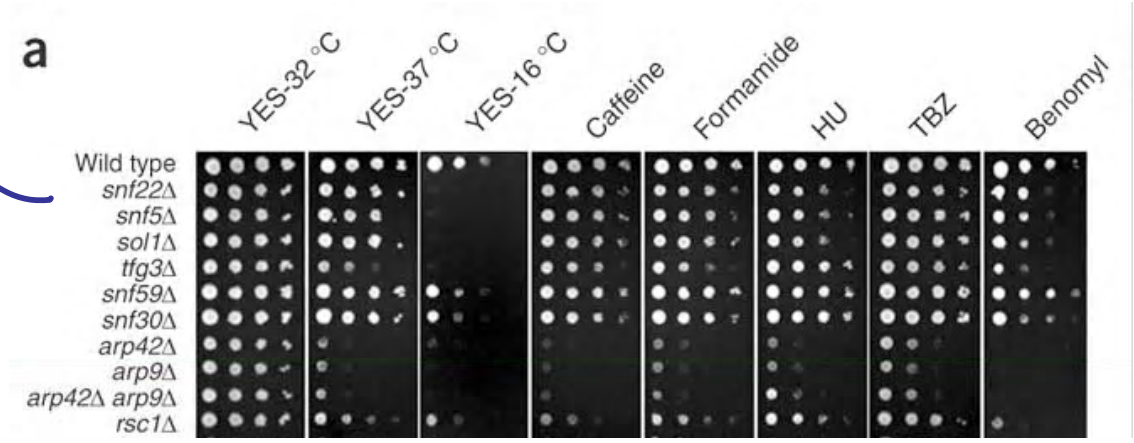
- Announcements, Old HW
- Lab Quiz
- Pre-lab Lecture
  - Microarray workflow
  - Intro to uArray analysis
  - Today in Lab, FNT

Old HW

- Figure comments from Natalie

"at-a-glance" genotype labeling

First sentence tells how you got the data



(**a**) Growth phenotypes of the viable SWI/SNF and RSC mutants. Wild-type (FWP52), *snf22*Δ (FWP229), *snf5*Δ (FWP231), *sol1*Δ (FWP245), *tfg3*Δ (FWP246), *snf59*Δ (FWP247), *snf30*Δ (FWP248), *arp42*Δ (FWP239), *arp9*Δ (FWP240), *arp42*Δ *arp9*Δ (FWP249), *rsc1*Δ (FWP234) and *rsc4*Δ (FWP242) strains were grown in liquid YES medium to stationary phase, subjected to ten-fold serial dilutions and spotted onto solid YES medium containing caffeine, formamide, hydroxyurea (HU), thiabendazole (TBZ) or benomyl as indicated (see Methods for concentrations). The leftmost spot for each came from a culture at 1×10^8 cells ml^{-1}, and approximately 3 μl were spotted. (**b**) Cell elongation, chromosomal

- Additional comment from me
  - In the final report, don't contradict yourself! If growth phenotype suggests TAP-TRP incorporation, and Western doesn't, find an explanation consistent with both results.

Announcements

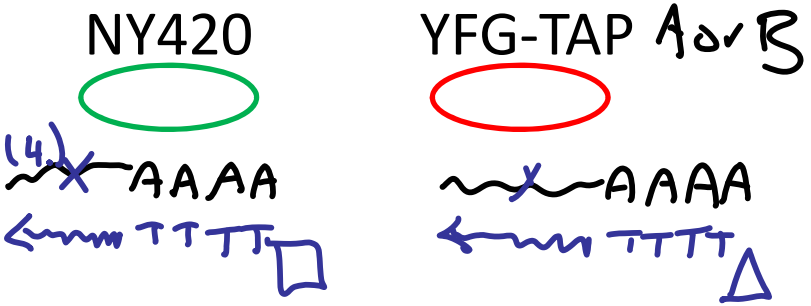
- BE seminar this week is Prof. Han, u/nfluidics
- Office hours
  - Natalie (in lab) Sunday 3-5 pm, Monday 2-4 pm
  - Agi (in 16-336) Tue 4-6 pm, other by appt.

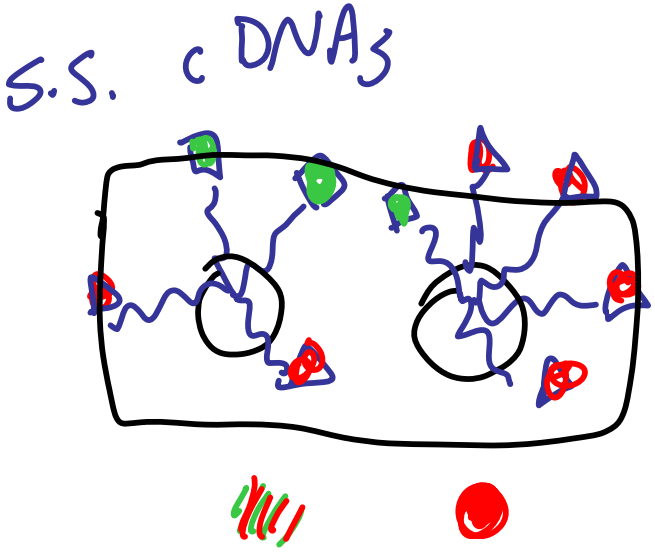
- Brief discussion of mid-term feedback

Microarray Workflow

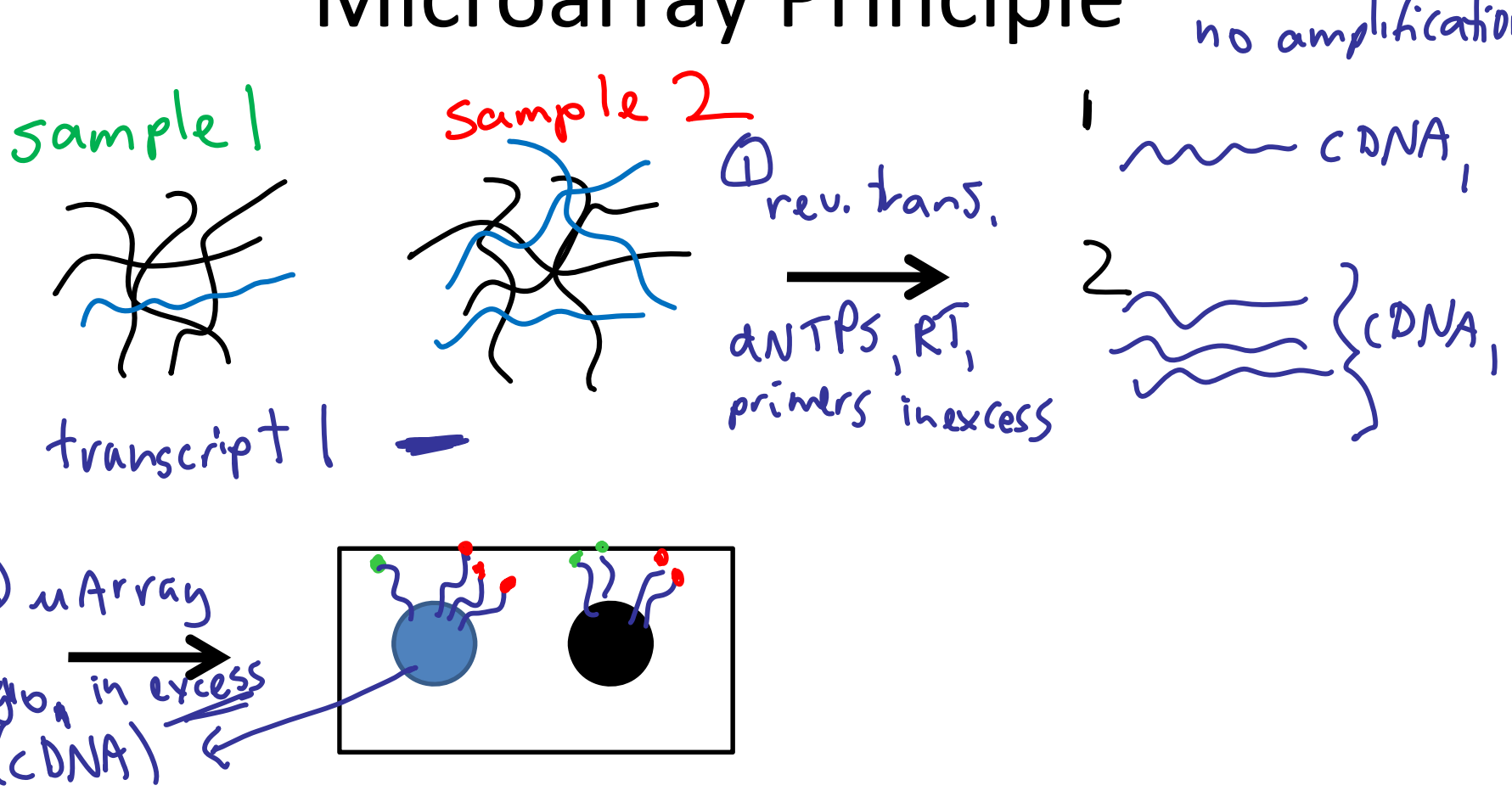
1. Isolate RNA from yeast
2. Anneal primers to RNA (w/capture sequences)
   80°C, 10'
3. Extension step w/RTase
   42°C, 1.5 hrs
4. Destroy RNA → NaOH
5. Hybridize cDNAs to array
6. Wash, add fluorophores

7 Wash, scan





Microarray Principle



Intensity of fluorescence at a particular spot is proportional to the original amount of the relevant mRNA in that sample. Next time you'll analyze 44,000 green and red intensity values!

yeast ~6K ORFs

Microarray Analysis

Analysis	What/why/how	Typical #'s
Background Subtraction	 intensity of b/w spot areas reflects noise $I - BI$ sometimes < 0 → change it = BI Excel =IF(I<0, BI, I) or 0	bkgnd. ~50-100 gene spots ~ 10^2 - 10^5
Normalization	$R_I = K G_I$ Cy5 less incorporation, less stable (but higher quantum yield than Cy3)	< 2-fold
Log2 Transform ratio of $G_I : R_I$	"readability" "help (LOG)"	

Today in Lab

- Set up RT-PCR
  - With RNase-free technique!
- Meanwhile practice uArray analysis
- Hybridize cDNA to uArray slides

- FNT is recommended, but optional

b/w 1Δ 5ug RNA

note: red vial = Cy 3 = green/red

blue vial = Cy 5 = red/red

*need to know which is which