CRISPR Protocol: (Akhmetov et al., 2018)

Design the gRNA sequences using the yeast ALD2 and its corresponding primers

Amplify gRNA sequences using PCR

Transform competent E.coli bacteria, with pYTK050 plasmid with gRNA. These plasmids also contain a GFP gene that is silenced upon the successful incorporation of the gRNA gene.

Extract plasmids from successfully transformed bacteria

To create a cassette gRNA plasmid, transform competent bacteria using the gRNA incorporated pYTK050 plasmids and connector plasmids, along with an ampicillin selection gene (AmpR-ColE1) to check for successful transformation

Select for those bacteria that have been successfully transformed and extract the gRNA cassette plasmid

Construct a cassette Cas 9 plasmid using the same method as stated in step 5.

Construct the yeast compatible, self-contained CRISPR plasmid by transforming competent bacteria with both the gRNA cassette plasmid and the Cas 9 cassette plasmid with kanamycin selection

Select for those bacteria that have been successfully transformed and grow within a liquid culture then purify plasmid

Preparing the DNA template

Prepare 2 DNA templates. One for the wild type human ALDH2 enzyme, and one for the mutant human ALDH2*2 enzyme

Design PCR primers for each of these gene sequences

Use PCR to amplify amounts of DNA repair template

Extract the DNA repair template for use in the yeast transformation

Yeast transformation

Prepare competent yeast according to the manufacturer's guidelines

Prepare a transformation reaction that contains: competent yeats to be transformed, lithium acetate, the self-contained CRISPR plasmid and repair template

Incubate to allow for the growth of the transformed yeast colonies on a plate

Then take these yeast colonies and plate them according to the yeast culture protocol, under the different conditions stated.

Reference

Akhmetov, A., Laurent, J., Gollihar, J., Gardner, E., Garge, R., & Ellington, A. et al. (2018). Single-step Precision Genome Editing in Yeast Using CRISPR-Cas9. BIO-PROTOCOL, 8(6). doi: 10.21769/bioprotoc.2765