

- Talk by Atissa from WAC, on oral presentations (in 16-336)
- No Lab Quiz!
- Pre-lab Lecture
 - ❖ Colony PCR results
 - ❖ Today in Lab
 - SDS-PAGE
 - Yeast Phenotype

comments on methods so far

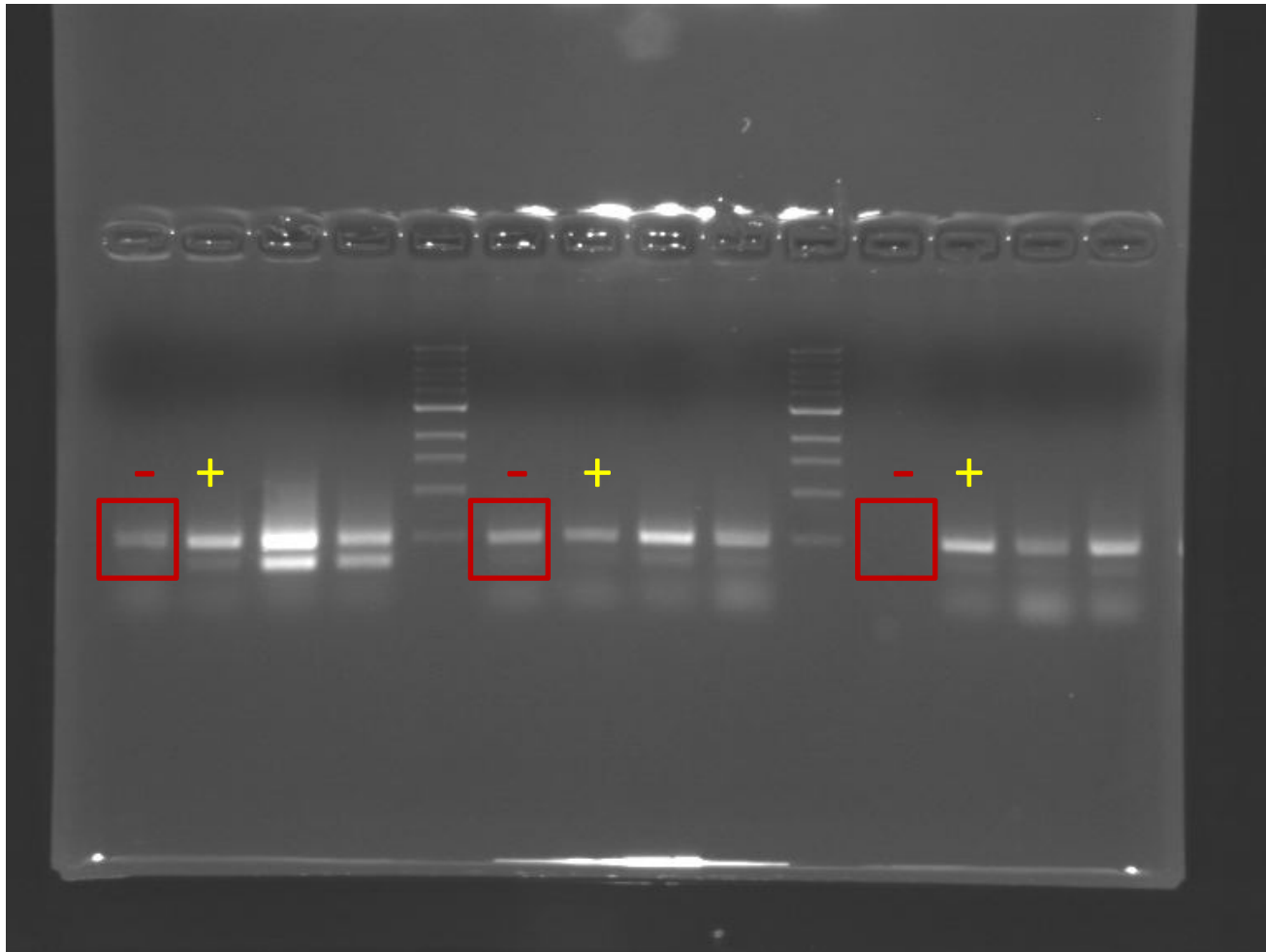
Announcements, old HW

* general: stick with essential information
(needed to repeat)

may include: cite wiki
emphasize TRP is for later selection

(PCR 2x conc., analytical gel, yeast
amount, plate composition)

Interpreting Colony PCR Results



(Available on M2D3 Talk page)

false + $\left\{ \begin{array}{l} \text{contamination} \\ \text{mis-priming} \end{array} \right.$

SDS-PAGE

acrylamide
TOXIC

leftovers → chemical waste

- You will make whole cell extracts with equal cell #s
 - Based on OD600 reading, normalize

- Glass beads // SDS, BME // boiling steps →

rid ~~high~~ higher-order structures

break open yeast

coat w/ uniform (-) charge

reduce S-S

- Gel separates proteins based on size
- After 1 hour run, blot to nitrocellulose and finally (we will) block with milk solution until next week.

Yeast Phenotype Testing (+FNT)

- Before loading gel, choose plates you will use and put them in the incubator to dry out
- For next time
 - Figure and interpretation for colony PCR
 - Calculation for RNA extraction
 - Prepare for Western analysis by calculating protein size (+ 20 KDa TAP tag)
- Spot test demonstration up front