CRISPR Constructs and Deletions of *BUL1*, *BUL2*, *Jen1*, *Art4* in *Saccharomyces cerevisiae*

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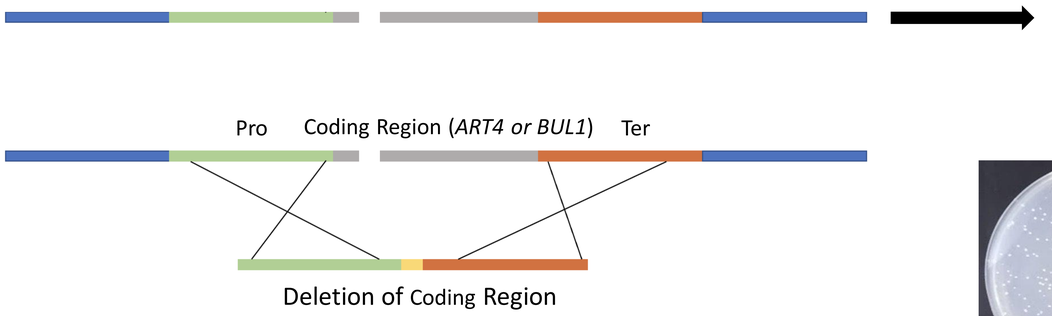
Background

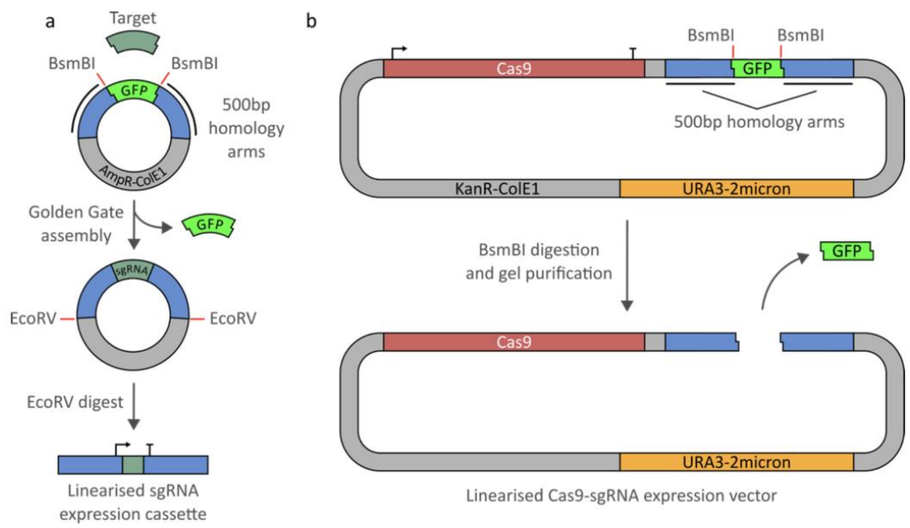
Art4, an alpha arrestin and adaptor protein, is involved in ubiquitin-dependent endocytosis of the Jen1 pump. In addition, Bul1 and Bul2 exhibit activity in the endocytosis of Jen1

Art1-Mup1 is the one of the best adaptor-pump pairs understood.

Art4-Jen1 is the best candidate for characterizing a second adaptor-pump pair.

Delete genes that encode these proteins to conduct a genetic screen.





https://benchling.com/pub/ellis-crispr-tools

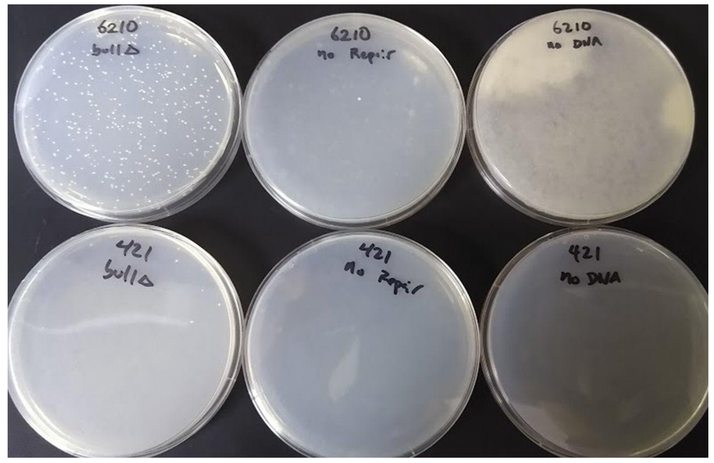
Findings

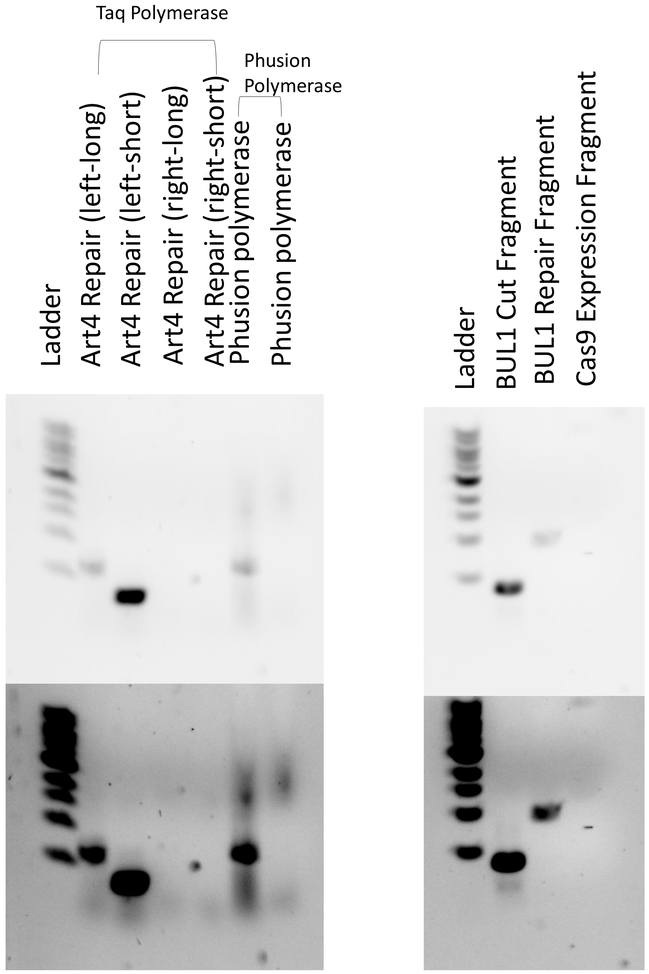
Art4 PCR did not yield desired products needed for Art4 deletion

Bul1/Bul2 repair fragment successfully created using PCR

CAS9 Expression Fragment

CRISPR constructs along with the repair fragments were put into yeast cells from 2 different strains and growth was observed.





Future Studies

Troubleshoot for Art4 deletion by varying magnesium concentration, annealing temperature. Try new genomic DNA preparation or ordering new primers if needed.

PCR characterization of colony growth amongst the two strains to check for correct integration location of the CRISPR repair