- Announcements, Review HW
- Lab Quiz
- Pre-lab Lecture
  - Your Colony Results
  - Tissue Culture
  - Safety + Technical Tips

Announcements, old HW

- OH will be in [redacted] (Su 7:30-9, Tu 4-5 pm)
- #1 – fine (units), #2 – careful with backbone size
- Methods are much improved! → structure/story
  - Explain controls (briefly), don't just list them * show your logic *
    e.g. single-enzyme digests, no ligase rxn.
  - Carefully consider the purpose of each gel
    first gel → isolate DNA fragments for cloning
    → verify that enzymes work
    * infer that double-digest worked, not directly see

single-cut plasmid, uncut

uncut plasmid

Interpreting Your Ligation Results

Group Colour	pCX-EGFP (#)	bkb + lig (#)	bkb + ins, no lig (#)	bkb + ins, lig 1 (#)	bkb + ins, lig 2 (#)
Hypothetical Data	1000	0	2	100	100
Yellow					
Green					
Blue					
Pink					
Purple					

Consider…

- Why did only the positive control work for many groups?
- What does the *no ligase* vs. the *no insert* sample control for? Which one do you expect to have more colonies? 2>3

  think of 1-2 reasons
- How big is the variation for identical samples, and what are the possible sources of error causing the variation?

Tissue Culture (TC) Environment

- What will "feel" physiological to a cell?

Temp = 37°C

pH = 7.2-7.4 ⟷ CO_2 = 5%

ambient O_2 levels

salts → (cells can burst or shrivel)

humidity · something to stick to

x sterility

Tissue Culture (TC) Medium

- What do cells need to survive?

food: glucose = C-source = energy
glutamine = alt. energy source
rxns. building blocks, cofactors: essential amino acids → (cells don't make them, varies by cell type)
vitamins, minerals, lipids
optional: non-essential aa
Na pyruvate = alt. energy
serum = growth factors, cytokines
non-food: antibiotics P/S (PA - 1°)
phenol red = pH indicator

Today in Lab

- Set up gels with diagnostic digest samples
- While the gels run, you will join me for a TC demonstration and practice lab
- At 3:30 pm, you will discuss the paper by Sonoda, et al. with Prof. Bevin

Announcements, old HW

- OH will be in 16-336 (Su 7:30-9, Tu 4-5 pm)
- #1 – fine, #2 – careful with backbone size
- Methods are much improved!
  - Explain controls (briefly), don't just list them
  - Carefully consider the purpose of each gel

Interpreting Your Ligation Results

Group Colour	pCX-EGFP (#)	bkb + lig (#)	bkb + ins, no lig (#)	bkb + ins, lig 1 (#)	bkb + ins, lig 2 (#)
Hypothetical Data	1000	0	2	100	100
Yellow	4000	0	0	0	0
Green	1884	1	144	380	656
Blue	2000	0	168	520	1031
Pink	426	0	0	0	0
Purple	300	0	0	0	0

Consider…

- Why did only the positive control work for many groups?
- What does the *no ligase* vs. the *no inse*rt sample control for? Which one do you expect to have more colonies?

- How big is the variation for identical samples, and what are the possible sources of error causing the variation?