Studying the Genes, Proteins, and Behaviors of S. cerevisiae (Bakers Yeast)

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ABSTRACT
In this experiment, we will cause a mutation in the HAO strain of S. cerevisiae resulting in the HB2 (red) strain. The HAO strain has 'normally' functioning Ade1 and Ade2 genes and when these genes are not functioning normally (due to UV light damaging the DNA), the HB2 strain is formed. As the length of UV light exposure increases, the mutation rate and death rate of cells increases. Unfortunately we did not obtain red colonies, as the UV light exposure resulted in cell death, so we obtained HAO and HB2 plated cultures from the professor to move forward (as the rest of the experiment needs red HB2 cells). A PCR gel we ran with the class exposed that we successfully obtained genomic HB2 DNA and further PCR gel analysis we ran the following week exposed that we successfully amplified the Ade1 and Ade2 genes and that these genes were in the HB2 strain.

INTRODUCTION
In this experiment, we will observe the genes, proteins, and behaviors of model organism, S.cerevisiae (bakers yeast). The goals of this experiment include: (1) determining if treating HAO (a white strain of yeast) with UV light (a DNA damaging agent) will produce HB2 (a mutant, red strain of yeast). (2) Observing colonies and the effect of UV mutagenesis and testing the nutritional requirements of HAO and HB2. (3) Observing the nutritional requirements of HAO and HB2 and extracting the genomic DNA of HB2. (4) Analyzing the genomic DNA of HB2 and isolating and amplifying Ade1 (~989bp) and Ade2 (~1.7kbp) genes from HB2. (5) Analyzing the amplified products of Ade1 and Ade2 genes from HB2. To mutate the HAO strain, we can expose the yeast cells to UV light (causing the HB2 strain to express). HAO cells are round and white, whereas HB2 cells are abnormally shaped and red/pink - these HB2 cells are a result of damaged purine metabolism.

MATERIALS
- Gloves
- 30W germicidal UV lamp
- Micropipettes
- Micropipette tips
- Biohazard receptacle
- YED plates (x10)
- Liquid culture of HAO yeast cells
- Sterile plastic spreaders

- Bunsen burner
- Stopwatch/timer
- Marker
- Sterile loop tools
- Liquid culture of mixed (HAO & HB2) yeast cells
- Vortexer
- Incubator
- Cling wrap
- Minimal medium plate (x1)
- Minimal plus adenine plate (x1)
- Plated HAO yeast
- Plated HB2 yeast
- Sterile test tubes containing YPD (x2)
- Metal loop tool
- Thermometer
- Water bath
- Microcentrifuge
- Qiagen DNeasy Kit (#69504)
- Ethanol (95-100%)
- Eppendorf tubes
- Liquid culture of HB2 yeast
- Sorbitol buffer
- Lyticase solution
- ATL buffer
- Proteinase K enzyme
- AL solution
- Column
- Collection tube (x2)
- AW1 buffer
- AW2 buffer
- AE solution
- PCR machine
- PCR gel
- Positive control genomic DNA
- E-gel 96 High Range DNA marker
- PCR test tubes (x8)
- BIOMIX (buffer, Mg^2+, dNTPs, Taq DNAP)
- Positive Control genomic DNA template
- HB2 genomic DNA template
- ADE1f primer (12.5 uM)
- ADE1r primer (12. uM)
- ADE2f primer (12.5 uM)
- ADE2r primer (12.5 uM)
- Mineral oil

- Sterile water
- Laminar airflow hood

METHODS

Week 1- Can UV Light Change the Apperance of Yeast?:

Obtained 9 YED medium plates, liquid culture of HAO cells, 30W germicidal ultraviolet light, sterile water, stopwatch, vortexer, and liquid mixed (HAO & HB2) yeast culture. Labeled each of the YED plates as follows: “0 sec”, “10 sec”, “20 sec”, “20 sec”, “40 sec”, “80 sec”, “1:10 HAO control”, “Mixed culture 1”, and “Mixed culture 2”. Micropipetted 100uL of HAO culture into the center of each YED plate with a lit bunsen burner. Spread gently with a sterile spreader. Exposed “10 sec” plate to UV light for 10 seconds, both “20 sec” plates to UV light for 20 seconds, “40 sec” plate to 40 seconds, and “80 sec” plate to UV light for 80 seconds, all exposed to UV light under a Laminar airflow hood. “0 sec” plate not exposed to UV light. Inverted culture tube thoroughly to resuspend cells. With a lit bunden burner, added 100uL culture to eppendorf tube with 900uL sterile water. Mixed by inverting eppendorf tube. Added 100uL of diluted cells to “1:10 HAO control” plate and spread evenly. With a lit bunsen burner and sterile loop tools, streaked mixed culture on “Mixed culture 1” and “Mixed culture 2” plates. Inverted plates, wrapped with cling wrap, and labled with our initials, then stored for 1 week in an incubator at 30C.

Week 2- Nutritional Requirements of HB2 and HAO yeasts:

Examined plates from week 1 and took down observations. Obtained a minimal medium plate, a minimal medium plus adenine plate, a YED plate, a YED HAO plate (from professor), and a YED HB2 plate (from professor). Labeled our 3 plates as follows: “HAO HB2 YED”, “HAO HB2 Minimal”, and “ HAO HB2 Minimal plus adenine”. Used a marker to split each plate and labeled either HAO or HB2 (each of the 3 plates had an HAO section and a HB2 section). Used a sterile loop tool with a lit bunsen burner to transfer a colony from the professor HAO plate and streaked each of the 3 plates on the “HAO” half of the plate. Then used a sterile loop tool with a lit bunsen burner to transfer a colony from the professor HB2 plate and streaked each of the 3 plates on the “HB2” half of the plate.Obtained 2 sterile tubes from professor, 1 labeled “HB2 control” and the other “HB2 yeast culture”. Touched a sterile loop tool to the HB2 plate and dipped it into the “HB2 yeast culture” tube. Flamed the loop tool, touched it into the “HB2 control” tube, and flamed it again. Incubated tubes and inverted plates wrapped in cling wrap in an incubator at 30C for 1 week.

Week 3- Extraction of Genomic DNA from HB2 mutant strain:

Using the “HB2 yeast culture” tube, removed 2mL and disposed of. Vortexed the tube to resuspend cells. Transferred 1mL into an eppendorf tube and spun the sample for 5 minutes at 2000rpm. Removed supernatant (keeping pellet only) and added 600uL of Sorbitol buffer, gently pipetting up and down to mix. Then added 20uL Lyticase, inverting several times to mix. Place the tube into a 30-35C water bath to incubate for ~30 minutes. Place the tube into the minifuge and span for 3 minutes at 2000rpm. Removed supernatant from pellet and discarded. Added 180uL ATL buffer to pellet, pipetting up and down to mix. Added 20uL enzyme Protinase K and

vortexed for ~5 seconds. Placed the tube in a water bath at ~55C to incubate for 30 minutes. Vortexed tube for ~15 seconds. Added 200uL AL buffer, vortexed for ~15 seconds. Incubated for ~10 minutes in a water bath at ~70C. Added 200uL of pure (100%) Ethanol, vortexed. Obtained a column from the Qiagen DNA extraction kit. Labeled column cap and side, emptied column, then pipetted the eppendorf contents (DNA containing solution) onto the column membrane. Placed column with the DNA containing solution in the minifuge and spun for 1 minute at max speed. Discarded supernatant, and placed the column back into its collection tube. Added 500uL AW1 buffer to column, spun for 1 minute at max speed, discarded the liquid, and again placed the column back into its collection tube. Added 500uL AW2 buffer to column membrane and spun in a minifuge for 3 minutes on max speed. Discarded liquid and again placed the column back into its collection tube. Minifuged tube for 1 minute and discarded liquid. Placed the column in a new collection tube labeled with our initials, then added 200uL AE buffer to the column membrane. Left to incubate at room temperature for ~1 minute. Placed the column in the minifuge and spun for 1 minute on full speed. Transferred liquid sample to a clean eppendorf tube with our initials. Placed the empty column into the collection tube and added 200uL AE buffer, incubated at room temperature for ~1 minute, spun in minifuge on max speed for 1 minute, and added the spun liquid to the eppendorf tube. Eppendorf tube and column then frozen for 1 week.

Week 4- Analysis of HB2 Genomic DNA and Isolation of Ade1 and Ade2:
Shared a PCR gel with the class. Loaded 15uL of our genomic DNA sample into a well in the gel and ran for 26 minutes then removed for analysis. Labeled 4 PCR tubes as follows: “T1”, “T2”, “T3”, and “T4”. T1 received 8.5uL of positive control genomic DNA, 2.0uL of ADE1f primer, 2.0uL of ADE1r primer, 12.5uL of BIOMIX, and 15uL mineral oil. T1 was then vortexed briefly to mix and set aside for 1 week. T2 received 8.5uL of positive control genomic DNA, 2.0uL of ADE2f primer, 2.0uL of ADE2r primer, 12.5uL of BIOMIX, and 15uL mineral oil. T2 was then vortexed briefly to mix and set aside for 1 week. T3 received 8.5uL of HB2 genomic DNA, 2.0uL of ADE1f primer, 2.0uL of ADE1r primer, 12.5uL of BIOMIX, and 15uL mineral oil. T3 was then vortexed briefly to mix and set aside for 1 week. T4 received 8.5uL of HB2 genomic DNA, 2.0uL of ADE2f primer, 2.0uL of ADE2r primer, 12.5uL of BIOMIX, and 15uL mineral oil. T4 was then vortexed briefly to mix and set aside for 1 week.

Week 5- PCR Analysis of Ade1 and Ade2 products:
Shared a PCR gel with another group. Loaded 15uL of each sample and of the ladder into each well. Ran the gel for 26 minutes and removed it for analysis.

RESULTS
Week 1- Can UV Light Change the Apperance of Yeast?:

Table 1- Expected Results of S.cerevisiae (HAO) growth when exposed to UV light

Length of UV light exposure (in seconds):	**Number of cells surviving:**	**% of cells surviving:**
0	~1,540	100

10	~1,386	90
20 (1 of 2 plates)	~890	58
20 (2 of 2 plates)	~890	58
40	~139	9
80	0	0

Graph 1- Expected Results of S.cerevisiae (HAO) growth when exposed to UV light

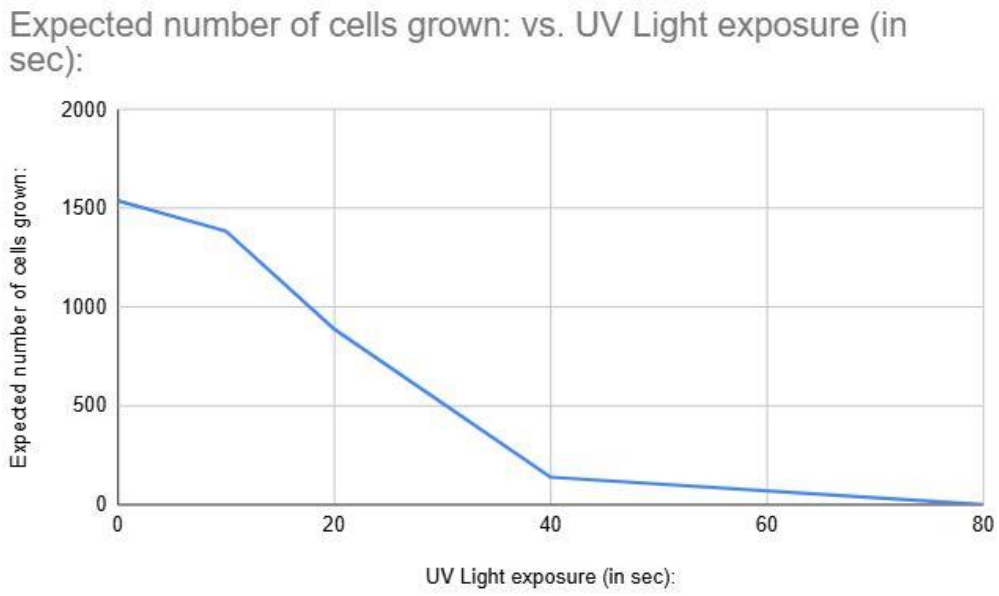


Table 2- Expected Growth Results of Plated Cultures

Plate Description:	Growth of white cells: (Y/N)	Growth of red cells: (Y/N)
1:10 HAO control (spread)	Yes	No
Mixed culture (1-streak)	Yes	Yes
Mixed culture (2-streak)	Yes	Yes

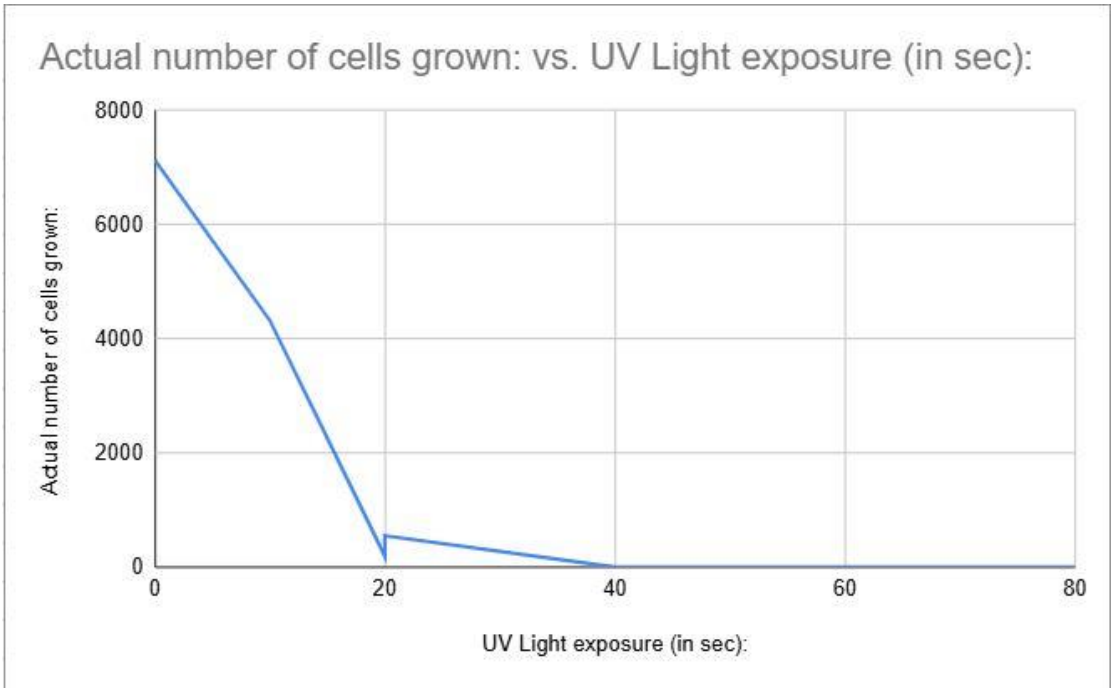
Week 2- Nutritional Requirements of HB2 and HAO yeasts:

Table 3- Actual S.cerevisiae (HAO) growth when exposed to UV light

Length of UV light	**Number of cells surviving:**	**% of cells surviving:**

exposure (in seconds):		
0	~7,132.2	100
10	~4,324.8	~60.64
20 (1 of 2 plates)	~185	~2.59
20 (2 of 2 plates)	~550	~7.71
40	0	0
80	0	0

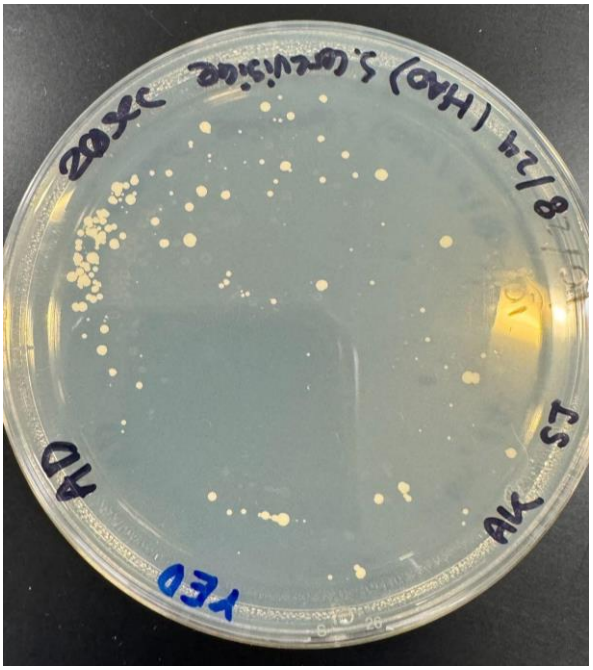
Graph 2- Actual S.cerevisiae (HAO) growth when exposed to UV light



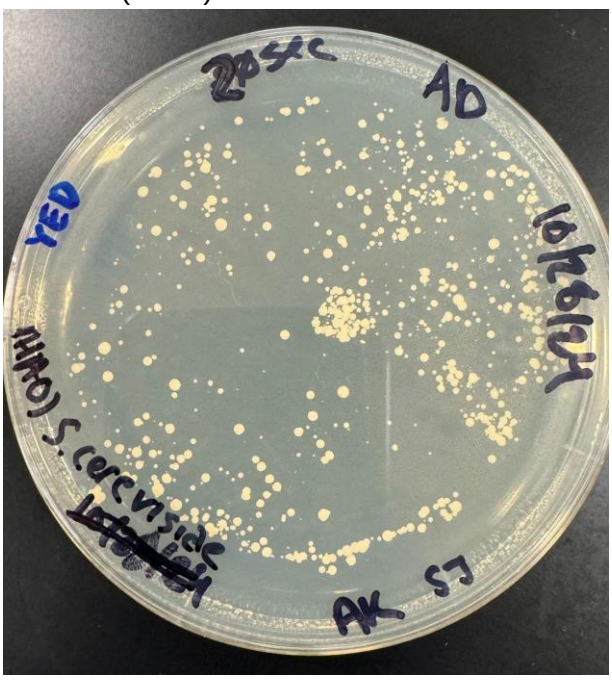
Below is the 0 second Plate:

Below is the 10 second Plate:

Below is (1 of 2) 20 second Plates:



Below is (2 of 2) 20 second Plates:



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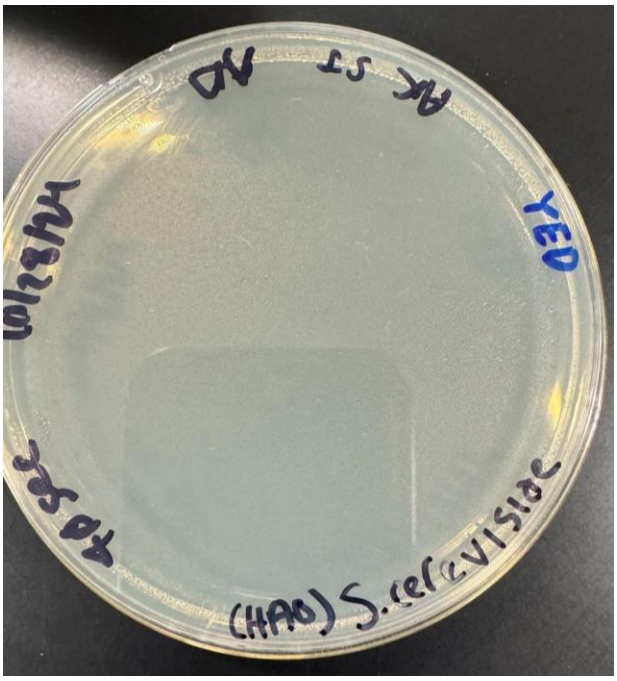


Below is the 80 second Plate:

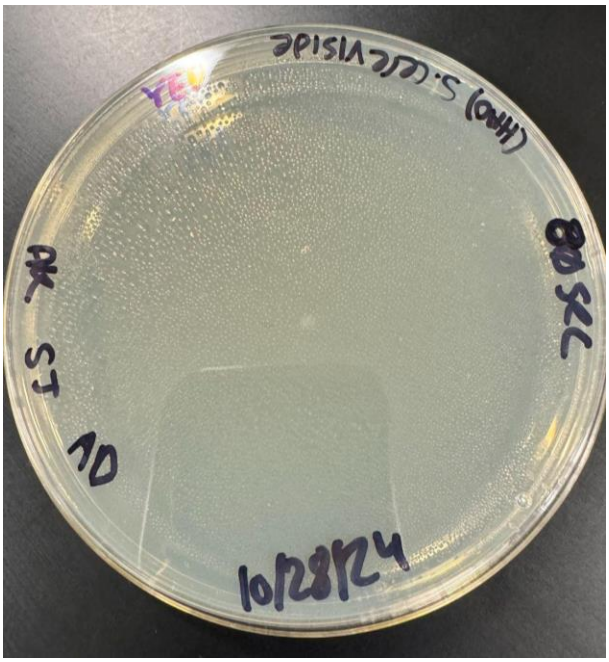


Table 4- Growth Results of Plated Cultures

Plate Description:	Number of white cells:	Number of red cells:
1:10 HAO control (spread)	~7,132.2	0
Mixed culture (1-streak)	TNTC	~105
Mixed culture (2-streak)	TNTC	~125

Table 5- Expected growth on medium plates of varying nutritional benefits, comparing strains

Strain:	Minimal medium:	Minimal medium + adenine:
1	No growth	No growth
2	Growth	Growth
3	No growth	Growth

Table 6- Expected growth in test tubes

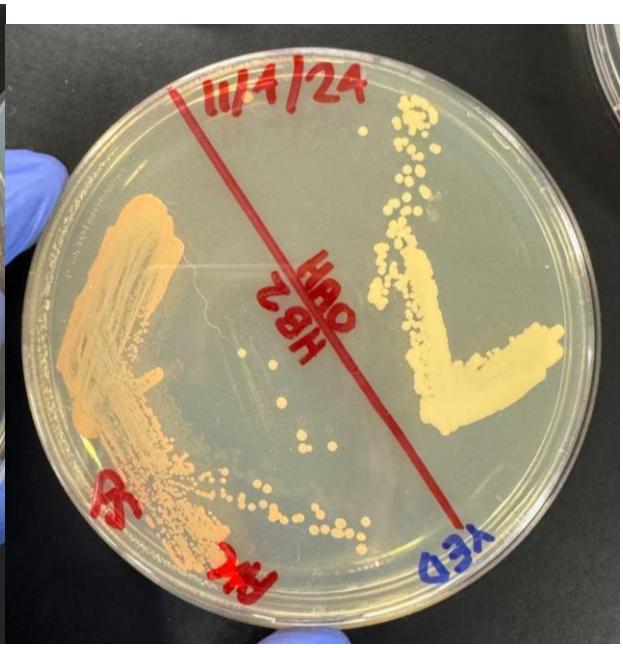
Tube description:	Growth?:
HB2 control	No
HB2 yeast culture	Yes

Week 3- Extraction of Genomic DNA from HB2 mutant strain:

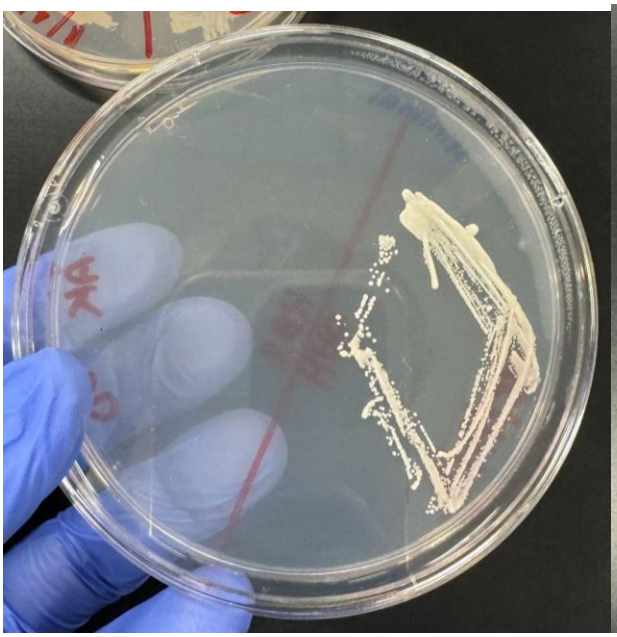
Table 7- Growth on medium plates of varying nutritional benefits

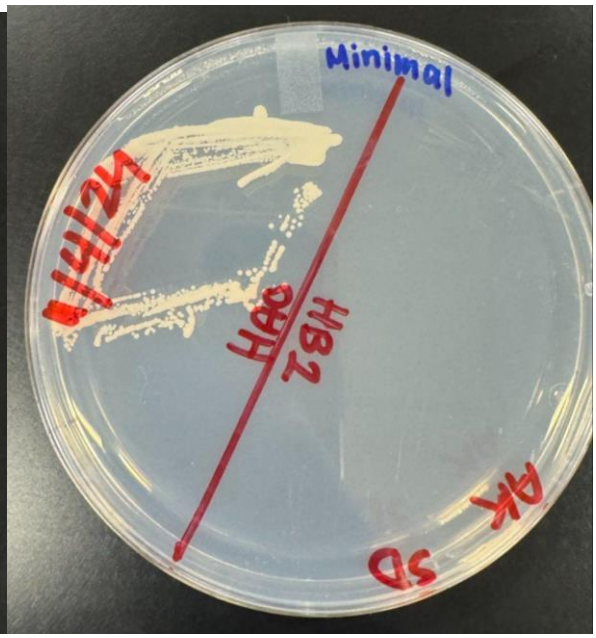
Plate description:	HAO Growth?:	HB2 Growth?:
HAO and HB2 on YED	Yes, TNTC white lawn with some individual white cells	Yes, TNTC red lawn with some individual red cells
HAO and HB2 on Minimal medium	Yes, TNTC white lawn with some individual white cells	No, no growth at all
HAO and HB2 on Minimal medium + adenine	Yes, TNTC white lawn with some individual white cells	Yes, TNTC white lawn with some individual white cells
HAO on YED	Yes, TNTC white lawn with some individual white cells	No, no growth at all
HB2 on YED	No, no growth at all	Yes, TNTC red lawn with some individual red cells

Below is the front and back of the HAO and HB2 of YED control plate:

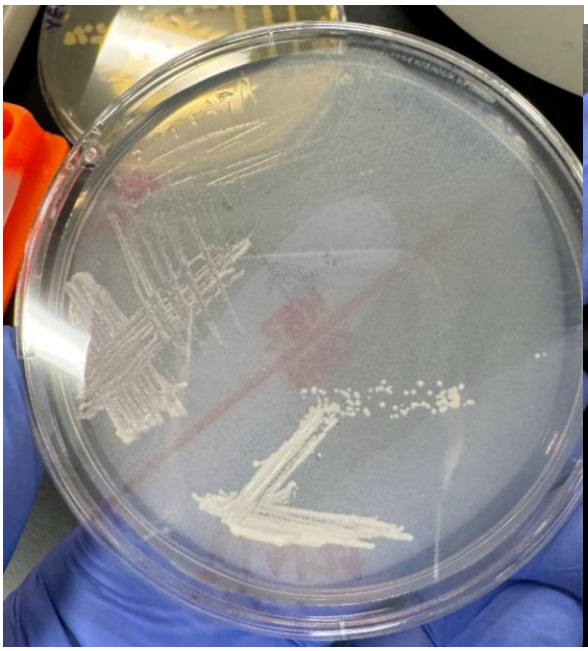


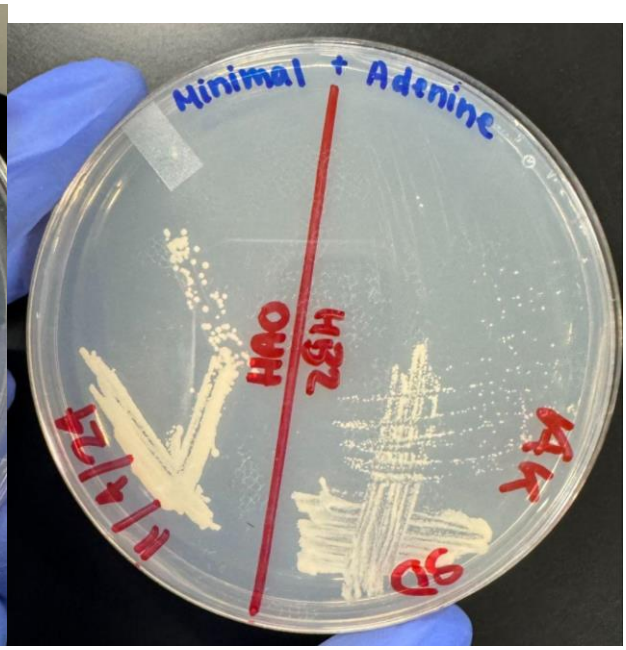
Below is the front and back of the HAO and HB2 on Minimal medium plate:



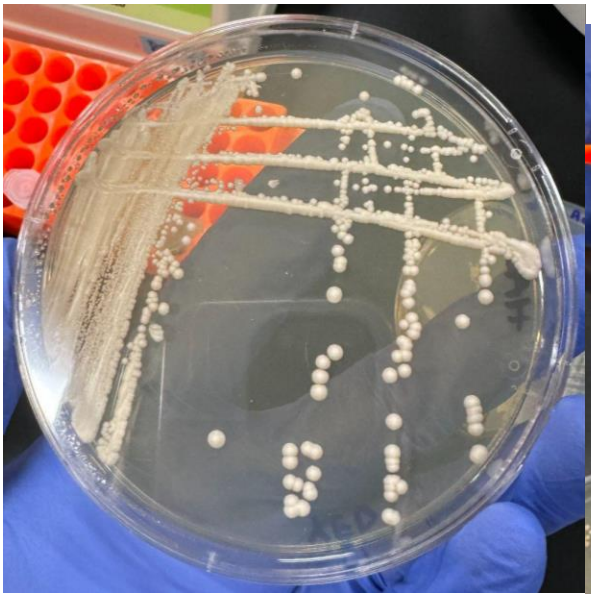


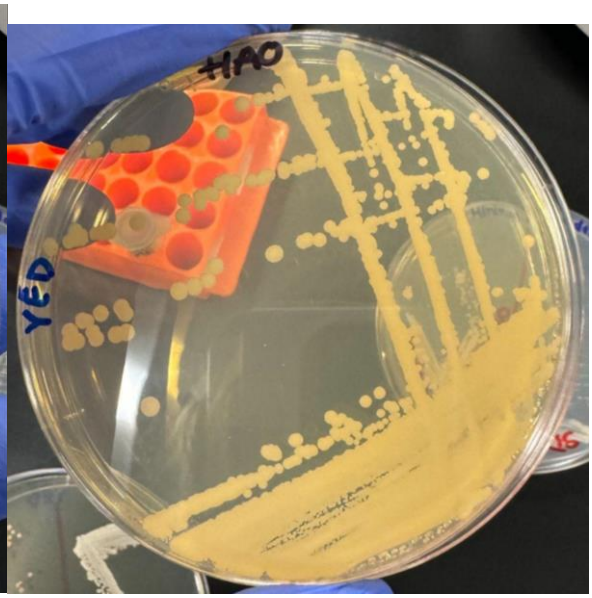
Below is the front and back of the HAO and HB2 on Minimal medium + adenine plate:





Below is the front and back of the HAO on YED plate from the professor:





Below is the front and back of the HB2 on YED plate from the professor:

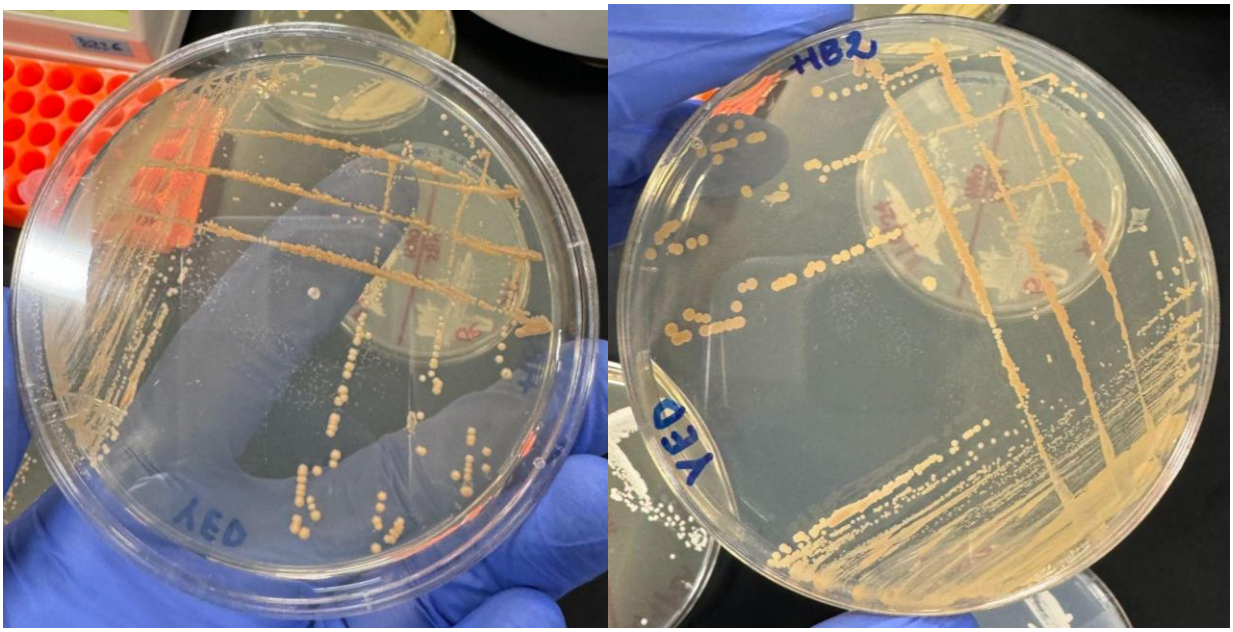


Table 8- Actual growth on medium plates of varying nutritional benefits, comparing strains

Strain:	Minimal medium:	Minimal medium + adenine:
1	No growth	No growth
2	Growth	Growth
3	No growth	Growth

Table 9- Results of Growth in test tubes

Tube description:	Growth?:
HB2 control	No
HB2 yeast culture	Yes, at the base of the tube there were red/pink tinge of cells

Week 4- Analysis of HB2 Genomic DNA and Isolation of Ade1 and Ade2:

Below is the class PCR results:

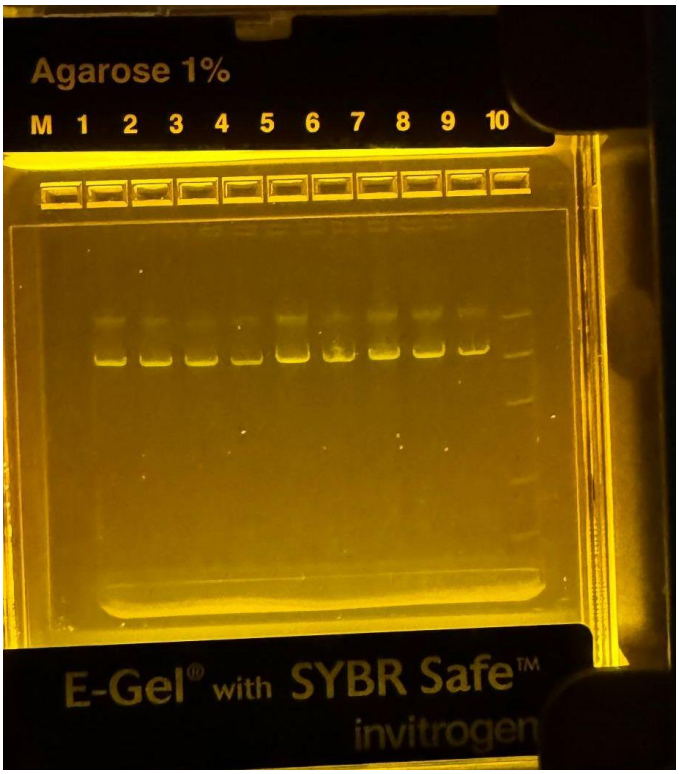


Table 10- Conents of class PCR Gel:

Well:	M	1	2	3	4	5	6	7	8	9	10
Contents:	Ladder	Positive control	YL + AY sample	Marta's sample	Our sample	AD + SJ sample	AE sample	Anna + Shaday sample	Irving + Liam sample	Positive control	Ladder

Below is the Ade1 Genomic DNA sequence:

```
>YAR015W_S288C ADE1 SGD:S000000070 ORF N-succinyl-5-aminoimidazole-4-carboxamide ribotide synthetase
  1 ATGTCAATTA CGAAGACTGA ACTGGACGGT ATATTGCCAT TGGTGGCCAG AGGTAAAGTT
 61 AGAGACATAT ATGAGGTAGA CGCTGGTACG TTGCTGTTTG TTGCTACGGA TCGTATCTCT
121 GCATATGACG TTATTATGGA AAACAGCATT CCTGAAAAGG GGATCCTATT GACCAAACTG
181 TCAGAGTTCT GGTTCAAGTT CCTGTCCAAC GATGTTCGTA ATCATTTGGT CGACATCGCC
241 CCAGGTAAGA CTATTTTCGA TTATCTACCT GCAAAATTGA GCGAACCAAA GTACAAAACG
301 CAACTAGAAG ACCGCTCTCT ATTGGTTCAC AAACATAAAC TAATTCCATT GGAAGTAATT
361 GTCAGAGGCT ACATCACCGG ATCTGCTTGG AAAGAGTACG TAAAAACAGG TACTGTGCAT
421 GGTTTGAAAC AACCTCAAGG ACTTAAAGAA TCTCAAGAGT TCCCAGAACC AATCTTCACC
481 CCATCGACCA AGGCTGAACA AGGTGAACAT GACGAAAACA TCTCTCCTGC CCAGGCCGCT
541 GAGCTGGTGG GTGAAGATTT GTCACGTAGA GTGGCAGAAC TGGCTGTAAA ACTGTACTCC
601 AAGTGCAAAG ATTATGCTAA GGAGAAGGGC ATCATCATCG CAGACACTAA ATTCGAATTC
661 GGTATTGACG AAAAGACCAA TGAAATTATT CTAGTGGACG AGGTGCTAAC GCCAGACTCC
721 TCTAGATTCT GGAACGGTGC CTCTTATAAG GTAGGAGAAT CCCAAGATTC TTACGATAAG
781 CAATTTTTAA GAGACTGGCT TACTGCTAAT AAGTTGAACG GTGTTAACGG CGTCAAAATG
841 CCCCAAGACA TTGTCGACAG GACAAGGGCC AAATATATAG AGGCTTATGA AACATTGACA
901 GGGTCTAAAT GGTCTCACTA A
```

Below is the Ade1 Coding DNA sequence:

```
>YAR015W_S288C ADE1 SGD:S000000070 ORF N-succinyl-5-aminoimidazole-4-carboxamide ribotide synthetase
ATGTCAATTACGAAGACTGAACTGGACGGTATATTGCCATTGGTGGCCAGAGGTAAAGTT
AGAGACATATATGAGGTAGACGCTGGTACGTTGCTGTTTGTTGCTACGGATCGTATCTCT
GCATATGACGTTATTATGGAAAACAGCATTCCTGAAAAGGGGATCCTATTGACCAAACTG
TCAGAGTTCTGGTTCAAGTTCCTGTCCAACGATGTTCGTAATCATTTGGTCGACATCGCC
CCAGGTAAGACTATTTTCGATTATCTACCTGCAAAATTGAGCGAACCAAAGTACAAAACG
CAACTAGAAGACCGCTCTCTATTGGTTCACAAACATAAACTAATTCCATTGGAAGTAATT
GTCAGAGGCTACATCACCGGATCTGCTTGGAAAGAGTACGTAAAAACAGGTACTGTGCAT
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CCATCGACCAAGGCTGAACAAGGTGAACATGACGAAAACATCTCTCCTGCCCAGGCCGCT
GAGCTGGTGGGTGAAGATTTGTCACGTAGAGTGGCAGAACTGGCTGTAAAACTGTACTCC
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TCTAGATTCTGGAACGGTGCCTCTTATAAGGTAGGAGAATCCCAAGATTCTTACGATAAG
CAATTTTTAAGAGACTGGCTTACTGCTAATAAGTTGAACGGTGTTAACGGCGTCAAAATG
CCCCAAGACATTGTCGACAGGACAAGGGCCAAATATATAGAGGCTTATGAAACATTGACA
GGGTCTAAATGGTCTCACTAA
```

Below is the Ade1 Protein sequence:

```
>YAR015W_S288C ADE1 SGD:S000000070 ORF N-succinyl-5-aminoimidazole-4-carboxamide ribotide synthetase
  1 MSITKTELDG ILPLVARGKV RDIYEVDAGT LLFVATDRIS AYDVIMENSI PEKGILLTKL
 61 SEFWFKFLSN DVRNHLVDIA PGKTIFDYLP AKLSEPKYKT QLEDRSLLVH KHKLIPLEVI
121 VRGYITGSAW KEYVKTGTVH GLKQPQGLKE SQEFPEPIFT PSTKAEQGEH DENISPAQAA
181 ELVGEDLSRR VAELAVKLYS KCKDYAKEKG IIIADTKFEF GIDEKTNEII LVDEVLTPDS
241 SRFWNGASYK VGESQDSYDK QFLRDWLTAN KLNGVNGVKM PQDIVDRTRA KYIEAYETLT
301 GSKWSH*
```

Below is the Ade2 Genomic DNA sequence:

```
>YOR128C_S288C ADE2 SGD:S000005654 ORF Phosphoribosylaminoimidazole carboxylase
   1 ATGGATTCTA GAACAGTTGG TATATTAGGA GGGGGACAAT TGGGACGTAT GATTGTTGAG
  61 GCAGCAAACA GGCTCAACAT TAAGACGGTA ATACTAGATG CTGAAAATTC TCCTGCCAAA
 121 CAAATAAGCA ACTCCAATGA CCACGTTAAT GGCTCCTTTT CCAATCCTCT TGATATCGAA
 181 AAACTAGCTG AAAAATGTGA TGTGCTAACG ATTGAGATTG AGCATGTTGA TGTTCCTACA
 241 CTAAAGAATC TTCAAGTAAA ACATCCCAAA TTAAAAATTT ACCCTTCTCC AGAAACAATC
 301 AGATTGATAC AAGACAAATA TATTCAAAAA GAGCATTTAA TCAAAAATGG TATAGCAGTT
 361 ACCCAAAGTG TTCCTGTGGA ACAAGCCAGT GAGACGTCCC TATTGAATGT TGGAAGAGAT
 421 TTGGGTTTTC CATTCGTCTT GAAGTCGAGG ACTTTGGCAT ACGATGGAAG AGGTAACTTC
 481 GTTGTAAAGA ATAAGGAAAT GATTCCGGAA GCTTTGGAAG TACTGAAGGA TCGTCCTTTG
 541 TACGCCGAAA AATGGGCACC ATTTACTAAA GAATTAGCAG TCATGATTGT GAGATCTGTT
 601 AACGGTTTAG TGTTTTCTTA CCCAATTGTA GAGACTATCC ACAAGGACAA TATTTGTGAC
 661 TTATGTTATG CGCCTGCTAG AGTTCCGGAC TCCGTTCAAC TTAAGGCGAA GTTGTTGGCA
 721 GAAAATGCAA TCAAATCTTT TCCCGGTTGT GGTATATTTG GTGTGGAAAT GTTCTATTTA
 781 GAAACAGGGG AATTGCTTAT TAACGAAATT GCCCCAAGGC CTCACAACTC TGGACATTAT
 841 ACCATTGATG CTTGCGTCAC TTCTCAATTT GAAGCTCATT TGAGATCAAT ATTGGATTTG
 901 CCAATGCCAA AGAATTTCAC ATCTTTCTCC ACCATTACAA CGAACGCCAT TATGCTAAAT
 961 GTTCTTGGAG ACAAACATAC AAAAGATAAA GAGCTAGAAA CTTGCGAAAG AGCATTGGCG
1021 ACTCCAGGTT CCTCAGTGTA CTTATATGGA AAAGAGTCTA GACCTAACAG AAAAGTAGGT
1081 CACATAAATA TTATTGCCTC CAGTATGGCG GAATGTGAAC AAAGGCTGAA CTACATTACA
1141 GGTAGAACTG ATATTCCAAT CAAAATCTCT GTCGCTCAAA AGTTGGACTT GGAAGCAATG
1201 GTCAAACCAT TGGTTGGAAT CATCATGGGA TCAGACTCTG ACTTGCCGGT AATGTCTGCC
1261 GCATGTGCGG TTTTAAAAGA TTTTGGCGTT CCATTTGAAG TGACAATAGT CTCTGCTCAT
1321 AGAACTCCAC ATAGGATGTC AGCATATGCT ATTTCCGCAA GCAAGCGTGG AATTAAAACA
1381 ATTATCGCTG GAGCTGGTGG GGCTGCTCAC TTGCCAGGTA TGGTGGCTGC AATGACACCA
1441 CTTCCTGTCA TCGGTGTGCC CGTAAAAGGT TCTTGTCTAG ATGGAGTAGA TTCTTTACAT
1501 TCAATTGTGC AAATGCCTAG AGGTGTTCCA GTAGCTACCG TCGCTATTAA TAATAGTACG
1561 AACGCTGCGC TGTTGGCTGT CAGACTGCTT GGCGCTTATG ATTCAAGTTA TACAACGAAA
1621 ATGGAACAGT TTTTATTAAA GCAAGAAGAA GAAGTTCTTG TCAAAGCACA AAAGTTAGAA
1681 ACTGTCGGTT ACGAAGCTTA TCTAGAAAAC AAGTAA
```

Below is the Ade2 Coding DNA sequence:

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>YOR128C_S288C ADE2 SGD:S000005654 ORF Phosphoribosylaminoimidazole carboxylase
   1 ATGGATTCTA GAACAGTTGG TATATTAGGA GGGGGACAAT TGGGACGTAT GATTGTTGAG
  61 GCAGCAAACA GGCTCAACAT TAAGACGGTA ATACTAGATG CTGAAAATTC TCCTGCCAAA
 121 CAAATAAGCA ACTCCAATGA CCACGTTAAT GGCTCCTTTT CCAATCCTCT TGATATCGAA
 181 AAACTAGCTG AAAAATGTGA TGTGCTAACG ATTGAGATTG AGCATGTTGA TGTTCCTACA
 241 CTAAAGAATC TTCAAGTAAA ACATCCCAAA TTAAAAATTT ACCCTTCTCC AGAAACAATC
 301 AGATTGATAC AAGACAAATA TATTCAAAAA GAGCATTTAA TCAAAAATGG TATAGCAGTT
 361 ACCCAAAGTG TTCCTGTGGA ACAAGCCAGT GAGACGTCCC TATTGAATGT TGGAAGAGAT
 421 TTGGGTTTTC CATTCGTCTT GAAGTCGAGG ACTTTGGCAT ACGATGGAAG AGGTAACTTC
 481 GTTGTAAAGA ATAAGGAAAT GATTCCGGAA GCTTTGGAAG TACTGAAGGA TCGTCCTTTG
 541 TACGCCGAAA AATGGGCACC ATTTACTAAA GAATTAGCAG TCATGATTGT GAGATCTGTT
 601 AACGGTTTAG TGTTTTCTTA CCCAATTGTA GAGACTATCC ACAAGGACAA TATTTGTGAC
 661 TTATGTTATG CGCCTGCTAG AGTTCCGGAC TCCGTTCAAC TTAAGGCGAA GTTGTTGGCA
 721 GAAAATGCAA TCAAATCTTT TCCCGGTTGT GGTATATTTG GTGTGGAAAT GTTCTATTTA
 781 GAAACAGGGG AATTGCTTAT TAACGAAATT GCCCCAAGGC CTCACAACTC TGGACATTAT
 841 ACCATTGATG CTTGCGTCAC TTCTCAATTT GAAGCTCATT TGAGATCAAT ATTGGATTTG
 901 CCAATGCCAA AGAATTTCAC ATCTTTCTCC ACCATTACAA CGAACGCCAT TATGCTAAAT
 961 GTTCTTGGAG ACAAACATAC AAAAGATAAA GAGCTAGAAA CTTGCGAAAG AGCATTGGCG
1021 ACTCCAGGTT CCTCAGTGTA CTTATATGGA AAAGAGTCTA GACCTAACAG AAAAGTAGGT
1081 CACATAAATA TTATTGCCTC CAGTATGGCG GAATGTGAAC AAAGGCTGAA CTACATTACA
1141 GGTAGAACTG ATATTCCAAT CAAAATCTCT GTCGCTCAAA AGTTGGACTT GGAAGCAATG
1201 GTCAAACCAT TGGTTGGAAT CATCATGGGA TCAGACTCTG ACTTGCCGGT AATGTCTGCC
1261 GCATGTGCGG TTTTAAAAGA TTTTGGCGTT CCATTTGAAG TGACAATAGT CTCTGCTCAT
1321 AGAACTCCAC ATAGGATGTC AGCATATGCT ATTTCCGCAA GCAAGCGTGG AATTAAAACA
1381 ATTATCGCTG GAGCTGGTGG GGCTGCTCAC TTGCCAGGTA TGGTGGCTGC AATGACACCA
1441 CTTCCTGTCA TCGGTGTGCC CGTAAAAGGT TCTTGTCTAG ATGGAGTAGA TTCTTTACAT
1501 TCAATTGTGC AAATGCCTAG AGGTGTTCCA GTAGCTACCG TCGCTATTAA TAATAGTACG
1561 AACGCTGCGC TGTTGGCTGT CAGACTGCTT GGCGCTTATG ATTCAAGTTA TACAACGAAA
1621 ATGGAACAGT TTTTATTAAA GCAAGAAGAA GAAGTTCTTG TCAAAGCACA AAAGTTAGAA
1681 ACTGTCGGTT ACGAAGCTTA TCTAGAAAAC AAGTAA
```

Below is the Ade2 Protein sequence:

```
>YOR128C_S288C ADE2 SGD:S000005654 ORF Phosphoribosylaminoimidazole carboxylase
  1 MDSRTVGILG GGQLGRMIVE AANRLNIKTV ILDAENSPAK QISNSNDHVN GSFSNPLDIE
 61 KLAEKCDVLT IEIEHVDVPT LKNLQVKHPK LKIYPSPETI RLIQDKYIQK EHLIKNGIAV
121 TQSVPVEQAS ETSLLNVGRD LGFPFVLKSR TLAYDGRGNF VVKNKEMIPE ALEVLKDRPL
181 YAEKWAPFTK ELAVMIVRSV NGLVFSYPIV ETIHKDNICD LCYAPARVPD SVQLKAKLLA
241 ENAIKSFPGC GIFGVEMFYL ETGELLINEI APRPHNSGHY TIDACVTSQF EAHLRSILDL
301 PMPKNFTSFS TITTNAIMLN VLGDKHTKDK ELETCERALA TPGSSVYLYG KESRPNRKVG
361 HINIIASSMA ECEQRLNYIT GRTDIPIKIS VAQKLDLEAM VKPLVGIIMG SDSDLPVMSA
421 ACAVLKDFGV PFEVTIVSAH RTPHRMSAYA ISASKRGIKT IIAGAGGAAH LPGMVAAMTP
481 LPVIGVPVKG SCLDGVDSLH SIVQMPRGVP VATVAINNST NAALLAVRLL GAYDSSYTTK
541 MEQFLLKQEE EVLVKAQKLE TVGYEAYLEN K*
```

Week 5- PCR Analysis of Ade1 and Ade2 products:

Below is the PCR results of our samples:

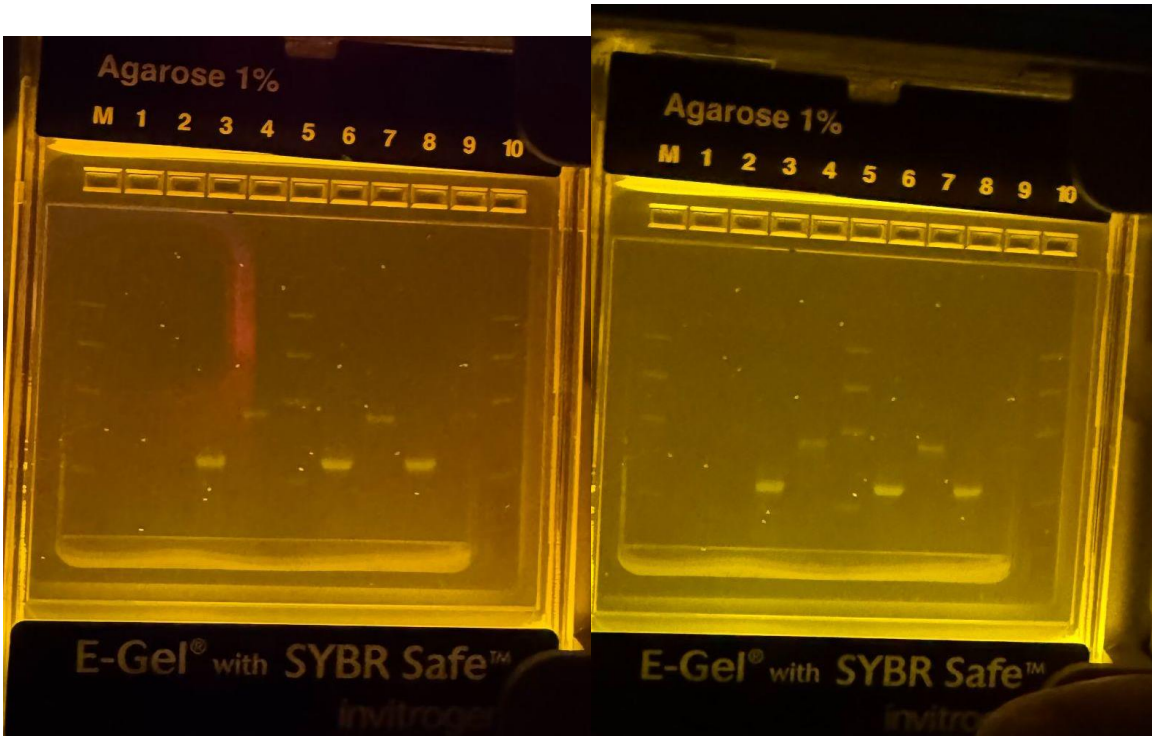

Table 11- Conents of our PCR gel:

Well:	M	1	2	3	4	5	6	7	8	9	10
Contents:	Ladder	T1 (other group)	T2 (other group)	T3 (other group)	T4 (other group)	Ladder	T1 sample	T2 sample	T3 sample	T4 sample	Ladder

DISSCUSSION

During the 5 week lab, our hypothesis was supported.

Week 1- Can UV Light Change the Apperance of Yeast?:

As we observed in the UV exposure plate, as the time the HAO yeast was exposed to UV light increases, the chances of mutation and cell death increases. Though none of our plates produced a HB2 (red yeast) mutation, our plates did grow fewer colonies as UV light exposure increased in time.

Week 2- Nutritional Requirements of HB2 and HAO yeasts:

HAO was able to grow on both minimal medium and minimal medium + adenine plates (and YED control plate). HB2 was only able to grow on the minimal medium + adenine plate. The control test tube had no growth but the yeast culture test tube successfully grew red/pink cells at its base. The HB2 that grew on the minimal medium + adenine plate was white/pink, and all the HAO that grew on all plates were white. Only on the control plate did the HB2 grow red. This is because HB2 is an auxotroph, so it cannot produce adenine. The HAO yeast cells are white because the adenine synthesis pathway doesn't occur when the adenine is supplied. HB2 will always grow red/pink on YED because it attempts adenine synthesis.

Week 3- Extraction of Genomic DNA from HB2 mutant strain:
The genomic DNA sample was successfully extracted and ready for PCR testing. To successfully isolate DNA, one must obtain a sample of cells and suspend them, disrupt cellular structure (creating a lysate), separate the soluble DNA from cellular debris and other insoluble materials, then bind the DNA of interest to a column, wash away proteins and other contaminants from the matrix, and elute the DNA. The DNA will then be ready for purification. Before DNA can get out of a cell (intact), it must pass through 3 barriers: cell wall, plasma membrane, and nuclear membrane. The genomic DNA post extraction was a milky/foamy liquid in appearance. When research scientists trying to identify a new flu virus, they would extract DNA from human tissues. The method of DNA extraction would differ, as the extraction would include 2 types of DNA (the human DNA and virus DNA). The 2 DNAs could be separated and would both be collected in the collection tube, whereas we only collected 1 yeast DNA.
Week 4- Analysis of HB2 Genomic DNA and Isolation of Ade1 and Ade2:
The PCR gel ran with the class successfully showed our genomic DNA of HB2 mutant red yeast and contained Ade1 and Ade2 genes. The first 3 bases of Ade1 AND Ade2 in the coding sequence(s) are 'ATG'. The last 3 nucleotides of Ade1 AND Ade2 are 'TAA', which is a stop codon (so it doesn't code for an amino acid). A total of 307 amino acids are connected to make the protein encoded by both Ade1 AND Ade2. Both Ade1 and Ade2 genes have 921 nucleotides required to code for all 307 amino acids. Using our 5 week experiment and the yeast genome database (https://www.yeastgenome.org/), there is evidence of DNA mutation of S. cerevisiae on its Ade1 and Ade2 gene that affected purine base metabolism. Red/pink color was expressed due to AIR (converted into red polymer that colors the HB2 yeasts red) and CAIR accumulation. Due to this, it is fair to say using the evidence collected in our experiments (and the evidence in the database), that the (HB2) cells that express red colonies are affected in purine base metabolism.
Week 5- PCR Analysis of Ade1 and Ade2 products:
Both the Ade1 and Ade2 genes were amplified, as expected. Well 6 and 8 contained Ade1 primers and well 7 and 9 contained Ade2 primers. The wells containing Ade1 primers expressed Ade1 presence in the gel and the wells containing Ade2 primers expressed Ade2 presence in the gel.

SOURCES:

- Lab manual
- Class data
- https://dnalc.cshl.edu/resources/animations/pcr.html
- https://www.yeastgenome.org/
- www.promega.com
- https://digication.com