Using CRISPR to Model Acute Intermittent Porphyria in Yeast

Kirsten Alsua OMS II*, Donna Oh OMS II*, and Brian M. Wasko, Ph.D.
*Equally contributing authors
Department of Basic Medical Sciences
Western University of Health Sciences, Lebanon, OR 97355

Western University of Health Sciences
College of Osteopathic Medicine of the Pacific
COMP-Northwest

Abstract

Acute Intermittent Porphyria (AIP) is an autosomal dominant genetic disorder resulting from the dysfunction of hydroxymethylbilane synthase (HMBS) in the heme biosynthetic pathway. The dysfunction presents as severe, acute, and recurrent attacks, albeit with non-specific general symptoms which mask the prevalence of the disease and promote misdiagnosis in its early stages. Undiagnosed or late diagnosed disease states can have exceptionally high cost of healthcare, with end-stage diagnoses requiring liver transplant as the only recourse of treatment. AIP is relatively rare with low penetrance levels and less than 1% of those affected with the mutation express the severe acute phenotypic traits. How additional genetic and environmental factors influence disease penetrance is poorly understood. Our goal is to explore factors that influence cellular phenotypes resulting from AIP using an in vivo yeast model containing AIP-associated mutations in the conserved HMBS gene (*HEM3*) that are known to have strong effects on enzymatic function.

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) was utilized to induce three independent mutations (hem3Δ1, hem3ΔC230, hem3-Q194R) in yeast cells and the mutations were confirmed with genetic sequencing.

Yeast growth was quantified using optical density (OD) measurements over a 24 hour period. The *hem3* loss of function mutants required exogenous hemin for growth. The hem3-Q194R missense mutant did not display hemin dependent growth and did not exhibit increased sensitivity to paraquat. We aim to utilize the hem3 mutants to determine if sensitivities to exogenous stressors exist, which will then be used as phenotypes to initiate genetic suppression screens. The goal of the project is to identify genetic factors that can modulate phenotypes arising from AIP causing mutations, in order to identify genes and pathways that may play a role in the etiology of human AIP disease penetrance.

Background

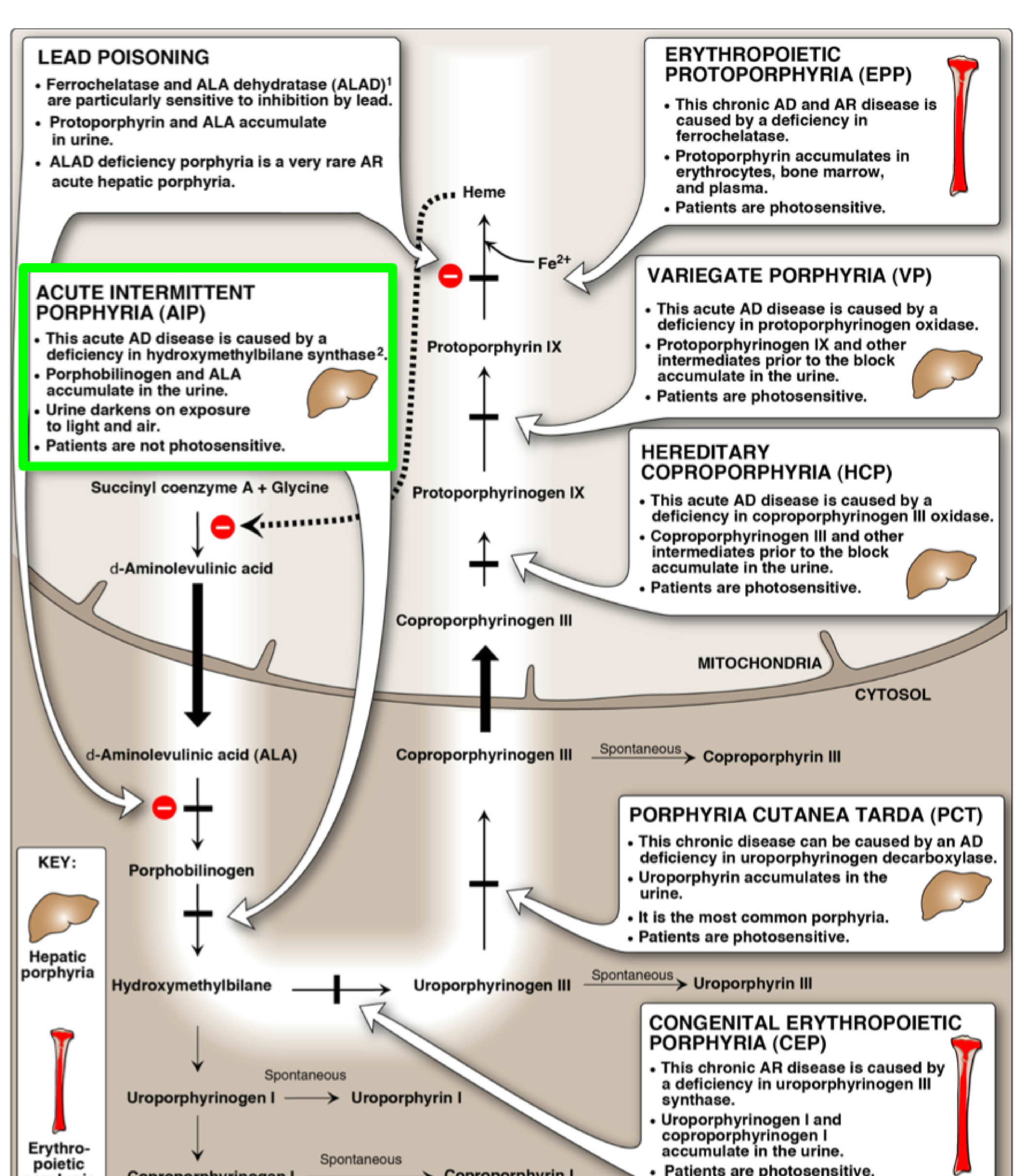

Figure 1. Defects in heme biosynthesis result in porphyrias. Mutations in the HMBS gene that result in enzymatic defects result in acute intermittent porphyria (AIP).

Results

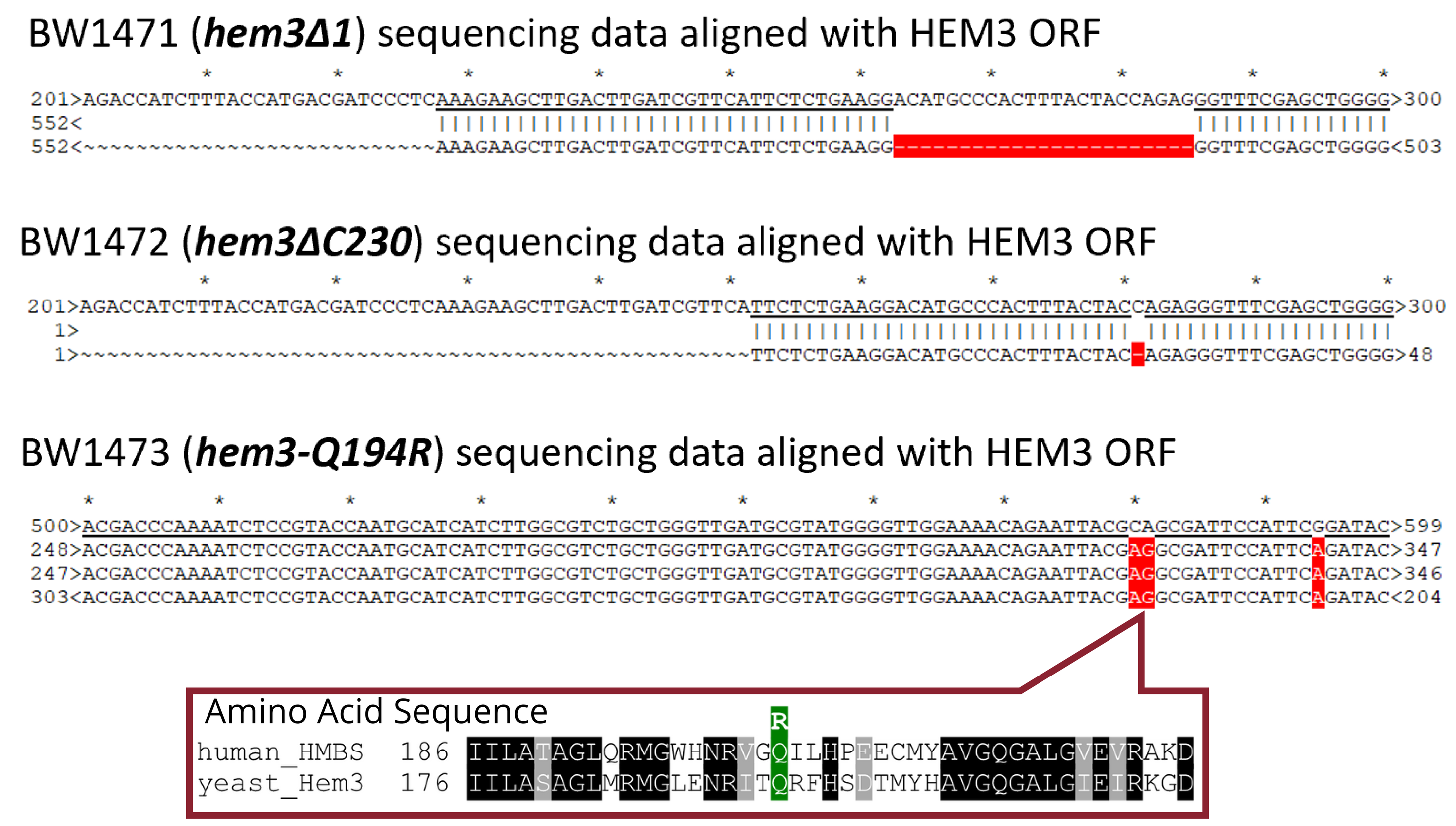

Figure 2. Nucleotide alignments of a portion of the *HEM3* gene with sequencing results in the newly generated yeast strains annotated hem3Δ1, hem3ΔC230, hem3-Q194R. The wildtype gene sequence is indicated on the top lines of the alignments. A partial amino acid sequence alignment of human HMBS and yeast Hem3 protein is also indicated.

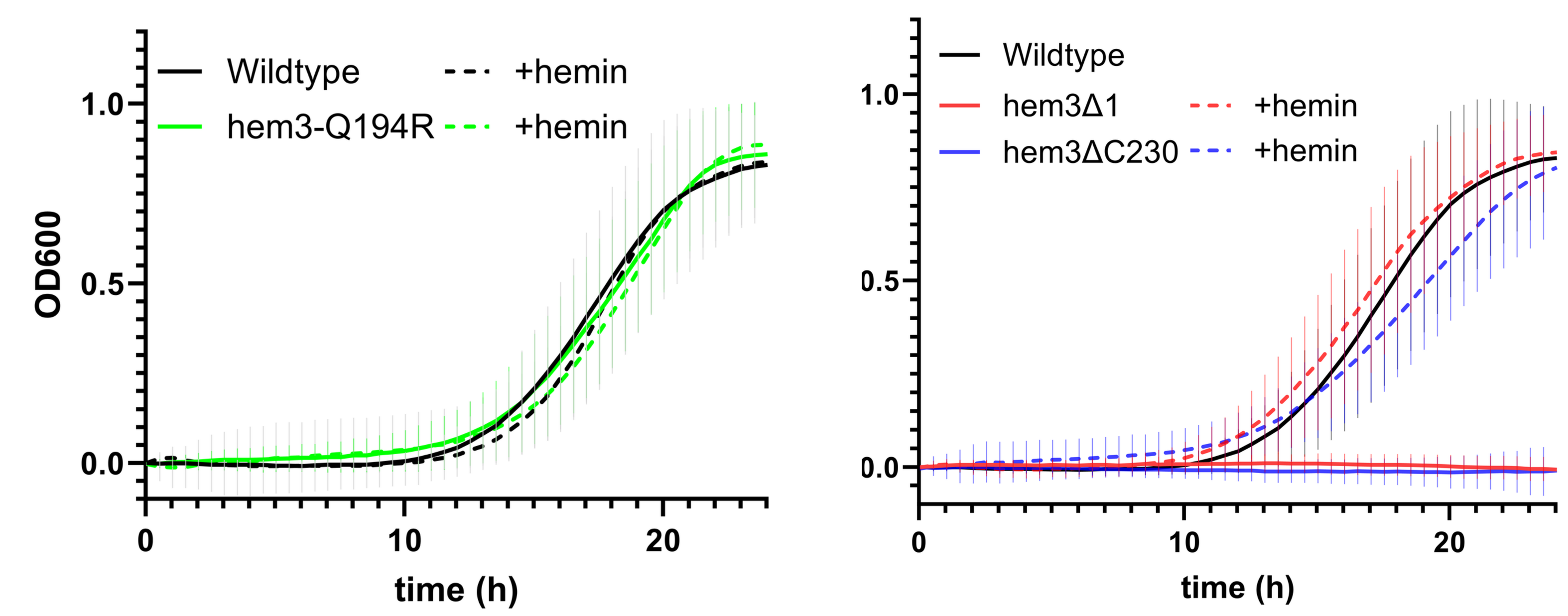

Figure 3. Hemin dependent growth is observed in mutants with loss of function of the *hem3* gene. Optical density at 600 nM (OD600) is measured over time to assess the growth of yeast in the presence and absence of 5 μM hemin in YPD growth media. The wildtype control and hem3-Q194R strain grew independent of hemin, while both deletion strains exhibited hemin dependent growth.

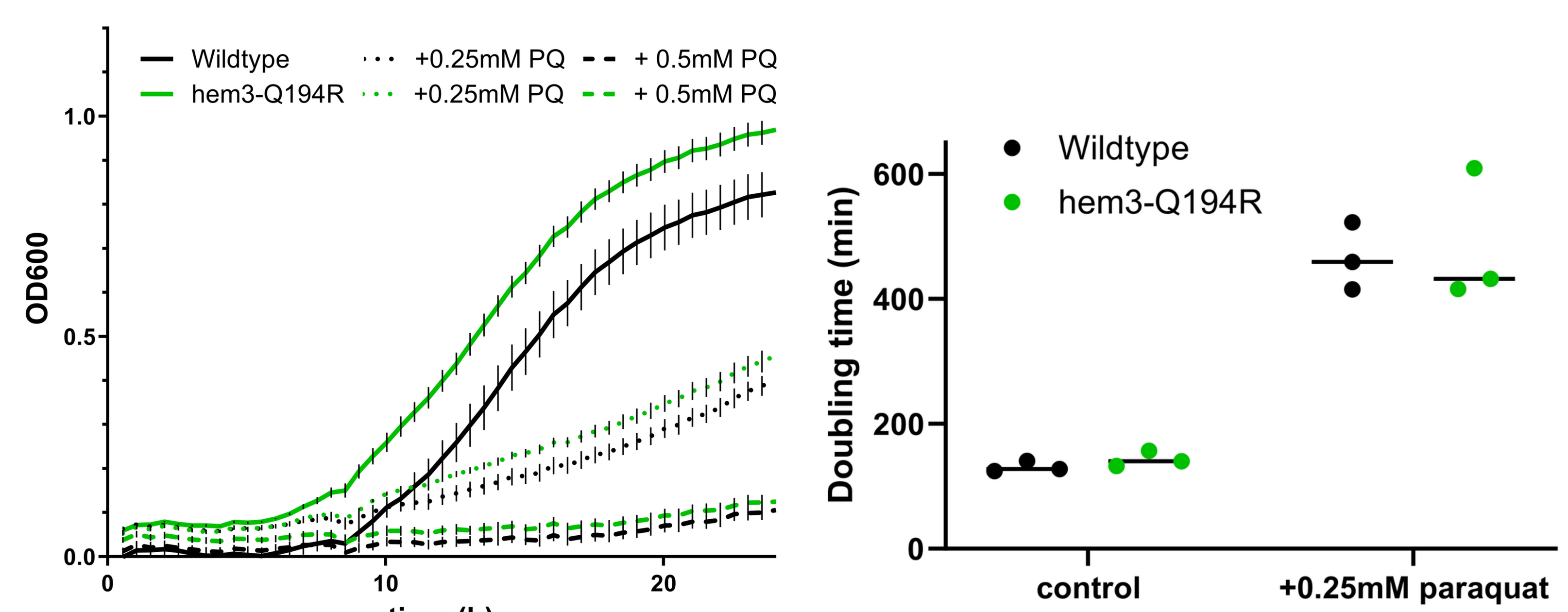

Figure 4. hem3-Q194R is not more sensitive than wildtype yeast to paraquat (PQ), a chemical that induces reactive oxygen species (ROS). Growth curves and calculated doubling times for yeast strain BW1473 (hem3-Q194R) and a wildtype control strain (BW4742) in the presence or absence of paraquat, indicates that paraquat impairs growth similarly in both strains.

Methodology

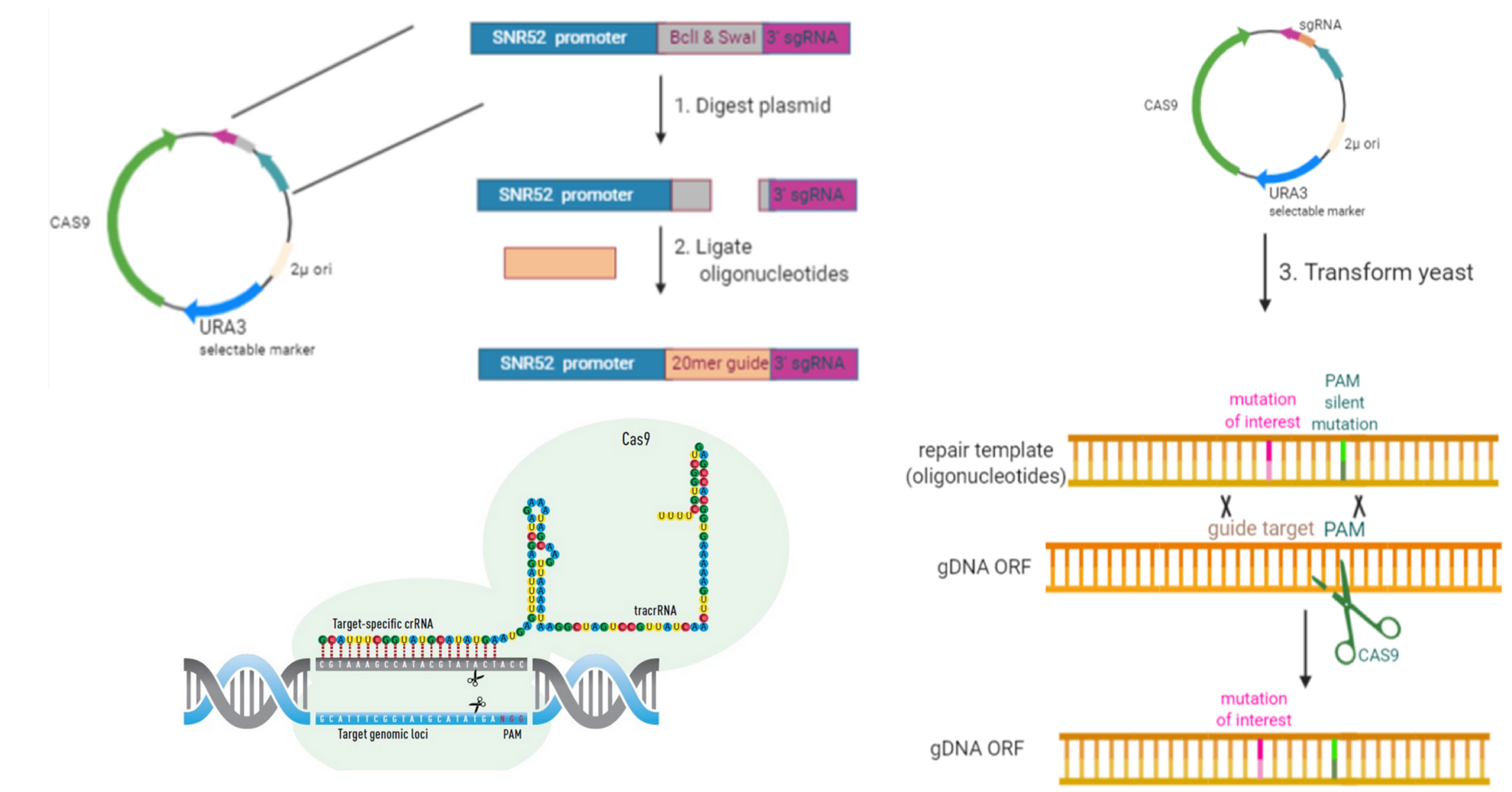

Figure 5. Schematic of CRISPR strategy to introduce mutations, based on Laughery et al.

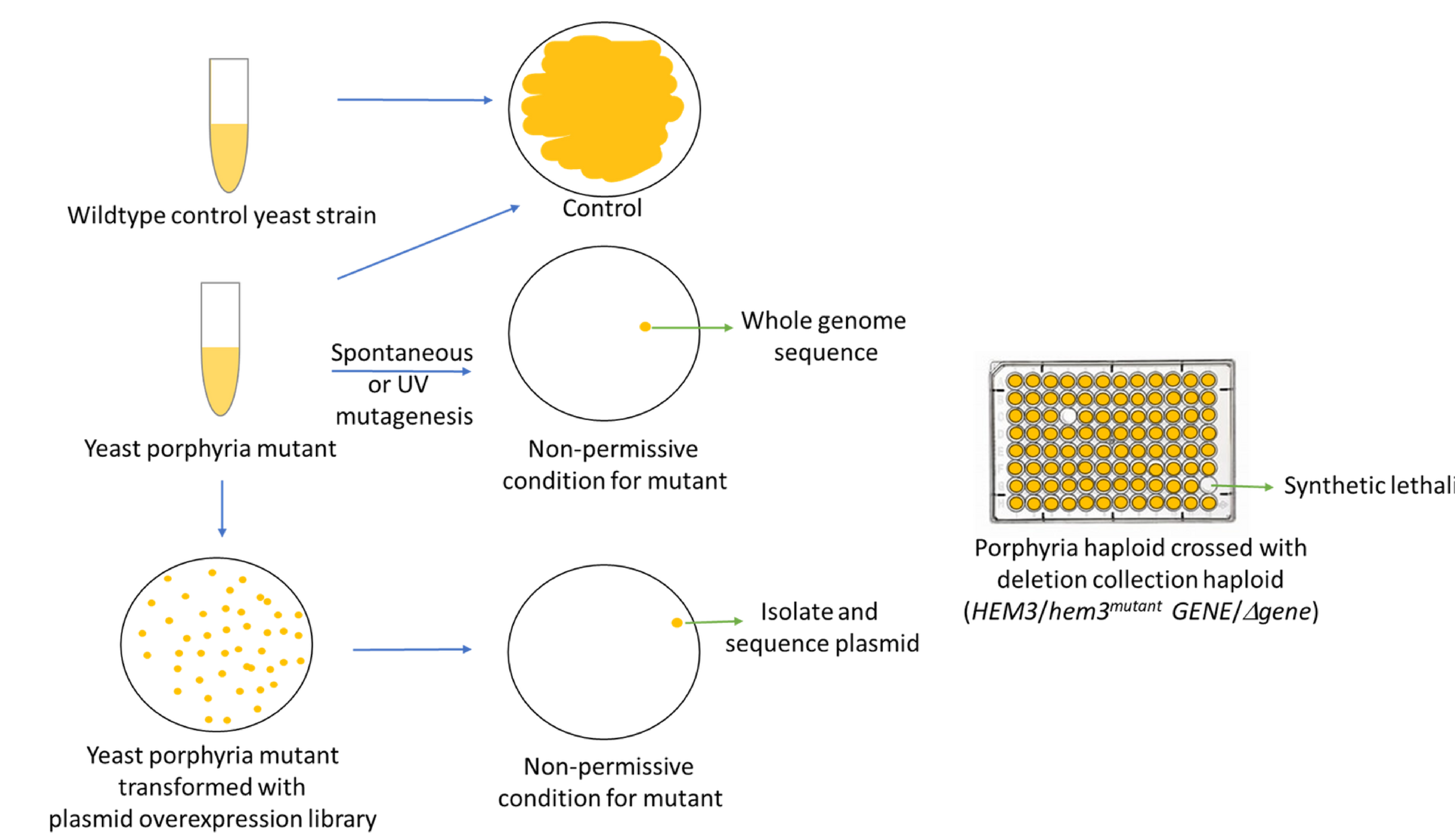

Figure 6. Future studies will involve using the newly generated strains to perform genetic screens to identify genes that when mutated or overexpressed can suppress sensitivity of hem3 mutants to an exogenous stressor yet to be determined (left side). Additionally, double heterozygotes of hem3 and the entire yeast deletion collection will be generated to identify genes that induce synthetic lethality or stress sensitvity when combined with AIP mutations.

Discussion

The autosomal dominance and low penetrance of acute intermediate porphyria poses an interesting challenge to identify the additional driving forces that underly the clinical manifestation of the disease symptoms. By establishing yeast models of AIP and identifying genetic factors that can influence cellular AIP phenotypes, we hope to identify conserved genes and pathways that might influence the penetrance of AIP. We envision future studies using yeast to model conserved mutations in other porphyria inducing genes to assess for similarities and differences among porphyrias that may help elucidate potential drug targets that contribute to disease presentation and progression.

References

- Laughery et al. New Vectors for Simple and Streamlined CRISPR-Cas9 Genome Editing in Saccharomyces cerevisiae. *Yeast.* 2015.
- Harvey, Richard A., Ph. D. Lippincott's Illustrated Reviews: Biochemistry. Philadelphia :Wolters Kluwer Health, 2011.
- Christie, M. S., Laitaoja, M., Aarsand, A. K., Kallio, J. P., & Bustad, H. J. (2022). Characterisation of a common hotspot variant in acute intermittent porphyria sheds light on the mechanism of hydroxymethylbilane synthase function. FEBS Open Bio, 12(12), 2136–2146.
- Ulbrichova, D., Hrdinka, M., Saudek, V., & Martasek, P. (2009). Acute intermittent porphyria - impact of mutations found in the hydroxymethylbilane synthase gene on biochemical and enzymatic protein properties: Mutations found in the HMBS gene. FEBS Journal, 276(7), 2106–2115