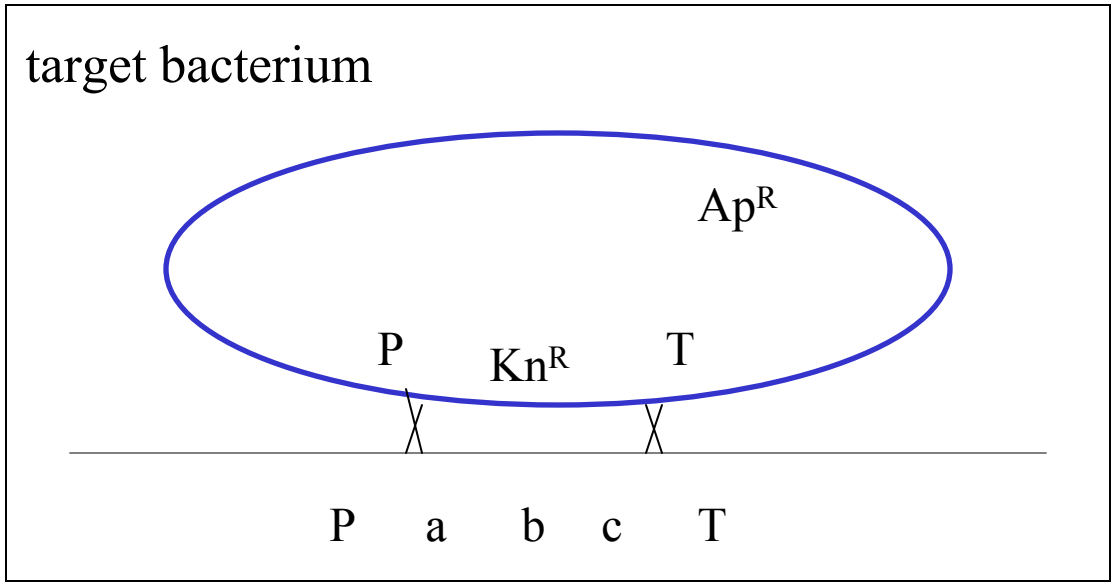
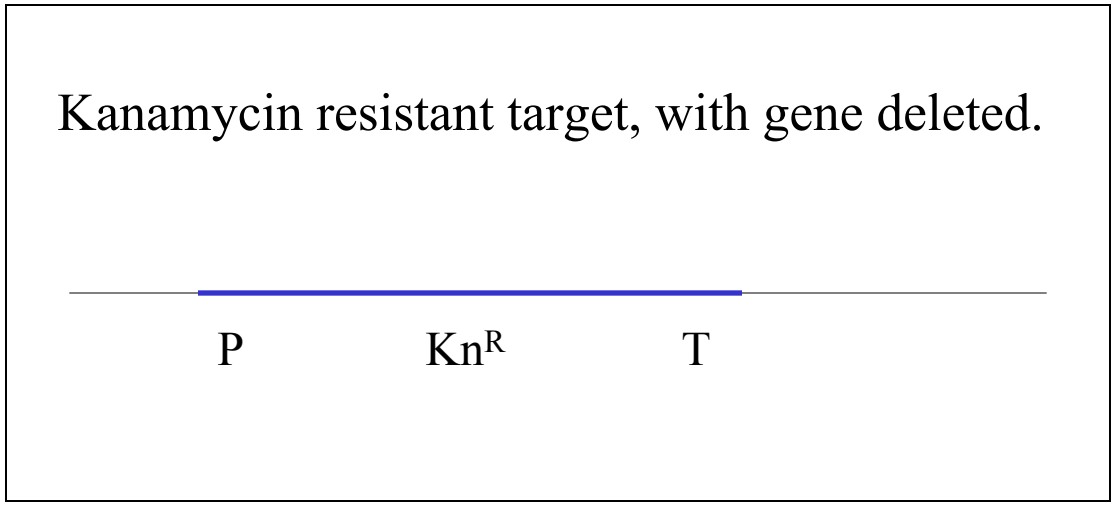
Marker exchange





Consider a bacterial target gene beginning with P, the promoter, with parts a, b, c and ending with T, the terminator.

For marker-exchange, any part of the target gene is cloned. In this case, the entire gene, including parts P & T, are cloned into a vector that can replicate in *E. coli*, but not the target bacterium.

In place of the coding region (abc), a marker gene is cloned (in this case, Kn^R, or Kanamycin resistance). This vector will be used to transform the target.

In two homologous recombination steps, the plasmid is lost. By keeping selection pressure on (ie., growing in presence of kanamycin), it will force exchange of the Kn^R gene for the target region. In the first step, the plasmid, a "suicide vector" that is unable to replicate in the target strain, integrates at either P or at T. In the second step, it recombines at the other region of homology and is lost. This step can be selected by including a levan sucrase (sacB) gene on the plasmid.