7.02/10.702 Spring 2005

Genetics Day 2 (TR section)
(Eric Sullivan)

Overview of GEN Module:

ara::lacZ^{+} **Q: What does this mean? A: *lacZ* gene is inserted and expressed in the *ara* operon**
:: - insertion delta– deletion
lowercase italics – genotype (DNA)
Regular case – phenotype (protein) (recommend appendix)

(show picture of *E. coli* genome – conjugation map) point out *ara* "this is what we want to change"
(show picture of *E. coli* LacZ protein (Bgal)– PDB 1DP0) "this is what we want to make instead"

Q: how do we accomplish this goal?
A: Genetics! ;-)

Look at it from "central dogma" perspective:

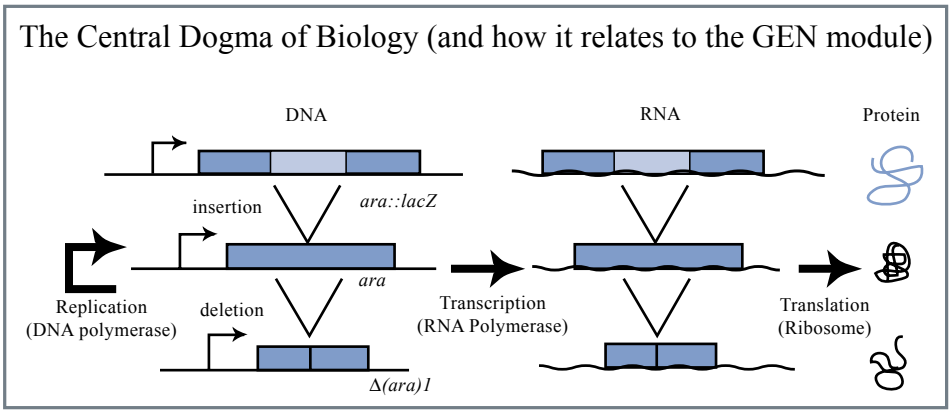

Figure by MIT OCW.

Insertion:

Q: What genetic element will insert the *lacZ* gene? A: mini-Tn*10*, *lacZ*, *kan* transposon (miniTn*10*)

Q: What is the transposon composed of?
A: inverted repeats – required for transposition
selectable marker (*kan*) – so only transpositions survive (are selected)
lacZ – reporter gene, so we can reach our goal

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

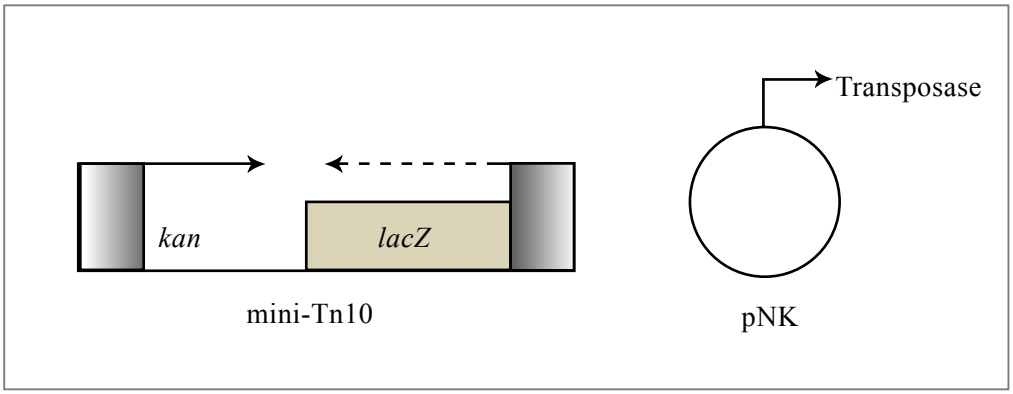

Figure by MIT OCW.

Expression:

Q: Assuming it integrates into an active ORF, what events must occur for LacZ sythesis?

A: Requires correct orientation (1/2) and reading frame (1/3) = (1/6)

(Product is called a translational fusion)

Ara operon structure – For regulation of *ara* operon see the appendix or, for more details, a molecular biology textbook

Inducible vs. Constitutive:

Inducible – transcription is off (or low), until an inducer (small molecule) is added – (*araBA::lacZ*$^+$)

Constitutive – transcription is always on, regardless of inducer presence – (*araC::lacZ*$^+$)

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

Q: Think about which genes have which control?

Hint: should a regulator protein always be made? What about a gene that breaks down arabinose?

Note: *araD*$^-$ - arabinose processing causes a lethal product to accumulate

Mutagenesis Strategy:

Delivery vector:

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

lambda 1205 vector – 1) Carries miniTn10 transposon – allows delivery of *lacZ* gene
2) No lysis – that would kill all the cells
3) No lysogeny – that would create Kan^R but not Ara- (attachment site in *E. coli* is not in any of the *ara* genes)

lysis – (lytic cycle) phage replicates and destroys the cell

lysogeny – the phage enters a dormant state and enters the bacterial chromosome – it waits until the environment is more favorable then it will excise itself and enter the lytic cycle

Conditions for mutagenesis:

Maltose (receptors) & Mg^{2+} – required for lambda1205 attachment

IPTG – lactose operon inducer (transposase on pNK is under the control of the lac operon's promoter)

(step 5) sodium citrate – chelator, binds Mg+2 to stop further infections

(step 7) 1hr recovery period – to allow Kan^R expression (or all cells would die when plated on Mac Ara Kan plates)

Isolation of Ara$^-$:

Screen – an assay is done selection – only what you want lives

Q: What phenotype are we selecting for?

A: Kan resistance – a transposition event

Q: What phenotype are we screening for?

A: Ara$^-$ - really white phenotype

Q: Are the Red colonies WT?

A: no, they had to get Kan^R through transposition

Q: Will only white colonies be LacZ$^+$?

A: no, any can be LacZ+ if transposon lands downstream of an active promoter, in the correct orientation and reading frame