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REKAYASA GENETIKA
IBD 131

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Pertemuan 4

VEKTOR KLONING

Universitas Esa Unggul

Sasaran Perkuliahan

- Mahasiswa dapat Mamahami dan menjelaskan tentang pembuatan vektor rekombinan sebagai wahana pengklonan DNA dalam rekayasa genetika
- Memahami dan menjelaskan tentang Vektor rekombinan
- Memahami dan mampu menjelaskan jenis – jenis vektor sesuai dengan sisipannya

CLONING STRATEGY

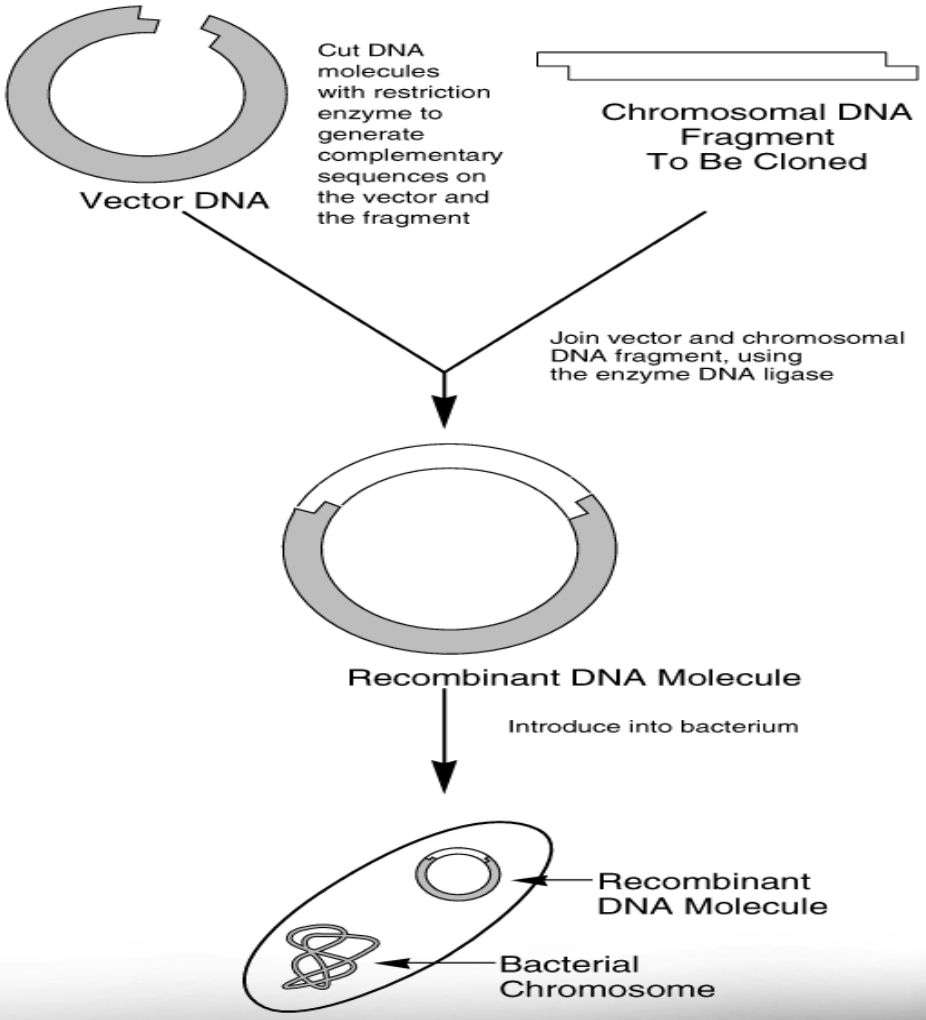
- **Strategy** depends on the starting information and desired endpoint.
- **Starting Information or Resources:**
  - Protein sequence
    - Positional cloning information
  - mRNA species / sequence
  - cDNA libraries
  - DNA sequence known or unknown
  - genomic DNA libraries
  - PCR product

CLONING

- **DNA cloning is** a technique for reproducing DNA fragments.
- It can be achieved by two different approaches:
  - cell based
  - using polymerase chain reaction (PCR).
- a vector is required to carry the DNA fragment of interest into the host cell using enzyme Ligase

CLONING PROCESS

- Gene of interest is cut out with RE
- Host plasmid is cut with same RE
- Gene is inserted into plasmid and ligated with ligase
- New plasmid inserted into bacterium (transform)



PLASMID CLONING STRATEGY

- **Involves five steps**:

  Enzyme restriction digest of **DNA sample.**

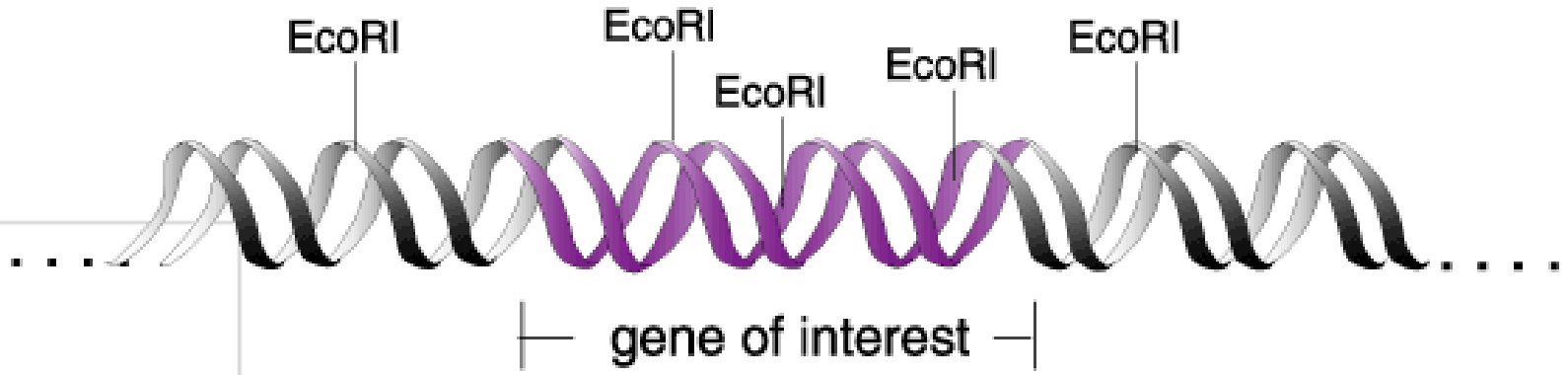
  Enzyme restriction digest of **DNA plasmid vector.**

  Ligation of DNA sample products and plasmid vector.

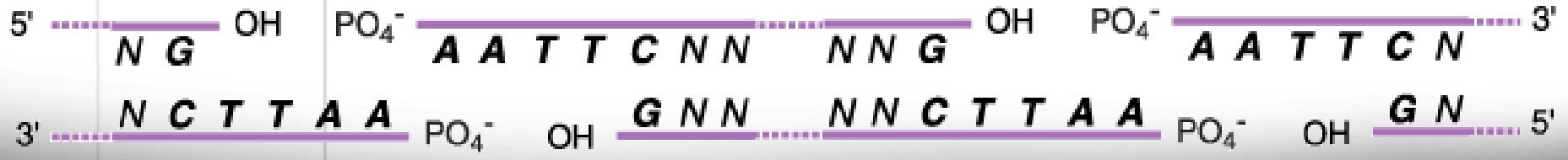
  Transformation with the ligation products.

  Growth on agar plates with selection for **antibiotic resistance**.

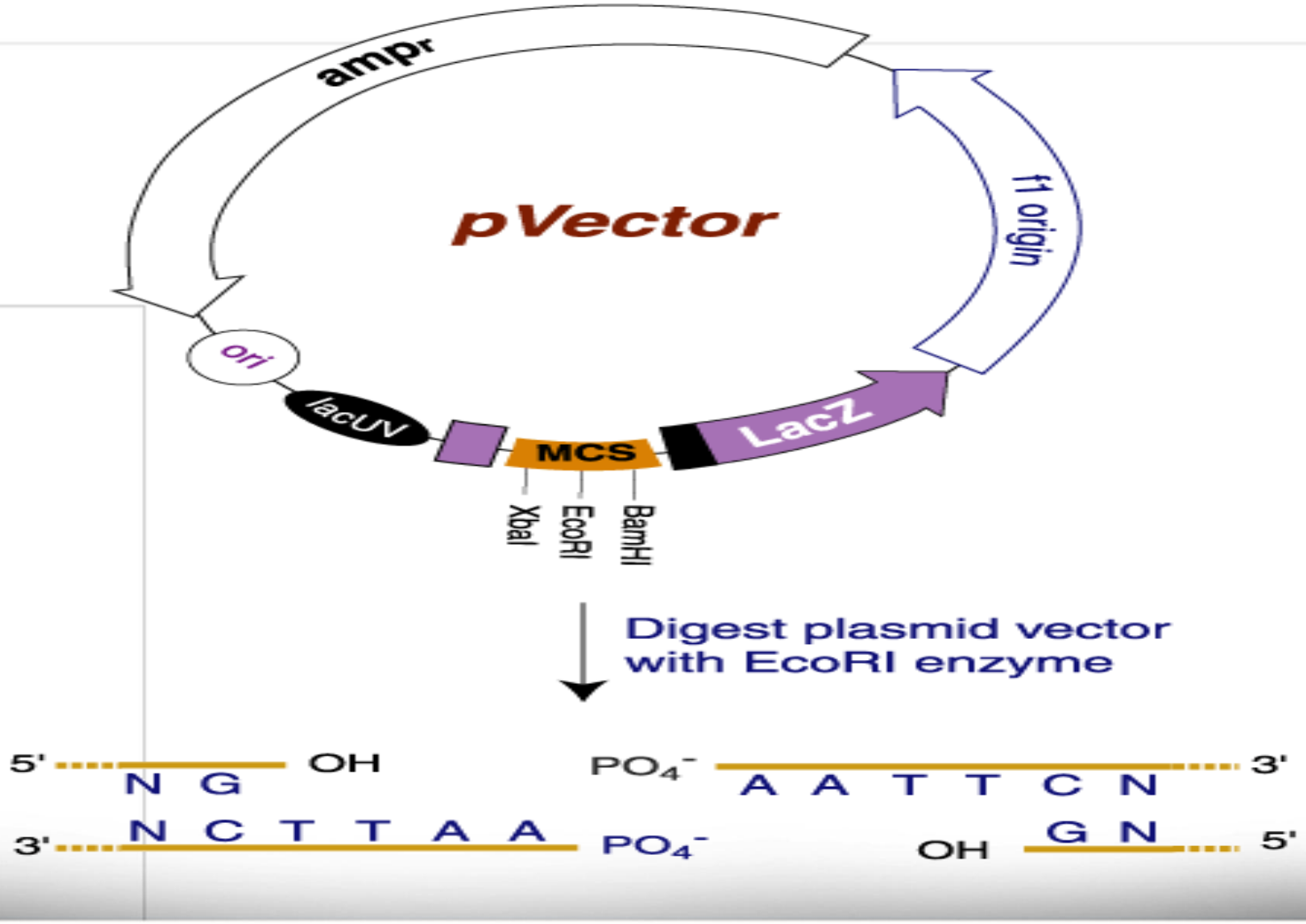
STEP 1. RE DIGESTION OF DNA SAMPLE



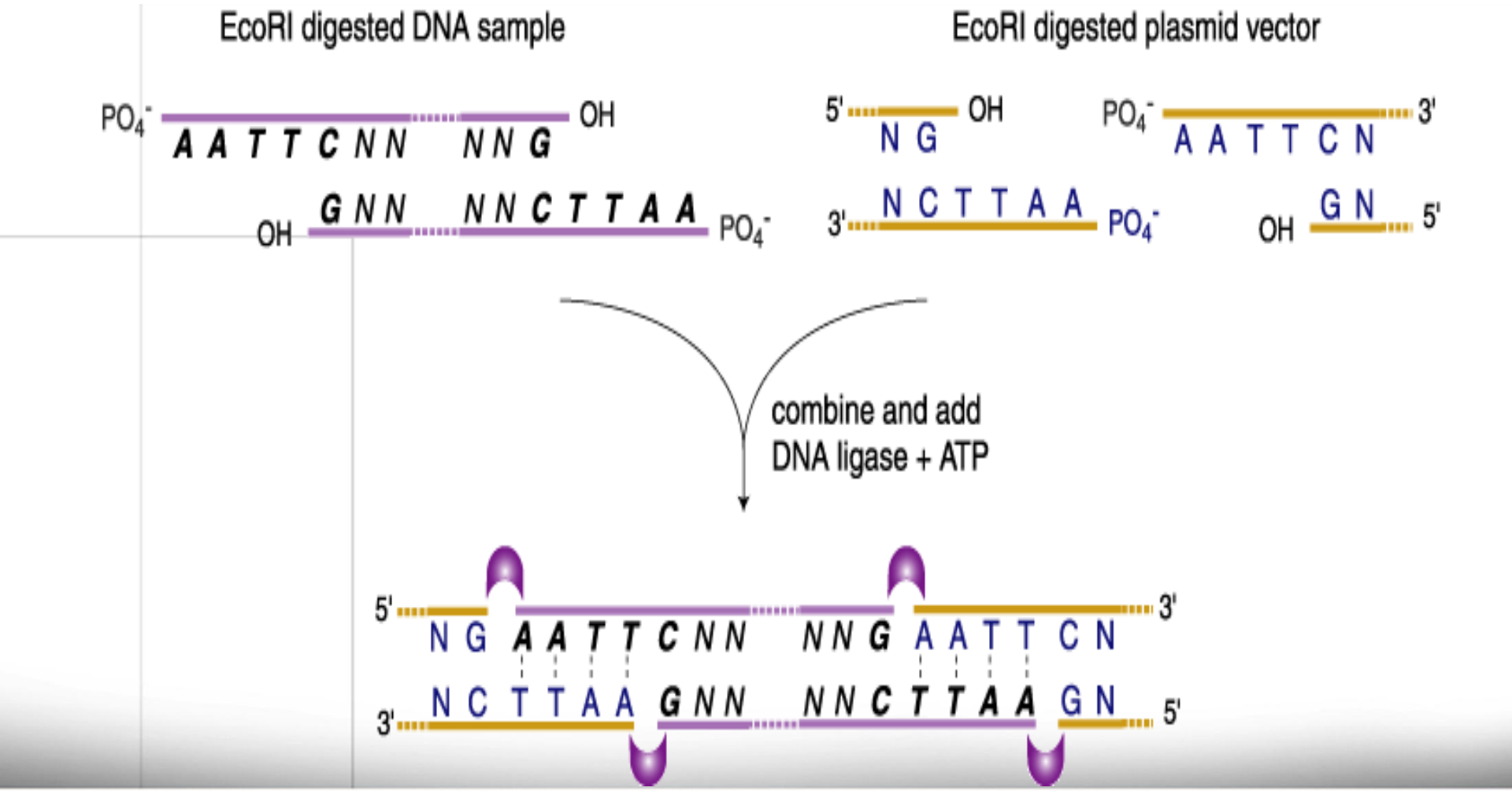
Digest DNA sample
with EcoRI enzyme



STEP 2. RE DIGESTION OF PLASMID DNA



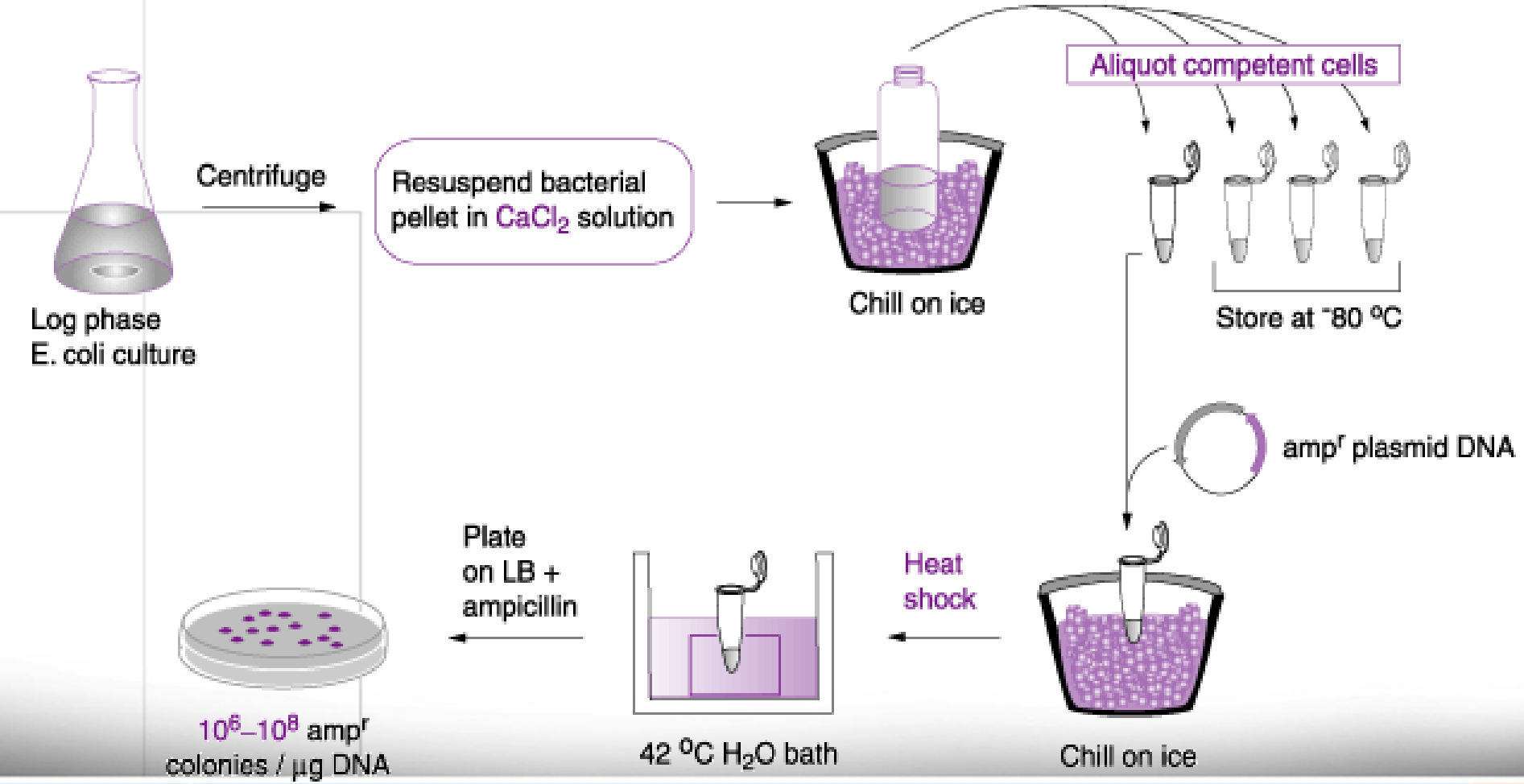
STEP 3. LIGATION OF DNA SAMPLE AND PLASMID DNA



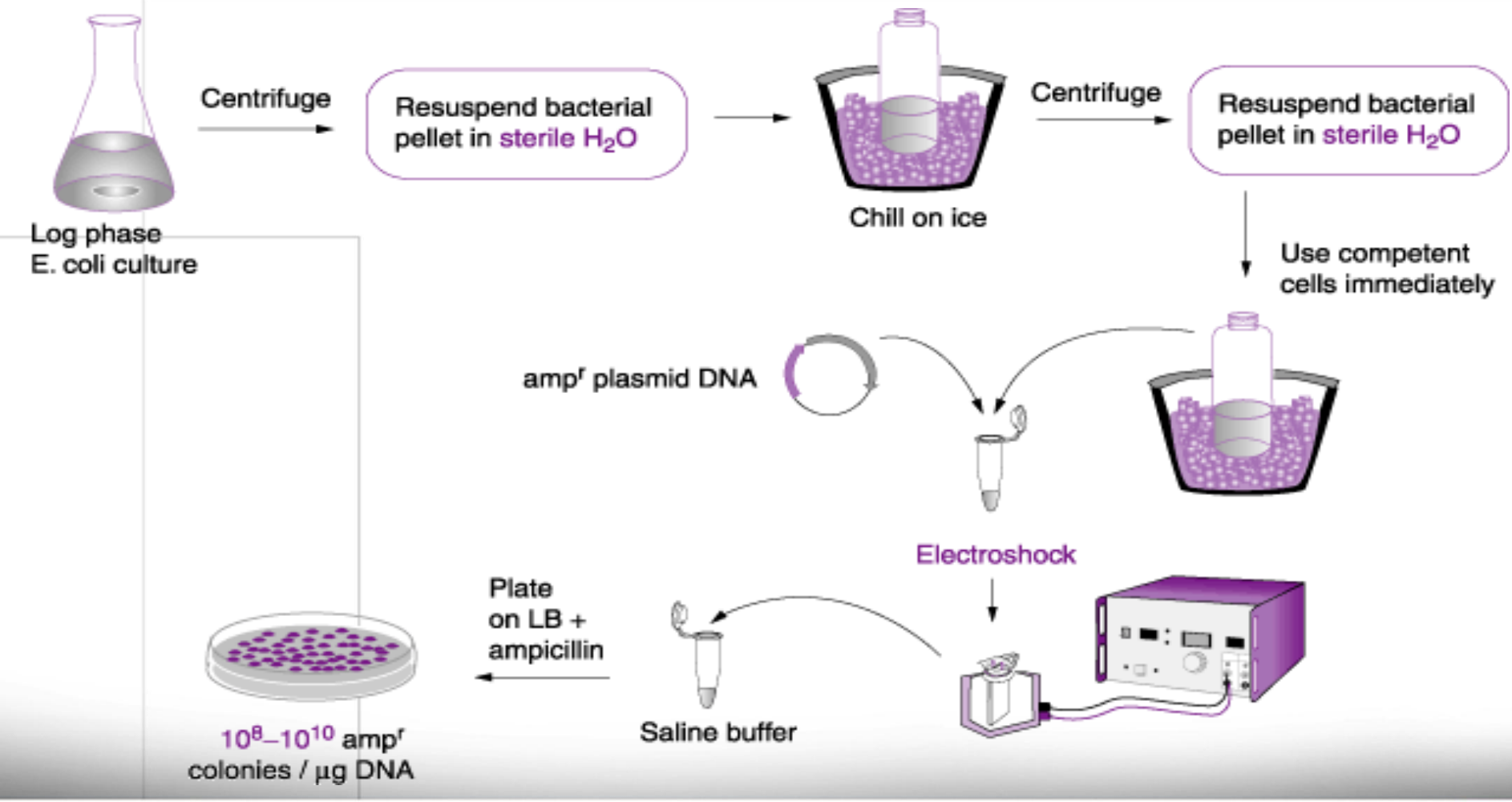
STEP 4. TRANSFORMATION OF LIGATION PRODUCTS

- The process of transferring exogenous DNA into cells is call "transformation"
- There are basically two general methods for transforming bacteria. The first is a chemical method utilizing CaCl2 and heat shock to promote DNA entry into cells.
- A second method is called electroporation based on a short pulse of electric charge to facilitate DNA uptake.

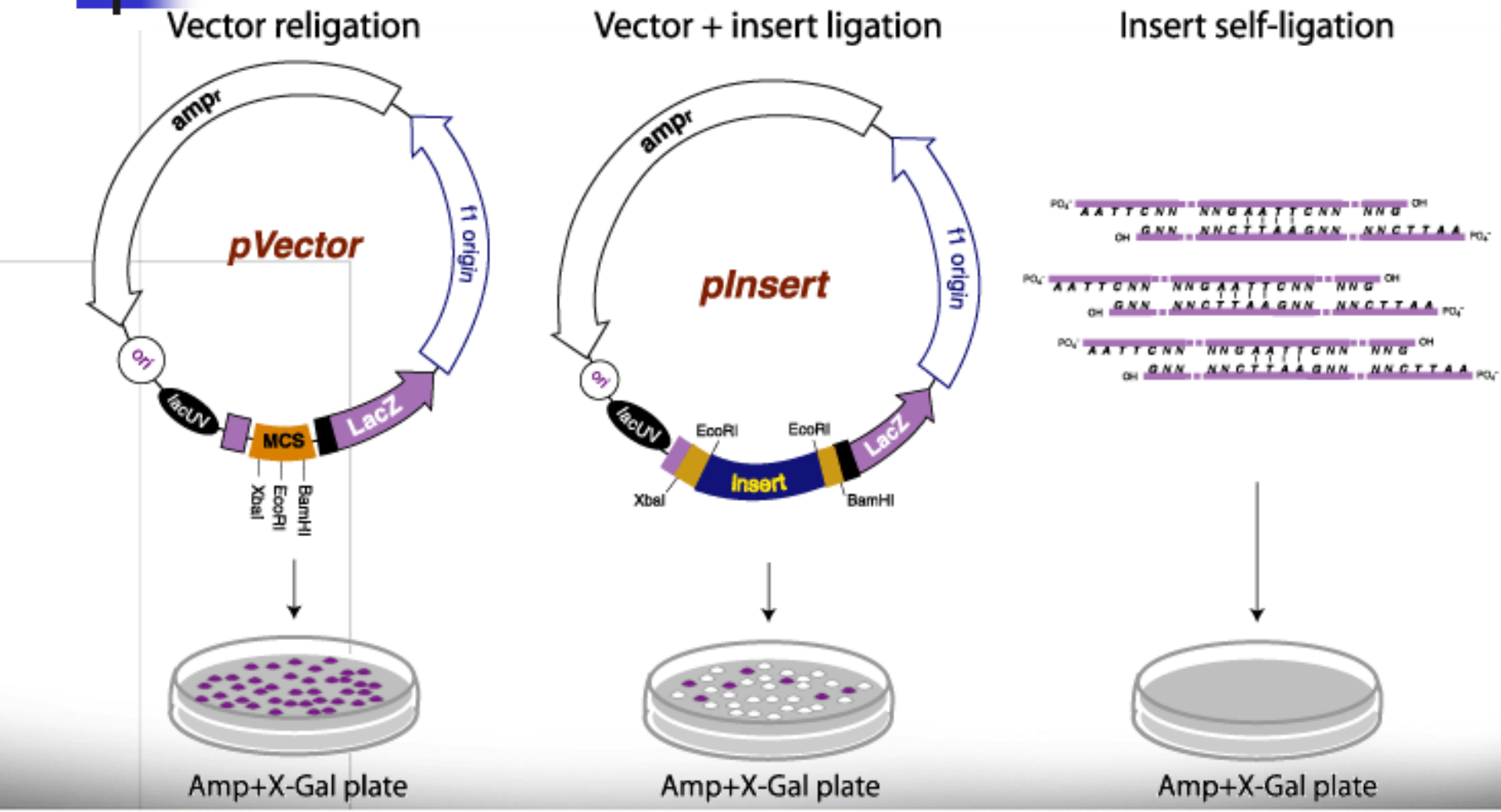
CHEMICAL TRANSFORMATION WITH CALCIUM CHLORIDE



TRANSFORMATION BY ELECTROPORATION



STEP 5. GROWTH ON AGAR PLATES



STEP 6. Blue white Scrining

- Koloni berwarna biru timbul karena pada vektor terdapat gen *lacZ* yang mengkode enzim β-galaktosidase.
- Protein ini bereaksi dengan X-gal yang mudah teroksidasi menjadi pigmen warna biru yang tidak larut
- isopropyl β-D-1-thiogalactopyranoside
- (IPTG) berfungsi sebagai induser yang akan mengaktifkan operon *lac* sehingga mengekspresikan gen *lacZ*
- Bila fragmen DNA sisipan menyisip pada gen *lac*Z, maka gen *lac*Z tidak dapat diekspresikan untuk menghasilkan enzim β-galaktosidase sehingga koloni *E. coli* berwarna putih dan positif membawa DNA sisipan

CLONING VECTORS

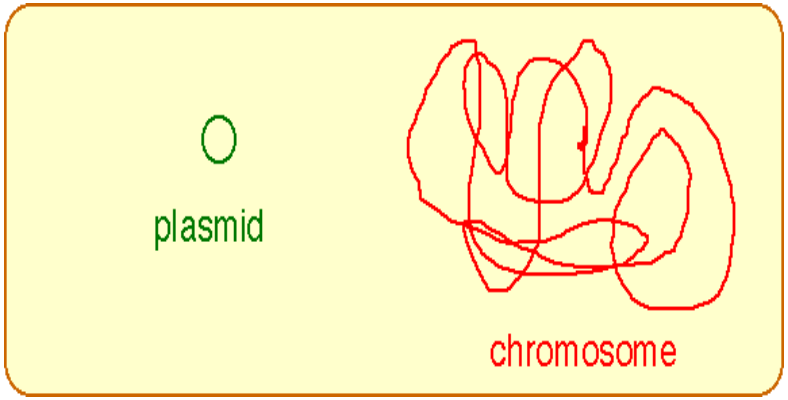
- **Cloning vectors** are DNA molecules that are used to "transport" cloned sequences between biological hosts and the test tube.

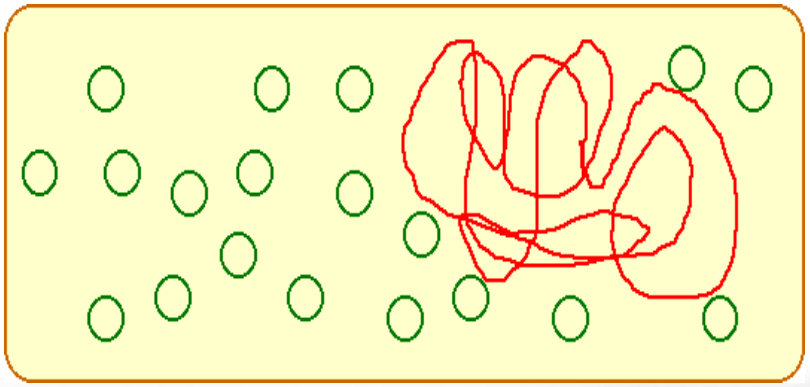
Cloning vectors share four common properties:

1. Ability to replicate.
2. Has a Origin of Replication site (ORI)
3. Contain a genetic marker selection.
4. Has a small molecular weight
5. Unique restriction sites to facilitate cloning of insert DNA
6. Can be induction for high copy number

PLASMIDS

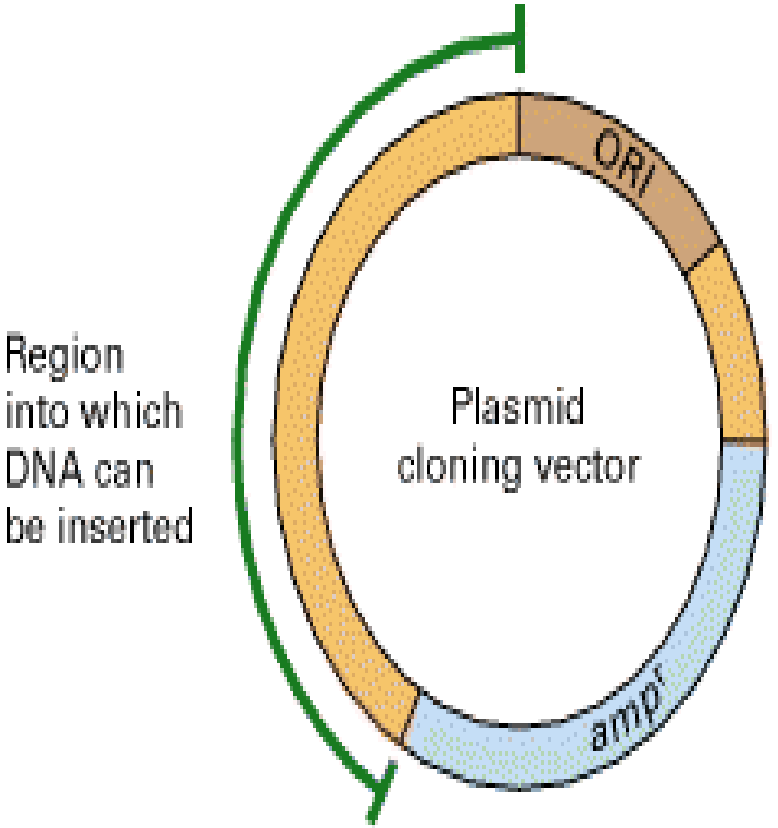
- Bacterial cells may contain extra-chromosomal DNA called plasmids.
- Plasmids are usually represented by small, circular DNA.
- Some plasmids are present in multiple copies in the cell





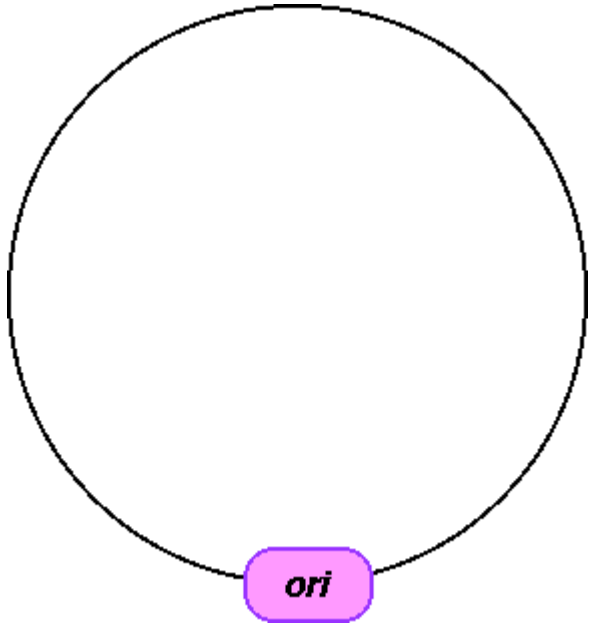
PLASMID VECTORS

- Plasmid vectors are ≈1.2–3kb and contain:
- replication origin (ORI) sequence
- a gene that permits selection,
- Here the selective gene is *ampr*; it encodes the enzyme b-lactamase, which inactivates ampicillin.
- Exogenous DNA can be inserted into the bracketed region .



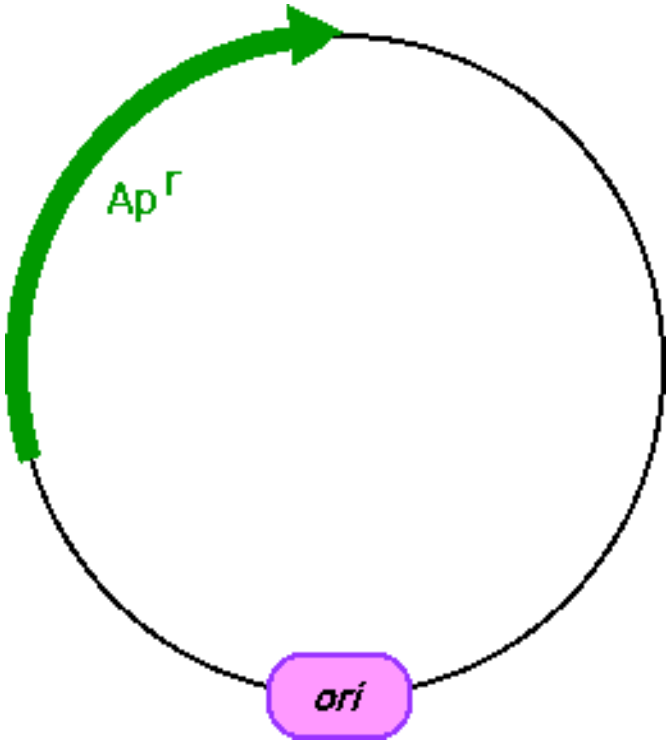
ORIGIN OF REPLICATION (ORI)

- **Origin of replication** is a DNA segment recognized by the cellular DNA-replication enzymes.
- Without replication origin, DNA cannot be replicated in the cell.



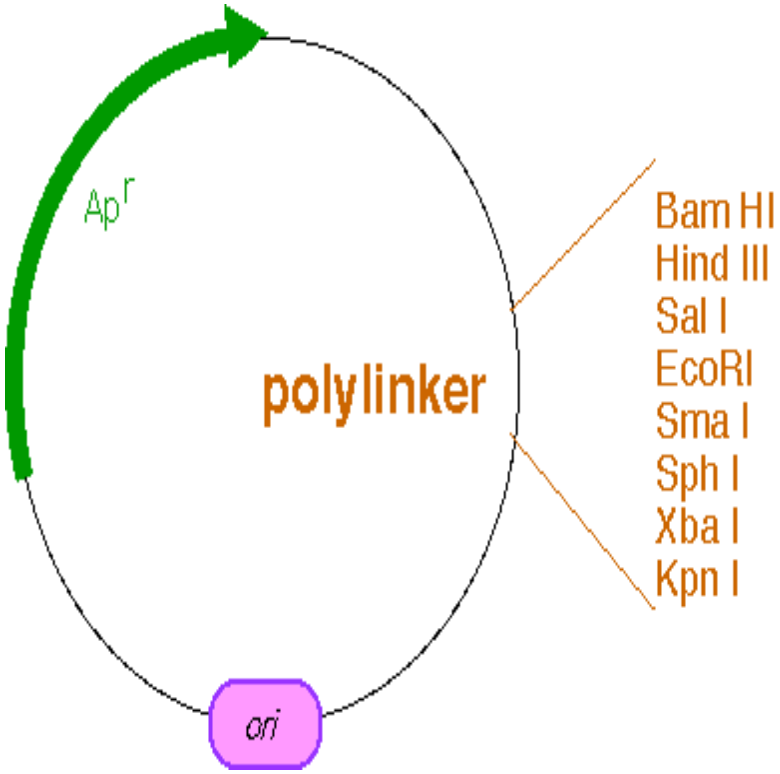
SELECTIVE MARKER

- **Selective marker** is required for maintenance of plasmid in the cell.
- Because of the presence of the selective marker the plasmid becomes useful for the cell.
- Under the selective conditions, only cells that contain plasmids with selectable marker can survive
- Genes that confer resistance to various antibiotics are used.
- Genes that make cells resistant to ampicillin, neomycin, or chloramphenicol are used



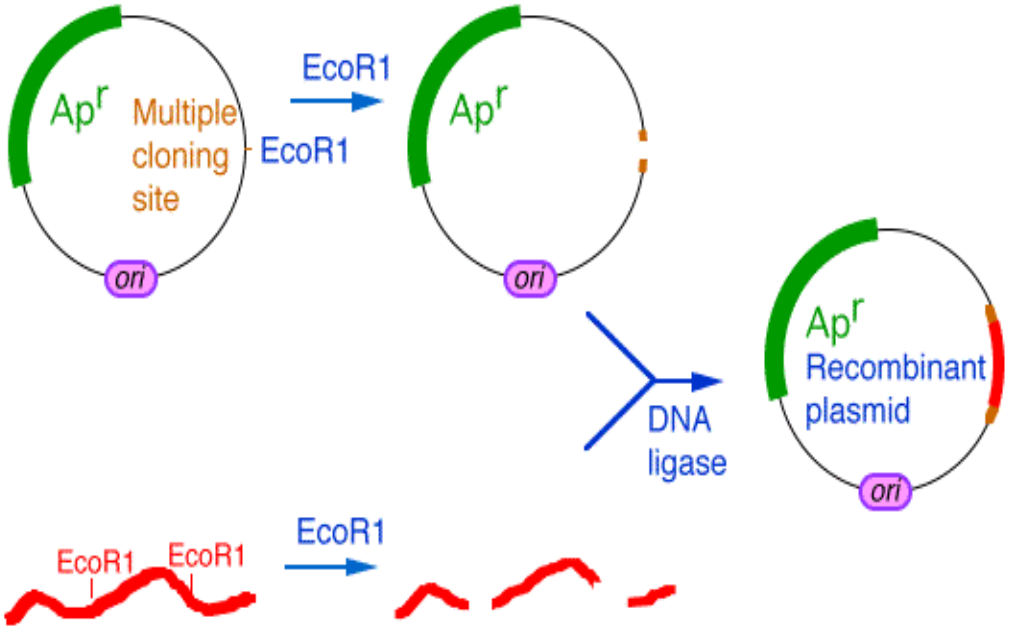
MULTIPLE CLONING SITE (MCS)

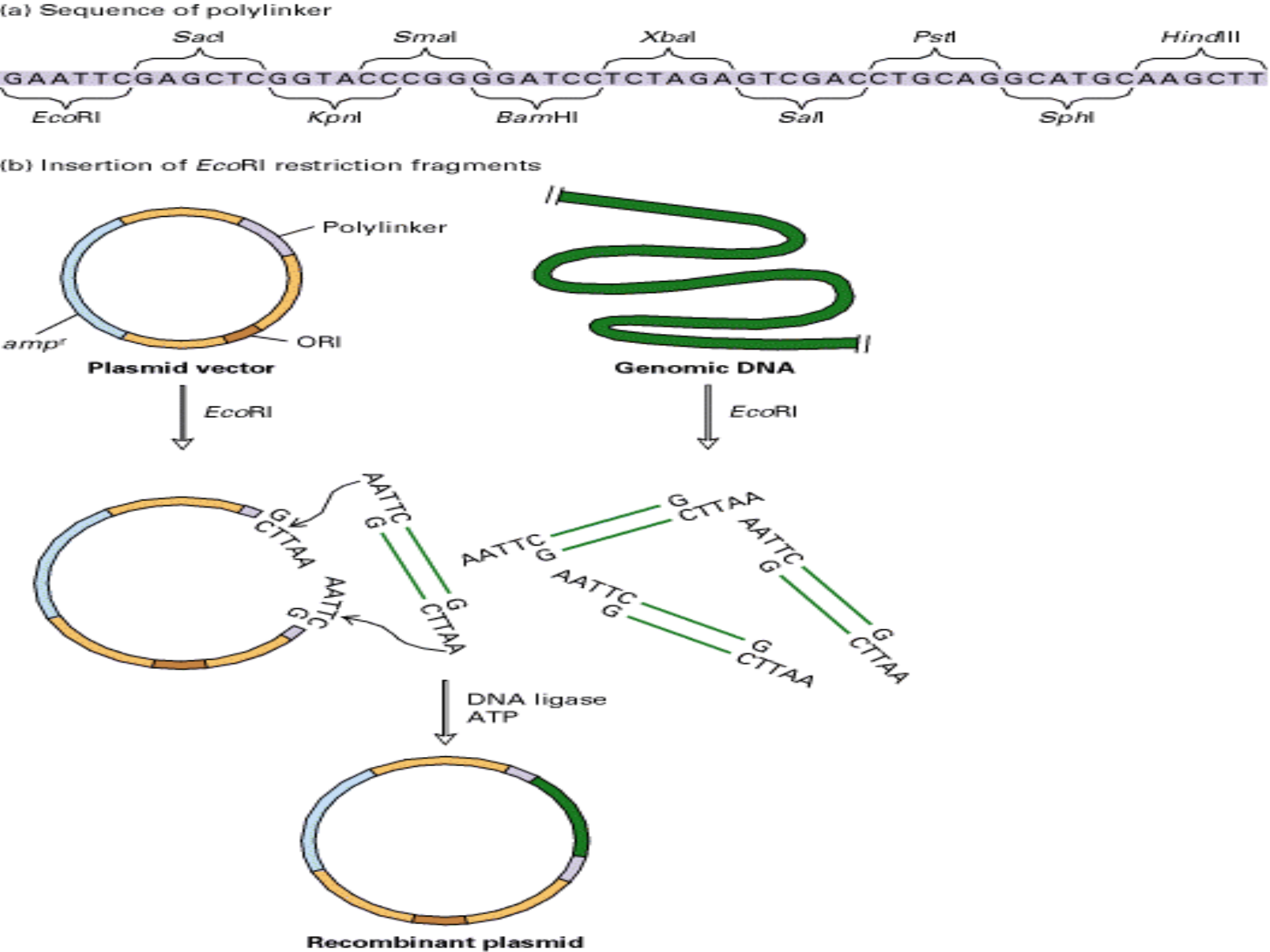
- disebut juga polylinker, merupakan situs yang dikenali oleh restriksi endonuklease
- Bersifat spesifik
- Tempat penyisipan gen



MULTIPLE CLONING SITE

- Gene to be cloned can be introduced into the cloning vector at one of the restriction sites present in the polylinker




(a) Sequence of polylinker
SacI
SmaI
XbaI
PstI
HindIII
GAATTCGAGCTCGGTACCCGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTT
EcoRI
KpnI
BamHI
SalI
SphI
(b) Insertion of EcoRI restriction fragments
Polylinker
ampr
ORI
Plasmid vector
Genomic DNA
EcoRI
EcoRI
AATTC
G
CTTAA
DNA ligase
ATP
Recombinant plasmid

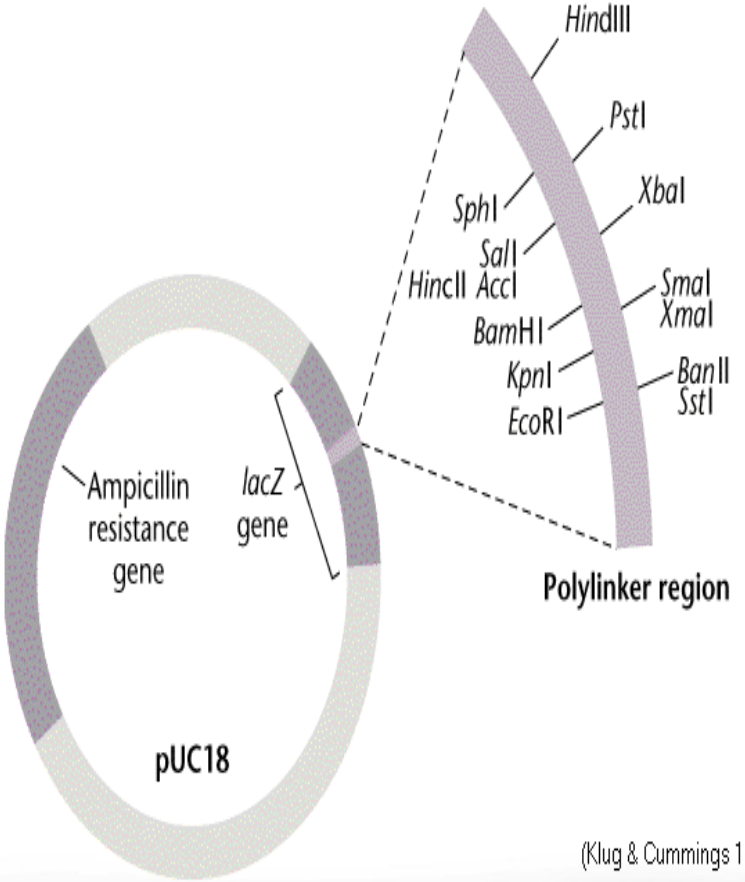
TYPES OF CLONING VECTORS

CLONING VECTORS

- Different types of cloning vectors are used for different types of cloning experiments.
- Tiap tipe digunakan untuk tujuan eksperimen yang berbeda. Dipilih berdasarkan :
- Ukuran fragmen
  - ✓ Ukuran vektor o
  - ✓ Situs restriksi
  - ✓ Jumlah kopi
  - ✓ Efisiensi kloning
  - ✓ Kemampuan utk ditapiskan
  - ✓ Tujuan eksperimen

PLASMID VECTORS

- Plasmid vectors are used to clone DNA ranging in size from several base pairs to several thousands of base pairs (100bp -10kb).
- Generally circular plasmids
- Ex. pGEM3, pBlueScript, pGEM-T easy



(Klug & Cummings 1997)

Disadvantages using plasmids

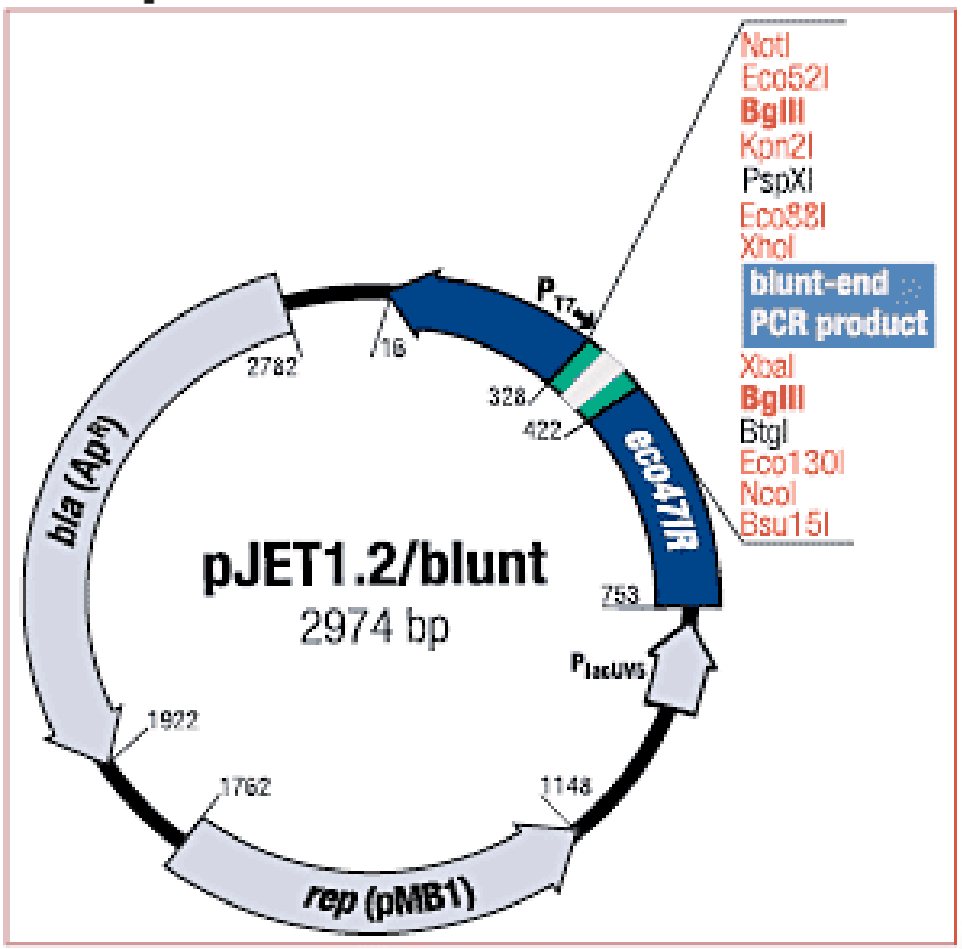
Kelemahan: o

- Tidak untuk fragmen DNA besar
- Ukuran 0-10 kb

Kelebihan:

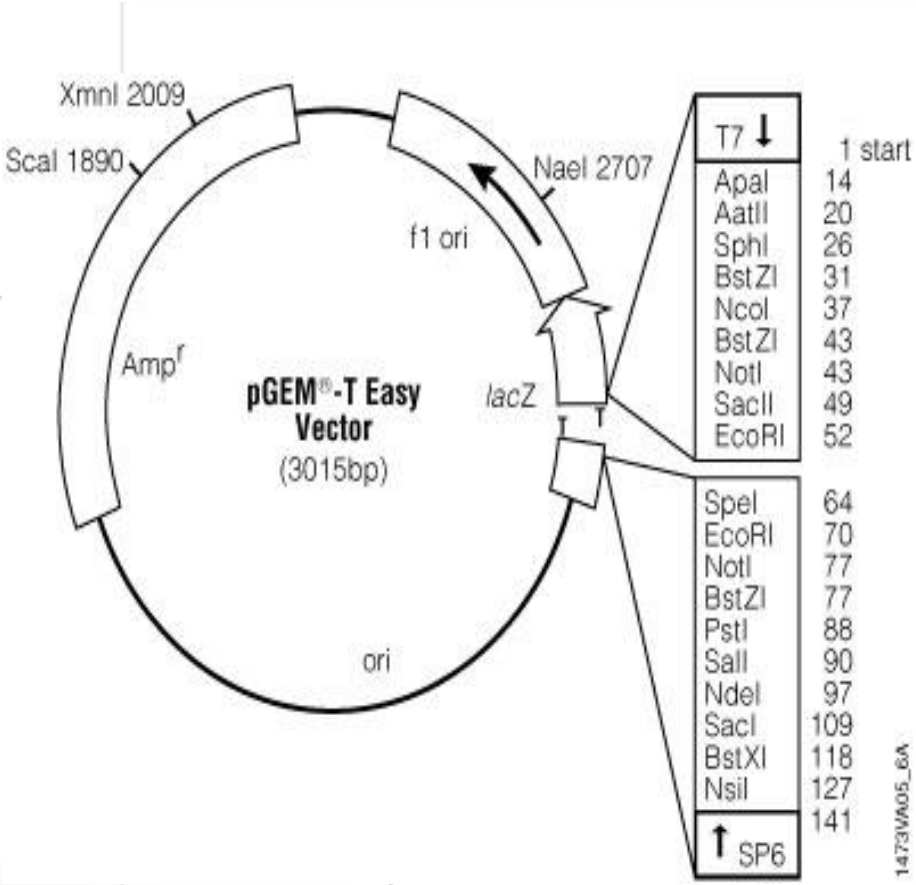
- Berukuran kecil,
- mudah dihandle
- Cocok untuk kloning fragmen kecil
- Transformasi efisien

pJET1.2/blunt untuk pustaka Genom



- bersifat linear
- dapat menerima sisipan dari 6 kb sampai 10 kb
- Pada ujung 5’ vektor mengandung gugus fosforil sehingga forforilasi primer tidak diperlukan
- mengandung *lethal gene*(gen mematikan)

pGEMT-Easy Vektor

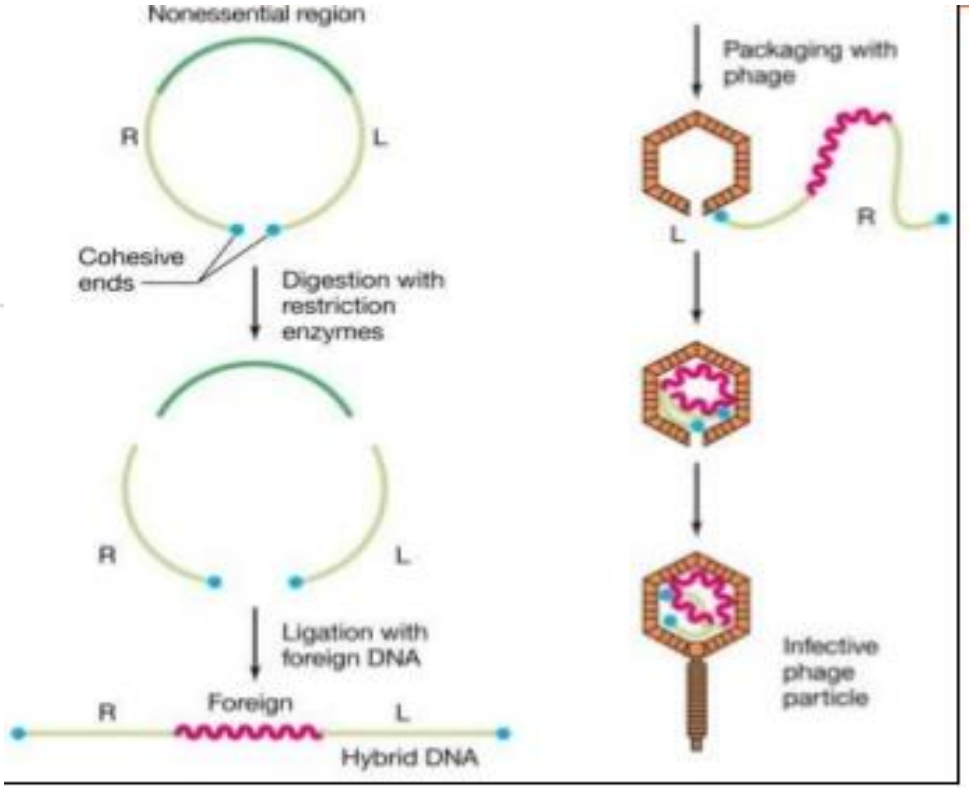


- bersifat Sirkular
- dapat menerima sisipan dari 100 bp – 1000 bp
- ukuran yang relatif kecil,
- mempunyai ori untuk replikasi dalam E. coli,
- membawa gen ketahanan untuk ampisilin
- Mempunyai multiple cloning site MCS
- Ada penambahan basa timin (T overhang) pada ujung 3 sehingga produk PCR dapat langsung di ligasikan tanpa ada perlakukan RE

BACTERIOPHAGE LAMBDA

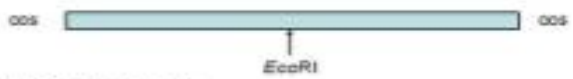
- Virus penginfeksi E.coli
- Ukuran fragmen 5-25 kbp ds DNA
- Memiliki 5’ twelve-base-pair sticky end (cos site)
- Replikasi di dalam inang
- Harus membawa satu atau lebih marker selektif
- Harus memiliki situs restriksi pada bagian DNA

BACTERIOPHAGE LAMBDA



Lambda vectors

- 1) Insertion vectors



- 2) Replacement vectors

- http://www.sh.lsuhsc.edu/gradcore/IDSP117/16

VEKTOR BERKAPASITAS BESAR

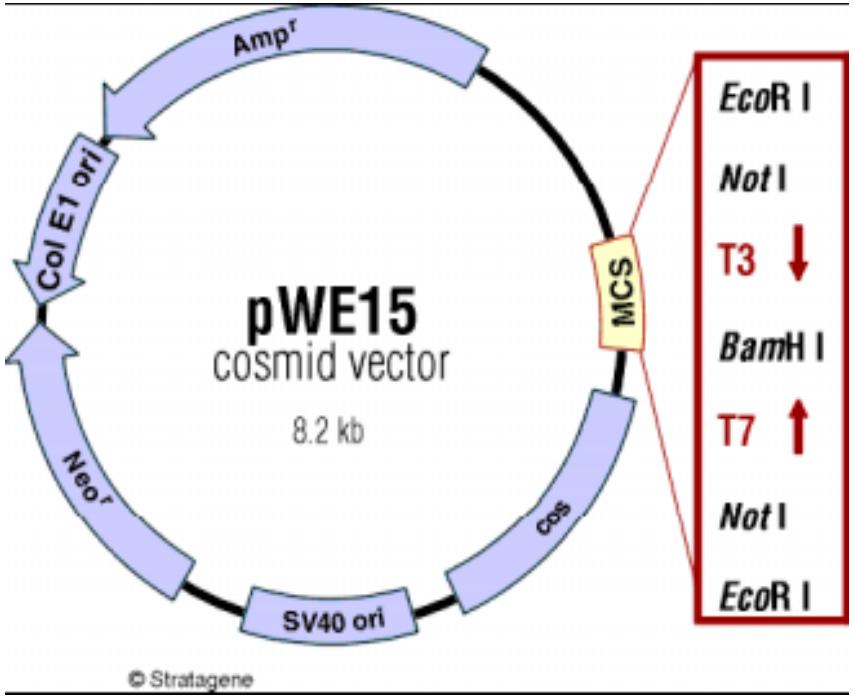
Cosmid (<50kbp), PAC dan BAC (100-300kbp), YAC (20- 2000kbp)

Manfaat :

- Memperkecil jumlah klon pustaka DNA yang harus dibuat
- Memudahkan penyimpanan.
- Mempercepat proses seleksi transformant target

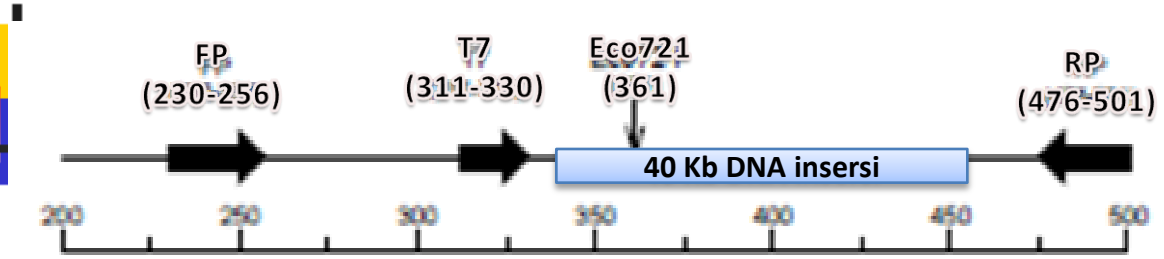
COSMID VECTOR

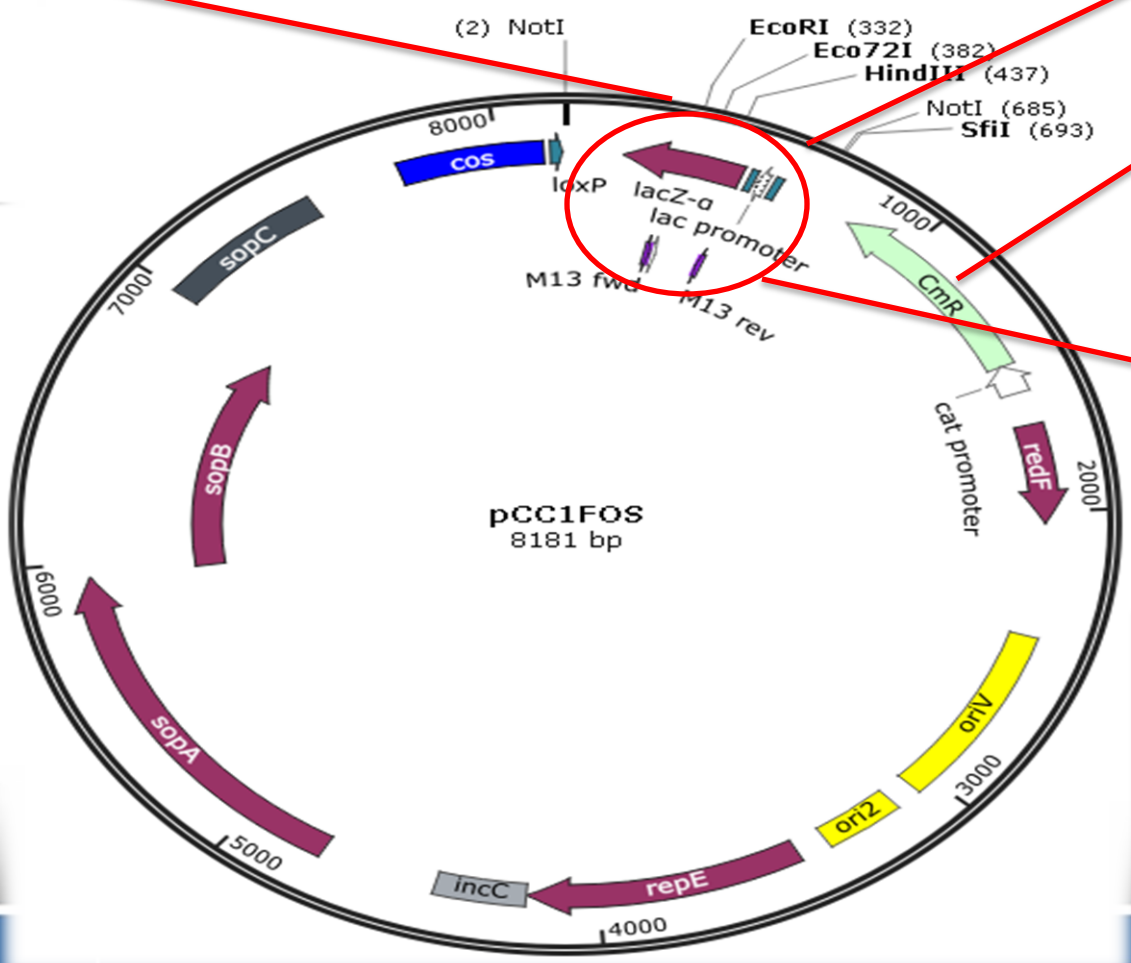
- **Purpose:**
  1. Clone large inserts of DNA: size ~ 45 kb
- **Features:**
  Cosmids are Plasmids with one or two Lambda Cos sites.
- Presence of the Cos site permits *in vitro* packaging of cosmid DNA into Lambda particles



FOsmid VECTOR

- Vektor sintetis yang merupakan gabungan antara plasmid dan fag l.
- Vektor yang dinamakan fosmid ini membawa segmen DNA l yang berisi tempat att
- Vektor ini memiliki ukuran 8.1 kb
- Bersifat linear
- dapat menerima sisipan 40 kb fragmen DNA
- membawa gen resisten kloramfenikol sebagai seleksi antibiotik dan gen LacZ sebagai seleksi biru putih
- dapat diinduksi untuk mendapatkan *high copy number*





Seleksi antibiotik cloramphenicol (*Cam*)

Seleksi biru putih

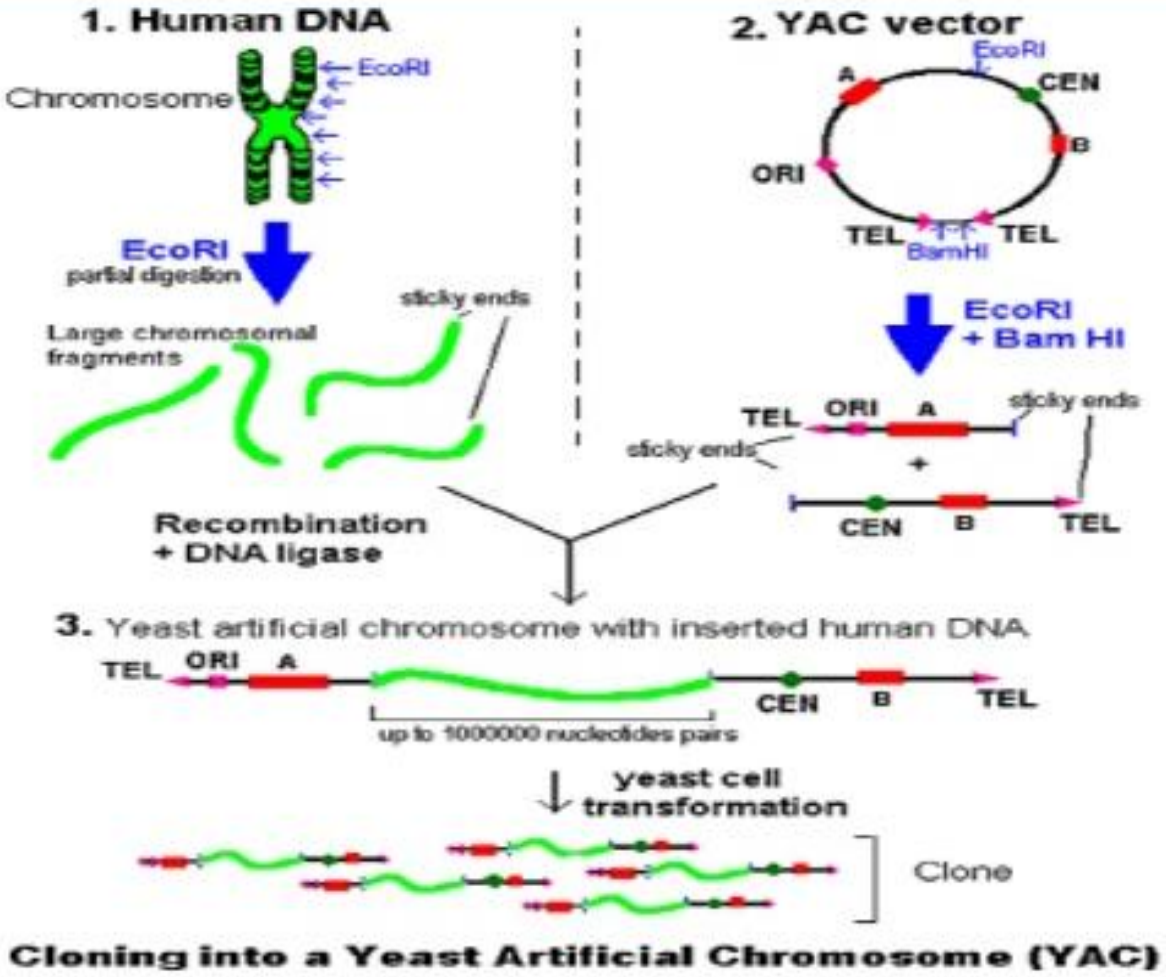
Peta CopyControl™ Fosmid Library pCC1FOS™ (Epicentre)

Yeast Artificial Chromosomes (YAC)

- Cloning vehicles that propogate in eukaryotic cell hosts as eukaryotic Chromosomes
- Linear DNA vektor
- Yeast Artificial Chromosome containing :
    - Yeast ORI/ARS
    - Centromer o
    - Telomer pada tiap ujung
    - Marker selektif
- Clone **very** large inserts of DNA: 100 kb - 10 Mb
- **YAC** vektor can carry handreds of thousands of base pairs foreign DNA

Yeast Artificial Chromosomes (YAC)

- **Additional features:**
- Often have a selection for an insert
- Sering ditemukan khimaera (DNA yang disisipkan dalam vektor kloning berasal dari 2 sumber/ area kromosom yang berbeda)
- The YAC can use both yeast and bacteria as a host



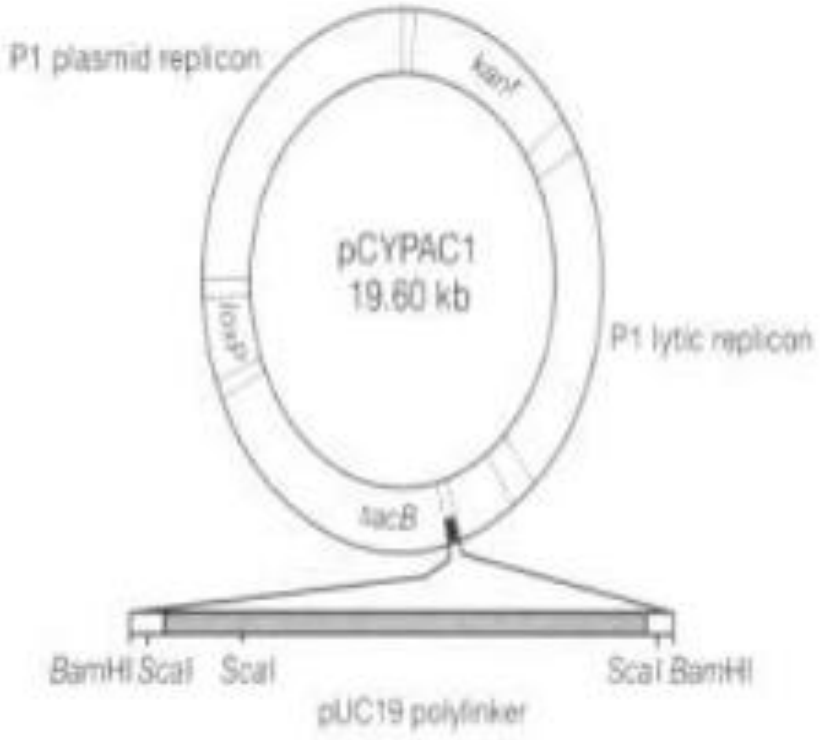
Cloning into a Yeast Artificial Chromosome (YAC)

PACs and BACs

- PAC (P1-derived Artificial Chromosomes)
- sistem kloning untuk isolasi DNA genomik berdasarkan F-factor plasmid
- mengandung beberapa elemen dari klon bakteriofag P1 (recombination or packaging site)
- Dapat membawa sekuens insersi hingga beratus kbp. Perkembangbiakan di dlm sel E.coli

- BACs - Bacterial Artificial Chromosomes
- Sistem kloning utk isolasi DNA berdasarkan F-faktor plasmid dengan jumlah kopian rendah
- Dapat diklon seperti plasmid dalam sel bakteri dan dapat membawa sekuens insersi beberapa ratus kbp (300kbp)
- lebih sedikit mengandung khimera dan efisiensi transformasi 100x dibanding YAC.

PACs and BACs



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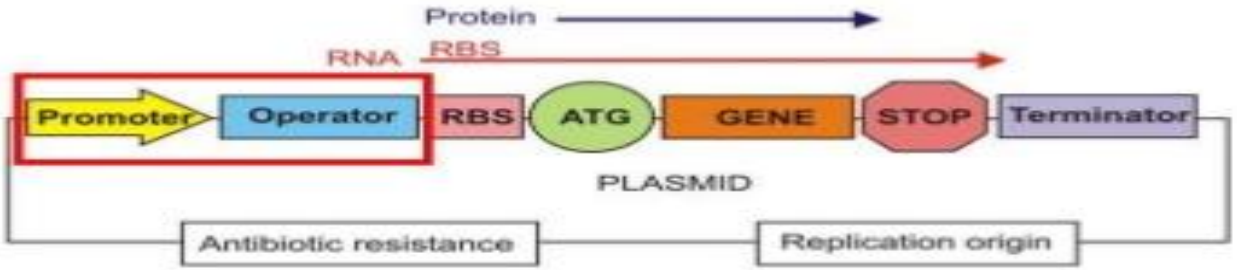
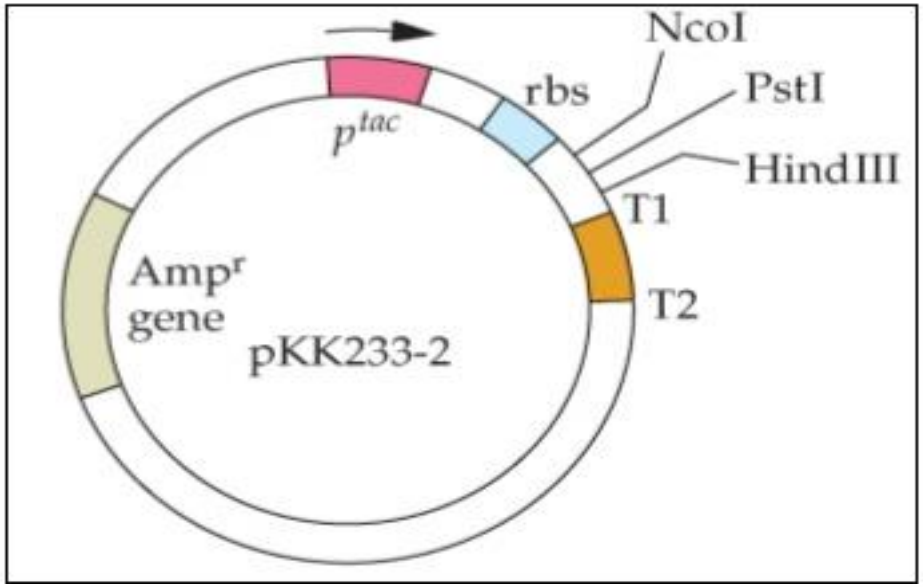
VEKTOR EKSPRESI

- Plasmid atau virus yang didesain untuk ekspresi protein.
- Digunakan misalnya untuk menghasilkan protein, misalnya insulin.
- Vektor ekspresi harus memiliki element ekspresi seperti:
  - Promotor,
  - RBS (ribosome binding site)
  - terminator,
  - start codon.



SISTEM EKSPRESi pada prokariot

- *E. coli, Bacillus subtilis, Staphylococcus carnosus, Streptomyces lividans*
- Prokaryotic promoter—ribosome binding site—MCS—transcription termination site
- Prokaryotic selectable marker

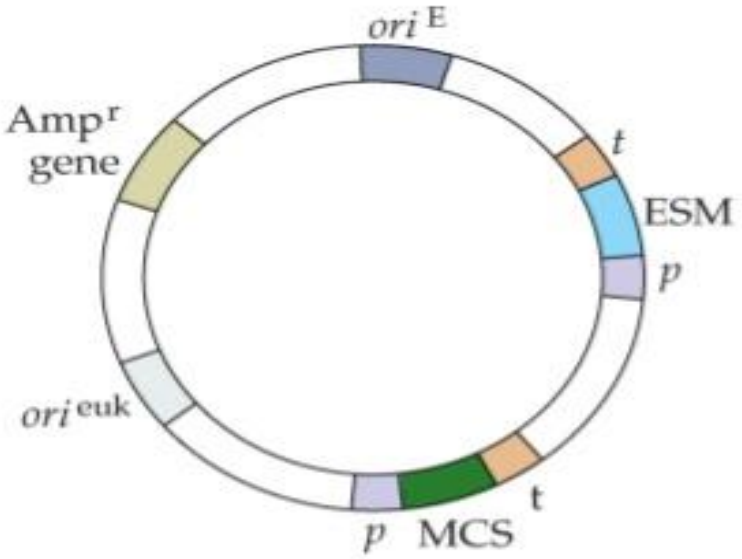


SISTEM EKSPRESi pada Eukariot

- Yeast, *Aspergillus niger*, Baculovirus-Insect Cells, Mammalian Cells (e.g. Chinese Hamster Ovary cells)
- Eukaryotic promoter-MCS-transcription termination site
- Eukaryotic selectable marker

Dapat dilakukan post translational modification.

- Glikosilasi
- Fosforilasi
- Hidroksilasi



RETROVIRAL VECTORS

- Vektor Retroviral digunakan untuk memperkenalkan gen baru atau diubah ke dalam genom sel manusia dan hewan.
- Retrovirus adalah virus RNA.
- RNA virus diubah menjadi DNA oleh reverse transcriptase virus dan kemudian diintegrasikan secara efisien ke dalam genom inang
- Gen asing atau bermutasi dimasukan ke dalam genom retroviral

SHUTTLE VECTORS

- Shuttle vectors dapat mereplikasi dalam dua organisme yang berbeda, mis. bakteri dan ragi, atau sel mamalia dan bakteri.
- Mereka memiliki asal-usul replikasi yang tepat.
- Oleh karena itu seseorang dapat mengkloning gen pada bakteri, mungkin memodifikasinya atau bermutasi dalam bakteri, dan menguji fungsinya dengan memasukkannya ke dalam ragi atau sel hewan


THANK
YOU
Novi Jan's Blog