

- **Announcements: OH** NK Sat, 3-5 in lab
ANS Sun 4-5:30 16-336
(ANS Tue 3-4 16-319)
- **Lab Quiz**
- **Pre-lab Lecture** + comments on Drafts
 - ❖ Your Colony Results
 - ❖ Tissue Culture
 - ❖ Safety + Technical Tips

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
Red	756	6	0	69	56
Green	1600	2	0	1	0
Blue	2740	6	1	0	3
Pink	1696	10	0	13	4
Purple	2192	48	6	188	232

Consider...

- Why might some groups not have gotten many exptl colonies?
- What does the *no ligase* vs. the *no insert* 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?

Tissue Culture (TC) Environment

- What will “feel” physiological to a cell?

$T = 37^{\circ}\text{C}$
humidity
pH 7.2-7.4 \longleftrightarrow CO_2 (often 5%)
ambient O_2 *STERILITY
sticky surface
salts (cells could burst or collapse)

Tissue Culture (TC) Medium

- What do cells need to survive?

food/life: C-source glucose and/or glutamine energy

essential amino acids
(often non-essential)
vitamins, minerals, lipids } building blocks
or co-factors
for rxns.

serum - cytokines (growth factors) proteins, lipids

non-food: antibiotics (Pen/Strep) pH indicator (phenol red)

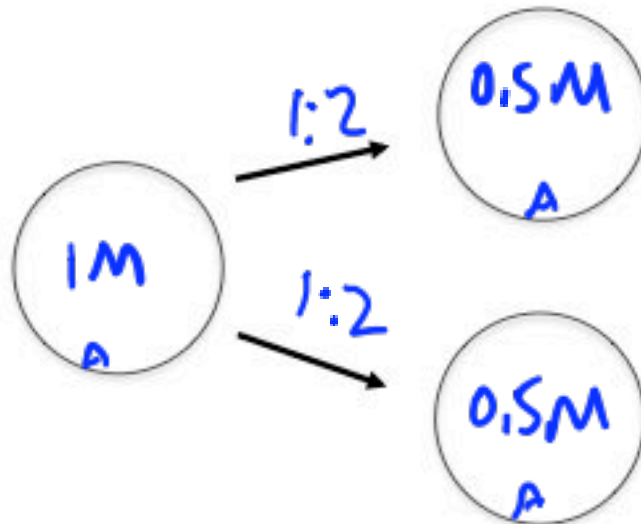
Passaging Cells

- Cells need room to grow
- They are split or “passaged” every 1 to N days

Example

Cells: $1M$
 (10^6)
 Area = A

$V_{cell} = 1mL$



$V_{cell} = 0.5mL$

$V_{medium} = 0.5mL$

ditto

general case, cell # and area matter $1M \xrightarrow{1:2} 2 \times 0.5M$

Today in Lab

- Join me for a TC demo and practice lab
- Set up gels with diagnostic digest samples
- While the gels run, you will discuss writing issues with Neal and Linda (~3:30 pm)