CRISPR-Cas9 ribonucleoprotein-mediated gene editing in the plant-pathogenic fungus *Magnaporthe oryzae*

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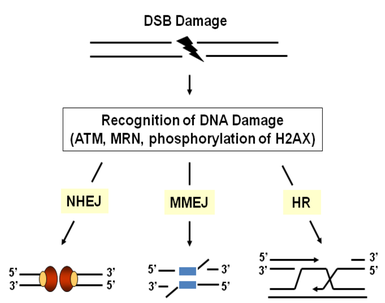
Background

DNA double-strand breaks (DSBs), a type of DNA damage, can be lethal or cause genome instability if left unrepaired.

There are three major DNA DSB repair pathways characterized from animal systems.

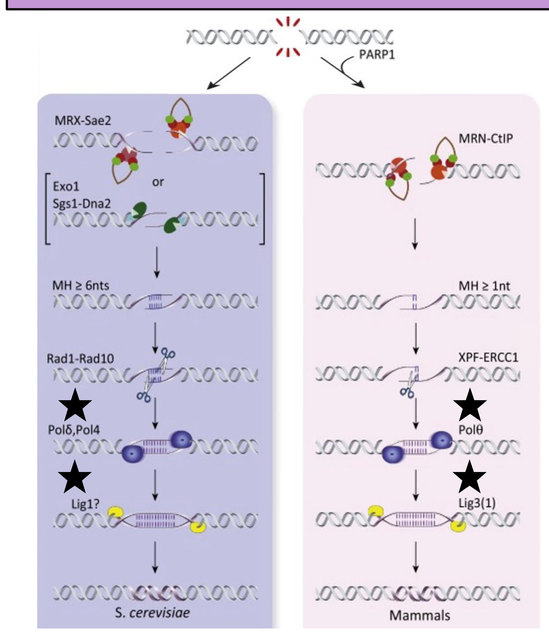
However, filamentous fungi lack the known genes involved in microhomology mediated end joining (MMEJ)



Three major DSB repair pathways in animals (Kim et al, 2013)

Research Question: What genes in *M. oryzae* are involved in MMEJ?

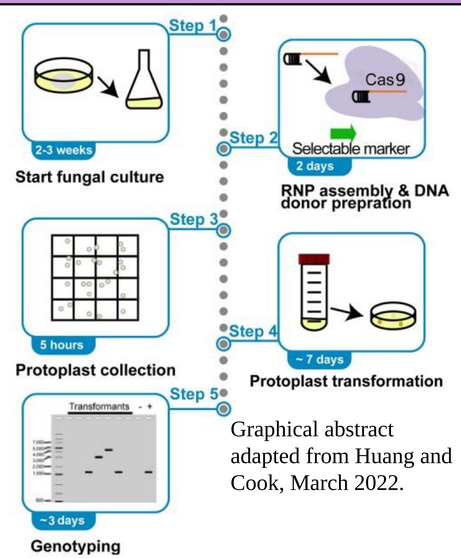
Methods



MMEJ DSB repair pathway (Sfeir and Symington, 2016)

Genes of interest	
Lig1A	Ligase 1 paralogs
Lig1B	
Polθ	Polymerases for DSB repair
Pol3	
Pol4	

We want to test if genes involved in other DNA DSB repair pathways have separate functions in MMEJ in *M. oryzae*.



Graphical abstract adapted from Huang and Cook, March 2022.

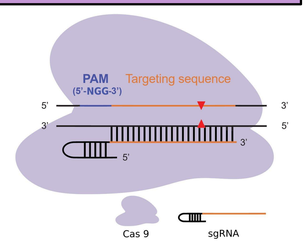
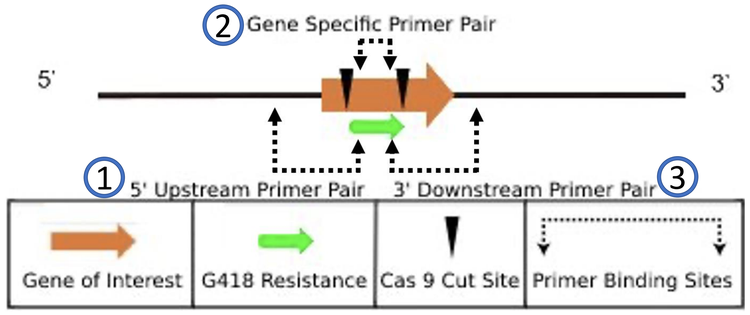


Diagram of Cas9 RNP, adapted from Huang and Cook, March 2022.

Link to protocol

We created knockouts for homologs of DNA repair genes, using CRISPR-Cas9 ribonucleoproteins (RNPs) to make cuts in the DNA surrounding our genes of interest (GOI). Donor DNA was added to allow antibiotic selection

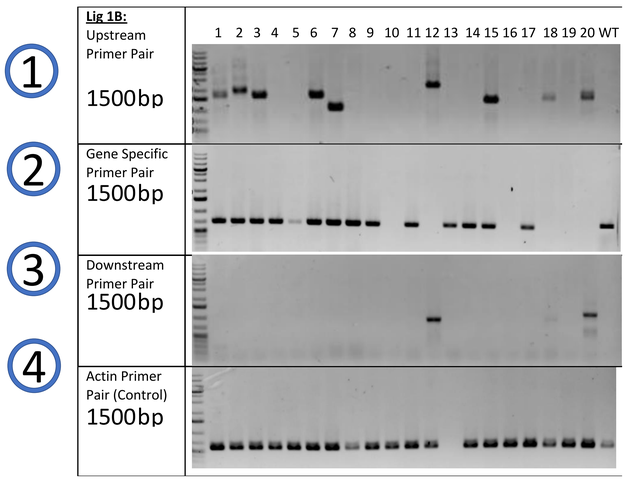
PCR genotyping to identify KOs



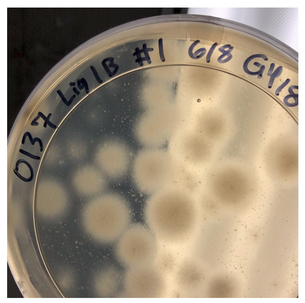
Schematic illustration of PCR primer pairs used for genotyping, adapted from Huang and Cook, March 2022.

1. The 5' upstream primer pair only amplifies a PCR product when G418 inserts at the GOI.
2. The gene specific primer pair only amplifies a PCR product in wildtype.
3. The 3' downstream primer pair only amplifies a PCR product when G418 inserts at the GOI.
4. Actin (not shown above) will act as a positive control to confirm that DNA extraction was successful.

Results



PCR genotyping of Lig1B transformants



Lig1B Transformants growing on complete media containing G418 antibiotic

Genes of Interest	# of transformants collected	# KOs based on PCR
Lig1A	13	0
Lig1B	20	3
Polθ	13	0
Pol3	14	TBD
Pol4	16	TBD

Results of PCR genotyping to identify knockouts.

One gene has been successfully knocked-out to date, although I am currently screening transformants of two additional genes.

Conclusion

The knockout strains created from this project will be used in future research projects to understand their impact on MMEJ

Acknowledgements

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