African Trypanosomes & HDAC3: what & why?

- **Causative agent of Human and Animal African Trypanosomiasis**
  - Fatal diseases if left untreated, very few successful treatment options once in brain
- **Main evasion mechanism:** surface protein variance in BF
  - Variant protein: VSG
  - Invariant protein: procyclin
- **Therapeutic Approach**
  - Manipulating this switch so that we can maintain invariant insect form, giving immune system time to launch antibody response

Chromatin Interacting Proteins!

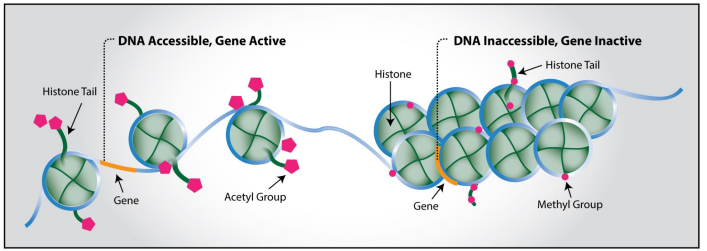


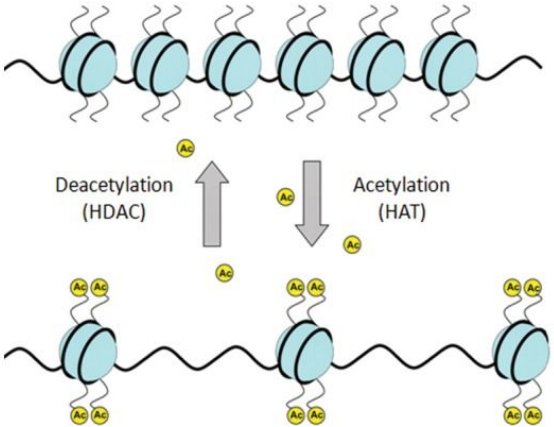
Image Source: EpigenTek

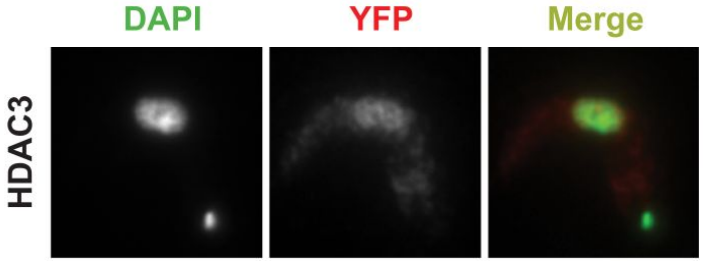
Genetic knock-down of HDAC3 to investigate effect on parasite differentiation!

HDAC3 (histone deacetylase 3)

Acetylation: decreases ability of histones to bind to DNA

HDACs: enzymes that remove acetyl group from lysine residues



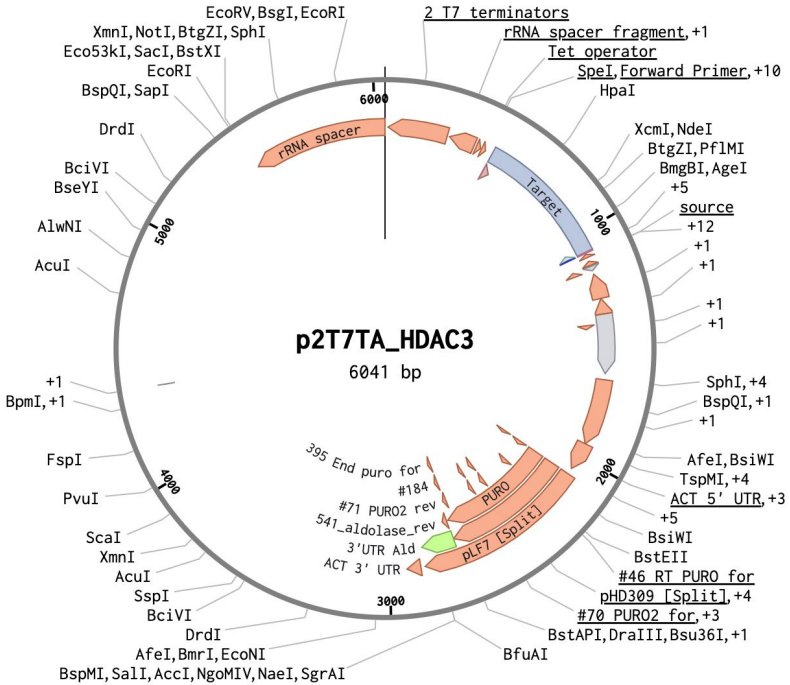


Staneva et al (2021):

- Predominantly nuclear
- HDAC3 enriched at known RNAII TSRs
- Strong association with TSR-enriched histone variants H2A.Z and H2B.V

How will knocking down HDAC3 affect the transition of bloodstream parasites to their invariant fly form?

Primer Design for RNAi



Vector: p2T7^{TABlue} Puro

Tet-On System: inducible by Dox (doxycycline)

- T7p promoters, Tet operator

Selective markers:

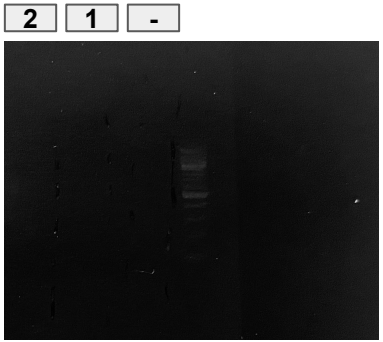
- Lactamase (pAmpR)
- Puro (rRNA)

Forward primer:
GCGA (clamp) + ACTAGT (SpeI) + GGTACC (KpnI) + TGGTACGGTGCCGAATGAAA

Reverse primer:
CGCT (r-clamp) + AAGCTT (HindIII) + CTTGCATGGAGCCAAGGAGA

Bacterial Transformation

PCR Amplification



Restriction Digest

- Plasmid: SpeI, HindIII, NdeI
- PCR: no NdeI
- Ligation of inserts to create expression vector

Bacterial Transformation

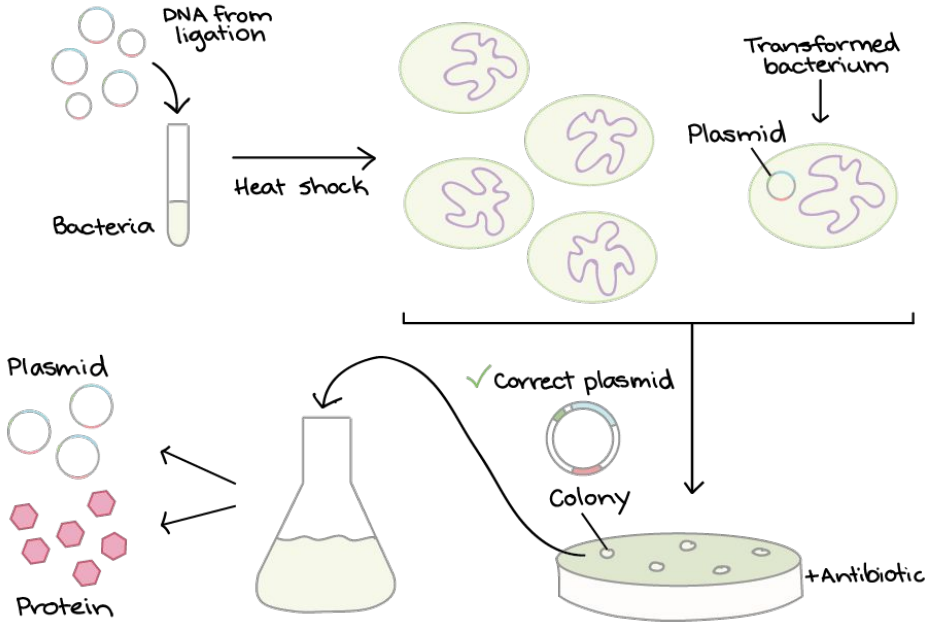


Image Source: KhanAcademy

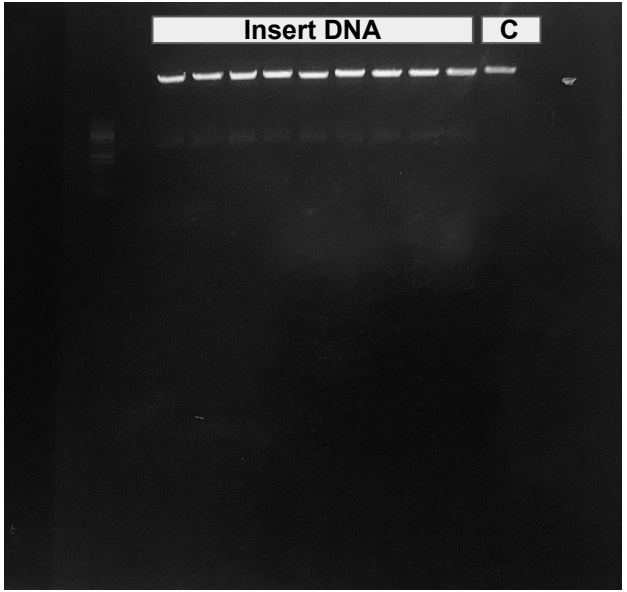
Miniprep: Plasmid Extraction

Resuspend pellet → **Alkaline Lysis** → **Neutralize** → **Wash/Elute**

<u>Spectrophotometry</u>
Culture #2: concentration = 506.151 ng/μL
Culture #7: concentration = 560.688 ng/μL

<u>Screening by PCR for presence of insert</u>
Restriction digest using KpnI and HindIII:
Control: original vector ($p2T7^{TABlue}$ Puro)

- Two bands for each reaction!
- Predicted length: ~600 bp
- Every culture is positive



Midiprep & Differentiation

50 mls of bacterial culture

Midiprep: using ZymoPURE kit

Scoring: 3 colonies chosen

- 2 grown in 1:100 & 1 grown in 1:10

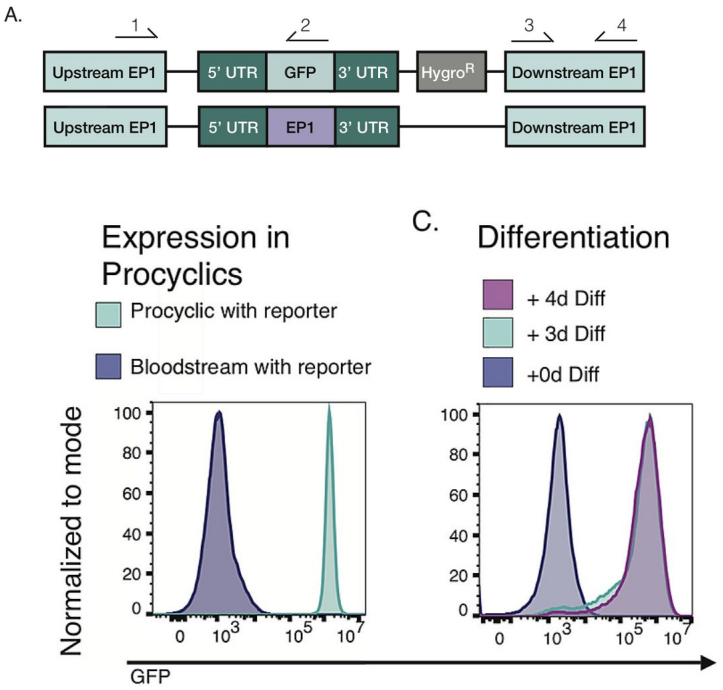
Vary levels of HDAC3: DOX

	+DOX	-DOX
Blood stream		
Insect		

Induce differentiation:

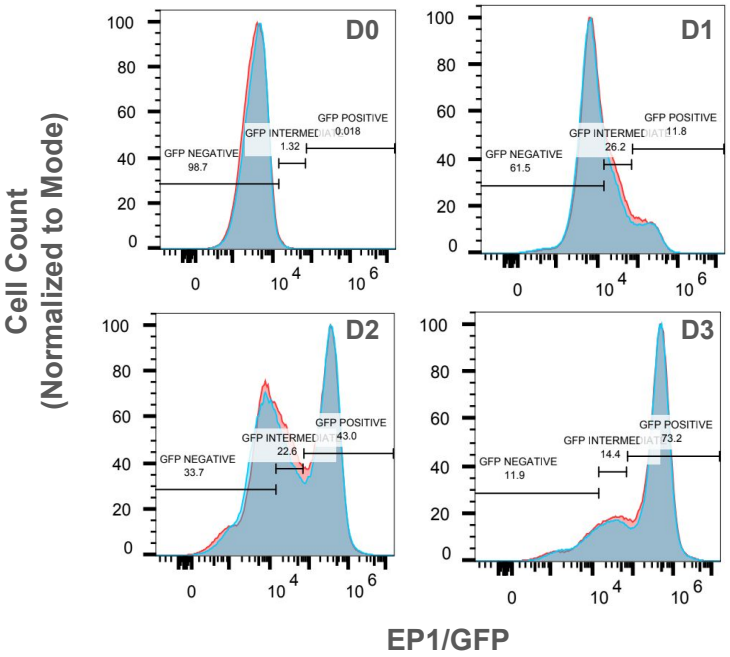
- temperature drop
- pH: cis-aconitate
- Media: DTM vs. HMI9

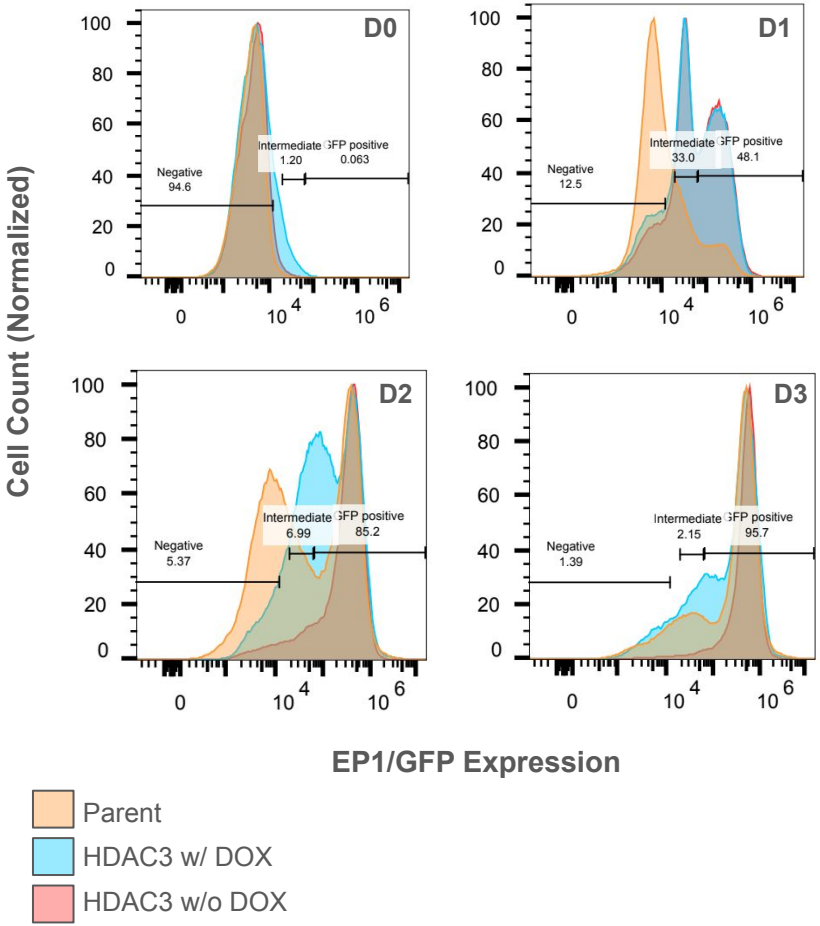
EP1/GFP Reporter System



Controls

Parent (+DOX vs. -DOX)





What do we expect?

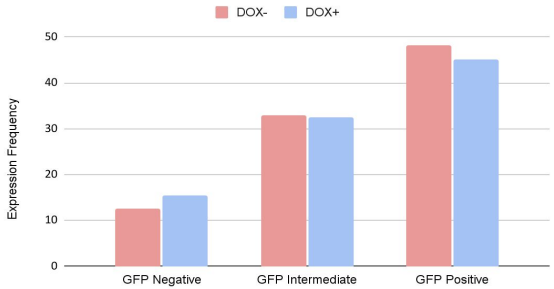
+DOX = Knockdown of HDAC3

HDAC3: decreases binding capability

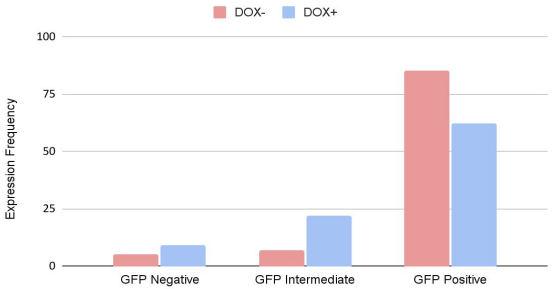
What do we see?

Results

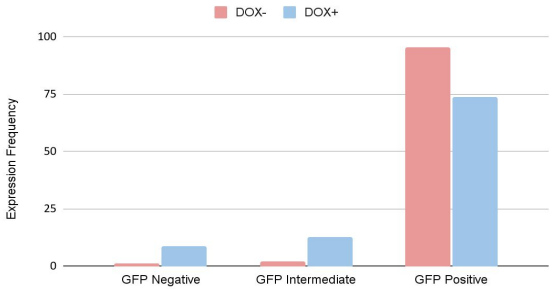
Day 1: EP1/GFP Expression in Parasites with HDAC3 Clone 2



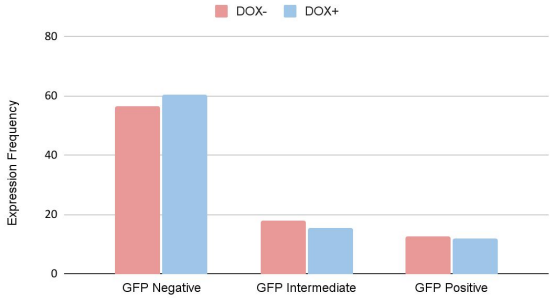
Day 2: EP1/GFP Expression in Parasites with HDAC3 Clone 2



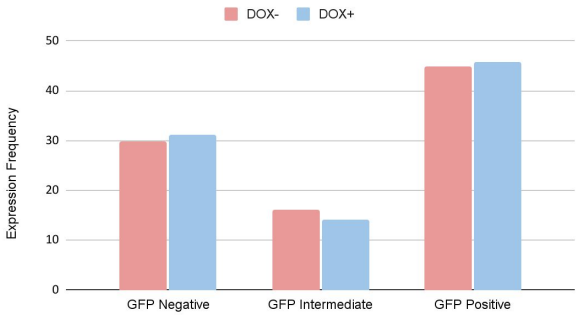
Day 3: EP1/GFP Expression in Parasites with HDAC3 Clone 2



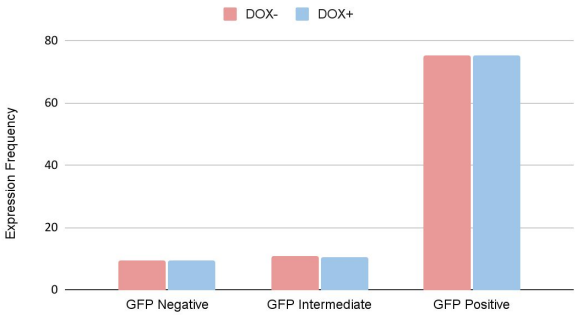
Day 1: EP1/GFP Expression in Parasites with No HDAC3



Day 2: EP1/GFP Expression in Parasites with No HDAC3



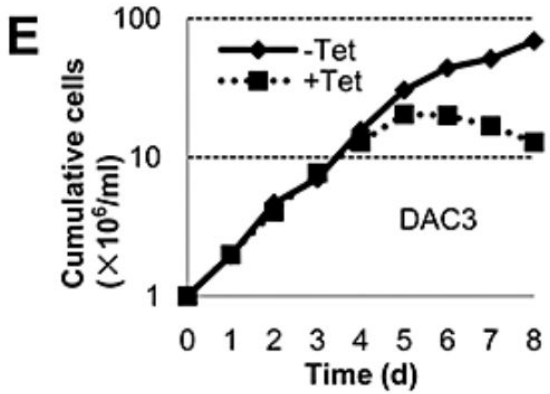
Day 3: EP1/GFP Expression in Parasites with No HDAC3



Interpretations

Wang et al. (2010):

- HDAC3: required for VSG expression site silencing
  - Coordinated switching of VSG coat (Faria et al. 2022)



- Cells w/ DOX (without HDAC3) cannot survive in insect form
- **HDAC3 is essential for the growth of insect form T. brucei**

Need for Replication

Human Errors: Accidentally pipetted parasites into tube that contained nucleofector solution during tryps purification

- No effect on transformation theoretically

Future Steps

- HDAC3 as a therapeutic target
  - Increasing HDAC3?
    - Kovářová et al. 2022
  - HAT + HDAC3 System
  - Association with BDF2

References

Faria, J., Briggs, E. M., Black, J. A., & McCulloch, R. (2022). Emergence and adaptation of the cellular machinery directing antigenic variation in the African trypanosome. *Current opinion in microbiology*, *70*, 102209.

Grozinger, C. M., & Schreiber, S. L. (2002). Deacetylase enzymes: biological functions and the use of small-molecule inhibitors. *Chemistry & biology*, *9*(1), 3-16.

Kovářová, J., Novotná, M., Faria, J., Rico, E., Wallace, C., Zoltner, M., ... & Horn, D. (2022). CRISPR/Cas9-based precision tagging of essential genes in bloodstream form African trypanosomes. *Molecular and Biochemical Parasitology*, *249*, 111476.

Schulz, D., Mugnier, M. R., Paulsen, E. M., Kim, H. S., Chung, C. W. W., Tough, D. F., ... & Debler, E. W. (2015). Bromodomain proteins contribute to maintenance of bloodstream form stage identity in the African trypanosome. *PLoS Biology*, *13*(12), e1002316.

Staneva, D. P., Carloni, R., Auchynnikava, T., Tong, P., Rappsilber, J., Jeyaprakash, A. A., ... & Allshire, R. C. (2021). A systematic analysis of Trypanosoma brucei chromatin factors identifies novel protein interaction networks associated with sites of transcription initiation and termination. *Genome Research*, *31*(11), 2138-2154.

Walsh, M. E., Naudzius, E. M., Diaz, S. J., Wismar, T. W., Martchenko Shilman, M., & Schulz, D. (2020). Identification of clinically approved small molecules that inhibit growth and affect transcript levels of developmentally regulated genes in the African trypanosome. *PLoS Neglected Tropical Diseases*, *14*(3), e0007790.

Wang, Q. P., Kawahara, T., & Horn, D. (2010). Histone deacetylases play distinct roles in telomeric VSG expression site silencing in African trypanosomes. *Molecular microbiology*, *77*(5), 1237-1245.

Zuma, A. A., & Souza, W. D. (2018). Histone deacetylases as targets for antitrypanosomal drugs. *Future Science OA*, *4*(8), FSO325.