



Inducing Mutations in *Pyricularia oryzae* to Observe Repair Pathways

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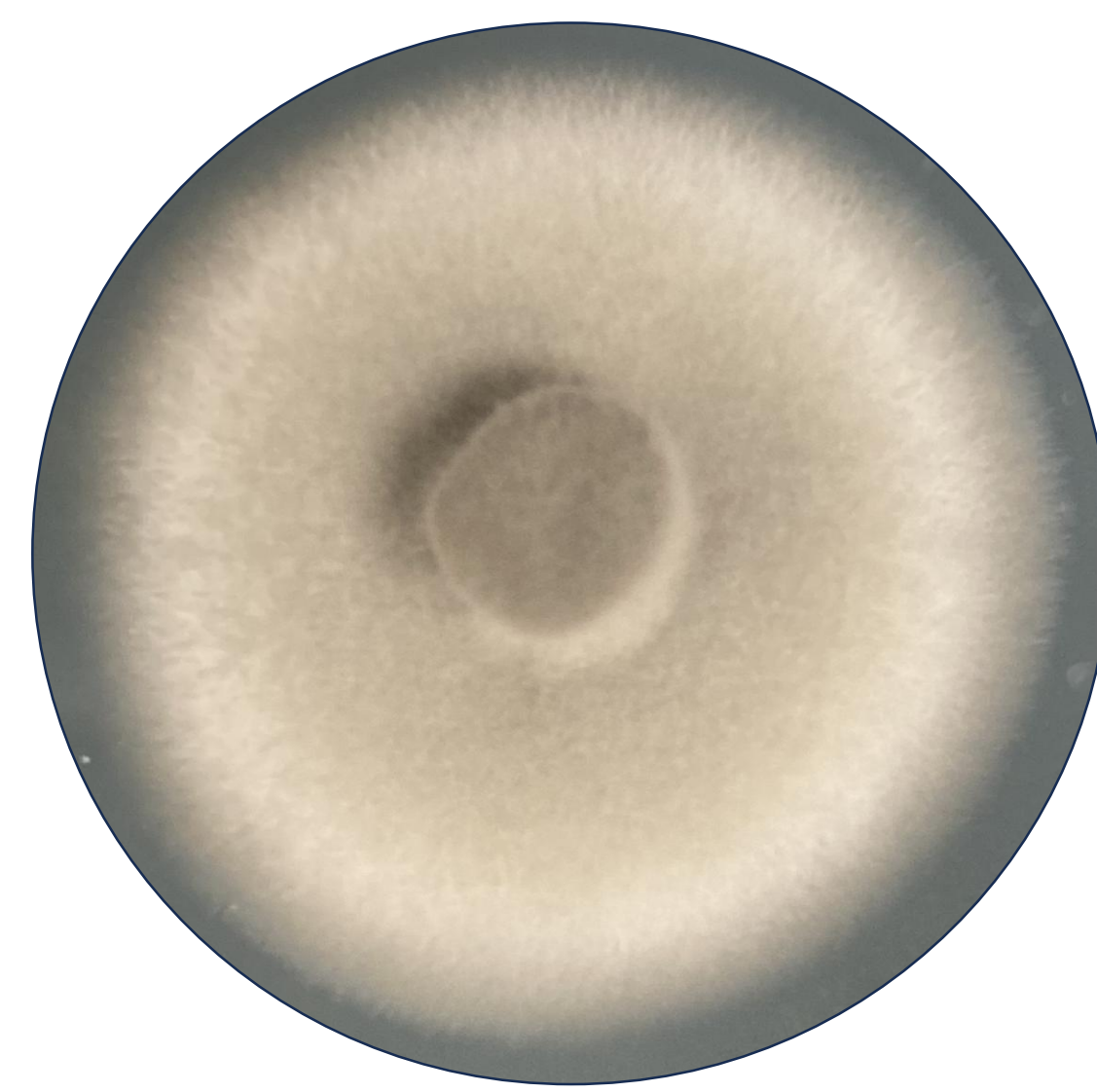


Background

Pyricularia oryzae



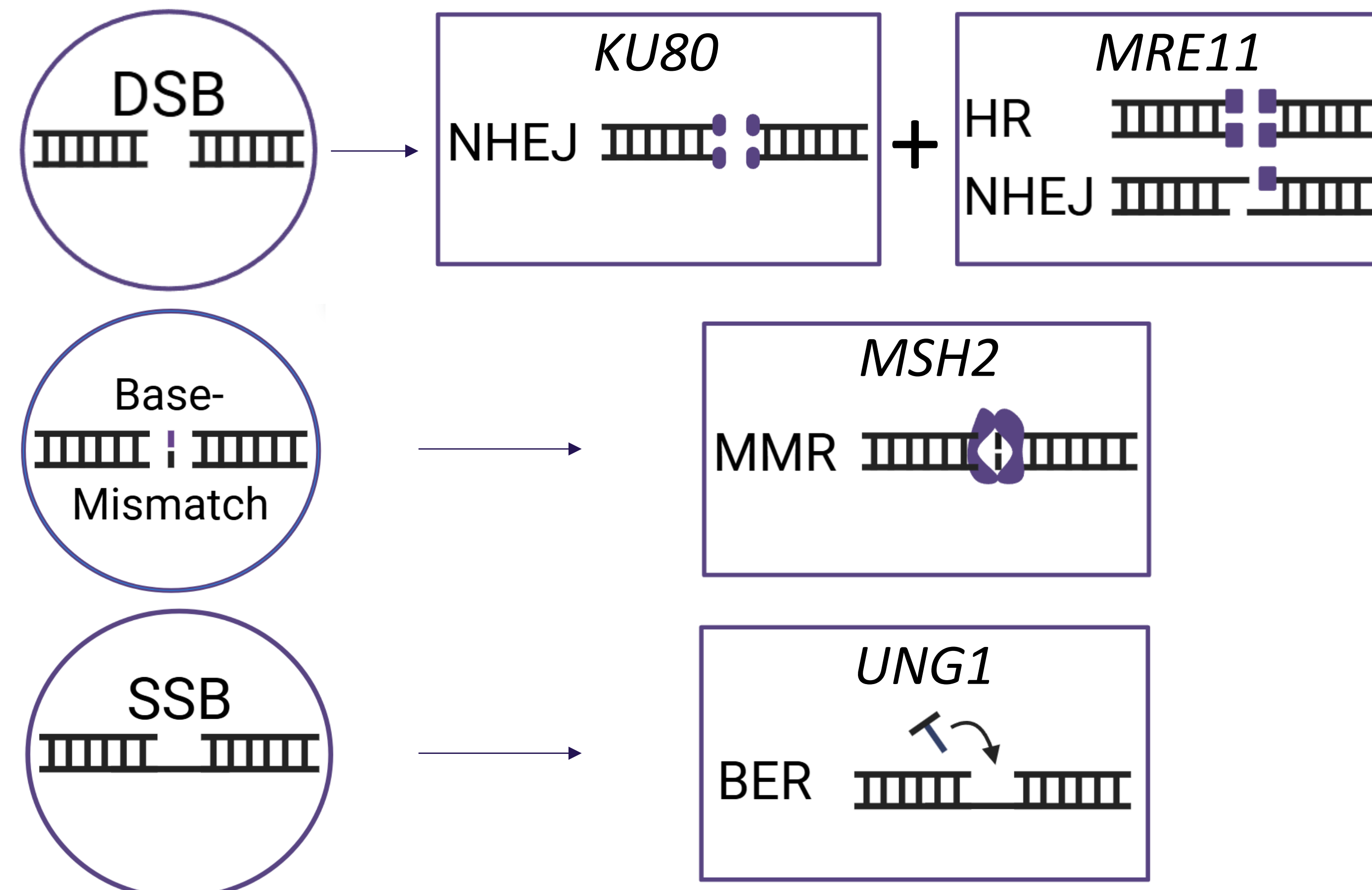
Rice infected with *P. oryzae*



P. oryzae colony

P. oryzae generates genome variation while reproducing asexually

Damage and repair bring variation to the genome:



Questions:

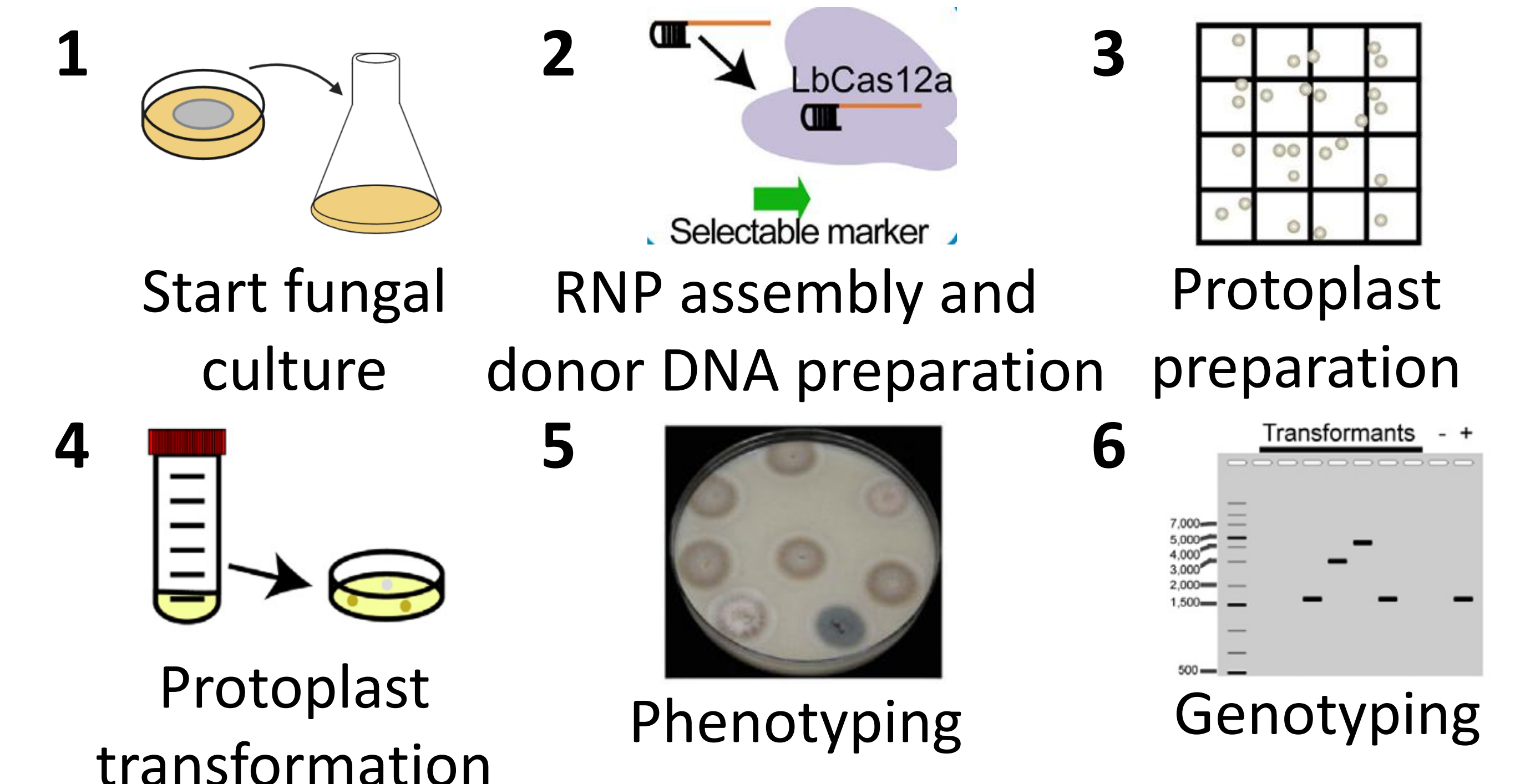
- Where do mutations occur in the genome?
- How do repair pathways shape mutation location?

Objectives:

- Accumulate mutations
- Knock out BER/MMR genes with Cas12a.

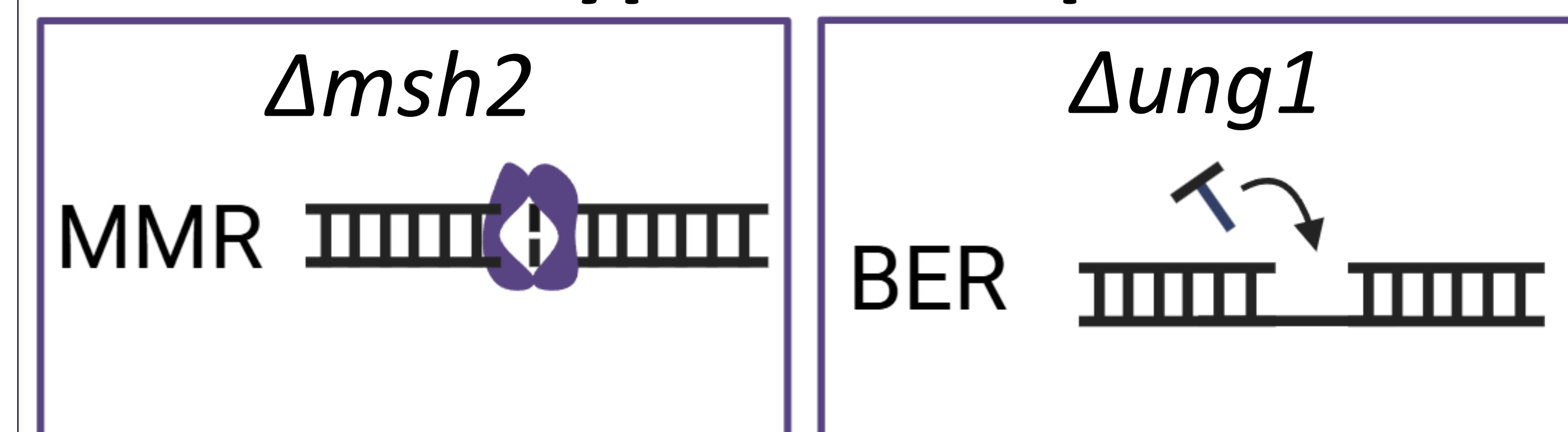
Knock-Out of Repair Genes

CRISPR-Cas12a Gene Editing:



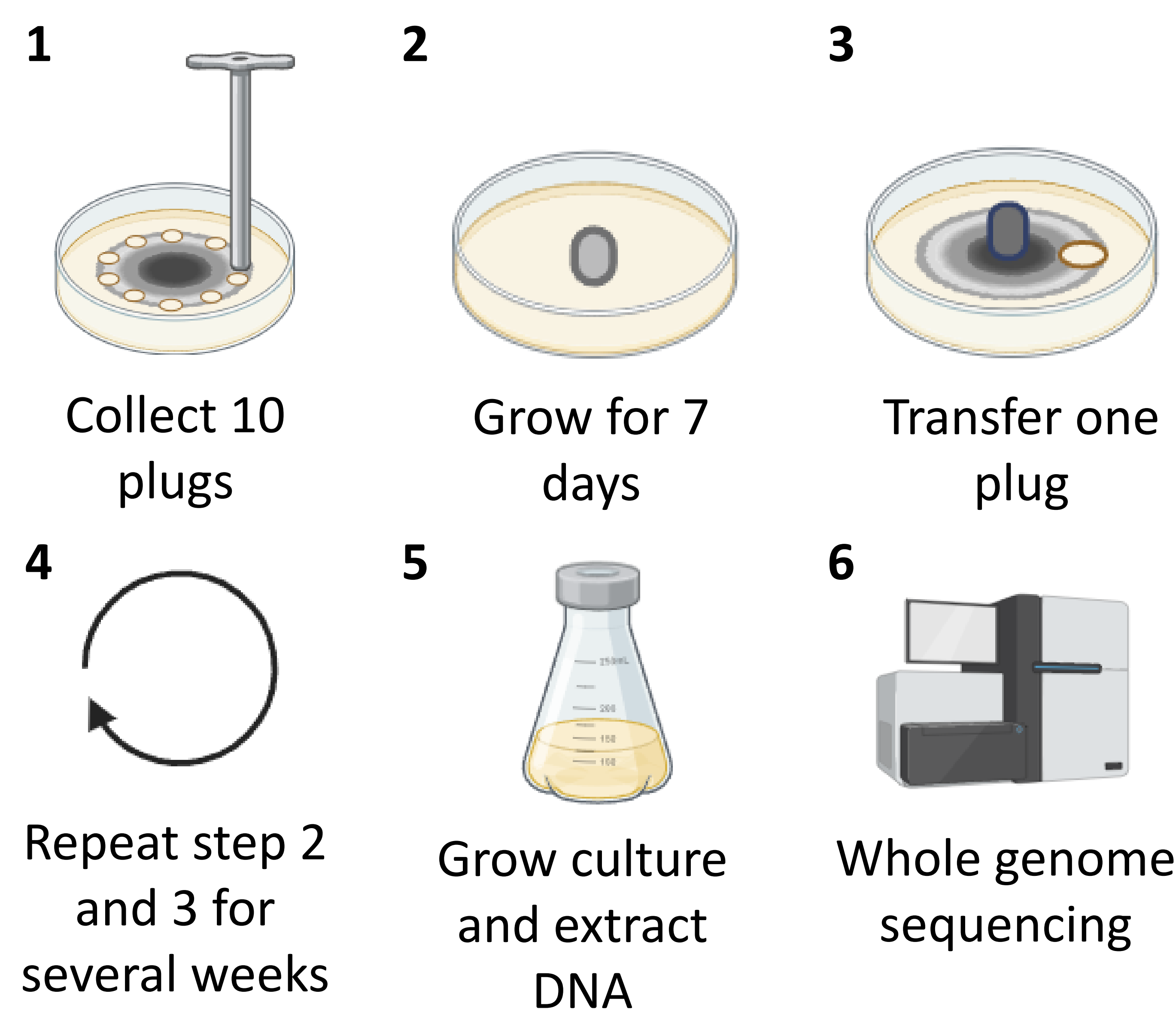
Huang & Cook, STAR Protocols 3, March 18, <https://doi.org/10.1016/j.xpro.2021.101072>

Genotypes attempted:

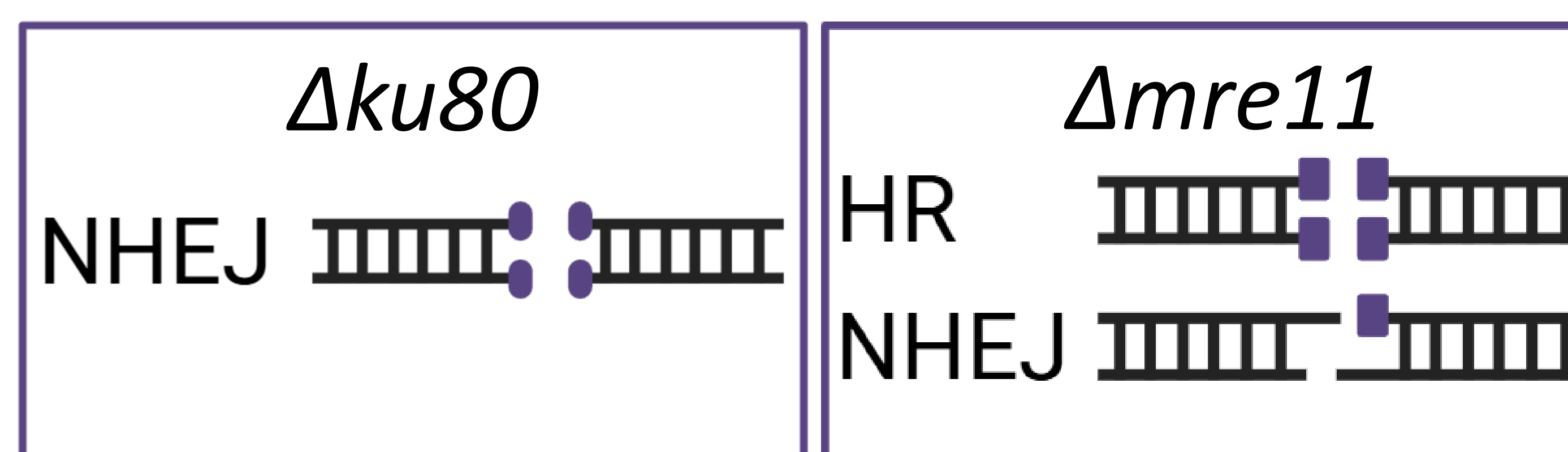


Mutation Accumulation

Mutation Accumulation:

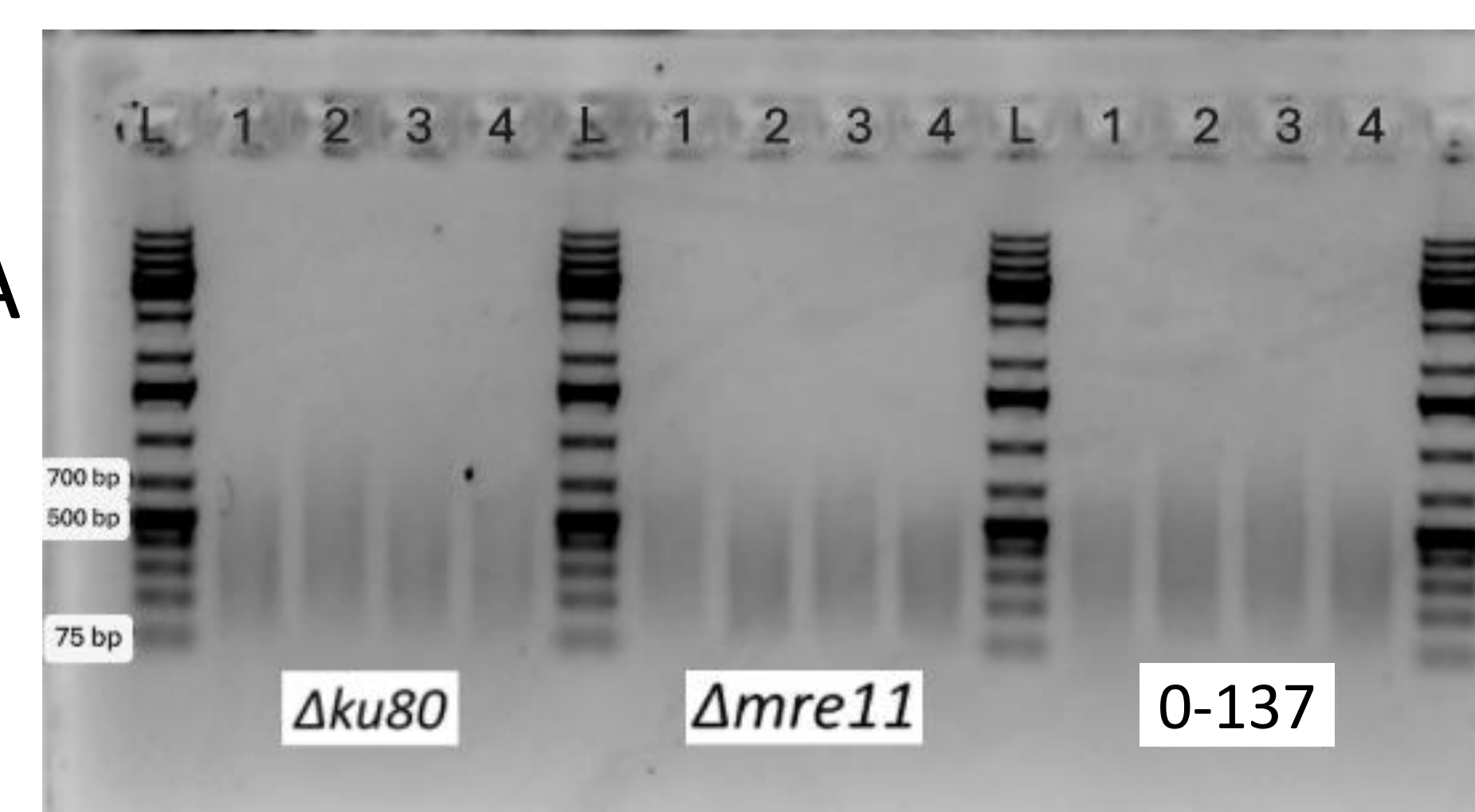


Gene-edited genotypes used:

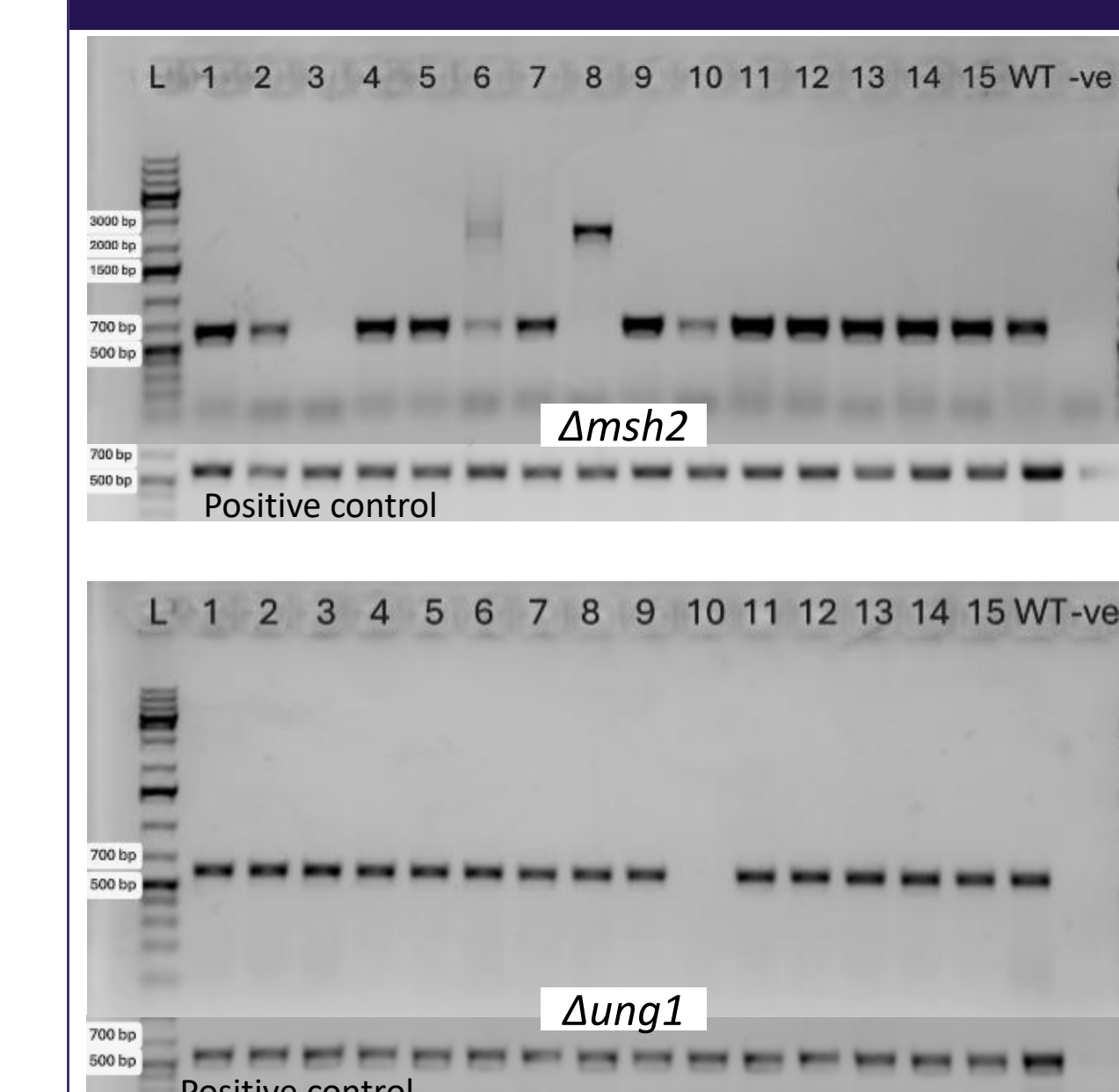


Results

The sheared DNA is the expected size of 300 bp.



Results



One MSH2 sample increased in size from a clean HYG gene insertion at the target site

No UNG1 samples showed an increase in size from the HYG insertion

Future Work

- Use repair gene knockout lines in future experiments.
- Sequence genomes from the mutation accumulation experiment.

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