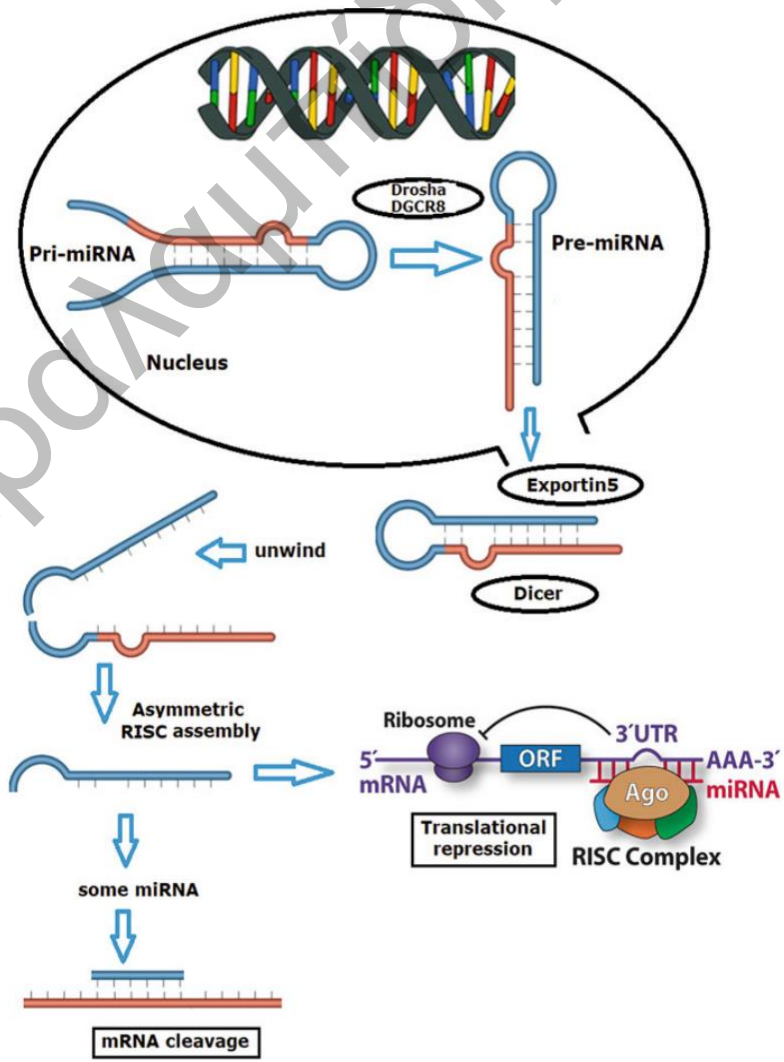


Gene silencing – PTGS

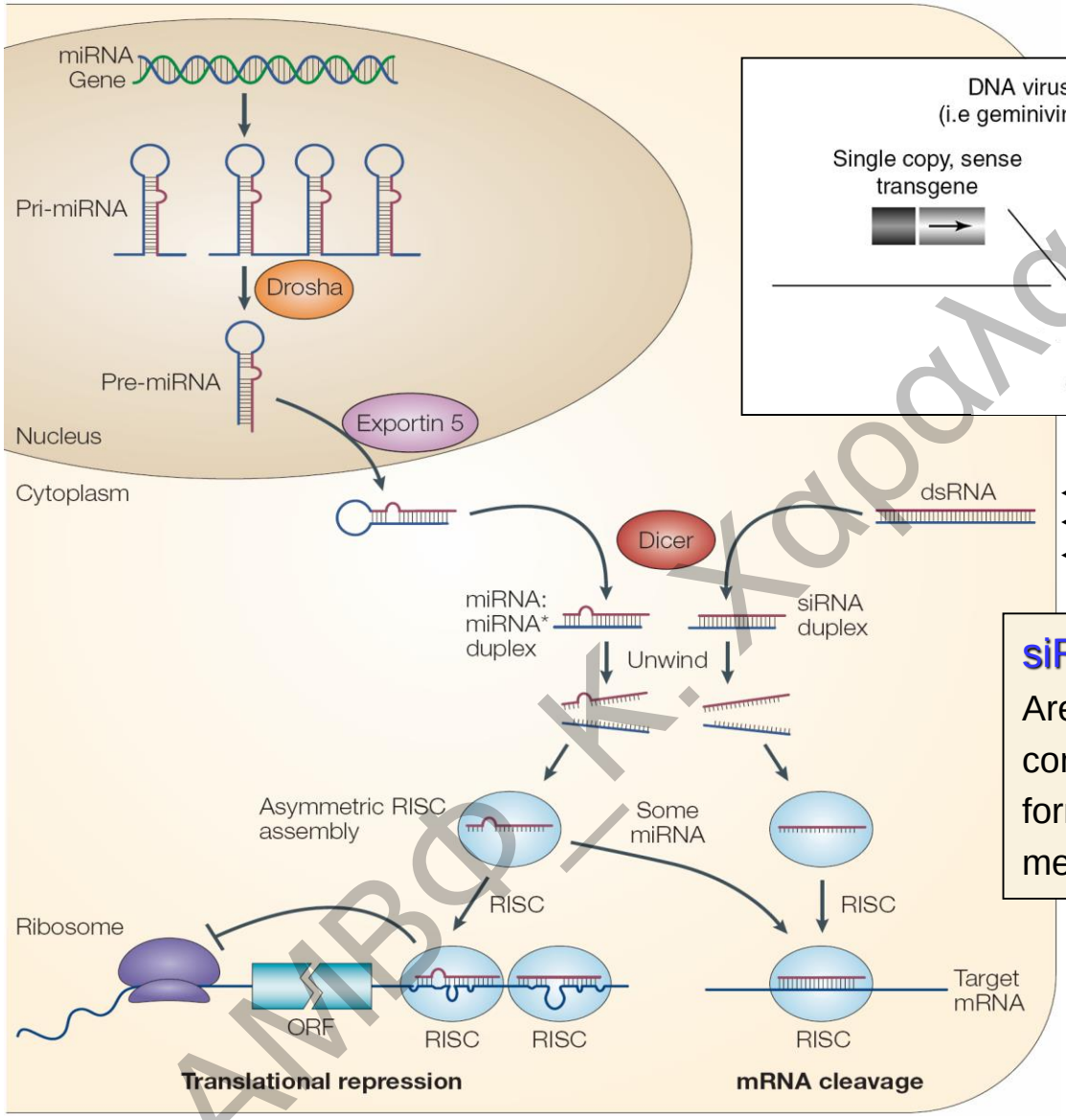
- Αποτελεί ένα είδος "αμυντικού μηχανισμού" των ευκαρυωτών
- Βασίζεται στην αλληλέπιδραση μεταξύ μορίων νουκλεϊνικών οξέων
- Προστασία από ιούς
- Ρύθμιση της γονιδιακής έκφρασης

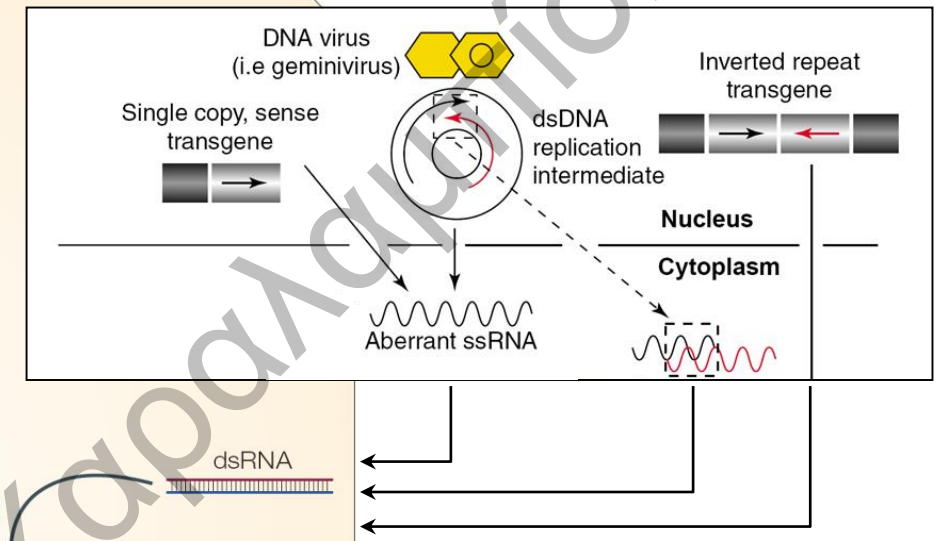
Gene regulation is a consequence of miRNA production





miRNA vs. siRNA



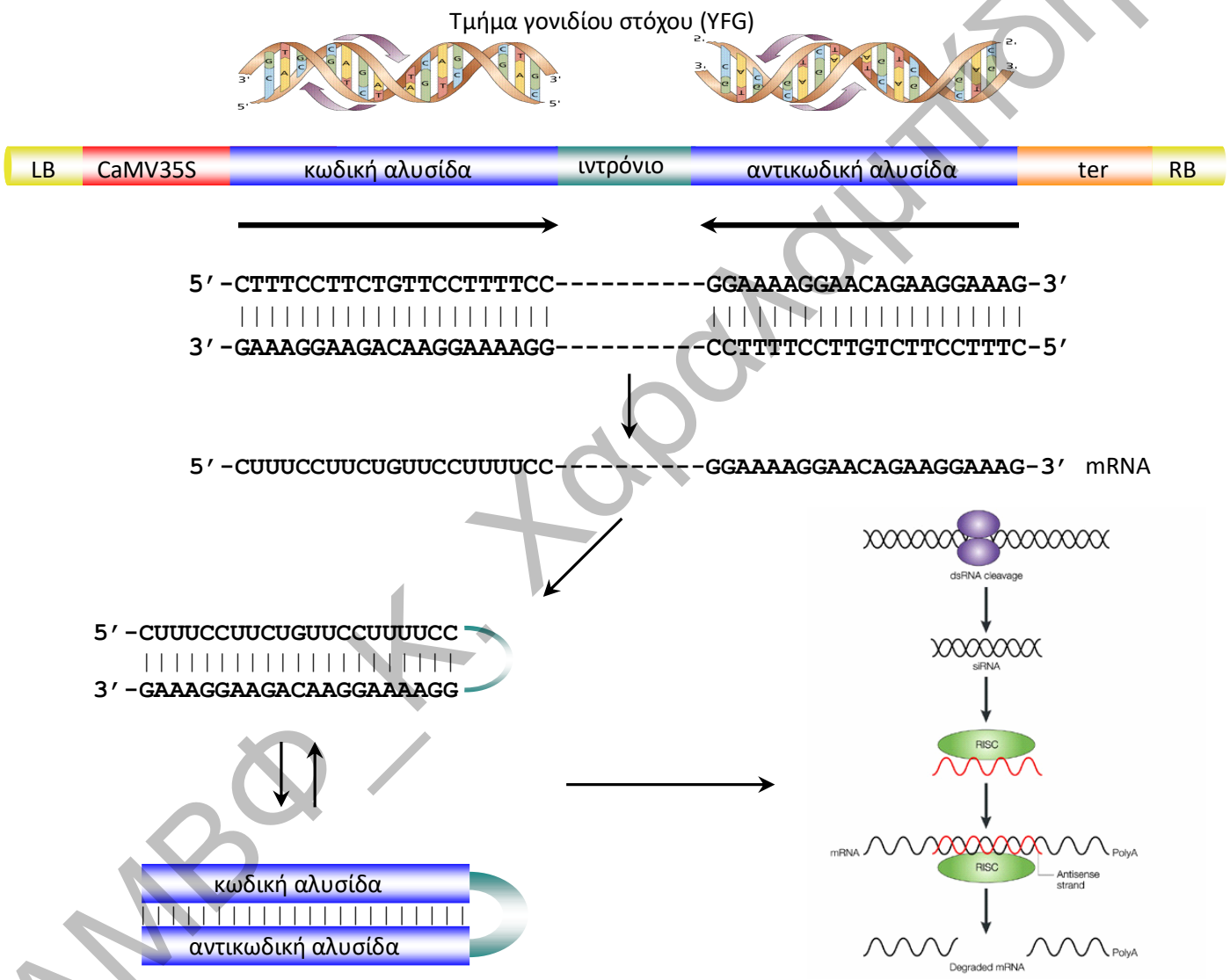


siRNAs

Are generated by aberrant, partial or completely overlapping ssRNAs. The formation of such dsRNAs triggers the mechanism of RNAi (PTGS)

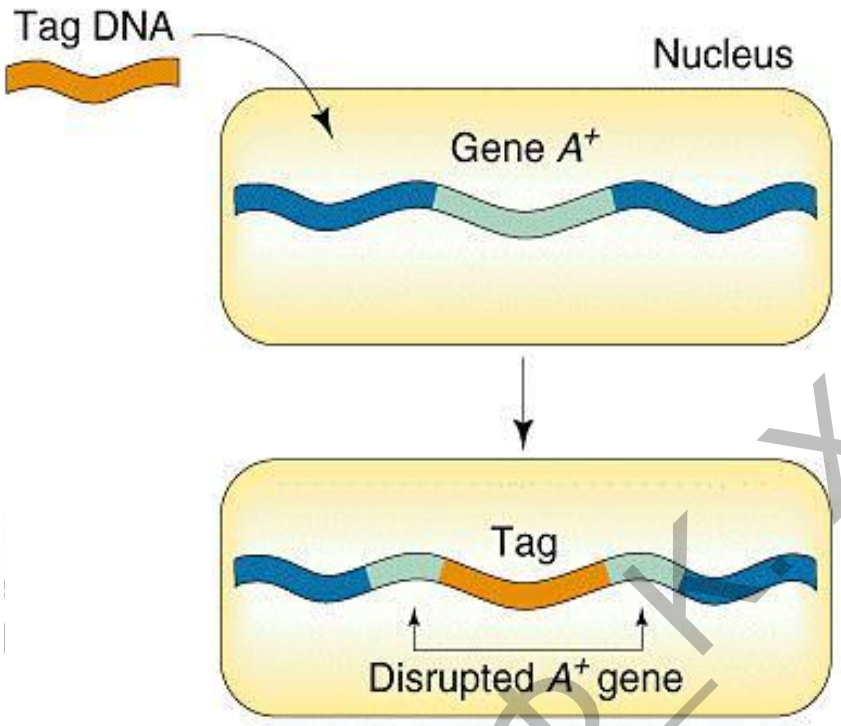
microRNA

Gene function studies through PTGS

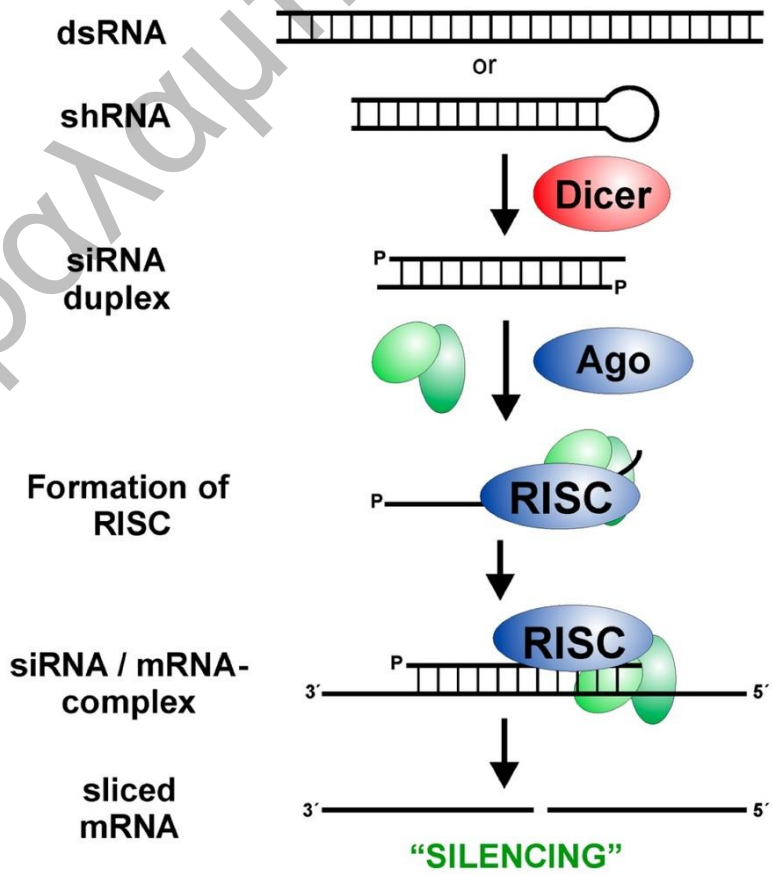


«Αντίστροφη γενετική» (reverse genetics)…

…from gene to mutant phenotype, to function

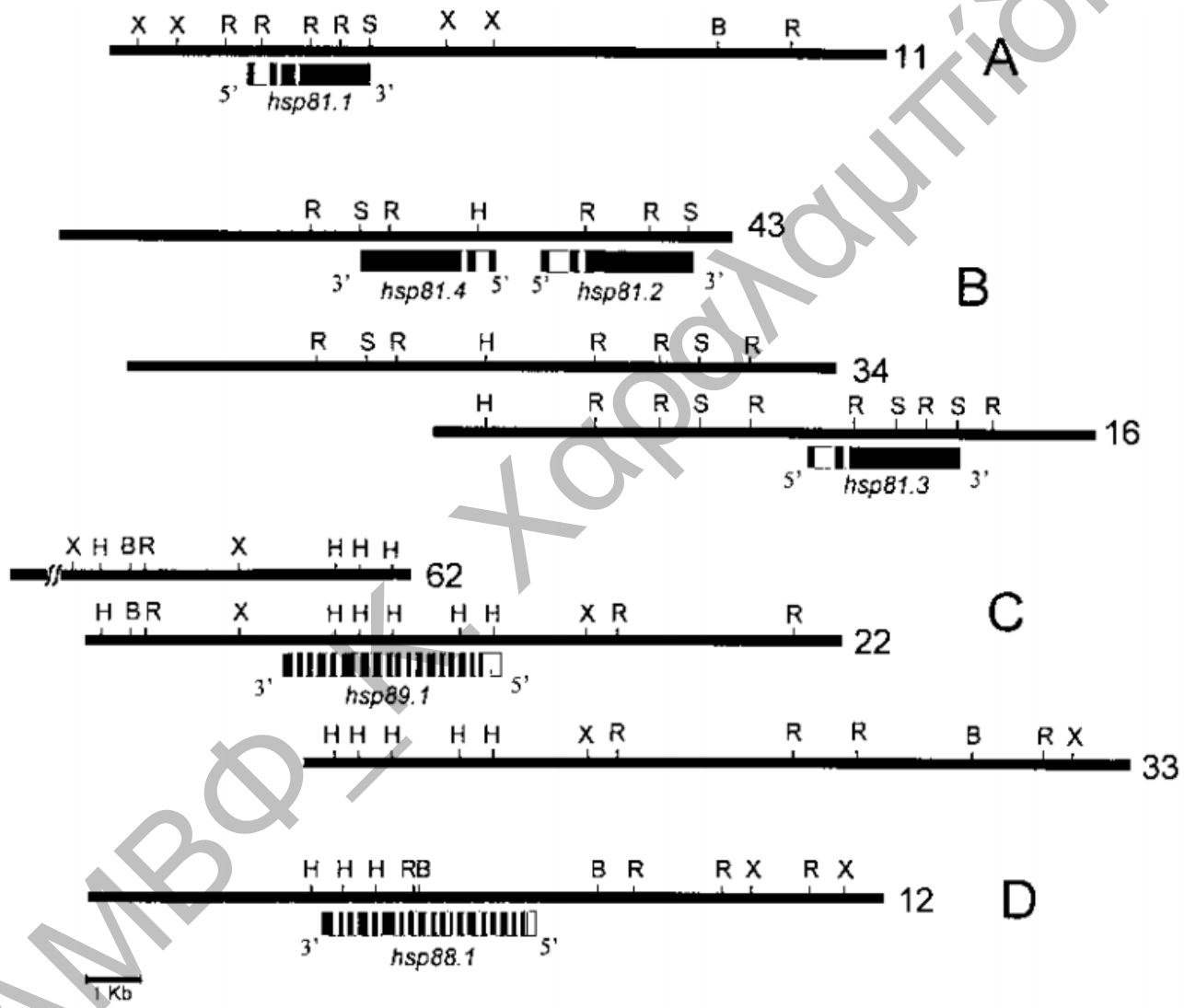


or

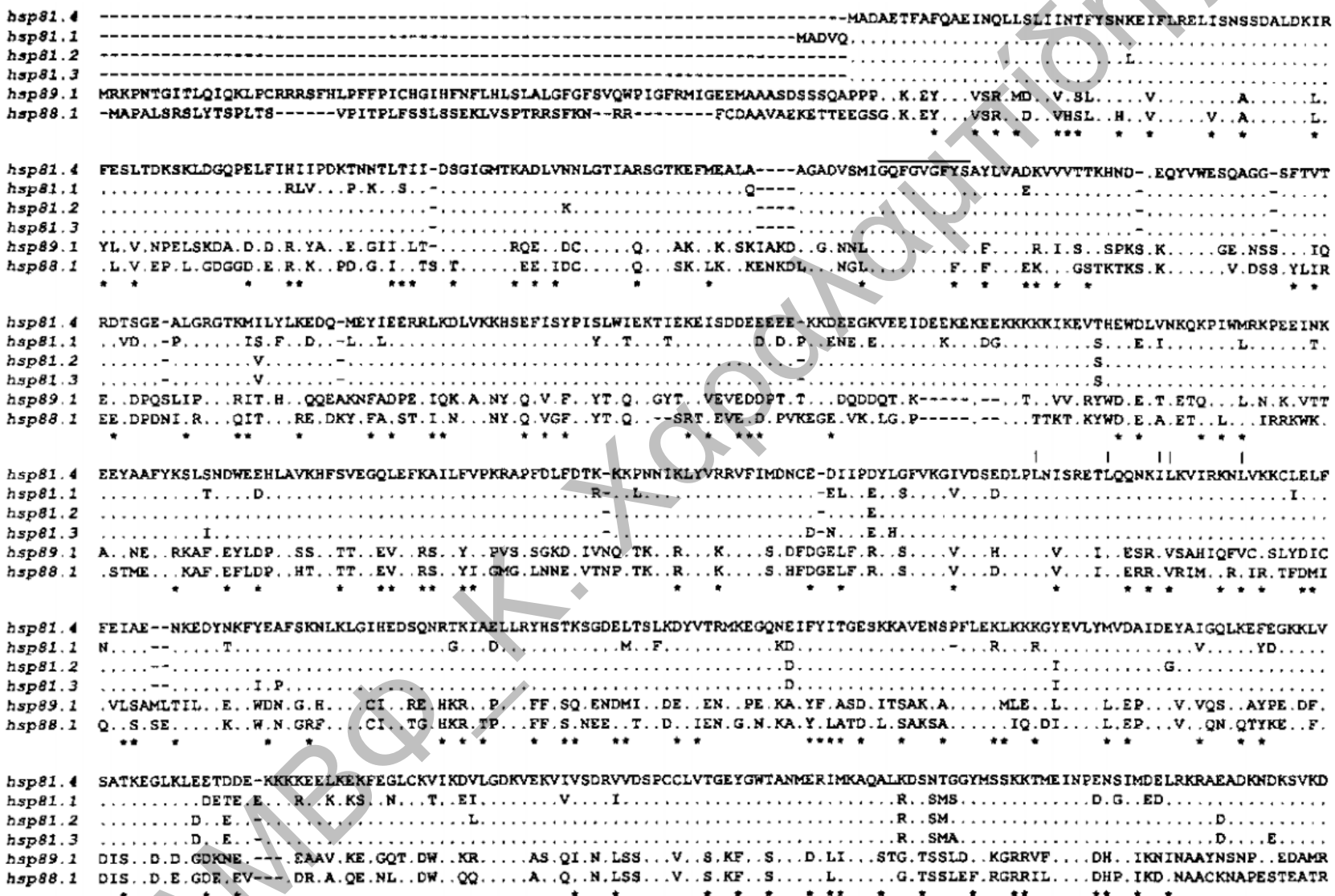


…cannot be applied if phenotype is lethal

Silencing of all gene family members or a single member…



Silencing of all gene family members or a single member…



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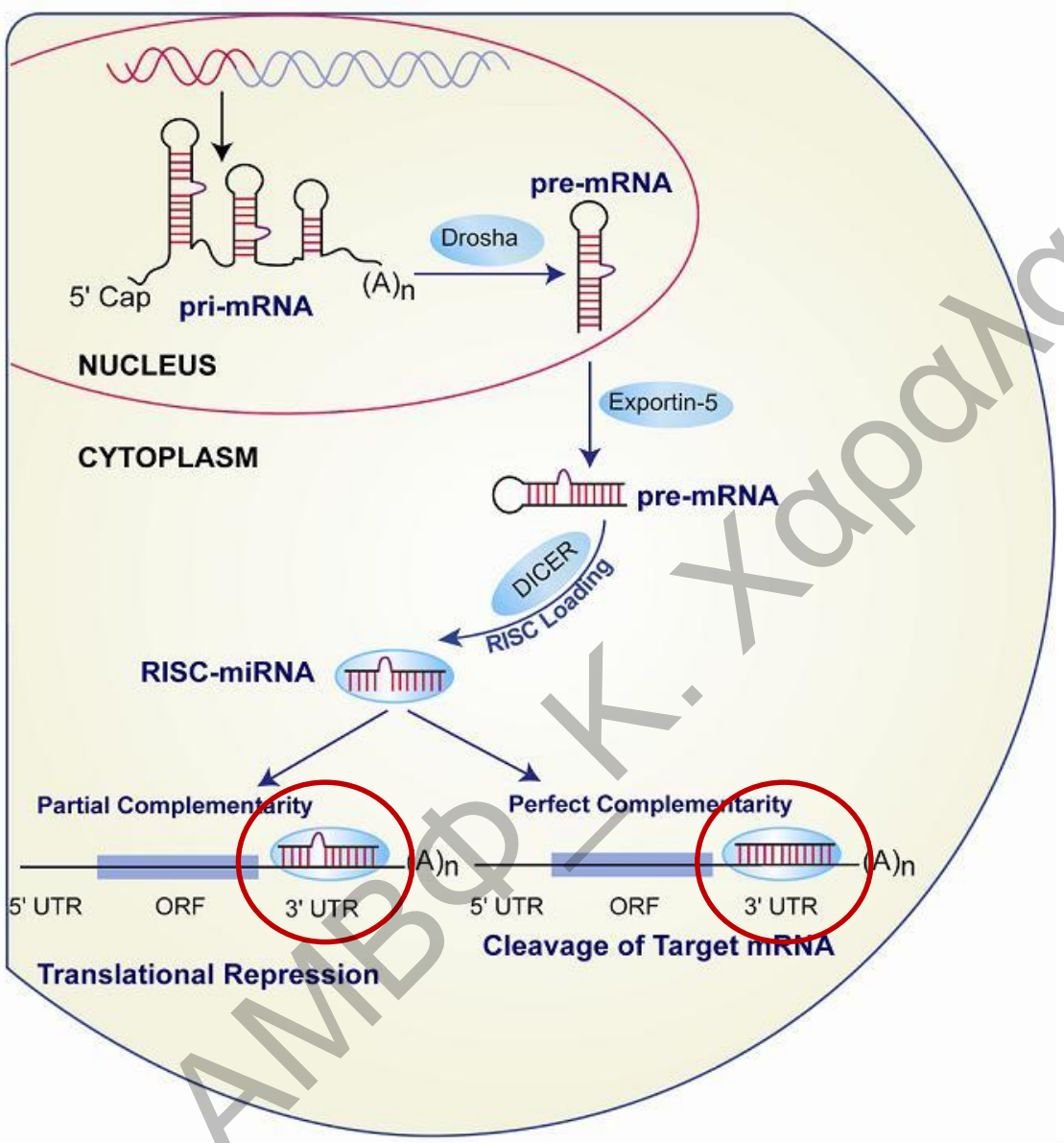
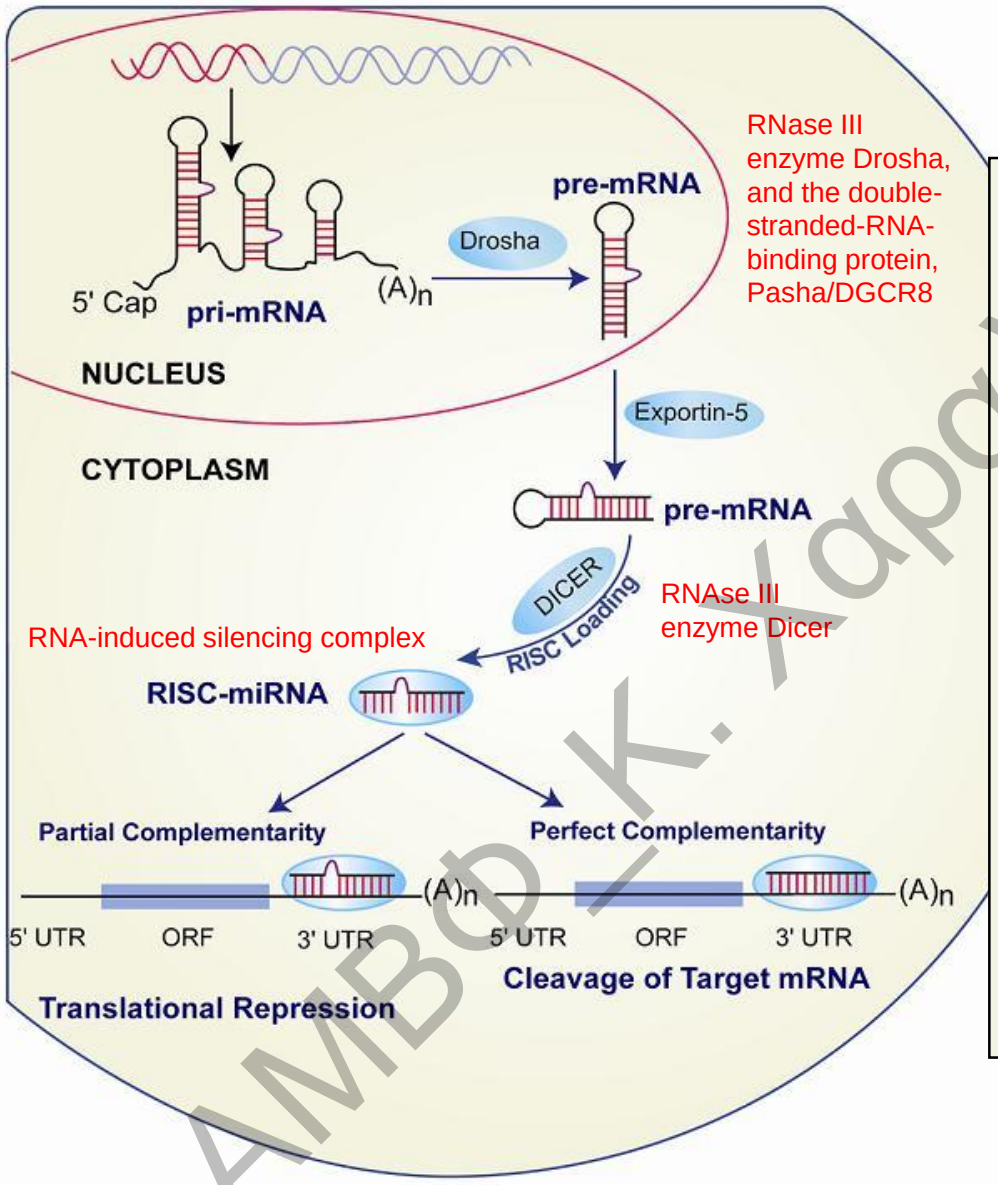


RNAi pros and cons

- Silencing rate variations
- Overcome lethality
- Can target gene families
- Can be designed to target specific gene family members

miRNA and gene regulation

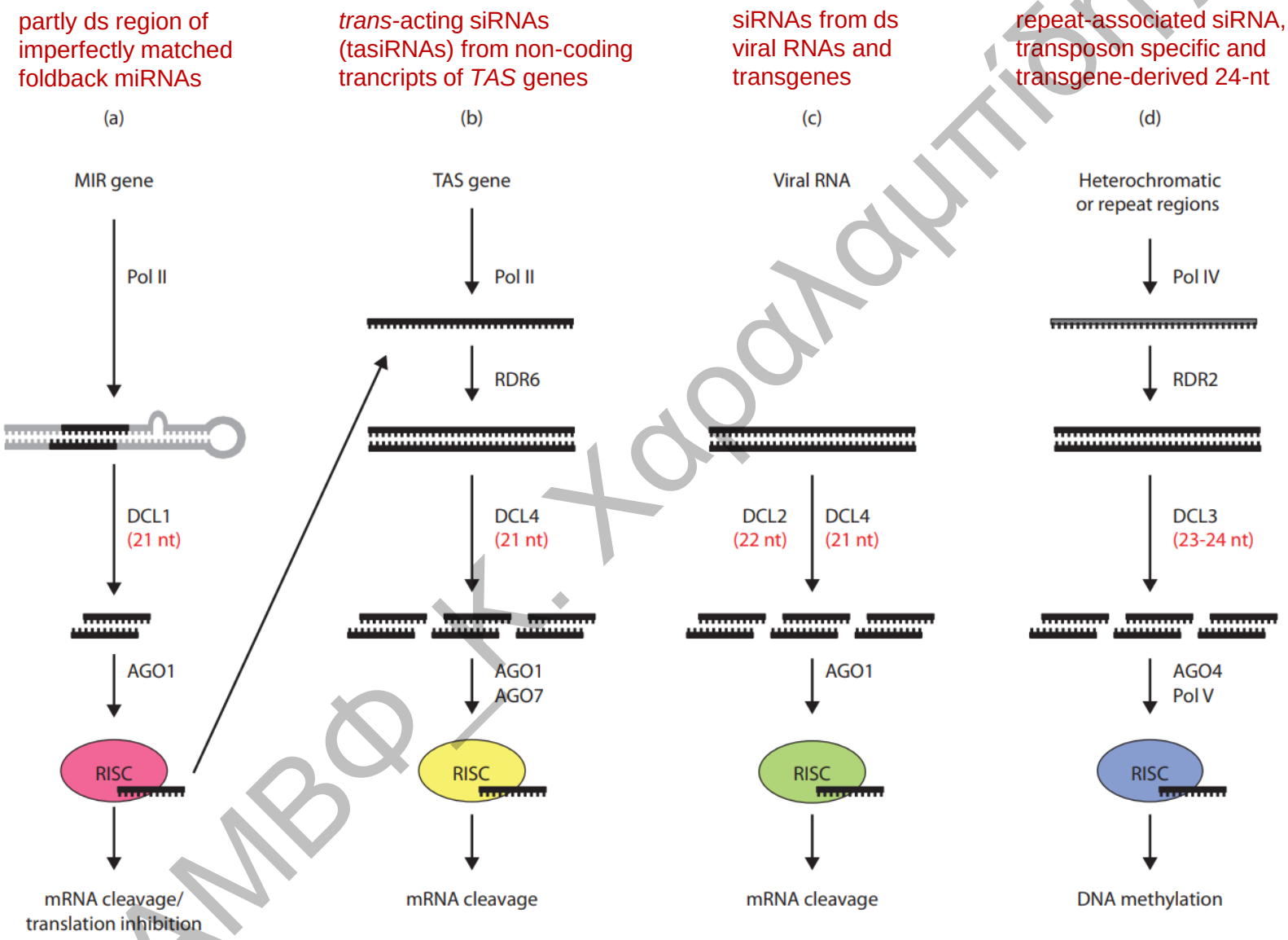


miRNAs

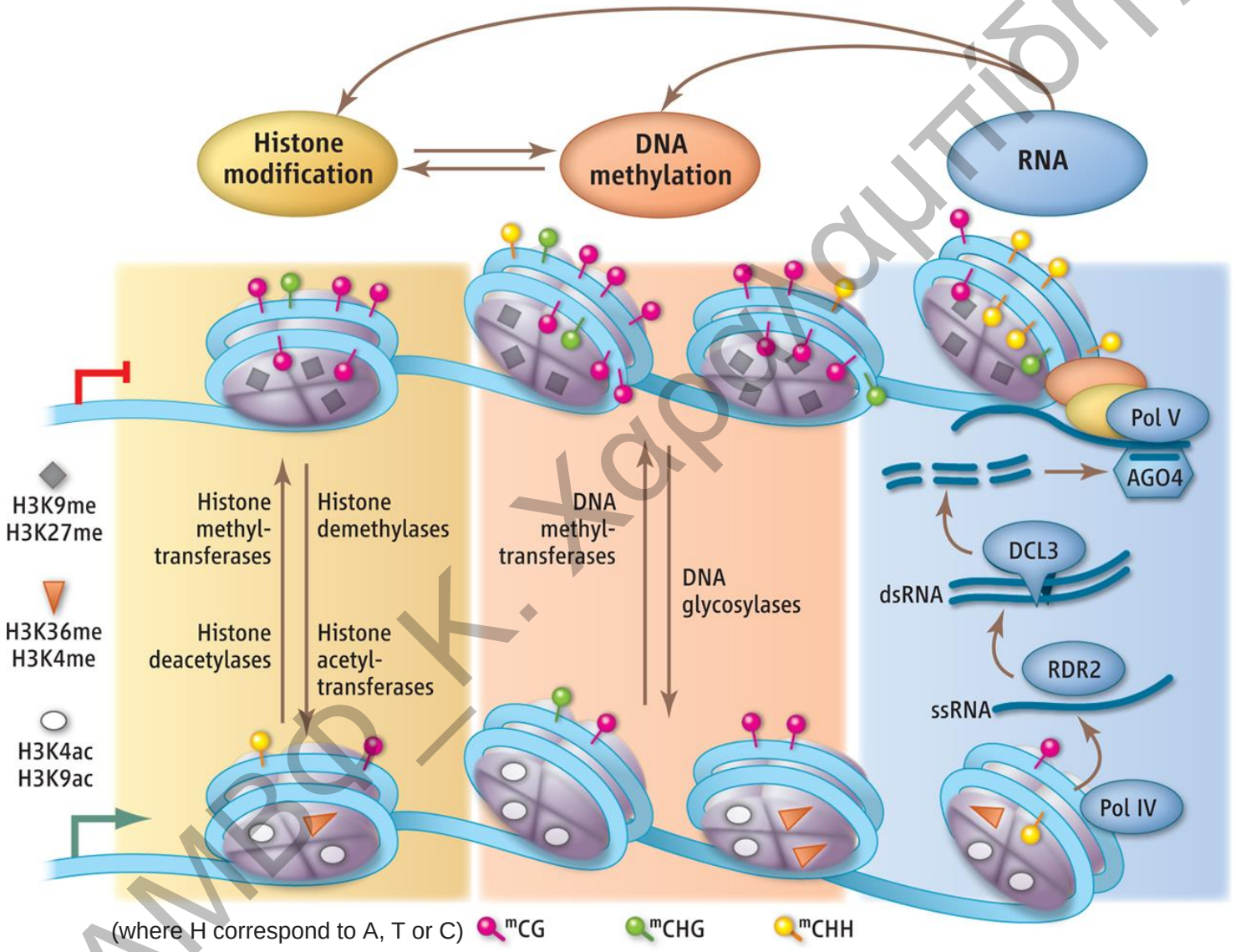
Regulate complex and diverse pathways governing aspects of

- Cell proliferation
- Cell death
- Early development
- Apoptosis
- Cell differentiation
- Fat metabolism
- Flower development
- Sex determiner *Bombyx mori*

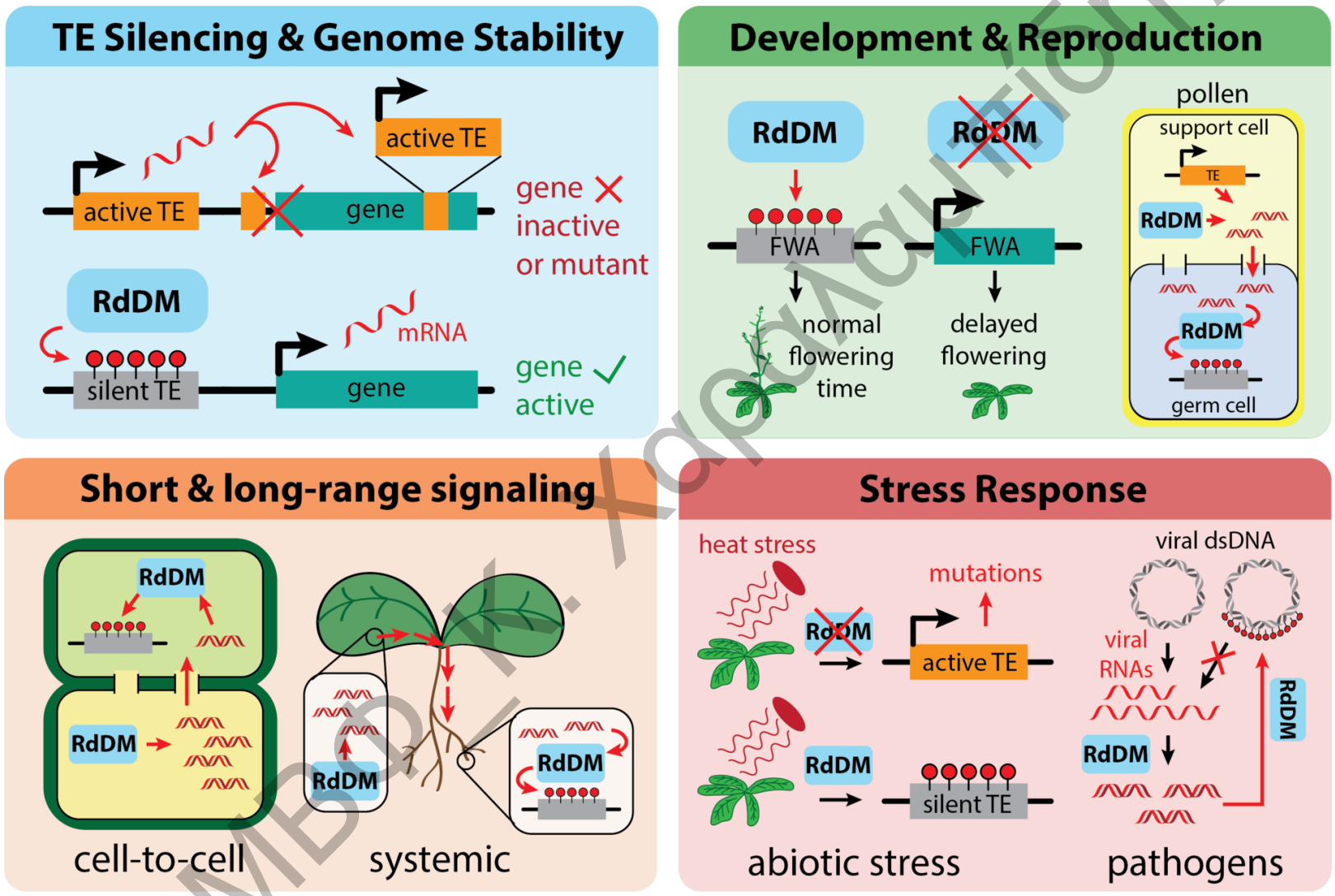
Silencing pathways in plants



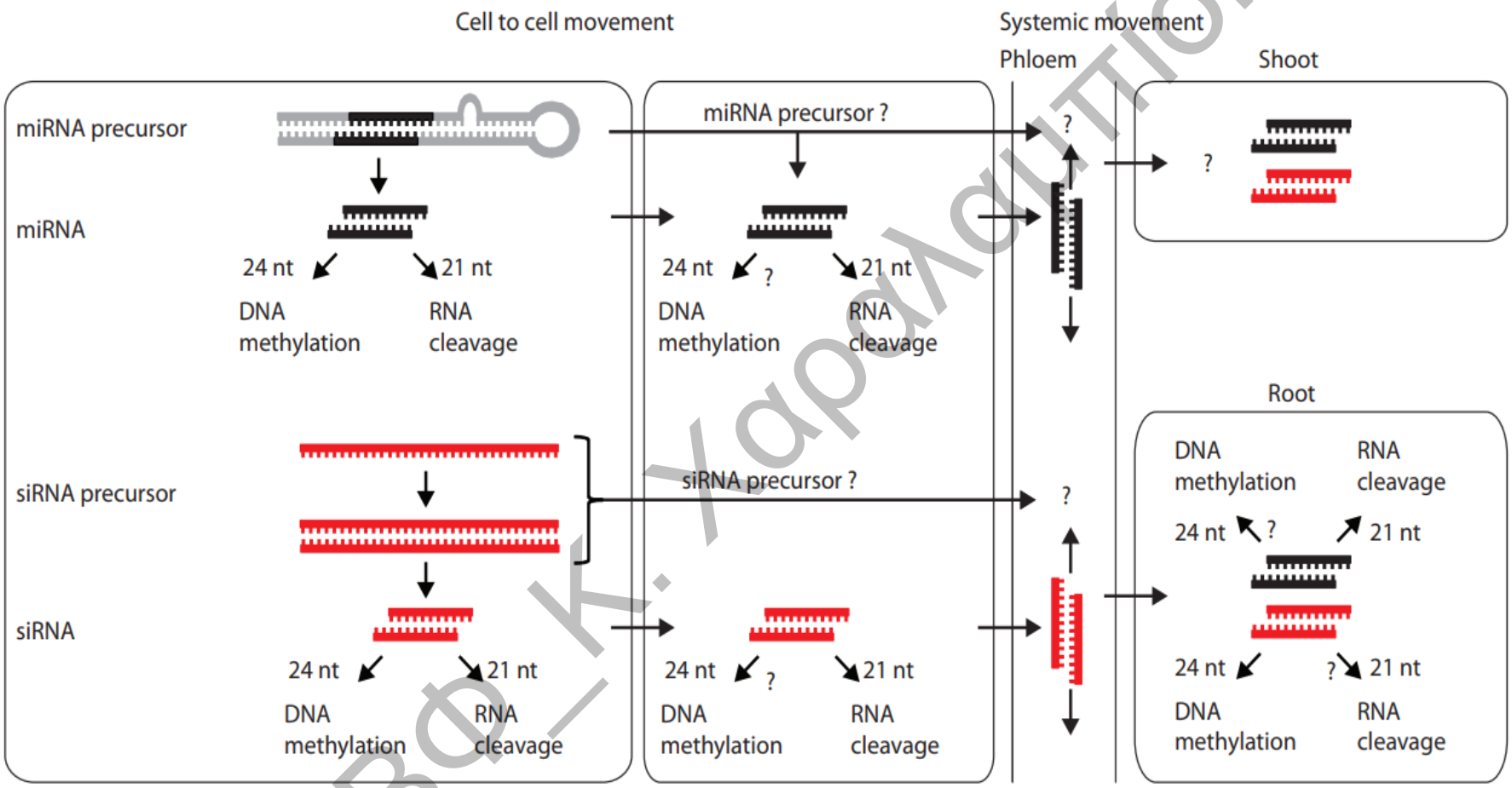
Silencing of genome loci by RNA vs. DNA and Histone modifications



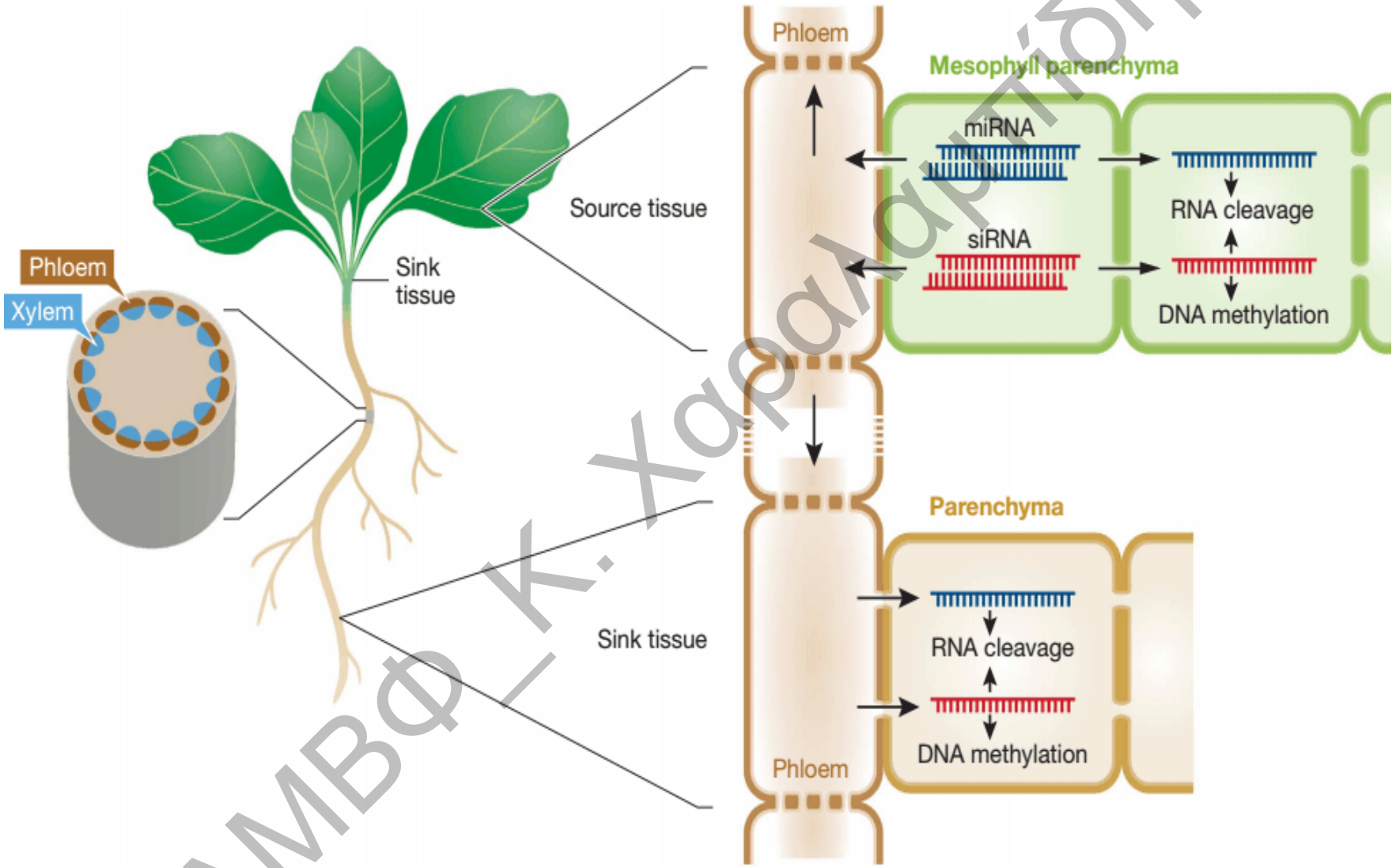
RNA-directed DNA methylation - RdDM pathway is unique to plants



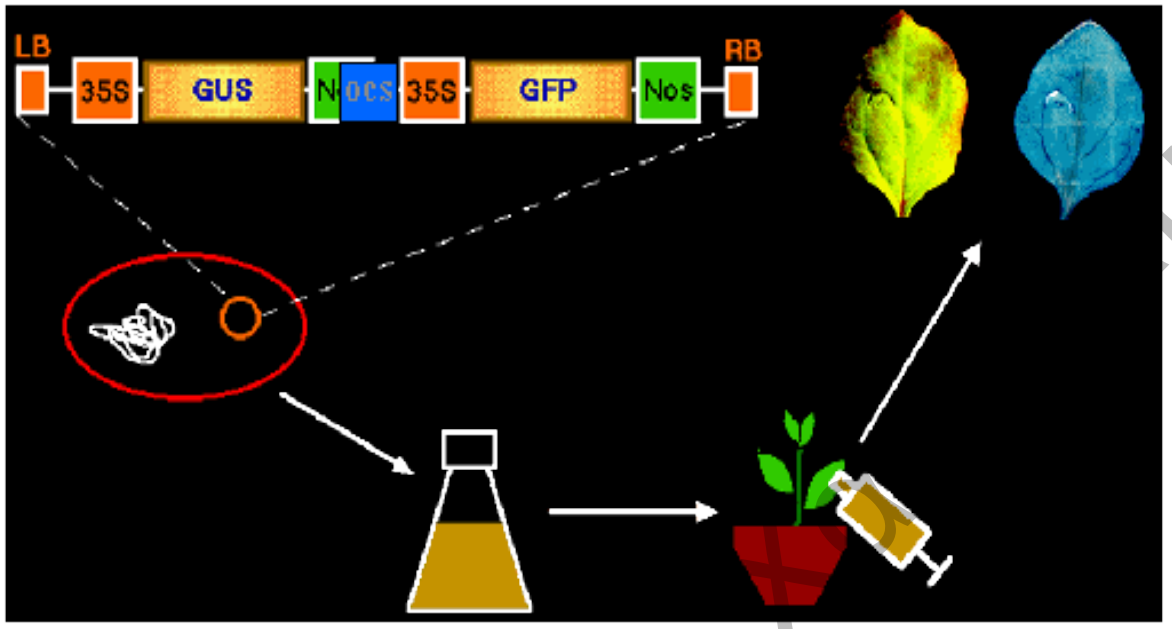
Mobile silencing RNAs and their activity

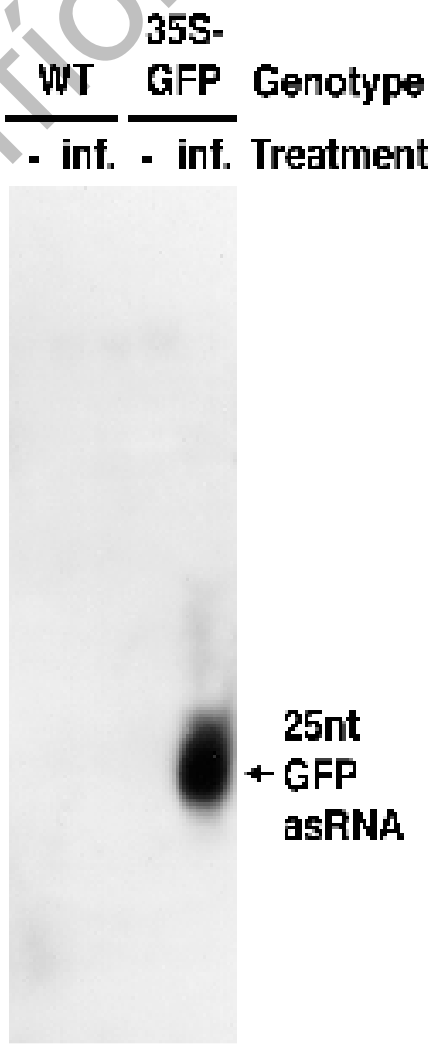


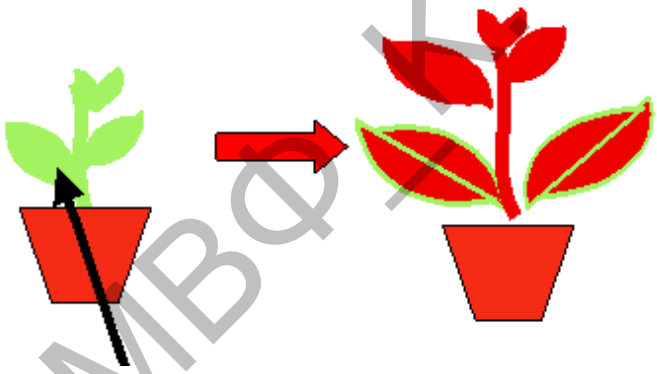
Systemic Gene Silencing



Systemic Gene Silencing by Agroinfiltration

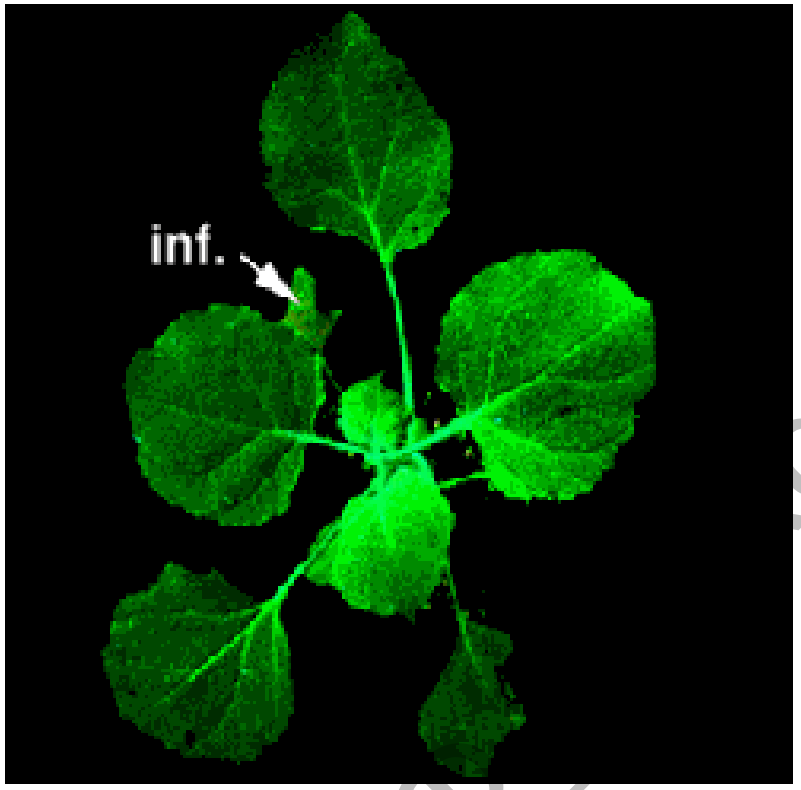


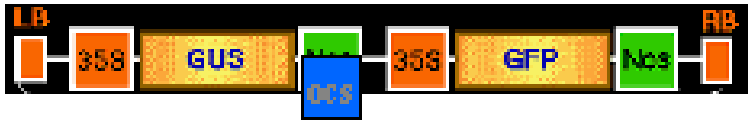




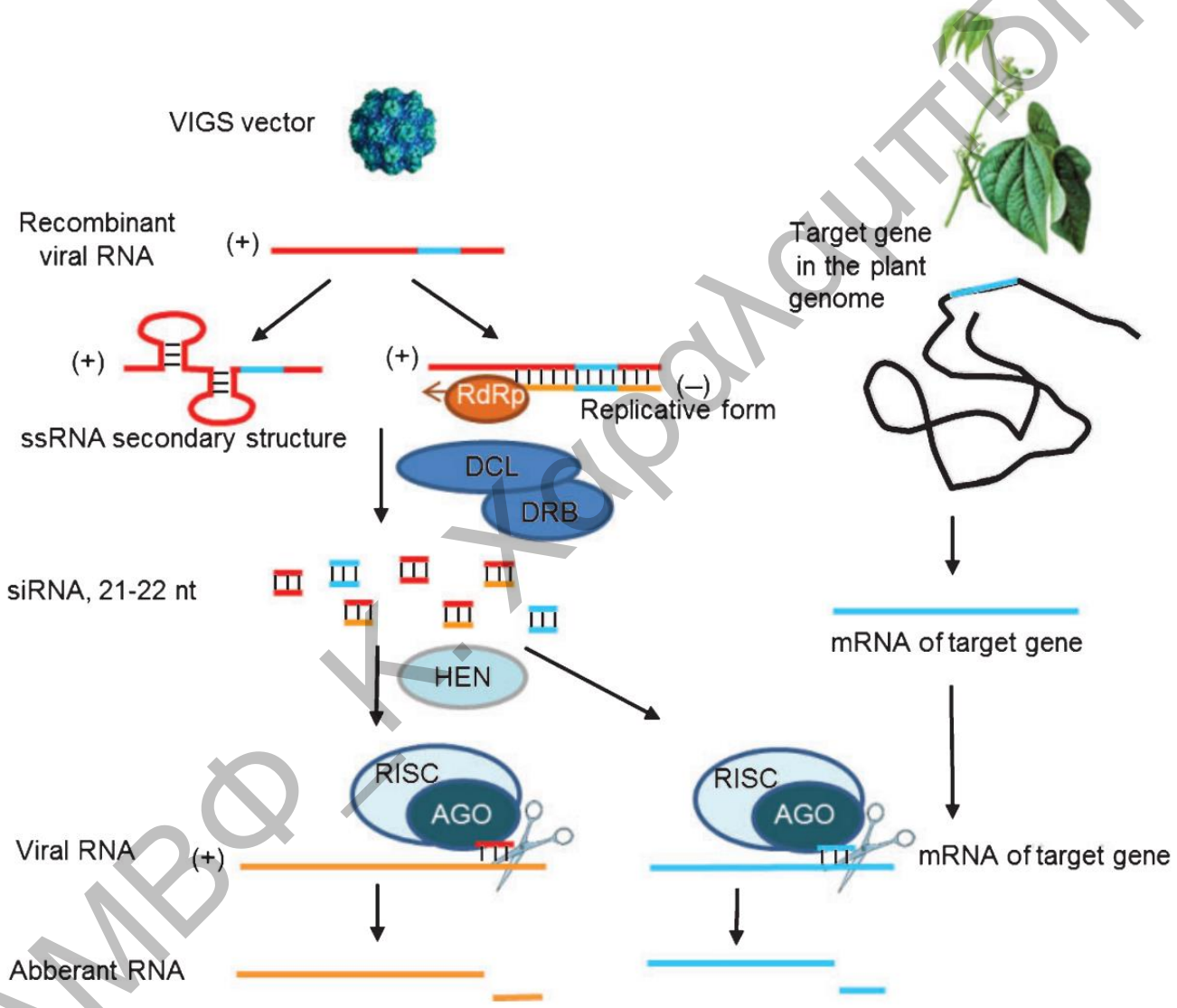
Agroinfiltrate with 35S-GFP

Systemic Gene Silencing

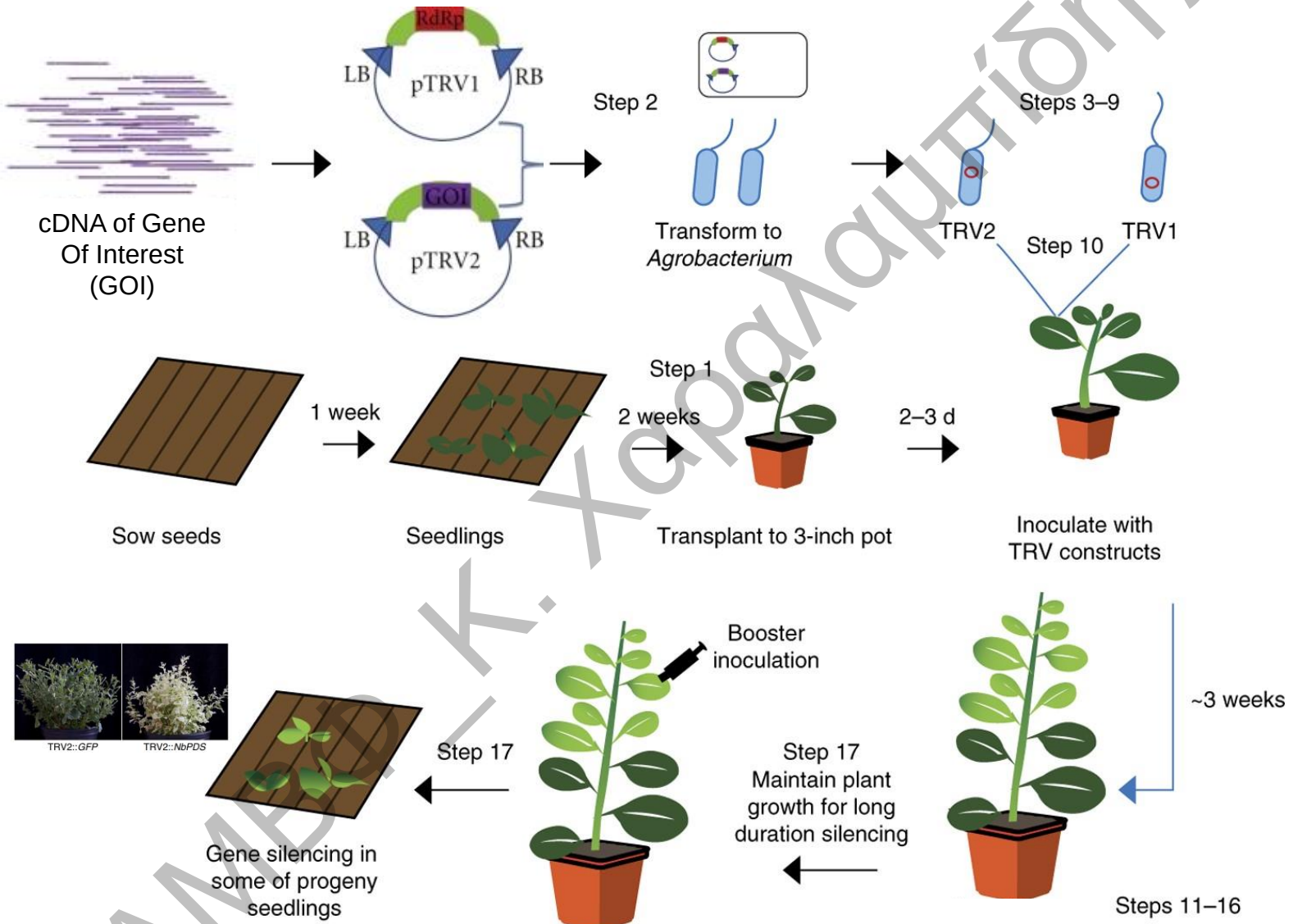




Molecular mechanism of Virus Induced Gene Silencing (VIGS)

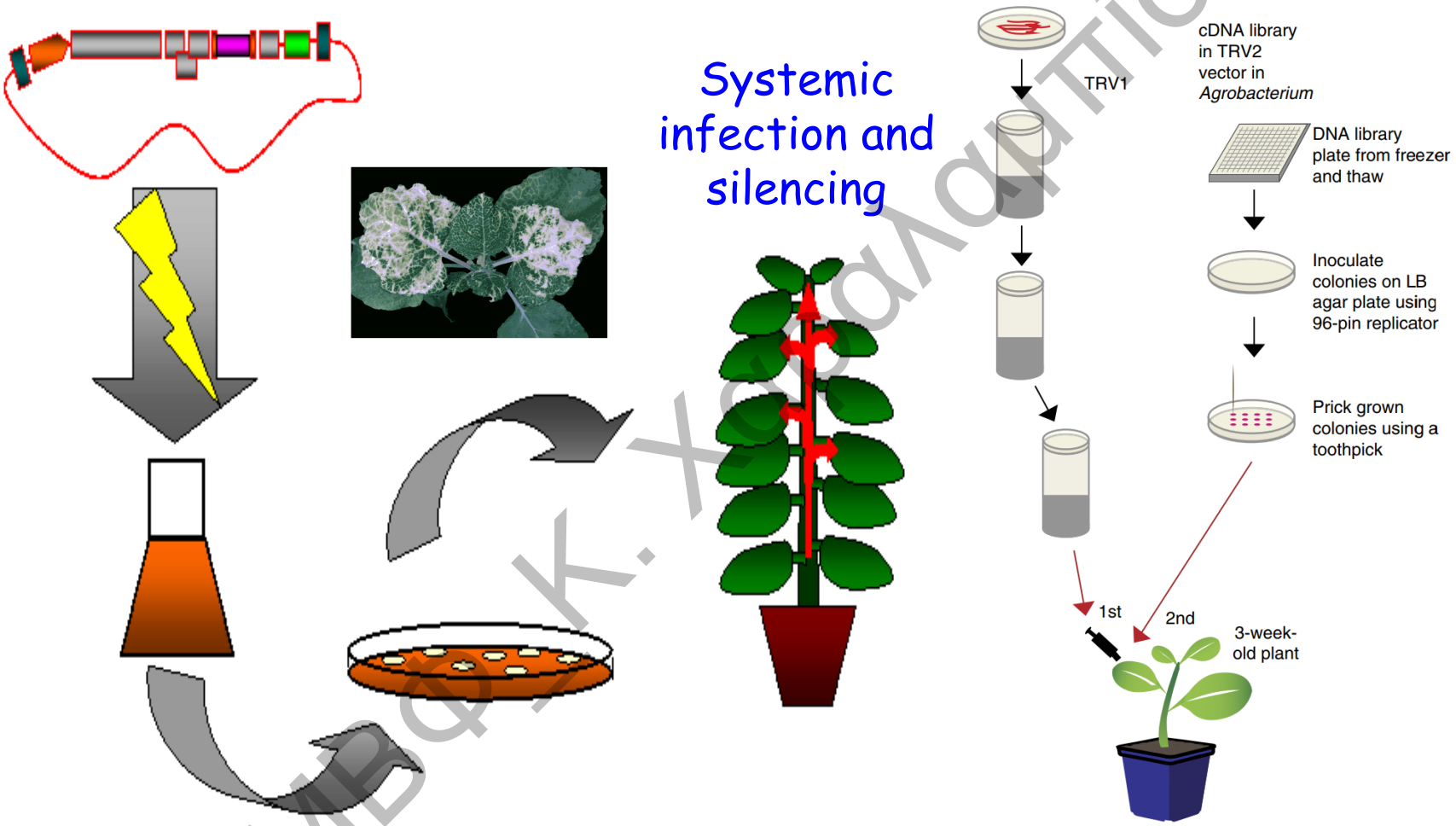


Systemic Gene Silencing by Virus Induced Gene Silencing (VIGS)



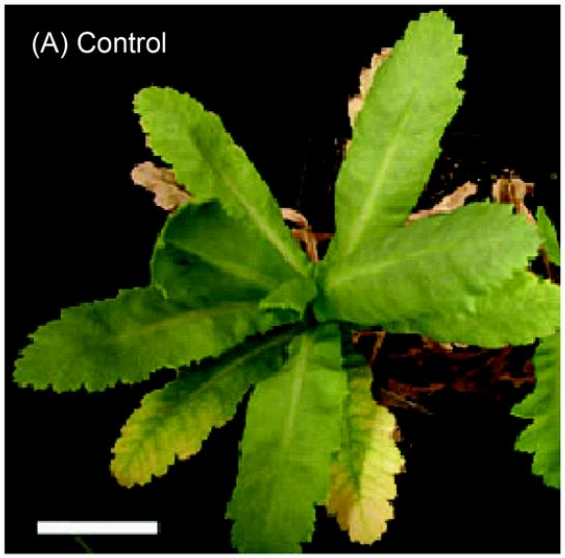
VIGS in Fast-Forward/Reverse Genetics

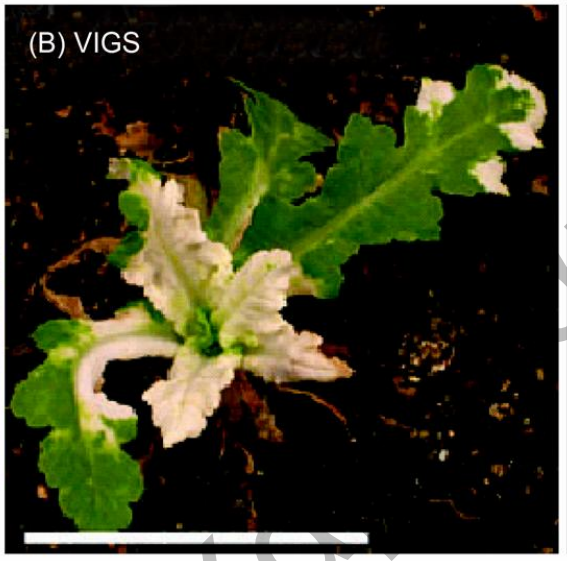
Forward Genetics since it is a kind of "mutagenesis"

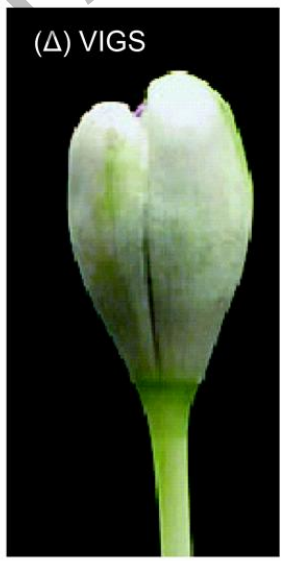


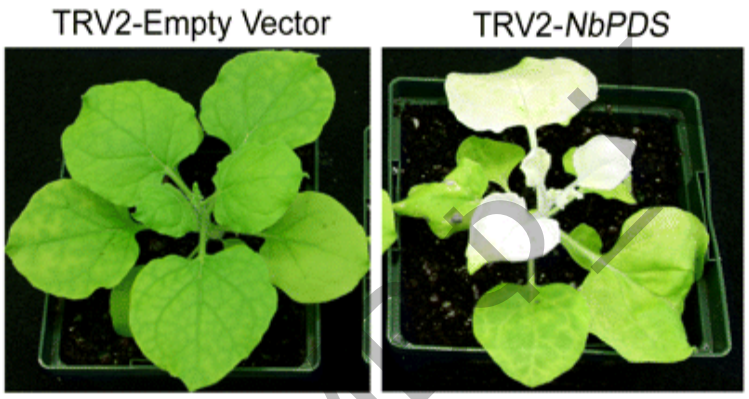
Reverse Genetics since the gene is somehow "known" from the Agrobacterium colony of the cDNA library on the plate.

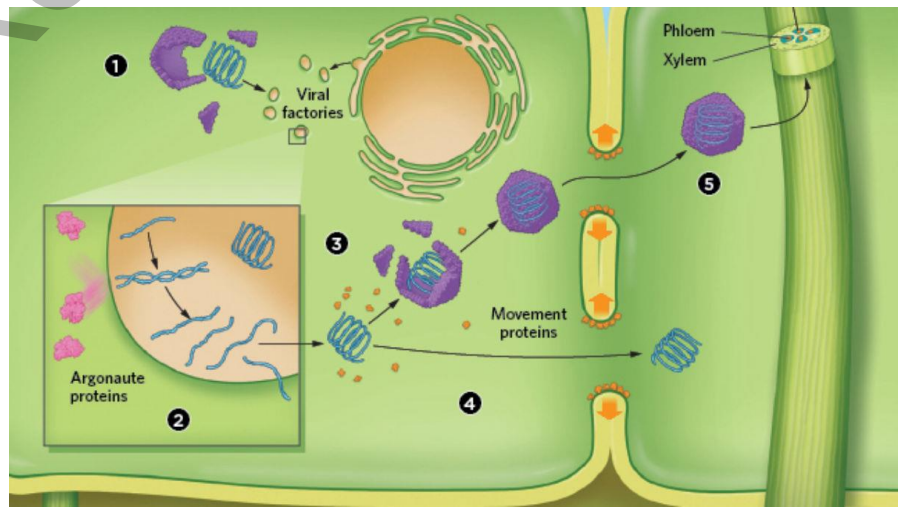
Phytoene desaturase VIGS in Forward Genetics of *P. somniferum*











Απόκτηση μεταλλαγμένων σειρών *Arabidopsis thaliana*

1. Λήψη ήδη δημιουργηθέντων μεταλλαγμένων σειρών που είναι καταχωρημένες σε τράπεζες γενετικού υλικού και σπερμάτων

2. Δημιουργία μεταλλαγμένων σειρών με τη μέθοδο της «προς τα εμπρός γενετικής» ή «προωθητικής γενετικής» (forward genetics)

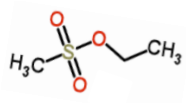
3. Δημιουργία μεταλλαγμένων σειρών με τη μέθοδο της «αντίστροφης γενετικής» (reverse genetics)

   - Στοχευμένη με RNAi «αντίστροφη γενετική» Δημιουργία μεταλλαγμένων σειρών με τη μέθοδο της μετα-μεταγραφικής γονιδιακής σίγησης (RNA gene silencing)

   - «Στόχευση επαγόμενων μεταλλάξεων σημείου στο γονιδίωμα» (Targeting Induced Local Lesions IN Genomes - TILLING)

   - Genome editing methods with nucleases – MN, ZGNS, TALEN, CRISPR) (Γονιδιωματική παρέμβαση/επεξεργασία)

Forward vs. Reverse Genetics



EMS

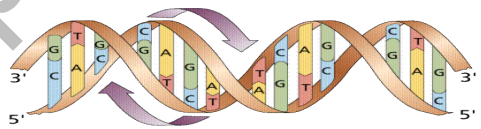
μεταλλαξιγέννεση

TILLING

Φαινότυπος

FORWARD GENETICS

Γονίδιο



TCGAGCGACATGACATGATCAGCA
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ATCGATTATATGCACGGCAGCAGA
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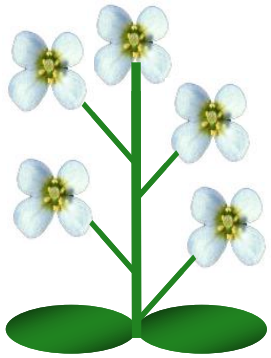


Προσεγγίσεις Μοριακή Γενετική Φυτών

TILLING

συμπληρωματικότητα

Φαινότυπος

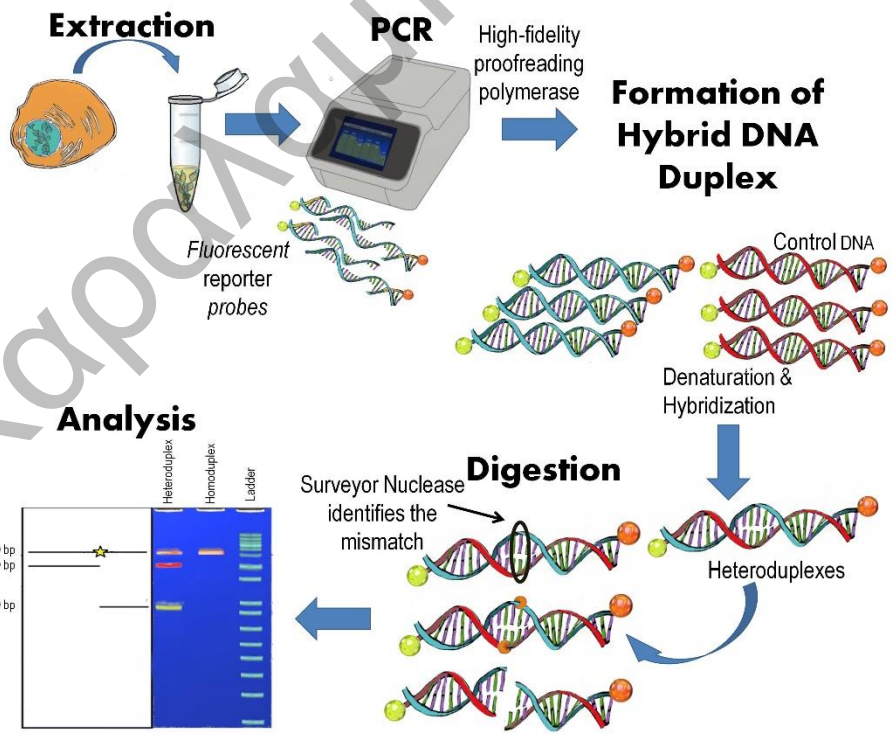
REVERSE GENETICS

TILLING (Targeting Induced Local Lesions IN Genomes)

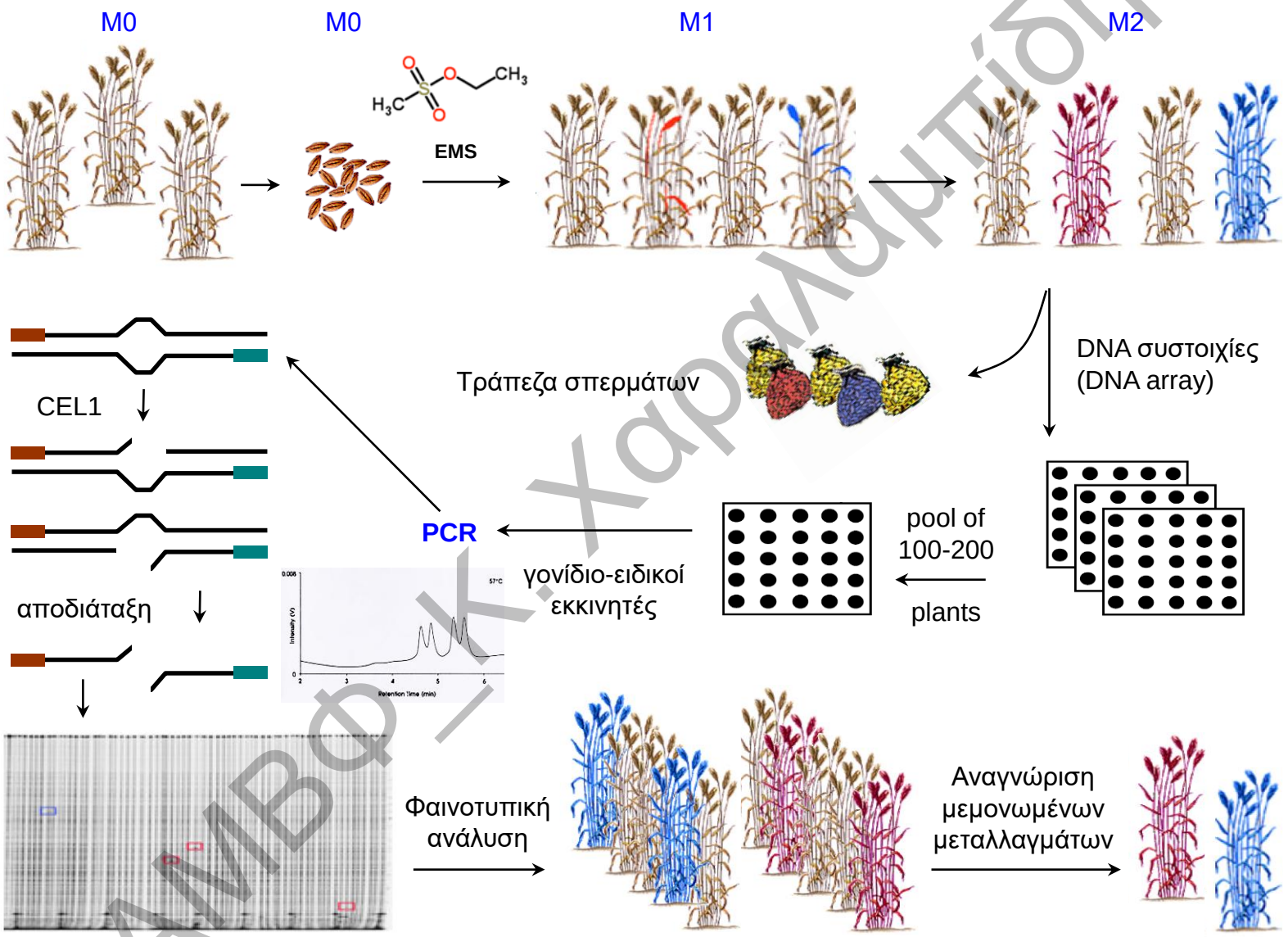
- TILLING is a general reverse genetic strategy that works with a mismatch-specific endonucleases to detect induced or natural DNA polymorphism in genes of interest.
- It combines chemical or physical mutagenesis and PCR based screening to identify mutations in one or more target genes.
- It is a non transgenic gene modification technique.

TILLING (Targeting Induced Local Lesions IN Genomes)

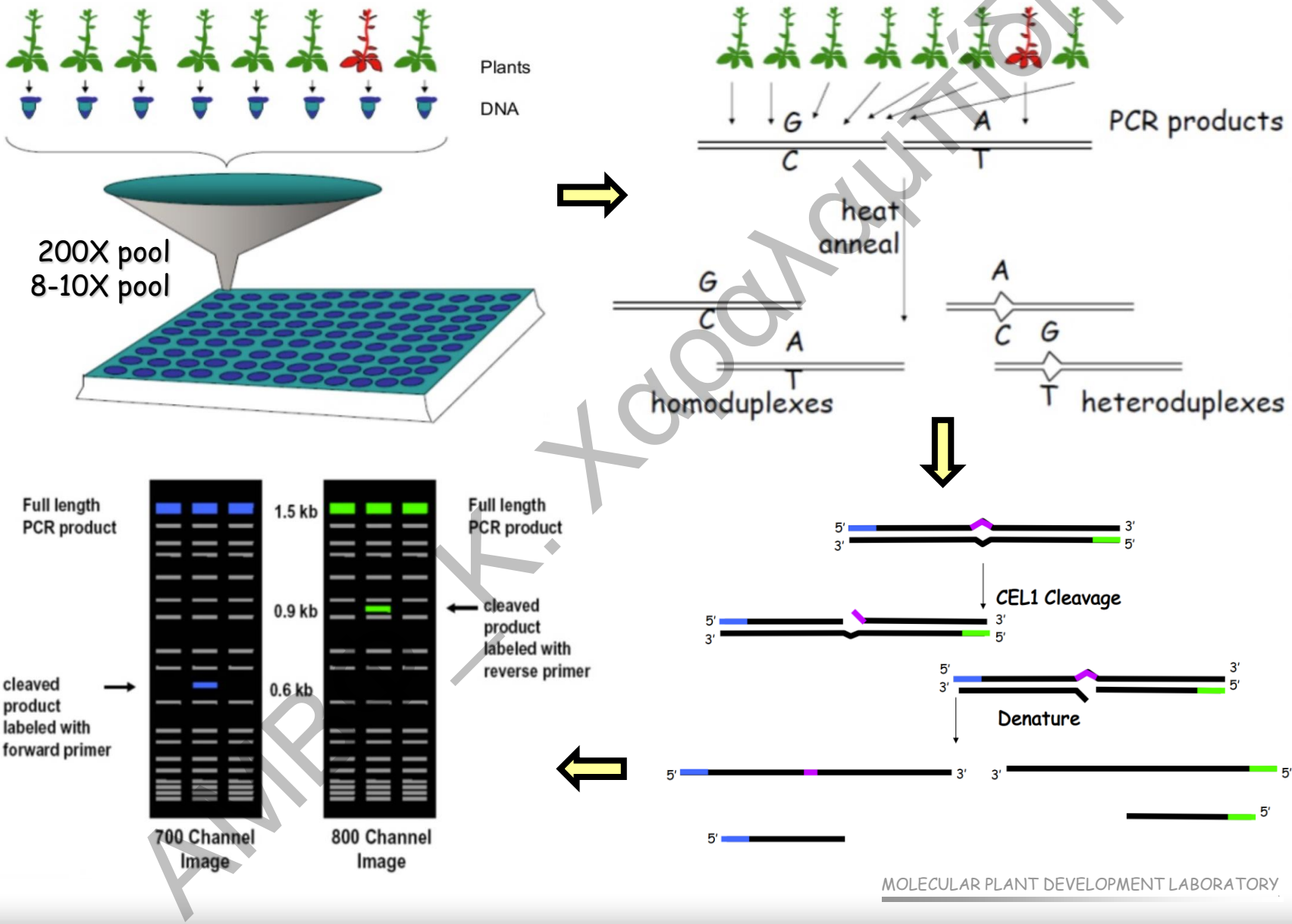
1. EMS mutagenesis.
2. Pooling of individuals (100-200) and DNA preparation.
3. PCR amplification of a region of interest with fluorescently tagged primers (e.g. blue and green).
4. Denaturation and annealing to follow formation of heteroduplexes at the site of mutation.
5. Resultant double-stranded products are digested with CEL1 or CJE (celery juice extract) nuclease, which cleaves one of the two strands at the heteroduplex mismatches.
6. Cleaved products are detected on polyacrylamide denaturing gels (Li-Cor) or by GC-MS/MS analytical methods.



TILLING (Targeting Induced Local Lesions IN Genomes)



TILLING (Targeting Induced Local Lesions IN Genomes)

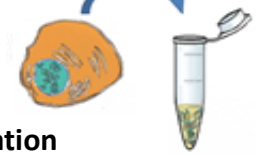


Detection of natural mutation or SNPs

ecoTILLING to look for natural mutations (SNPs) in individuals, usually for population genetics analysis.

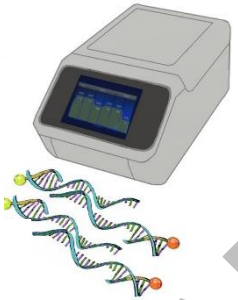
a) Collection of accessions

Extraction



b) DNA isolation

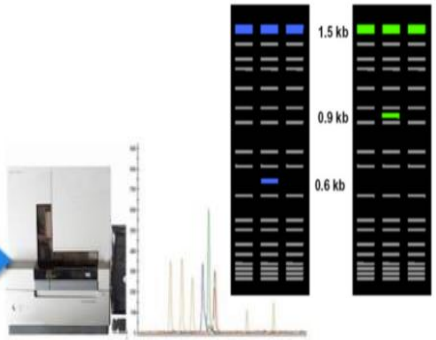
c)
PCR amplification



Denature, anneal

d)
Heteroduplex formation

e)
Digest mismatches
by applying CELI



f)
Capillary electrophoresis
and mutation discovery

TILLING (Targeting Induced Local Lesions IN Genomes)

1. It is independent of genome size, reproduction system or generation time.
2. High throughput and data analysis can be automated.
3. Valuable for essential genes, where sublethal alleles are required for phenotypic analysis.
4. TILLING is suitable for any organism that can be heavily mutagenized, even those that lack genetic toolboxes.
5. In organisms that do not have efficient transformation system, TILLING is the only practical choice.
6. Overcomes problems of transgenic approach as it is independent of transgene efficiency and regeneration of plants.

Απόκτηση μεταλλαγμένων σειρών *Arabidopsis thaliana*

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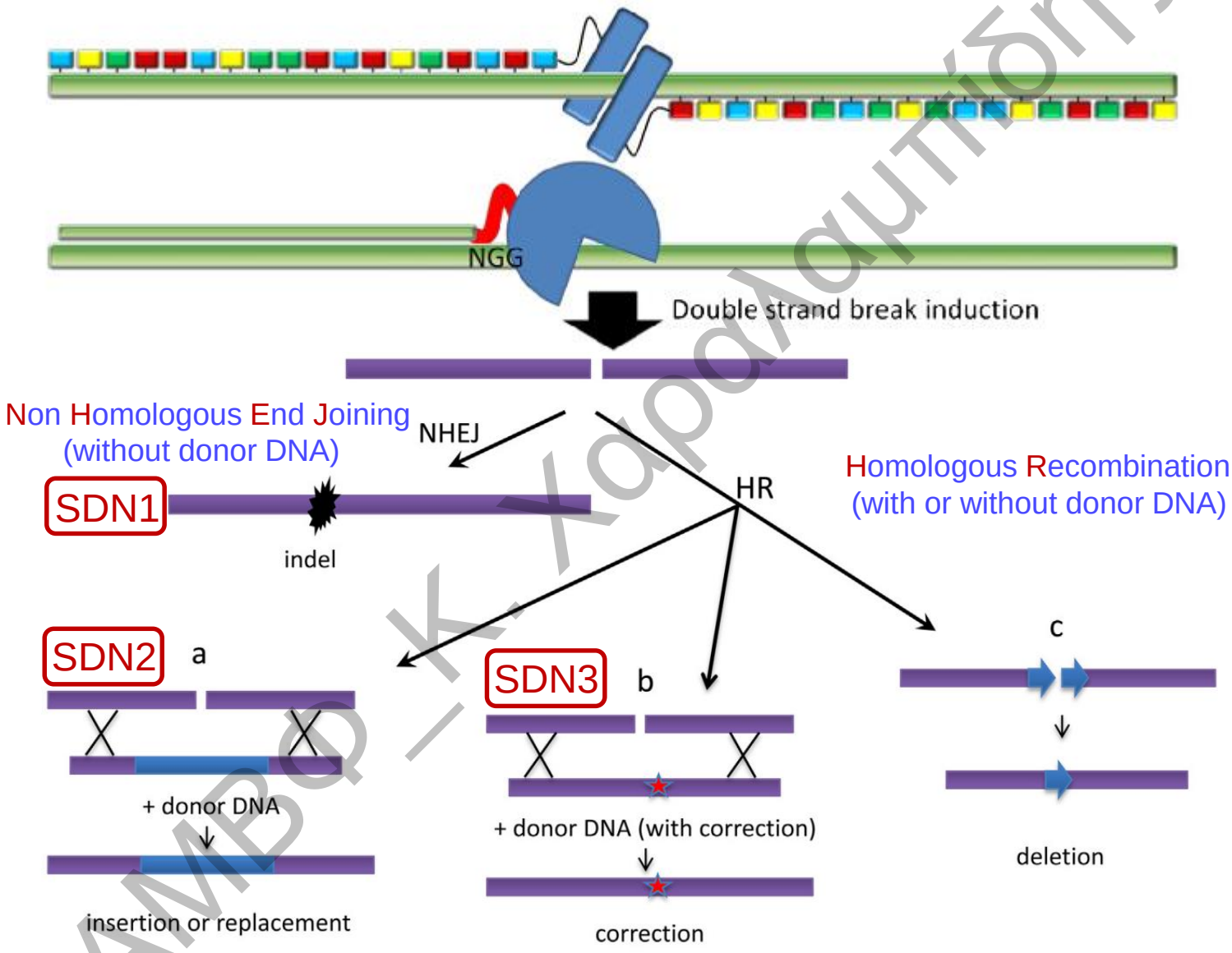
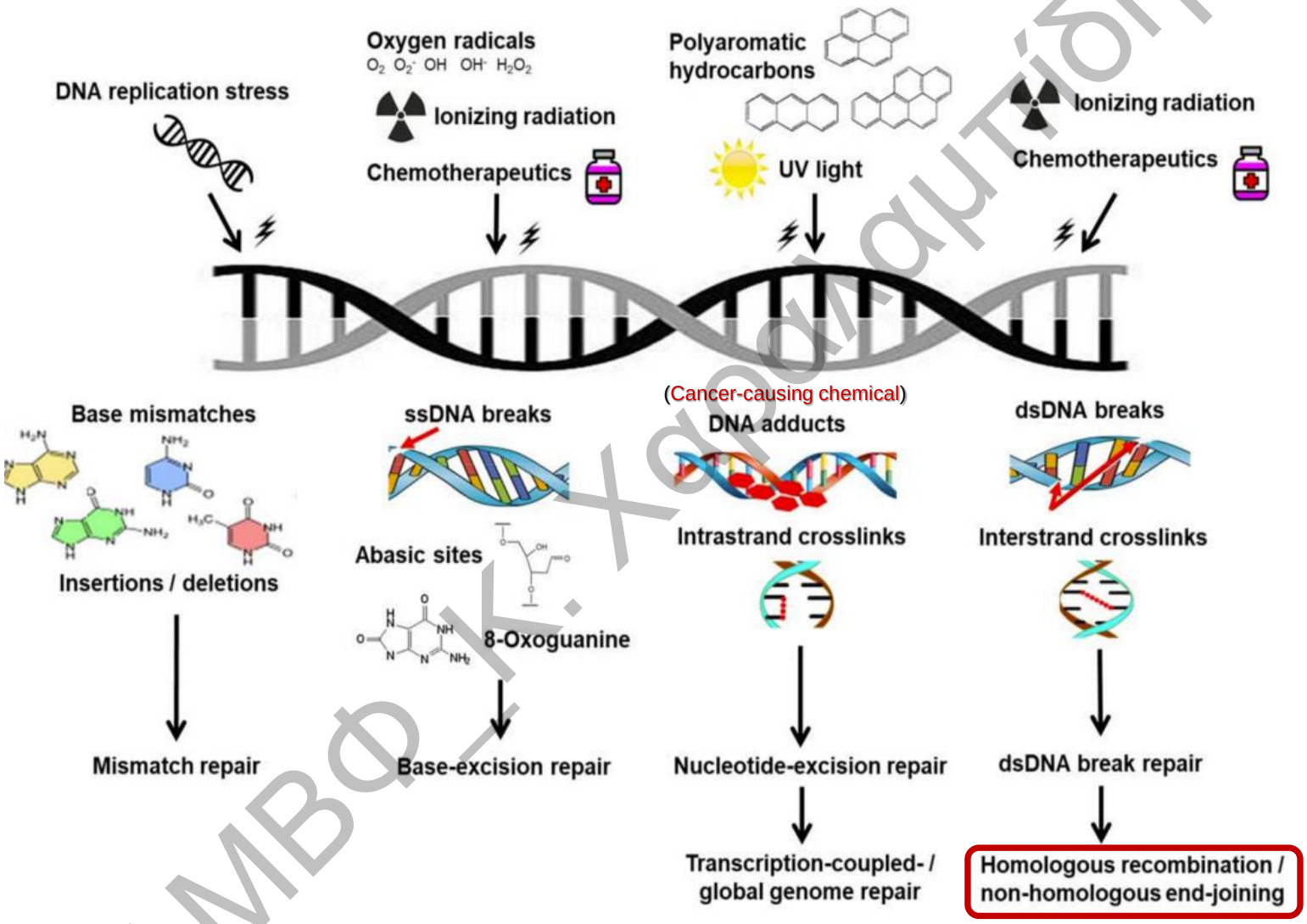
   - Genome editing methods with nucleases – MN, ZGNS, TALEN, CRISPR) (Γονιδιωματική παρέμβαση/επεξεργασία) – GENE DRIVES

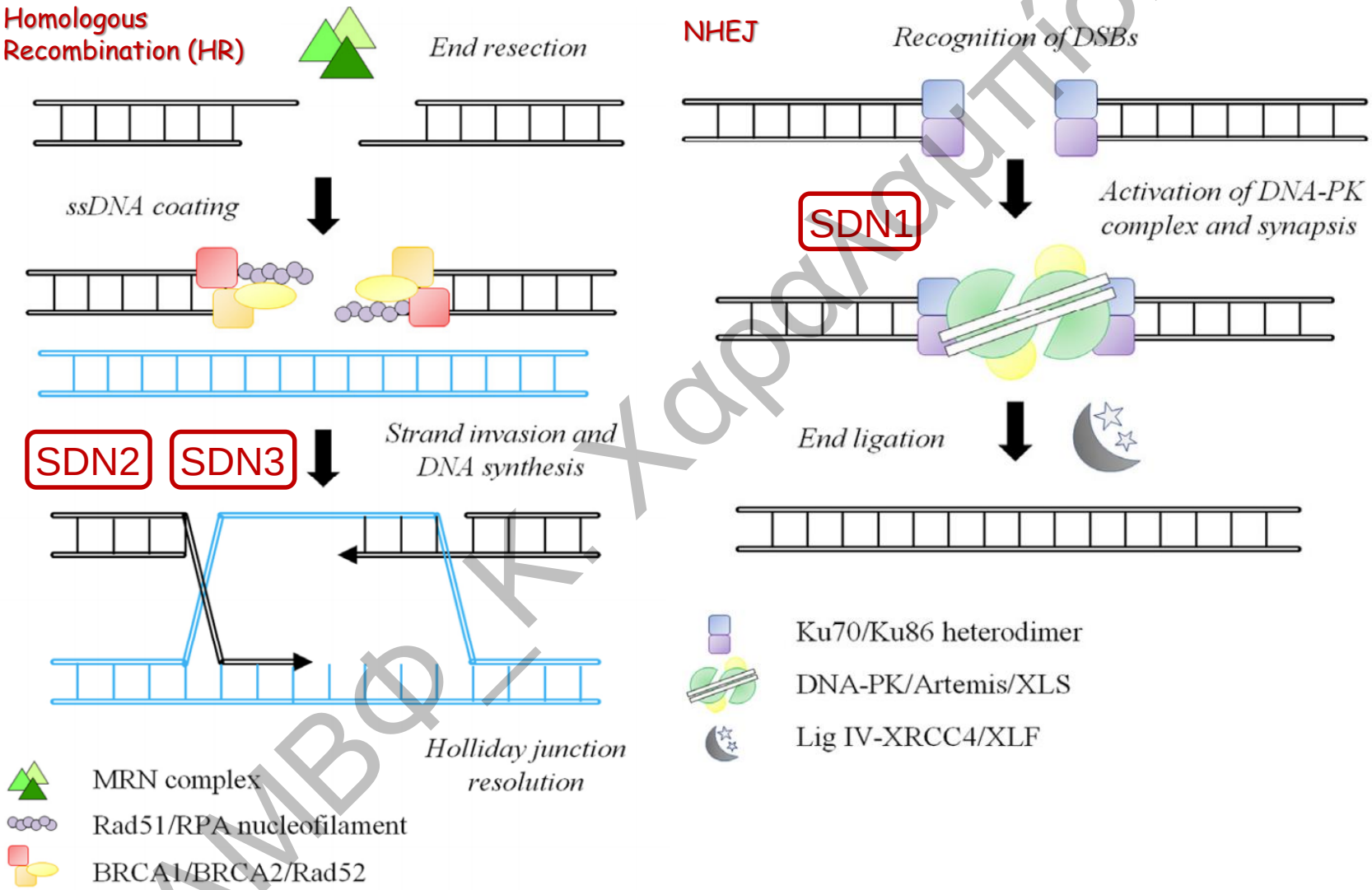
Genome editing by using Site-Directed Nuclease (SDN) technology



Deoxyribonucleic acid damage and repair mechanisms



HR vs. NHEJ DNA repair mechanism in eukaryotes



Genome editing by using Site-Directed Nuclease (SDN) technology

Tools mechanistically different from the earlier transgenic technologies used to create the largely controversial Genetically Modified Plants.

- **Spo11 endonuclease** initiates meiotic recombination by catalyzing the formation of double-strand breaks in DNA that is required for homologous chromosome pairing and synaptonemal complex formation.
- **Meganucleases (MN)** are endodeoxyribonucleases characterized by a large recognition site (double-stranded DNA sequences of 12 to 40 base pairs), which generally occurs only once in any given genome.
- **Zinc Finger Nucleases (ZFNs)** are a class of engineered DNA-binding proteins that facilitate targeted editing of the genome by creating double-strand breaks in DNA at user-specified locations.
- **Transcription activator-like effector nucleases (TALEN)** are nucleases engineered to cut specific sequences of DNA. They are made by fusing a TAL (from *Xanthomonas*) effector DNA-binding domain to a DNA cleavage domain.
- **CRISPR (clustered regularly interspaced short palindromic repeats)** are sequences found in bacteria and archaea, used to detect and destroy foreign DNA. They play a key role in the anti-phage defense system of prokaryotes.

Plant genome editing tools for generating GMPs (Cisgenics)

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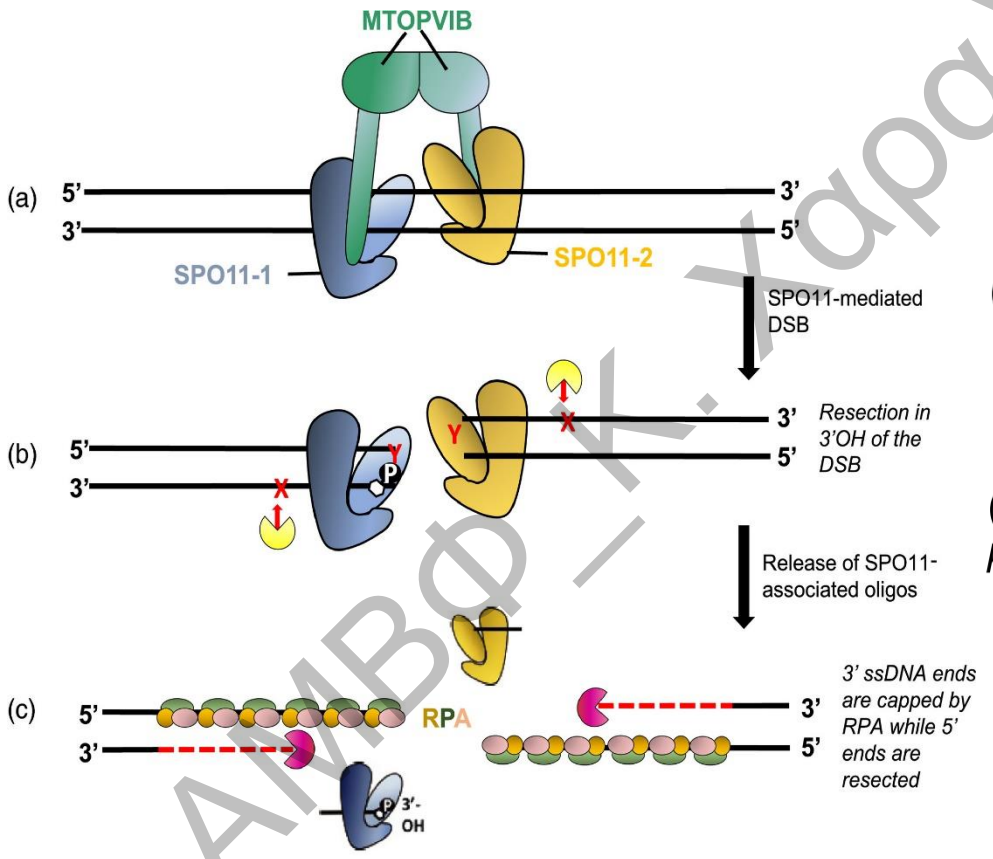
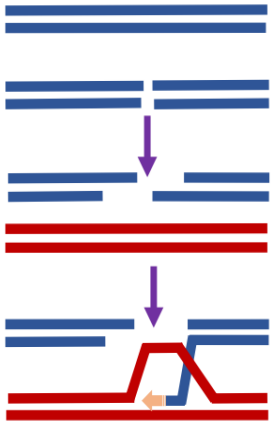
SPO11 Mediated Targeted Genome Editing

Meiogenix, based in Paris, has developed this gene-editing technology that that aims to make the natural process of meiotic recombination more efficient and increase the genetic diversity of plants. It is based on the fusion of the Spo11 endonuclease, responsible for initiating meiotic recombination, to a DNA binding domain, such as an inactivated Cas9 or TALEN protein in order to target meiotic recombination to the desired region.

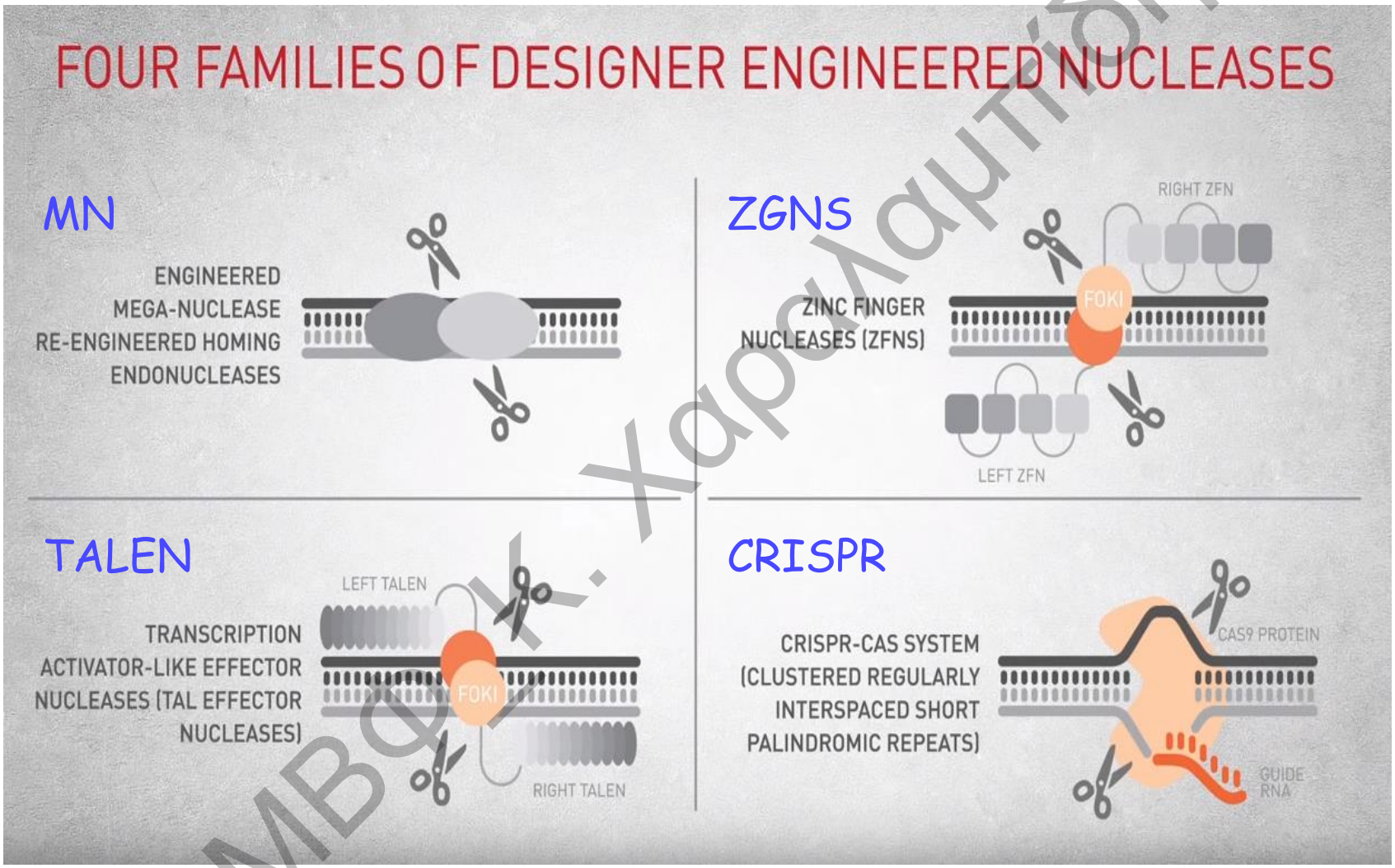


SPO11
(and associated proteins)

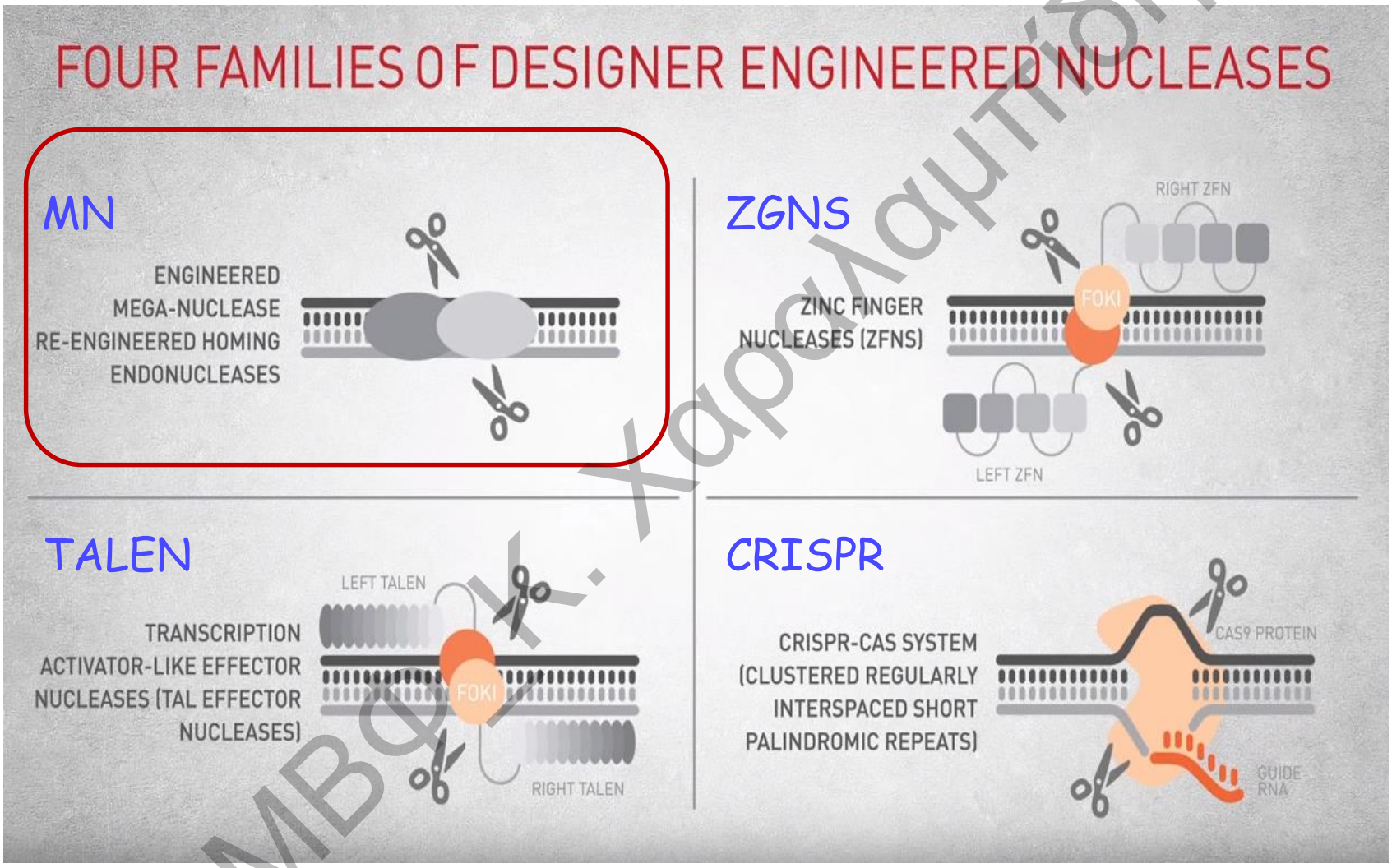
DMC1 , RAD51
(and associated proteins)



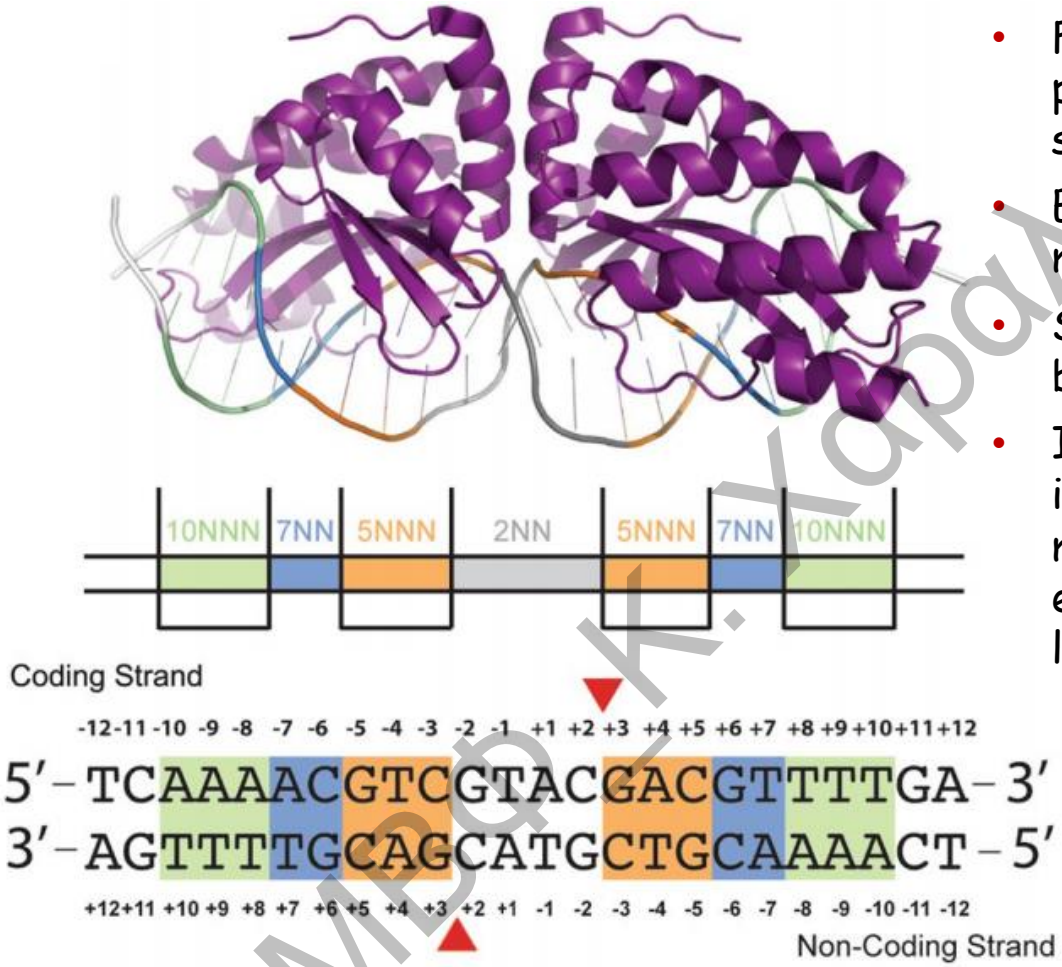
Genome editing methods with nucleases



Genome editing methods with nucleases



Characteristics of Meganucleases (MN or NM)

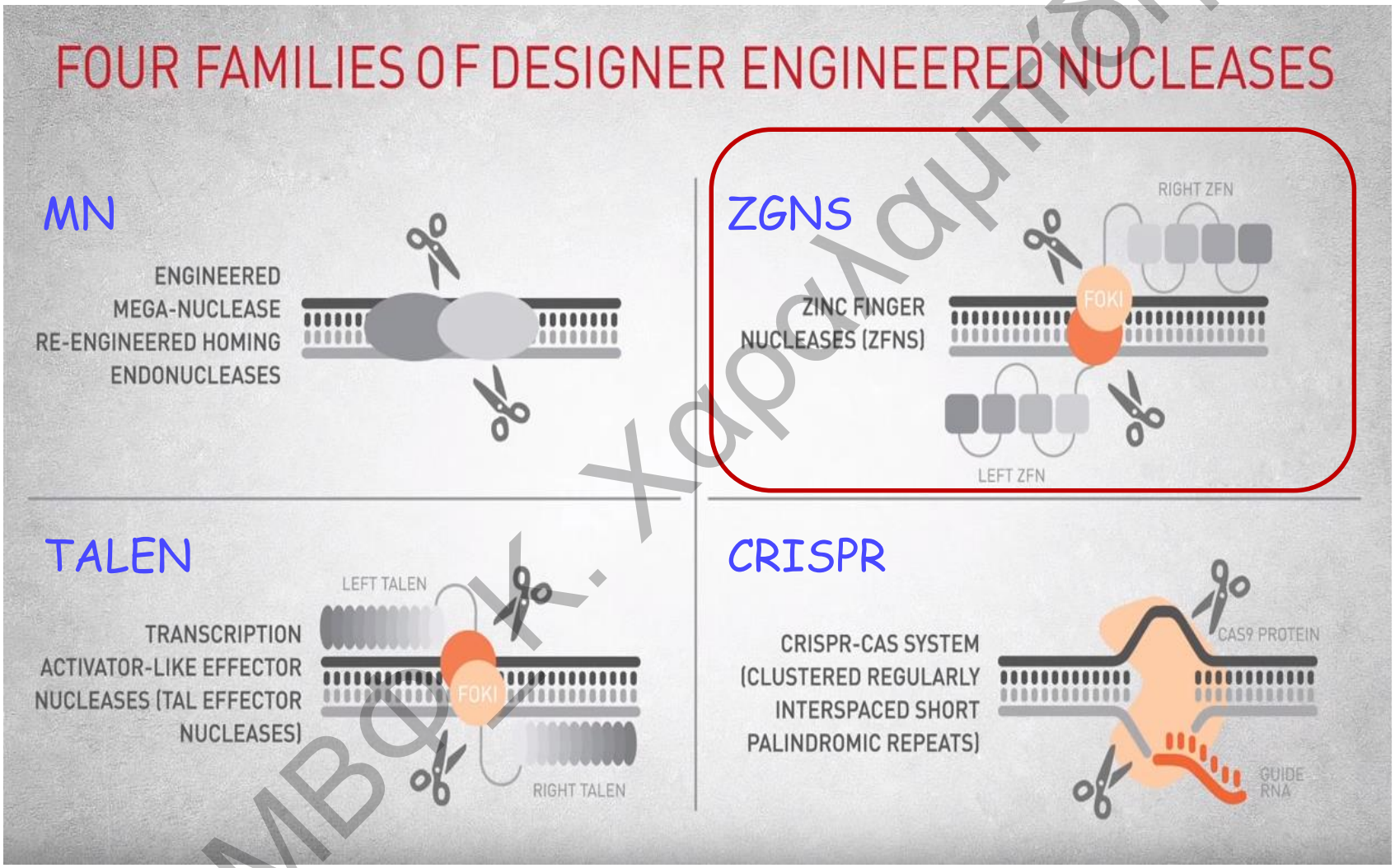


- Found in archaea, bacteria, phages, fungi, yeast, algae and some plants.
- Expressed in the nucleus, mitochondria or chloroplasts.
- Several hundred of them have been identified.
- In December 2014, the USPTO issued patent 8,921,332 covering meganuclease based genome editing in vitro. The patent was licensed exclusively to Cellectis.

Characteristics of Meganucleases (MN)

- Meganucleases are endodeoxyribonucleases characterized by a large recognition site (double-stranded DNA sequences of 12 to 40 base pairs).
- The site generally occurs only once in any given genome. For example, the 18-base pair sequence recognized by the I-SceI meganuclease would on average require a genome twelve times the size of the human genome to be found once by chance.
  - $4^{18} = 69X10^9$ (human genome is about $6X10^9$), $4^{15} = 10^9$ (6 times in human genome)
- Meganucleases are therefore considered to be the most specific naturally occurring restriction enzymes in living organisms.
- Among meganucleases, the LAGLIDADG family of endonucleases has become a valuable tool for genome engineering over the past fifteen years.
- Their recognition site can be modified through protein engineering, in order to target any given sequence in the genome of an organism (chimeric meganucleases).
- Meganucleases can therefore act as "molecular DNA scissors" that can be used to replace, eliminate or modify sequences in a highly targeted way.
- However, DNA methylation and chromatin structure affect the efficacy of meganuclease digestion. Consideration of the epigenetic context of a target sequence is therefore necessary for the practical application of these enzymes.
- Off target activity of engineered meganucleases is still a problem due to binding of the enzymes on similar sequences containing 1, 2 or 3 mismatches.

Genome editing methods with nucleases

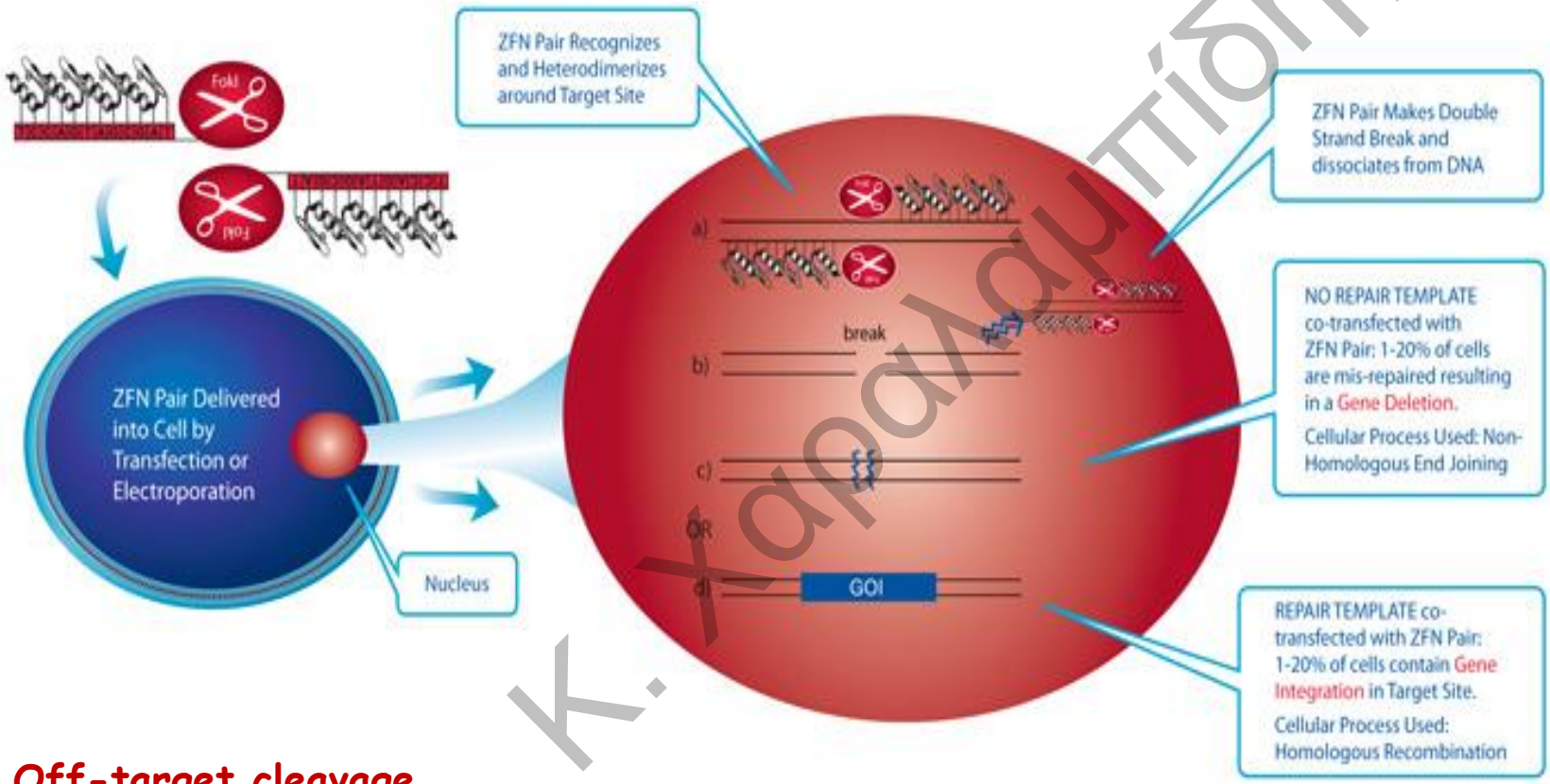


ZFN-Mediated Targeted Genome Editing

Zinc-finger nucleases (ZFNs) are artificial restriction enzymes generated by fusing a zinc finger DNA-binding domain to a DNA-cleavage domain (FokI bacterial nonspecific endonuclease). Zinc finger domains can be engineered to target specific desired DNA sequences. By taking advantage of endogenous DNA repair machinery (NHEJ), these reagents can be used to precisely alter the genomes of higher organisms.

Double-strand breaks are important for site-specific mutagenesis in that they stimulate the cell's natural DNA repair processes, namely homologous recombination and non-homologous end joining (NHEJ).

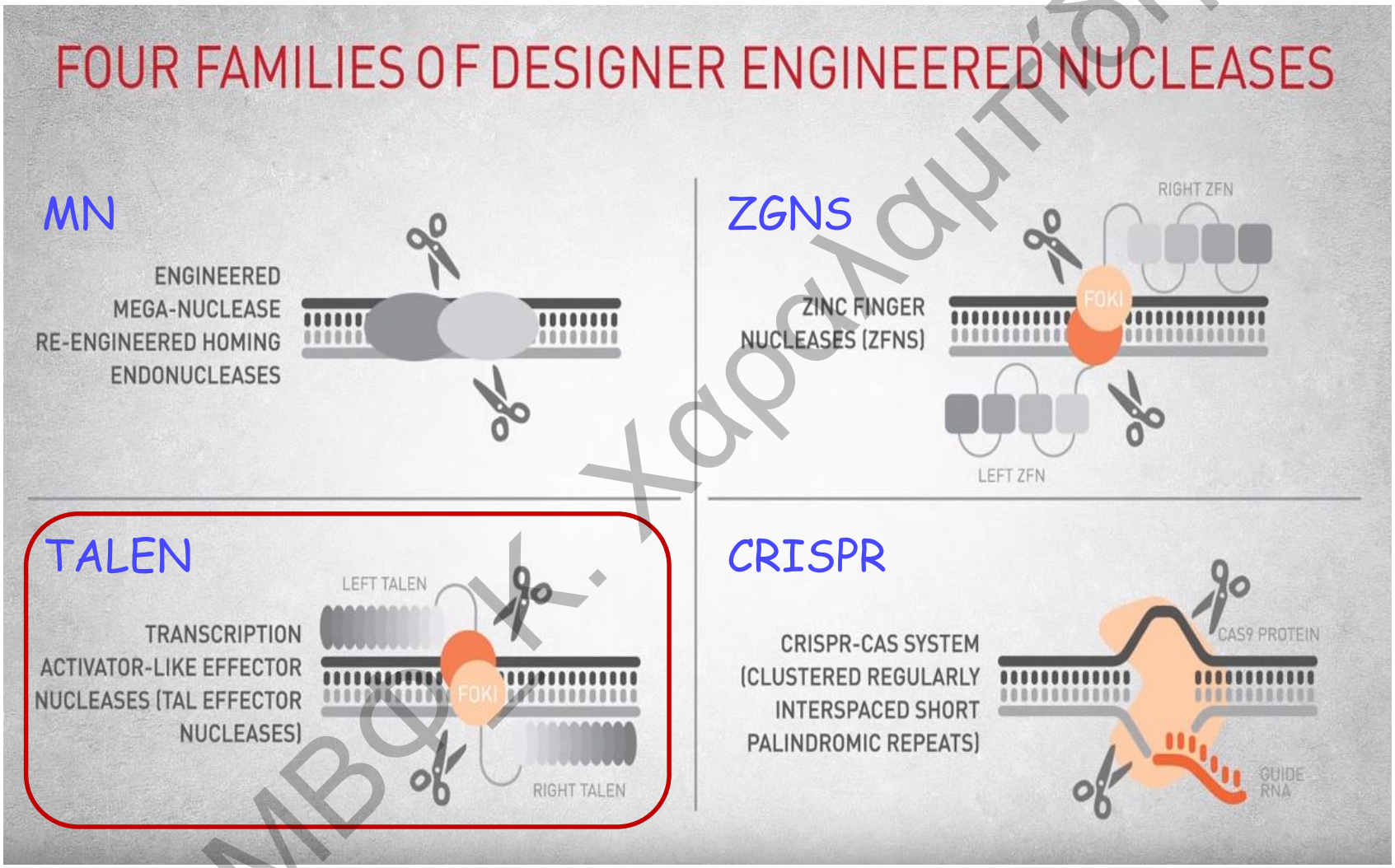
ZFN-Mediated Targeted Genome Editing by NHEJ



Off-target cleavage

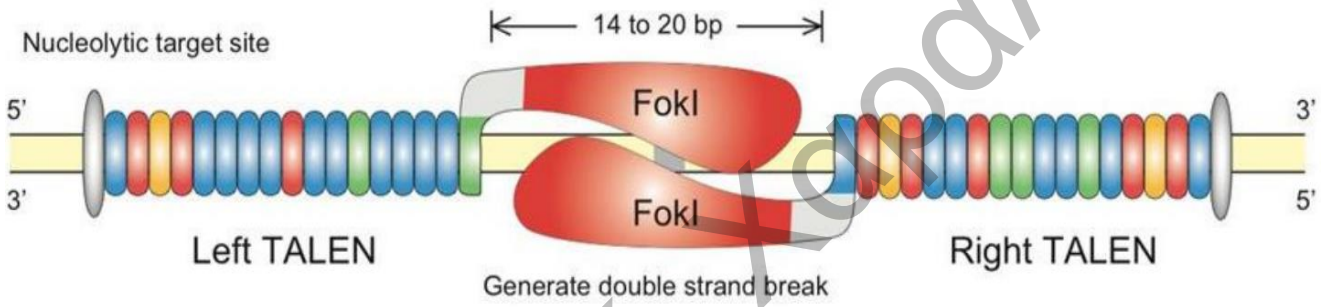
If the zinc finger domains are not specific enough for their target site or they do not target a unique site within the genome of interest, off-target cleavage may occur. Such off-target cleavage may lead to the production of enough double-strand breaks to overwhelm the repair machinery and, as a consequence, yield chromosomal rearrangements and/or cell death.

Genome editing methods with nucleases

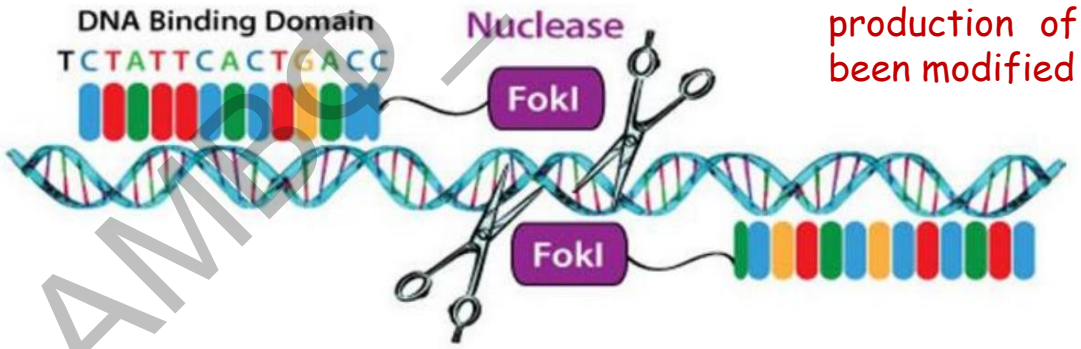


Transcription activator-like effector nucleases (TALEN)

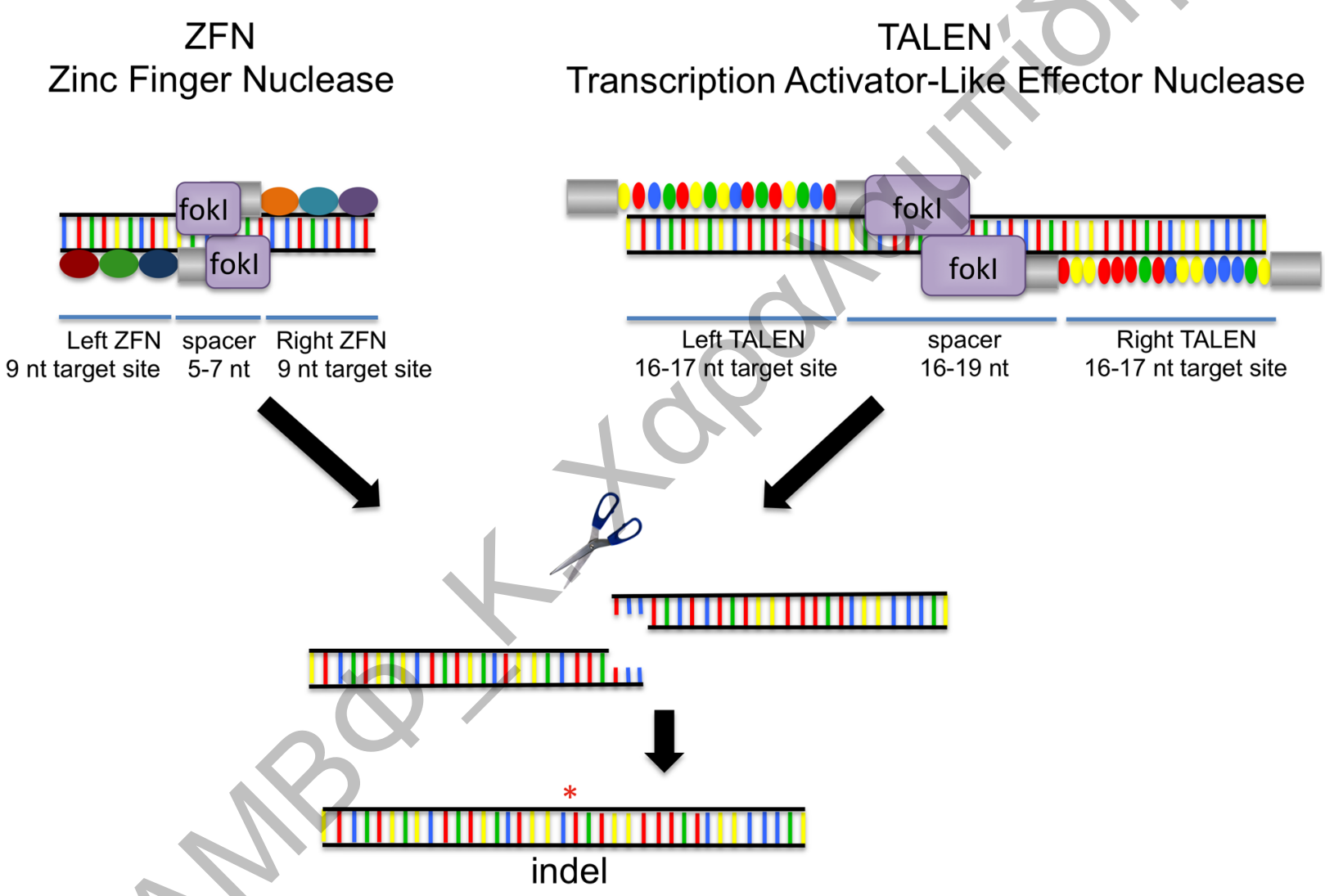
Transcription activator-like effector nucleases (TALEN) are restriction enzymes that can be engineered to cut specific sequences of DNA. They are made by fusing a TAL effector DNA-binding domain to a DNA nuclease domain (FokI). Transcription activator-like effectors (TALEs) can be engineered to bind to practically any desired DNA sequence and cut at specific locations.



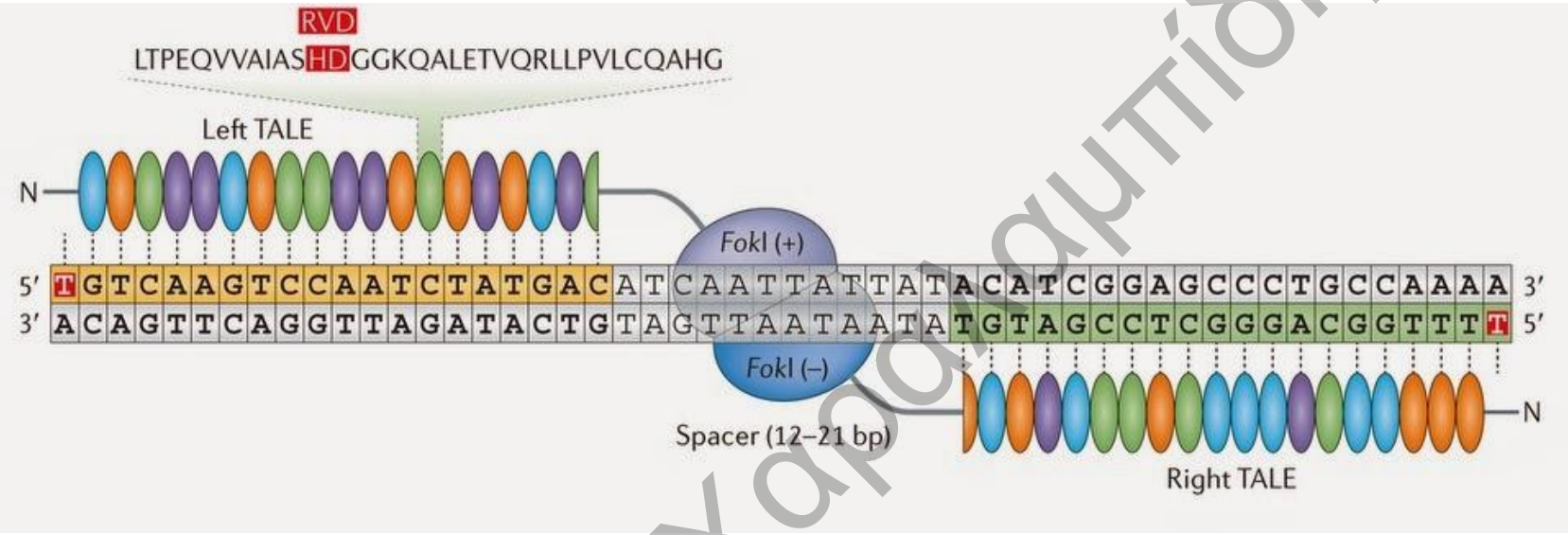
Cellectis is already growing gene modified soybeans that contain less saturated fatty acids for the production of oil. The soybeans have been modified by turning off two genes.



ZFN vs. TALEN gene editing nucleases by NHEJ and HR

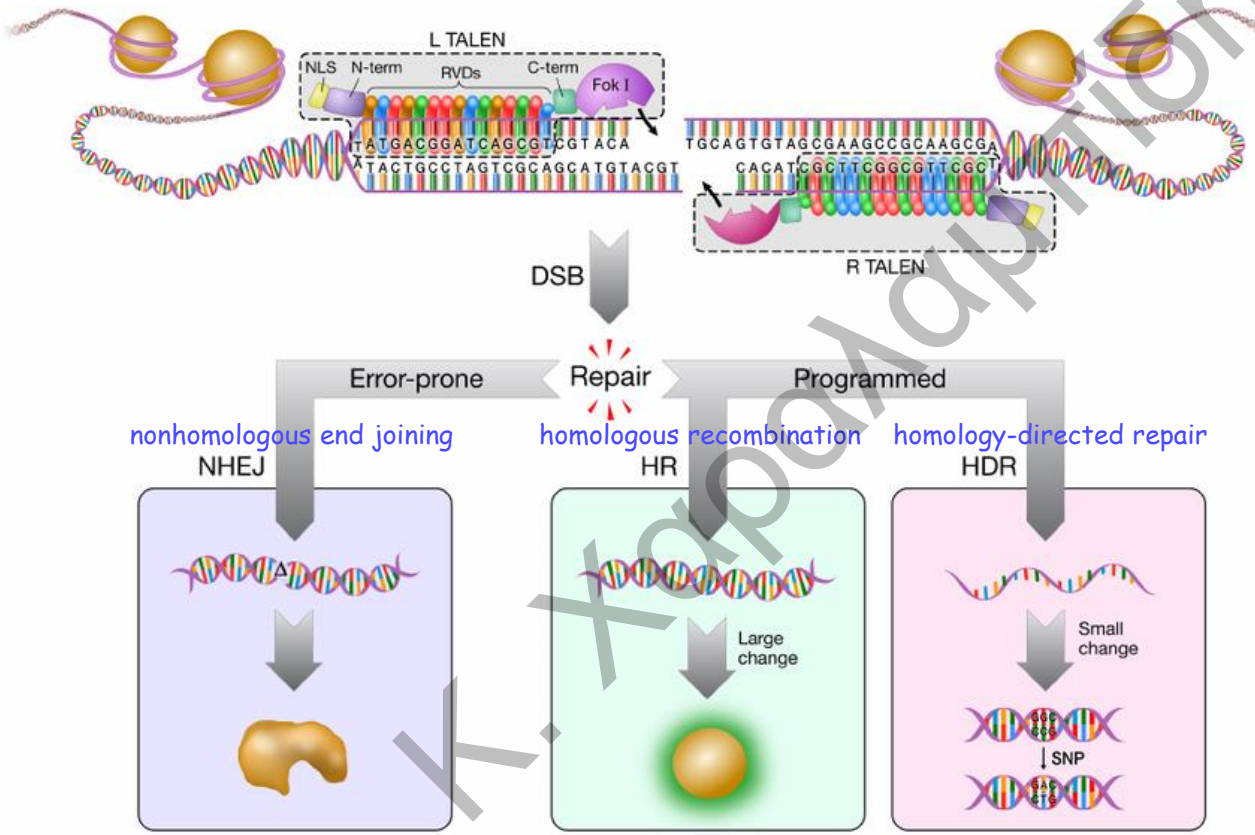


TALEN used in plants



Arabidopsis *(Arabidopsis thaliana)*	*adh1*	Knockout	[70]
Tobacco *(Nicotiana benthamiana)*	*surA, surB, hax3*	Knockout, insertion	[156, 157]
False brome grass *(Brachypodium distachyon)*	*aba1, cxk2, coi1, hta1, rht, sbp, smc6, spl*	Knockout	[154]
Rice *(Oryza sativa)*	*avrxa7, pthxo3, badh2, ckx2, dep1, sd1*	Knockout	[154, 155]

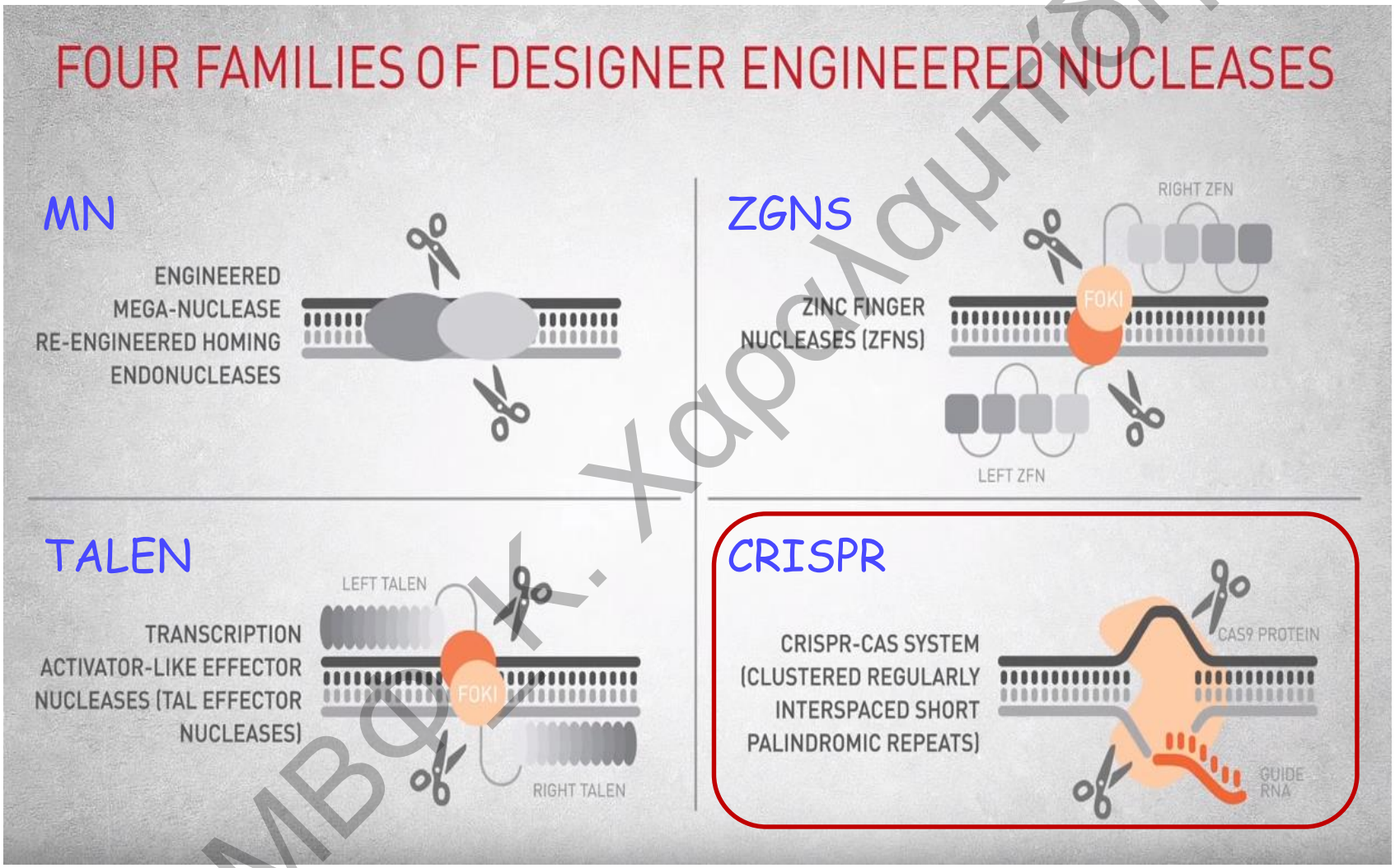
TALEN Mediated Targeted Genome Editing



The off-target activity

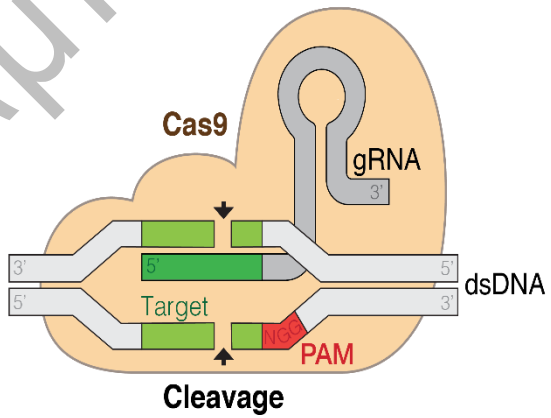
An active nuclease may lead to unwanted double-strand breaks and may consequently yield chromosomal rearrangements and/or cell death. TALEN constructs are believed to have the greatest precision of the currently available technologies (except CRISPR ?)

Genome editing methods with nucleases



CRISPR Technology (Clustered regularly-interspaced short palindromic repeats)

«Ομαδοποιημένων μικρών παλίνδρομων επαναλήψεων»



CRISPR brings an important advantage over TALEN and zinc-finger nucleases (ZFNs). It is much easier and faster to use and does not required creating a gene editing protein from scratch for each specific DNA modification. With CRISPR, the same Cas9 molecule can be directed to any sequence just by providing it with a guide RNA molecule, which is much easier to synthesize.

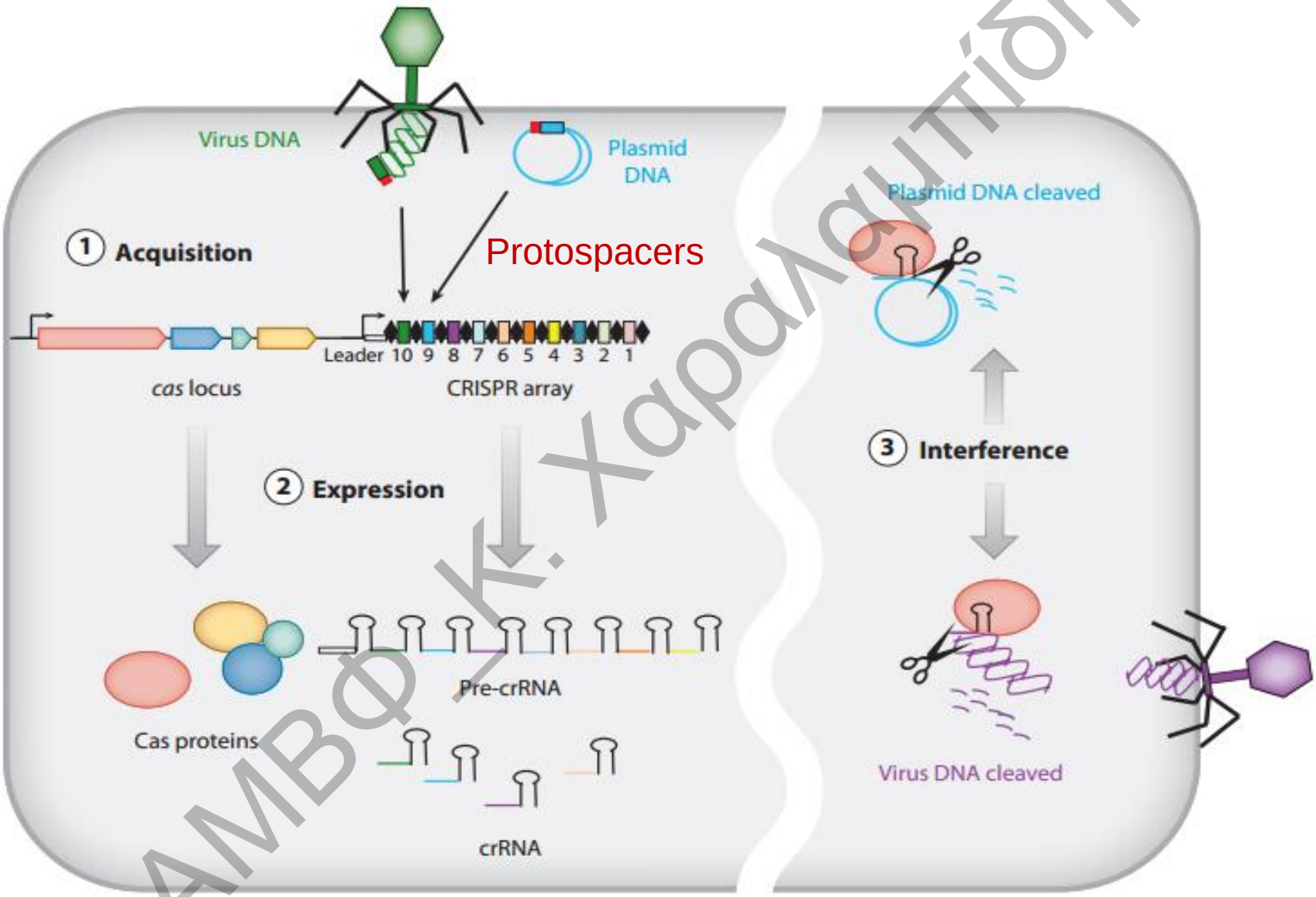
CRISPR history

In 1987, Japanese scientist Yoshizumi Ishino and his team at Osaka University discovered clusters of DNA in some bacteria that contained strange repeats. It wasn't known why they repeated or what the clusters did, if anything.

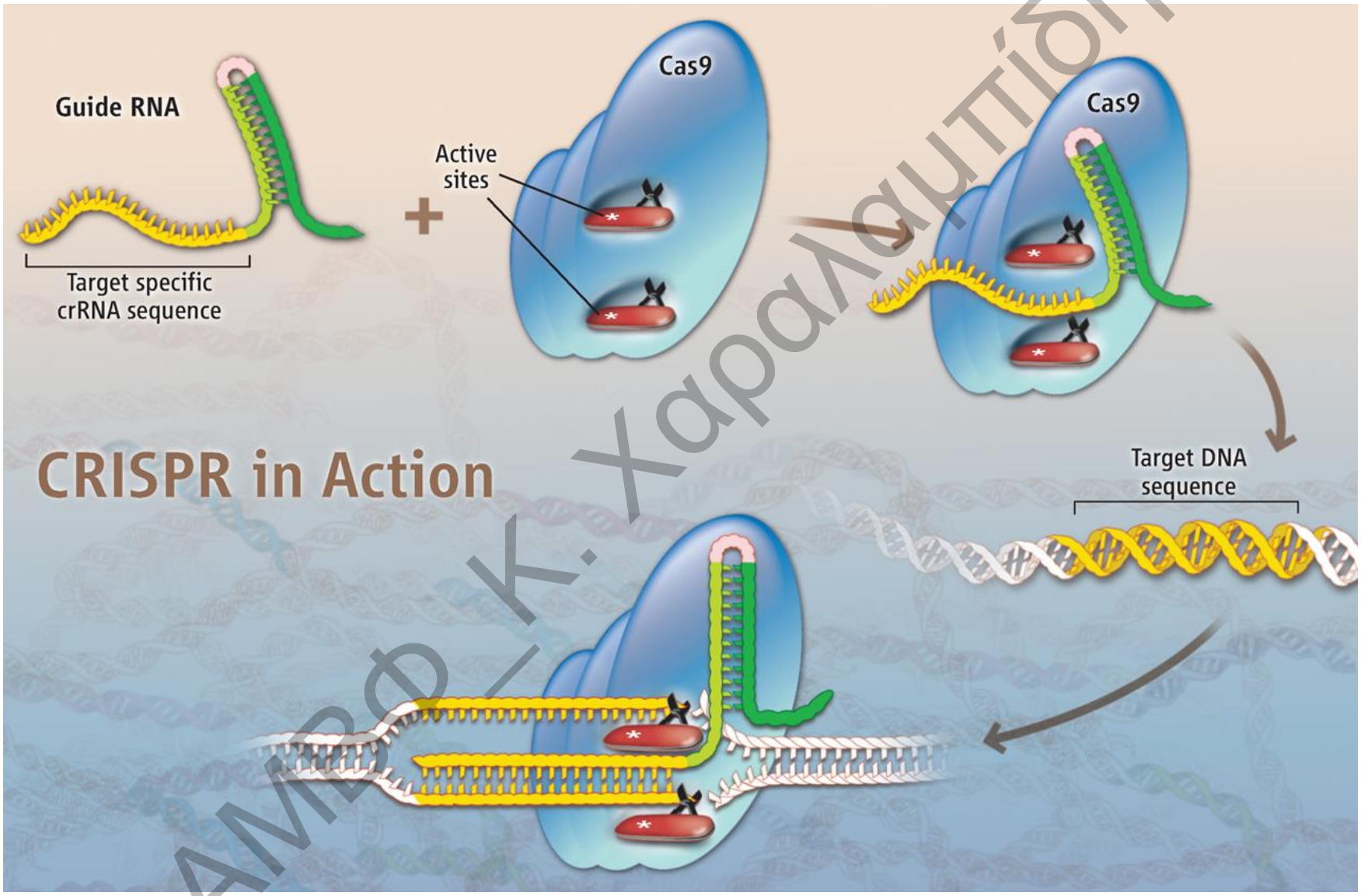
In 2001, researchers Francisco Mojica and Ruud Jansen coined a name for these repeating sections — CRISPRs, which stands for Clustered Regularly Interspaced Short Palindromic Repeats.

They realized that this bacteria was incorporating segments of DNA from viral invaders into its own genome and using it as an early warning system against attacks by the same virus. The CRISPR segments identify and flag the matching DNA in the viruses. But it's particular enzymes, including one called Cas9, that serve as weapons against the invaders. Cas9 carries a copy of the CRISPR sequence with it, forming together the CRISPR-Cas9 system.

CRISPR Technology (Clustered regularly-interspaced short palindromic repeats)



CRISPR Technology (Clustered regularly-interspaced short palindromic repeats)



Selective loading and processing of prespacers for precise CRISPR adaptation

Department of BioNanoScience, Applied Science
Laboratory of Chirlmin Joo

TU Delft

CRISPR history

In vitro editing (Patent in EU)

2012
Science

Jennifer Doudna
University of California, Berkeley

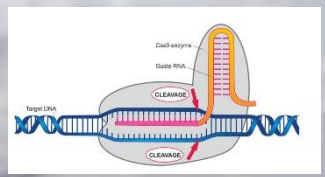
Emmanuelle Charpentier
Max Planck Institute for Infection Biology

In vivo editing (Patent in EU)

2013
Science

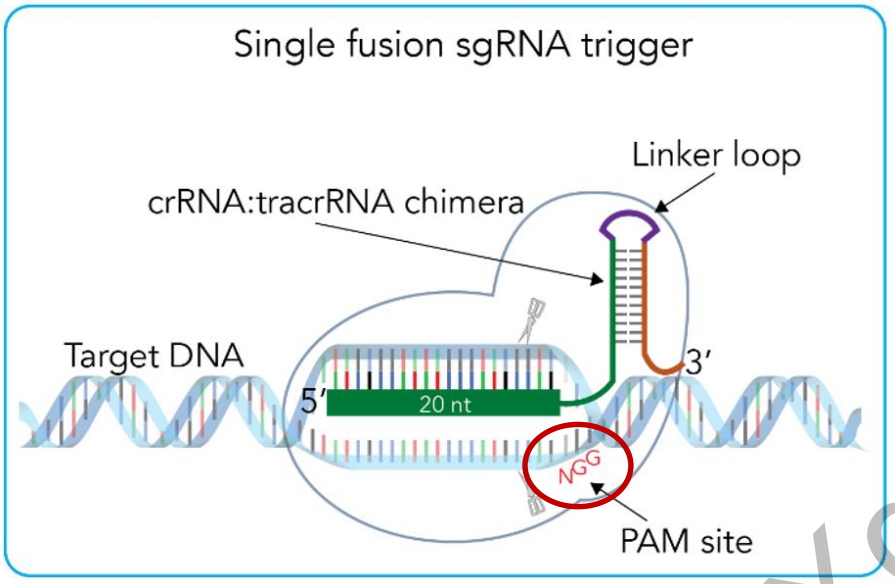
Feng Zhang
MIT, NY

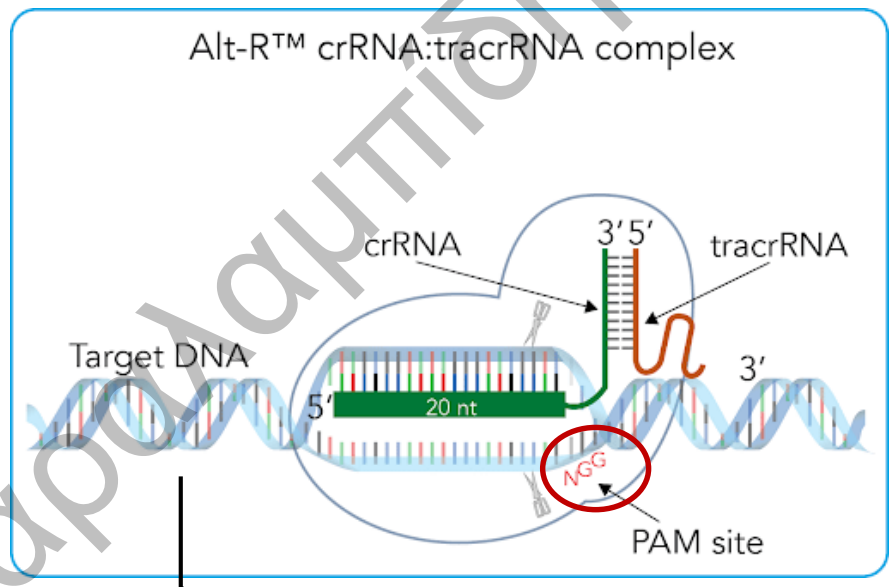
Doudna, Charpentier, and their colleagues published the first account of programming the CRISPR/Cas9 system to precisely cut naked plasmid and double-stranded DNA

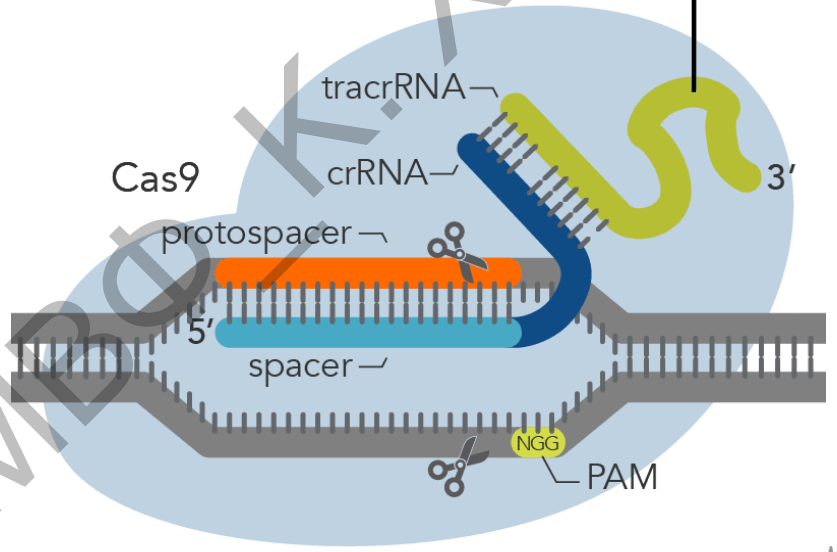


The Nobel Prize in Chemistry 2020

Single fusion sgRNA vs. 2-part crRNA::tracrRNA complex

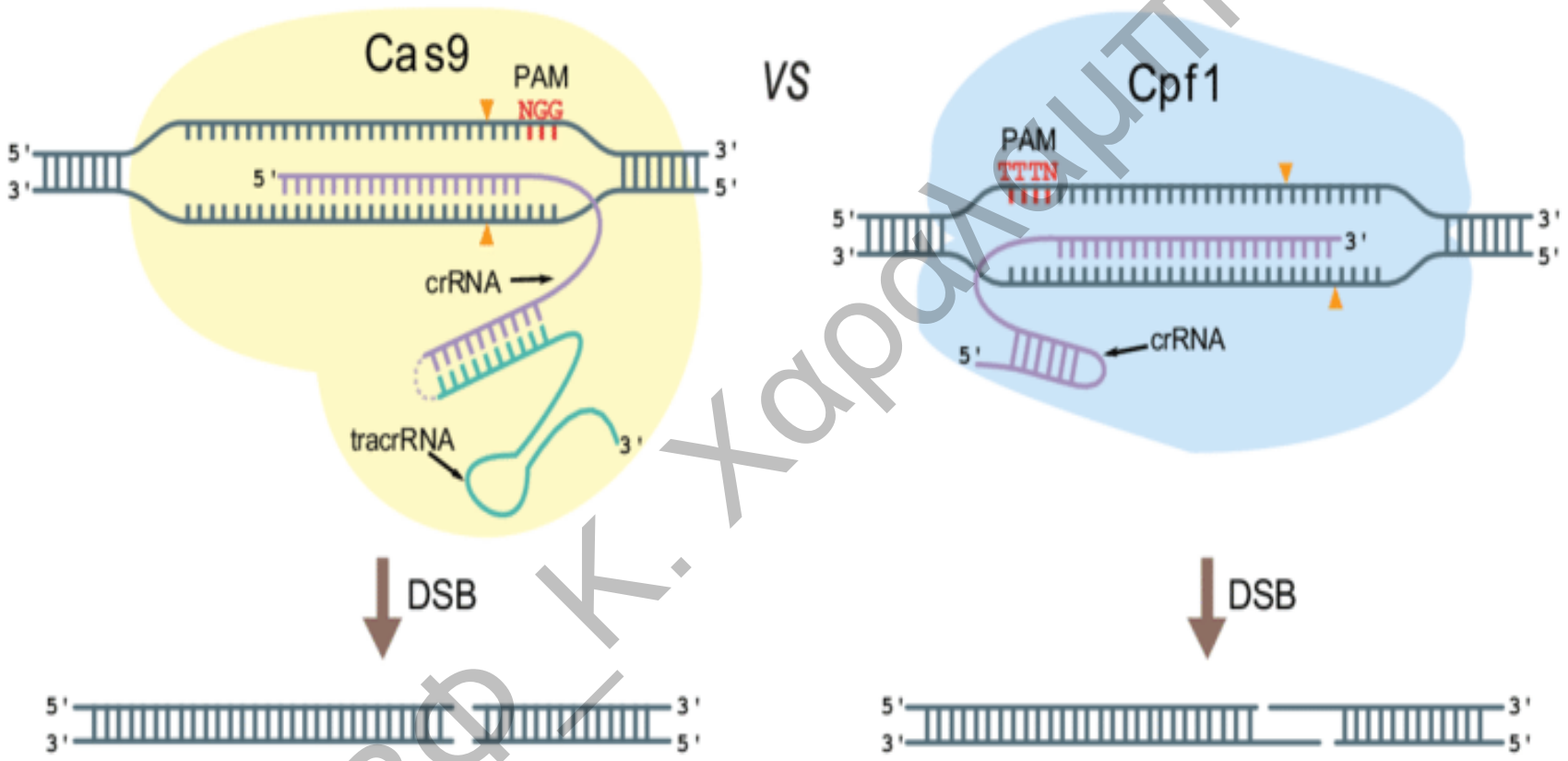




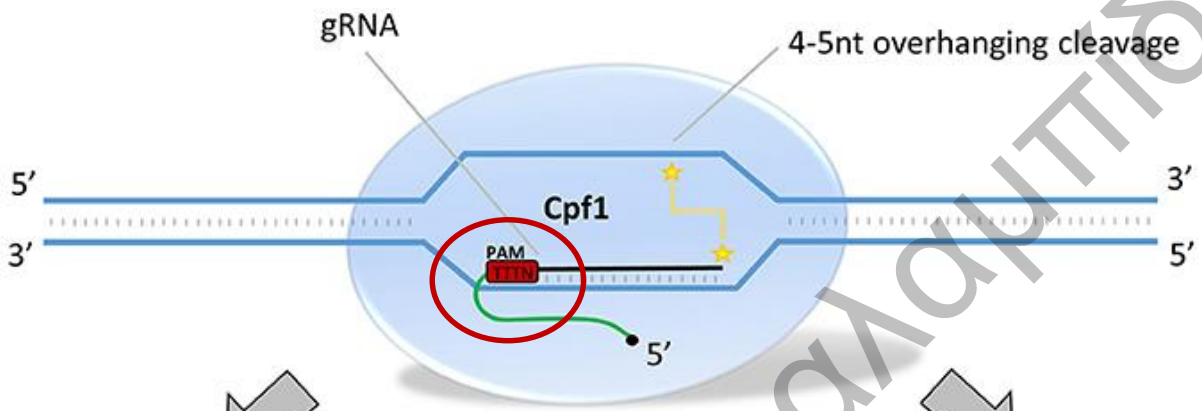


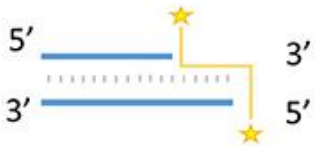
CRISPR CAS9 components

CRISPR Cas9 vs. CRISPR Cpf1 (CAS12a)



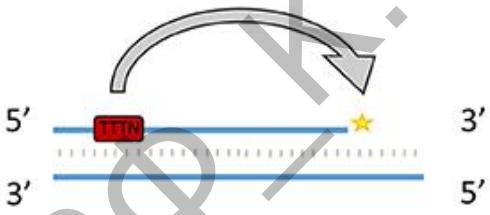
CRISPR Cpf1 (CAS12a) advantages over Cas9





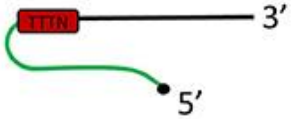
A Staggered overhanging DNA cleavage

CAS9 generated blunt end cuttings



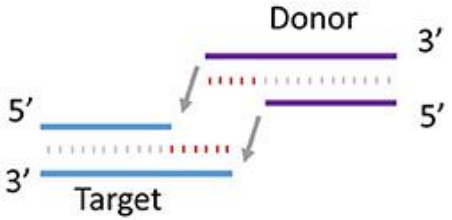
B Cleavage distal to T-rich PAM motif

No disruption of the PAM site due to cuts 18-23 bp downstream



C Shortened gRNA for efficient and compact delivery

Only requires crRNA (~42nt) CAS9 requires crRNA & tracrRNA (~100nt)



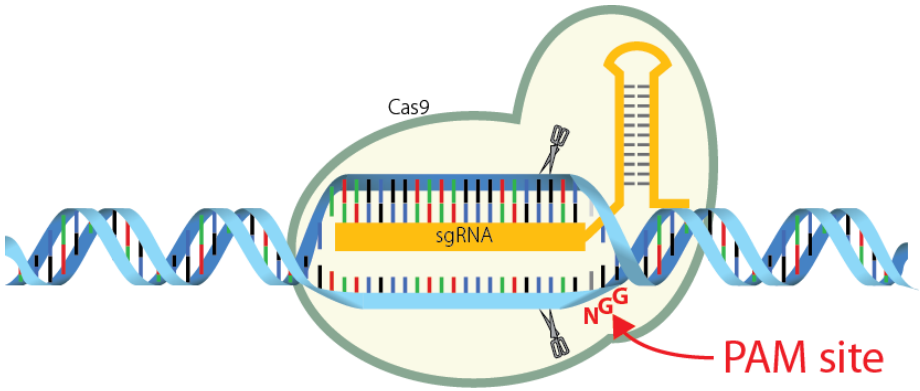
D NHEJ repair based insertion of heterologous genes

Resulting in a more specific NHEJ

Different PAM sequences from various CRISPR nucleases

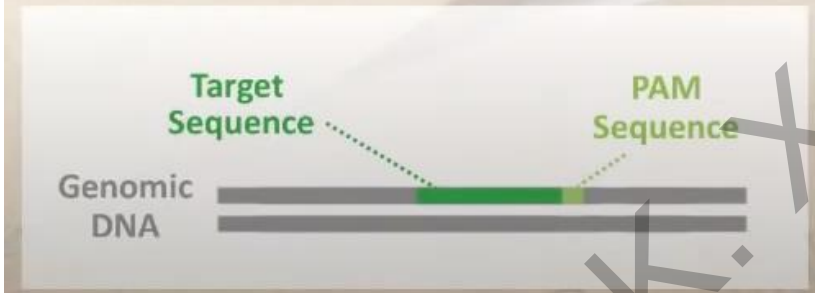
CRISPR Nucleases	Organism Isolated From	PAM Sequence (5' to 3')
SpCas9	*Streptococcus pyogenes*	NGG
SaCas9	*Staphylococcus aureus*	NGRRT or NGRRN
NmeCas9	*Neisseria meningitidis*	NNNNGATT
CjCas9	*Campylobacter jejuni*	NNNNRYAC
StCas9	*Streptococcus thermophilus*	NNAGAAW
LbCpf1	*Lachnospiraceae bacterium*	TTTV
AsCpf1	*Acidaminococcus* sp.	TTTV

Symbol[15]	Description	Bases represented				
A	**a**denine	A				1
C	**c**ytosine		C			
G	**g**uanine			G		
T	**t**hymine				T	
U	**u**racil				U	
W	**w**eak	A			T	2
S	**s**trong		C	G		
M	amino	A	C			
K	keto			G	T	
R	purine	A		G		
Y	pyrimidine		C		T	
B	not A (**B** comes after A)		C	G	T	3
D	not C (**D** comes after C)	A		G	T	
H	not G (**H** comes after G)	A	C		T	
V	not T (**V** comes after T and U)	A	C	G		
N	**n**y base (not a gap)	A	C	G	T	4



sgRNA design can be complicated

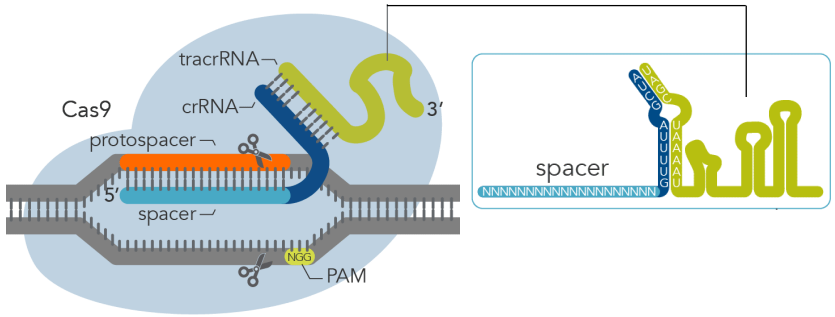
- Must target at a PAM sequence.
- PAM sequence depends on the Cas nuclease.



Cas Nuclease	PAM Sequence
spCas9, dCas9	5'-NGG-3'
spCas9 Nickase	2 x 5'-NGG-3', <20bp apart, on opposite strands
saCas9	5'-NNGRRT-3'
Cpf1	5'-TTTN-3'
nmCas9	5'-NNNNGATT-3'
cjCas9	5'-NNNNRYAC-3'

Bad sgRNA design can lead to:

- Off-target cleavage.
- No cleavage at all.



Genome Editing Glossary

Cas = CRISPR-associated genes

Cas9, Csn1 = a CRISPR-associated protein containing two nuclease domains, that is programmed by small RNAs to cleave DNA

crRNA = CRISPR RNA

dCAS9 = nuclease-deficient Cas9

DSB = Double-Stranded Break

gRNA = guide RNA

HDR = Homology-Directed Repair

HNH = an endonuclease domain named for characteristic histidine and asparagine residues

Indel = insertion and/or deletion

NHEJ = Non-Homologous End Joining

PAM = Protospacer-Adjacent Motif

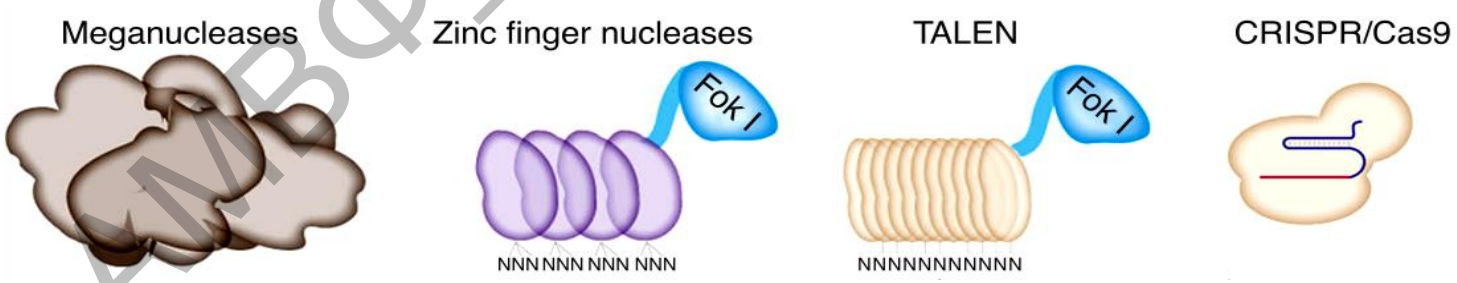
RuvC = an endonuclease domain named for an *E. coli* protein involved in DNA repair

sgRNA = single guide RNA

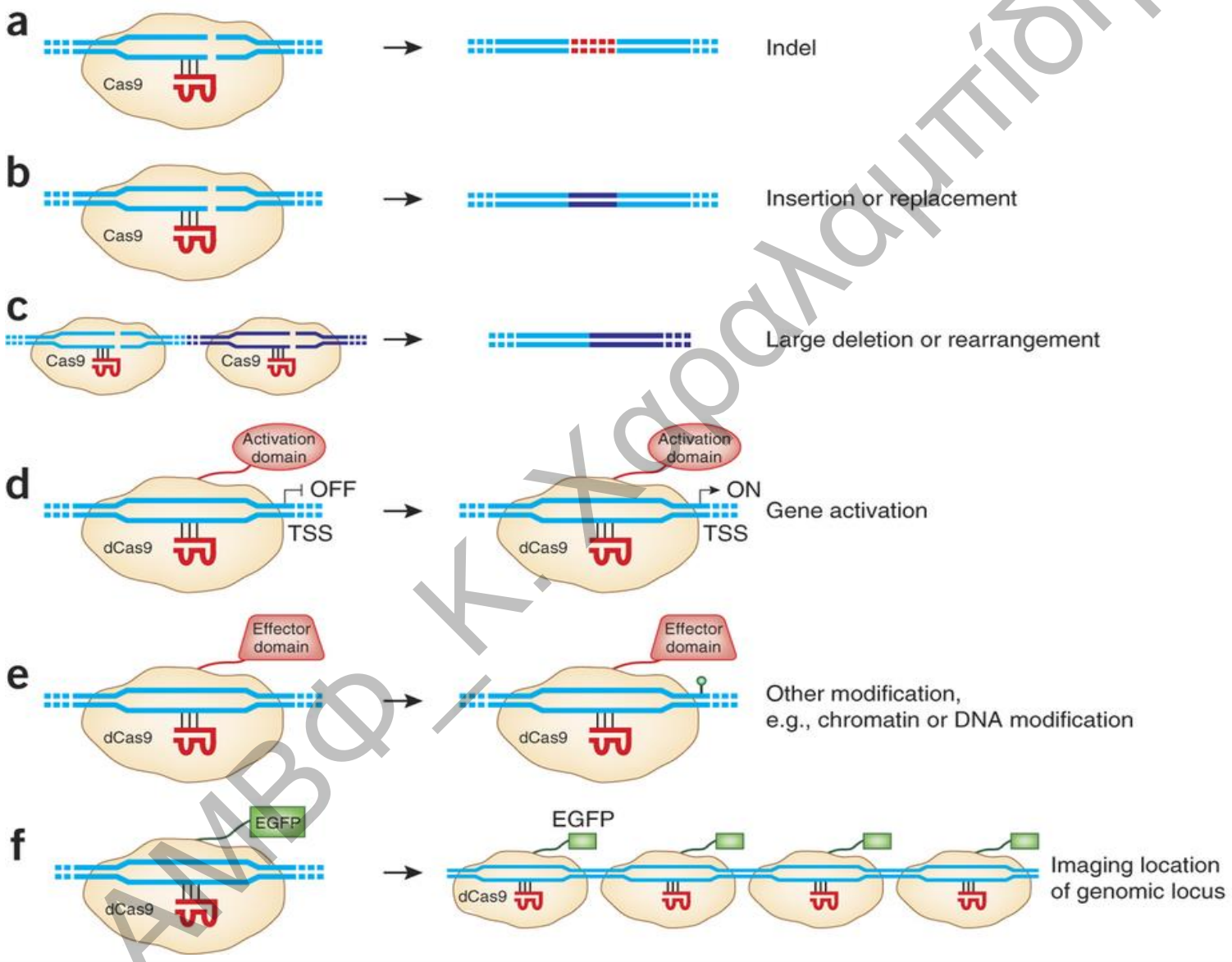
tracrRNA, trRNA = trans-activating crRNA

TALEN = Transcription-Activator Like Effector Nuclease

ZFN = Zinc-Finger Nuclease



CRISPR Technology (Clustered regularly-interspaced short palindromic repeats)



CRISPR Technology (Clustered regularly-interspaced short palindromic repeats)

Article | Published: 19 October 2020

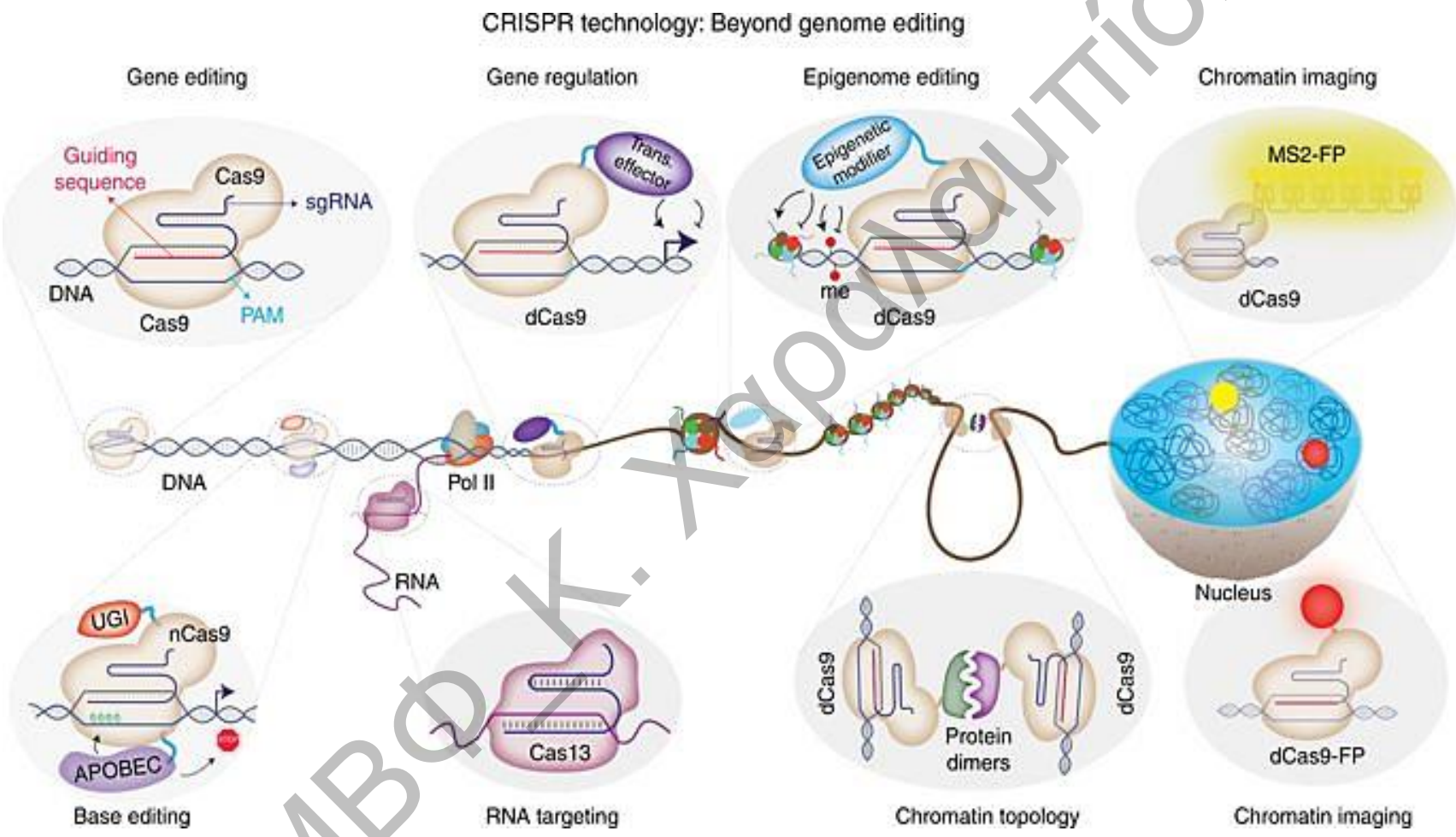
A compact Cascade–Cas3 system for targeted genome engineering

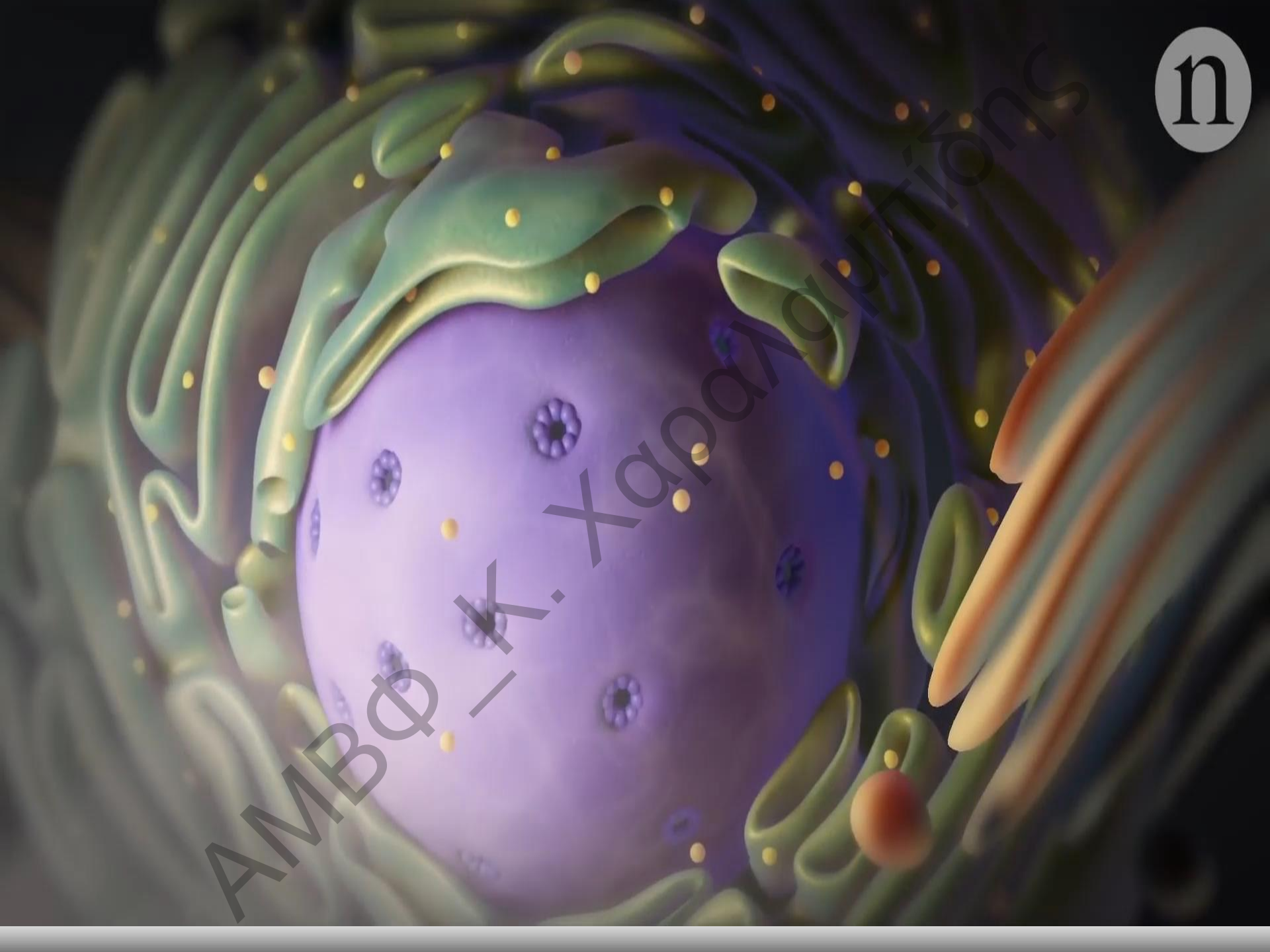
Bálint Csörgő, Lina M. León, Ilea J. Chau-Ly, Alejandro Vasquez-Rifo, Joel D. Berry, Caroline Mahendra, Emily D. Crawford, Jennifer D. Lewis & Joseph Bondy-Denomy

Nature Methods (2020) | Cite this article

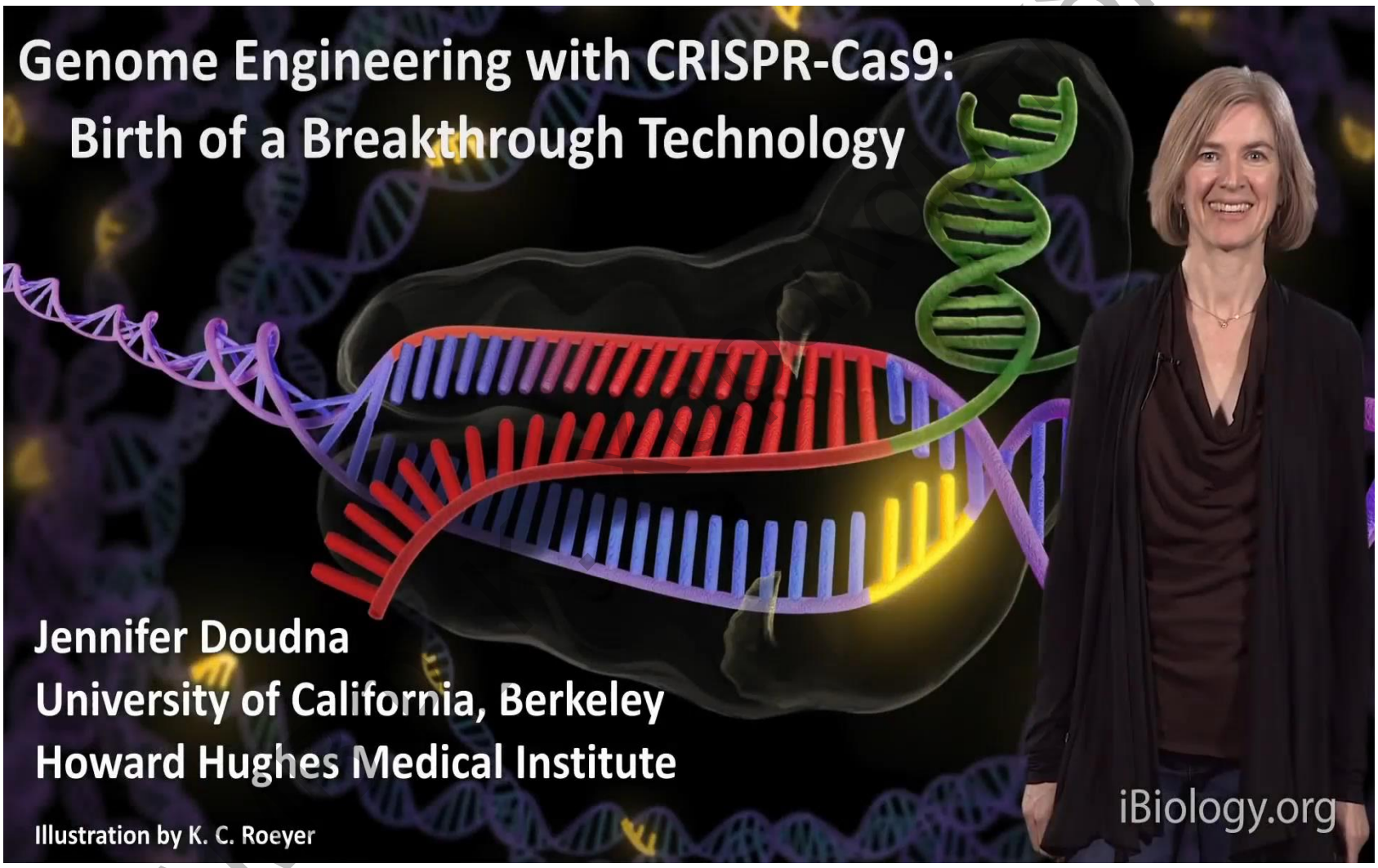
- Cas3 for targeted genome engineering in bacteria. DNA cleavage guided by a single CRISPR RNA generated large deletions (7–424 kilobases) in *Pseudomonas aeruginosa* with near-100% efficiency.
- *P. aeruginosa* Type I-C Cascade–Cas3 (*Pae*Cas3c) facilitates rapid strain manipulation with applications in synthetic biology, genome minimization and the removal of large genomic regions.

CRISPR Technology (Clustered regularly-interspaced short palindromic repeats)

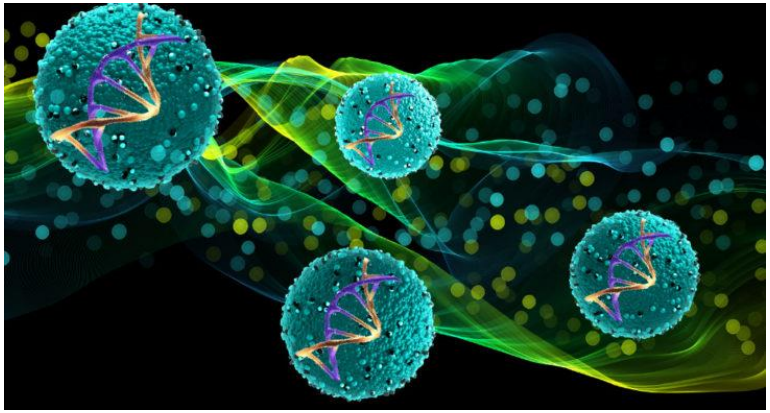




https://www.youtube.com/watch?v=SuAxDVBt7kQ&feature=emb_logo

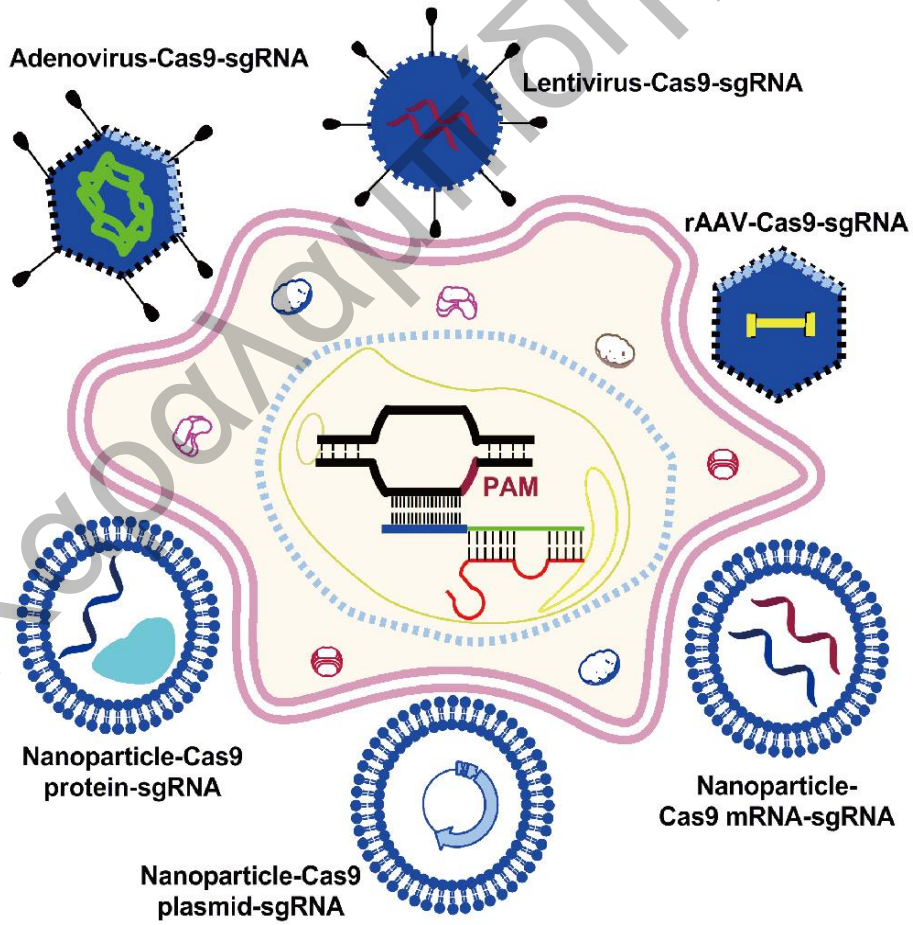


Nanoparticle mediated delivery of CRISPR for treating Tyrosinemia



Instead of using injections or viruses with ~6% success rate…

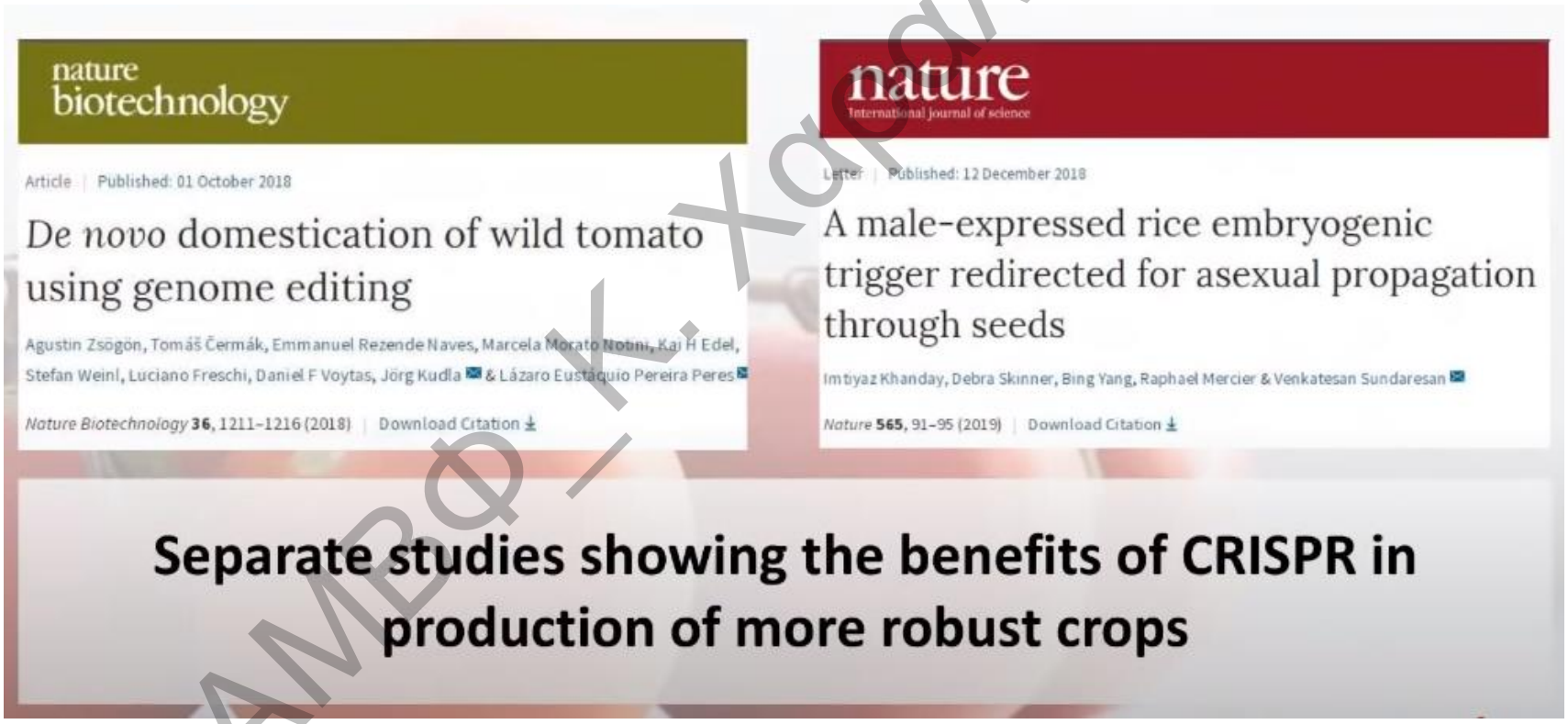
…a new study, MIT researchers have developed nanoparticles that can deliver the CRISPR genome-editing system and specifically modify genes in mice.



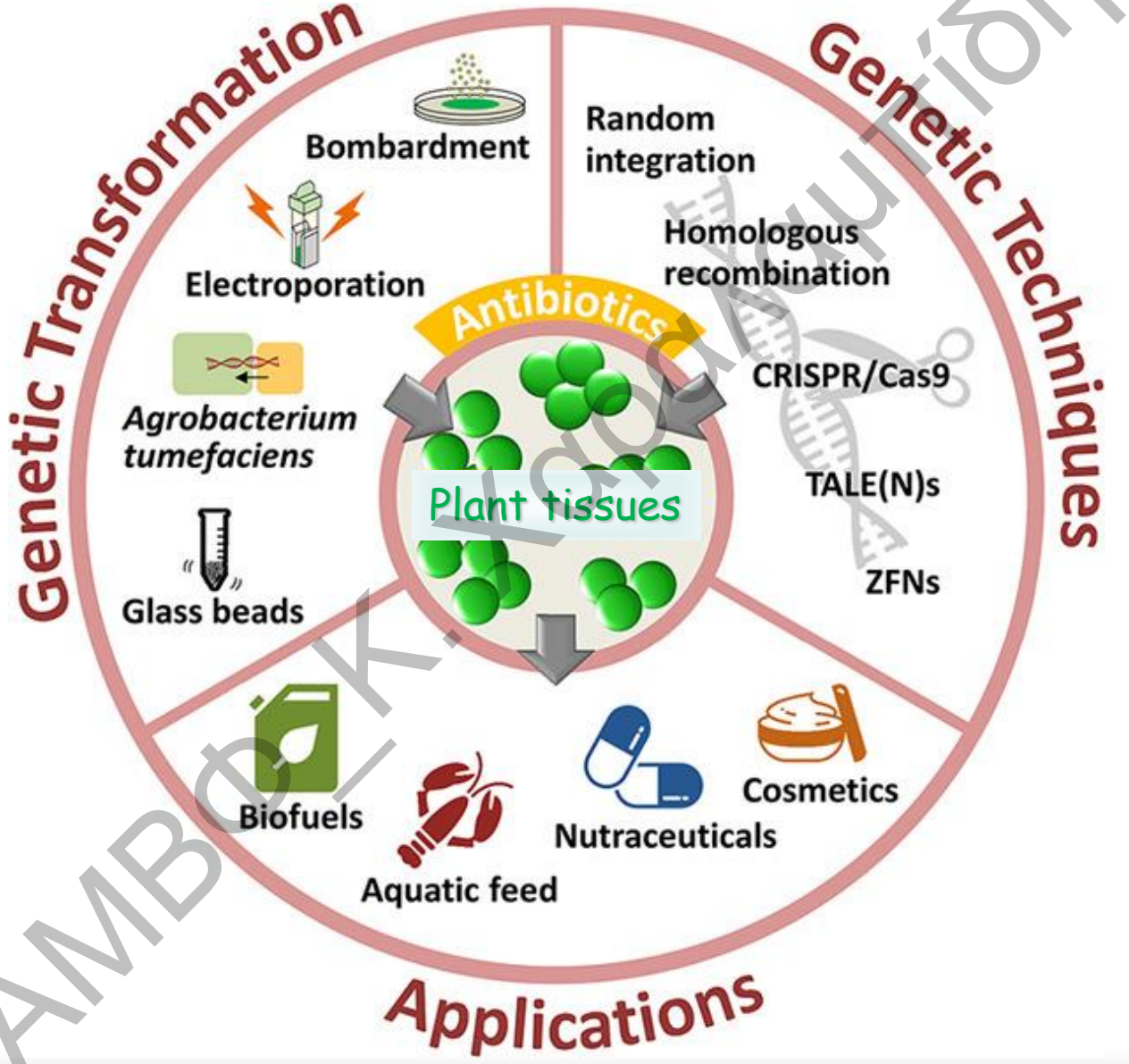
Using this delivery technique, researchers were able to edit certain genes in about 80% of liver cells, the best success rate ever achieved with CRISPR in animals.

Gene editing in plants (ZFN-TALENS-CRISPR)

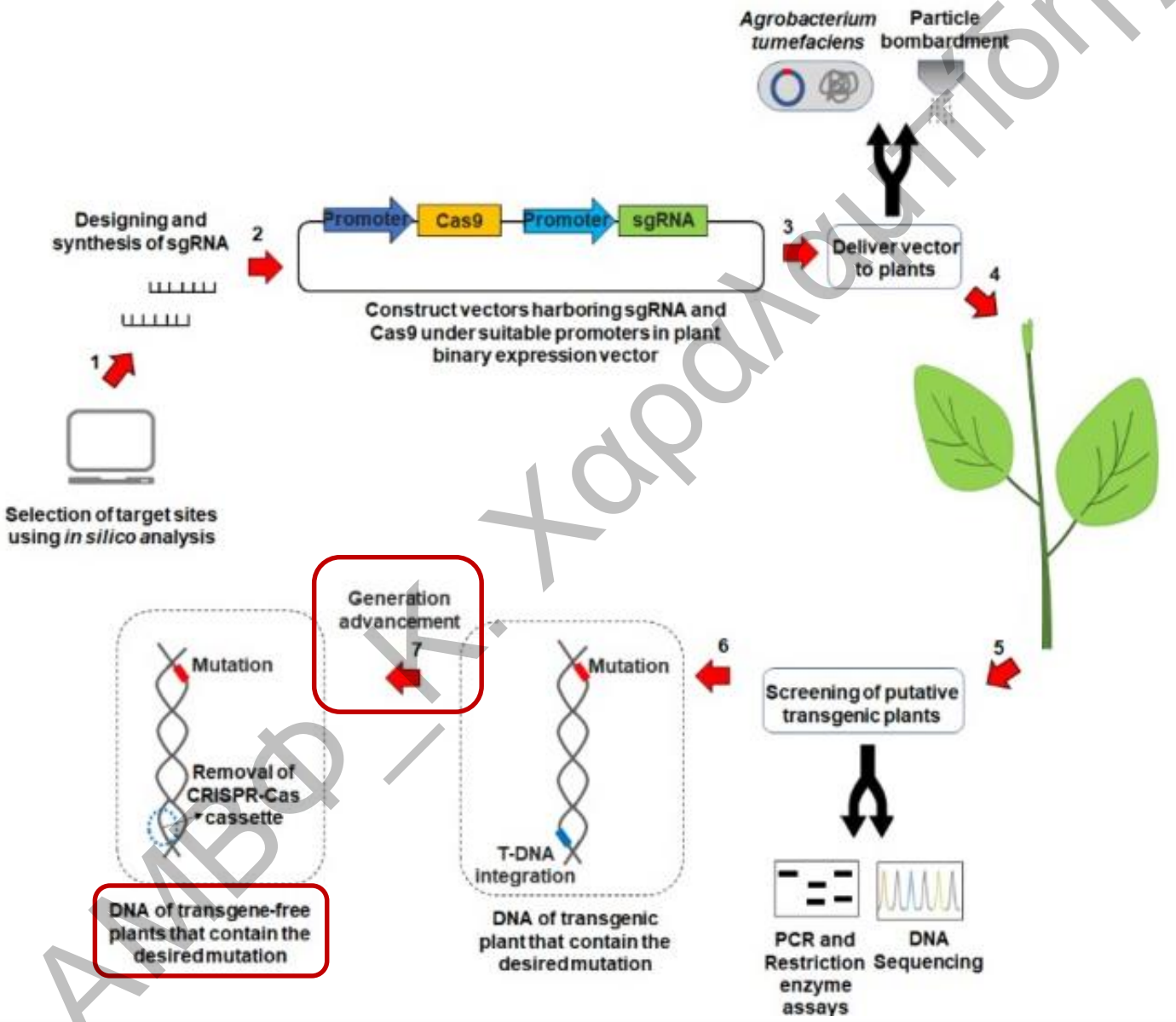
The first- and second-generation genome editing tools are zinc finger nucleases (ZFN), and transcription activator-like effector nuclease (TALENS), which were used in plants in 2005 and 2011, respectively. Yet Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) is the third generation tool used in plants in 2013.



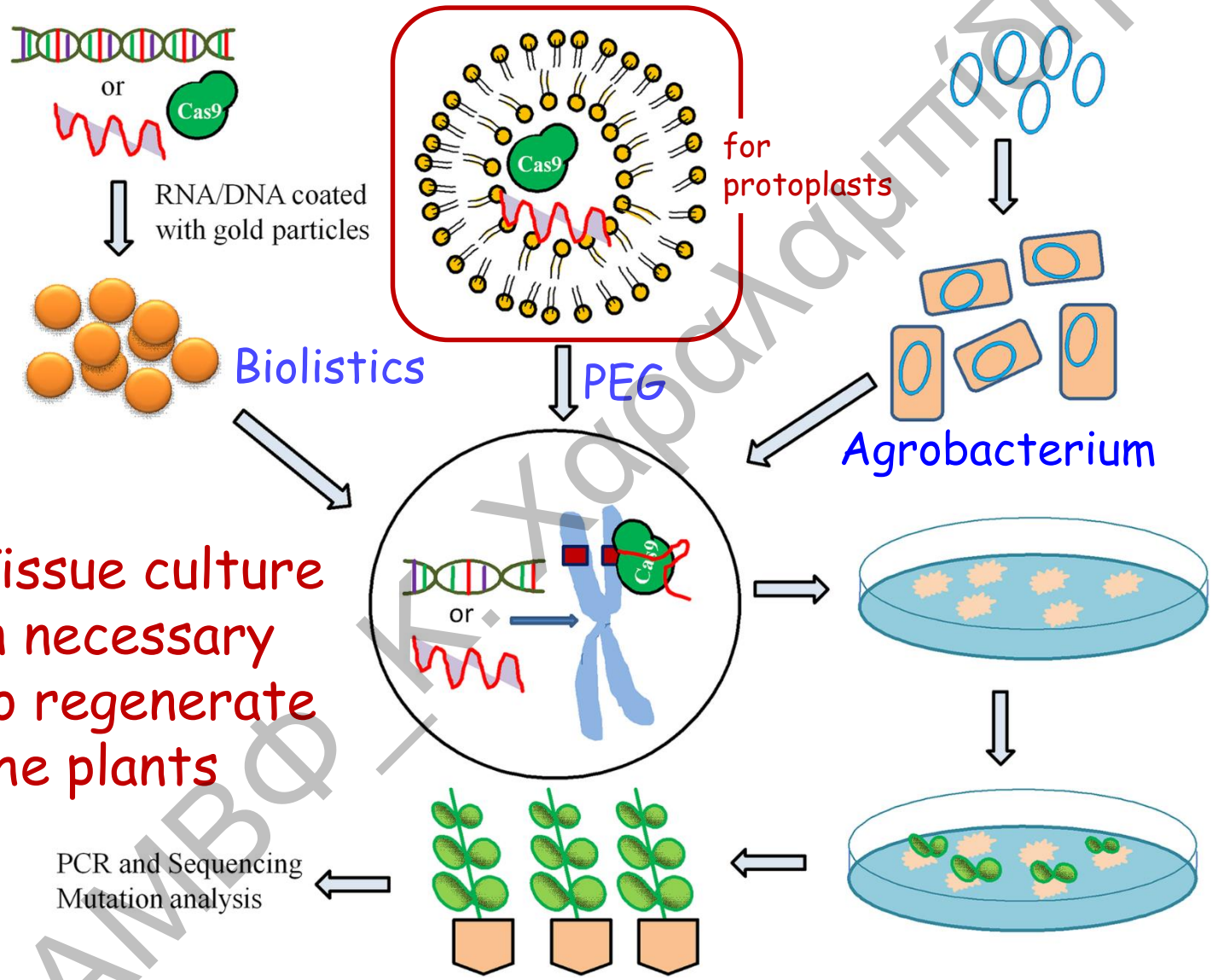
Gene editing in plants (ZFN-TALENS-CRISPR)



Gene Drive in plants (Agrobacterium & Biolistics)



Gene Drive in plants (Biolistics/PEG/Agrobacterium)



Gene Drive in plants (Biolistics/PEG/Agrobacterium)

Agrobacterium

Plant name	CRISPR/Cas9 vector	Selectable marker	Strain	Target genes	Reference
Arabidopsis thaliana	pUC119-RCS	Marker free	GV3101	*AtPDS3, AtFLS2, RACK1b, RACK1c*	[41]
Arabidopsis thaliana	pCAMBIA1300	*HptII*	GV3101	*BRI1, GAI, JAZ1*	[42]
Banana	pRGEB31	*HptII*	AGL1	*RAS-PDS*	[13]
Banana	pRGEB31	*HptII*	AGL1	*LCYε*	[43]
Citrus sinensis	pCas9-GN	*NptII*	LBA4404	*CsWRKY22*	[44]
Cucumis sativum	pRCS	*NptII*	EHA105	*eIF4E, eIF(iso)4E*	[45]
Glycine max	p201N Cas9	*NptII*	K599	*GFP transgene*	[46]
Kiwi fruit	pHLW-sgRNA-Cas9-AtU6-1, pPTG-sgRNA-Cas9-U6-1	*NptII*	EHA105	*AcPDS*	[47]
Lotus japonicus	pCAMBIA1300	*HptII*	EHA105	*LjLb1, LjLb2, LjLb3, LjSYMRK*	[48]
Marchantia polymorpha	pMpGE013 and pMpGE014	*HptII*	–	*MpARF1*	[49]
Medicago trancatula	pMDC32-AtU6-26	*HptII*	ARqual	*GUS*	[50]
Medicago truncatula	pFGC5941	*Bar*	–	*MtPDS*	[51]
Nicotiana benthamaina	pICH86966	–	AGL1	*NbPDS, PDS*	[14]
Nicotiana benthamaina	pUC19, pKQ334	*HptII*	GV3101	*NbPDS3, NbIspH*	[52]
Nicotiana tabaccum	pORE	*NptII*	LBA4404	*NtPDS, NtPDR6*	[53]
Oryza sativum	VK005	*HptII*	EHA105	*ISA1*	[54]
Populous tomentosa	pYLCRIPSR/Cas9, pUC18	*HptII*	–	*PtoPDS*	[55]
Salvia miltiorrhiza	pCAMBIA1300	*HptII*	C58C1	*SmRAS*	[56]
Solanum lycopersicum	pYLCRISPR/Cas9	*HptII*	–	*SGR1, LCY-E, Blc, LCY-B1, LCY-B2*	[57]
Solanum lycopersicum	pENTR-sgRNA: pMR290/Cas9	*NptII*	EHA105	*SlCCD8*	[58]
Solanum tuberosum	pMDC32	*HptII*	–	*StALS1*	[59]
Sorghum bicolor	pVS1 binary vector derived from pLH7500	*NptII*	Y158	*DsRED2*	[20]
Triticum aesituvam	pBI121	*NptII*	GV3101	*Inox, PDS*	[14]
Zea mays	pMCG1005	*Bar*	EHA101	*Argonaute 18, Dihydroflavonol-4-reductase*	[60]

Gene Drive in plants (Biolistics/PEG/Agrobacterium)

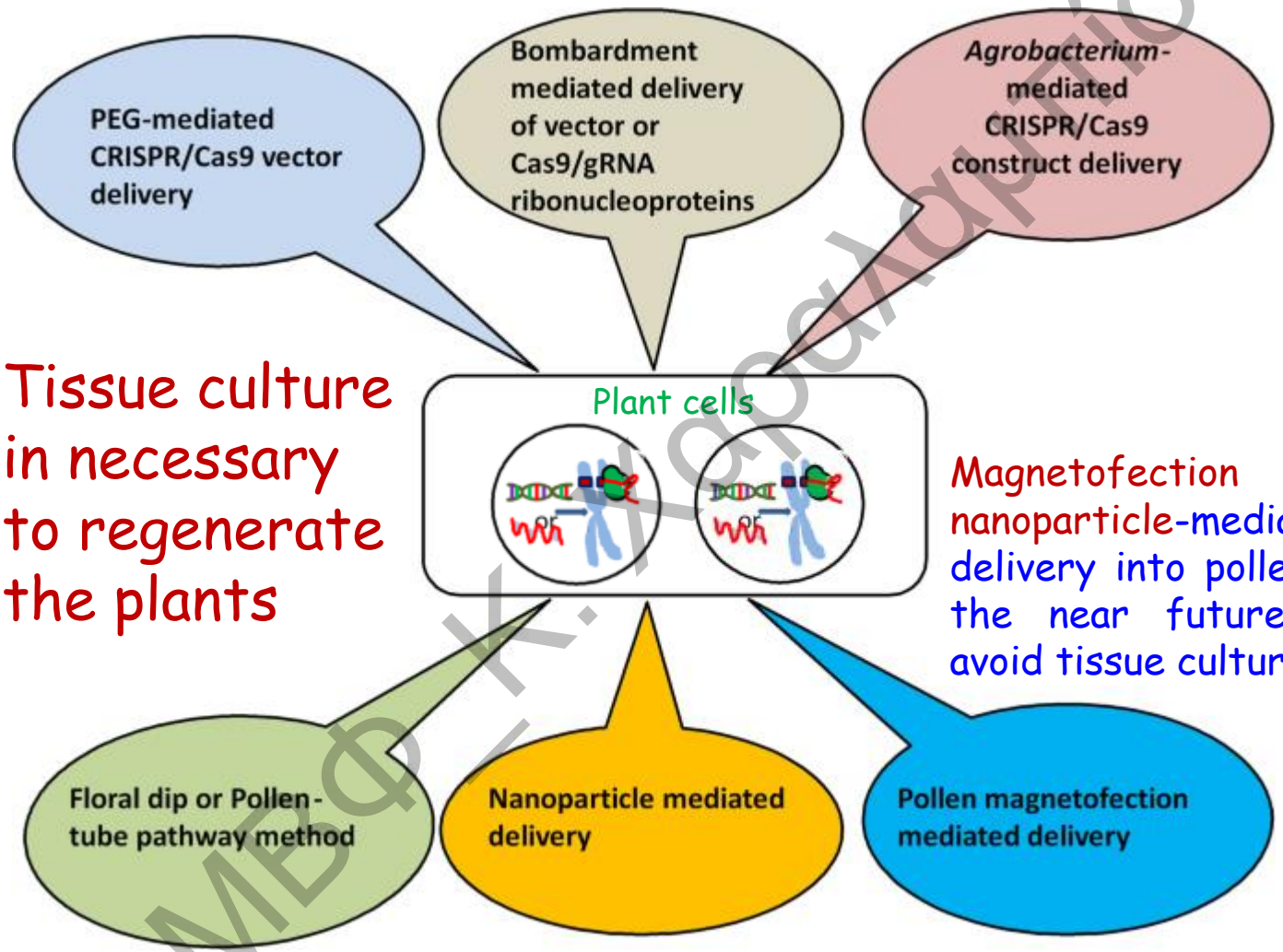
Biolistics

Plant name	CRISPR/Cas9 vector or RNP complex	Selectable marker	Target genes
Glycine max	QC810 and RTW830, QC799 and RTW831	*HptII*	*DD20, DD43*
Hordeum vulgare	pcas9:sgRNA	*HptII*	ENGase
Oryza sativum	pCam1300-CRISPR-B	*HptII*	*crtI, ZmPsy*
Oryza sativum	CRISPR-RNP complex	*HptII*	*OsPDS1*
Oryza sativum	pJIT163-2NLSCas9	*HptII*	*OsPDS, OsBADH2*
Oryza sativum	pOsU3-sgRNA, pJIT163-2NLSCas9	*HptII*	*OsPDS, OsDEP1*
Triticum aesituvam	pJIT163-Ubi	*bar*	*TaMLO-A1, TaMLO-B1, TaMLO-D1*
Zea mays	pSB11-Ubi:Cas9	*Pat*	*LIG1, Ms26, Ms45, ALS1, ALS2*

PEG (protoplasts)

Plant name	CRISPR/Ca9 vector or ribonucleoprotein complexes	Targeted genes
Apple	Cas9-sgRNA ribonucleoprotein complexes	*DIPM-1, 2,* 4
Brassica oleracea, Brassica rapa	Cas9-sgRNA ribonucleoprotein complexes	*FRI, PDS*
Citrullus lanatus	PHSN1, PHSN2	*ClPDS*
Glycine max	pCas9-GmU6-sgRNA, pCas9-AtU6-sgRNA	*Glyma08g02290, Glyma12g37050, Glyma06g14180*
Grapevine	Cas9-sgRNA ribonucleoprotein complexes	*MLO-7*
Oryza sativum	pRGE3, pRGE6	*OsMPK5*
Oryza sativum	pUC19-OsCas9	*OsSWEET14, OsSWEET11*
Oryza sativum	pJIT163-2NLSCas9	*OsPDS, OsBADH2*
Petunia	Cas9-sgRNA ribonuclease protein complexes (RNPs)	*PhACO1*
Physcomitrella patens	pAct-Cas9, psgRNA PpAPT-KO4, PpAPT-KO7	*PpAPT*
Solanum tuberosum	Cas9-sgRNA Ribonucleoprotein complexes (RNPs)	*GBSS (GT4)*
Triticum aestivum	pCR8-U6-gRNA	*TaEPSPS*
Zea mays	p ZmU3-gRNA	*ZmIPK*
Zea mays	CT-nCas9	*ZmALS1, ZmALS2*

Existing and potential future CRISPR/Cas9 delivery methods



Tissue culture in necessary to regenerate the plants

Magnetofection and nanoparticle-mediated delivery into pollen in the near future to avoid tissue culture

Multiplex genome editing studies in plants (2020)

Species	System and strategy	Number and identity of target genes	Number of target sites	Notes on mutations	Application	Reference
Dicots						
Arabidopsis	CRISPR/Cas9 with single gRNA	Three paralogous RPL10 subunit genes	One in each gene	Notable because the target genes had different PAMs	Methodology	[11•]
	CRISPR/Cas9 with six gRNAs	Six *PYR/PYL* paralogs	One in each gene	Primarily indels. One of 15 lines mutated in all six targets	Hormone signaling	[27]
Tobacco (*N. tabacum*)	CRISPR/Cas9 with nine gRNAs	Six (2x XylT and 4x FucT) but exist as homeoalleles so 12 in total	Three consensus sites each in the XylT and FucT.AB FucT.CD sequences	Primarily indels. Lines recovered with mutations in all 12 targets	Molecular farming	[37•]
	CRISPR/Cas9 with five gRNAs	Seven (2x XylT and 5x FucT) but exist as homeoalleles so 14 in total	Five (one consensus for XylT, two consensuses for FucT.A-C, two consensuses for FucT.DE)	Primarily indels. Lines recovered with mutations in all 14 targets	Molecular farming	[38•]
Tobacco (*N. benthamiana*)	Two TALEN pairs	Two XylT genes and two FucT genes	One module for each gene family	Primarily indels. Mutations recovered in all targets	Molecular farming	[36•]
	CRISPR/Cas9 with seven gRNAs	Two XylT genes and four FucT genes	Three consensus sites each in the XylT and FucT.12 sequences. One further target in the conserved part of all four FucT genes	Primarily indels. Mutations recovered in all targets	Molecular farming	[39•]
Tomato	CRISPR/Cas9 with six gRNAs	Five genes: *GABA-TP1*, *GABA-TP2*, *GABA-TP3*, *CAT9* and *SSADH*	One in each gene except GABA-TP1 with two	*GABA-TP2* which was not edited perhaps because of the high GC content of the target site	Metabolism	[21]
	CRISPR/Cas9 with six gRNAs	Five genes: *SGR1*, *LCY-E*, *Blc*, *LCY-B1* and *LCY-B2*	One in each gene except SGFR1 with two	Indels recovered in all five genes	Metabolism	[22]
	CRISPR/Cas9 with eight gRNAs	*CLV3* promoter	Eight targets in promoter	Allelic series recovered	Development	[40••]
Potato	CRISPR/Cas9 with nine gRNAs	*St16DOX*	Nine	Indels and larger deletions spanning target sites	Metabolism	[23]
	CRISPR/Cas9 with three gRNAs	*GBSS1*	Three	Primarily indels	Metabolism	[24]
Rapeseed	CRISPR/Cas9 with two gRNAs	Four paralogs of RGA family	Two sites in each gene	Primarily indels. All four genes mutated in 86% of the recovered lines	Hormone signaling	[26]
	CRISPR/Cas9 with two gRNAs	BnaA9.FUL, BnaC2.FUL and BnaC7.FUL	Two, targeting conserved sites in each gene	Indels, more than half the recovered lined were mutated in all three genes	Development	[26]
	CRISPR/Cas9 with three gRNAs	Both homeologs of CLV1, CLV2 and CLV3	One in each gene	Primarily indels	Development	[31]
	CRISPR/Cas9 with single gRNA	Two homeologs of ALCATRAZ (ALC)	Two	Primarily indels	Development	[52]
Cotton	CRISPR/Cas9 with two gRNAs	Two homeologs: GhMYB25-like A and GhMYB25-like D	Two in each gene	Primarily indels	Development	[53]
	CRISPR/Cas9 with six gRNAs	Two homeologs of *GhCLA1*, also *GhEF1* and *GhPDS* simultaneously.	Two in each gene	Primarily indels	Development	[54]
	CRISPR/Cas9 with two gRNAs	Two homeologs of *GhCLA1*	Two in each gene	Primarily indels	Development	[55]
Grapevine	CRISPR/Cas9 with four gRNAs	*VvWRKY52*	Four	Indels and larger deletions	Fungal resistance	[20]

Multiplex genome editing studies in plants (2020)

Table 1 (*Continued*)

Species	System and strategy	Number and identity of target genes	Number of target sites	Notes on mutations	Application	Reference
Morning glory	CRISPR/Cas9 with multiple gRNAs	One: *EPHEMERAL1* (*EPH1*)	Three	Indels at individual targets and also deletions spanning the targets	Development	[56]
Monocots						
Rice	CRISPR/Cas9 with single gRNA	Three paralogous cyclin-dependent kinase paralogs	One in each gene		Development	[9]
	CRISPR/Cas9 with three gRNAs	*OsGSTU*, *OsMRP15* and *OsAnP*	One in each gene		Metabolism	[14•]
	CRISPR/Cas9 with three gRNAs	*OsWaxy*	Three	Lines recovered with mutations in one or two but not all three sites	Metabolism	[14•]
	CRISPR/Cas9 with 11 gRNAs	Eleven paralogs of the FT-like family	One in each gene	Indels recovered in 10 of 11 targets. Maximum 7 simultaneous mutations	Development	[14•]
	CRISPR/Cas9 with two gRNAs	*ALS1*	Two	HDR-mediated allele replacement in background of NHEJ events	Herbicide resistance	[18]
	CRISPR/Cas9 with two gRNAs	*ESPS*	Two	Target sites flanked an excision cassette which was replaced with a cassette released from the donor plasmid using the same gRNAs	Herbicide resistance	[19•]
	CRISPR/Cas9 with five gRNAs	*Hd2*, *Hd4* and *Hd5*	Two in *Hd2* and *Hd4*, one in *Hd5*	Mostly single-nucleotide indels. Among 18 lines, 14 carried mutations in all three targets	Development	[28]
	CRISPR/Cas9 with three gRNAs	*GW2*, *GW5* and *TGW6*	One in each gene	Mostly indels. Among 21 lines, 20 carried mutations in all three targets	Development	[29]
	CRISPR/Cas9 with eight gRNAs	*MPK1*, *MPK2*, *MPK5* and *MPK6*	Two in each gene	Mostly indels, individual, pairwise and quadruple mutants recovered	Development	[30]
Wheat	CRISPR/Cas9 with two gRNAs	The α-gliadin gene family (100 members)	Two consensus targets	Wide range of mutants recovered, including one line with simultaneous mutations in 35 different genes	Development	[33]
	TALENs, single module	Three *MLO* homeologs	One in each gene		Disease resistance	[8•]
	CRISPR/Cas9 with single gRNA	Three *EDR1* homeologs	One in each gene		Disease resistance	[10]
Barley	CRISPR/Cas9 with two gRNAs	Two paralogs of *HvPM19* gene family	One in each gene		Hormone signaling	[25]
Sorghum	CRISPR/Cas9 with single gRNA	Twenty paralogs of the *k1C* family	One conserved target	Among 26 events, 72 plants contained mutations in multiple targets including one line with 11 mutations in distinguishable k1C genes and another in the *k1C3–9* group	Development	[32•]
Sugarcane	One TALEN pair	The *COMT* gene family (109 unique copies)	One highly conserved site	Wide range of multiple mutations achieved, including one line with 107 co-mutations	Metabolism	[6••]

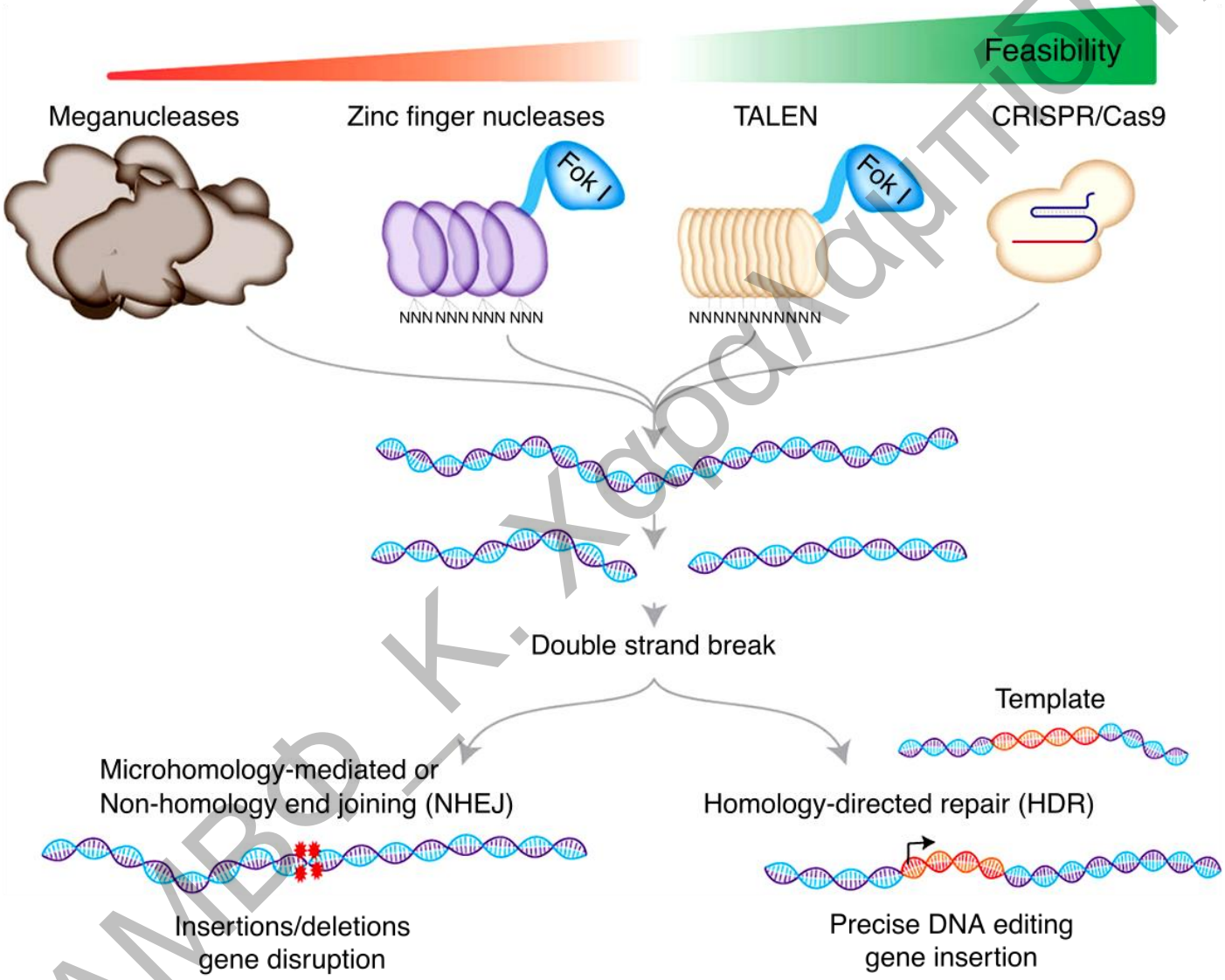
CRISPR Gene editing in plants for the environment

Gene-editing CRISPR technique can help us cut emissions from farming

There are risks to using CRISPR, but also to not embracing it, because it will be much harder to reduce greenhouse gas emissions from food production without gene editing

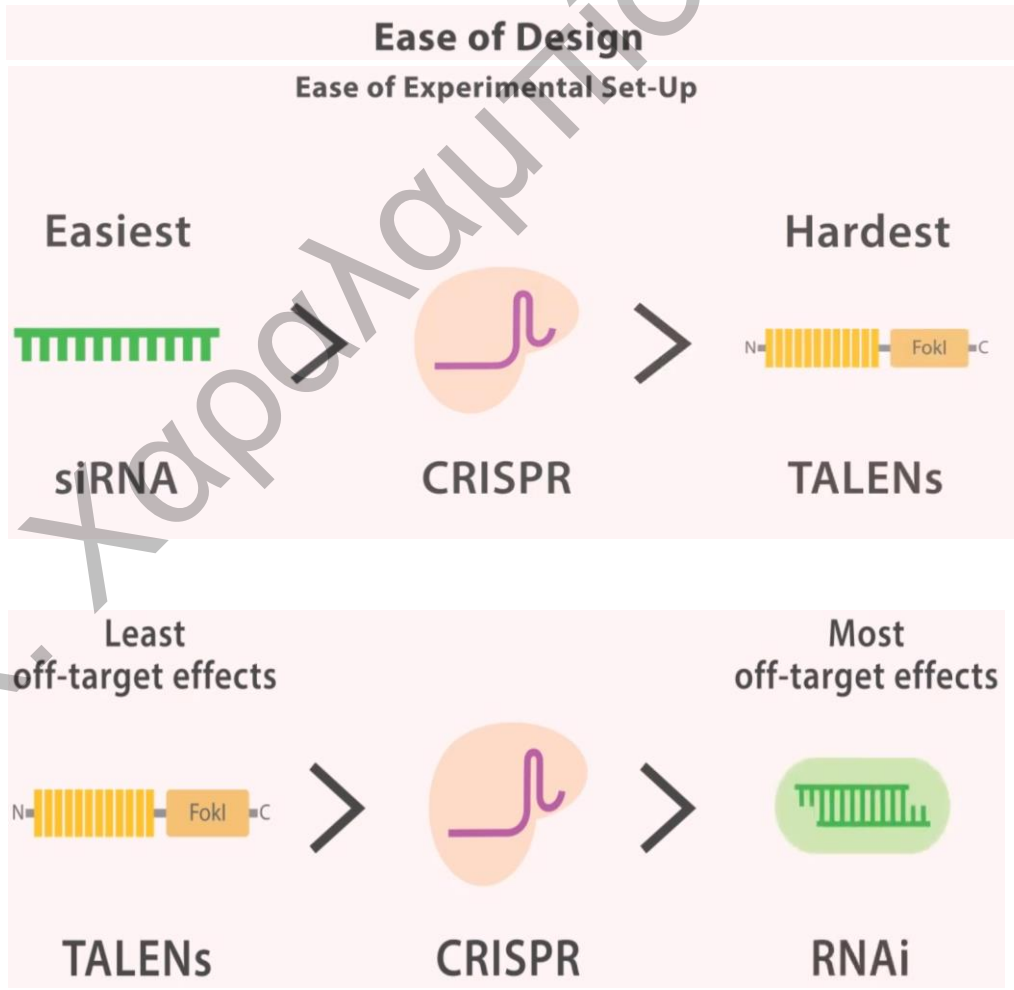
EARTH 7 October 2020

Nucleases used for genome editing and DNA repair



Gene editing in plants (RNAi-ZFN/TALENS-CRISPR)

CRISPR carries the most promise. TALENs produce fewer off-target edits than ZFNs or CRISPR-Cas9 or even RNAi, but that isn't a large enough benefit to offset the specificity of CRISPR-Cas9. ZFN and TALEN technologies require complex proteins to be engineered with a high cost of use. Thus, numerous investigators who previously used ZFNs and TALENs have switched to modern CRISPR platforms.



CRISPR legislation…

The 'problem' - transgenics to genome editing

2000

'recombinant DNA, genetic modification'

transgenics

= 'GMO' ✓

cisgenesis, intragenesis

2018

'genome editing'

oligo–directed mutagenesis (ODM),

NPBTs

CRISPR, ZFN (SDNs)

= 'GMO' ?

CRISPR legislation…

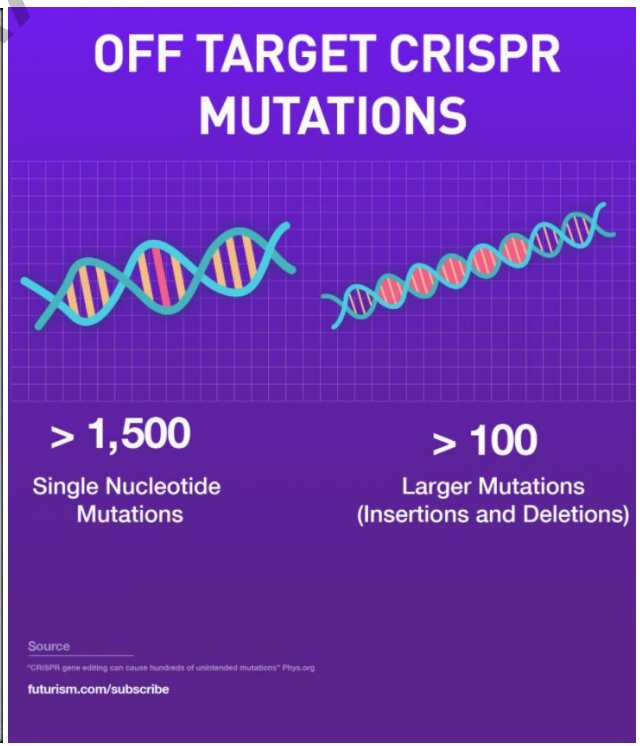
Following the earlier request from the French government, Advocat General Michal Bobek, who led the Court of Justice of the European Union case, released a statement in early 2018 regarding the proposed changes to the regulations. He suggested that while crops that have undergone gene editing should be considered GMOs, they could be exempted from strict regulation if no foreign DNA was inserted.

Despite this, and contrary to scientific advice, the court ruled that only techniques that have "conventionally been used in a number of applications and have a long safety record" should be exempt from GMO regulations.

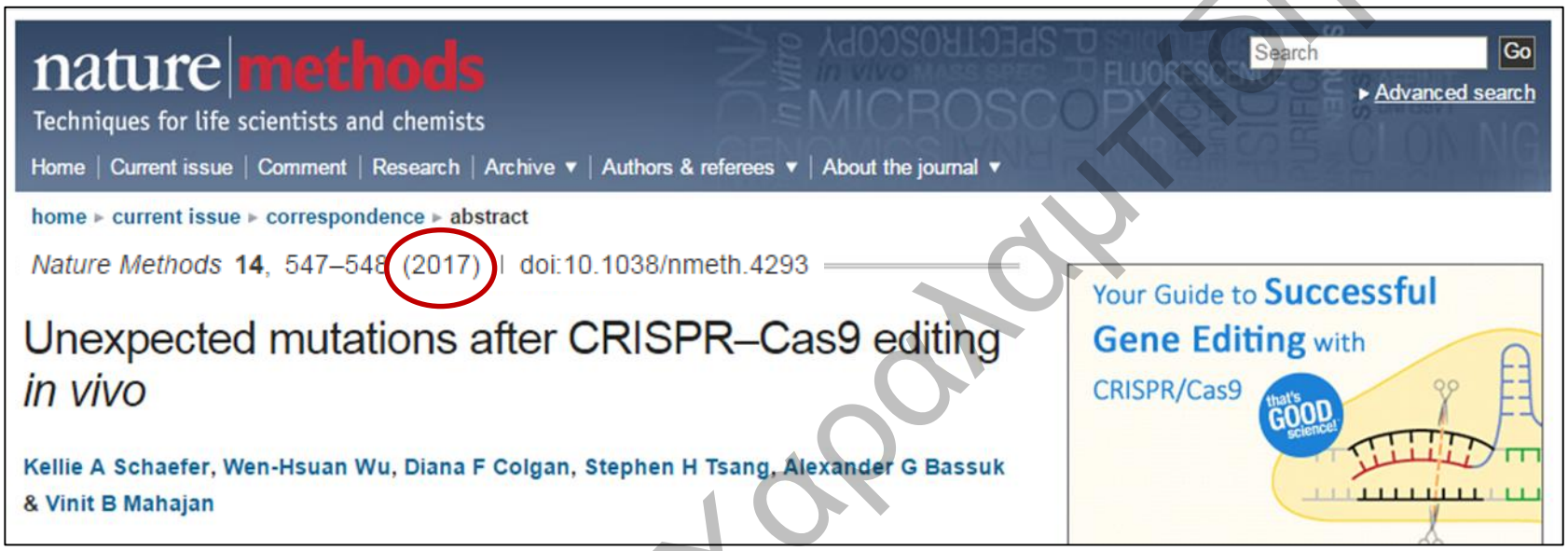
This means that any technique developed since the regulation came into being, including CRISPR gene editing, is now subject to the same regulations as GMOs. In contrast, older and less precise techniques, such as exposing plants to radiation to trigger random mutations, are not.

…however…

…scientists all over the world have questioned the precision of CRISPR/Cas9 gene editing technology, indicating undesired "**on target**" deletions and rearrangements (up to 25%) at the site of the gene of interest, as well as numerous "**off target**" edits.



CRISPR Technology pitfalls



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Techniques for life scientists and chemists

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Nature Methods **14**, 547–548 (2017) | doi:10.1038/nmeth.4293

Unexpected mutations after CRISPR–Cas9 editing *in vivo*

Kellie A Schaefer, Wen-Hsuan Wu, Diana F Colgan, Stephen H Tsang, Alexander G Bassuk & Vinit B Mahajan

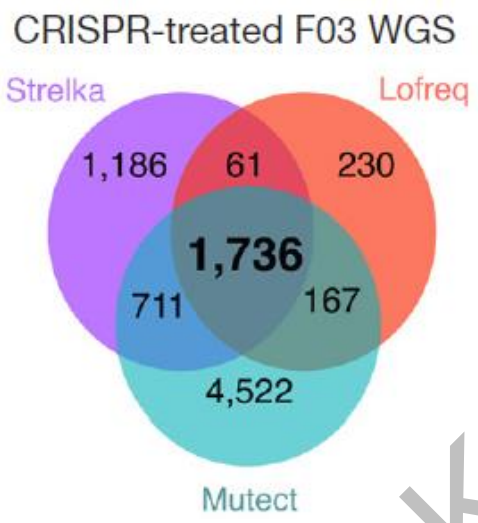
Your Guide to **Successful Gene Editing** with CRISPR/Cas9

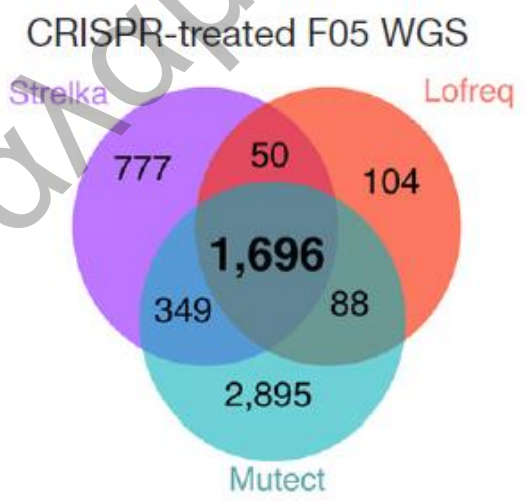
that's GOOD science!

- Whole-genome deep sequencing on CRISPR-treated mice, showed a very large number of single nucleotide variants (SNVs), about ~1700 per mouse, caused by CRISPR.
- Most of these mutations were not indels and were not associated with sites homologous to the CRISPR gRNA, many of which were in protein-coding and non-coding RNA genes.
- So it is very difficult to tell whether phenotypes found in a CRISPR-based approach are the result of the targeted mutation or off-target random non-relevant mutations.
- The new results by Schaefer et al. seriously challenge the utility of CRISPR as a tool.

Unexpected mutations after CRISPR–Cas9 editing *in vivo*

NATURE METHODS | VOL.14 NO.6 | JUNE 2017 | 547





Variant type	CRISPR-treated F03 mouse	CRISPR-treated F05 mouse	Identical variants in CRISPR-treated mice
WGS SNVs	1,736	1,696	1,397
WGS indels	164	128	117
Exon SNVs	60	51	39
Exon indels	6	3	2

CRISPR Technology pitfalls

MENU nature methods

Retraction | Published: 27 April 2018

Retraction Note: Unexpected mutations after CRISPR–Cas9 editing *in vivo*

Kellie A Schaefer, Wen-Hsuan Wu, Diana F Colgan, Stephen H Tsang, Alexander G Bassuk & Vinit B Mahajan

Nature Methods **15**, 394(2018) | Cite this article

The original article was published on 30 May 2017

CRISPR Technology pitfalls

GENETICS

New Study Finds Unintended Consequences of CRISPR Gene Editing

Ryan F. Mandelbaum
7/18/18 2:40pm • Filed to: CRISPR

34.3K 26 Save

Scientists and have heralded the CRISPR-Cas9 gene editing system as revolutionary way to edit DNA.

Studies found some potentially harmful unintended effects.

This isn't the first time a paper has found flaws in CRISPR's abilities, though one previous paper on the matter has been retracted. But many feel this new research is worth taking seriously, and that CRISPR might cause large, unexpected deletions to a cell's genome.

sciencealert

nature biotechnology

BREAKING: CRISPR Could Be Causing Extensive Mutations And Genetic Damage After All

PETER DOCKRILL 16 JUL 2018

Letter | Published: 16 July 2018

Repair of double-strand breaks induced by CRISPR–Cas9 leads to large deletions and complex rearrangements

Michael Kosicki, Kärt Tomberg & Allan Bradley ✉

Nature Biotechnology **36**, 765–771 (2018) | Download Citation ⬇

CRISPR has been heralded as one of the most important breakthroughs in modern science, but there could be a hidden and potentially dangerous side effect to the wonders of its genetic editing technology, a new study reveals.

CRISPR Technology pitfalls

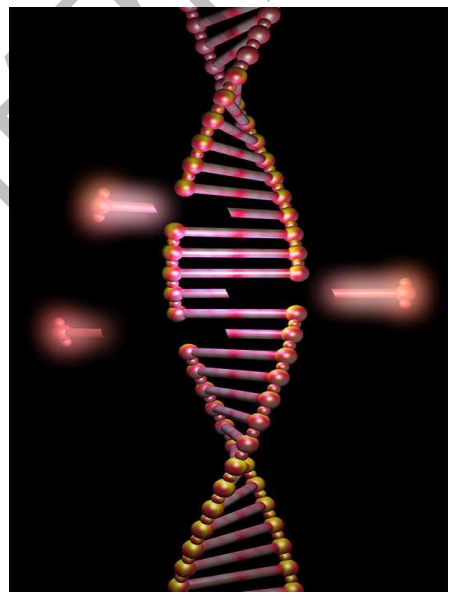
nature
International journal of science

Article | Published: 25 October 2017

Programmable base editing of A•T to G•C in genomic DNA without DNA cleavage

Nicole M. Gaudelli, Alexis C. Komor, Holly A. Rees, Michael S. Packer, Ahmed H. Badran, David I. Bryson & David R. Liu

Nature **551**, 464–471 (23 November 2017) | Download Citation



Liu's team has added a different enzyme to the modified CRISPR protein to create a base editor that changes A into G and T into C. The team evolved the enzyme by setting up a system in which bacteria had to evolve it, to survive.

The new base editor (ABE7) works extremely well. In a series of tests, it made the desired DNA letter change **in over half of human cells**, with **hardly** any unwanted mutations. This makes ABE7 **safer** than standard CRISPR, **minimising** the risk of mutations that could cause cancer, for instance.

CRISPR Technology pitfalls

Science

REPORTS

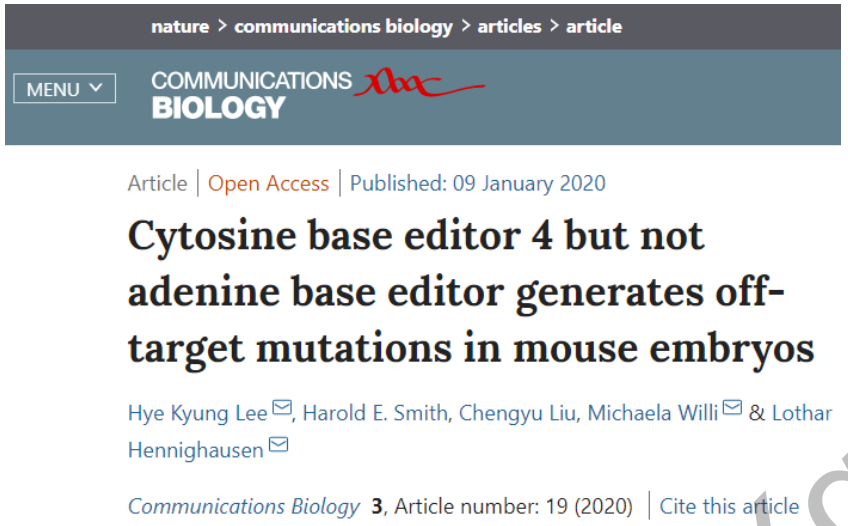
Cite as: E. Zuo *et al.*, *Science* 10.1126/science.aav9973 (2019).

Cytosine base editor generates substantial off-target single-nucleotide variants in mouse embryos

Erwei Zuo[1,2]*, Yidi Sun[3]*, Wu Wei[3,4,5]*, Tanglong Yuan[2]*, Wenqin Ying[1], Hao Sun[6], Liyun Yuan[3], Lars M. Steinmetz[4,7,8]†, Yixue Li[3,9,10]†, Hui Yang[1]†

Genome editing holds promise for correcting pathogenic mutations. However, it is difficult to determine off-target effects of editing due to single nucleotide polymorphism in individuals. Here, we developed a method named GOTI (Genome-wide Off-target analysis by Two-cell embryo Injection) to detect off-target mutations by editing one blastomere of two-cell mouse embryos using either CRISPR-Cas9 or base editors. Comparison of the whole genome sequences of progeny cells of edited vs. non-edited blastomeres at E14.5 showed that off-target single nucleotide variants (SNVs) were rare in embryos edited by CRISPR-Cas9 or adenine base editor, with a frequency close to the spontaneous mutation rate. In contrast, cytosine base editing induced SNVs with over 20-fold higher frequencies, requiring a solution to address its fidelity.

CRISPR Technology pitfalls



nature > communications biology > articles > article

MENU

COMMUNICATIONS BIOLOGY

Article | Open Access | Published: 09 January 2020

Cytosine base editor 4 but not adenine base editor generates off-target mutations in mouse embryos

Hye Kyung Lee, Harold E. Smith, Chengyu Liu, Michaela Willi & Lothar Hennighausen

Communications Biology **3**, Article number: 19 (2020) | Cite this article

Deaminase base editing[1,2] directly converts target C·G base pairs to T·A by cytosine base editors (CBE), or target A·T base pairs to G·C by adenine base editors (ABE), without inducing double-stranded DNA breaks[3]. Since the majority of known human pathogenic variants are single-nucleotide alterations[2,4], base editing has been heralded as a high-fidelity tool to correct single-nucleotide polymorphisms (SNPs) associated with many human disorders.

While exceptional precision is paramount in a quest to correct somatic and in particular germline mutations, recent studies have revealed that CBEs can induce bystander mutations, including deletions, in mouse zygotes[5] and plants[6]. In contrast, ABE displays a greater fidelity[5,7], even though unexpected C-to-T conversions have been observed with ABE at some target sites[5,8].

CRISPR Technology and its first applications (LCA)

SCIENCE ADVANCES | RESEARCH ARTICLE

October 2019

HEALTH AND MEDICINE Leber Congenital Amaurosis

CRISPR-Cas9–mediated therapeutic editing of *Rpe65* ameliorates the disease phenotypes in a mouse model of Leber congenital amaurosis

Dong Hyun Jo[1]*, Dong Woo Song[2]*, Chang Sik Cho[1], Un Gi Kim[2], Kyu Jun Lee[2], Kihwang Lee[3], Sung Wook Park[1†], Daesik Kim[4], Jin Hyoung Kim[1], Jin-Soo Kim[4], Seokjoong Kim[2], Jeong Hun Kim[1,5,6‡], Jung Min Lee[2,7‡]

Doctors try 1st CRISPR editing in the body for blindness

By MARILYNN MARCHIONE March 4, 2020

BioTechniques

March 2020

CRISPR pitfalls of EYS gene editing (Autosomal Recessive Retinitis Pigmentosa)

Cell

ARTICLE | ONLINE NOW

Allele-Specific Chromosome Removal after Cas9 Cleavage in Human Embryos

Michael V. Zuccaro [8] • Jia Xu [8] • Carl Mitchell • ... Rogerio Lobo • Nathan Treff • Dieter Egli [9] •
Show all authors • Show footnotes

Published: October 29, 2020 • DOI: https://doi.org/10.1016/j.cell.2020.10.025

Large-scale damage was found in a study that analyzed **human embryos** that had been edited with CRISPR-Cas9. The Columbia University Irving Medical Center, showed that applying CRISPR-Cas9 to repair **EYS gene** early in the development of a human embryo eliminates an entire chromosome or a large section of it.

About half the embryos seemed unable to cope with the trauma of the break. The genetic damage failed to heal, eventually forcing cells to tear off and toss aside large chunks of the chromosome that harbored the mutated EYS. In some cells, the entire chromosome was lost.

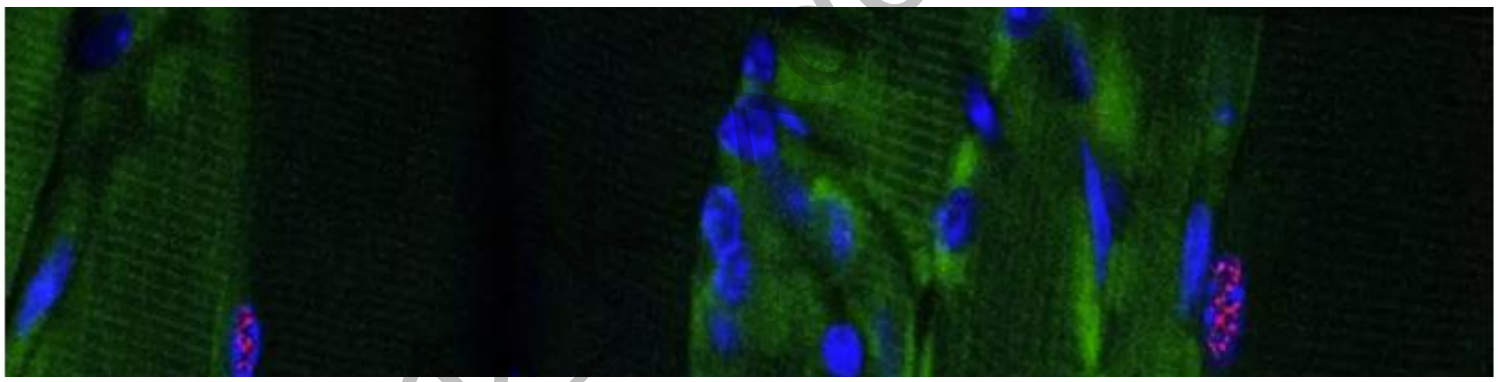
CRISPR Technology and its first applications (MD)

nature biomedical engineering

Article | Published: 14 September 2020

The sustained expression of Cas9 targeting toxic RNAs reverses disease phenotypes in mouse models of myotonic dystrophy type 1

Ranjan Batra, David A. Nelles, Daniela M. Roth, Florian Krach, Curtis A. Nutter, Takahiro



Sufferers of this disease inherit repeating DNA segments that cause a toxic accumulation of repetitive RNA, which causes the deterioration of muscles and increasing weakness characteristic of the disease.

Huntington's and ALS, which are also caused by similar RNA buildup, to follow.

CRISPR Technology and its first applications

CRISPR system in mice eliminates tumor cells without affecting healthy cells

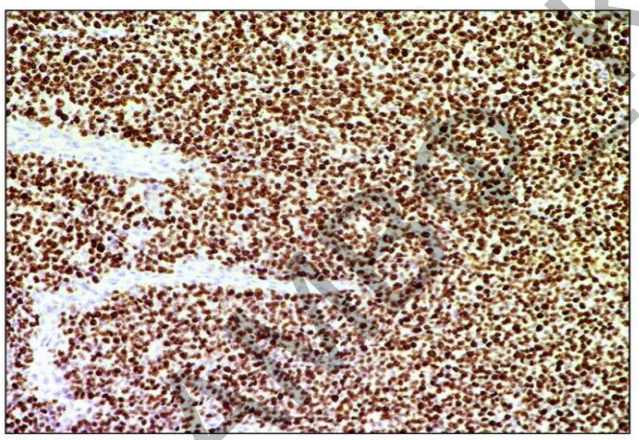
Article | Open Access | Published: 08 October 2020

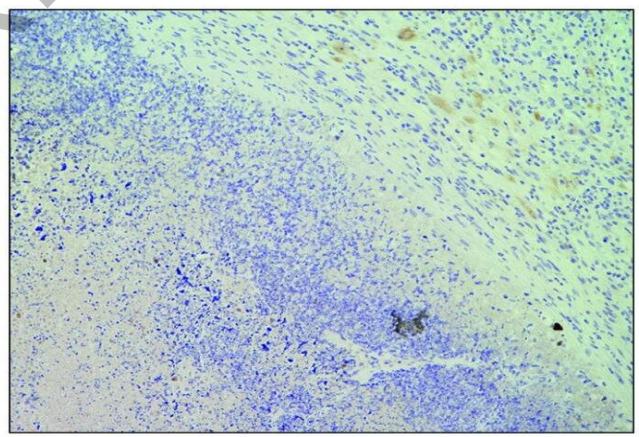
In vivo CRISPR/Cas9 targeting of fusion oncogenes for selective elimination of cancer cells

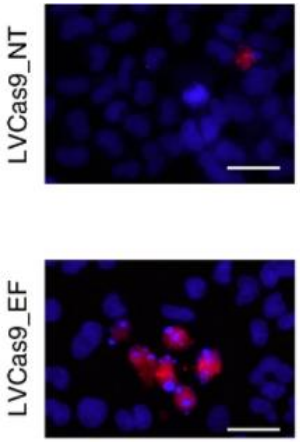
…by destroying the fused oncogene created by translocation

M. Martinez-Lage, R. Torres-Ruiz ✉, P. Puig-Serra, P. Moreno-Gaona, M. C. Martin, F. J. Moya, O. Quintana-Bustamante, S. Garcia-Silva, A. M. Carcaboso, P. Petazzi, C. Bueno, J. Mora, H. Peinado, J. C. Segovia, P. Menendez & S. Rodriguez-Perales ✉

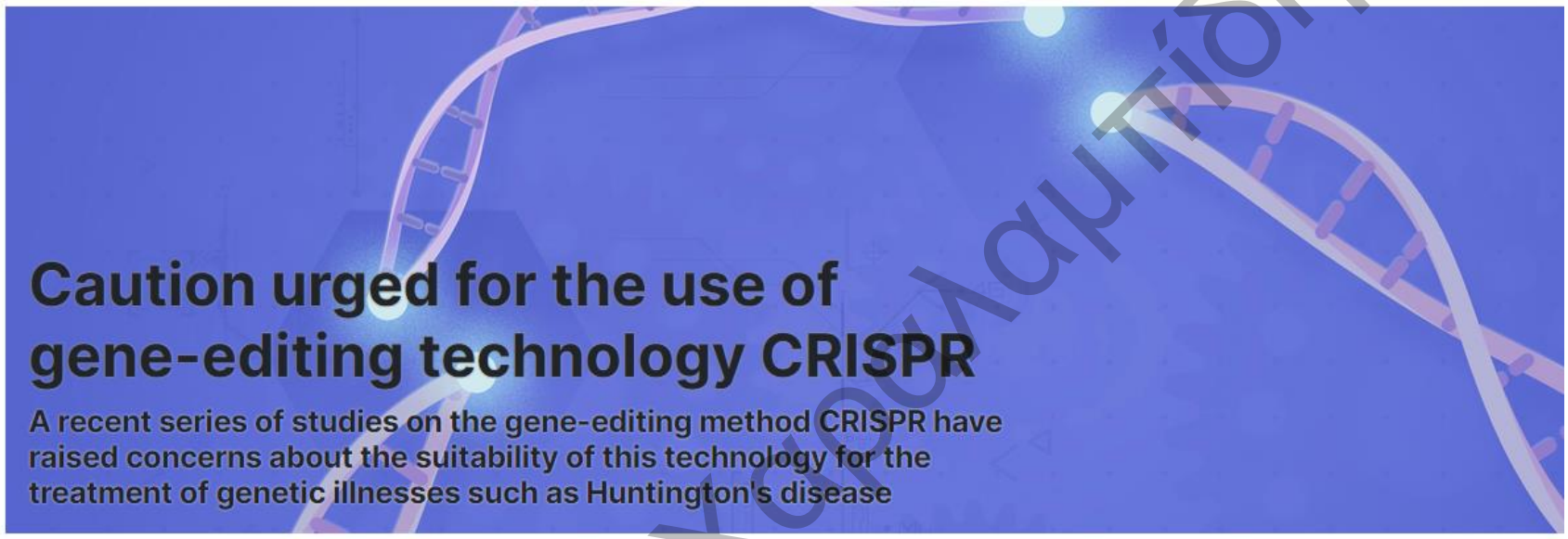
Nature Communications **11**, Article number: 5060 (2020) | Cite this article







… and the skepticism continues…



Caution urged for the use of gene-editing technology CRISPR

A recent series of studies on the gene-editing method CRISPR have raised concerns about the suitability of this technology for the treatment of genetic illnesses such as Huntington's disease

By Dr Rachel Harding | August 12, 2020 | Edited by Dr Leora Fox

A gene-editing tool known as CRISPR has been heralded as a breakthrough technology for scientists in the lab but also as a potential strategy to treat numerous genetic diseases, including Huntington's. But a series of recent studies has suggested that CRISPR is less precise than previously thought, leading to unintended changes in the genome. Three independent studies which each sought to edit a single gene have shown that other parts of the DNA were also unexpectedly changed.

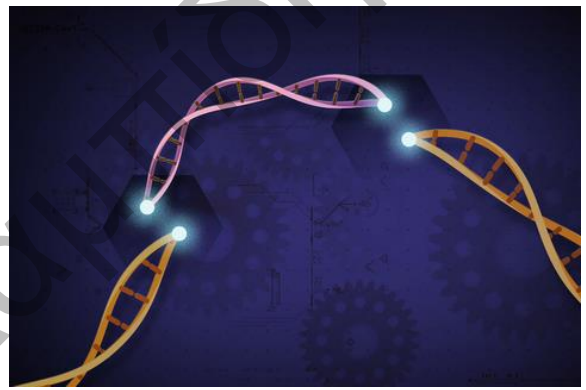
Κλινικές δοκιμές για CAR-T θεραπείες με CRISPR - pitfall

Biotech

CRISPR stock slides as CAR-T death overshadows signs of efficacy

FIERCE Biotech

by Nick Paul Taylor | Oct 21, 2020 9:05am



Chimeric antigen receptor T cell **Phase 1 trial for lymphoma.**
Four of 11 patients responded completely to infusions of T cells.

One of the first 11 patients to receive CRISPR Therapeutics' anti-CD19 allogeneic CAR-T cell therapy has **died**. The patient was hospitalized with febrile neutropenia in the weeks after receiving the drug and died 52 days post-treatment with the CAR-T therapy.

CRISPR makes its CAR-T candidate, CTX110, by using CRISPR/Cas9 to edit T cells from healthy donors (off-the-shelf or **allogeneic**). The process equips the T cells to target cancer cells that express CD19 and reduces the risk of the patient's immune system rejecting the T cells. Through the modifications, CRISPR may be able to create CAR-T cells without the complex, time-consuming process required to make **autologous** (patient's specific T-cells) therapies.

The phase 1 trial in patients with relapsed CD19+B-cell malignancies is an early test of that hypothesis as data on the first 11 people treated in the clinical trial showed.

CRISPR in humans is still not possible and illegal…

One Year After The "CRISPR AIDS Resistant Babies" Lulu and Nana Scandal, The Chinese Scientist Is Still Missing (26 NOVEMBER 2019)

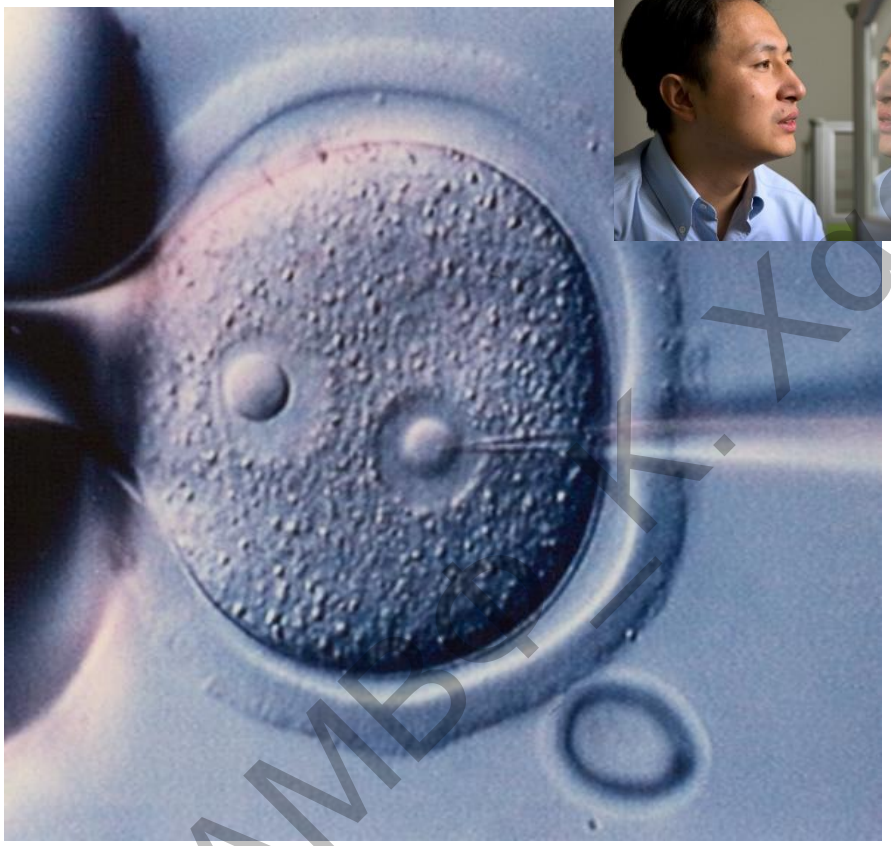
Chinese scientist He Jiankui shocked the world by claiming he had helped make the first gene-edited babies. One year later, mystery surrounds his fate as well as theirs. He has not been seen publicly since January, his work has not been published and nothing is known about the health of the babies.

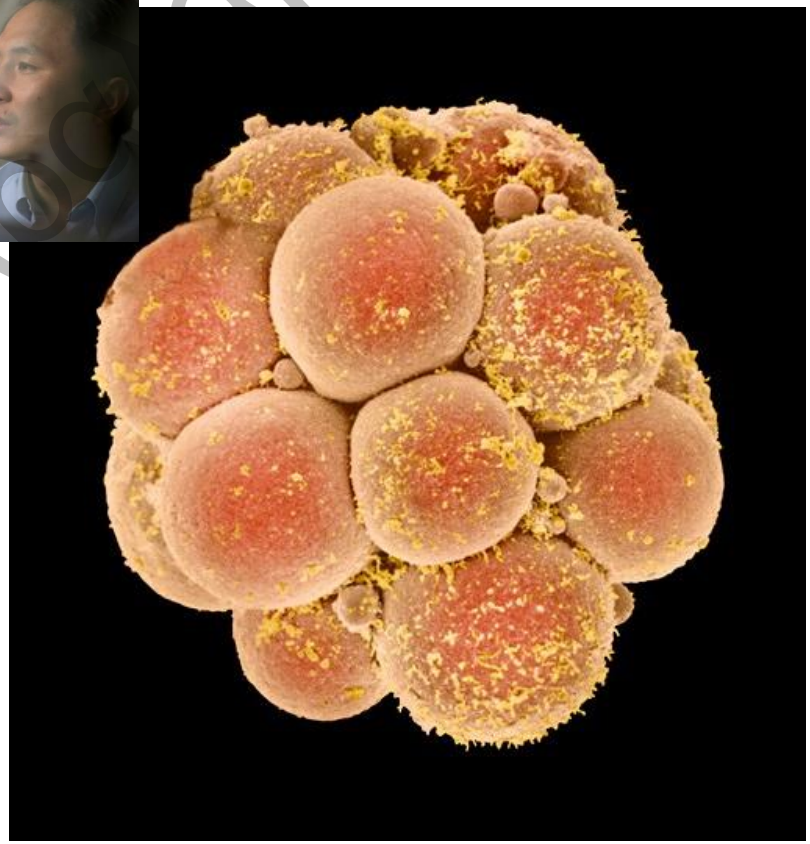
CRISPR in humans is still not possible and illegal…

MEGAN MOLTENI SCIENCE 09.03.2020 12:05 PM

Human Embryo Gene Editing Gets a Road Map—Not a Green Light

After the 2018 “Crispr baby” scandal, a global commission assessed the technology and set strict criteria for moving it toward clinical trials.





…and the skepticism continues…

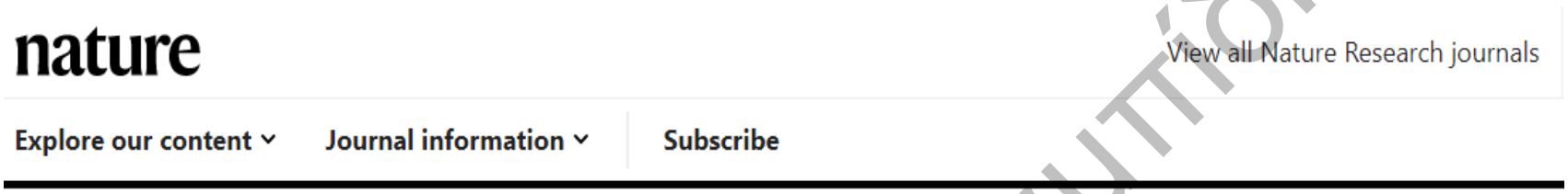
NEWS · 25 JUNE 2020

CRISPR gene editing in human embryos wreaks chromosomal mayhem

Three studies showing large DNA deletions and reshuffling heighten safety concerns about heritable genome editing.

Heidi Ledford

…and the skepticism continues…



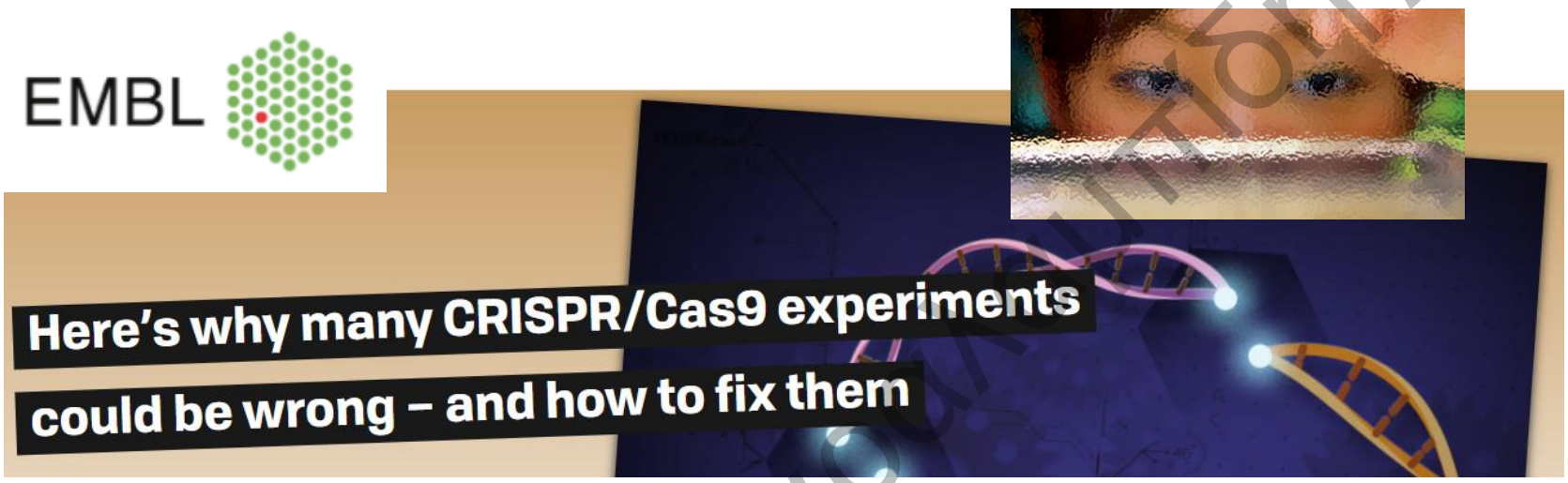
NEWS · 03 SEPTEMBER 2020

‘CRISPR babies’ are still too risky, says influential panel

The safety and efficacy of genome editing in human embryos hasn’t been proven, researchers warn.

Heidi Ledford

…and the skepticism continues…



Researchers at the European Molecular Biology Laboratory in Heidelberg, Germany used CRISPR to make cuts in 136 different genes. In about a third of cases, proteins were still produced from these "damaged" genes and, furthermore, many of the proteins remained partially functional. This strange phenomenon, of damaged DNA producing functional protein, does more than punctuate life's remarkable adaptability and resilience.

This means that dozens, or hundreds, of studies that used CRISPR/Cas9 to knock out genes, but failed to validate that the encoded protein was fully removed, could be incorrect or misleading.

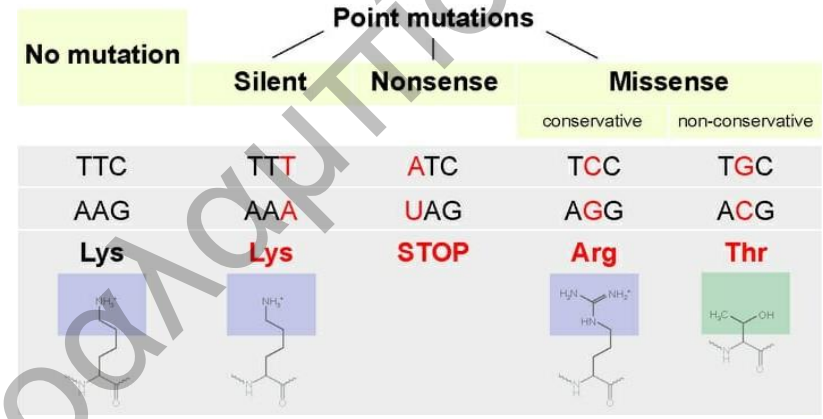
…and the skepticism continues…

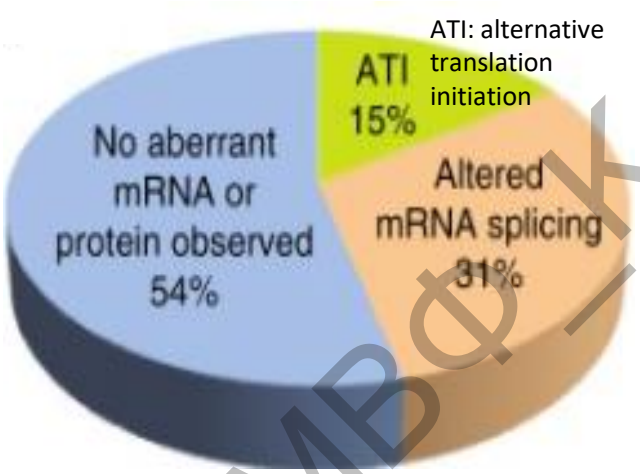
nature COMMUNICATIONS

Nat Commun. 2019; 10: 4056.
Published online 2019 Sep 6. doi: 10.1038/s41467-019-12028-5
PMCID: PMC6731291
PMID: 31492834

CRISPR-Cas9-based mutagenesis frequently provokes on-target mRNA misregulation

Rubina Tuladhar,[1] Yunku Yeu,[2] John Tyler Piazza,[1] Zhen Tan,[3] Jean Rene Clemenceau,[2]





ATI: alternative translation initiation

46% of CRISPR-edited cell lines unexpected changes in protein expression or mRNA splicing were observed.

Conceivably the mutant proteins could contribute to unwanted neomorphic cellular phenotypes.

CRISPR Correctives

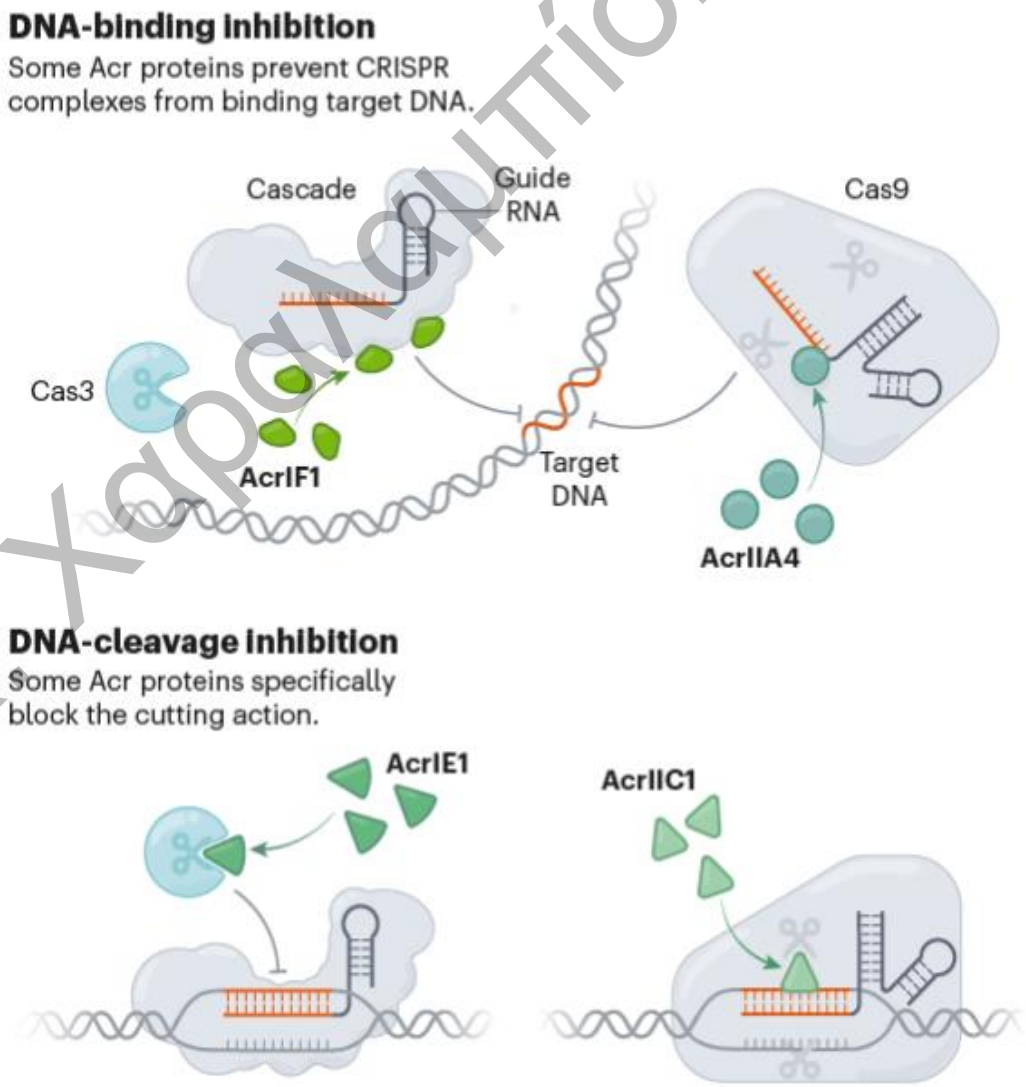
NEWS FEATURE · 15 JANUARY 2020 · CORRECTION 04 FEBRUARY 2020

The kill-switch for CRISPR that could make gene-editing safer

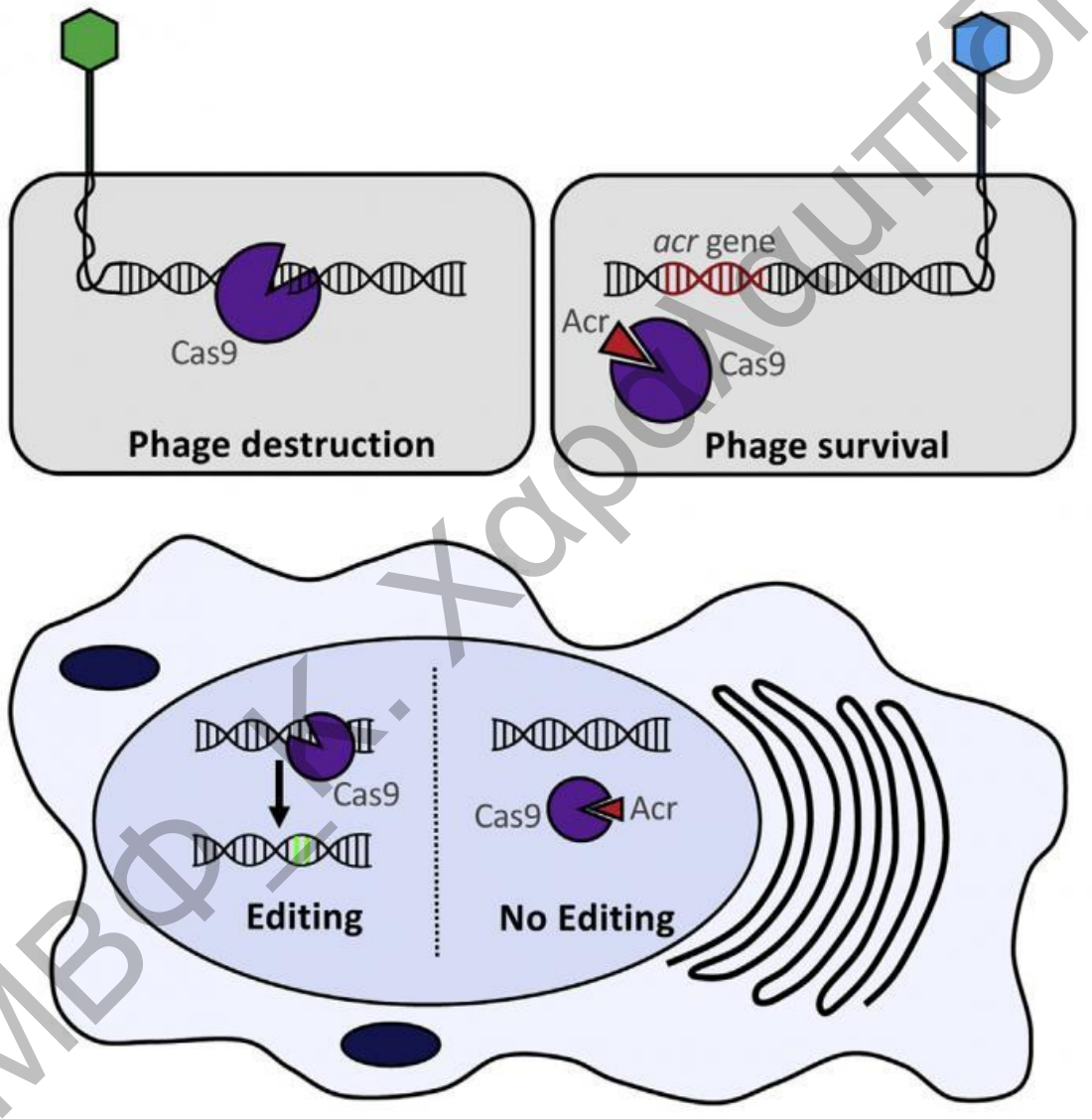
How anti-CRISPR proteins and other molecules could bolster biosecurity and improve medical treatments.

Anti-CRISPRs could also help to confine editing activity to particular cells and tissues. In 2019, research teams in Germany, Japan and the United States independently attempted to use the proteins in tandem with microRNAs to bring about tissue-specific editing.

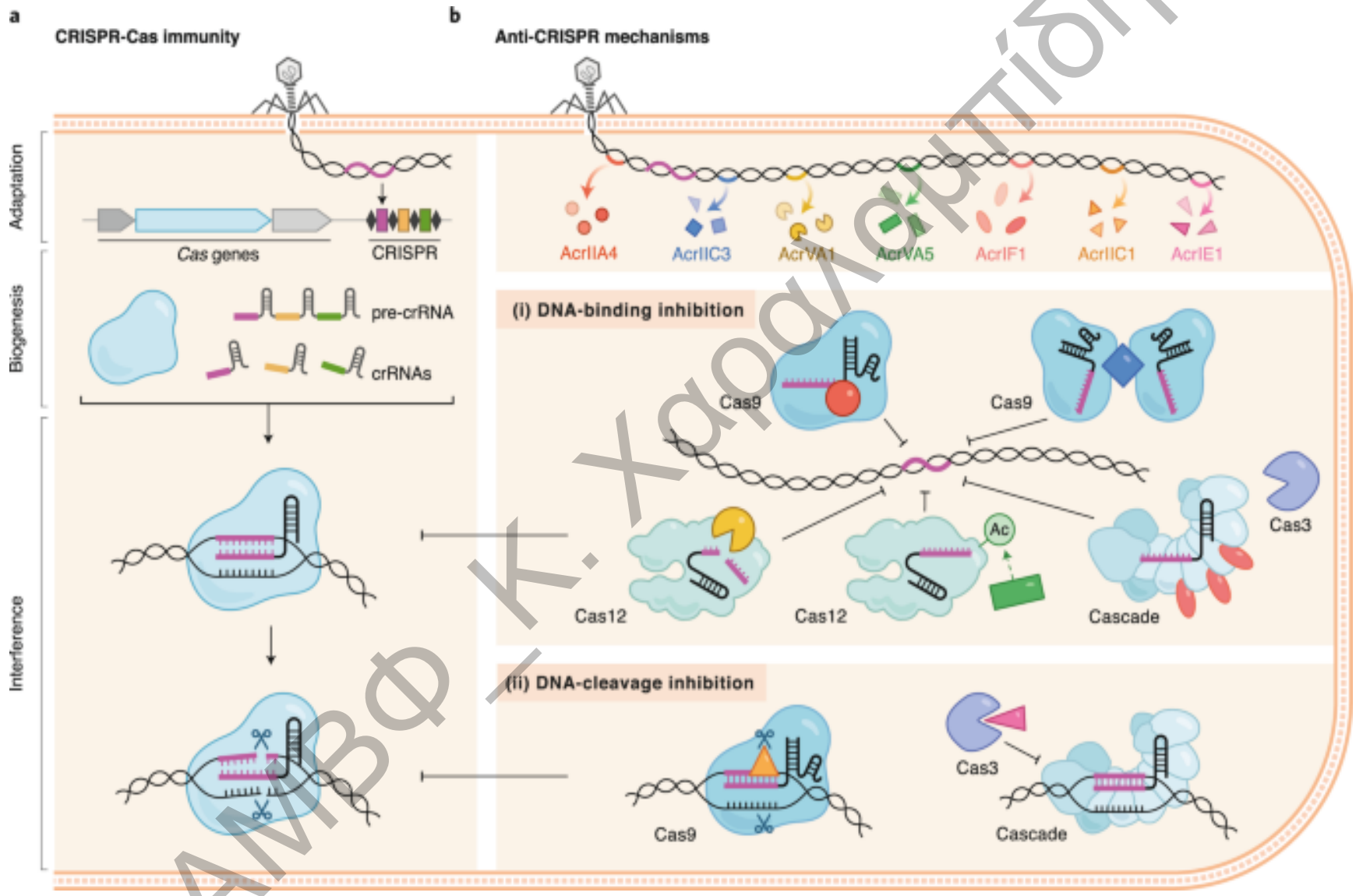
They wanted to allow editing in the liver while suppressing it in all other tissues of the mouse. So they designed an anti-CRISPR protein that would be active everywhere except in the presence of microRNA-122, which is found only in the liver. In the mice, the anti-CRISPR successfully blocked Cas9 editing throughout the body, except in liver.



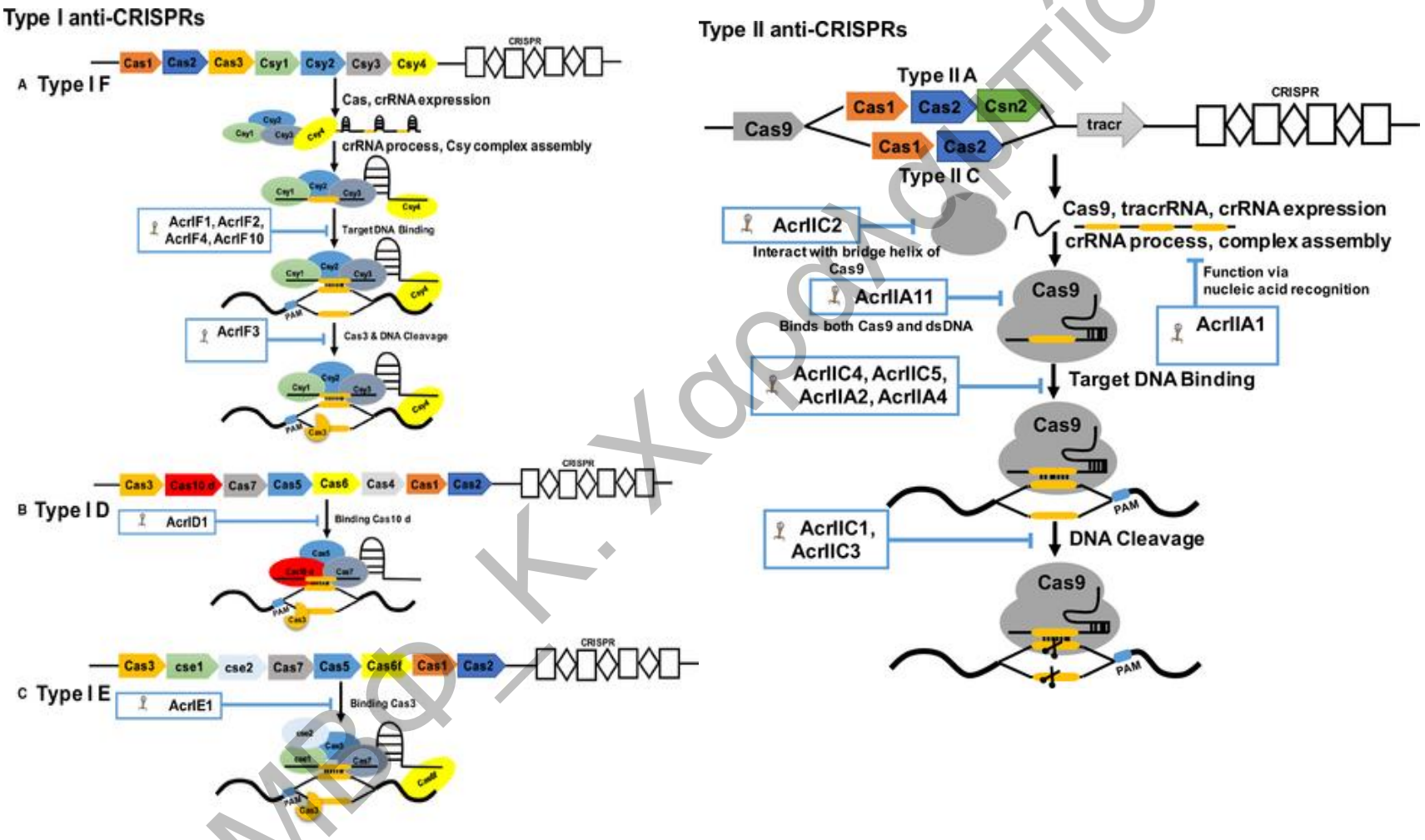
Anti-CRISPR Protein Is Gene Editing "Off-Switch"



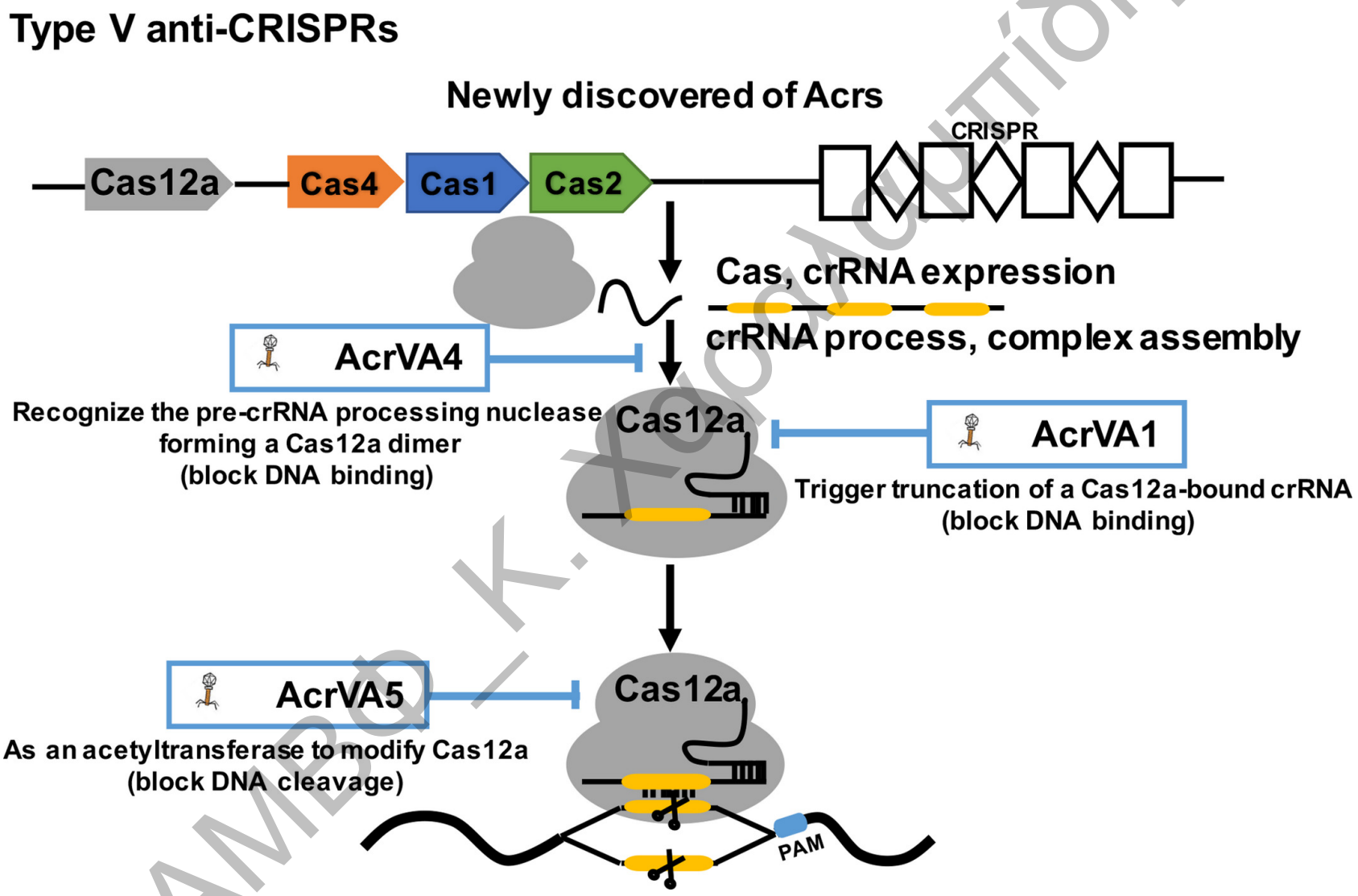
CRISPR Correctives



Anti-CRISPR proteins targeting the CRISPR-Cas system



Anti-CRISPR proteins targeting the CRISPR-Cas system



The Science Fiction Future of CRISPR…yet to come…

The first milestone was in 2003 when European scientists resurrected the Pyrenean ibex, a type of mountain goat that had gone extinct a few years earlier.

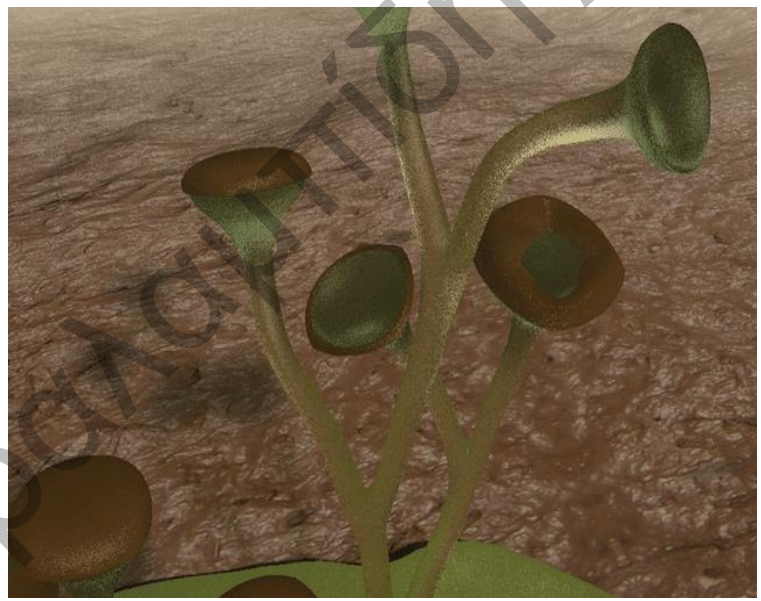
Sadly, the kid died a few minutes after she was born, so the ibex was not just the first animal to be brought back from extinction, but also the first to go extinct twice.

Although the dodo died out in the 16th Century, we don't have any DNA samples to clone it.

The Science Fiction Future of CRISPR…yet to come…

CRISPR to introduce genes from the passenger pigeons into its modern-day and hybrids will be bred for several generations. The first generation of 'revived' pigeons is expected to hatch in 2022.

A group at Harvard is now working on bringing back the mammoth that went extinct thousands of years ago.

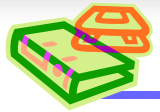
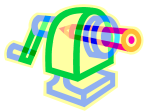


- Cooksonia inhabited the earth more than 400 million years ago.
- They were small in stature, no more than a few centimeters.
- The first plants to have a stem.
- The most primitive form of vascular land plant ever.

The Jurassic Park for real…?

Παρ' όλο που οι ΓΤΟ και οι μεθοδολογίες αυτές δεν είναι πανάκεια για την επίλυση όλων των προβλημάτων που αντιμετωπίζει η ανθρωπότητα, αποτελούν ωστόσο πανίσχυρα εργαλεία για την αύξηση της φυτικής και ζωικής παραγωγής, την προστασία του περιβάλλοντος και τη βελτίωση της υγείας και ποιότητας ζωής του ανθρώπου…

END OF PART II





Thanks for your attention