

# **ŽLAHTNJENJE RASTLIN Z UPORABO NOVIH TEHNIK TARČNEGA PREUREJANJA GENOMOV**

asist. dr. Ester Stajič

UNIVERZA V LJUBLJANI  
BIOTEHNIŠKA FAKULTETA  
ODDELEK ZA AGRONOMIJO

[ester.stajic@bf.uni-lj.si](mailto:ester.stajic@bf.uni-lj.si)

## PRESS RELEASE

7 October 2020

# The Nobel Prize in Chemistry 2020

The Royal Swedish Academy of Sciences has decided to award the Nobel Prize in Chemistry 2020 to

**Emmanuelle Charpentier**

Max Planck Unit for the Science of Pathogens, Berlin, Germany

**Jennifer A. Doudna**

University of California, Berkeley, USA

*"for the development of a method for genome editing"*

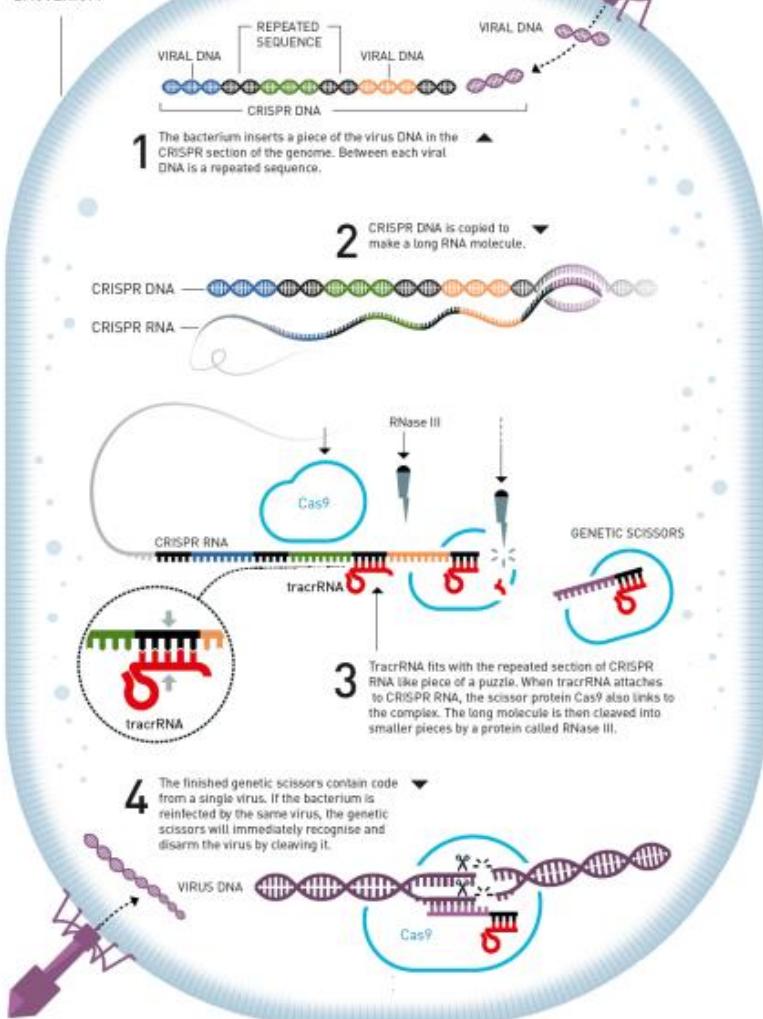
**Genetic scissors: a tool for rewriting the code of life**



### Streptococcus' natural immune system against viruses: CRISPR/Cas9

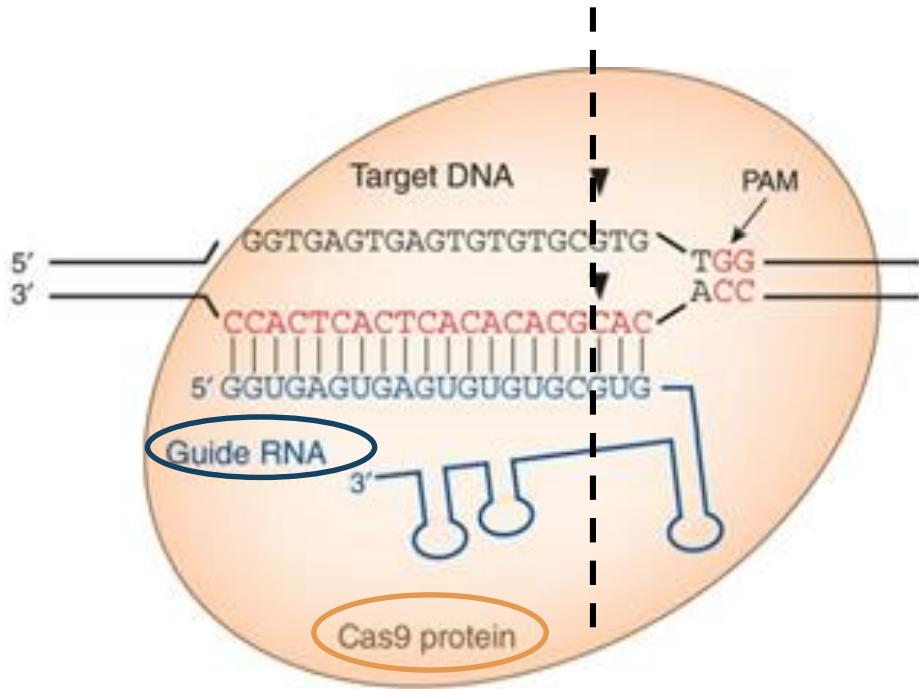
When viruses infect a bacterium, they send their harmful DNA into it. If the bacterium survives the infection, it inserts a piece of the virus DNA in its genome, like a memory of the virus. This DNA is then used to protect the bacterium from new infections.

STREPTOCOCCUS  
BACTERIUM



Johan Jarnestad/The Royal Swedish Academy of Sciences

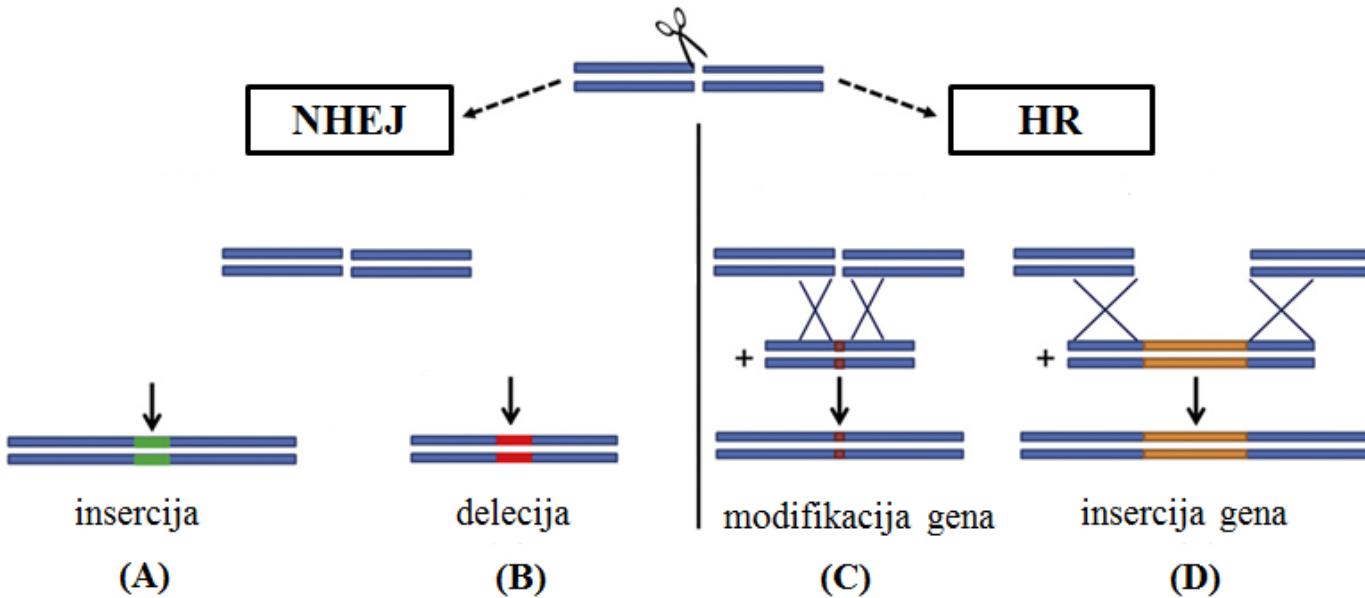
# CRISPR/Cas9



Caroll 2013; *Nat. Biotechnol.*

- vezava proteina Cas9 in sgRNA na tarčno zaporedje
- nastanek dvojnega preloma
- popravljalni mehanizmi vodijo v nastanek tarčnih mutacij

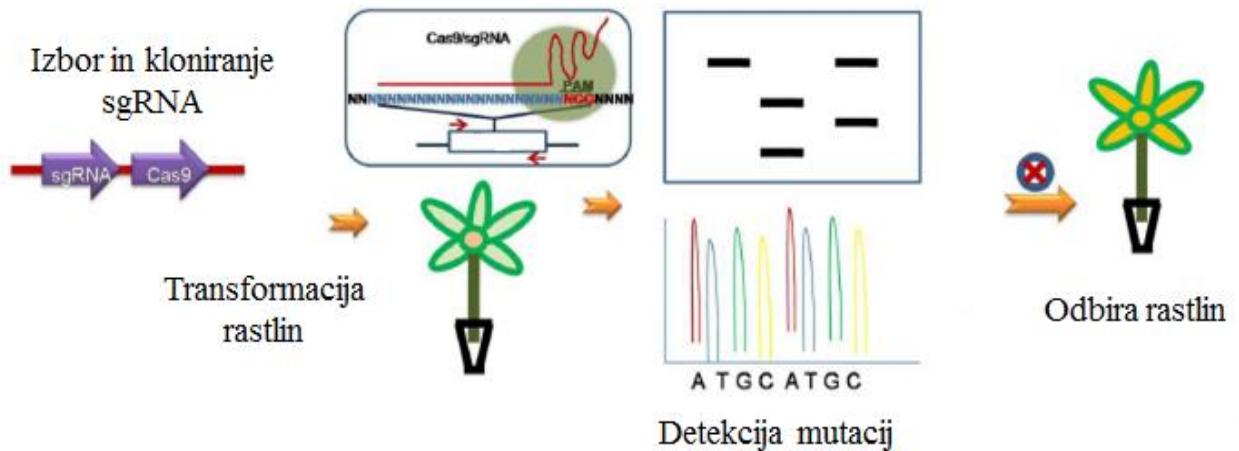
# Popravljanje dvoverižnih prelomov



prirejeno po [Bortesi in Fischer 2015; Biotech. Adv.](#)

- NHEJ = združevanje nehomolognih koncev
- HR = homologna rekombinacija

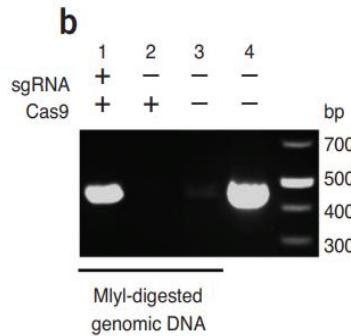
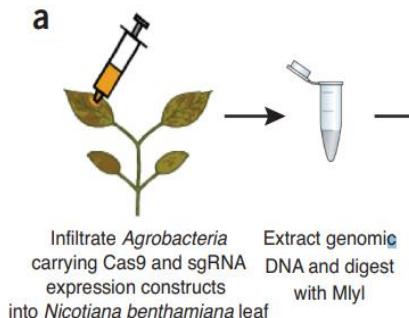
# Postopek pri rastlinah



prijevano po Khatodia in sod. 2016; *Front. Plant Sci.*

- Glavni koraki pri uporabi tehnologij tarčnega spremnjanja genomov pri žlahtnjenu rastlin:
    - identifikacija regij PAM v tarčnem genu,
    - priprava in kloniranje sgRNA v vektorje,
    - vnos vektorjev v rastline s transformacijo,
    - detekcija in identifikacija induciranih mutacij,
    - odbira rastlin z željenimi mutacijami v genomu.

# Prvi primeri pri rastlinah



**c**

	PDS	MlyI	PAM
1	AATGCCCAAATTGGACTTGTCT	-----	-----
2	GCCGTTAATTGAGAG-T-CCA	AGGTAAATTCA	CAGCTTATCTTGAGCTCGAGGT
3	AGGTAAATTCA	-----	-----
4	AGGTAAATTCA	-----	-----

sgRNA

Cas9

bp

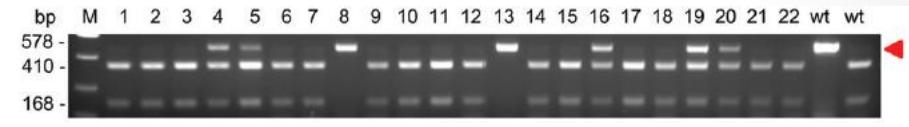
700  
500  
400  
300

MlyI-digested genomic DNA

Sequence details:

- PDS: AATGCCCAAATTGGACTTGTCT
- MlyI sites: -9, -7, -6, -5, -1, +1 T, -1 A
- PAM: -1, +1, +1, +1, +1, -4

2013 Nekrasov in sod., Nat. Biotechnol.



## OspDS-SP1

### Monoallelic mutant

TCCAAACCGTTCAATGCTGGAGTTGGCTTTGCTCCTG	AGAGG	WT
TCCAAACCGTTCAATGCTGGAGTTGGCTTTGCTCCTG	-1	-1
TCCAAACCGTTCAATGCTGGAGTTGGCTTTGCTCCTG	-3/+1	-3/+1
TCCAAACCGTTCAATGCTGGAGTTGGCTTTGCTCCTG	+1	+1
Homozygous biallelic mutant (no.8)		
TCCAAACCGTTCAATGCTGGAGTTGGCTTTGCTCCTG	aCAGAGGAATGGGTTGGACGGAGTGAC	+1
Homozygous biallelic mutant (no.13)		
TCCAAACCGTTCAATGCTGGAGTTGGCTTTGCTCCTG	tCAGAGGAATGGGTTGGACGGAGTGAC	+1
TCCAAACCGTTCAATGCTGGAGTTGGCTTTGCTCCTG	gCAGAGGAATGGGTTGGACGGAGTGAC	+1
TCCAAACCGTTCAATGCTGGAGTTGGCTTTGCTCCTG	cCAGAGGAATGGGTTGGACGGAGTGAC	+1



2013 Shan in sod., Nat. Biotechnol.

# Aplikacije

- višji pridelek
- kvaliteta pridelka
- odpornost na herbicide
- odpornost na škodljivce



**d**

	Target 1	PAM	PAM	Target 2
WT	ACATAGAAAAA	GGTGACCTG	GGGAGAC	TGGGACCATCTTCTGTTAACCGCCCTGCCCTTGCTCT
Plant 1	ACATAGAAAAA	GGTGACCTG	GGGAGAC	GGTGACCATCTTCTGTTAACCGCCCTGCCCTTGCTCT
Plant 2	ACATAGAAAAA	GGTGACCTG	GGGAGAC	-CTTGATTAACCTTGACTCTTTCAAGG -48
Plant 8	ACATAGAAAAA	GGTGACCTG	GGGAGAC	-CTTGATTAACCTTGACTCTTTCAAGG -48
Plant 10	ACATAGAAAAA	GGTGACCTG	GGGAGAC	-CTTGATTAACCTTGACTCTTTCAAGG -48
	ACATAGAAAAA	GGTGACCTG	GGGAGAC	TTGATTAACCTTGACTCTTTCAAGG -49



2017 Nekrasov in sod., *Scientific Reports*

Crop	Trait	Edited genes	Stage
Banana	Disease resistance (BXW, Fusarium wilt, BSV)	<i>DMR6</i> , BSV sequences	3,1
Cassava	Disease resistance (BB)	<i>SWEET</i> gene promoters	3
	Food safety (cyanide-free)	Linamarin synthase	3
	Quality (waxy starch)	<i>GBSS1</i>	3
	Disease resistance (MLN)	<i>C6 QTL</i>	1
Maize	Weed resistance ( <i>Striga</i> )	Strigolactone	3
Potato	Disease resistance (PVY <sup>a</sup> , late blight)	<i>eIF-4E</i> , <i>StDMR6-1</i> , <i>StCHL1</i>	2
Rice	Disease resistance (BLB, RHB)	<i>SWEET</i> gene promoters, <i>AGO4</i> , <i>STV11</i>	4,3
	Food safety (low arsenic and cadmium)	<i>OsNRAMP5</i> , <i>OsPT8</i> , <i>LS1</i> , <i>LS2</i>	3
	Nitrogen remobilization, and methane emission reduction	Unpublished	3
	Insect resistance <sup>a</sup> (BPH)	BPH resistance alleles	2
Wheat	Disease resistance (rusts, mildew) <sup>a</sup>	<i>Lr67</i> and others	3

2022 Pixley in sod., *Nat. Gen.*

# Zakonodaja, omejitve

Published: 14 April 2016

## Gene-edited CRISPR mushroom escapes US regulation

Emily Waltz

[Nature](#) 532, 293 (2016) | [Cite this article](#)

10k Accesses | 317 Citations | 1762 Altmetric | [Metrics](#)

A fungus engineered with the CRISPR–Cas9 technique can be cultivated and sold without further oversight.



The common white button mushroom (*Agaricus bisporus*) has been modified to resist browning.  
Credit: Jose A. Bernat Bacete/Getty Images

## Sanatech Seed launches world's first GE tomato

By Maura Maxwell | 16 March 2021

Japan's first gene-edited food is a tomato that contains four to five times more GABA, a substance reported to be effective in controlling high blood pressure.



CJEU (2018): ,Tarčno preurejanje je genetska modifikacija, ki se ne uvršča med izjeme GSO zakonodaje.'



EUROPEAN  
COMMISSION

Brussels, 5.7.2023  
COM(2023) 411 final  
  
2023/0226 (COD)

Proposal for a

**REGULATION OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL  
on plants obtained by certain new genomic techniques and their food and feed, and  
amending Regulation (EU) 2017/625**



**Objectives of the proposal:**

- Ensure high level of protection of health and the environment. These rules apply only for NGT plants which are as safe as conventionally-bred plants. These plants are safe for humans, animals and the environment. For any other NGTs, GMO rules will still apply.
- Contribute to sustainability in a wide range of plant species, especially for the agri-food system.
- Create opportunities for research and innovation, including for SMEs.

**WHAT ARE NEW  
GENOMIC TECHNIQUES?**

NGTs are techniques that can help breed new plant varieties faster, and with higher precision than classical breeding techniques, such as seed selection or cross-breeding. NGTs can produce a wide diversity of plant products. These plants may have only small changes that might also occur in nature or through classic breeding or they may have more complex modifications.

**Key elements of the new rules:**

Establishment of two categories of plants obtained by NGTs:

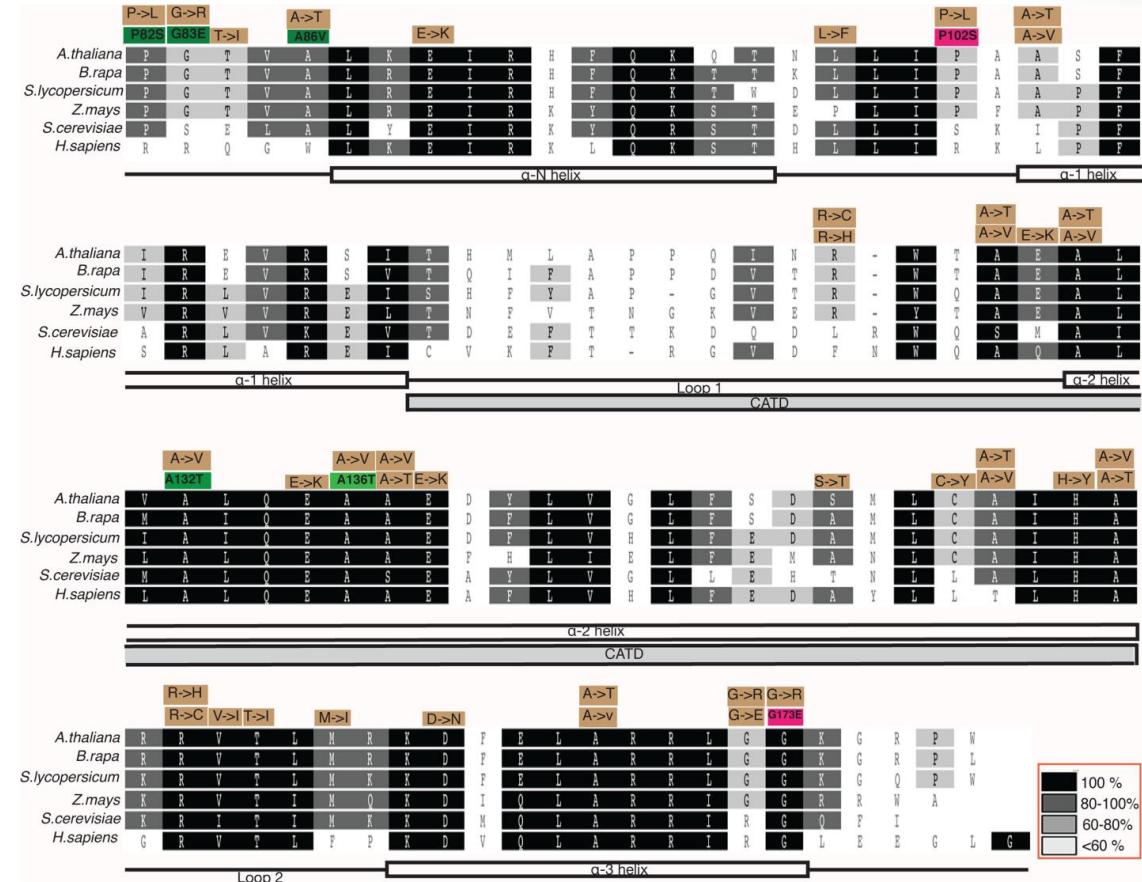
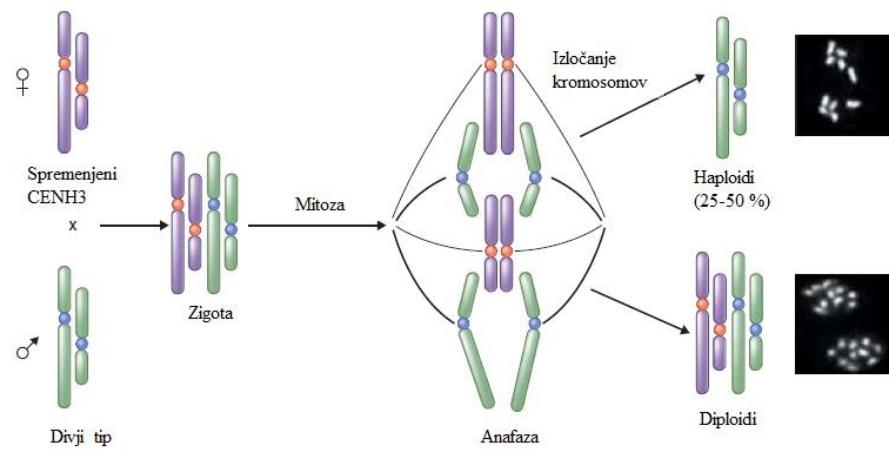
- Category 1: Plants that are comparable to naturally occurring variations will require notification (and central registration).
- Category 2: Plants with more complex modifications will go through the more extensive process of the GMO-regulation.

Incentives to steer development of plants in support of sustainability goals

Transparency about all NGT plants on the EU market (for e.g. through labelling of seeds)

Robust monitoring of economic, environmental and social impacts of NGT products

# Protein CENH3 in vpliv na indukcijo haploidov



- Centromerni protein CENH3 – del nukleosoma, omogoča pripenjanje niti delitvenega vretena
- Pri križanju z divjim tipom pride do nastanka spontanih haploidov pri navadnem repnjakovcu

2015 Kuppu in sod., PLOS

# Metode raziskovanja

## Rastlinski material:

štiri hibridne sorte rdečega zelja (Bejo, Syngenta)

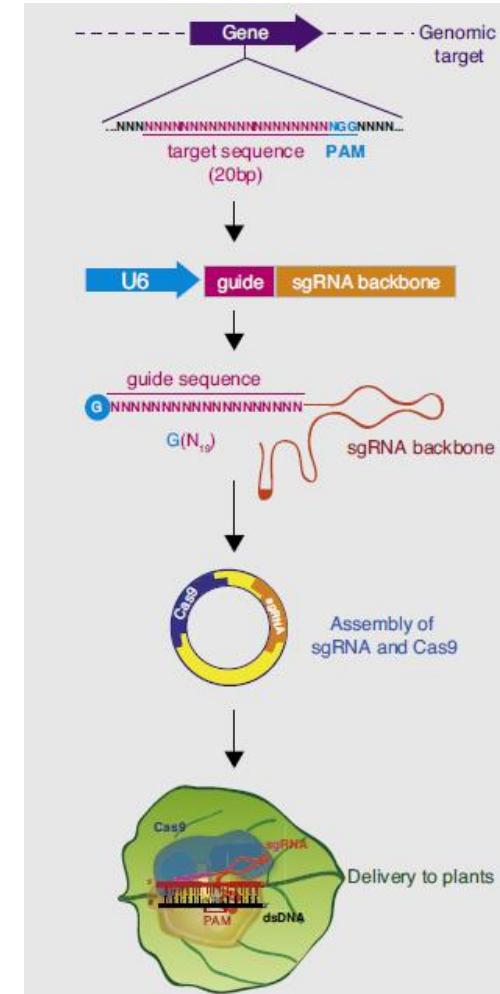
BIOINFORMACIJSKA ANALIZA  
podatkovne baze (EnsemblPlants, NCBI)  
Clustal Omega  
CHOPCHOP

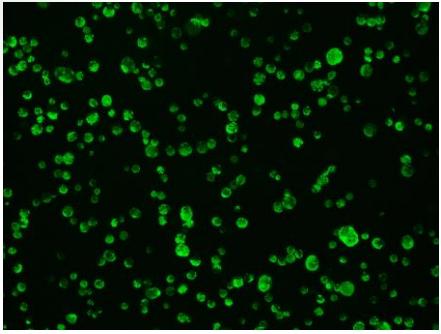


IZDELAVA EKSPRESIJSKIH VEKTORJEV  
tehnologija Gateway® in Golden Gate



VNOS VEKTORJEV V RASTLINSKE CELICE IN DETEKCIJA  
MUTACIJ





## VNOS VEKTORJEV V RASTLINSKE CELICE IN DETEKCIJA MUTACIJ

### VNOS VEKTORJEV

### DETEKCIJA MUTACIJ

- metode prehodne transformacije:
  - transformacija protoplastov
  - agroinfiltracija
- metode stabilne transformacije:
  - transformacija z uporabo bakterije *Agrobacterium tumefaciens*



Stajič 2023; MDPI Plants

Referenčno zaporedje	
T T A C A A G C C T G G A A C C G T T G   C C C T C A G A G A G A T T C G C C A T -	79,40 % (18978 odčitkov)
T T A C A A G C C T G G A A C C G T T G   C C C C T C A G A G A G A T T C G C C A -	2,74 % (654 odčitkov)
T T A C A A G C C T G G A A C C G T T G   C C C C T C A G A G A G A T T C G C C A T -	2,40 % (573 odčitkov)
T T A C A A G C C T G G A A C C G T T G   A C C C T C A G A G A G A T T C G C C A T -	2,26 % (541 odčitkov)
T T A C A A G C C T G G A A C C G T T G   - C C T C A G A G A G A T T C G C C A T -	1,40 % (335 odčitkov)
T T A C A A G C C T G G A A C C G T T G   C C C C T C A G A G A G A T T C G C C A -	0,79 % (189 odčitkov)
T T A C A A G C C T G G A A C C G T T G   - - T C A G A G A G A T T C G C C A T -	0,69 % (166 odčitkov)
T T A C A A G C C T G G A A C C G T T G   A C C C T C A G A G A G A T T C G C C A T -	0,52 % (124 odčitkov)
T T A C A A G C C T G G A A C C G T T G   - C T C A G A G A G A T T C G C C A T -	0,51 % (122 odčitkov)
T T A C A A G C C T G G A A C C G T T G   - - - A G A G A T T C G C C A T -	0,48 % (114 odčitkov)
T T A C A A G C C T G G A A C C G T T G   G C C C T C A G A G A G A T T C G C C A -	0,39 % (94 odčitkov)
T T A C A A G C C T G G A A C C G T T G   - - - - A G A T T C G C C A T -	0,39 % (93 odčitkov)
T T A C A A G C C T G G A A C C G T T G   - - - A G A G A G A T T C G C C A T -	0,35 % (84 odčitkov)
T T A C A A G C C T G G A A C C G T T G   - - - C A G A G A G A T T C G C C A T -	0,23 % (56 odčitkov)
T T A C A A G C C T G G A A C C G T T G   - - - - A T T C G C C A T -	0,20 % (48 odčitkov)

Stajič in sod. 2019; PCTOC

# Zaključek

- številne aplikacije pri žlahtnjenju rastlin
- spremembe pri sproščanju tudi v EU



## Zahvala

- program [P4-0077](#): 'Kmetijske rastline - genetika in sodobne tehnologije'
- doktorski projekt [Z4-3215](#): 'Optimizacija protokola za CRISPR/Cas9 in tarčno preurejanje nukleotidov pri zelju'

**ARIS**

Javna agencija za znanstvenoraziskovalno in inovacijsko dejavnost Republike Slovenije

**HVALA ZA POZORNOST!**