**Research Plan**

A. **Rational:** Huntington's disease patients typically die within 17 years of diagnosis from various complications such as accidents, aspiration and dysphagia. Huntington's disease is a result of the expansion of glutamine repeats in the Huntingtin protein (Htt) found on the fourth chromosome. Huntington's disease is one of many neurodegenerative diseases with an autosomal dominant manner of inheritance. The prevalence of Huntington's disease is 10.6–13.7 individuals per 100,000. This research is important as it delves deeper into the unfolding of the huntingtin protein which is the first physical change seen in the body as a result of the huntingtin gene mutation. Studying how the unfolded proteins interact in the cell can ultimately help us find a treatment for this disease to keep it at bay and allow those who suffer from it to have the severity of their symptoms reduced. Previous research indicated that the unfolded protein aggregate together to form an inclusion body in the cell and that other proteins help form this inclusion. This study was done to investigate how other proteins impact the creation of this inclusion body.

B. **Research Questions, Hypothesis, Engineering Goals and Expected Outcome:** The engineering goals were to properly genetically engineer the yeast strains with mutant huntingtin tagged with mCherry for the purpose of observing these cells with unfolded huntingtin protein. When huntingtin inclusion bodies form, are there other proteins that are necessary for the formation and regulation of the inclusion body? Additionally, do these proteins colocalize to the inclusion body or are they simply just interacting with it from the cytoplasm? We expected some of the chaperone proteins in our clone collection to colocalize and be present in the inclusion bodies as the role of chaperone proteins are to interact with unfolded protein and either refold them or aggregate them to prevent them from disturbing the cell's homeostasis.

C. **Procedure, Risk and Safety, Data Analysis:**

**I. Procedures:**

Yeast Transformation-

The process used for Yeast transformation began by ensuring that the cells are in mid-log phase (OD 0.1-0.3 at 600nm). The original clone collection will be purchased from Yeast GFP Clone Collection, Thermofisher Scientific. 1.5 mL of the cell suspension will spin for 5 minutes at 5000rpm in a tabletop centrifuge to pellet the cells. This will create a supernatant to be decanted and the pellet will be resuspended in 1.0 mL of .1 M LiAc. Then, the cells will pellet again in the same tabletop centrifuge for 5 minutes at 5000rpm with the residual LiAc removed after pelleting. These cells will then be resuspended in 240 μL of Polyethylene glycol (PEG), first using a pipette, and then using a vortex. After, 36 μL of 1.0 M LiAc, 25 μL of SS-DNA and 1 μL of the plasmid containing mHtt-mCherry and the Lew 2 gene will be added to the solution. This solution will be vortexed 3 times for 2-3 seconds as not to break the SS-DNA. The cells will be incubated

at 30 °C for 30 minutes in a water bath. Next, to break the membranes in the cells, they will be heat shocked in a water bath at 42 °C for 45 minutes. This solution was then added to a microfuge at 5000rpm for 2 minutes and all the transformation mix was removed with a micropipette. The pellet created from the microfuge will be resuspended in 100 μL of water and spread on a warmed plate using a glass spreader. The glass plate must contain selectable media that is devoid of Lew 2 to ensure that only cells that were transformed and have the plasmid survive as those cells are able to create Lew 2. Finally, parafilm will be added to the edge of plates to prevent them from drying out and incubated upside down for 2-4 days at 30 °C.

Inoculation of Cell Cultures-

After the cells grow in the plates, one colony will be selected and added to YPD medium to be inoculated in a falcon tube. If there is a relatively large difference between the sizes of the colonies present in one plate, then two colonies will be chosen, one small and one large and both will be inoculated in separate falcons tubes to be observed separately. These tubes will be incubated overnight at 30 °C and will be ready for slide preparation the next morning.

Slide Preparation-

The cells will be used from cultures inoculated the previous day and will be spun in a microcentrifuge for 2 min 30 sec at 5000rpm. The majority of supernatant will be decantanded and the pellet will be resuspended in in the small amount of residual liquid. This creates a small liquid with a large amount of cells and about 2 μL of the liquid will be added to the slide and covered with a coverslip. A small drop of oil is added to the lenses of the microscope and slide must be placed upside down on the lense before being viewed.

**II. Risk and Safety:** The procedures will be completed while wearing the correct personal protective equipment such as a lab coat, gloves and safety glasses to be cautious of the dangerous chemicals used in these procedures. Additionally, all the excess liquid will be cleaned with ethanol before being disposed of correctly, either in regular trash or with other biohazards which were then autoclaved. Moreover, a mentor will be present during all the procedures done to ensure that the correct safety protocols are being followed.

1. **Human Participant Research:** No humans were used in this research project.
2. **Vertebrate Animal Research:** No vertebrate animals were used in this research project.
3. **Potentially Hazardous Biological Agent Research:**
    a. The PHBA used in my project was a non pathogenic Saccharomyces cerevisiae cell line with recombinant DNA. The original clone collection will be purchased from Thermofisher and will be genetically engineered using the process described above in the lab. These are classified as BSL 1 and were disposed of as such.

Additionally, while handling these cells, correct personal protective equipment will be used while handling these cells with care.

b. After the cells were done being observed, the recombinant yeast will be washed with ethanol and contained in a jar with bleach. Then, it will be disposed of in red biohazardous waste bags and sent off-site to be autoclaved and properly treated.

4. **Hazardous Chemicals, activities and devices:**

Chemicals will be handled delicately with extreme care and will only be worked with under the correct conditions while using proper protective gear. This includes but is not limited to tying back long hair, wearing gloves and, no loose clothes and being supervised by a mentor at all times. Moreover, specific procedures will be used for disposal of the dangerous chemicals and PHBAs in this project.

Lithium Acetate-

This chemical will be used in the Yeast transformation and it is a potential skin irritant and a serious eye irritant. If it makes contact with skin or eyes, wash thoroughly with water and if irritation persists, seek medical attention. If LiAc is inhaled, get fresh air and seek medical attention if there is trouble breathing. These dangerous acts can be prevented by wearing gloves, safety glasses and working in an open area with plenty of fresh air.

Polyethylene Glycol (PEG)-

This chemical will be used in the Yeast transformation and it is a potential skin irritant and a serious eye irritant. One should wear gloves and safety glasses while working with PEG. If it makes contact with skin or eyes, wash thoroughly with water and if irritation persists, seek medical attention. Moreover, PEG can be damaging when inhaled which can be prevented by working in an open area with plenty of fresh air.

Yeast Extract Peptone Dextrose (YEPD)-

YEPD is not as dangerous as other chemicals but can be very hazardous when ingested or inhaled. If ingested or inhaled, contact a medical professional and seek their opinion based on the severity of the contact. YPD is used throughout the laboratory experiment as it houses the cells in the incubator and on the slides.

**III. Data Analysis:** After viewing on a Nikon Eclipse Ti-S inverted epifluorescence microscope, the data will be analysed using FIJI ImageJ and recorded every day. The data was measured quantitatively as well as qualitatively and results are shown in the experiment paper. The size and number of inclusion bodies will be recorded quantitatively while the colocalization of the tagged protein with the mutant huntingtin is observed qualitatively.

**D. Bibliography:**


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