Designing a Novel Yeast Brewing Strain Using CRISPR-Cas9 Genome Engineering
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How do specific genes in yeast contribute to flavor profiles?

- During fermentation, yeast produce a number of aromatic compounds that contribute to beer flavor.
- Isoamyl alcohol is catalyzed by alcohol acetyl transferase (ATF1 & ATF2) to form isoamyl acetate, an ester with a banana aroma.
- Yeast strains for particular beer styles have evolved to produce specific, desirable flavors. High levels of isoamyl acetate is a hallmark of a great Hefeweizen, while being undesirable in many other styles.

How do ATF1, ATF2, and IAH1 work together?

- When factoring in the balance of ATF1 & ATF2 driving the forward reaction, and IAH1 driving the reverse reaction, there is a strong linear correlation (r = 0.8301) when comparing (ATF1+2)/IAH1 ratio vs. isoamyl acetate isoamyl alcohol ratio.
- ATF1 has a relative good correlation of r=0.6021, but not as good as the gene fold expression vs. isoamyl acetate isoamyl alcohol ratio.

This means….

1) The expression of any one gene is not indicative of isoamyl acetate production.
2) The relative expression of all three genes factored together is indicative of isoamyl acetate production. There is a strong correlation between ATF1+ATF2:IAH1 expression and isoamyl acetate production.

Hypothesis:
By lowering (or completely removing) IAH1 production, there will be an increase in isoamyl acetate.

Modifying the Genes

- Now knowing this gene ratio, we aim to modify isoamyl acetate production by genetically modifying IAH1.
- Goal: Increase the amount of isoamyl acetate in beer by knocking out IAH1 via the use of CRISPR-Cas9.
- CRISPR-Cas9 is a novel engineering technique that allows for change genes on a base precision level.

Plasmid Design

- Step 1: Designing sgRNA site
  - Using E-Crisp, design the site that would be inserted into Cas9 in order to show the target site.
  - Using the web protocol, the sgRNA was designed to avoid the mutation found within the IAH1 sequence within our genome.
  - Newmer was designed in order to work around the mutations found in:
    - A3 [ATT-AC] missense mutation
    - A181 (GCC>GCT) silent mutation
    - X163N (AAA>ATT) missense mutation
  - Identified mRuby gene in one of the plasmids ordered in order to add to our sgRNA.
  - The following oligos were created:
    - Forward oligo: GJM120-5'-GACCTTGGCAAGAACATGATGCTTCT-3'
    - Reverse oligo: GJM121-5'-AAAGACAGCATATGACATTCCTGACCA-3'
  - Synthesis and annotation of oligos
  - Assemble oligos in NEB protocol for 1 day

- Step 2: Prepping for CRISPR editing
  - Prepare plasmids:
    - pYTK050, pYTK003, pYTK068, pYTK959, pYTK-DN1, pYTK-DN2, pYTK-DN4, and pYTK-DN5
  - Prepare plates and media

- Step 3: Nicking sgRNA oligos
  - Prepare 100 uM stock of oligos in molecular grade dH2O
  - Mix 10 uL of forward and 10 uL of reverse oligo in PCR tube.
  - Anneal the oligos using the thermal cycler under the following conditions:
    - 95 C for 5 min
    - 55 C for 15 min
    - 25 C for 15 min
    - 4 C forever

Future Steps

- Run 1st golden gate reaction
  - Create 2 reaction mixes: a negative control and the golden gate
  - Normal reaction has the sgRNA in each one, add:
    - 1 uL 10x T4 DNA ligase Buffer
    - 0.5 uL T7 DNA ligase
    - 0.5 uL BsmBI-V2
  - Run in thermal cycler under the following conditions:
    - 42 C for 1 hr
    - 16 C for 1 hr (ligation step not included in NEB protocol for 1 insert, can’t hurt)
    - 60 C for 10 min
    - 80 C for 10 min
    - 4 C forever
- Plate samples and perform extraction to see if the first editing work
- Run a mini prep to confirm the knockout of GFP gene

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