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Genetics Day 6 Recitation Notes
(Eric Sullivan)

I. Genetic Linkage
II. Metabolism: catabolism vs. anabolism
III. Cotransduction mapping

I. Genetic Linkage

- two genes which are extremely close together on the chromosome are said to be "linked"
- Draw two genes, AraC and GeneX interrupted with miniTn10 transposon
    - If a piece of DNA containing ara::miniTn10 gets transferred to recipient, then cells will be Ara- and KanR
    - If a piece of DNA containing geneX::miniTn10 gets transferred to recipient, then cells will be Ara+ and KanR.
    - We used a low MOI in our mutagenesis to ensure that only one transposon should occur in our donor (mutant) strains, and therefore we hope that all our transductants are Ara- and KanR

II. Metabolism

- Anabolism: the ability to synthesize molecules
    - ex. Leu- mutant cannot synthesize leucine, so we must provide a Leu- mutant with leucine for it to grow

- Catabolism: the ability to break down molecules
    - ex. Ara- mutant cannot break down arabinose (for use as a carbon source). so if arabinose is the only carbon source provided (like on an M9 Arabinose plate), an Ara- mutant won't grow.

Experimentally:

Plate	Ara+ strain	Ara+ Explanation	Ara- strain	Ara- Explanation
M9 Ara Leu Kan	growth	strain can use the arabinose on the plate as a carbon source	no growth	can't use the arabinose on the plate as a carbon source
M9 Glu Leu Kan	growth	strain can use the glucose on the plate as a carbon source	growth	strain can use the glucose on the plate as a carbon source

Plate	Thr+ strain	Thr+ Explanation	Thr- strain	Thr- Explanation
M9 Glu Leu	growth	strain can make its own threonine, so none needs to be provided	no growth	strain cannot make its own threonine, and none is provided
M9 Glu Leu Thr	growth	threonine is provided on the plate, but strain can also make its own	growth	threonine is provided on the plate for cells to use

III. Cotransduction Mapping

- Donor and recipient must be different at all 3 markers
  - Donor: $Ara^-(Kan^R)$, $Leu^-(Cm^R)$, Thr^+
  - Recip.: $Ara^+(Kan^S)$, $Leu^-(Cm^S)$, Thr^-

- Select for one, screen for the other two (example: select KanR, screen for CmR/CmS and Thr+/Thr-)

- Cotransduction frequency – probability of having two genes transduced on the same DNA
  - The larger the number, the closer the two are (lower map distance)
  - Similarly, lower frequencies are further apart (high map distance)

Example 3-factor cross from the appendix:

Donor: Tet^R, Met^+, Thr^+
Recipient: Tet^S, Met^-, Thr^-

Select for Tet, screen for Thr and Met

Plate used	Met^+/Thr^+	Met^-/Thr^+	Met^+/Thr^-	Met^-/Thr^-	
M9 Glu Met Tet	+	+	-	-	
M9 Glu Thr Tet	+	-	+	-	
M9 Glu Thr Met Tet	+	+	+	+	
	548	579	3	90	= 1220

CTF of Tet and Met = (548 + 3)/1220 = 45%
CTF of Tet and Thr = (579 + 548)/1220 = 92%

Three possible orders:
1. Tet, Met, Thr
2. Thr, Tet, Met
3. Met, Thr, Tet

The CTF data allows us to eliminate order #1
-- CTF of Tet and Thr is larger than the CTF of Tet and Met, therefore Tet and Thr must be closer than Tet and Met.

Rarest class – Tet^R, Met^+, Thr^-
- If order is Thr, Tet, Met: to get the rare class requires a double crossover event
- If order is Met, Thr, Tet: to get the rare class requires a quadruple crossover event

- Since rare classes arise from rare events—and a quadruple crossover event is more rare than a double crossover event—the gene order is Met, Thr, Tet.