Developing near isogenic *Magnaporthe oryzae* lines to evaluate genome position effects

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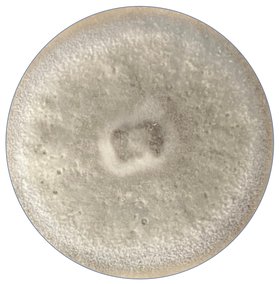
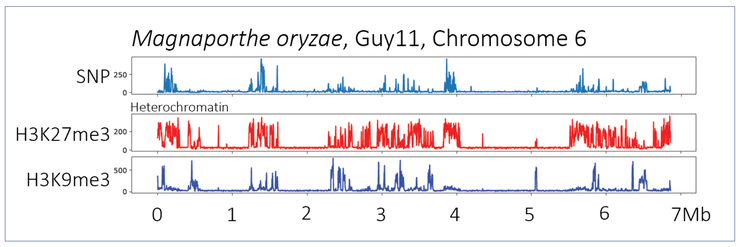
Background

Magnaporthe oryzae is a filamentous fungal pathogen that poses a major threat to global agriculture.

DNA variation between *M. oryzae* strains contributes to differences in host range and pathogen success.


An *M. oryzae* colony

Regions of the genome are more variable than others.



Regions with many single nucleotide polymorphisms (SNPs) occur in heterochromatic regions.

Question:
- How could genome position effects in *M. oryzae* be evaluated?

Objective:
- Use a CRISPR-Cas12 system to develop near isogenic *M. oryzae* lines with the selectable marker gene *URA3* at different loci

Creating Near Isogenic Lines

Guide RNA (gRNA) for each of 11 targeted loci were identified as a 23 base pair sequence and combined with the Cas12a protein to form a ribonucleoprotein (RNP) which cuts the DNA at the targeted sequence

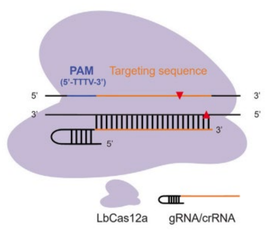
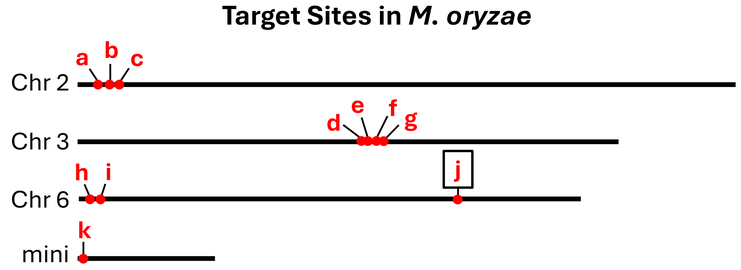


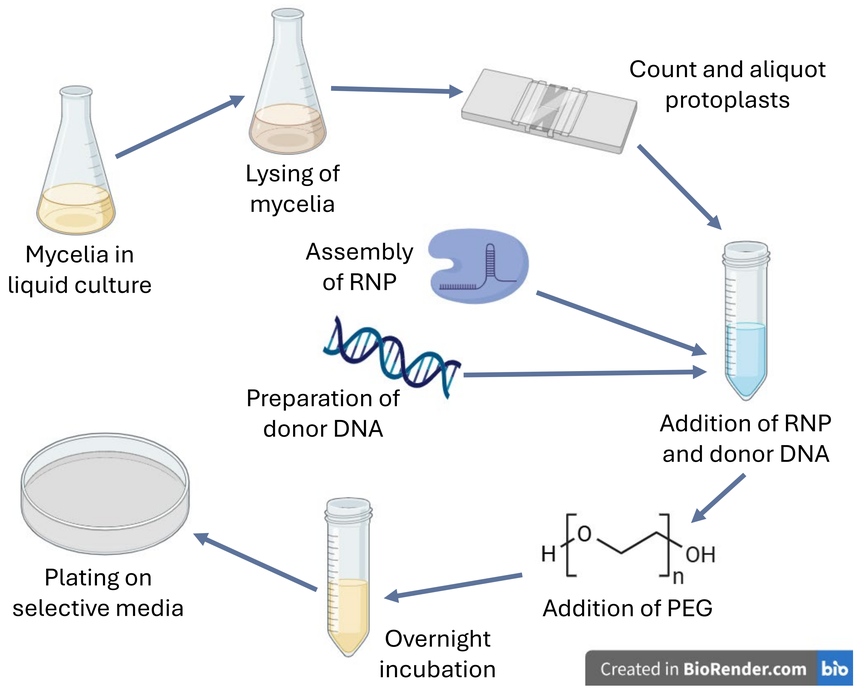
Diagram from Huang and Cook, 2022.

Target Sites in *M. oryzae*



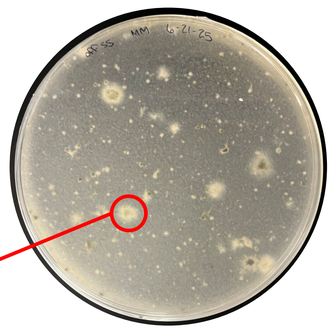
The target sites are shown in their approximate locations in the genome. The wild-type locus is marked with a box.

Transforming *M. oryzae*



Phenotypically Selecting Putative Transformants

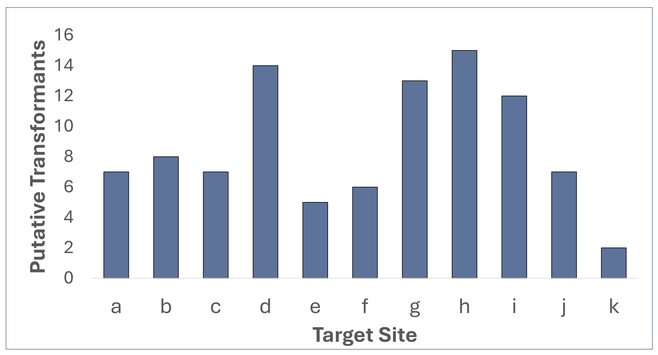
Transformed cells were phenotypically selected on media lacking uracil and uridine which selects for colonies with a working copy of *URA3*.


Colonies growing on selective media.


Isolated colony on selective media.

Putative transformants were isolated onto selective media to confirm the phenotypic selection.

The number of putative transformants varied between target sites.

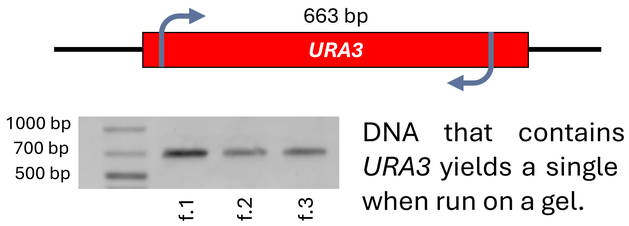


Genotyping Near Isogenic Lines

DNA was extracted from the isolated colonies and used for polymerase chain reaction (PCR) genotyping.

Prescence of *URA3*

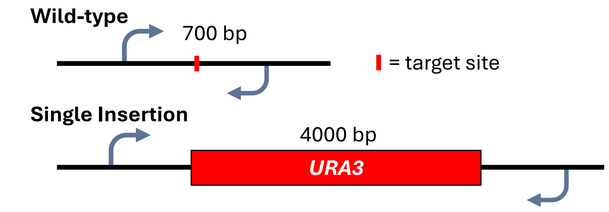
Primers amplify a 663 base pair (bp) section within the donor DNA.

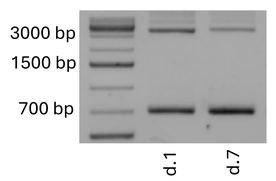


DNA that contains the *URA3* yields a single band when run on a gel.

Single Insertion at the Locus

Primers flank the target site. The wild-type product is 700 bp and a single insertion of the donor results in a 4000 bp product.

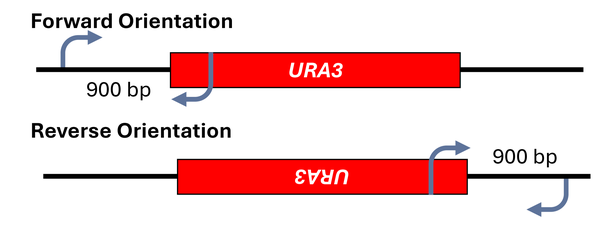


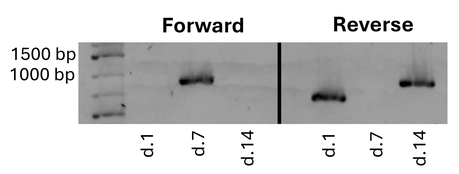


DNA from mixed samples yields a single insertion and wild-type bands.

Donor DNA Orientation

One primer is located inside the donor and amplifies out, and the other is found near the target site.





Single insertions show a forward or reverse band, indicating the donor direction.

Conclusions

- One near isogenic line has been confirmed with *URA3* on chromosome 6.
- Other lines are currently in the process of being genotyped.
- Transformations that resulted in no successful new lines are currently being repeated
- These lines will be used in future experiments to evaluate potential genome position effects in *M. oryzae*.

Acknowledgements

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