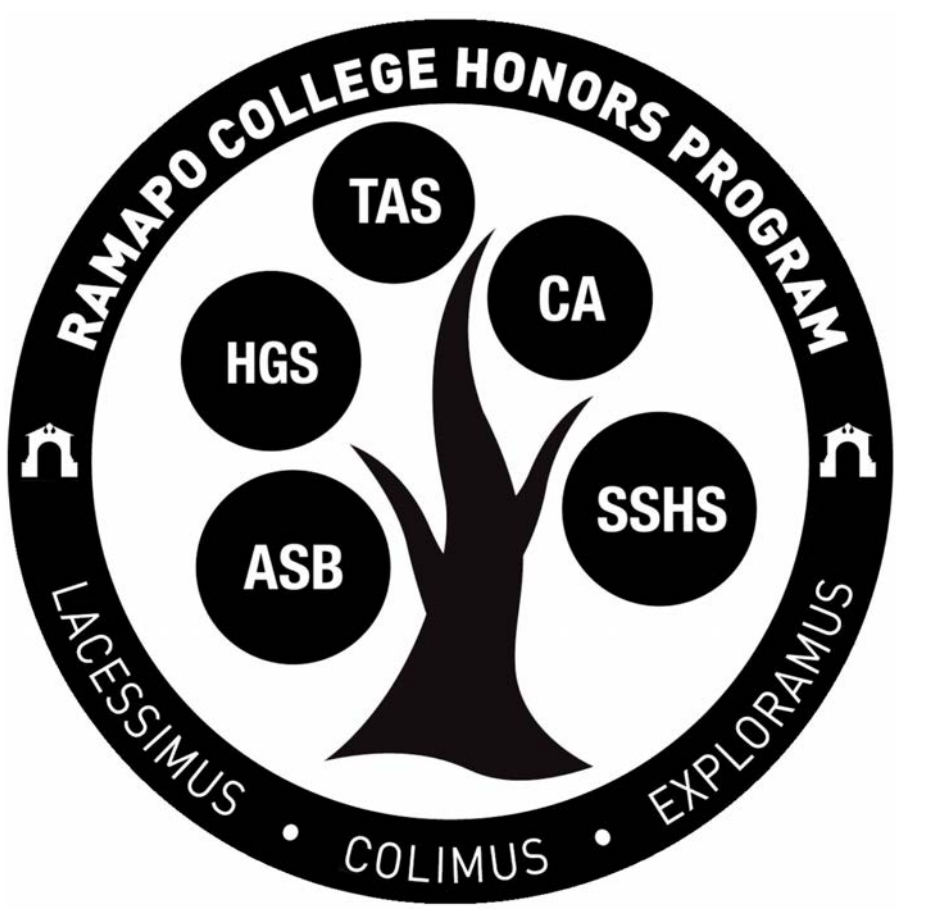


# The Genetics of Brewing: Characterizing Yeast (*Saccharomyces cerevisiae*) Genes that Influence Flavor and Aroma

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## Introduction

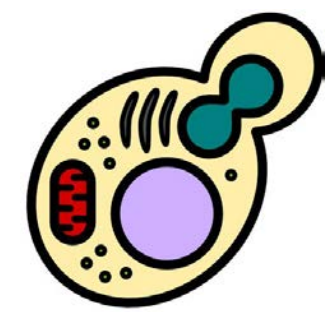
### Background

- The **craft beer industry**: booming and **lucrative** business due to **global demand** of new beer flavors (projected to be worth **\$190 billion in 2027**).<sup>1</sup>
- Brewers are **globally motivated to improve their beer** (select different combinations of ingredients).<sup>1</sup>

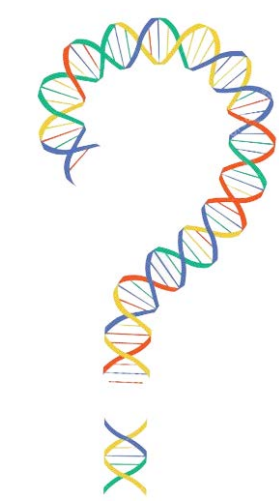


**Yeast (*Saccharomyces cerevisiae*):** produces the alcohol and contributes to the overall **beer flavor/aroma** profile.<sup>1</sup>

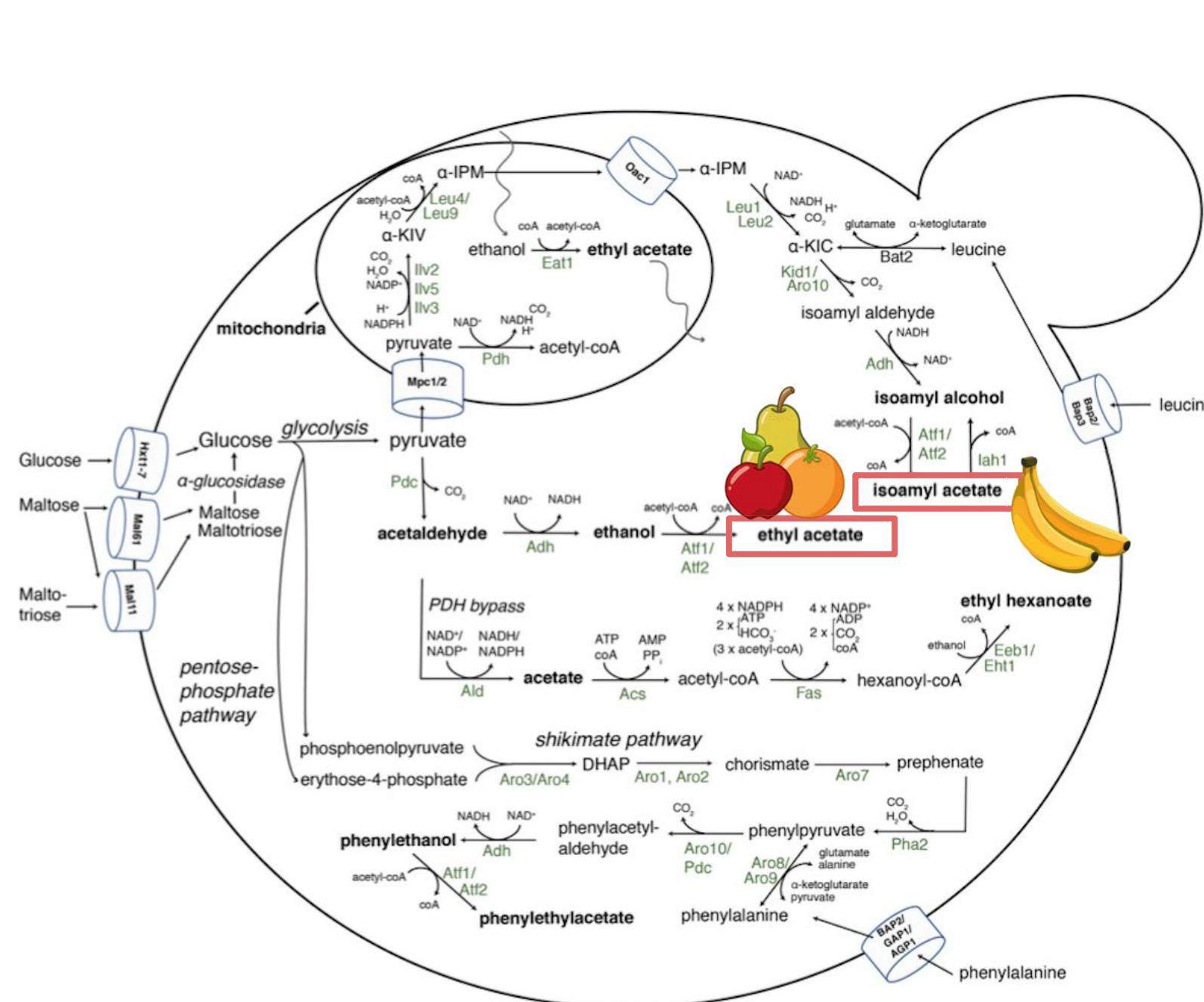
- Yeast creates **desirable/undesirable aromatic compounds** via metabolic pathways.<sup>3</sup>



Brewers have artificially selected for yeast strains that impart the traits they are looking for in their beer. However, the **genetic background of yeast** remains largely **uncharacterized**.<sup>3</sup>



- Understanding the **role that yeast genetics** play in imparting **flavor/aroma** during fermentation will allow brewers to refine their craft.<sup>1</sup>



**ATF1, ATF2, and IAH1** genes in yeast are involved in biochemical pathways producing **ethyl acetate** (fruity) and **isoamyl acetate** (banana) profiles.<sup>2,3</sup>



| Yeast Strains Selected            | Beer Description   |
|-----------------------------------|--|
| 1. California Ale Yeast           | Used in many kinds of beer. Clean flavor.  |
| 2. Hefeweizen Ale Yeast           | High levels of isoamyl acetate (banana notes)                                      |
| 3. Dry English                    | Dry taste. Used in American and English beers.                                     |
| 4. German/Kölsch Ale Yeast        | Bitter notes. Crisp and clean characteristics.                                     |
| 5. San Diego Super                | Dry and bitter flavor. Versatile strain.   |
| 6. Monastery Ale                  | Plum and cherry notes.   |
| 7. Belgian Ale                    | Phenol prominent flavors/aromas of clove, allspice and peppercorns.                |
| 8. Belgian Wit                    | Herbal aroma/flavor. Dry beer.   |
| 9. English Cider                  | Dry beer with apple flavor profile.  |
| 10. Belgian Saison I Ale          | Earthy, peppery and spicy notes.   |
| 11. Dusseldorf Alt Ale            | Clean and malty ales. Emphasizes the bitterness of hops and the sweetness of malt. |
| 12. Saccharomyces brut-like Trois | Wild yeast. Sour beer with tropical/fruity notes.                                  |

12 yeast strains selected for experimentation

### Objective

To determine if there are differences in the DNA sequence between yeast strains for the **ATF1, ATF2, and IAH1** genes which produce esters that are desirable (and undesirable) in a variety of beers.

### Hypothesis

Yeast strains have been artificially selected for based on the flavor/aromatic traits of the beer, so there may be differences in alleles between strains due to mutations (that arise as the yeast are reused in batches) affecting the expression of **ATF1, ATF2, and IAH1** enzymes.

## Experimental

1. Strains purchased from **White Labs** (yeast distributor).

2. Yeast grown on agar plates and as liquid cultures.

3. Yeast DNA isolated and extracted to analyze genes of interest.

4. Primers designed to direct DNA synthesis in prep for PCR.

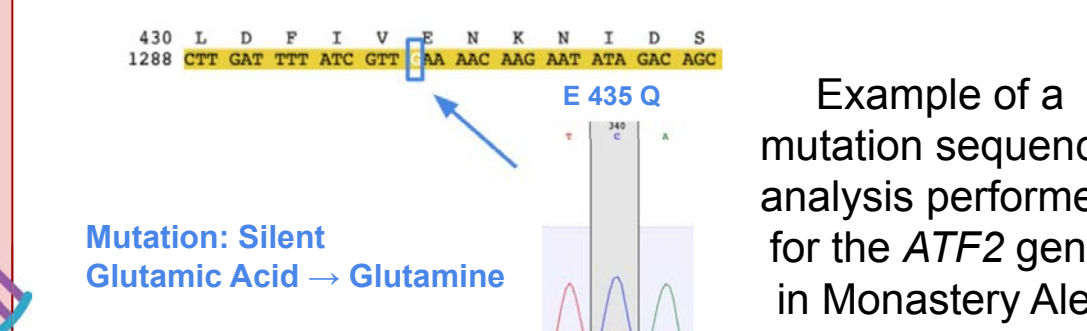
5. To **amplify the DNA** and copy a specific portion of interest, **polymerase chain reaction (PCR)** was performed.

6. **Gel electrophoresis** performed to visualize PCR results.

7. Successful strains sent out for **sequencing** to **Psomagen** to determine the nucleotide sequence of DNA.

8. **ApE** (Bioinformatic Tool) used to **analyze** sequencing files for **mutations**.

| Corresponding Gene | Primers Designed (F/R) | Sequence            |
|--------------------|------------------------|---------------------|
| ATF1               | uM109 (ATF1_F)         | ctttaggagcaacacac   |
| ATF1               | uM110 (ATF1_R)         | tccttcaacacacacac   |
| ATF2               | uM111 (ATF2_F)         | agggatgctcctgagca   |
| ATF2               | uM112 (ATF2_R)         | ccatgctgctcctgagca  |
| IAH1               | uM113 (IAH1_F)         | taaaagggagcaataaag  |
| IAH1               | uM114 (IAH1_R)         | ctctgaaagctgctgagca |



## Conclusion

All of the mutations for **ATF1** were synonymous mutations. The silent mutations in **ATF1** could indicate its important role in all strains examined.



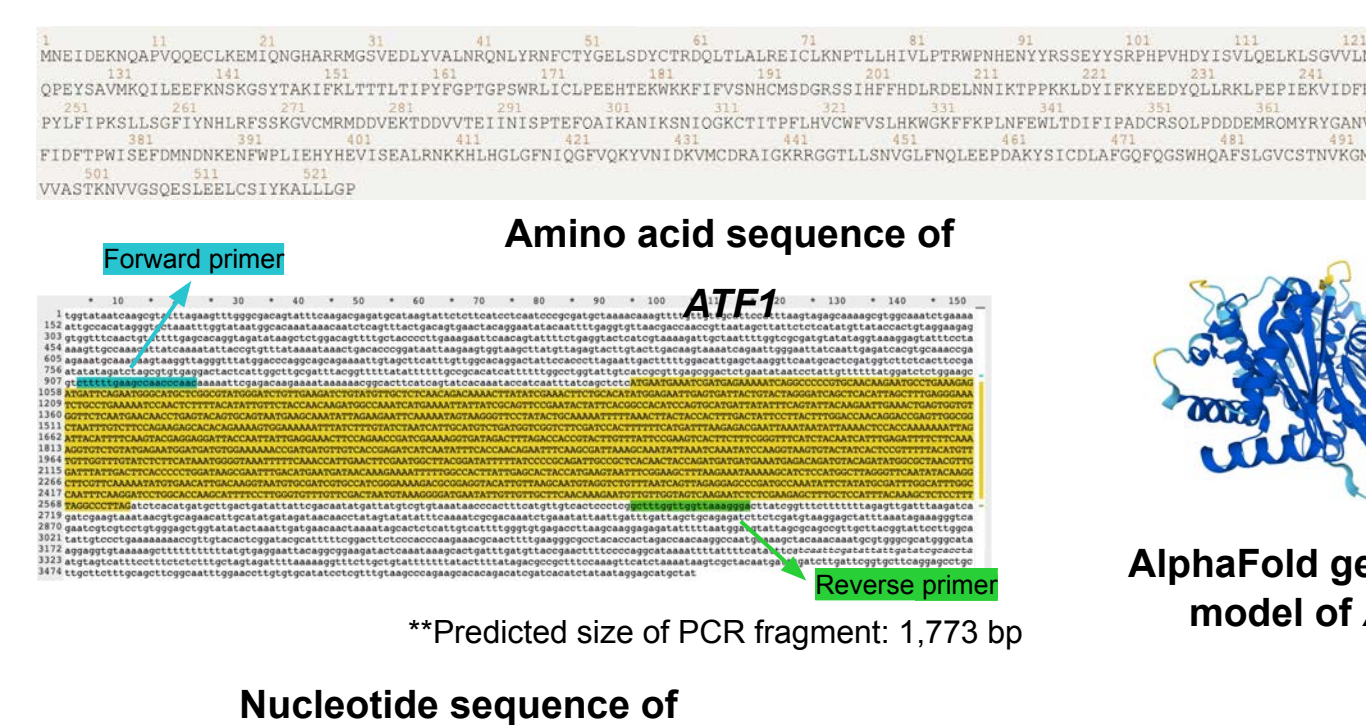
The missense mutations found in **ATF2** and **IAH1** alter the primary amino acid sequence in some strains, but the functional consequence requires further experimentation.



There were no mutations found that would indicate a clear functional change in any of the genes examined (i.e. deletions, frameshift, or nonsense mutations). It is clear these genes are playing an important role in all strains, but gene expression experiments will help determine to what extent.

## Results

### ATF1:

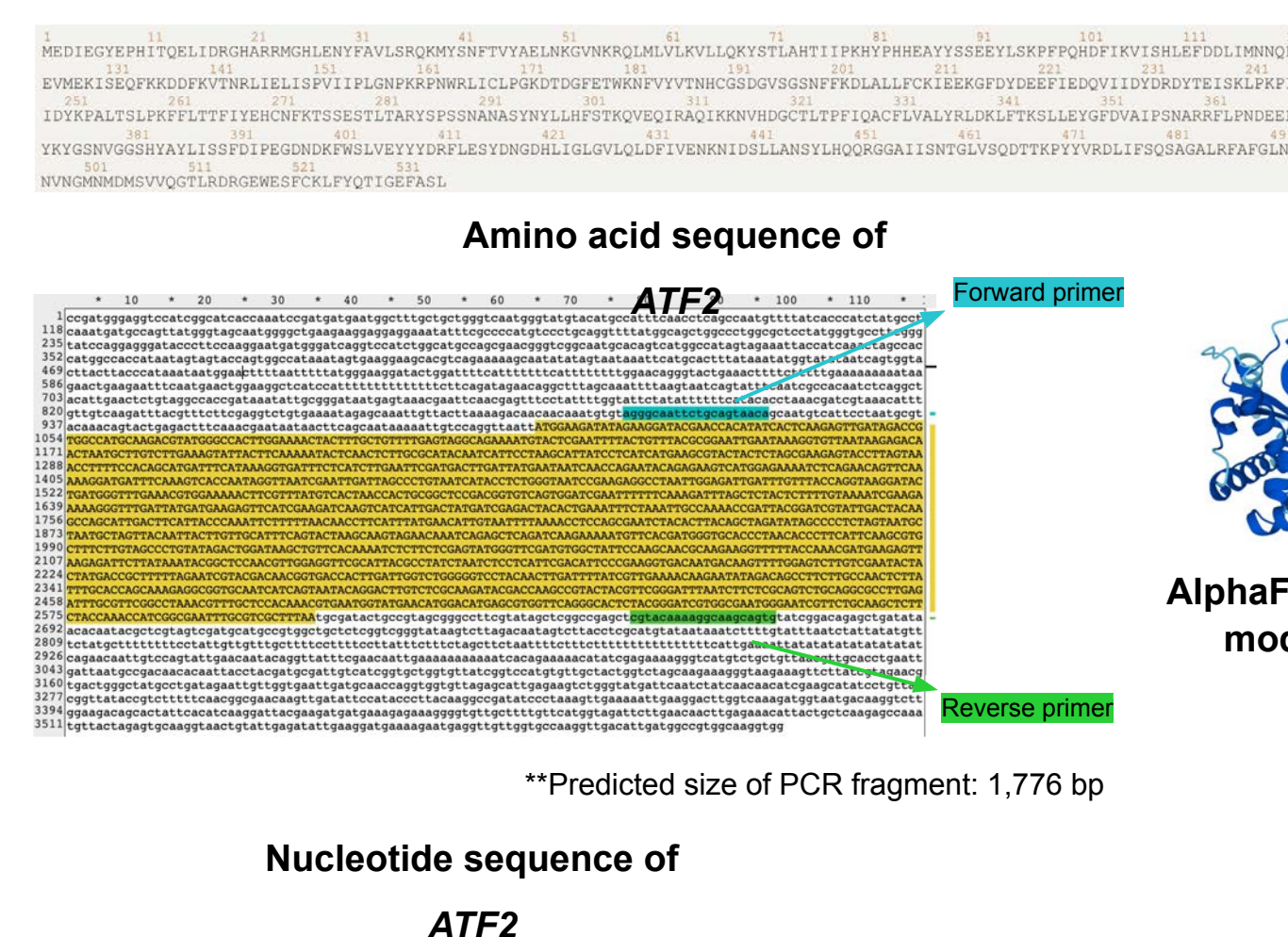


### Mutations/Position for all Strains

| Strain                        | Mutations  |
|-------------------------------|--|
| California Ale Yeast          | A 10 A, P 11 P, R 60 R, G 417 G                                      |
| Hefeweizen Ale Yeast          | R 46 R, Q 113 Q, L 232 L, S 404 S, N 429 N                           |
| Dry English                   | F 203 F, G 417 G   |
| San Diego Super               | A 10 A, P 11 P, R 60 R, F 418 F                                      |
| Monastery Ale                 | I 70 I   |
| Belgian Saison I Ale          | No mutations found   |
| Saccharomyces brut-like Trois | A 10 A, R 46 R, Q 113 Q, I 309 I, S 404 S, N 429 N, K 502 K, Y 518 Y |

All of the mutations identified in the **ATF1** genes between the strains were **silent**.

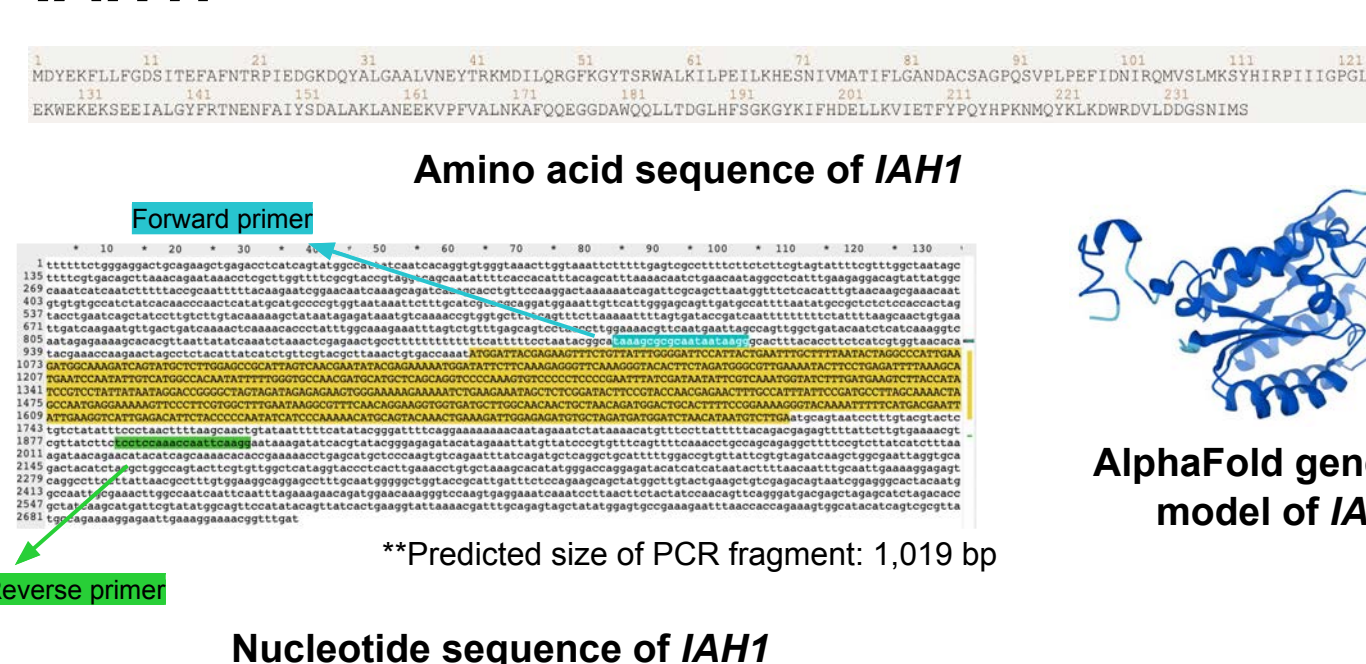
### ATF2:



| Strain                        | Mutations   |
|-------------------------------|---|
| Dry English                   | V 64 L, F 112 F, L 156 V, L 166 L, Q 226 Q, S 279 S, S 289 T, Q 305 E, A 311 A, L 338 L, K 344 K, E 435 Q |
| Belgian Wit                   | T 12 T, V 64 L, Q 67 Q, L 156 V, Q 226 Q, S 279 S, S 289 T, Q 305 E, A 311 A, L 338 L, K 344 K, E 435 Q   |
| German/Kölsch Ale             | V 64 L, F 112 F, L 156 V, L 166 L, Q 226 Q, S 279 S, S 289 T, Q 305 E, A 311 A, L 338 L, K 344 K, E 435 Q |
| San Diego Super               | V 64 L, F 112 F, L 156 V, Q 226 Q, S 279 S, S 289 T, Q 305 E, A 311 A, L 338 L, K 344 K, E 435 Q, S 530 S |
| Belgian Saison I Ale          | E 435 Q, G 530 S  |
| English Cider                 | V 306 G, C 330 W  |
| Monastery Ale                 | E 435 Q, G 530 S  |
| Saccharomyces brut-like Trois | E 435 Q, G 530 S  |

Various **missense** and **silent** mutations found in **ATF2**.

### IAH1:



| Strain            | Mutations                        |
|-------------------|----------------------------------|
| Dry English       | I 23 T, A 82 A, K 163 N          |
| Belgian Wit       | I 23 T, A 31 A, K 163 K, V 164 V |
| German/Kölsch Ale | I 23 T, A 82 A, K 163 N          |
| Yeast             | I 23 T, A 82 A, K 163 N          |
| San Diego Super   | D 11 A, A 82 A, K 163 N          |
| Monastery Ale     | I 23 T, A 82 A, K 163 N          |

Multiple **missense** and **silent** mutations also found in **IAH1**.

## Future Directions

- Re-extract yeast DNA and redo PCR** for unsuccessful strains for further analysis.
- Repeat sequencing** to dismiss/assert the presence of PCR induced mutations compared to potential allelic differences between strains.
- Examine differences in the **expression of the ATF1, ATF2, and IAH1** genes using RNA extraction, qPCR and Western blotting techniques.
- Use **computational models** such as AlphaFold 2 to predict/visualize how the mutations we found affect their respective proteins.

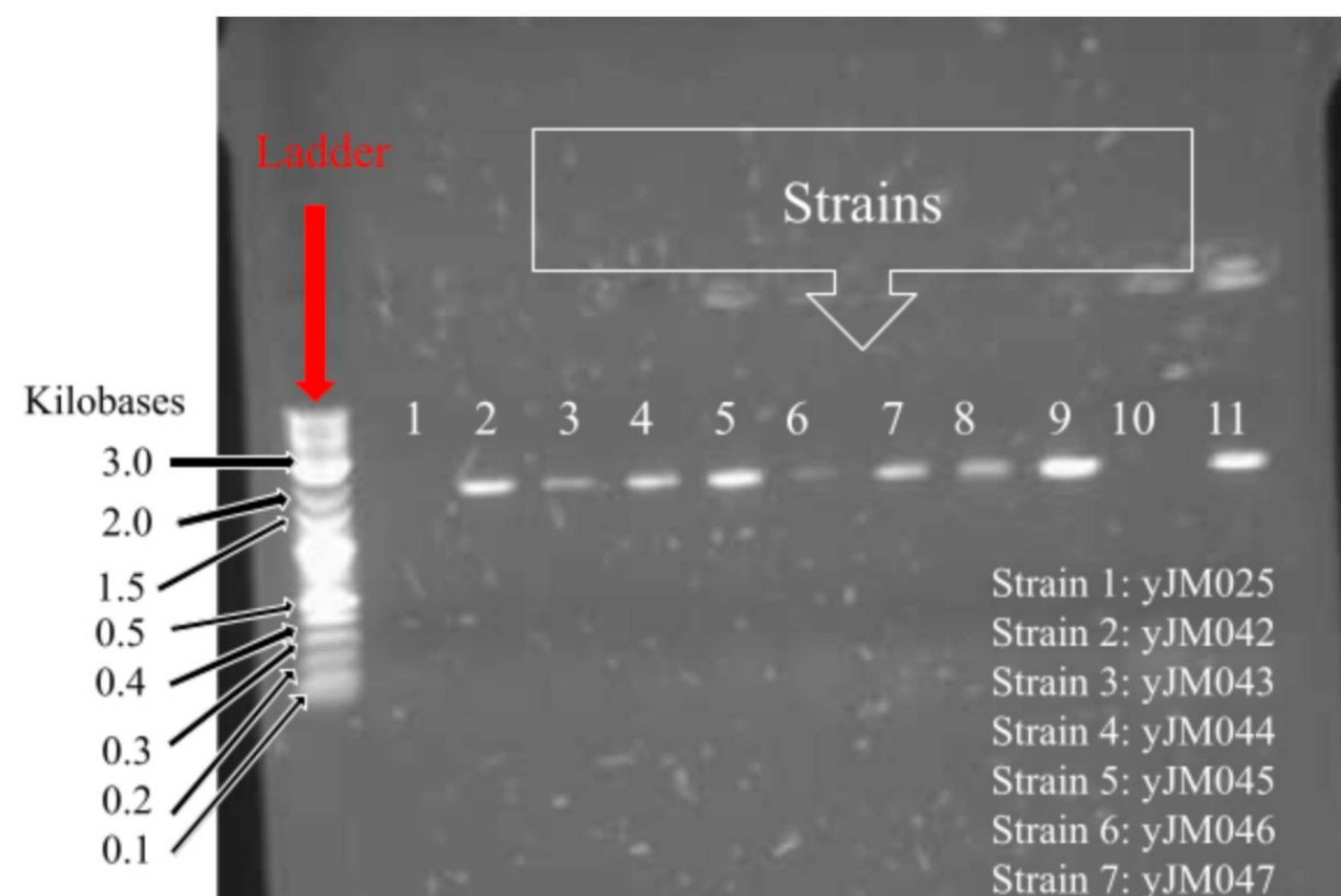
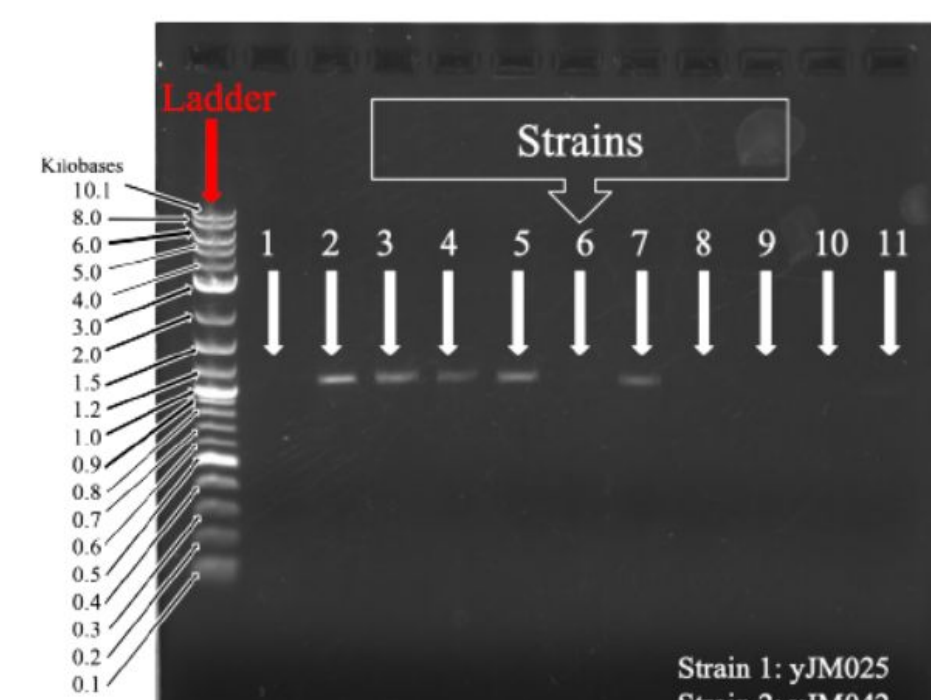
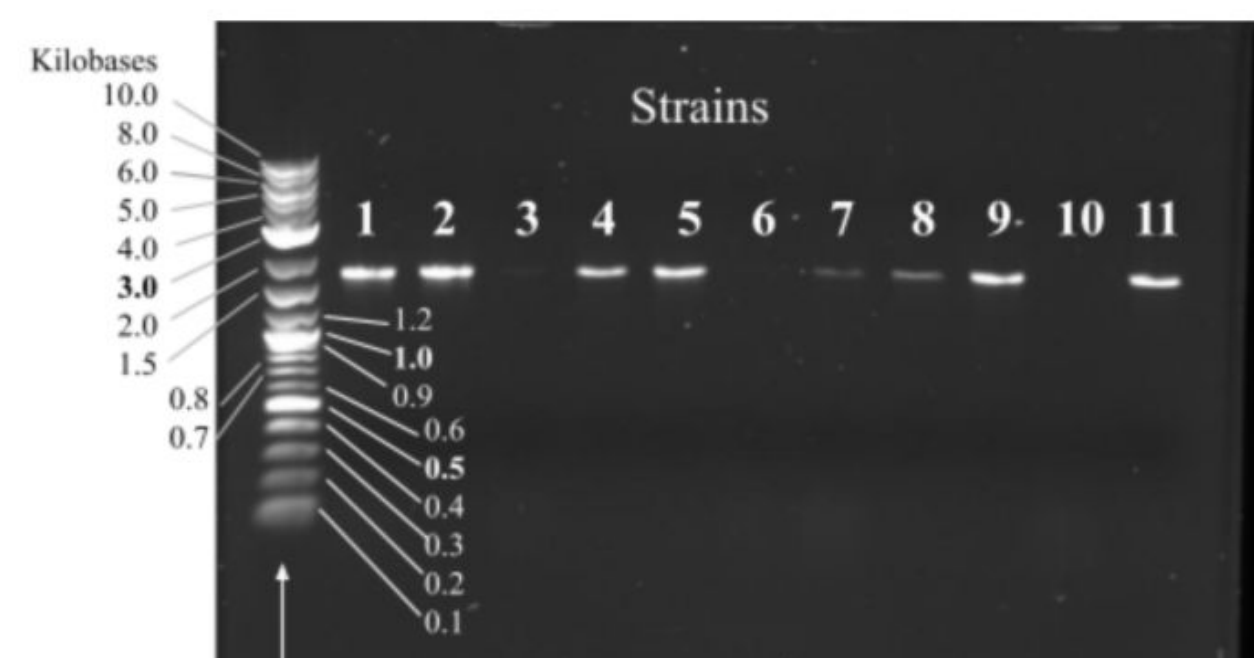
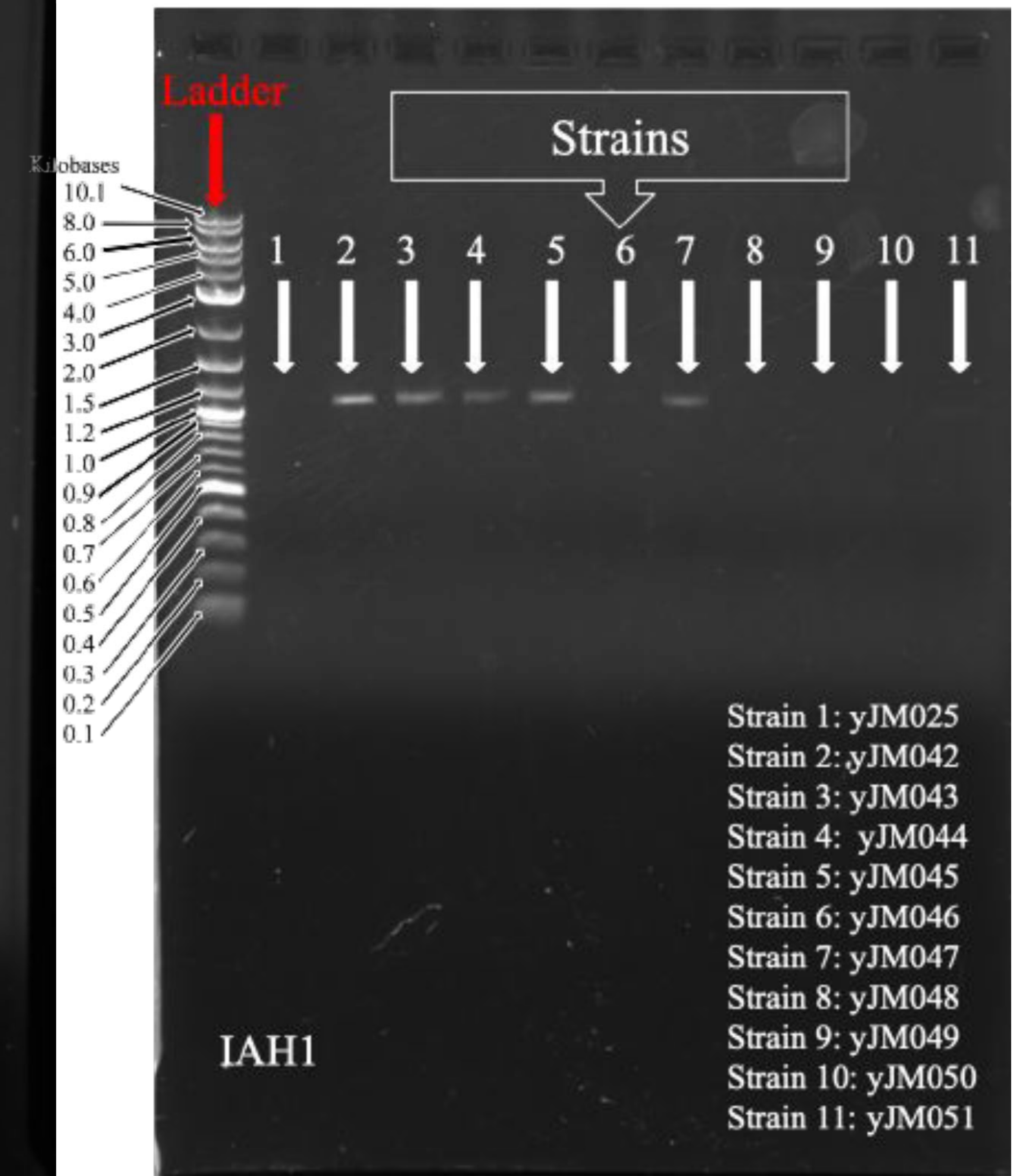
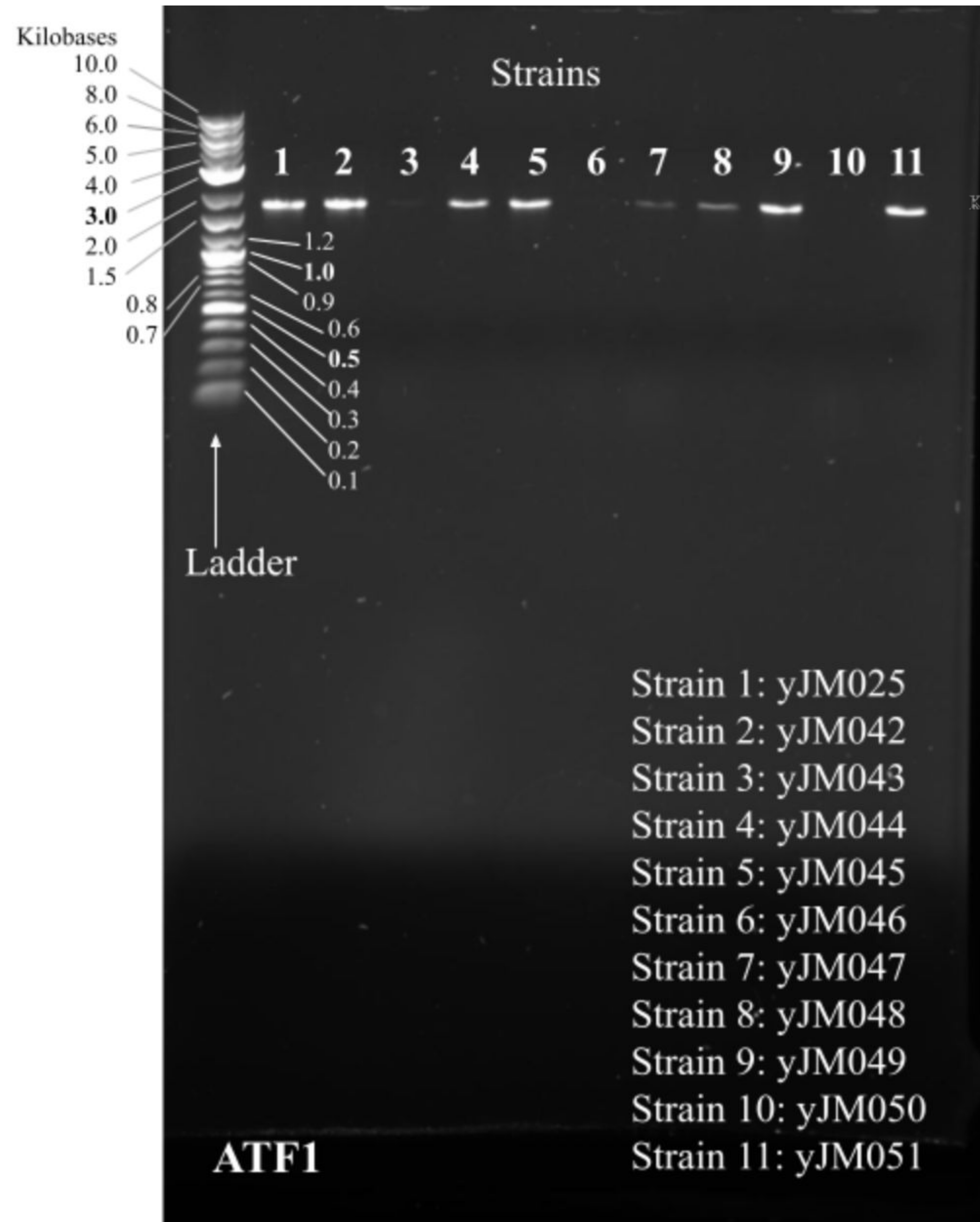
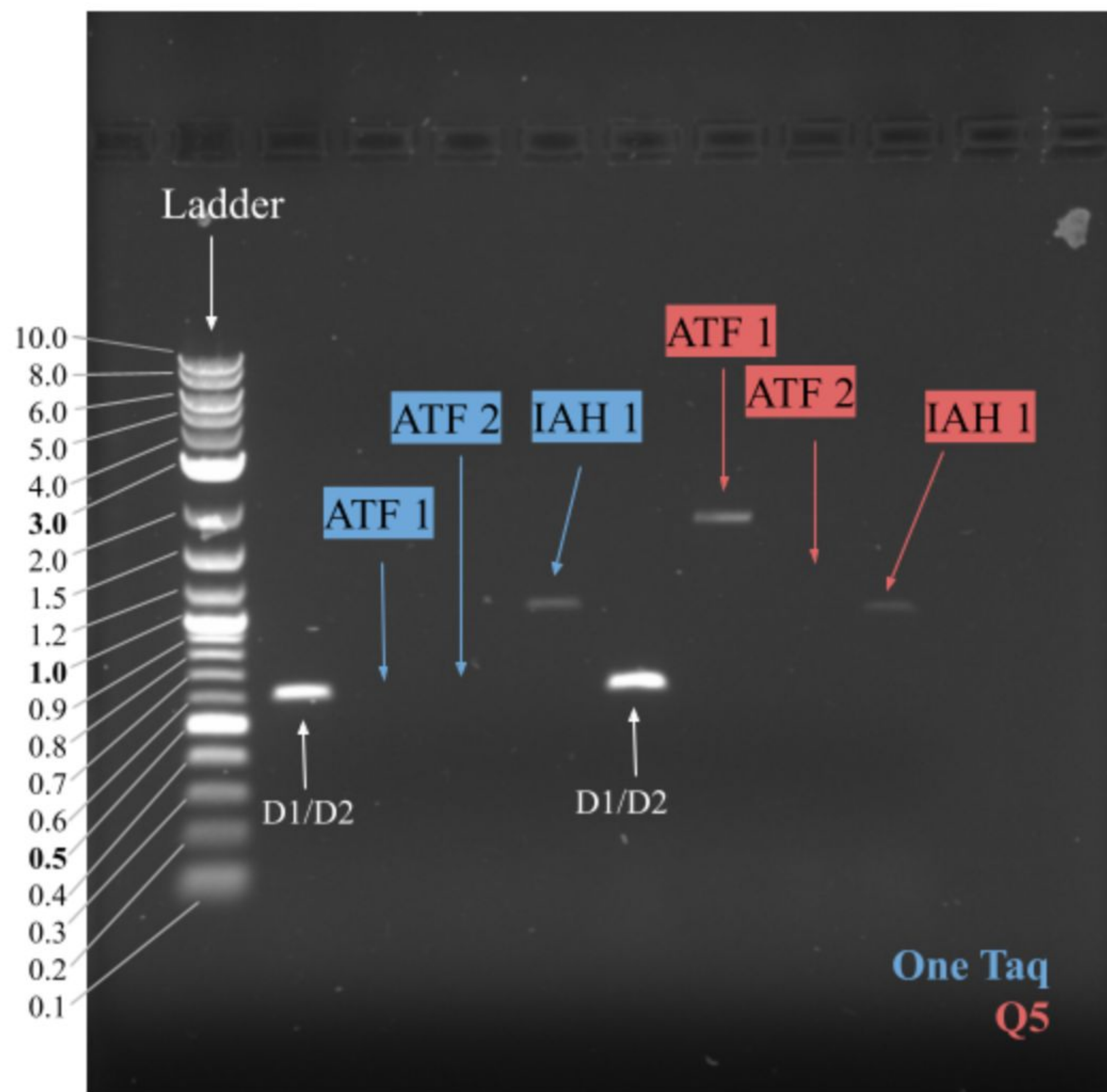
## Citations

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## Acknowledgments

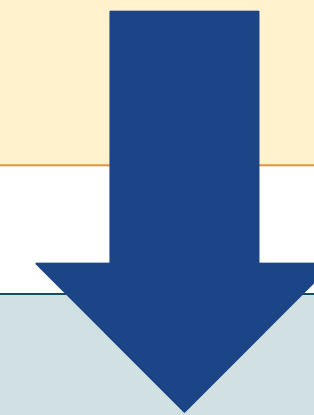
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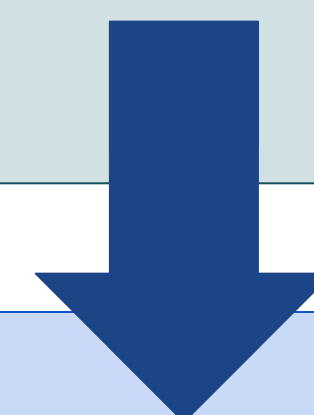




Missense mutations observed in the *ATF2* and *IAH1* genes may play a role in the enzymatic activity of their gene products



No frameshift/nonsense mutations found, suggesting that the genes may play an important metabolic role (despite different levels of aromatic compounds generated by different strains).



The results demonstrate the promising outcomes of studying the genetic profiles of yeast for brewing, a process that will continue to flourish as brewing practices improve to produce more favorable/controlled beer characteristics

- Investigate **other species of yeast** and the countless strains capable of producing **favorable beer flavors/aromas**

All of the mutations identified in the *ATF1* genes between the strains were **silent**.  
A number of **missense mutations** were seen in the *ATF2* and *IAH1* genes.

KEY:

■ Silent Mutations

■ Missense Mutations