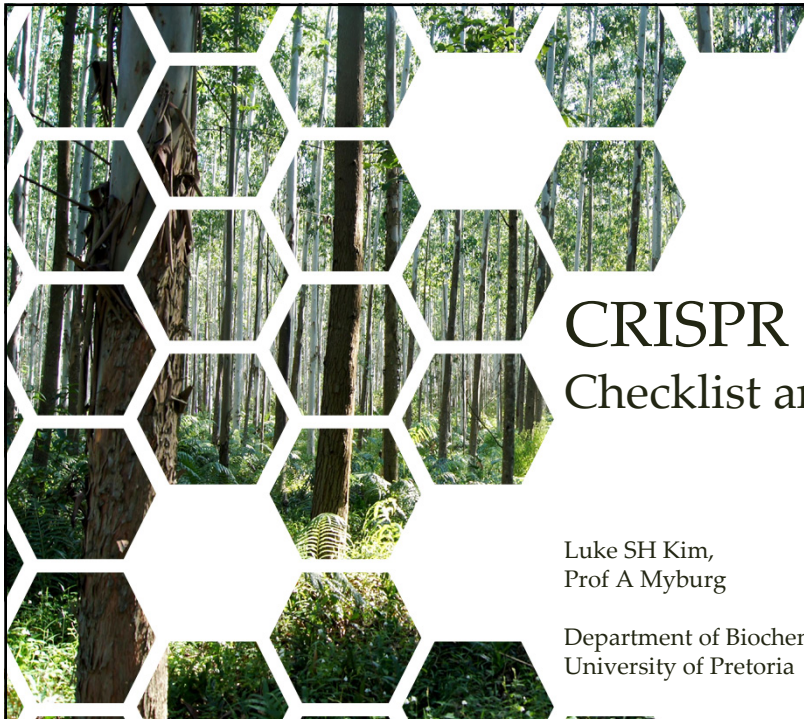




CRISPR in Forest Crops

Checklist and Do-nots

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1

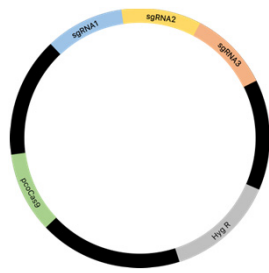


CRISPR to understand wood formation

- Woody biomass play a substantial role in the Bioeconomy of SA
- Candidate genes involved in wood formation were identified
- **Xylan biosynthesis genes**
- What is the biological function of xylan biosynthesis genes in wood formation?
- How does genetic manipulation of the xylan biosynthesis genes affect wood formation and processing?

2

2



Challenges with sgRNA

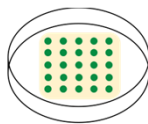
- Online tools (CRISPOR; Concordet *et al.* 2018)
- sgRNA efficiency tested using *in vitro* assay
- Efficiency variation
- Optimisation is key!

3

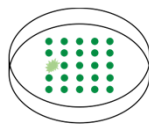
3



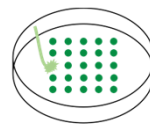
Agrobacterium
transformation



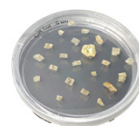
Co-cultivation



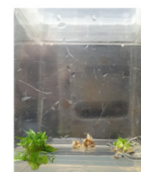
Callus formation



Shoot formation



Callus formation



Shoot initiation



Shoot elongation

Challenges with leaf disc transformation

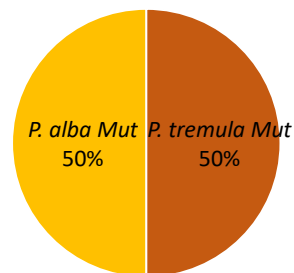
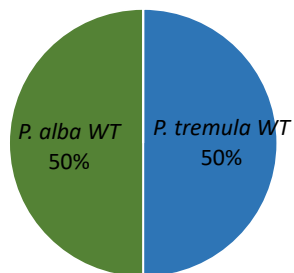
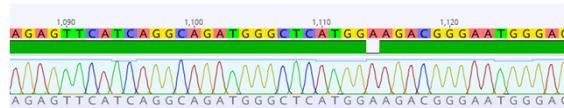
- *Agrobacterium*-mediated leaf disc transformation
- Low efficiency (0~2.4%)
- Optimisation of media
- Success in mass!

4

4

Challenges with mutation screening methods

○ Sanger sequencing

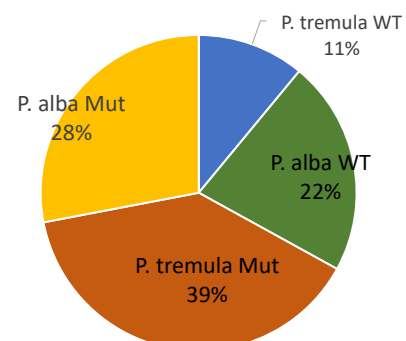


5

5

Genetic mosaicism in CRISPR mutants

- Illumina MiSeq
- Allele-specific mutation
- Percentage mutation
- **Genetic mosaicism**
- Implications?



6

6

Summary



Experimental limitations

sgRNA - Variation in efficiency

Transformation - Recalcitrance

Mutation screening - Limited detection power



Implications

Unsuccessful or partial mutagenesis

Low transformation efficiency & limited transformation methods

Misinterpretation of mutagenesis



What to do?

Validate the efficiency *in silico* and *in vitro* prior to *in planta* experiment

Explore novel transformation approaches

Thorough investigation using **NGS**

7

7



What is your experience with CRISPR?

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