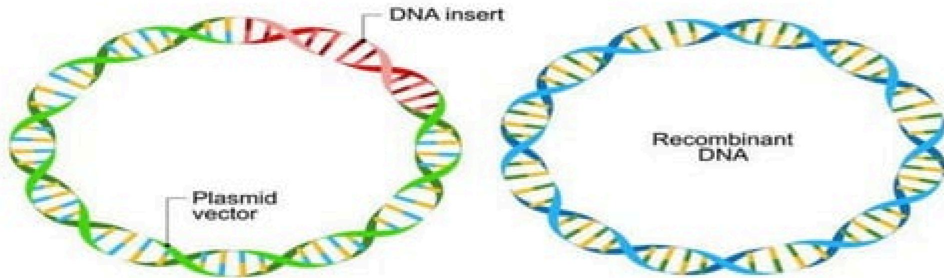
CLONING VECTORS



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- Bacterial Artificial Chromosome (BAC)
- Yeast Artificial Chromosome (BAC)
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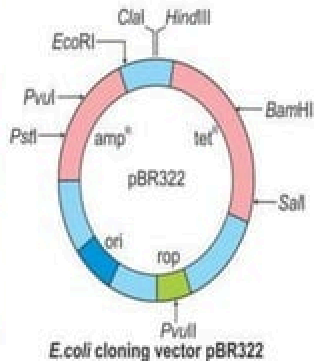
CLONING VECTOR

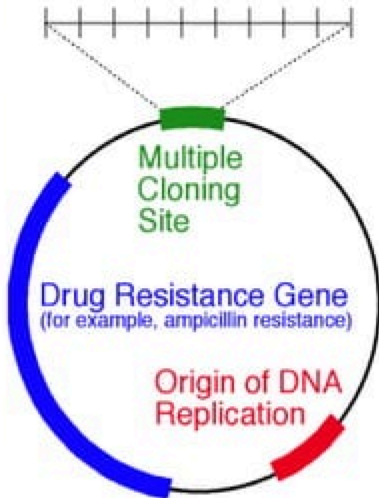
- In molecular cloning, a **vector is a DNA molecule used as a vehicle** to artificially carry foreign genetic material into another cell, where it can be replicated or expressed.
- For gene cloning, the two molecules are required: **DNA to be cloned** and a **cloning vector**.
- A **cloning vector** is a **small piece of DNA** taken **from a virus, a plasmid** or the **cell of a higher organism,** that can be **stably maintained** in an organism and into which a foreign DNA fragment can be inserted for cloning purposes.
- Most vectors are **genetically engineered**, are chosen according to the **size and type of DNA to be cloned**.
- The vector should allows **convenient** insertion of DNA fragment **in the vector** & removal of DNA fragment **out of the vector**. It can be done by treating vector and foreign DNA with **same restriction enzyme** and then **ligating** the fragments together **by DNA ligase.**
- **Without cloning vector, molecular gene cloning is totally impossible.**
- **Insertion of the vector** can be done by 3 ways: **Transformation, Transfection & Transduction**

HISTORY



E.coli cloning vector pBR322

- Scientists **(Herbert Boyer, Keiichi Itakura and Arthur Riggs)** working in Boyer's lab (University of California, San Francisco) recognized a **general cloning vector** with unique restriction sites for cloning in foreign DNA and the expression of antibiotic resistance genes for selection of transformed bacteria.
- In **1977**, they described the **first vector** designed for cloning purposes, **pBR322 – a plasmid**.
- The p stands for "plasmid," and **BR for "Bolivar" and "Rodriguez."**
- This vector was small, ~4 kb in size, and had two antibiotic resistance genes for selection.



FEATURES OF A CLONING VECTOR

1. Origin of replication (ori):

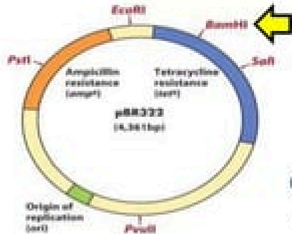
- This is a sequence from where replication starts.
- Any piece of **DNA when linked to this sequence** can be made to replicate within the host cells.
- **Copy number** of linked DNA.
- Vector **whose ori supports high copy number**, many copies of target DNA can be obtained.

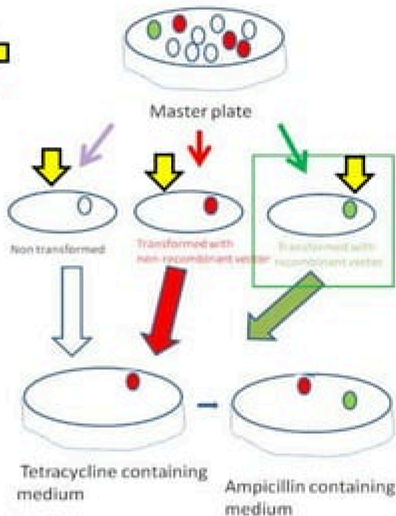
2. Cloning sites:

- Cloning site is a place where the **vector DNA can be digested** and **desired DNA can be inserted by the same restriction enzyme.**
- It is a point of **entry or analysis** for genetic engineering work.
- Recently, recombinant plasmids contain a **multiple cloning site (MCS)** which have many (up to ~20) restriction sites.

3. SELECTABLE MARKER

- Selectable marker is a gene that confers **resistance to particular antibiotics.**
- Eg. Genes encoding R to amp, tet or kena





- **Two functions:**

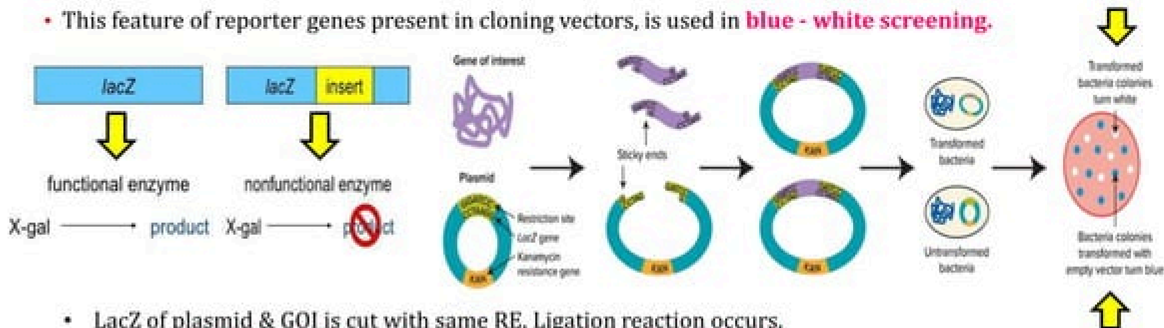
1. Helpful in identifying & eliminating non-transformants.
2. Helpful in selection of recombinants.

- **How to do selection of recombinants:**
- GOI is inserted in **BamHI site of tet R gene in vector pBR322**.

1. **Non-transformed:** Cannot grow on A & T medium.
2. **Transformed:** Grow on A & T medium.
3. **Transformed with non-recombinant vector:** Grow on both A & T medium.
4. **Transformed with recombinant vector:** Grow on A medium only. Do not grow on T medium because **tet R gene** is inactivated. Further, recombinants can be selected.

4. REPORTER GENE OR MARKER GENE

- **Reporter genes** are used in cloning vectors **to facilitate the screening of successful clones.**
- By using these genes, successful clone can be easily identified.
- This feature of reporter genes present in cloning vectors, is used in **blue - white screening.**

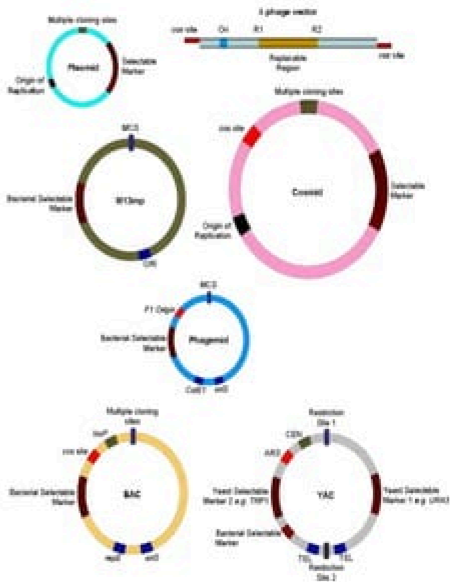


- LacZ of plasmid & GOI is cut with same RE. Ligation reaction occurs.
- Plated on **LB broth, Kanamycin plates overlaid with IPTG & X gal.**
- Incubated overnight at 37° C.
- **Transformed bacteria or Recombinants**: White colonies
- **Untransformed bacteria or Non-recombinants:** Blue colonies, because vector has not taken GOI.

ADDITIONAL PROPERTIES OF VECTORS

- Easy to isolate & purify.
- Compatible with host cell.
- High in copy number.
- Able to express itself utilizing the host machinery.
- Able to move under two system (Prokaryote and Eukaryote system).
- Less than **10 kb in size** because large DNA molecules are broken during purification procedure.

	Cloning vector	Expression vector
Definition	Small DNA molecule that carries a foreign DNA fragment into the host cell	Vector that facilitates the introduction, expression of genes & production of proteins.
Major function	Used to introduce a foreign DNA fragment into the host	Used to express the introduced gene by producing the relevant proteins
Features	Consists of ori, restriction site & a selection marker	Contains enhancers, promoter regions, termination codon, transcription codon, transcription initiation sequence, ori, restriction sites, & selectable marker
Examples	Plasmids, Bacteriophages, BAC, Cosmids	Mostly plasmids



TYPES OF CLONING VECTORS

- Plasmid
- Bacteriophage
- Cosmid
- Bacterial Artificial Chromosome (BAC)
- Yeast Artificial Chromosome (BAC)
- Human Artificial Chromosome (HAC)
- Retroviral Vectors

'HoneySweet' (C5), The First Genetically Engineered Plum Pox Virus–resistant Plum (Prunus Domestica L.) Cultivar

- **'HoneySweet'** was originally selected in vitro as a **regenerated shoot** from a hypocotyl slice, that had been transfected with **Agrobacterium tumefaciens** EHA 101 carrying the **plasmid pGA482GG / PPV-CP-33** (Scorza *et al* 1994).
- **Regenerated, transgenic shoot, coded as C5,** along with other transgenic shoots, was rooted in vitro and transferred to a greenhouse.
- Tested by giving **aphid inoculations** with the different strains of **Plum pox virus (PPV)** in greenhouse.
- After field evaluation of transgenic plants for **12 years** in aphid vectored conditions, it was released as **C5.**
- **Later patented as 'HoneySweet'.**

Scorza *et al* (2016)
US Kearneysville

Fig.
Transgenic papaya line 55-1 showing resistance to PRSV compared to infected non transgenic papaya.

Transgenic Virus Resistant Papaya: From Hope to Reality for Controlling Papaya Ringspot Virus in Hawaii

How transgenic papaya formed ?

- **Coat protein (CP) gene** was isolated from **mild mutant PRSV HA 5-1.**
- CP gene was inserted into **DNA plamisd by vector pGA482GG**
- Plasmid DNA was delivered via **high velocity micro-projectiles gun** into the papaya cells **(Sunset and Kapoho).**
- As a result, sufficient number of transgenic papaya plants were obtained.
- **Micropropagated plants (R0)**, designated as **55-1**, showed **excellent resistance against PRSV.**
- Furthur, **Line 55-1** was crossed with **non-transgenic Sunset** (50 % progenies being transgenic) to produce **R1 plants.**
- Later named as '**SunUp**'.

Gonsalves *et al* (2004)
U.S. Pacific Basin Agricultural Research Center, Hawaii

THANK YOU