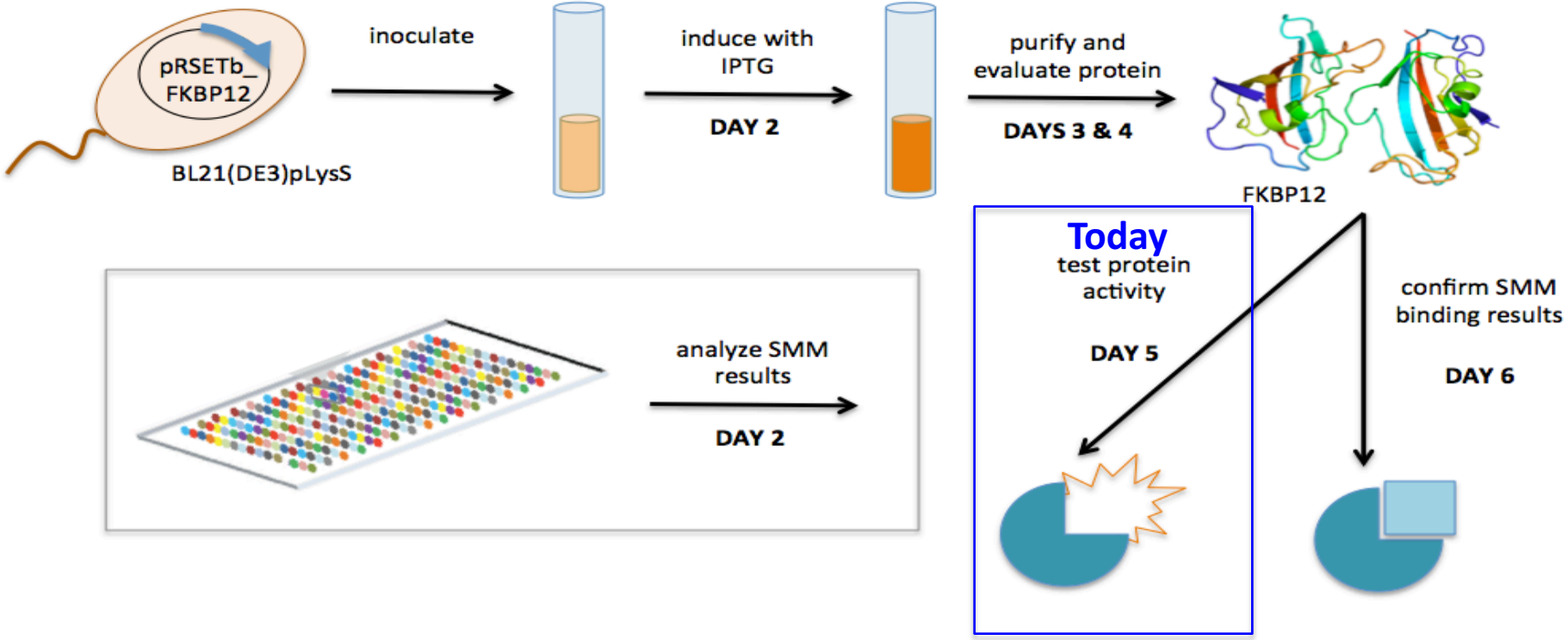
M1D5: Test protein activity using peptidyl-prolyl cis-trans isomerase assay

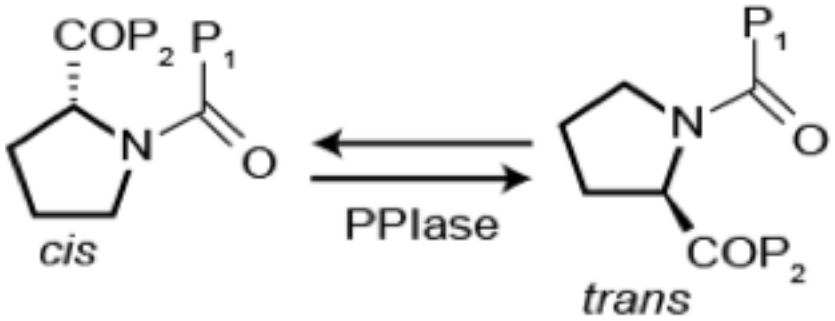
Announcements/Reminders:
3/1-2: Comm lab workshop at 3pm
3/6-7: M1 Quiz 2
3/12: Data Summary due

1. Prelab discussion
2. Set up “master-mixes” for PPIase Assay
3. Load 96-well plate for absorbance reading
   - 3 groups at a time, 30 min per plate
   - During down time: work on Methods, Casper will go over quiz, Sean will come by to answer figures questions

Test protein activity using peptidyl-prolyl cis-trans isomerase assay



FKBP12 is a protein (enzyme) with peptidyl prolyl cis-trans isomerase activity



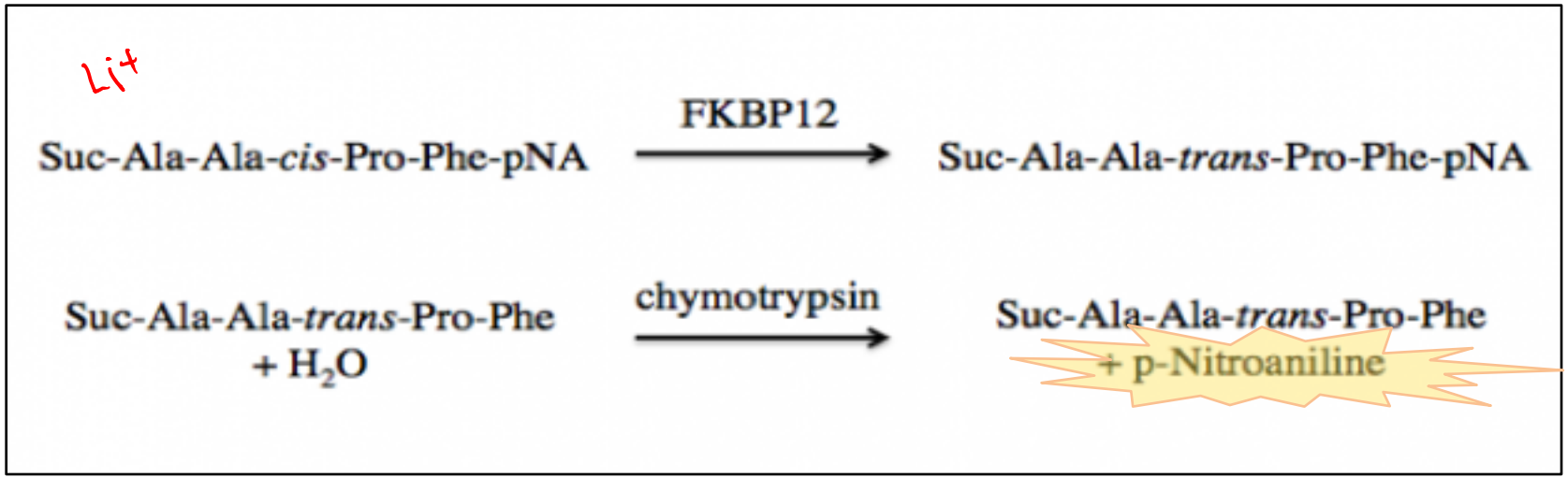
PPIase = Peptidyl prolyl isomerase

- PPIase Catalyzes cis-trans isomerization
- Aids in protein folding

How can we measure this activity?

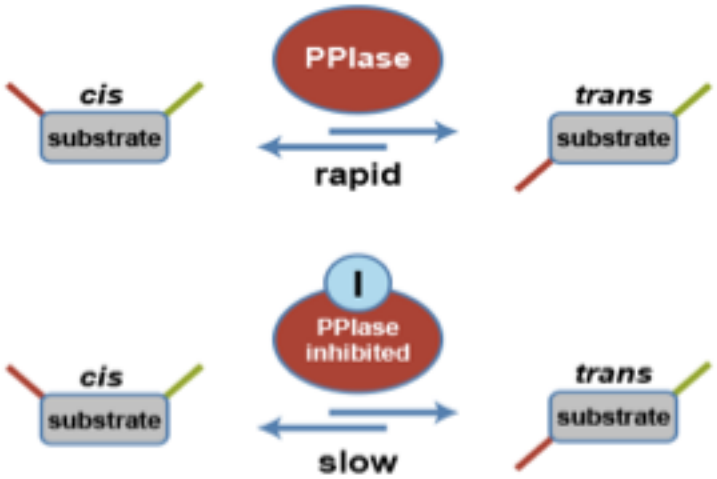
Image credit: http://www.selcia.com/ppiase-services/range-of-services/functional-ppiase-assay

PPIase assay measures rate of conversion from cis to trans isomer



- Substrate to test FKBP12 activity: suc-AAFP-pNA
- Use chymotrypsin to cleave p-Nitroaniline (pNA)
- pNA is chromogenic: absorbs 405 nm (looks yellow)
- Track presence of pNA over time ($\Delta A/\Delta t$)

Assess FKBP12 activity and affect of compounds using PPIase Assay



- Is the protein you purified active?

  Substrate only vs. Substrate + protein

- Positive control—known binder to FKBP12 (and inhibits isomerase activity)

  protein + Rapamycin (0.1μM) vs. protein + DMSO

- Unknown results: compounds you chose from the SMM screen

Image credit: http://www.selcia.com/ppiase-services/range-of-services/functional-ppiase-assay

You will set up 9 “master mixes”

At your bench:

9 x  : Assay buffer + DMSO **or** Rapamycin **or** Compound #1 **or** Compound #2

- Tris-HCl (pH8)
- chymotrypsin
- Water

Only add protein when told by instructor!

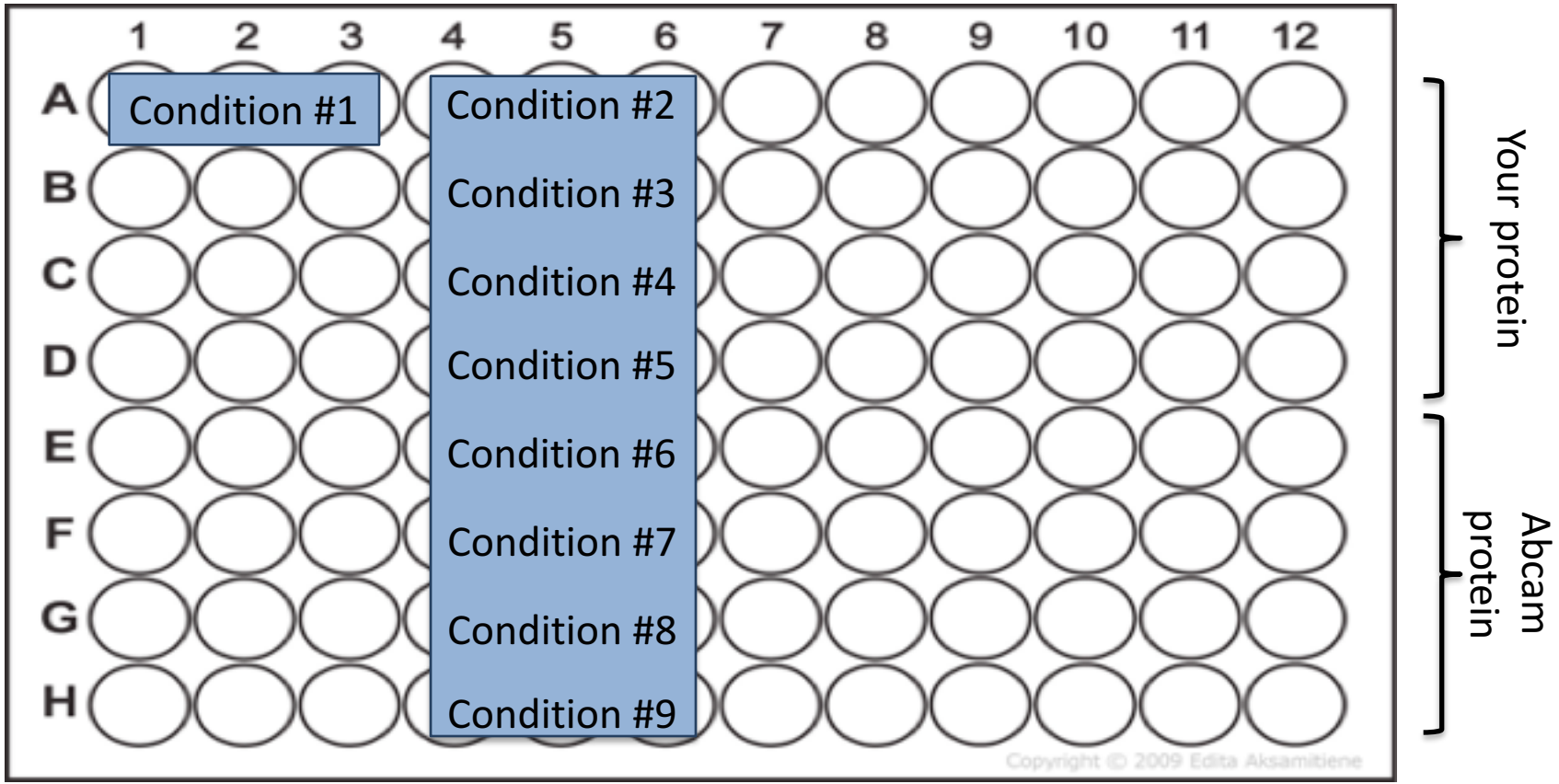
When told by instructor: + Your team’s FKBP12 **or** Abcam FKBP12

Instructors will add suc-AAFP-pNA right before measurement

- One tube is background control with buffer and suc-AAFP-pNA only
- Each tube has enough volume for 3.25 reactions (accounts for pipetting error)
- Each reaction is 200 uL
- Each reaction will be placed in 1 well of a 96 well plate

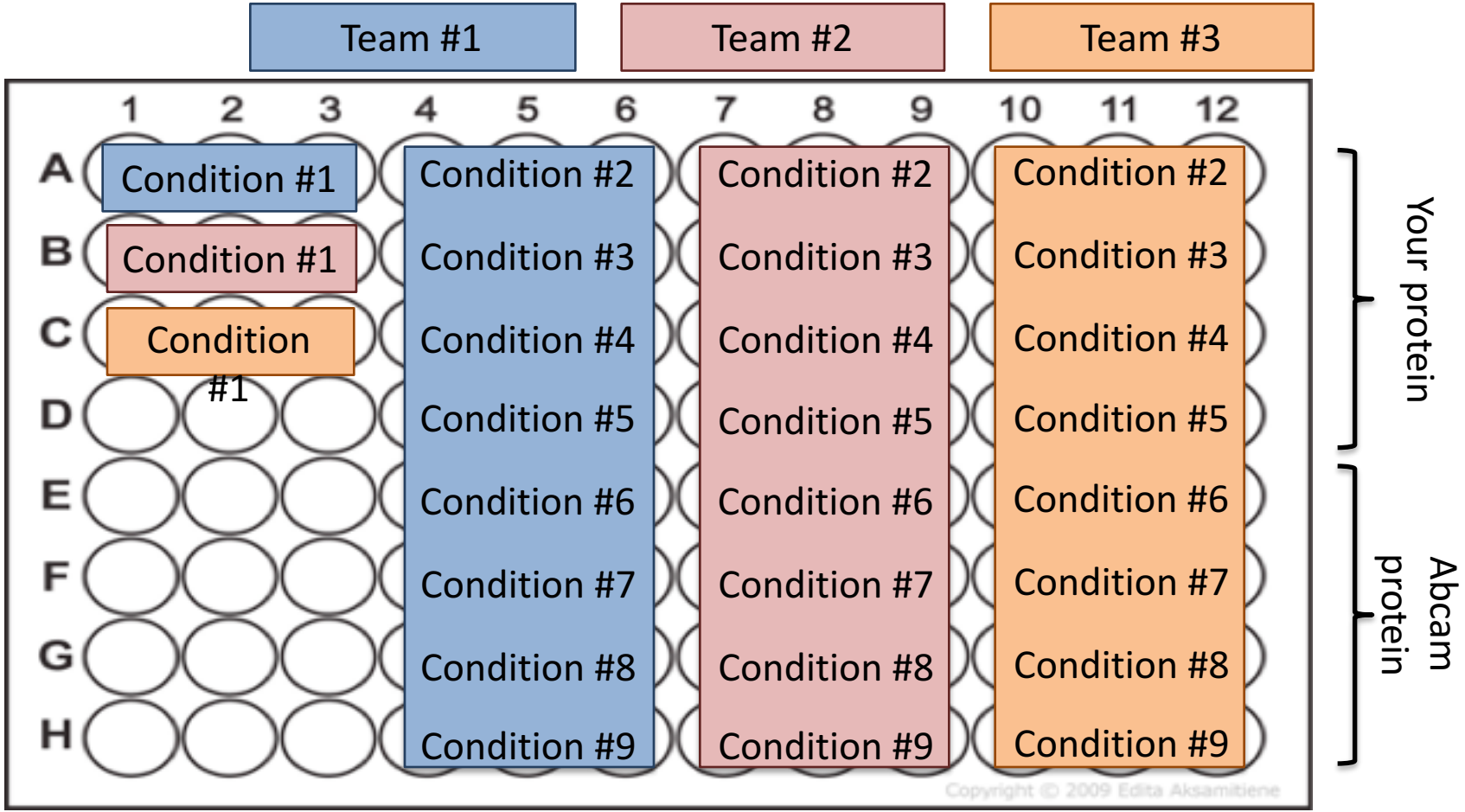
Load 96-well plate for your group

Read absorbance at 405 nm every minute for 30 minutes



Instructors load three groups on one 96-well plate

Read absorbance at 405 nm every minute for 30 minutes



Quantify the specific activity of the protein

Specific activity: nmol substrate/min/mg of protein

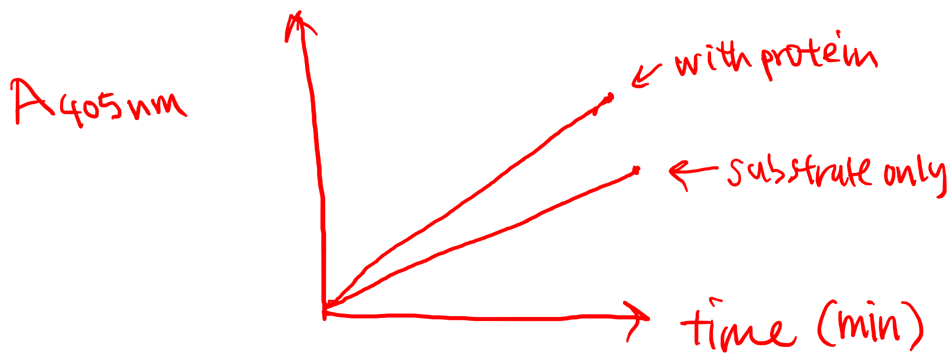
$\frac{A_{final} - A_{initial}}{30 min}$

$\frac{\text{Concentration of pNA}}{min} \sim \left[\frac{mM}{min}\right] * \text{volume} = \frac{moles}{min}$

$$\text{Specific activity} = \frac{(A_{405\_Test}/\text{min} - A_{405\_Blank}/\text{min})(\text{reaction volume})}{(\text{volume of FKBP12})(\varepsilon_{pNA})}$$

ε_{pNA} (extinction coefficient for pNA) ~ 9.3 mM^{-1}

Convert volume to mg based on known protein concentration



Homework due M1D6

- Mini-presentation outline
  - Bullet/outline form
  - Follow time and content guidelines
  - Introduce yourself and your research
  - Clearly state your hypothesis to identify main question
  - Be quantitative when stating findings (NOT “this was more/less than...”)
  - For this HW assignment, put placeholder statements for key findings

Category	Approximate worth	Elements of a strong presentation
Content	50%	• Did you introduce your research? • Did you include the key findings (and the techniques used to gather these results, if necessary)? • Was the importance of your project clear?
Organization	25%	• Is the presentation logical and easy-to-follow? • Are the main points emphasized? • Did you include transition statements such that the presentation 'flows' and is easily followed/understood?
Delivery	25%	• Do you show confidence and enthusiasm? • Did you use appropriate language (technical or informal, as appropriate)? • Is your speech clear?

http://engineerbiology.org/wiki/20.109(S18):_M1_Mini-presentation

- Consider getting started on M1D7 homework
- Data summary will be due Monday 3/12 at 10pm

Today in lab:

- Do all calculations before beginning bench work—check with teaching staff!
- During down time
  - Casper will go over quiz
  - Sean will be around to answer questions
  - Work on Methods, homework and data summaries