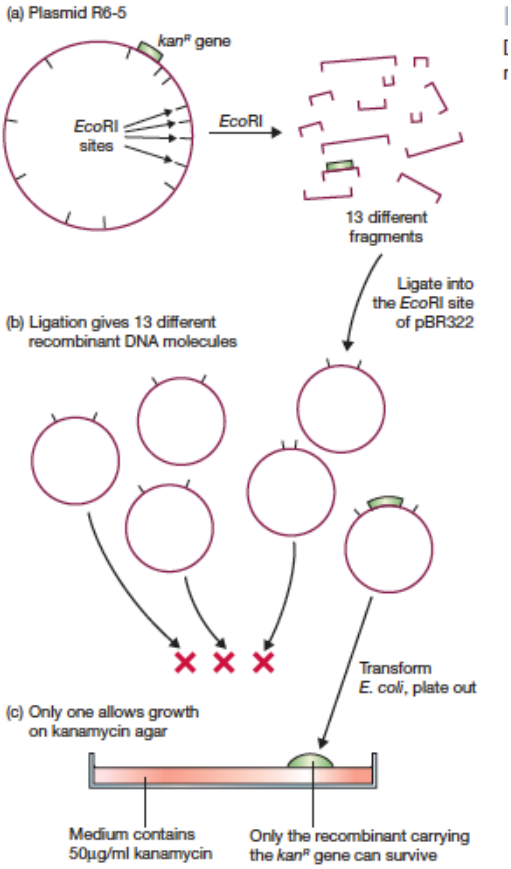
Direct selection

To be able to select for a cloned gene it is necessary to plate the transformants onto an agar medium on which only the desired recombinants, and no others, can grow. The only colonies that are obtained will therefore be ones that comprise cells containing the desired recombinant DNA molecule. The simplest example of direct selection occurs when the desired gene specifies resistance to an antibiotic. As an example we will consider an experiment to clone the gene for kanamycin resistance from plasmid R6-5. This plasmid carries genes for resistances to four antibiotics: kanamycin, chloramphenicol, streptomycin, and sulphonamide. The kanamycin resistance gene lies within one of the 13 EcoRI fragments To clone this gene, the EcoRI fragments of R6-5 could be inserted into the EcoRI site of a vector such as pBR322. The ligated mix will comprise many copies of 13 different recombinant DNA molecules, one set of which carries the gene for kanamycin resistance Insertional inactivation cannot be used to select recombinants when the EcoRI site of pBR322 is used. This is because this site does not lie in either the ampicillin or the tetracycline resistance genes of this plasmid . But this is immaterial for cloning the kanamycin resistance gene because in this case the cloned gene can be used as the selectable marker. Transformants are plated onto kanamycin agar, on which the only cells able to survive and produce colonies are those recombinants that contain the cloned kanamycin resistance gene

figure: Direct selection of the cloned R6-5 Kanamycin resistance gene



Marker Rescue

Marker rescue is the restoration of gene function by replacement of a defective gene with a normal one by recombination. Marker rescue extends the scope of direct selection Direct selection would be very limited indeed if it could be used only for cloning antibiotic resistance genes. Fortunately the technique can be extended by making use of mutant strains of E. coli as the hosts for transformation. As an example, consider an experiment to clone the gene trpA from E. coli. This gene codes for the enzyme tryptophan synthase, which is involved in biosynthesis of the essential amino acid tryptophan. A mutant strain of E. coli that has a non-functional trpA gene is called trpA–, and is able to survive only if tryptophan is added to the growth medium. E. coli trpA– is therefore another example of an auxotroph (p. 106).
The E. coli trpA– auxotroph can be used to clone the correct version of the trpA gene. Total DNA is first purified from a normal (wild-type) strain of the bacterium. Digestion with a restriction endonuclease, followed by ligation into a vector, produces numerous recombinant DNA molecules, one of which may, with luck, carry an intact copy of the trpA gene . This is, of course, the functional gene, as it has been obtained. from the wild-type strain.

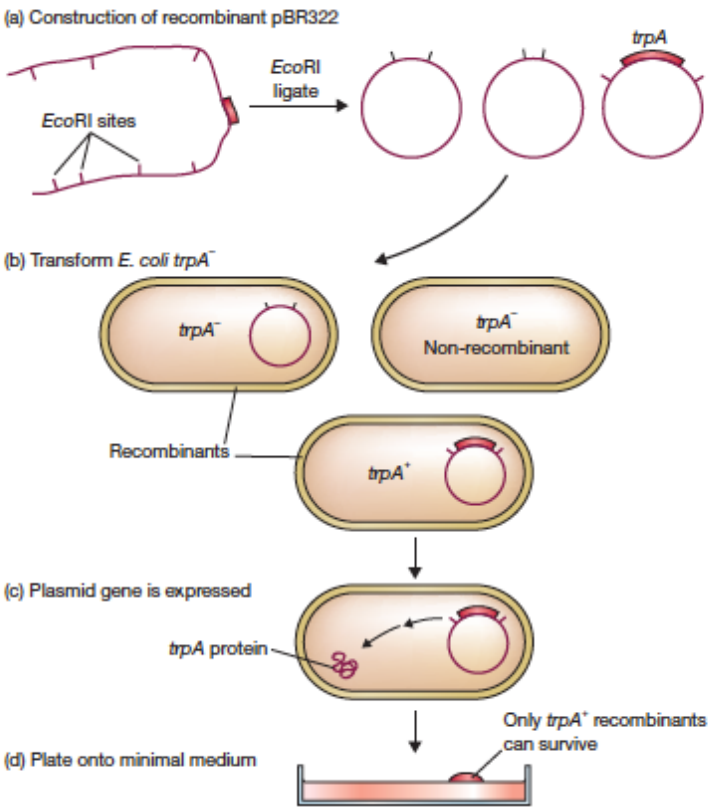


Figure : Ditect selection of *Tryp A* gene cloned in trypA - gene strain of *E.coli*

Vertical and horizontal gene transfer

Transmission of genetic information from parent to offspring is termed **vertical gene transfer**. Lateral movement, or movement of genetic information from a donor to an unrelated recipient, is called **horizontal gene transfer** The transfer of genetic material horizontally usually involves the use of viruses, plasmids, or mobile elements such as transposons . Horizontal gene transfer occurs not only for unrelated species, but for related species as well.