- Announcements
- Lab Quiz
- Pre-lab Lecture
  - Yeast Strain Overview
  - Growth Curves
  - Yeast Transformation
  - Today in Lab

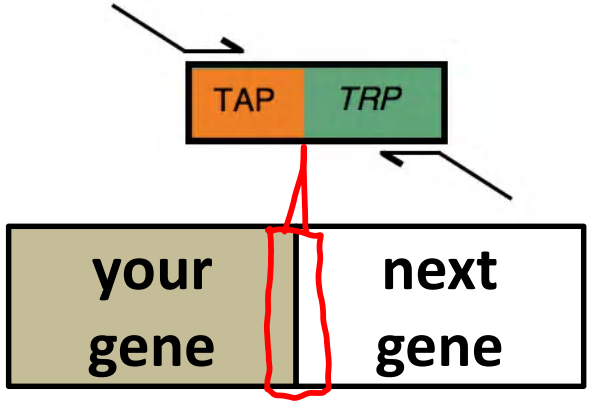
Announcements

- Prof. Bevin's office hours
  - Room 16-743, Monday 4:30-6 pm

- Module 1 lab report revision due ***Wednesday***
  - Great care was taken to comment on your work – embrace the opportunity to improve your writing!
  - Up to 1.5 letter grade improvement

Module 2: where are we/going?

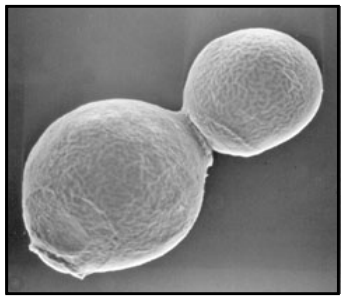
- Last time:



PCR product

- Today: transform yeast with linear PCR product
  ↓ rely on HR to incorporate
- Next time: evaluate which colonies have tag

A little more about your yeast



»NY411 → lab shorthand

genotype

MAT(A) his4-917d, lys2-173R2, leu2d1, ura3-52, trp1d63

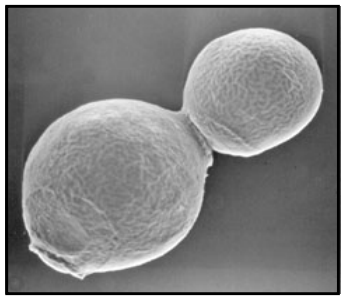
two mating types

haploid < A / α

diploid — A/α

- why use haploid?
  - all protein is tagged
  - diploid useful if lethal

A little more about your yeast



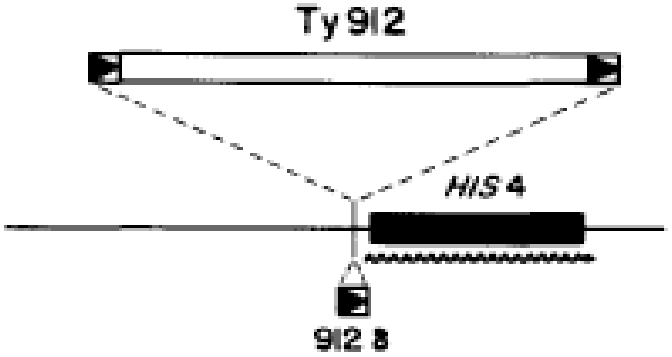
»NY411

defects in biosynthesis

δ

MAT(A) his4-917d, lys2-173R2, leu2d1, ura3-52, trp1d63

not delete

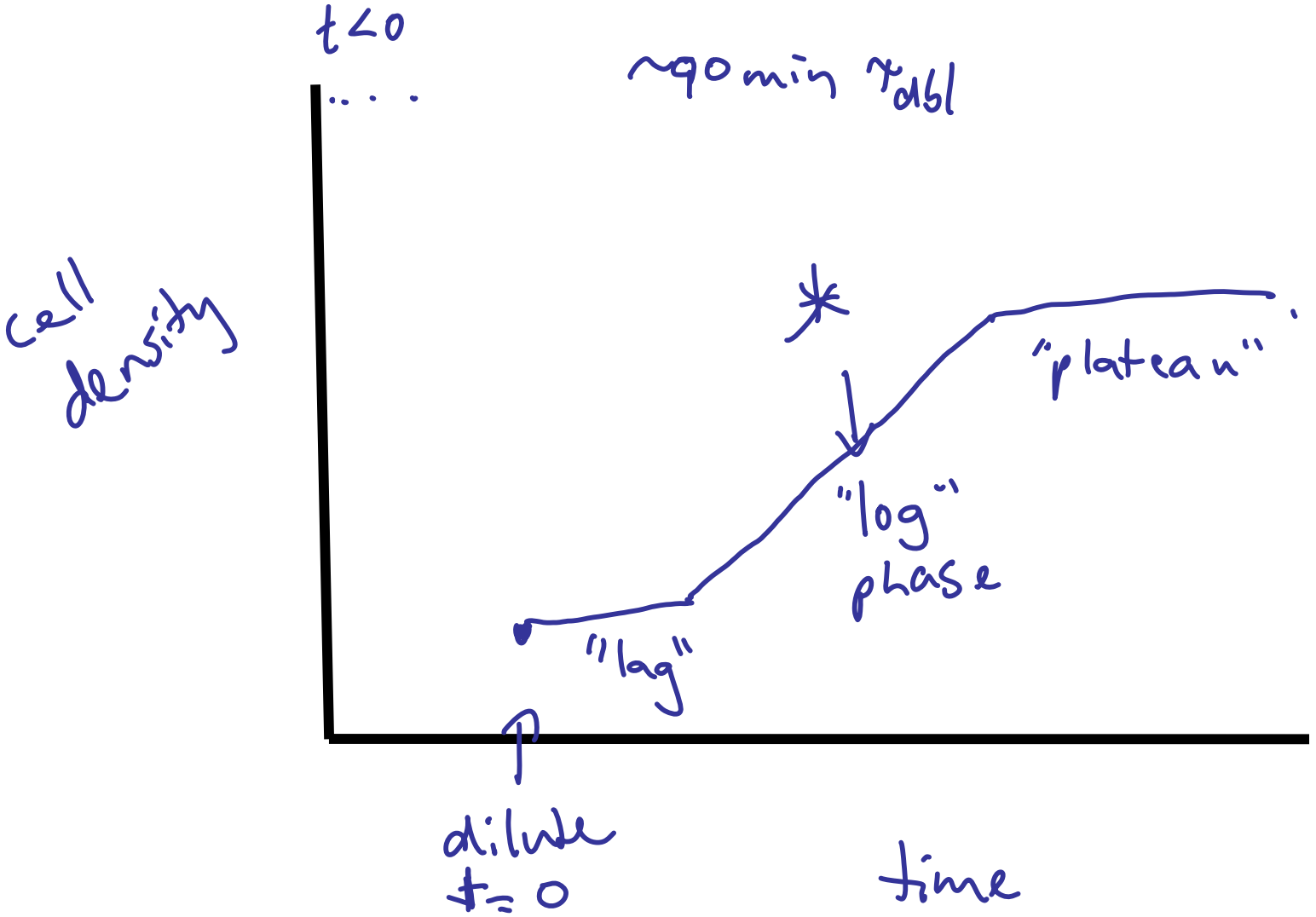


Ty -element

present, can't grow w/out His

* Sometimes SAGA mutations * suppress this

Yeast Growth Cycle



Yeast Transformation

- You will make your own competent cells today!
  - Competent yeast are more stable than competent bacteria

- Wash the cells with Wash Sol'n (= water?)
- Resuspend the cells in Competent Sol'n (= LiAc?)
- Mix with your DNA samples

  No DNA, pRS414, concentrated/cleaned PCR
- Resuspend in Transformation Sol'n (= PEG?)
- 1 hr, 30°
- Plate on SC-trp

Transformation Controls + Outcomes

Sample	Expectation (# colonies)	Role
no DNA	no colonies	(-) control for contamination
pRS414 (TRP)	lots of colonies	(+) control for transformation → efficiency
PCR product linear	some	experiment

What if… selecting for TRP transformants

Today in Lab

- Clean PCR product, transform yeast
  - Be careful with alcohol burners
- During incubation
  - Read sample module 1 assignments (at teaching bench)
  - Work on for next time assignment
    - Materials and methods
    - Read article to be discussed next time
    - Module 1 lab report revision
    - (Start thinking about presenting in Mod2 vs. Mod3…)

- Enjoy the long weekend – you've worked so hard and learned so much already!