- Announcements
- Pre-lab Lecture
  - ❖ Intro to Module 2 and SAGA
  - ❖ Gene Modification Choices
  - ❖ Primer Design Overview
  - ❖ Today in Lab

Announcements, old HW

- BE Seminar tomorrow by Prof. Darrell Irvine
  - Topic: immune bioengineering
  - My old lab!

32-141 @ 4pm

- Introducing… Brian, TA for Module 2

- Assignment for Friday is long, but also integrated with today's work
- Module 2 pre-lab lectures will be closely adapted from N. Kuldell

Module 2: Protein Engineering

- Central question: is "tagging" a protein functionally neutral
- Proteins of interest: SAGA-related (part of, or regulated by)
- Why yeast? model system
  - eukaryotic
  - easy to work with
  - well-organized nomenclature

* SAGA is well-conserved *

ADA3 = gene
Ada3(p) = protein
ada3 = mutation (recessive)

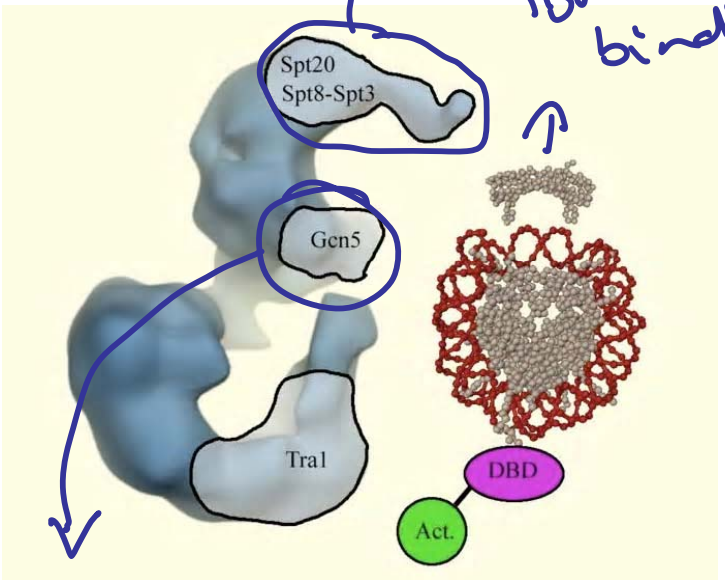
Quick introduction to SAGA

19 subunits in SAGA

Subunit	size,chromosome,null p-type
Ada subunits	
Ada1 (aka HFI1, SUP110, SRM12, GAN1)	1.467 kb=489 aa, Chr. XVI, viable
Ada2 (aka SWI8)	1.305 kb=434aa, Chr. IV, viable
Ada3 (aka NGG1, SWI7)	2.109 kb=702aa, Chr. IV, viable
Gcn5 (aka ADA4, SWI9)	1.32 kb=439aa, Chr. VII, viable
Ada5 (aka SPT20)	1.815 kb=604aa, Chr. XV, viable
Spt subunits	
Spt3	1.014 kb=337aa, Chr. IV, viable
Spt7 (aka GIT2)	3.999 kb=1332aa, Chr. II, viable
Spt8	1.809 kb=602aa, Chr. XII, viable
Spt20 (aka Ada5)	1.815 kb=604aa, Chr. XV, viable
TAF subunits	
TAF5 (aka TAF90)	2.397 kb=798aa, Chr. II, inviable
TAF6 (aka TAF60)	1.551 kb=516aa, Chr. VII, inviable
TAF9 (aka TAF17)	0.474 kb=157aa, Chr. XIII, inviable
TAF10 (aka TAF23, TAF25)	0.621 kb=206aa, Chr. IV, inviable
TAF12 (aka TAF61, TAF68)	1.620 kb=539aa, Chr. IV, inviable

interacts with TBP

TBP = TATA-binding protein



enzymatic domain (HAT)
interact w/ nucleosome

size ~ 19 nm
(working ~ 1 nm)

You might choose a SAGA subunit…

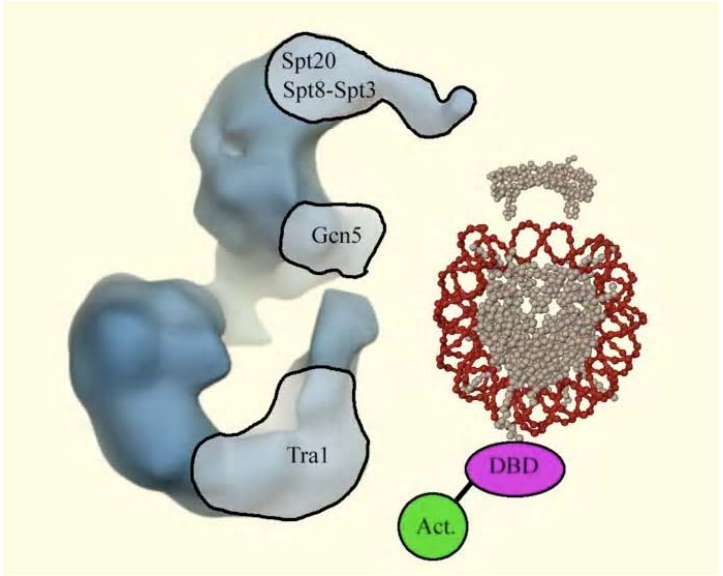
Why might a particular subunit deletion render the yeast inviable?

- necessary for SAGA stability
- has another (non-SAGA) essential function

Tra subunit	
Tra1	11.235 kb=3744aa, Chr. VIII, inviable
Other subunits	
Sgf73	1.974 kb=657aa, Chr. VII , viable
Sgf29	0.779 kb=259aa, Chr. III, viable
Sgf11	0.3 kb=99aa, Chr.XVI, viable
Ubp8	1.416 kb=471aa, Chr. XIII, viable
Sus1	gene with intron, Chr. II, viable

unknown function

5 choices



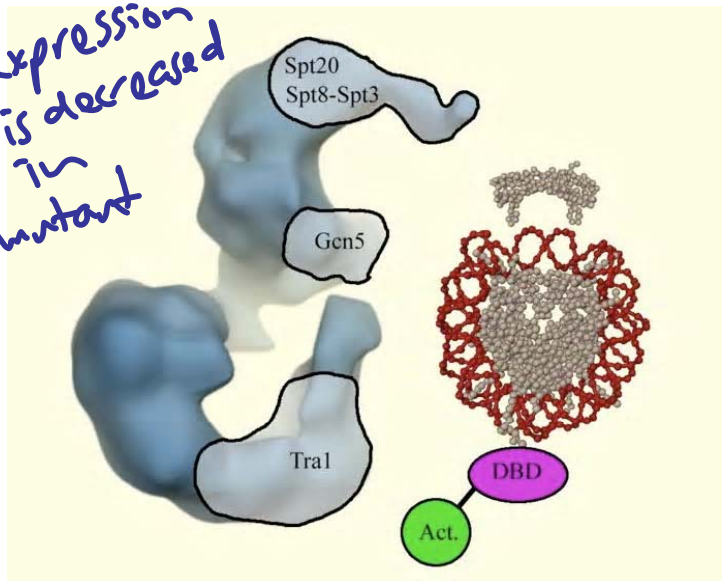
Alternatively, a SAGA-regulated gene….

Unknown	ORF	SGF73 green signal	sgf73 red signal	log2 (green/red)
1	YHR033W	38938 and 69586	285 and 570	7.1 and 6.9
2	YOR302W	3374 and 6054	49 and 167	6.1 and 5.2
3	YJR097W	524 and 1052	13 and 28	5.3 and 5.2
4	YBL028C	1146 and 2706	32 and 323	5.2 and 3.1
5	YDR034W-B	17290	447	5.3
6	YGR067C	12025	320	5.2
7	YKL037W	8340	282	4.9
8	YER067W	6296 and 12450	82556 and 81036	-3.7 and -2.7

WT

deletion

expression is decreased in mutant



- How were these ORFs discovered?
  - deletion of (non-essential) SAGA subunit SGF73
- You can become the world expert on these uncharacterized open reading frames!

*SGD

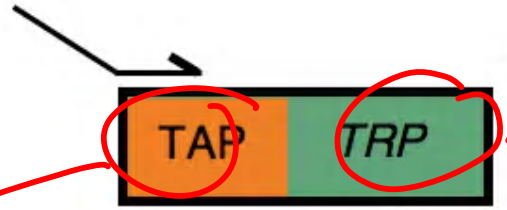
The MOST IMPORTANT thing to know about your work for this module…

Things may go wrong.

→ You may have to switch to a different gene.

(2-3 piloted)

Multi-component tag insertion



Tandem 3- 2-step

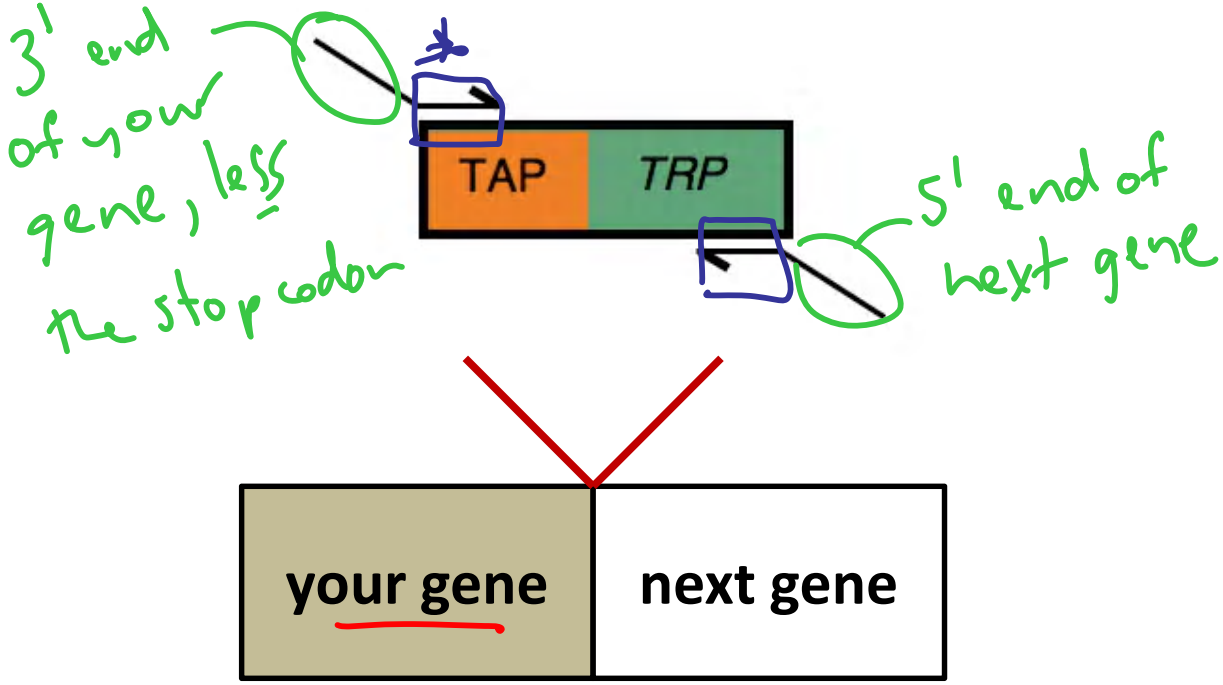
Affinity Purification

Select a protein (from a mixture)

gene needed for tryptophan biosynthesis

TRP rescues yeast with a trp phenotype

Designing your primers today



* landing sequence = universal, 20 bp each

Today in Lab

- Choose a gene, design primers, and set up PCR
- The module 2 assessment is a research article, so…
- Start learning about your chosen gene, and the SAGA complex in general, in some depth
  - Begin writing your draft introduction, **due Friday**
  - Start a wiki page to keep track of your references
  - Read the abstract of at least 5 relevant papers
- Try to imagine what total success as well as total failure could look like (and know that we have alternatives at the ready in the instances that experiments are pesky)