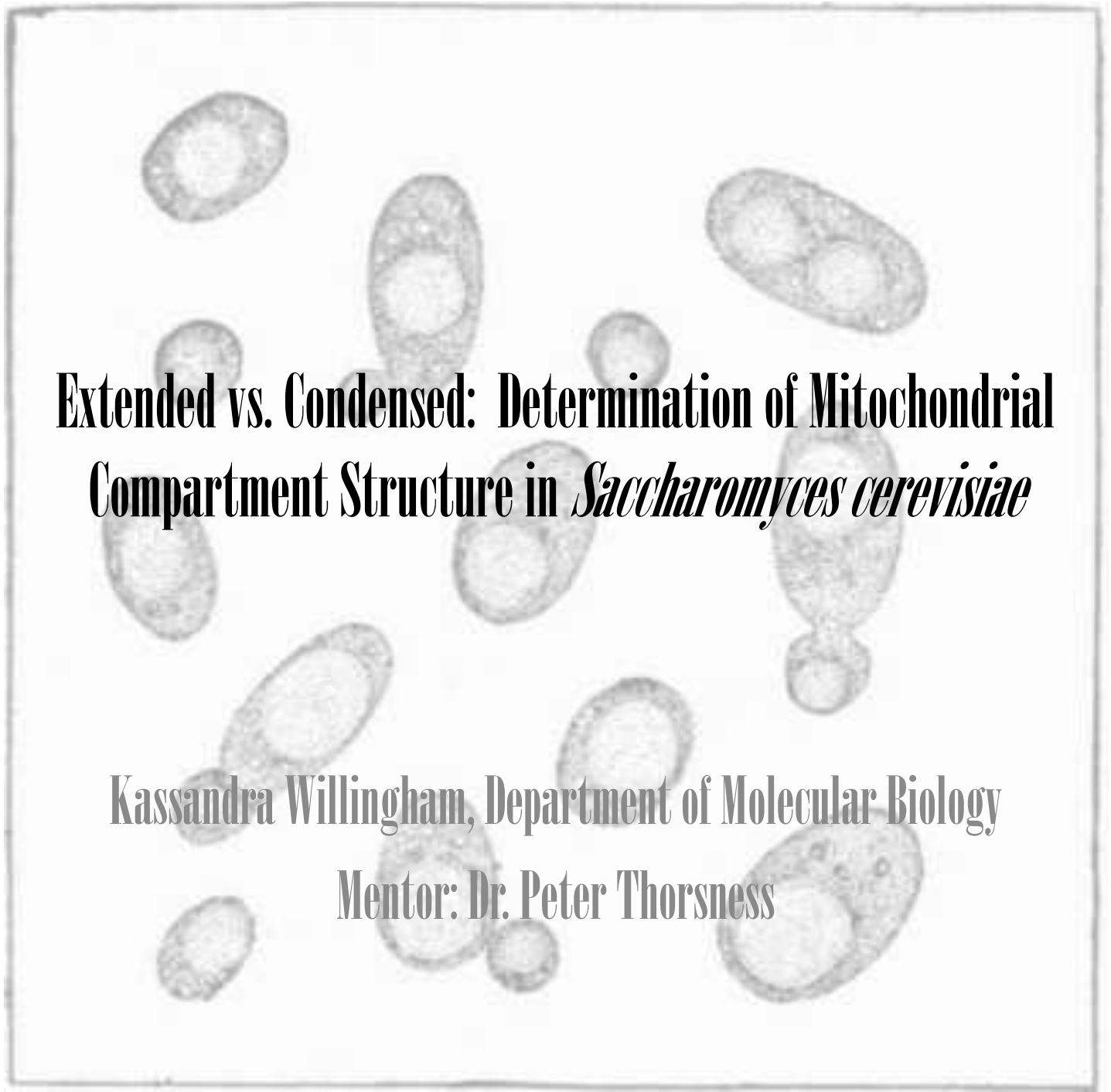

# Extended vs. Condensed: Determination of Mitochondrial Compartment Structure in *Saccharomyces cerevisiae*

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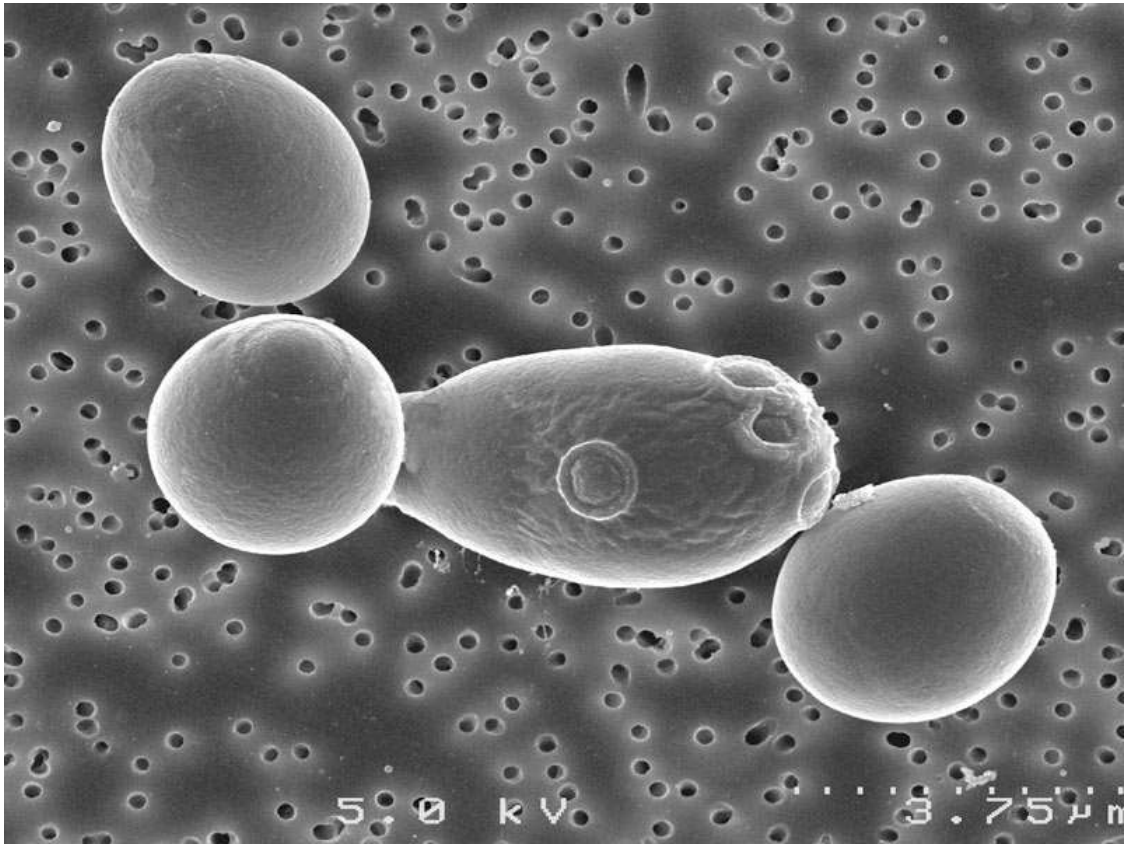
# *Saccharomyces cerevisiae*

- Model organism for eukaryotes
- A species of budding yeast
- About 3-5 μm
- Small genome: $1.4 \times 10^7$ basepairs

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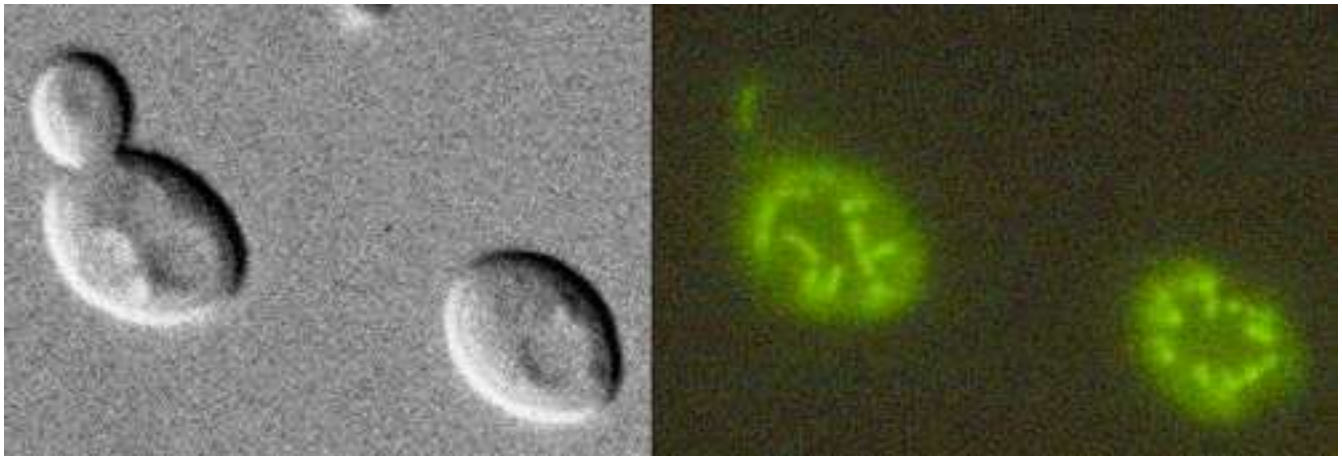
# Mitochondria

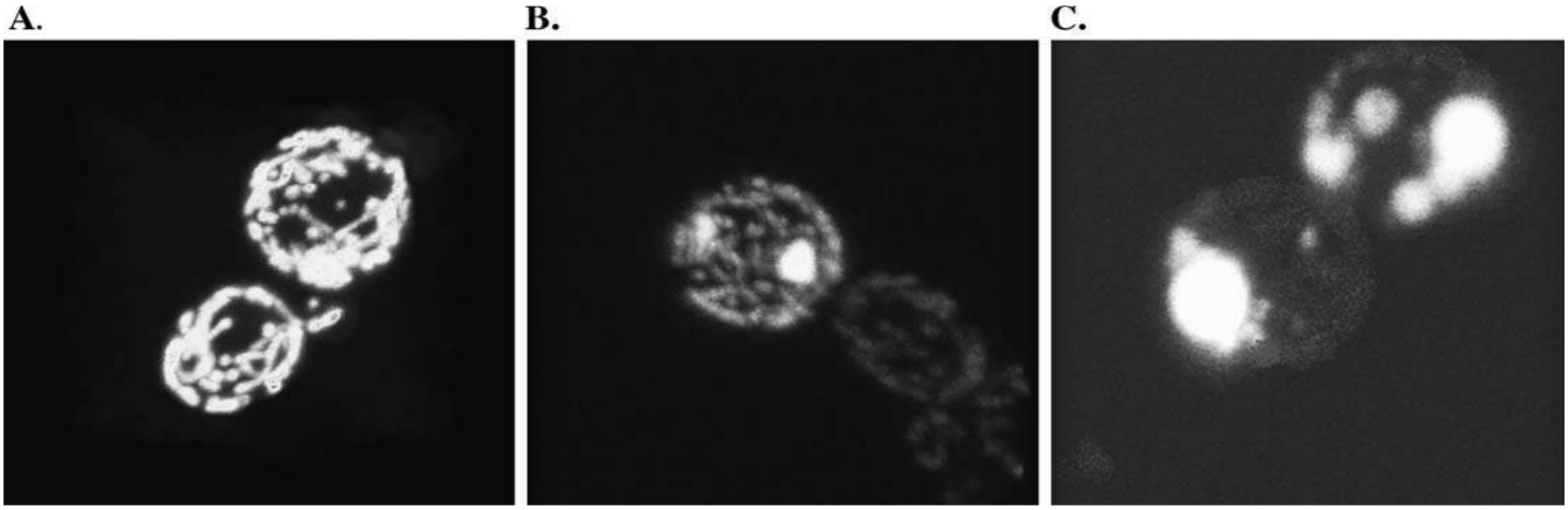
- Often referred to as the powerhouse of cells
- Membrane enclosed organelles that are responsible for the generation of a cell's energy through the production of ATP



# Mitochondrial Morphology

•Mitochondrial compartments contained inside the cells of a wild-type yeast generally exhibit extended mitochondrial structures (figure A)

- •Condensed structures are also present (figure C)
- •Also mixed structures
- •Mitochondria shown below were observed by using fluorescent microscopy and mitochondrial targeted Green Fluorescent Protein (photo provided by Peter Thorsness)

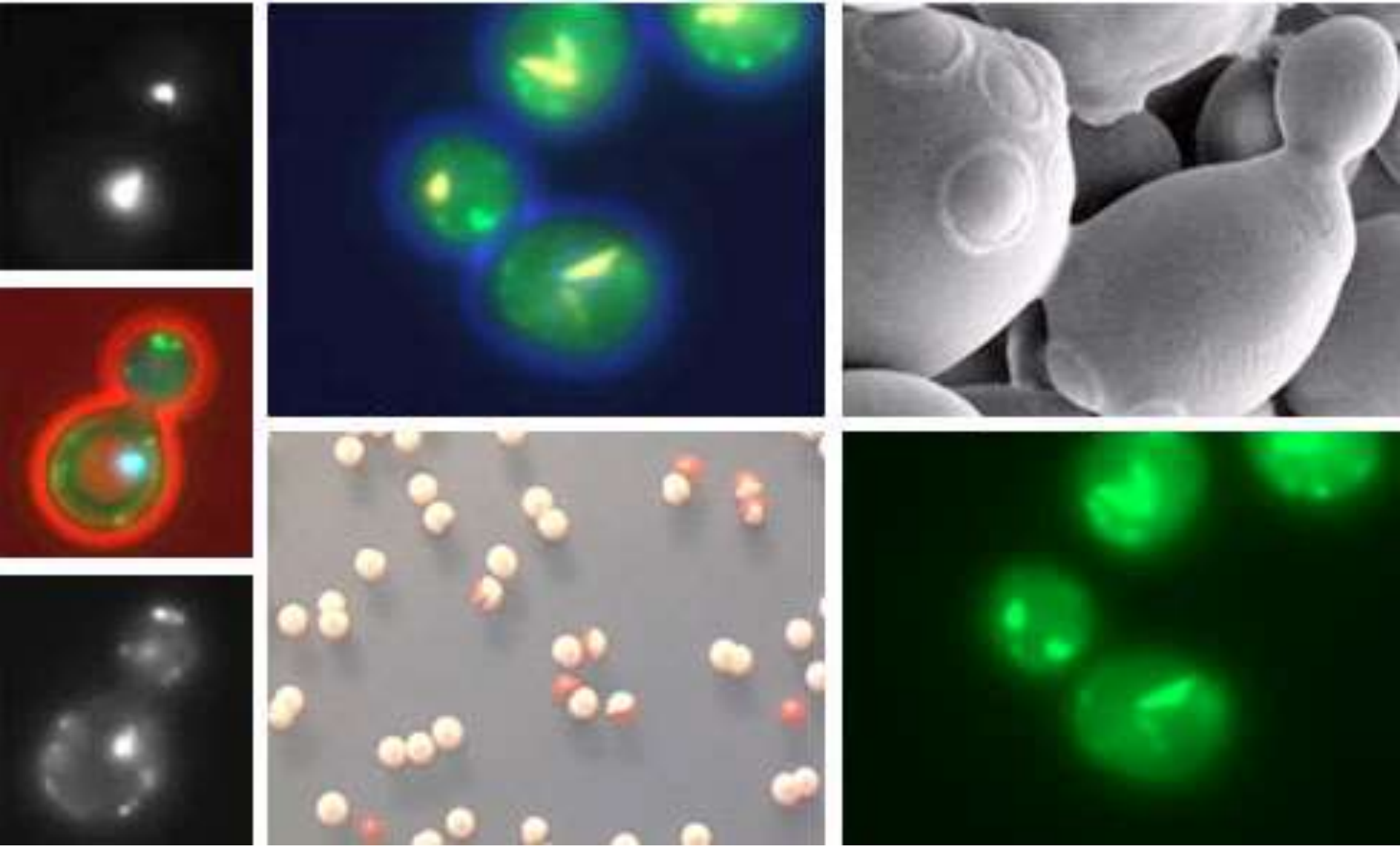


# Extended vs. condensed morphologies

- Although genetic makeup is identical, condensed, extended, and mixed morphologies are observed in wild type yeast.

- Important question:  If the genes are identical, causes morphological distribution?

- Hypothesis: The morphology of a daughter cell is highly dependent on the morphology of the mother cell, due to yeast budding.



http://www.kent.ac.uk/bio/styles/Images/staff/tuite/Slide1.jpg

# Research Procedures

# Strain Construction

- Goal: construct a strain of yeast that is likely to contain a significant percentage of condensed mitochondrial compartments, while still containing mitochondrial DNA.

| Strain Name | Phenotype |
|---|---|
| NXY-25 | Mat α, lys2 ura 3-52 leu 2-3,112 trp 1-Δ1 aac 2-1 [ρ+, pDO12] |
| SPY-28 | Mat a, ade2 ura 3-52 leu 2-3,112 trp 1-Δ1 gem1Δ::kanMX aac 2-1 [ρ+] |
| KWY-66 | Mat a, ade2 ura 3-52 leu 2-3,112 trp 1-Δ1 mdm33Δ::kanMX aac 2-1 [ρ+] |
| KDY-3 (NXY 25 X SPY-28) | Mat α lys2 ura 3-52 leu 2-3,112 trp 1-Δ1 mdm33Δ::kanMX aac 2-1 [ρ+, pDO12] |
| KDY-4 (NXY 25 X KWY-66) | Mat α lys2 ura 3-52 leu 2 trp1 gem1Δ::kanMX aac 2-1 [ρ+, PDO12] |

# Transformation with pDO12 Plasmid

- NXY25 originally contained pDO12
  - pDO12 is responsible for the expression of mt GFP*

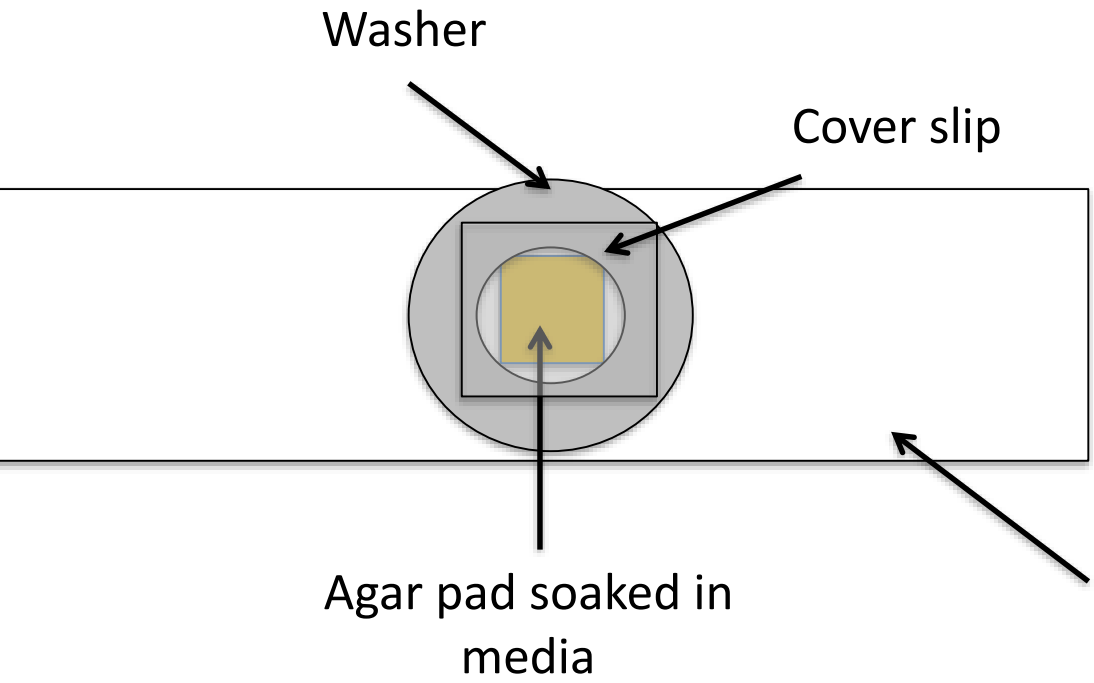
- Uracil is genetic marker
- KDY 3 and 4 both plated on SD-ura plate
  - No growth = loss of plasmid
  - Transformation to obtain “glowing structures”
    - Protocol:  Modified Yeast Transformation with LiOAC by Fred Ponticelli

# Determination of Mitochondrial Morphology Distribution

- pDO12 transformants are grown overnight at 30 ° C
- Heavy smear represent “mother cells”
- Colonies arisen from heavy smear represent “daughter cells”
- Hanging drop method

# Hanging Drop Method

- Agar pad soaked in liquid media facilitates observation of **living cells**




•Cells are dropped onto a small agar pad that has been soaked in media.
•Yeast cells live off of the agar pad, and the counts will more closely represent a living population

# Liquid Media vs. Solid media

- Solid Media:
  - Easier to count
  - The count obtained may depend on the location of the cells with respect to the colony
- Liquid media
  - We can excise a colony, ensuring that we are getting a representative count of the entire colony
  - Excise colony, drop into TSB, count, incubate 4 hours, count again

# Results!

# Solid Media

| Name of Strain | Colony Number | % extended | % condensed |
|---|---|---|---|
| KDY 4 | "Parent" | 59.8 | 40.2 |
| | 1 | 60.0 | 40.0 |
| | 2 | 65.7 | 34.3 |
| | 3 | 46.0 | 54.0 |
| | 4 | 60.7 | 39.3 |
| | 5 | 51.2 | 48.1 |
| | 6 | 61.5 | 38.5 |
| | 7 | 46.1 | 53.9 |
| | 8 | 42.6 | 57.4 |
| | 9 | 53.5 | 46.5 |
| | 10 | 43.8 | 56.2 |

* If you hadn't noticed, morphologies range from 42.6-65.7% extended structures, and 34.3-57.4% condensed structures

# Liquid Media

**Morphology Distribution (Preliminary count)**

| Name of Strain | Colony Number | % extended | % condensed |
|---|---|---|---|
| KDY 4- grown in liquid SD-ura media | "Parent" | 69.8 | 30.2 |
| | 1 | 64.5 | 35.5 |
| | 2 | 50.7 | 49.3 |
| | 3 | 64.0 | 36.0 |
| | 4 | 50.3 | 49.7 |
| | 5 | 55.8 | 44.2 |

**Morphology Distribution After 4 Hour Incubation at 30 C**

| Name of Strain | Colony Number | % extended | % condensed |
|---|---|---|---|
| KDY 4- grown in liquid SD-ura media | "Parent" | 67.0 | 33.0 |
| | 1 | 72.6 | 27.4 |
| | 2 | 66.2 | 33.8 |
| | 3 | 58.8 | 41.2 |
| | 4 | 61.8 | 38.2 |
| | 5 | 71.7 | 28.3 |

**The percentage of extended morphology increases in most colonies after 4 hour incubation

# So, what does it all mean?

- Varying Percentages
  - Possible issues with counting bias

- Liquid vs. solid media
  - Why did numbers of extended morphologies increase after 4 hours incubation in liquid media?

# Future Experiments

- Transformation of wild-type PTY 33 with TOM70 GFP
  - Because the GFP is located next to a protein, the brightness of the glow is **not** dependent on membrane permeability

Questions?

# Acknowledgements


- Peter and Mary Thorsness
- Karen White
- Fay Lab
- NSF and EPSCoR


# Thank You!!

# References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