7.02/10.702 recitation notes Spring 2005

Genetics Day 3
(Eric Sullivan)

- There was some confusion about how we're going to disrupt the *ara* genes, so I created a handout (posted on 7.02 website on the Recitation notes page)

I. Day 2.5 results:

Q: (refresher) what was the selection?
A: Kan^R (transposition)

Q: (refresher) what was the screen?
A: white (Ara^-) versus red (Ara^+)

- You'll get 3-4 plates with white (actually translucent) colonies, named w/ bench#W# (and red controls)
- There were many more than expected (80-90 vs. 0-4) - Means everyone gets their own mutants that were picked from single isolated colonies

II. Characterization of putative Ara^- mutants:

Q: Why are we characterizing the mutants?
A: Allows us to study regulation of the *ara* operon
A2: Also, because not all whites are Ara^-

M9 Ara Leu Kan	**(-)**	Ara^- won't grow
M9 Glu Leu Kan	**(+)**	Should all grow (unless a gene required for growth on minimal media or for using glucose as a carbon source is mutated)
Mac Ara Kan	**(W)**	Reconfirm white phenotype Sugar fermentation – acidic byproducts – red Amino acid breakdown – basic byproducts- white
LB X-gal Kan	**(B)**	Proper insertions into constitutive genes (*araC*) will be blue
LB Ara X-gal Kan	**(B)**	Proper insertions into constitutive (*araC*) and Inducible genes (*araAB*) will be blue
LB CM	**(+)**	All Cm^R

Q: Go through the list for a mutant
H33 Ara^- Lac^- $LacZ^+$-constitutive Kan^R Leu^- Cm^R
delta(*lac)U169*, *ara*::miniTn10(*LacZ*$^+$, *kan*), leu-60::*cm*
A: results are listed above

- Use 1 stick to inoculate all 6 plates, repeat 3 more times for a single strain.
- Patching order is important!

Q: What if you patch backwards i.e. rich to minimal?
A: Ara^- might be fake results—perhaps nothing grew because you ran out of cells! Very important when replica plating, that you have a non-selective media last. Also, don't want to carry over nutrients from rich plate onto minimal plates.

III. *lacZ* expression (to produce β-galactosidase)

Q: assuming it integrates into an active ORF, what events must occur for LacZ synthesis?
A: Requires insertion of the *lacZ* in the correct orientation (1/2) and reading frame (1/3) = (1/6). This allows the *lacZ* gene to be transcribed under the control of that gene's promoter, and translated using the start codon and RBS of that gene. This product is called a translational fusion.

Figure removed due to copyright reasons.
Please see:-

Figure 29-28 in Voet, D., and J. Voet. *Biochemistry*. New York: J. Wiley & Sons, 2004. ISBN: 0471250902.

Definitions:

1. Inducible – transcription is off (or low), until an inducer (small molecule) is added –example *araBAD*
2. Constitutive – transcription is always on, regardless of inducer presence – example *araC.*

(Careful with definitions – pos. and neg. control use different terminology—i.e. activated, repressed)

Q: Which genes have which control?
A: *araC* expression is constitutive because you always want a regulator to have a chance to regulate. *araBAD* expression is inducible, since they should only be present w/ arabinose (not energetically favorable to the cell to have proteins needed to breakdown arabinose present when arabinose is not present).

araD$^-$: we won't obtain these mutants in our selection/screen, because arabinose processing causes a lethal product to accumulate in strains carrying a mutation in *araD*.

IV. Lambda titration:

Q: Why perform the titer?
A: so we can calculate MOI of mutagenesis experiment

- MOI – multiplicity of infection (ratio of phage to bacteria)
- Poisson distribution describes the behavior of the population at different MOIs (see appendix pages 60).
- Determination of lambda titer is similar to bacterial titer, except we're looking for regions that lack bacterial growth (plaques) instead of colonies (growth of bacteria).

Q: Are we growing lambda1205 on pNK / KBS1 cells?
A: no, LE392

Q: Why *can* they grow on LE392?
A: These strains carry an amber suppressor tRNA. This is a special tRNA that recognizes the amber stop codon (see below), but is charged with an amino acid. (Usually, tRNAs that recognize stop codons are not charged with amino acids, and therefore cause termination of protein synthesis.) The amber suppressor tRNA recognizes the amber stop codon and adds the amino acid leucine, which allows protein synthesis to continue. (Note: this only happens about 10% of the time. Why would it be problematic if it occurred more often?)

Amber UAG
Ochre UAA
Opal UGA

In lambda1205, there is an amber mutation in the gene encoding DnaP, a protein required for phage DNA replication and the lytic life cycle. In pNK/KBS1 cells (no amber suppressor tRNA), this mutation leads to the production of a truncated (shortened), non-functional DnaP protein—and therefore the phage cannot lyse the cells.

When lambda1205 infects LE392, the amber suppressor tRNA allows production of the full-length, functional DnaP protein. This allows DNA replication to proceed, and the phage can undergo the lytic life cycle.

Q: Why are we using TMG for dilutions (Tris pH7.5, $MgSO_4$, gelatin)?
A: Increases the stability of the phage