Overview of GEN Module:

\( \text{ara}::\text{lacZ} \) Q: What does this mean? A: \( \text{lacZ} \) gene is **inserted** and **expressed** in the \( \text{ara} \) operon

:: - insertion  delta–deletion

*lowercase italics* – genotype (DNA)

Regular case – phenotype (protein) (recommend appendix)

(show picture of \( \text{E. coli} \) genome – conjugation map) point out \( \text{ara} \) “this is what we want to change”

(show picture of \( \text{E. coli} \) 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:

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The Central Dogma of Biology (and how it relates to the GEN module)

<table>
<thead>
<tr>
<th>DNA</th>
<th>RNA</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>replication (DNA polymerase)</td>
<td>transcription (RNA polymerase)</td>
<td>translation (Ribosome)</td>
</tr>
<tr>
<td>insertion</td>
<td>ara::lacZ</td>
<td>deletion</td>
</tr>
<tr>
<td>ara</td>
<td>delta</td>
<td>D(ara)-1</td>
</tr>
</tbody>
</table>
```

Figure by MIT OCW.

**Insertion:**

Q: What genetic element will insert the \( \text{lacZ} \) gene? A: mini-Tn10, \( \text{lacZ} \), kan transposon (miniTn10)

Q: What is the transposon composed of?

A: inverted repeats – required for transposition

selectable marker (\( \text{kan} \)) – so only transpositions survive (are selected)

\( \text{lacZ} \) – 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 synthesis?
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<sup>R</sup> 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<sup>2+</sup> – 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<sup>2+</sup> to stop further infections
(step 7) 1hr recovery period – to allow Kan<sup>R</sup> expression (or all cells would die when plated on Mac Ara Kan plates)

Isolation of *Ara<sup>−</sup>*:
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<sup>−</sup>* - really white phenotype

Q: Are the Red colonies WT?
A: no, they had to get Kan<sup>R</sup> 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