

# Assessment of Histone acetyltransferase Homologs in *Cryptococcus neoformans*

\*Yuqi Wang,

\*Phoebe Bridy, Felice Kho,

Gary D. Isaacs, Ph.D.

# How our project started - Why Cryptococcus Neoformans?

Infection in lungs and brains



Grows well in cerebral spinal fluid (rich in sugar)

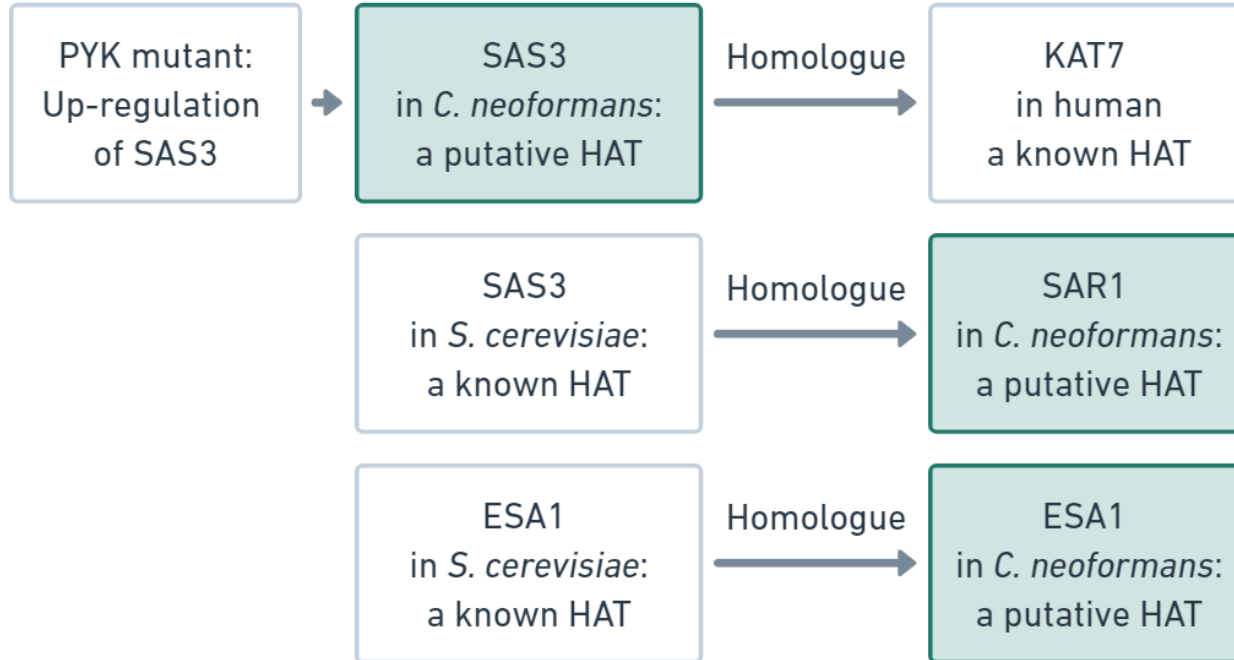


What if it cannot digest sugar?



PYK mutant - Upregulation of SAS3 gene

# Why SAS3? SAR1? ESA1?



# What are Histone Acetyltransferases (HATs)?



Image from <https://www.rcsb.org/3d-view/1FY7/1>

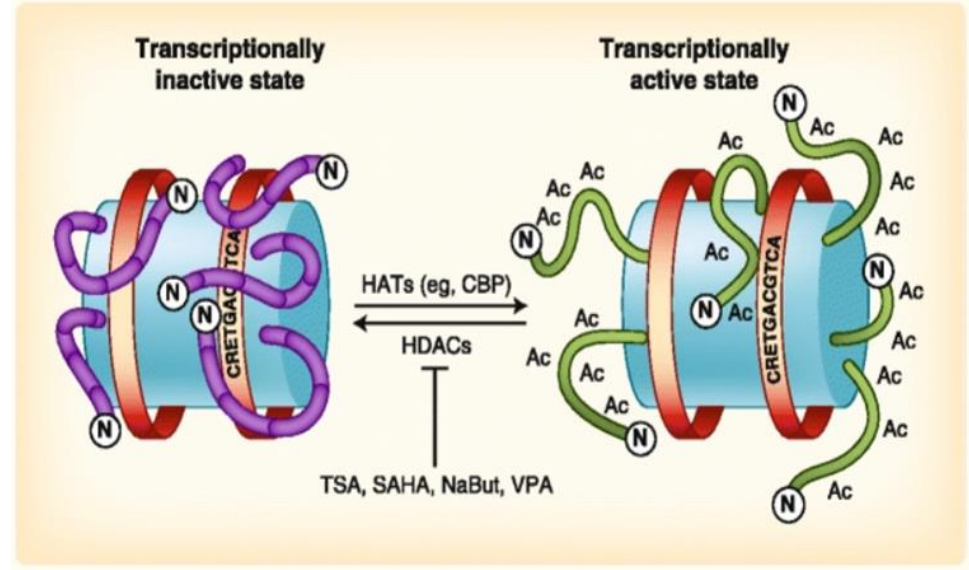
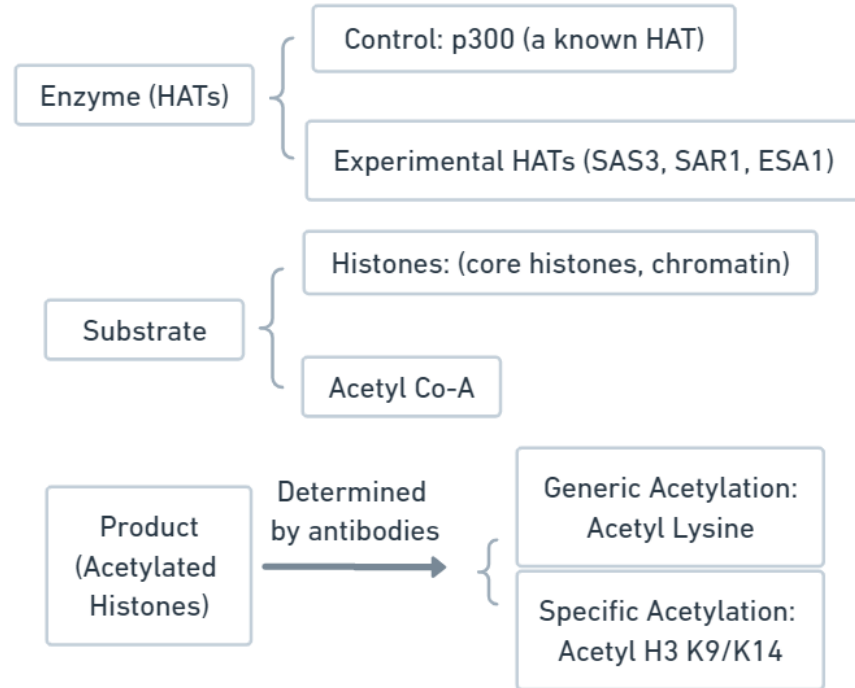


Image from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2847696/>

# Project Overview

## Histone Acetylation Reaction (HAT Assay)

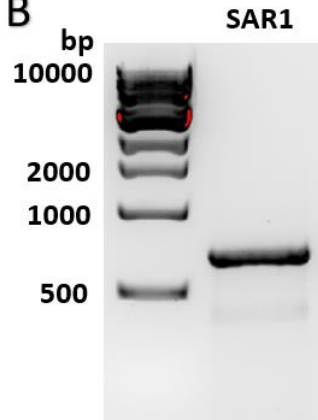


# Cloning (HAT enzymes)

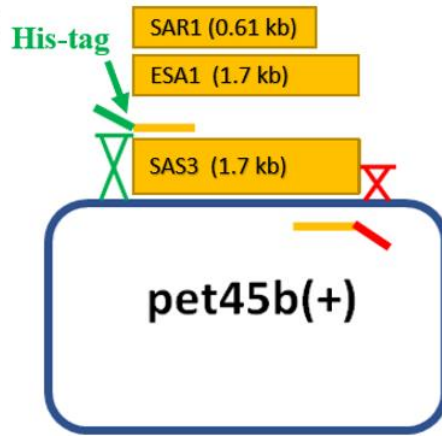
A



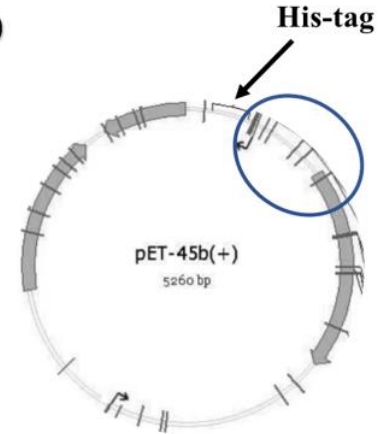
B



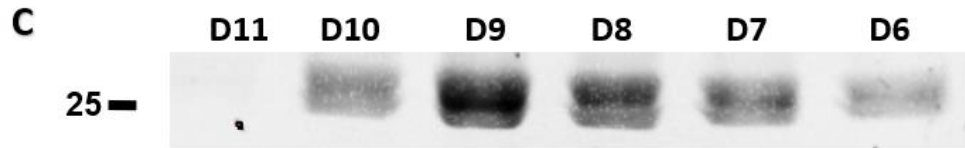
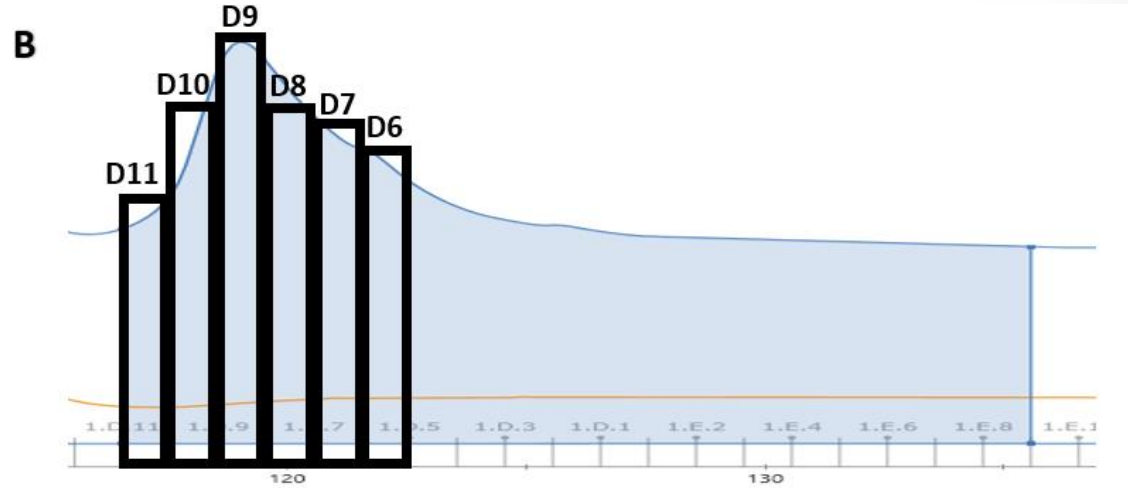
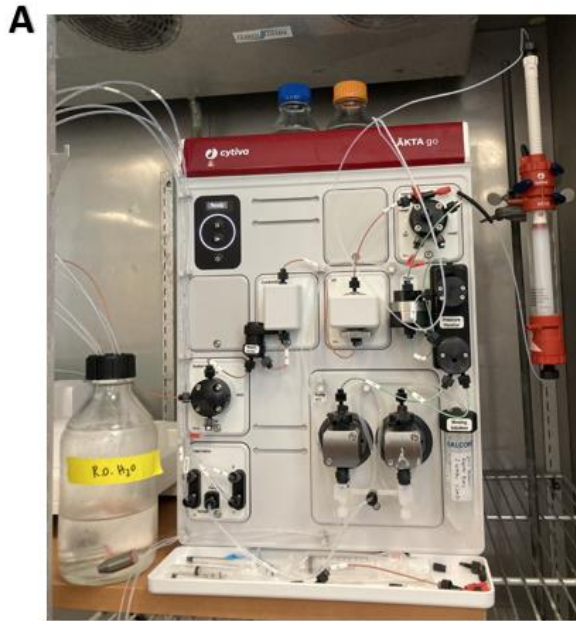
C



D



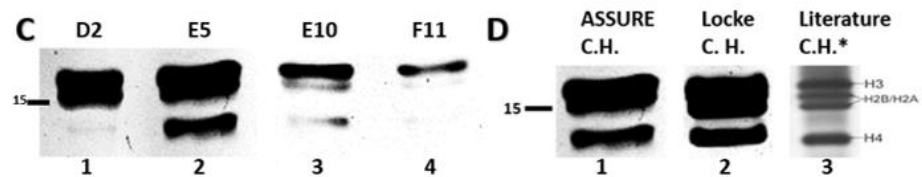
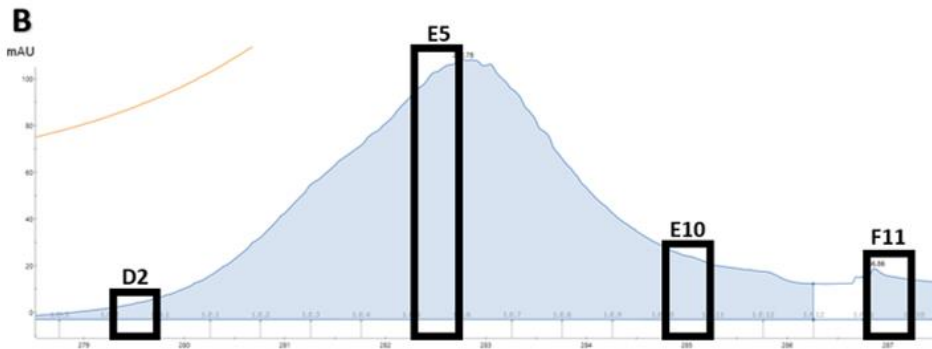
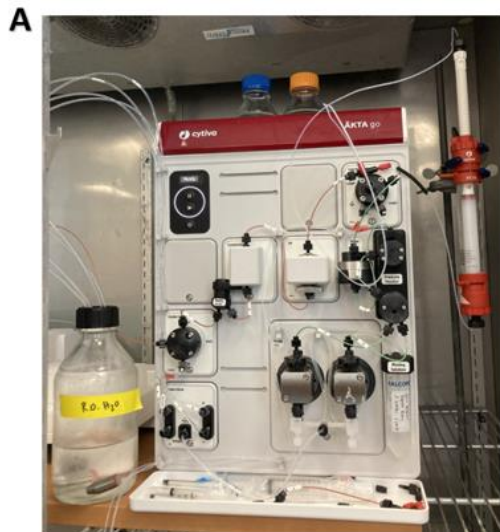
# AKTA-Go FPLC: His-prep of SAR1



# **SAR1 His-prep Round 2**

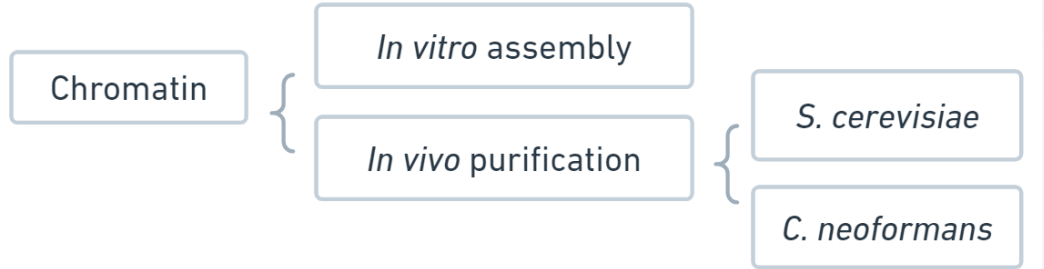


# AKTA-FPLC: Core Histone Extraction (Substrate)



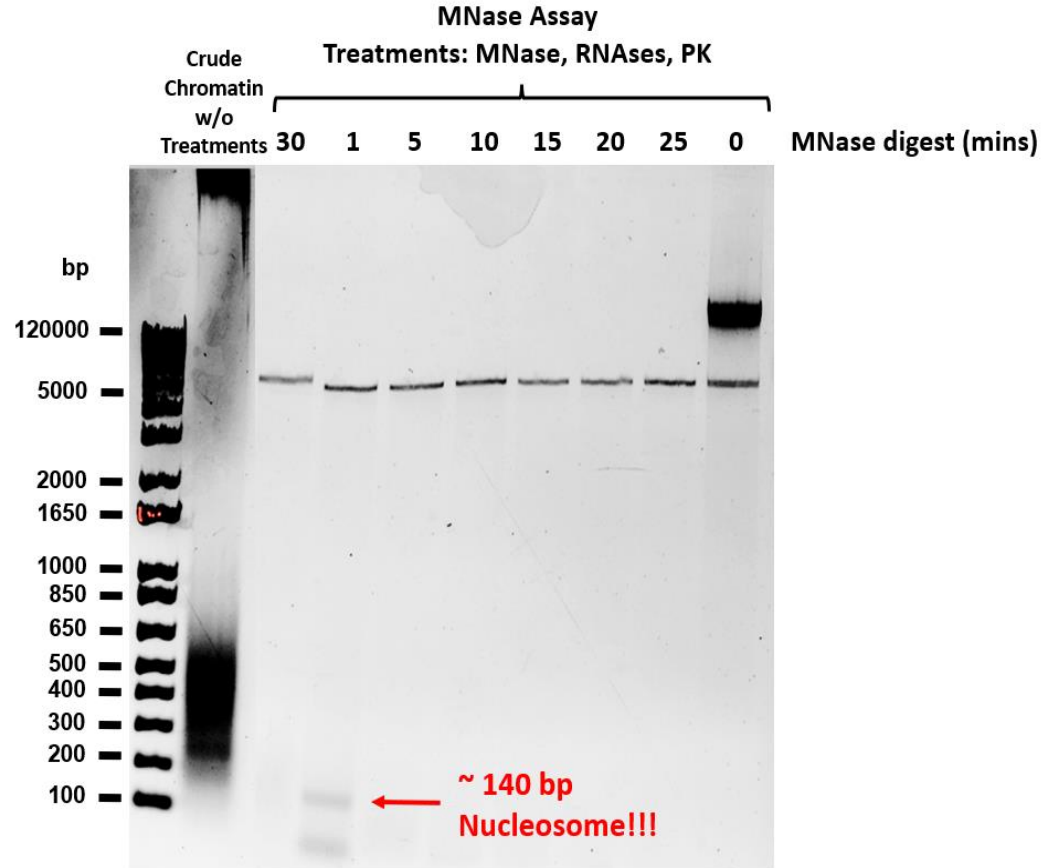
\*Gel image of literature C.H. was adapted from [Doninck et al. \(2009\)](#) [Phylogenomics of Unusual Histone H2A Variants in Bdelloid Rotifers](#).

# Chromatin



- MNase assay:
  - MNase degrades free DNA
  - Nucleosome size of *S. cerevisiae* ~140 bp
- Why chromatin?

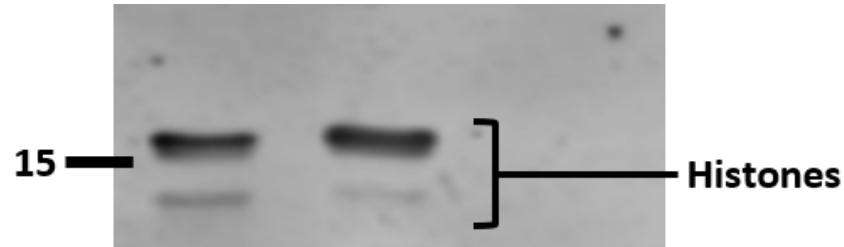
# Chromatin: Yeast Nuclei Extraction



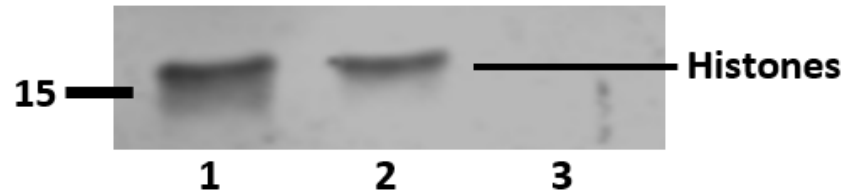
# HAT Assay (Histone Acetylation Reaction)

	p300	Full length	HAT d.	HAT d.
C. Histones		+	+	+
Acetyl-CoA		+	+	-

Antibody  
Acetyl Lysine

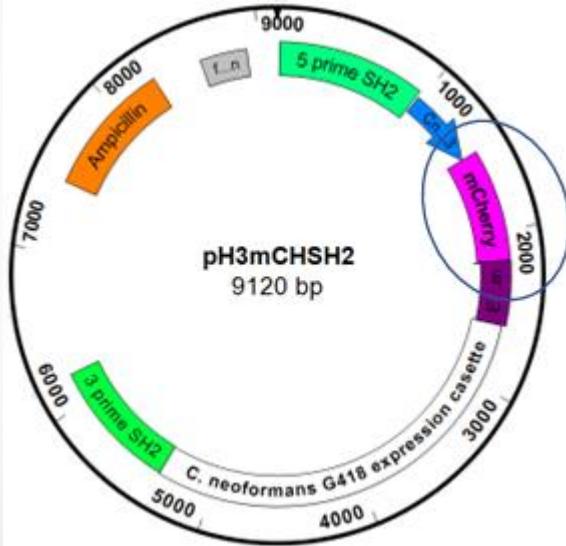


Antibody  
Acetyl H3 K9/K14



# **SAR1 HAT Assay- Western Blot**

# Future directions



- ESA1 and SAS3 clones from *E. coli*
- SAR1 clone from *C. neoformans*
- Chromatin from *C. neoformans*
- *In vitro* chromatin assembly

# Troubleshooting

- Changing PCR conditions
- Varying HAT assay substrates

**Questions?**



**Thank you for listening!**