ŽLAHTNJENJE RASTLIN Z UPORABO NOVIH TEHNIK TARČNEGA PREUREJANJA GENOMOV

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PRESS RELEASE

7 October 2020

The Nobel Prize in Chemistry 2020

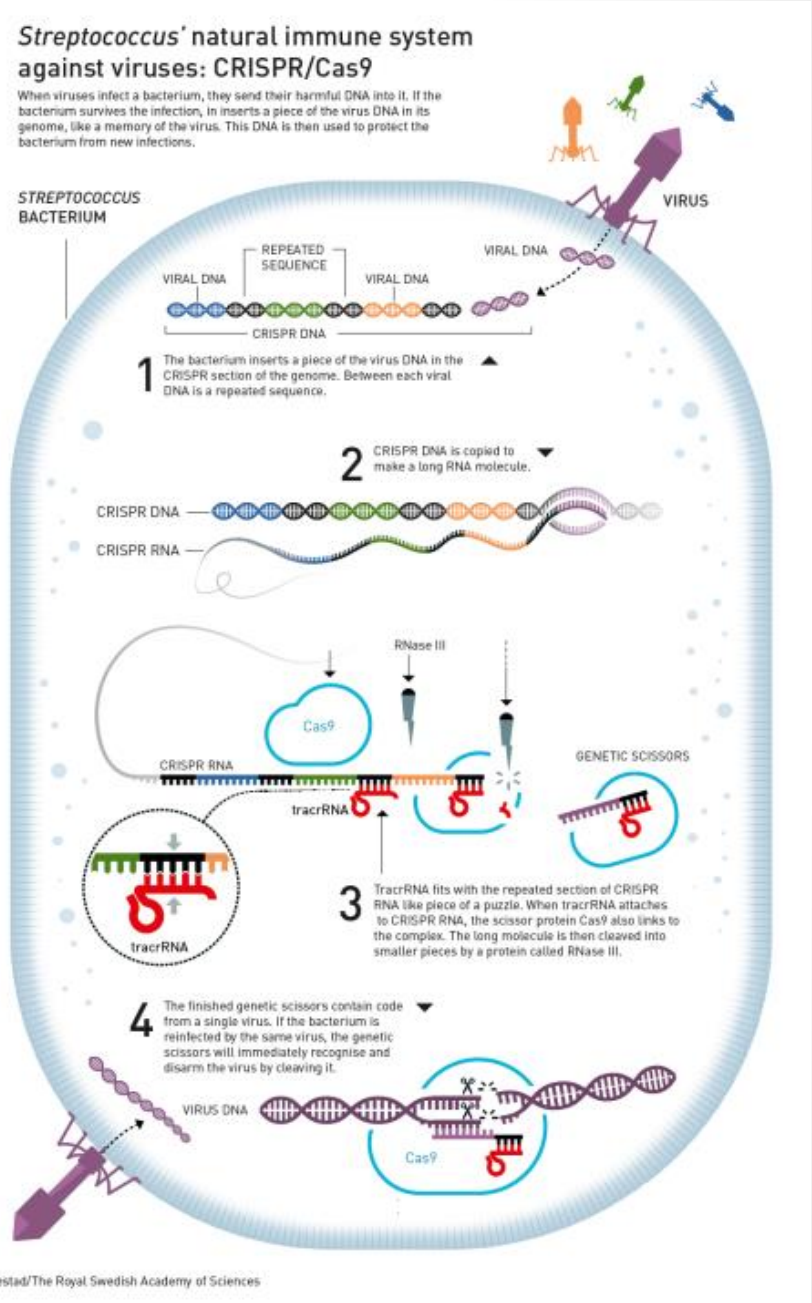
The Royal Swedish Academy of Sciences has decided to award the Nobel Prize in Chemistry 2020 to

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

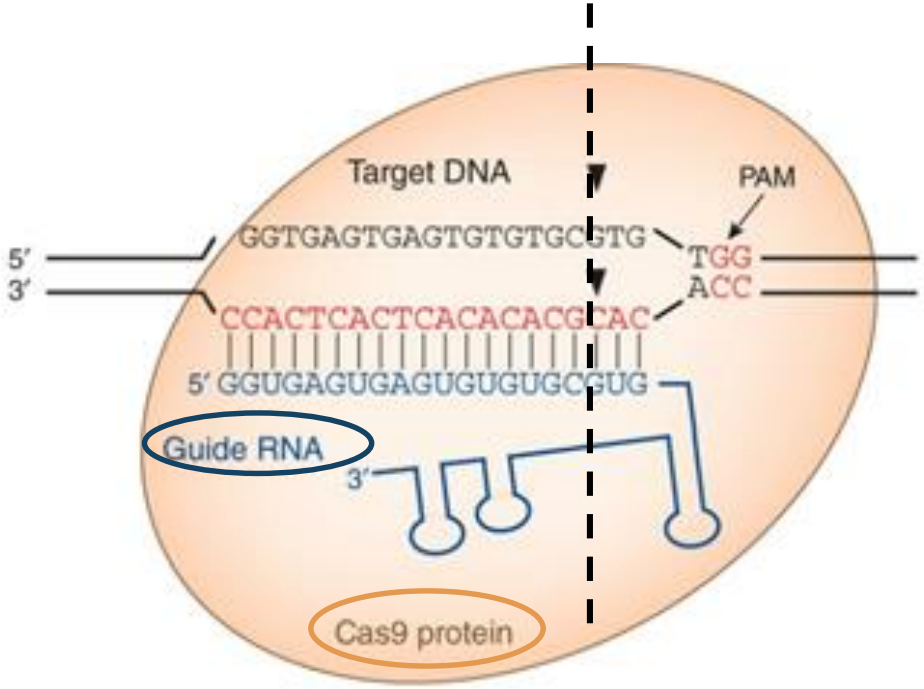
Jennifer A. Doudna
University of California, Berkeley, USA

"for the development of a method for genome editing"

Genetic scissors: a tool for rewriting the code of life



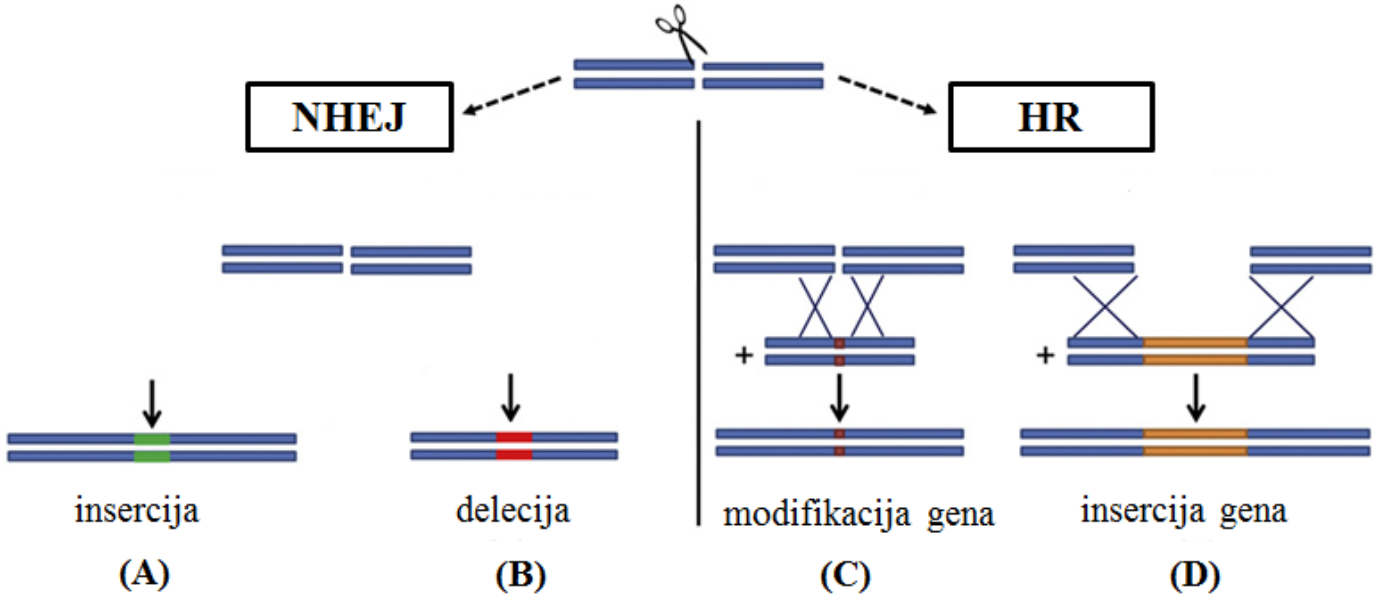
CRISPR/Cas9



Caroll 2013; *Nat. Biotechnol*.

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

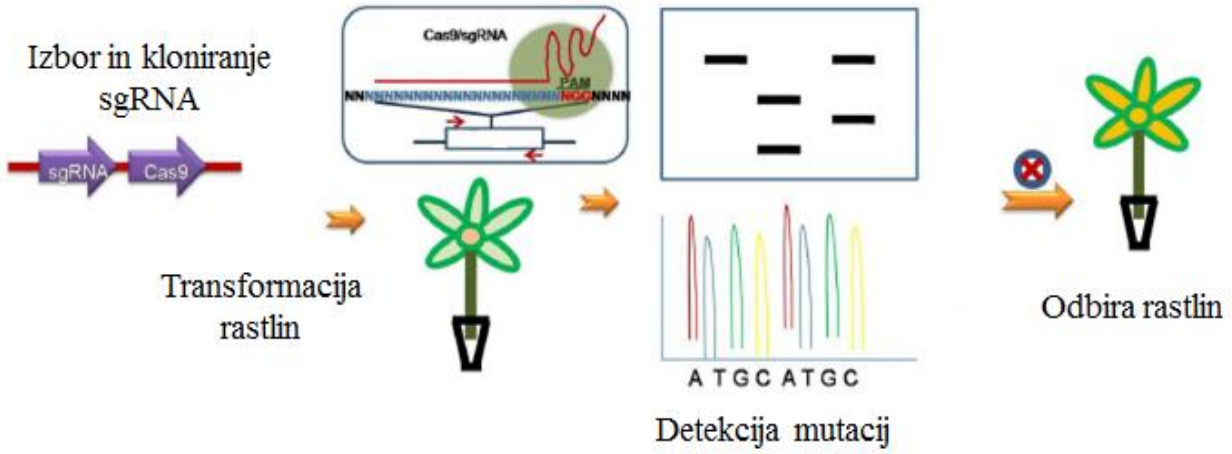
Popravljanje dvoverižnih prelomov



prirejeno po Bortesi in Fischer 2015; *Biotech. Adv*.

- NHEJ = združevanje nehomolognih koncev
- HR = homologna rekombinacija

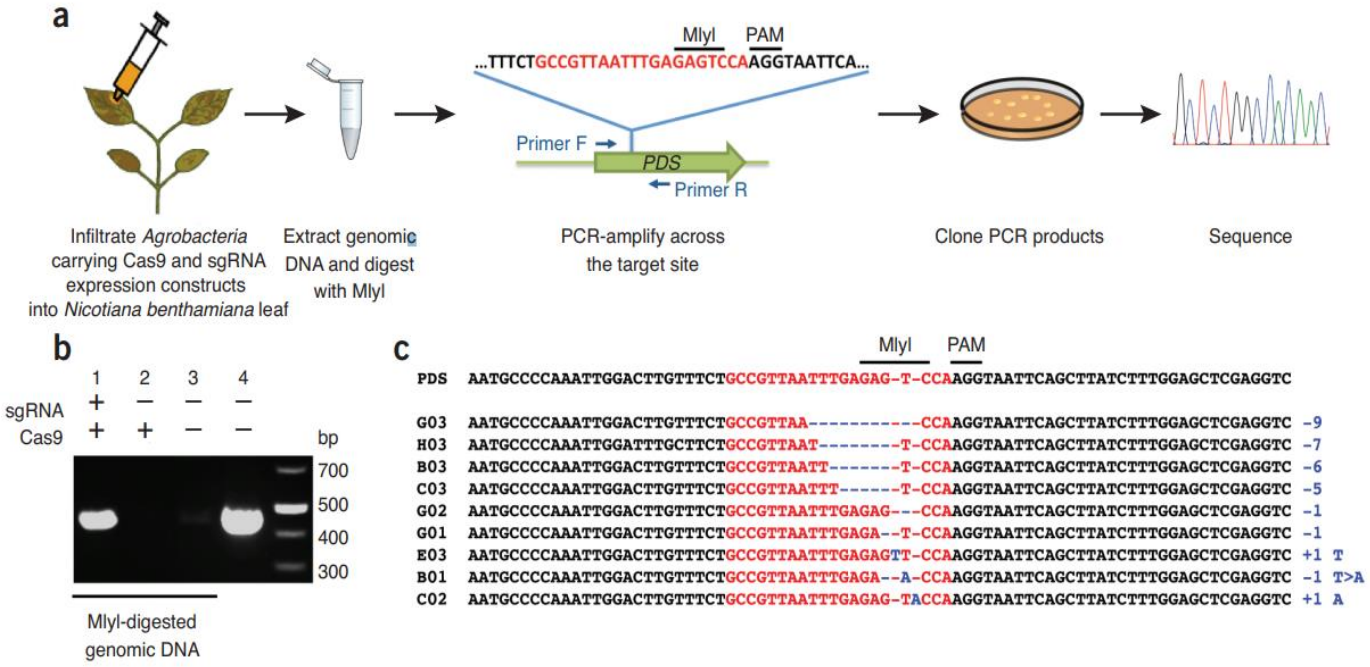
Postopek pri rastlinah



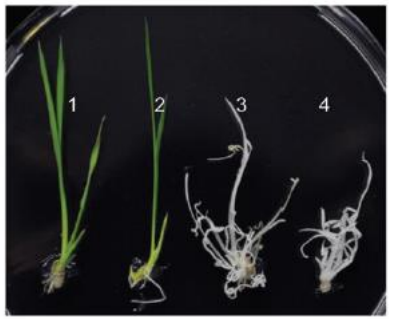
prirejeno po Khatodia in sod. 2016; *Front. Plant Sci.*

- Glavni koraki pri uporabi tehnologij tarčnega spreminjanja genomov pri žlahtnjenju 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



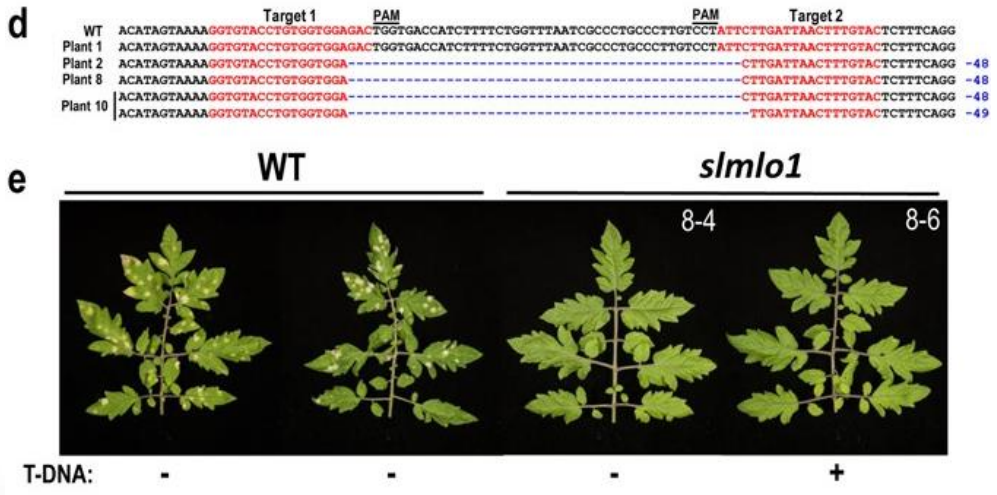
2013 Nekrasov in sod., *Nat. Biotechnol*.



2013 Shan in sod., *Nat. Biotechnol.*

Aplikacije

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



2017 Nekrasov in sod., *Scientific Reports*

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

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:

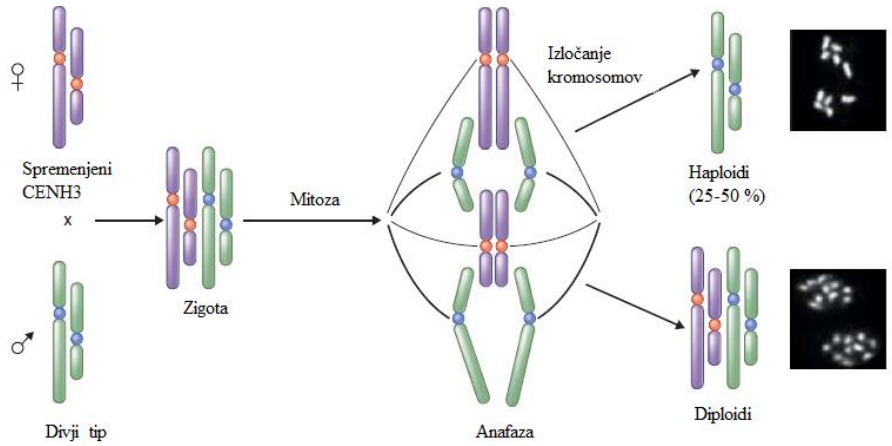
- Category 1: Plants that are comparable to naturally occurring variations will require notification (and central registration).
- Category 2: Plants with more complex modications will go through the more extensive process of the GMO-regulation.

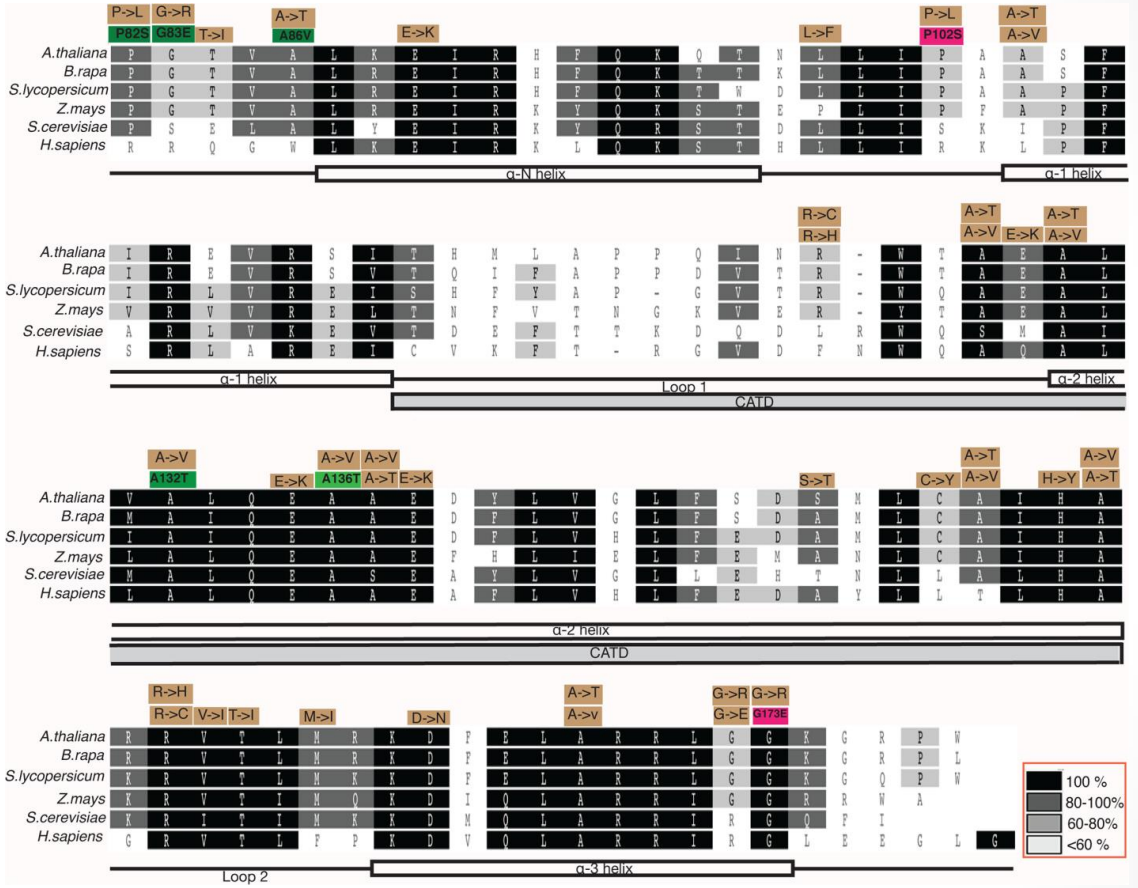
Incentives to steer development of plants in support of sustainability goals

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

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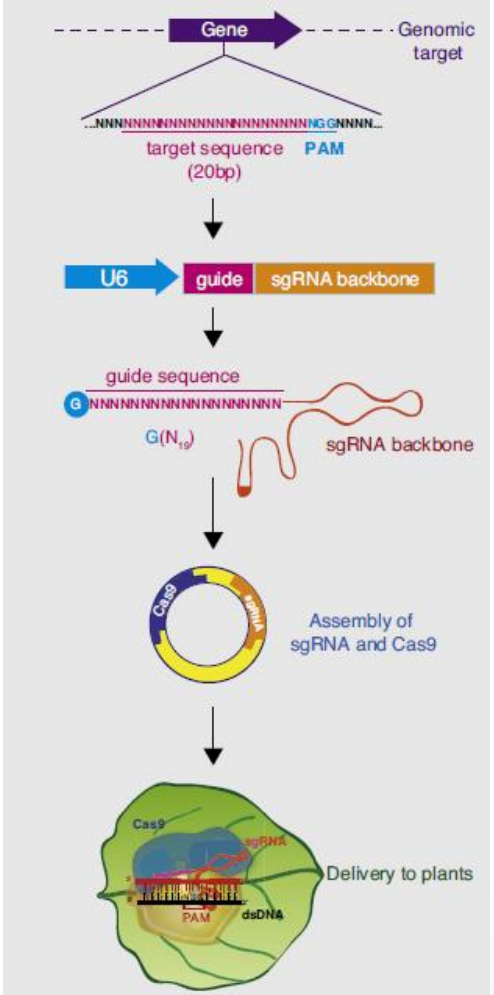
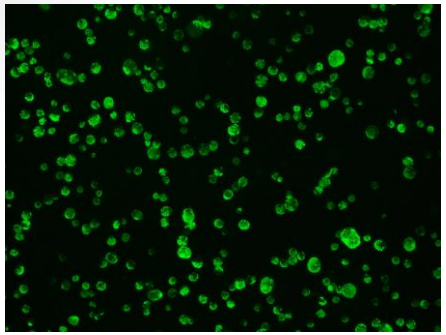
VNOS VEKTORJEV V RASTLINSKE CELICE IN DETEKCIJA MUTACIJ



2015 Belhaj in sod., *Current Opinion in Biotechnology*



VNOS VEKTORJEV V RASTLINSKE CELICE IN DETEKCIJA MUTACIJ

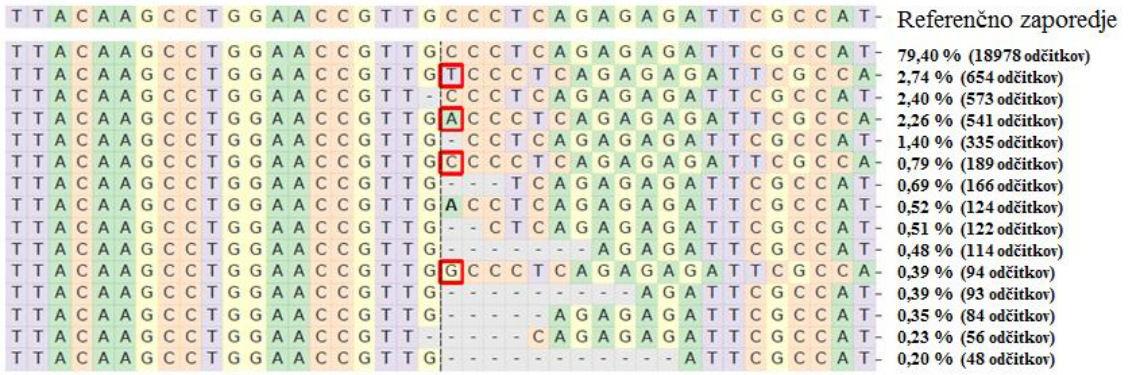
VNOS VEKTORJEV

- <u>metode prehodne transformacije</u>:
  - transformacija protoplastov
  - agroinfiltracija
- <u>metode stabilne transformacije</u>:
  - transformacija z uporabo bakterije *Agrobacterium tumefaciens*

<u>Stajič 2023; *MDPI Plants*</u>

DETEKCIJA MUTACIJ

- <u>izolacija DNA</u>
- <u>določanje tarčnih mutacij:</u> NGS, T7E1, sekvenciranje po Sangerju



<u>Stajič in sod. 2019; *PCTOC*</u>

Zaključek

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

Zahvala

- program P4-0077: 'Kmetijske rastline - genetika in sodobne tehnologije'
- podoktorski 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!