Positive & Negative Selection in Targeted Gene Insertions

Aim: To perform a targeted gene insertion into genome and select those cells that has undergone such transformation.

Approach: Use positive negative selection vectors (PNS vectors)

What are these PNS vectors? PNS vectors are those special vectors used for targeted gene insertion. PNS vectors have the following functional elements for site specific gene insertion.

- A positive selection marker
- A negative selection marker
- Homologous recombination sites and
- The desired gene to insert.

The following scheme shows the arrangement of these 4 components in PNS vector.

GOI = gene of interest, PSM = positive selection marker; NSM = negative selection marker

(Blue regions: homologous recombination sites)

Description of functional elements:

The vector comprises of first DNA sequence (homologous recombination site 1) which contains at least one sequence portion which is substantially homologous to a portion of a first region of a target DNA sequence. The vector also includes a second DNA sequence (homologous recombination site 2) containing at least one sequence portion which is substantially homologous to another portion of a second region of a target DNA sequence. The gene of interest (altered gene) is located in between these two homologous recombination sites, as shown in figure above.

Positive selection marker is positioned **in-between** the first and second homologous recombination sites (see figure) and encodes an antibiotic resistance (eg; neomycin) which when expressed is functional in the target cell in which the vector is used. On the other hand, negative selection marker is positioned **outside** the homologous recombination site (see figure) and generally codes for a suicide gene (eg; thymidine kinase) also functional in the target cell. This sequence is substantially incapable of homologous recombination with the target DNA sequence.

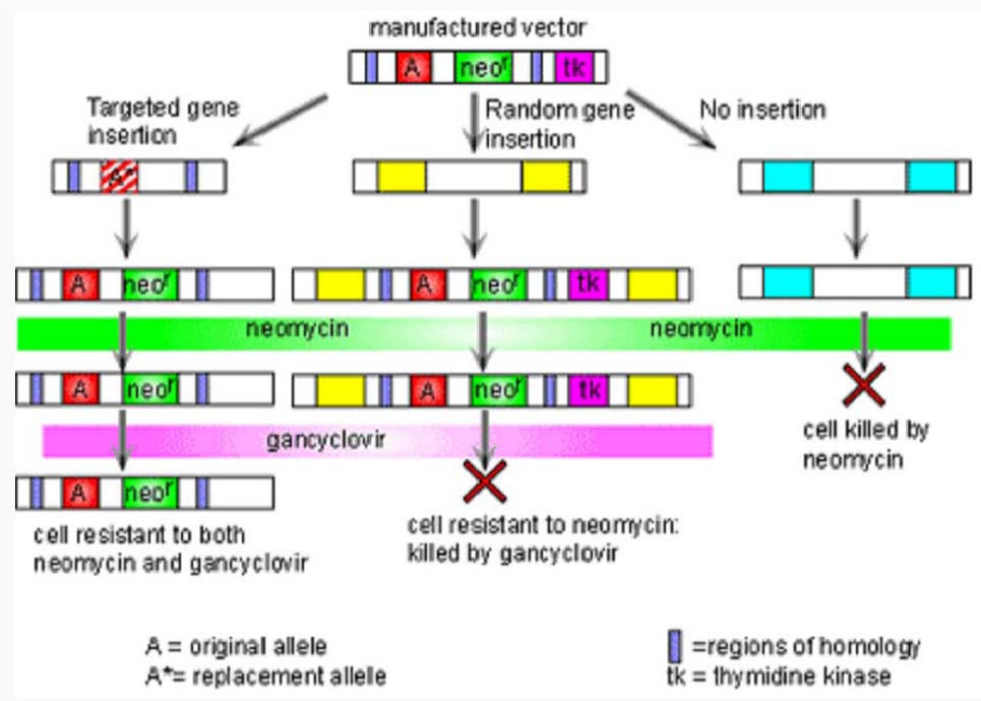
Functions at one glance:

- Homologous recombination sites – enable site specific gene insertion by homologous recombination events.
- Positive selection marker – **removes** those cells that have **not** undergone any insertion.
- Negative selection marker – **removes** those cells that have undergone **random gene insertion**.

The following scheme illustrates the details of selection process.

PSM: Neomycin

NSM: Thymidine kinase



Note: The herpesvirus thymidine kinase gene – commonly used as “suicide gene” in safety system in gene therapy experiments, allows cells expressing the gene to be killed using gancyclovir.