CRISPR for dummies:

Stellenbosch UNIVERSITY IYUNIVESITHI UNIVERSITEIT forward together sonke siya phambili saam vorentoe

an introduction to CRISPR technologies

Manuela Campa

4th Sustainable Bio-Innovation Symposium

"Enabling Genome Editing Based Innovation in SA"

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Genome editing

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A tool for precise modification of the genome of a cell/organism

- meganucleases
- zinc finger nucleases (ZFNs)
- transcription activator-like effector nucleases (TALENs)

- CRISPR/Cas (Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) / CRISPR-associated protein (Cas))

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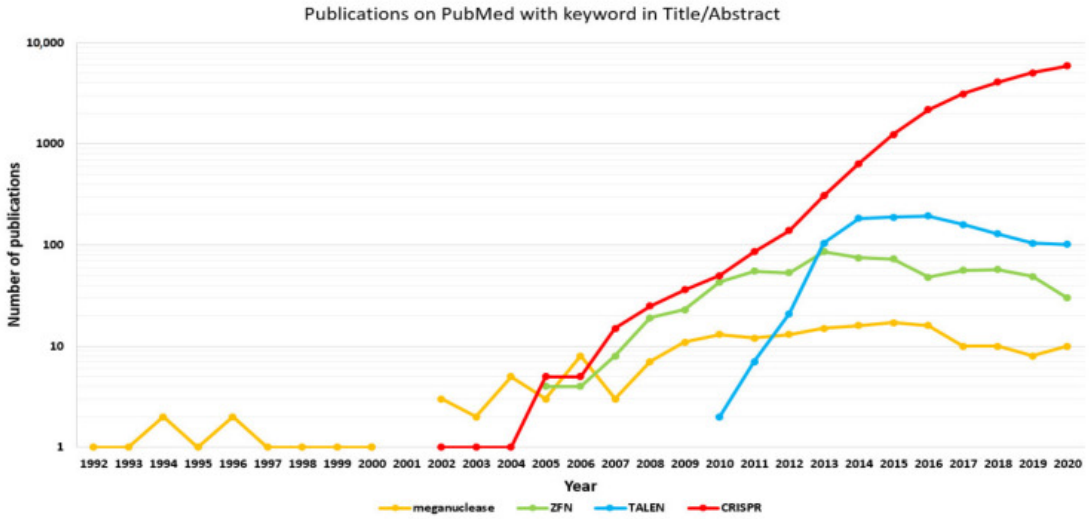
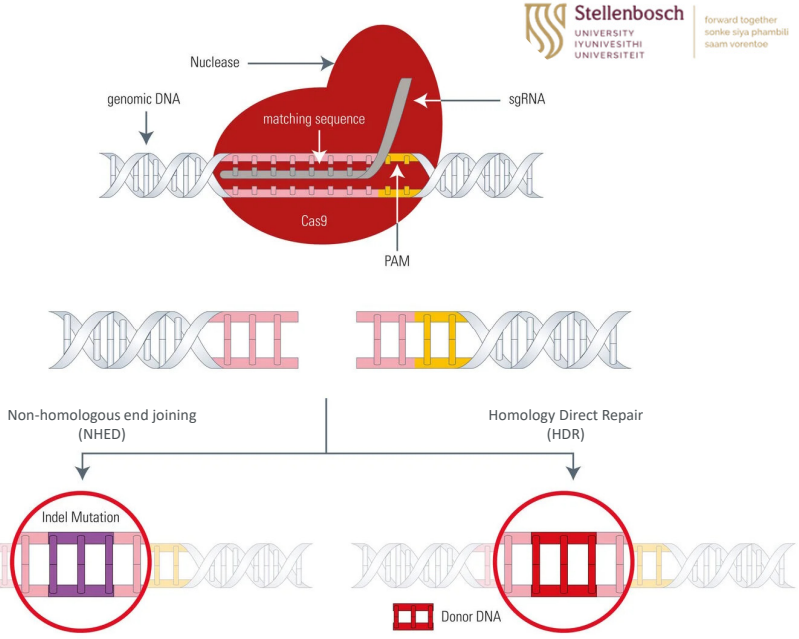
Genome editing



CRISPR/Cas9

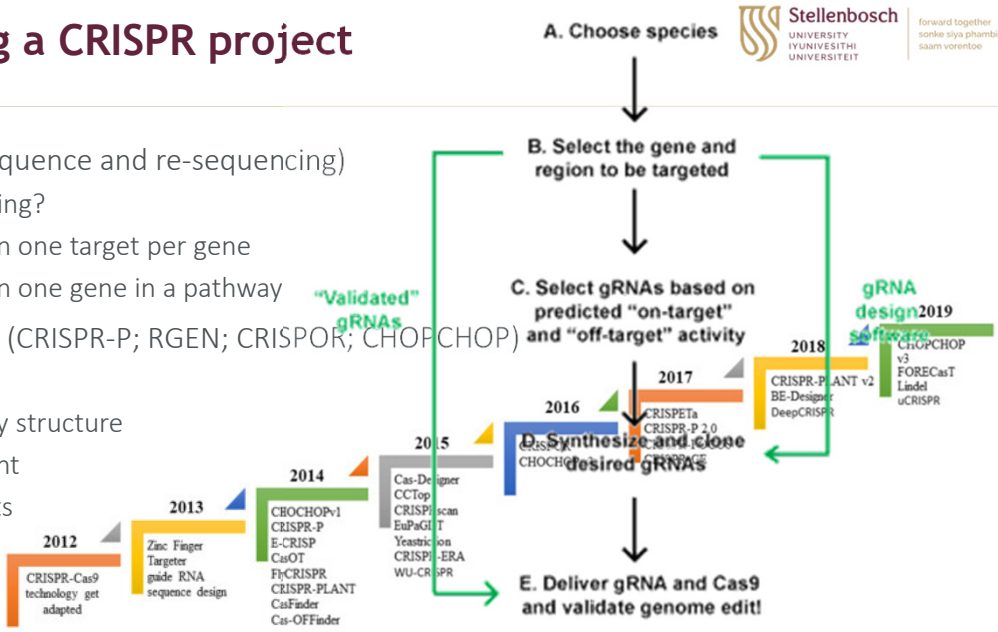
- Bacterial natural immune system
- 2012: tool for genome editing by Jennifer Doudna and Emmanuelle Charpentier
- Two components: endonuclease (scissor) and guide RNA (direct the scissor to the target DNA)



Executing a CRISPR project

Design

- Target ID (sequence and re-sequencing)
  - Multiplexing?
  - More than one target per gene
  - More than one gene in a pathway
- gRNA design (CRISPR-P; RGEN; CRISPOR; CHOPCHOP)
  - PAM
  - Secondary structure
  - GC-content
  - Off-targets



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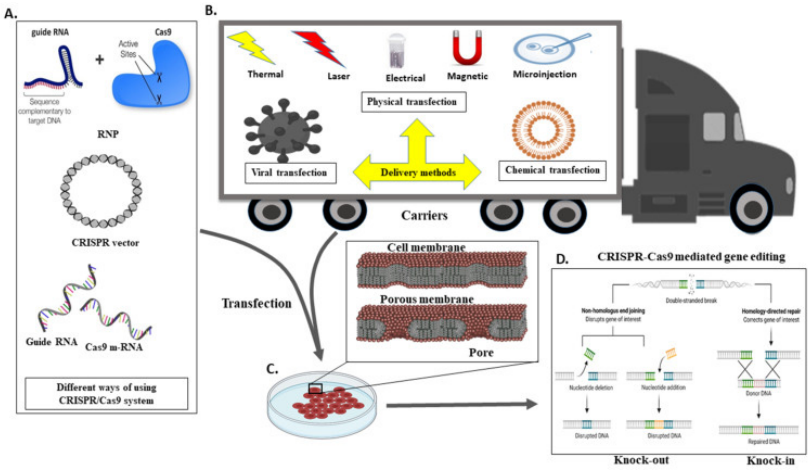
Perform: deliver Cas and gRNA into a cell

Delivery

- Ribonucleoprotein complexes (RNPs) – DNA-free
  - Cas9, Cas12: Thermo, IDT, NEB …
  - gRNA: Sigma, IDT …
- expressed from a plasmid under the control of an appropriate promoter
  - Addgene https://www.addgene.org/crispr/
- *in vitro* transcribed capped mRNA



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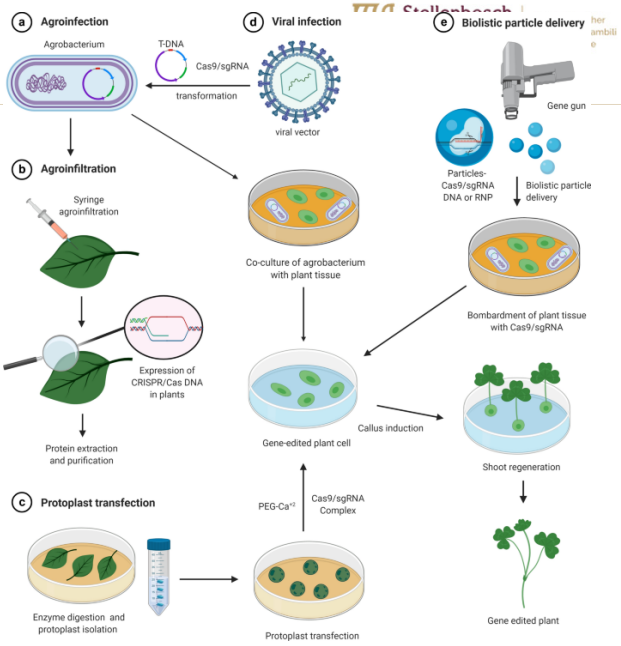

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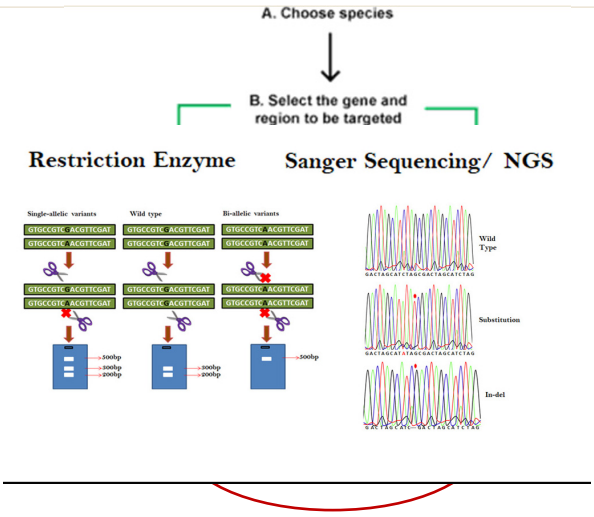
Evaluate: editing analysis

- Enzyme Mismatch Cleavage Assays, like the T7E1 endonuclease assay
- PCR amplification, Sanger sequencing and Tracking of Indels by Decomposition (TIDE) or Inference of CRISPR Edits (ICE)
- PCR amplification and next-generation sequencing (Illumina)



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Editing analysis

Method	Readout	Recommended Uses	Specialized Equipment	Bioinformatic Analysis Required	Turnaround Time	Cost
T7E1	Rough indel size and efficiency estimate	Optimization of experimental design; evaluation of several gRNA sequences	No	No	Same day	$
Sanger	Detailed sequence information at target site	Confirmation of T7E1 results with detailed sequence information	Yes	Yes	2-14 days	$$
NGS	Detailed sequence information including off-target sites and frequency	Large number of samples; Evaluation of off-target editing; Ideal for preparing regulatory reports	Yes	Yes	3-5 days	$$$$

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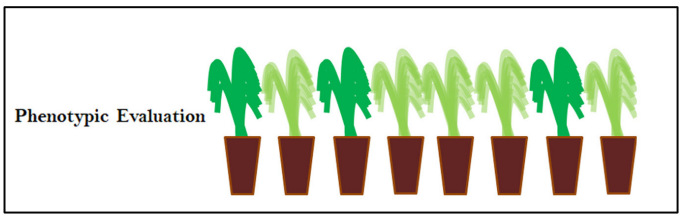
Phenotype/Functional characterization



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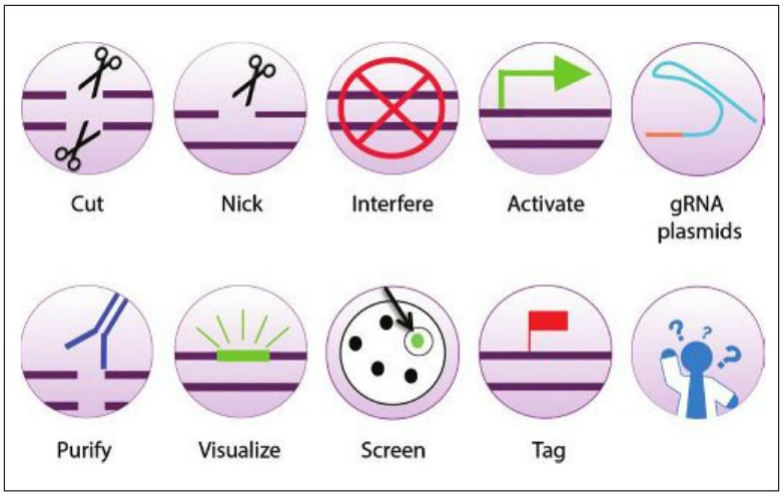
Applications

- Many Cas proteins
  - Different PAM
  - DNA and RNA
- dCas9 (expression regulation)
- nCas9 (increase specificity)
- Base editing
- Prime editing
- Epigenetic
- Visualisation



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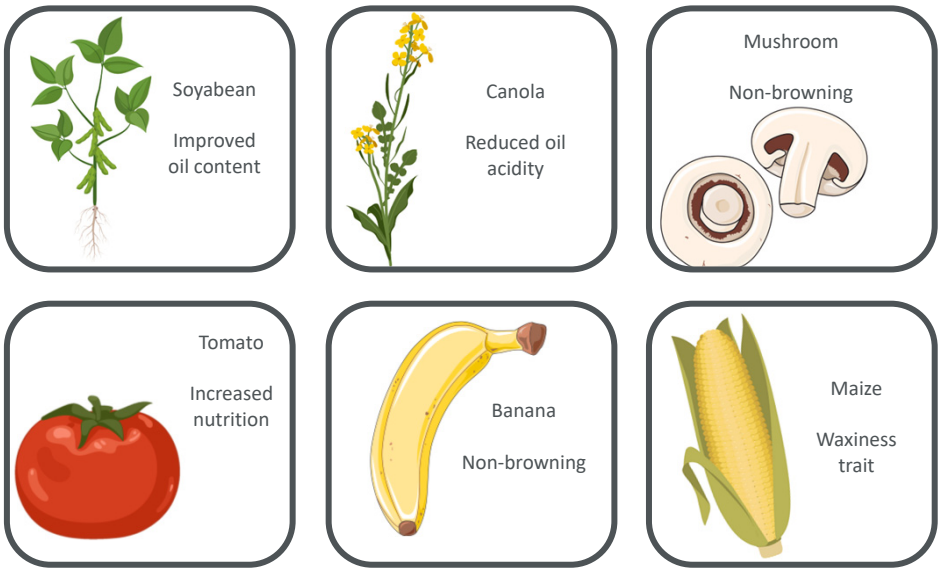
Thanks!



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