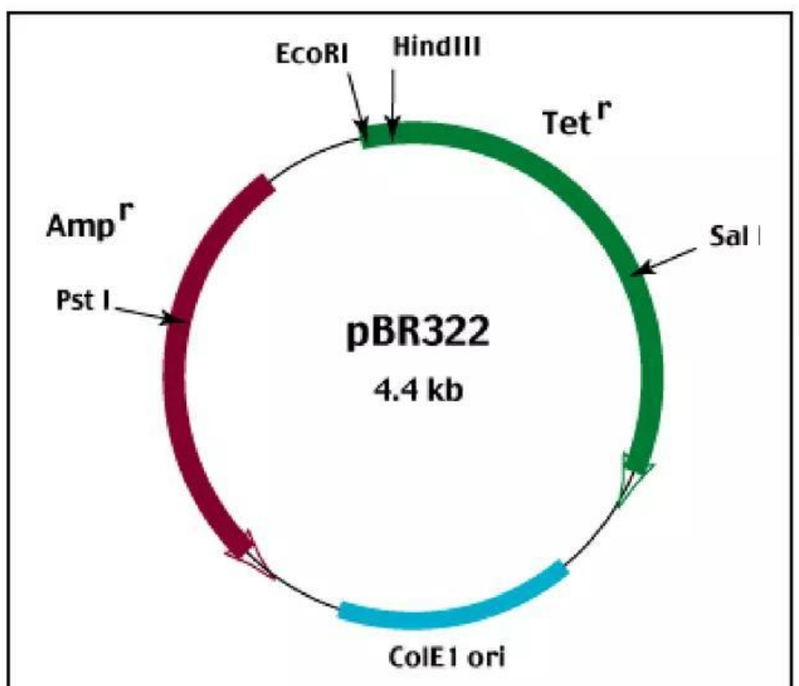
pBR322

Introduction:

pBR322 is a purpose built plasmid vector and was one of the first widely used cloning vector. It has a relatively small size of 4,363 bp. This is important because transformation efficiency is inversely proportional to size and above 10 kbp is very low.

The nomenclature of pBR 322:

- Created in 1977 in the laboratory of Herbert Boyer at the University of California, San Francisco.
    - 'p' indicates as a plasmid
    - 'BR' identifies Bo-liver and Rodriguez, the two researchers who developed it
    - '322' distinguishes those plasmids from others (like pBR 325, pBR 327, etc.) developed in the same laboratory.
- pBR322 is 4361 base pairs in length and has two antibiotic resistance genes:
    - The gene *bla* encoding the ampicillin resistance (Amp^R) protein
    - The gene *tetA* encoding the tetracycline resistance (Tet^R) protein.
- It contains the origin of replication of pMB1, and the *rop* gene, which encodes a restrictor of plasmid copy number.
- The plasmid has unique restriction sites for more than 40 restriction enzymes
- 11 of these 40 sites lie within the Tet^R gene
- There are two sites for restriction enzymes HindIII and ClaI within the promoter of the Tet^R gene.
- There are six key restriction sites inside the Amp^R gene.



- It is isolated from *E.coli*
- Size: 4361 bp
- Cloning limit: 0.1-10 kb
- Marker gene: Ampicillin and Tetracycline resistant gene
- Restriction site for various restriction endonucleases

Construction of pBR322:

pBR322 is NOT a naturally occurring plasmid.

It is manufactured by following steps:

The plasmid was constructed with genetic material from 3 main

- The amp^R gene originally resided on the plasmid RSF 2124 (a naturally occurring antibiotic resistant plasmid in *E. coli*)
- The tet^R is derived from pSC101 (a second antibiotic resistant plasmid)
- The origin of replication is derived from pMB1, which is closely related to the Colicin producing plasmid ColE1.

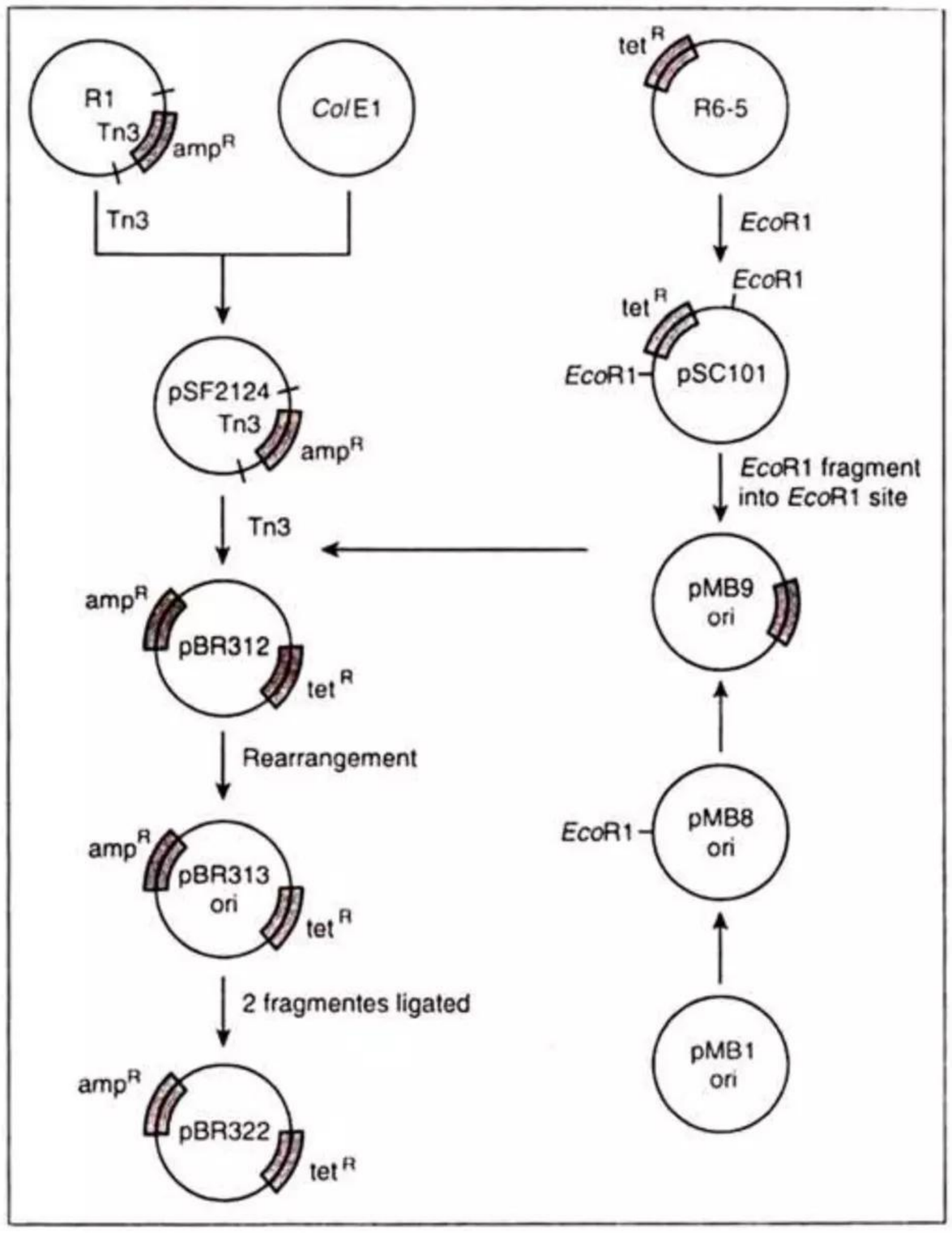


Fig. 4.15: The Pedigree of pBR322

Recombinant selection with pBR322 is done by **insertional inactivation of an antibiotic resistance gene.**

For example, A recombinant pBR322 molecule, one that carries an extra piece of DNA in the BamHl site is no longer able to confer tetracycline resistance on its host, as one of the necessary genes is now disrupted by the inserted DNA. Cells containing this recombinant pBR322 molecule are still resistant to ampicillin, but sensitive to tetra cycline (amp^R tef^s).

Uses of pBR322:

It is widely used as a cloning vector. In addi-tion to this, it has been widely used as a model system for study of prokaryotic transcription and translation.

Advantages of pBR322:

1. Small size (~ 4.4 kb) enables easy purifi-cation and manipulation.

2. Two selectable markers (amp and tet) al-low easy selection of recombinant DNA.

3. It can be amplified up to 1000-3000 copies per cell when protein synthesis is blocked by the application of chloramphenicol.

Disadvantages of pBR322:

1. It has very high mobility i. e; it can move to another cell in the presence of a conjugative plasmid like F-factor. Due to this, the vector may get lost in a population of mixed host cells.

2. There is a limitation in the size of the gene of interest that it can accommodate.

3. Not a very high copy number is present as is expected from a good vector.

4. The screening process time- consuming and laborious.