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## Fluorescent Analysis of Protein Localization in *Saccharomyces cerevisiae*

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The background of the slide is black, decorated with numerous out-of-focus fluorescent spots. Most of these spots are green, while a few are blue. They are scattered across the slide, with a higher concentration on the right side.

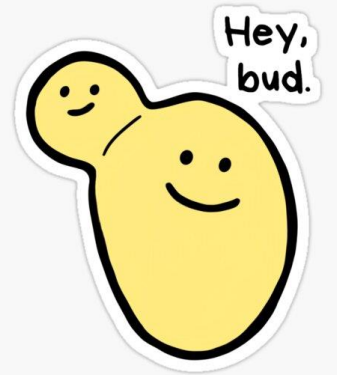
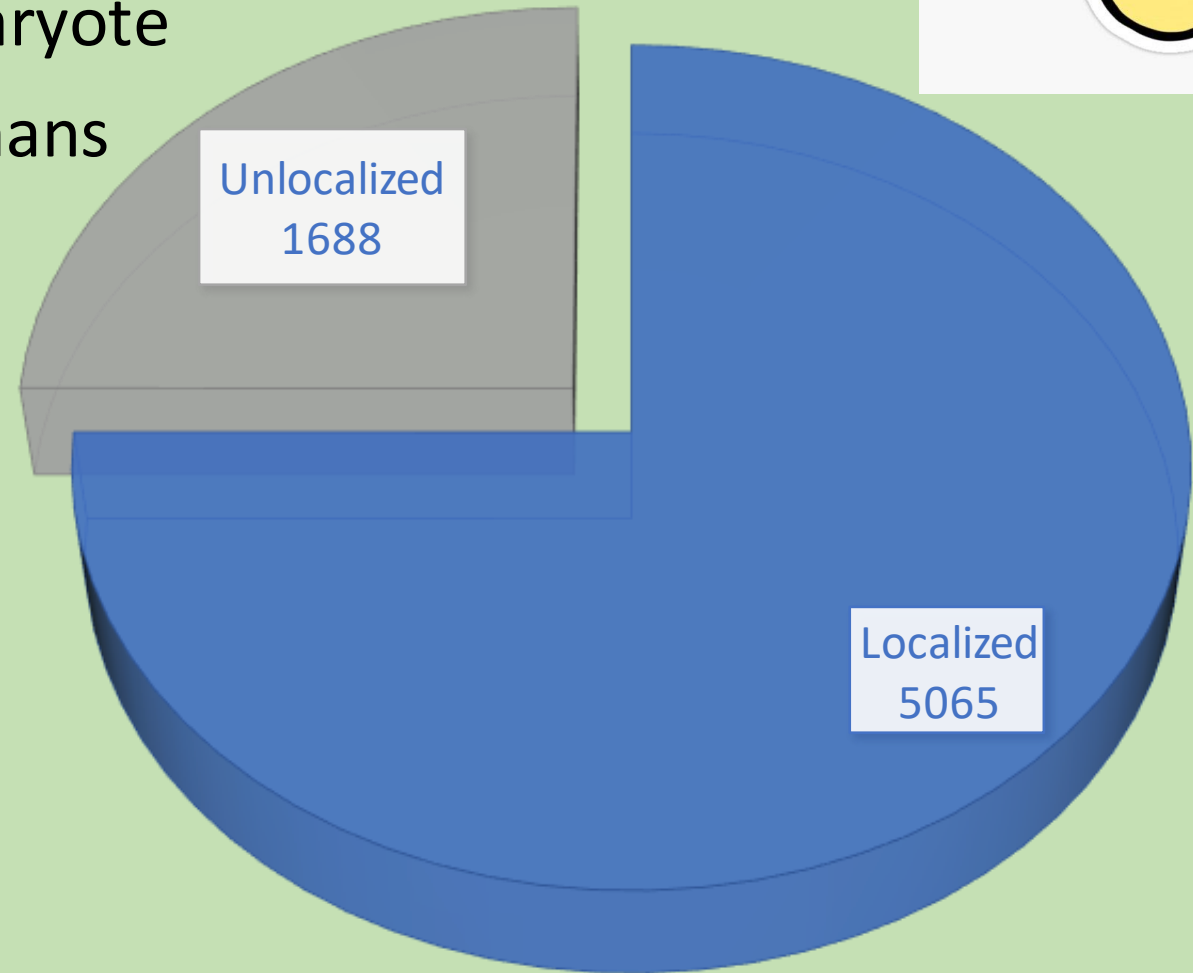
# Fluorescent Analysis of Protein Localization in *Saccharomyces cerevisiae*

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# *Saccharomyces cerevisiae*

- Most studied single-celled eukaryote
- 23% homologous genes to humans
- Fully sequenced genome
- 6,753 potential proteins
- Data collected in the *Saccharomyces* Genome Database
- 75% of proteome localized



# Selected Open Reading Frames (ORFs)

## Fsh3/YOR280C

- Human ortholog- Ovca2
- Serine hydrolase
- Tumor suppressor in ovarian cancer

## Ade13/YLR359W

- Human ortholog- Adsl
- Adenylosuccinate lyase
- Autistic features

## Gid10/YGR006C

- Human ortholog- Gid4
- Ubiquitin ligation
- Smith-Magenis syndrome

## Question:

Where does each protein localize within the cell?



# Comparative Analysis

- Inferred phylogenetic relationships from maximum likelihood estimation
- Known localization of orthologous proteins compared to unknown localizations
- Localization in nucleus, cytoplasm, and mitochondria

## Localization

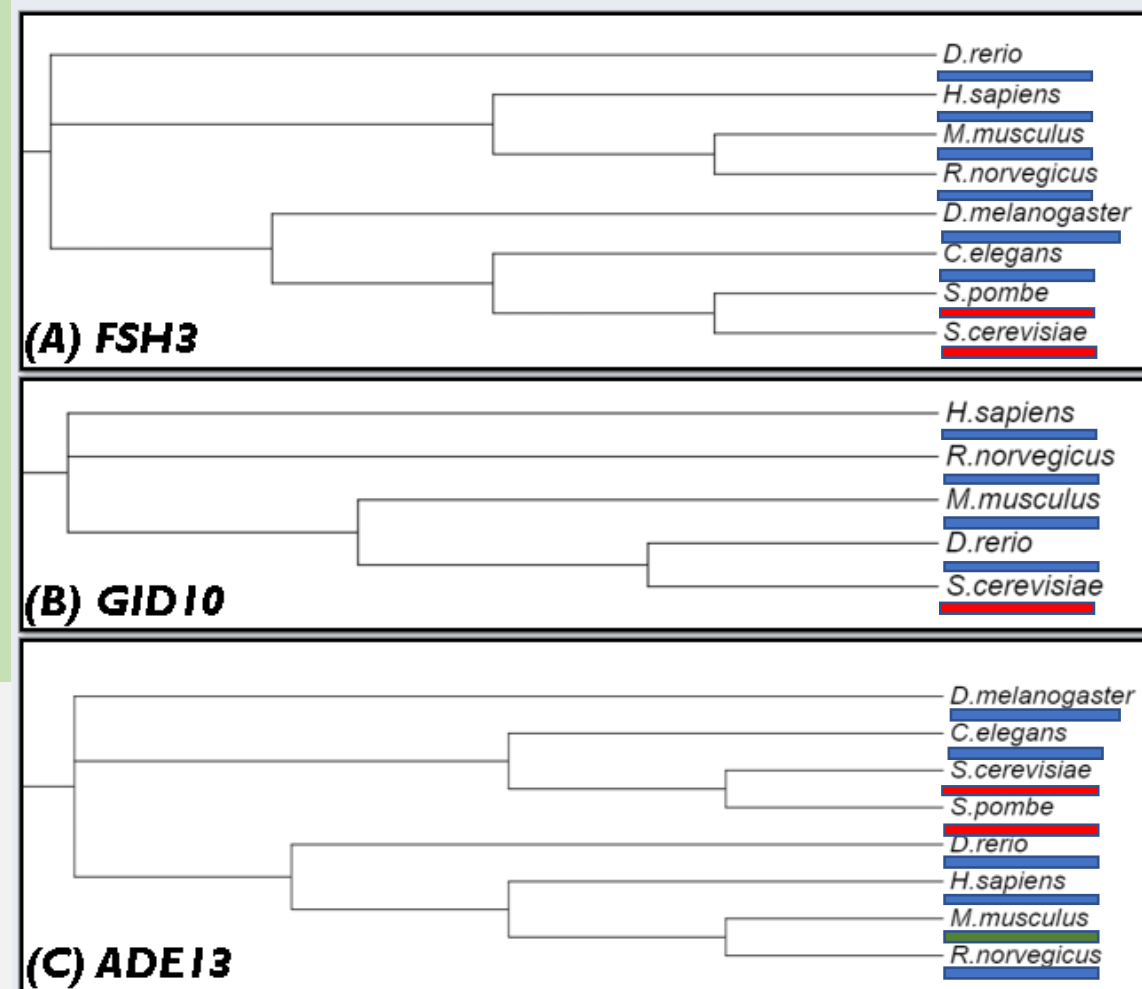
Unknown localization -  
Cytoplasm and nucleus -  
Mitochondria -



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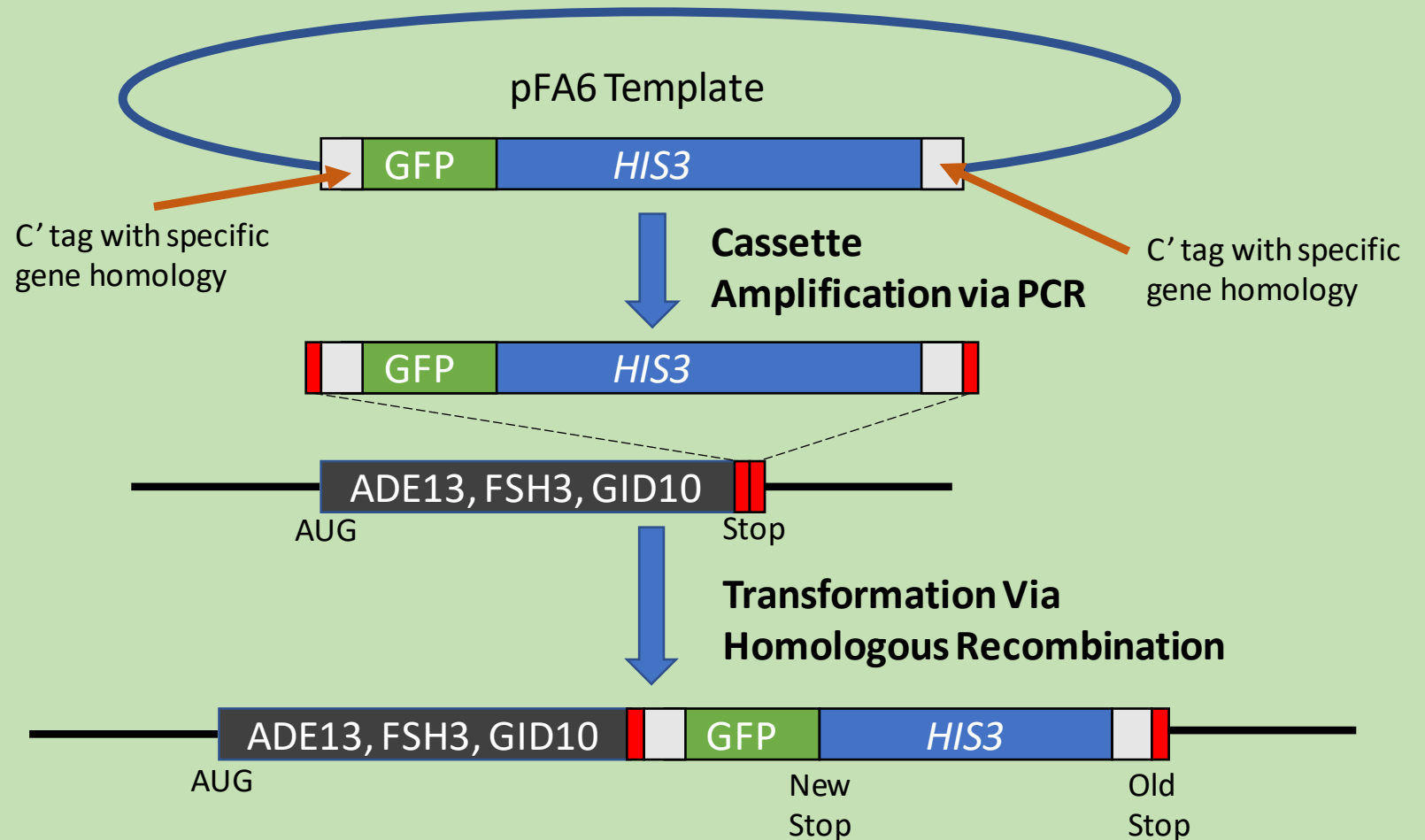


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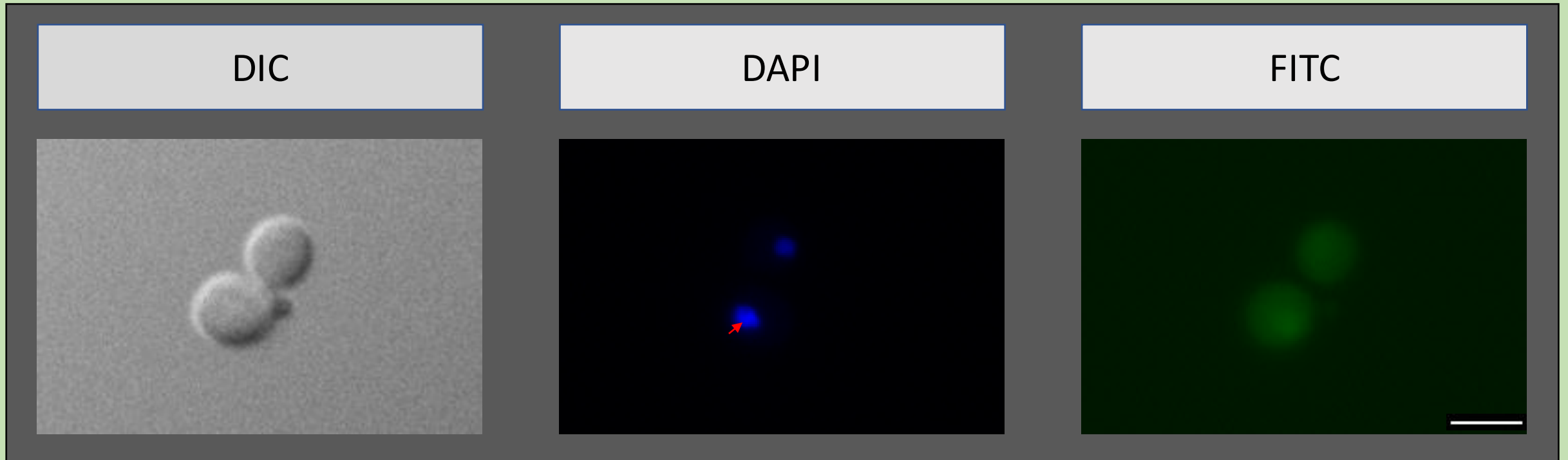


# Methods: C'-GFP tagged strain Construction

- *HIS3* used as selective marker
- Selected by growth on SC-His media
- Integration confirmed via colony PCR



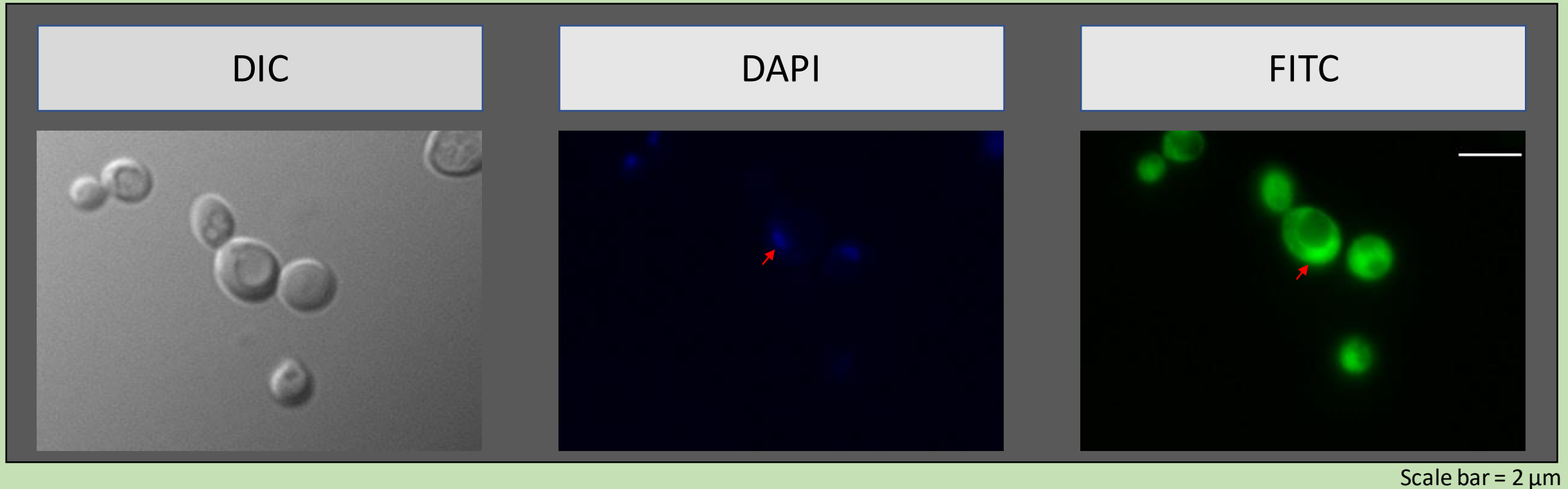
# Fsh3 protein localization



Scale bar = 2  $\mu\text{m}$

GFP fluorescence shows cytoplasmic and unique puncta localization.

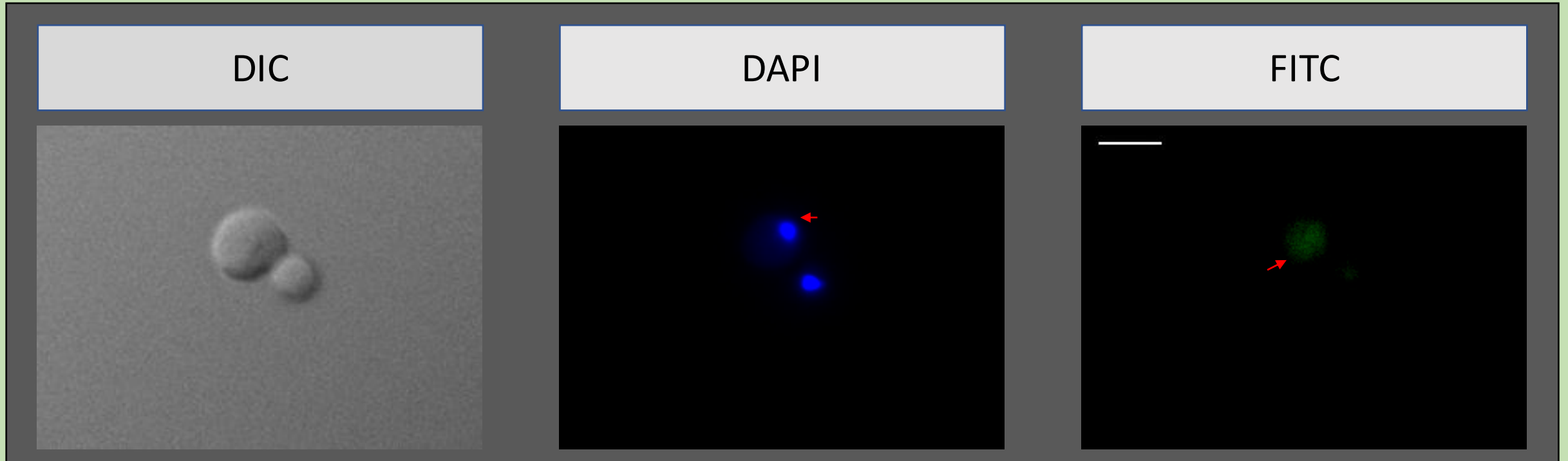
# Ade13 protein localization



GFP fluorescence shows cytoplasmic localization.



# Gid10 protein Localization

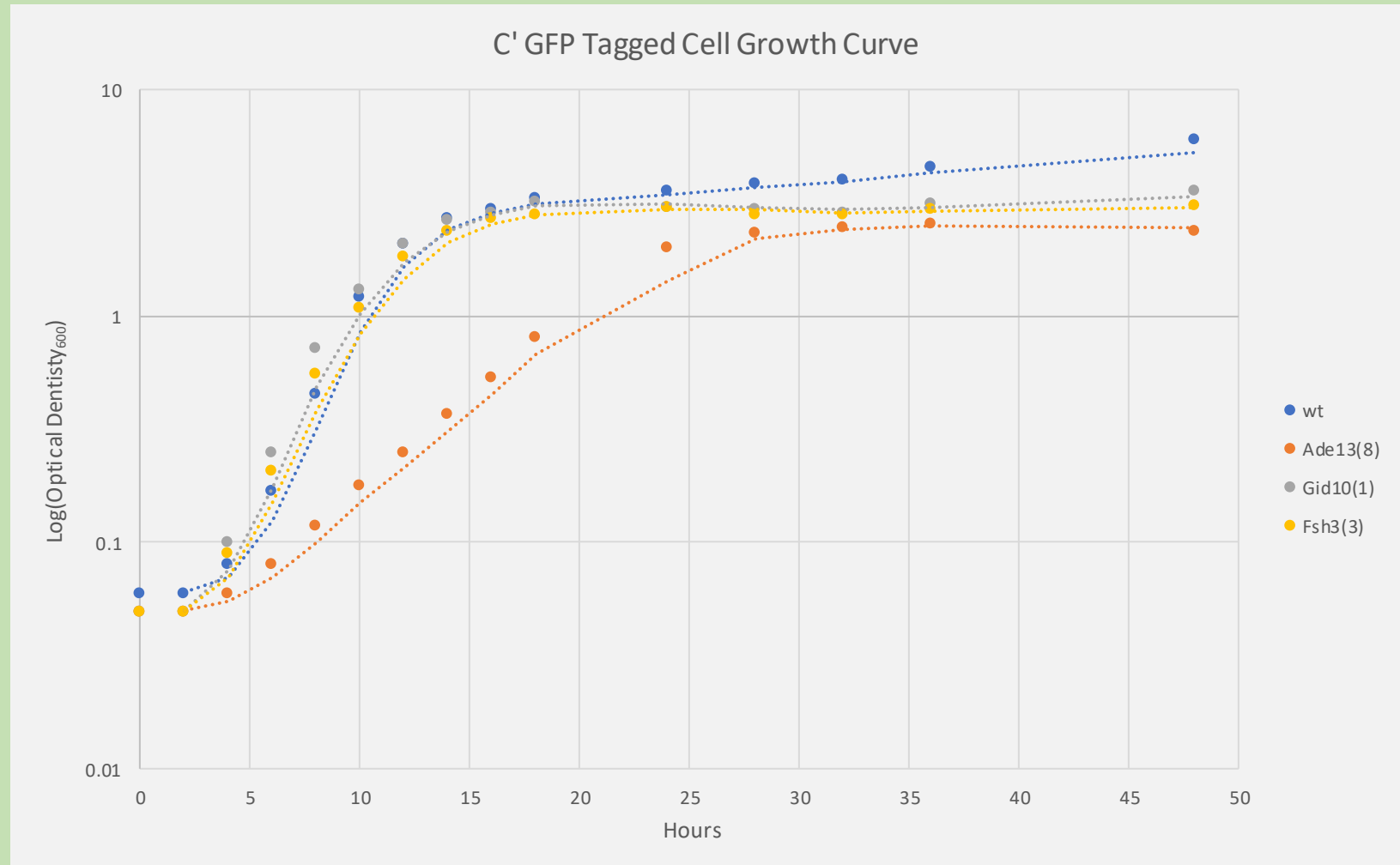


Scale bar = 2  $\mu\text{m}$

GFP fluorescence shows cytoplasmic localization.

# Growth Curve

- Optical Density at 600nm wavelength measured over 48 hours at 30°C
- C' GFP tag affecting essential protein
- Ade13-GFP cells have delayed log phase



# Discussion

## Fluorescent Analysis

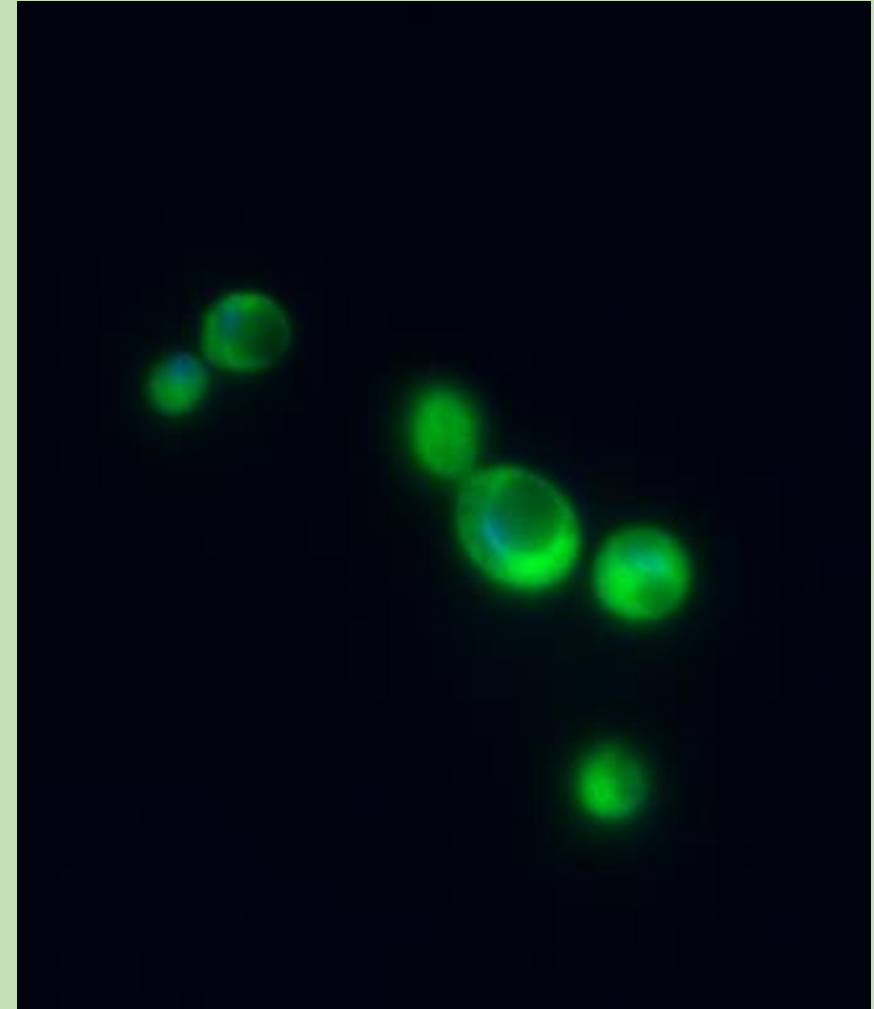
- Fsh3- Cytoplasmic localization and unique puncta
- Ade13- Cytoplasmic localization excluding vacuole
- Gid10- Cytoplasmic localization

## Growth Curve

- Delayed log phase of *ADE13-GFP* cells

## Further Directions

- Compare growth curve of other C' GFP tagged
- Western blotting to confirm proper expression of proteins
- Colocalization studies using other organelle marker proteins (especially the puncta)



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Questions?

# References

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