7.02/10.702 Spring 2005

Genetics Day 2 (WF section)
(Eric Sullivan)

Overview of GEN Module:

Arabinose is a sugar source for *E. coli*.
Therefore, it has genes that make proteins which can break it down.
Draw example w/ theoretical *ara* gene

Q: Important elements for transcription (synthesis of mRNA)?
A: promoter (site at which RNA polymerase binds)

Q: Important elements for translation (synthesis of protein)?
A. start codon, ribosome binding site (RBS)

(also notice italics for genotype versus cap letters for phenotype – see manual appendix for more information)

Q: How can you study how the organism utilizes it?
A: "Break" the pathway by making a mutation
(Show chromosome) If you don't know the gene(s), genetics is the easiest way to identify them

Q: What genetic element are we using to 'break' the genes?
A: mini-Tn10 transposon

Q: What is the transposon composed of?
A: inverted repeats – required for transposition
selectable marker (*kan*) – so only transpositions survive (are selected) (expressed whenever inserted because has promoter, start codon, RBS)
a reporter *(lacZ)* – to study the regulation of the disrupted gene (requires fusion to another gene for expression because has no promoter, start codon, or RBS)

Q: Is that enough to get integration?
A: no, also needs a transposase – located on pNK

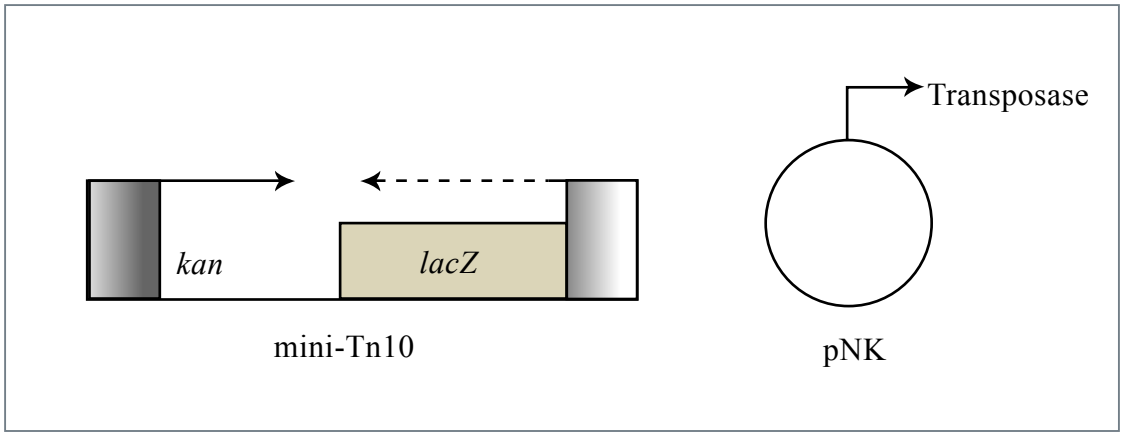

Figure by MIT OCW.

Q: How do we identify potential mutants?
A: Do a selection and a screen

Def: Screen – an assay is done to distinguish different phenotypes
Def: Selection – only what you want (that is, what you selected for) lives

Q: What phenotype are we selecting for?
A: Kan resistance – a transposition event

Q: What phenotype are we screening for?
A: Ara^- vs. Ara+ - white colonies (Ara-) vs. red colonies (Ara+) on Mac Ara Kan

Q: Are the Red colonies WT?
A: no, they had to get Kan^R through transposition

Q: if we're trying to screen for Ara^-, and selecting for Kan^R, what must be the phenotype of the original cells?
A: Ara^+ and Kan^S

Q: What plate will allow us to see a change in both phenotypes?
A: Mac Ara Kan

Q: How are we going to deliver the transposon to the cells?
A: lambda 1205 vector

1) Carries miniTn10 transposon – allows delivery of *lacZ* gene and *kanR* gene
2) No lysis – that would kill all the cells
3) No lysogeny – that would create Kan^R but never Ara^- (integration at *att* site is much more common than tranposition; the *att* site in *E. coli* is NOT in any of the *ara* genes)

Protocol review:

Q: Cells were grown o/n in LBMM, why?
A: Maltose (receptors) & Mg^{2+} – required for phage attachment

Q: Then, IPTG was added, why?
A: lactose promoter inducer (transposase gene on pNK is under control of the promoter for the lac operon)

Q: Mix cells and virus, let sit for 30' w/o shaking, why?
A: To allow for adsorption (phage to stick to cell surface)

Q: Then, (step 5) sodium citrate added, why?
A: chelator, binds Mg+2 ions to stop further infections

Q: (step 7) 1hr recovery period, why?
A: to allow Kan^R expression before plating on Mac Ara Kan (otherwise all cells would die)

Today's Tips:
Semi-log paper- log growth will be a straight line. Use <u>that</u> for doubling time. Ask TA for help using semi-log paper
Divide cfu/ml by OD550 to get the cfu/ml that's equal to one OD.
Start with mutagenesis, then do counting of colonies (use marker to keep track)