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BMT/15/17
ORIGINAL: English
DATE: May 20, 2016

INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

Geneva

WORKING GROUP ON BIOCHEMICAL AND MOLECULAR TECHNIQUES AND DNA PROFILING IN PARTICULAR

Fifteenth Session

Moscow, Russian Federation, May 24 to 27, 2016

GENE AND GENOME EDITING WITH CRISPR-CAS9

Document prepared by an expert from the Netherlands

Disclaimer: this document does not represent UPOV policies or guidance

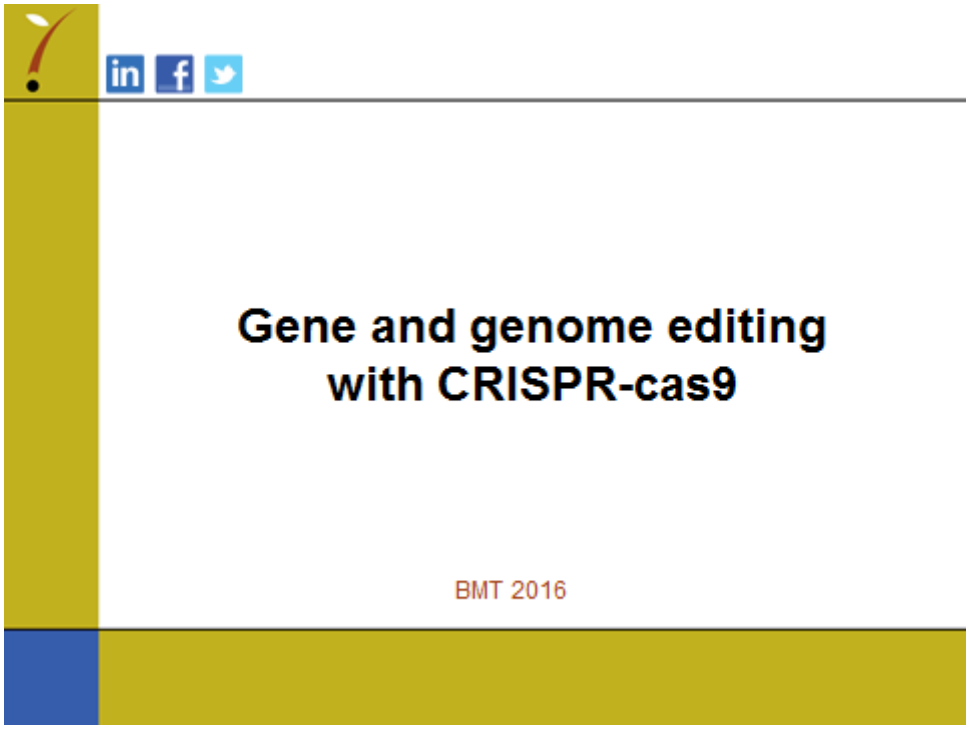
The Annex to this document contains a copy of a presentation “Gene and genome editing with CRISPR-cas9” to be made at its fifteenth session of the Working Group on Biochemical and Molecular Techniques and DNA-Profiling in particular (BMT).

Hedwich Teunissen, Molecular Biologist, Naktuinbouw

[Annex follows]

ANNEX

GENE AND GENOME EDITING WITH CRISPR-CAS9



Content

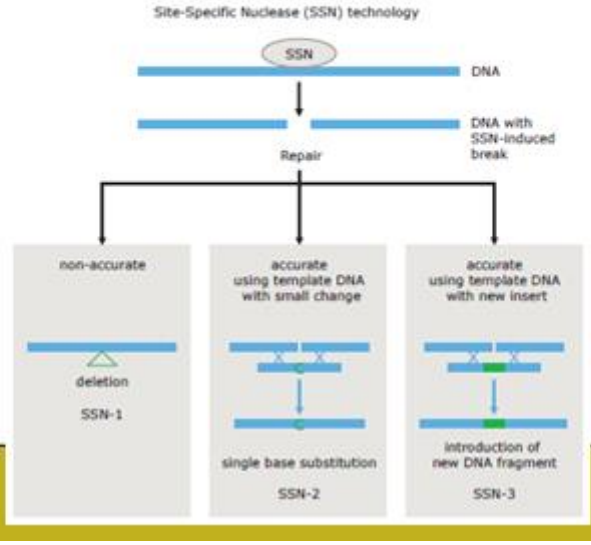
- Gene/Genome Editing
- History – development of technologies
- CRISPR-cas – How does it work
- Applications
- Social/political/legal/UPOV discussion

Definitions

Gene editing or Genome editing
Container name for techniques capable to introduce ***targeted*** changes of the genetic material.

SSN

Sequence-specific nuclease (SSN) technology



SSN-history

Zinc-finger nucleases (ZFN) 2002

Transcription Activator-Like Effector Nucleases (TALEN) 2010

CRISPR-Cas-based RNA-guided DNA endonucleases published in Science in 2012

Jennifer Doudna

Emmanuelle Charpentier

Break through

Tabel 1: Aantal wetenschappelijke publicaties over het CRISPR-Cassysteem[1]	
2010	0
2011	1
2012	3
2013	100
2014[2]	235

1) zoektermen in PubMed: CRISPR Cas9
2) tot 30/9/2014

In PubMed (for the 2012 paper) it's exponential: 120 citations the first year, 400 in 2013, 600 in 2014, and more than 1200 as of October 2015.

There was a pent-up need for the technology to manipulate genomes to be easy, and that's what we're seeing now.

CRISPR-cas9

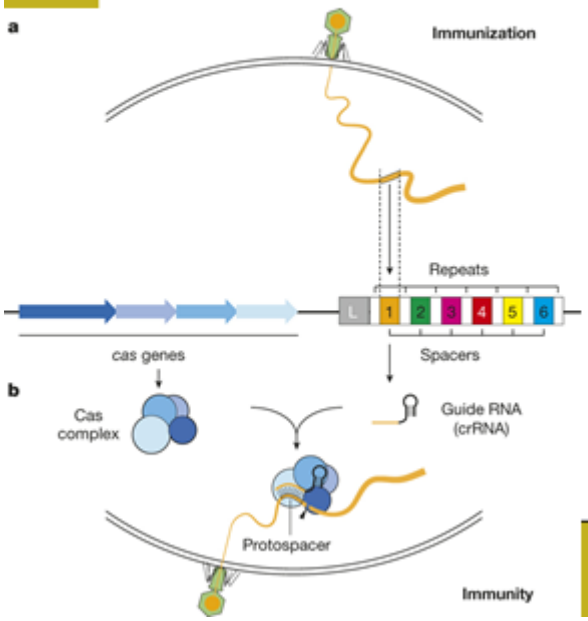
Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR)

Cas = CRISPR-associated; genes that code for nucleases

Together these form the immuunsystem of bacteria against alien (viral) DNA

It is a RNA-driven genetic silencing mechanism that resembles eukaryotic microRNA silencing mechanisms

CRISPR-cas9



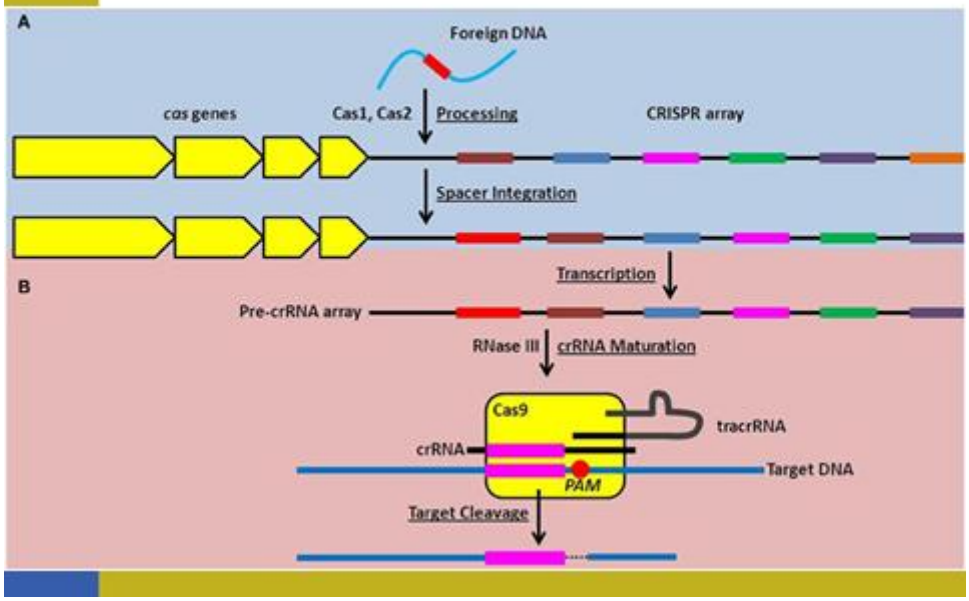
Cas genes code for cas-nuleases

CRISPR-locus consists of repeats and spacers

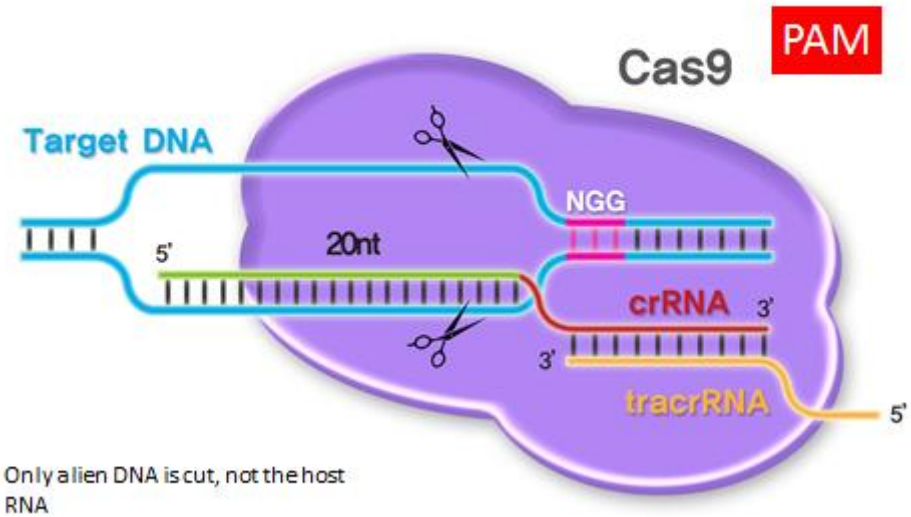
Spacers are templates for short CRISPR-derived RNA (crRNA) 20 nucleotides

Together with tracrRNA, crRNA forms a hybrid RNA

CRISPR-cas9



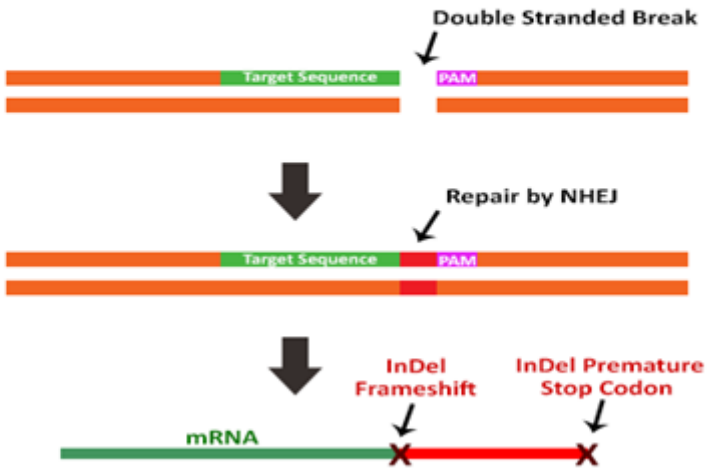
CRISPR-cas9



Only alien DNA is cut, not the host RNA

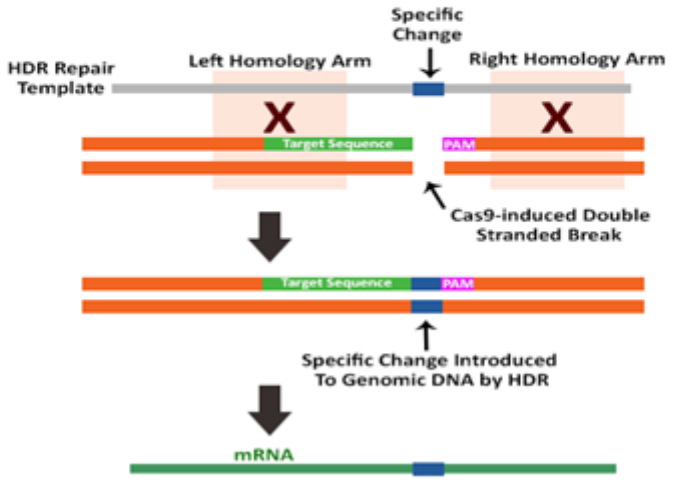
Repair mechanism

1. Non-homologous end- joining



Repair mechanism

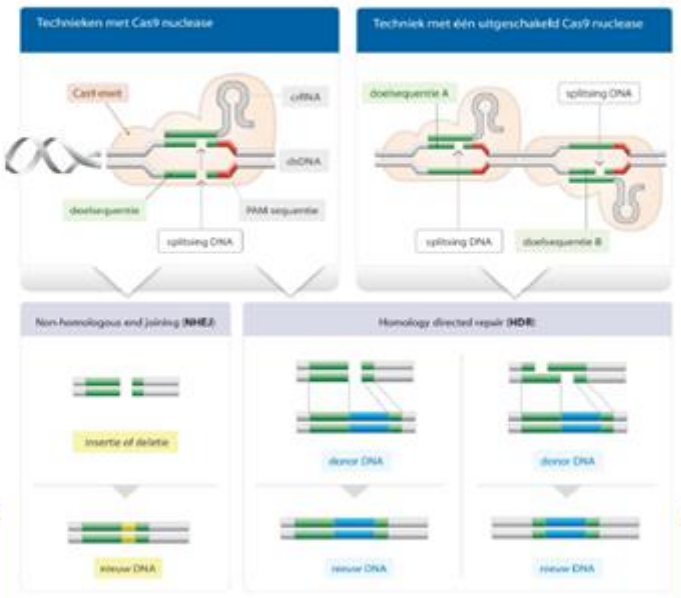
2. Homologous-directed repear



Also applicable in Eukaryotes

- crRNA and tracrRNA is combined to form a chimaere RNA (single guide RNA = sgRNA)
- CRISPR-Cas system can be introduced into plant cells:
  - Agrobacterium tumefaciens or Rhyzobium radiobacter
  - Protoplasts
  - Virusses used as vector
- Construct has a signal to lead Cas9 to the cel nucleus.

Variations



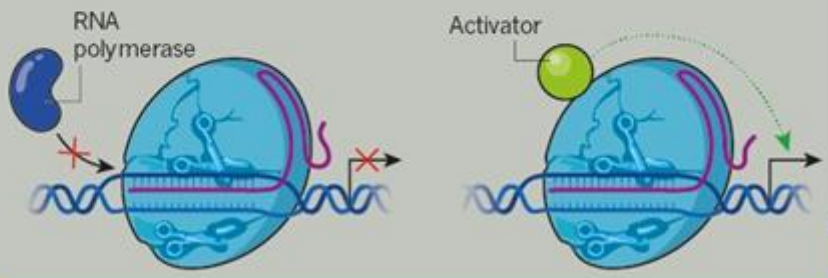
Null-variant I

Broken scissors

The Cas9 enzyme can be broken so that it no longer cuts DNA. But with the right guide RNA, it can still attach to specific parts of the genome.

CRISPR inhibition
A broken, or 'dead', Cas9 enzyme will block the binding of other proteins, such as RNA polymerase, needed to express a gene.

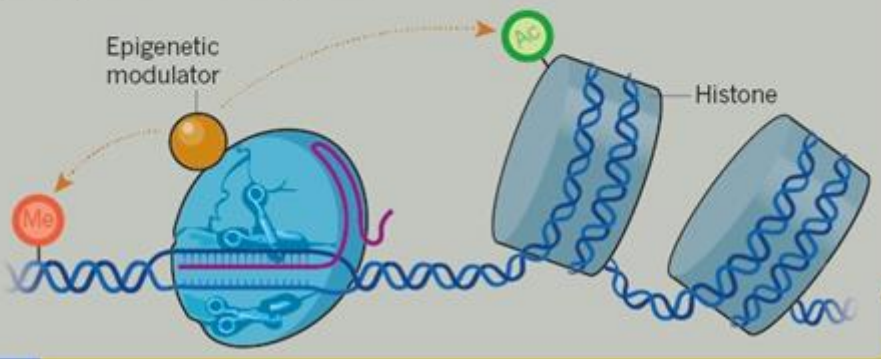
CRISPR activation
An activating protein can be attached to a dead Cas9 protein to stimulate expression of a specific gene.



Null-variant II

CRISPR epigenetics

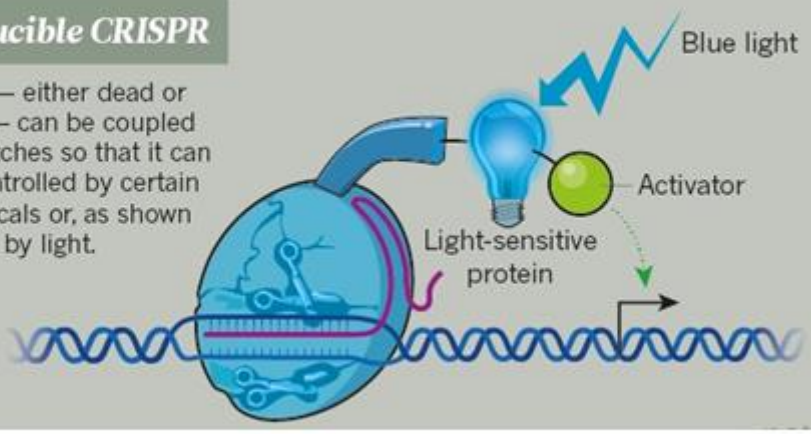
A broken Cas9 enzyme can be coupled to epigenetic modifiers, such as those that add methyl groups (Me) to DNA or acetyl groups (Ac) to histone proteins. This will allow researchers to study how precisely placed modifications affect gene expression and DNA dynamics.



Biological switch

Inducible CRISPR

Cas9 — either dead or alive — can be coupled to switches so that it can be controlled by certain chemicals or, as shown below, by light.



Advantages

- Gene-editing is possible on very specific locations in all living cells.
- Fast, cheap, effective and 'Dummy' proof.
- Changes with CRIPSR-Cas9 do not leave sequence and vector sequencies. Therefore not different from 'normal' mutations.
- Single locus or multi-locus
- On different levels
  - Targeted changes in DNA
  - Regulation (inhibit or stimulate – on/off switch)
  - Epigenetic processes

Example

- **Resistence for Powdery mildew in polyploid crop.**

Powdery mildew resistance in wheat using SSN-1

SSN-mediated mutation was used to knock-out the expression of the susceptibility gene mlo in bread wheat. Silencing of this gene led to resistance to powdery mildew. Wang, Y.P. et al. (2014) Simultaneous editing of three homoeoalleles in *hexaploid* bread wheat confers heritable resistance to powdery mildew. Nat. *Biotechnol*. 32, 947-951

Risks

- Off-target effects
  - Not enough specificity in sgRNA (only 20 nt and adjacent to PAM sequence)
  - Experimental conditions can effect off-target binding

Social, political, legal discussion

- Are CRISPR-Cas techniques GM events?
- In USA first (mushroom) variety released as non GM variety.
- Other commercial varieties (Mais) on the way….

DuPont Pioneer to Commercialize First CRISPR-Cas Product

Social, political, legal discussion

- Advisory board Dutch government on GM (COGEM):
  CRISPR-Cas products cannot unambiguously be assessed:
  - Transgene (GM)
  - Mutations/Indels (sometimes GM / sometimes non-GM)
  - Regulation of expression (non-GM)

Social, political, legal discussion

- Regulatory system: *Process-based vs Product-related*
  - **Process:** the process and used techniques are subject for regulation
  - **Product:** properties of a product or organism determine need for regulation. In this case the regulation does not need to be adjusted following technological and scientific developments
- Revision of European regulations needed
- Gap between science and legislation is widening rapidly. This may have huge consequences for industry, science and society

Social, political, legal discussion

- GMO in EU: Genetic modification in agricultural crops is relatively limited. Conventional breeding is still a good alternative.
- Techniques as CRISPR-Cas affect the whole biotechnological industry, the medical sector included. Major economic consequences.
- Ethical questions: 'enhancement' vs eradicating hereditary diseases.

UPOV type discussion

- Potential to produce large volumes of varieties, targeted to specific applications?
- Targeted editing to create a clear distinct variety with same market properties?
- Tiny or small differences; undermining the Right?
- Not only breeding companies can do it?
- Interesting for patenting?
- Should UPOV be part of the debate?

[End of Annex and of document]