

HHMI

Presentation

Lab Group
Three

Two

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& Natalie

What have we
learned so far?

- We determined the gene that we are going to knock out: IRA2
- Began looking for primers that would bracket our sequence of DNA during PCR
- Prepared our PCR and modified the IRA2
- Tested our PCR with gel electrophoresis to see if it was successful
- Took cultured yeast and inoculated it in an agar filled petri dish to allow them to grow
- Transformed yeast via the lithium acetate model

How has our
hypothesis
developed?

We originally hypothesized that the defects in yeast from the knockdown of IRA2 will cause yeast to respond poorly to stressors. Additionally, the budding would be increased and rates of recombination would be lowered in comparison to the control, due to the lack of inhibition in the cAMP pathway. We also hypothesized that MSL1 would have an effect on recombination because one of its functions is the splicing of RNA.

We did not have sufficient time and resources at our disposal to knock out both IRA2 and MSL1. Therefore, we decided to retract a portion of our original hypothesis and gear our experimental design toward the disabling of the IRA2 gene.

Once we refined our plan, we began determining exactly what we are hoping to discover and how we were going to do so.

According to scientific research, knocking out genes can increase or decrease the lifespan of yeast. We believe that knocking out the IRA2 gene will decrease the lifespan of our yeast because IRA2 responds to stress. Our prediction is that this is because IRA2 genes are involved in the ras-camp pathway, which allows proteins in and out of the cell and responds to stress. IRA2 also plays a role in sporulation which is a protective mechanism of the yeast. IRA2 responds to extreme temperature change, subjection to hydrogen peroxide, nitrogen starvation and oxidative stress. We plan to test our yeast at extreme temperature change because it is the easiest stressor to replicate in a lab and we hope to elucidate the mechanisms through which IRA2 helps yeast survive stressors.

Our Hypothesis:

If we knock out IRA2 in *saccharomyces cerevisiae*, the morphant yeast will exhibit a less efficient response to stress, specifically extreme temperature change, than the non-morphant. We believe the morphant yeast will have a shorter lifespan and lower rate of budding when subjected to the temperature change.

Experimental Design:

- Knock out the IRA2 gene from our yeast
- Culture the yeast
- Subject the yeast to extreme temperature change

For all strains of *Saccharomyces cerevisiae*,

The optimum temperature: 30 to 35C

The maximum temperature: 37 to 40C

Yeast cannot grow at higher than 40C

Yeast would die at higher than 60C after 10 to 15 min.

- Observe the yeast under a microscope and measure survival rates

Why is this
important?

Wine

- *Saccharomyces cerevisiae* is the most widespread ingredient in wine fermentation
 - This is what converts grape juice to wine
 - Yeast consume the sugar and release alcohol and carbon dioxide as byproduct
- Possible link to how temperature changes affect fermentation of yeast
 - Find ideal temperature for wine and alcohol production

Mammal

- Optimal temperature for yeast growth is same as our body temperature.
- IRA1 and 2 are homologous to human neurofibromin in sequence and function
- A product of the IRA2 gene exhibits GAP activity similar to that of the mammalian GAP protein.