Advance Genetic Engineering

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GENETIC ENGINEERING VECTORS

PART II : COSMIDS VIRUSES, EXPRESSION VECTORS & ARTIFICIAL CHROMOSOMES

Cosmids

-first described by Collins and Hohn in 1978

-hybrid plasmid that contains a Lambda phage *cos sequence*

-Cosmids can contain 37 to 52 (normally 45) kb of DNA,

- limits based on the normal bacteriophage packaging size

-They can replicate as plasmids if they have a suitable origin of replication

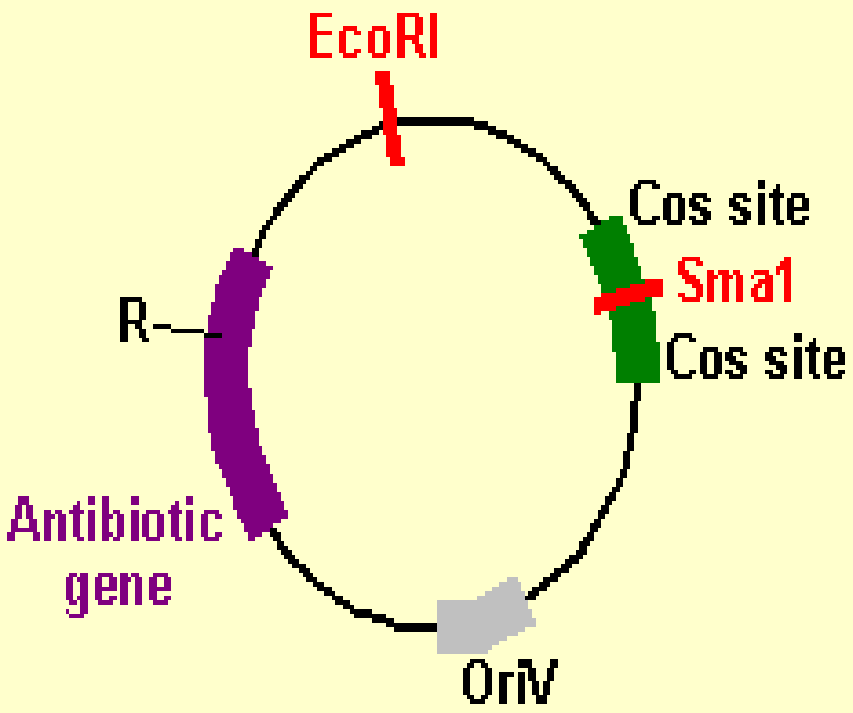
-contain a gene for selection such as antibiotic resistance

-Unlike plasmids, they can also be packaged in phage capsids

-*Cos* sequences are ~200 base pairs long and essential for packaging.

-They contain a *cosN* site where DNA is nicked at each strand, 12 bp apart, by terminase. This causes linearization of the circular cosmid with two "cohesive" or "sticky ends" of 12bp.

Basic Features of a Cosmid

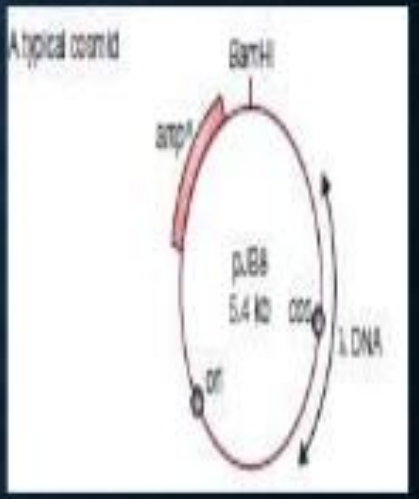


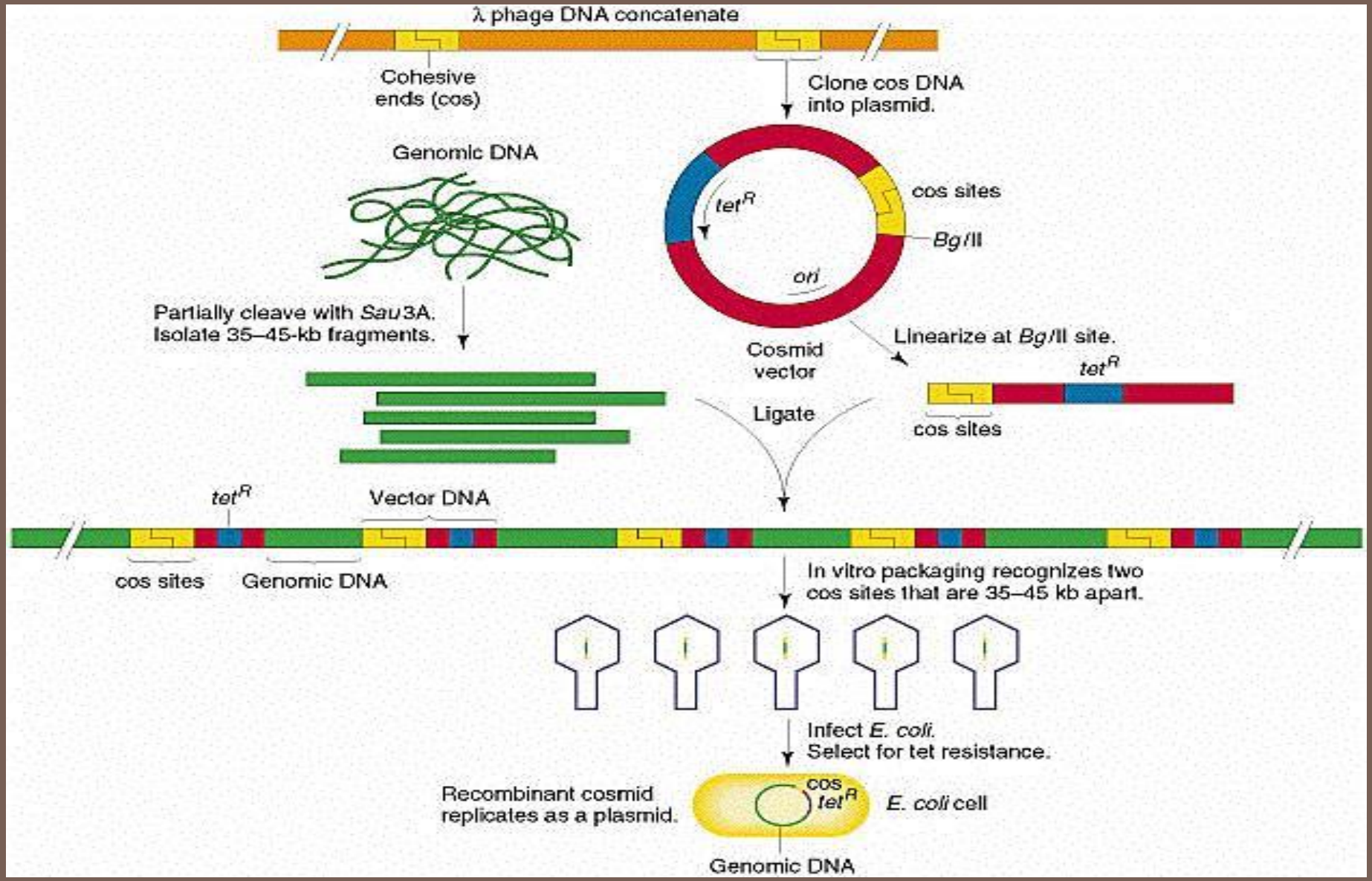
KEY

OriV - origin of replication.
Cos sites - provide blunt ends.
R - recombinant site
EcoRI, SmaI } - Restriction endonuclease recognition sequence.

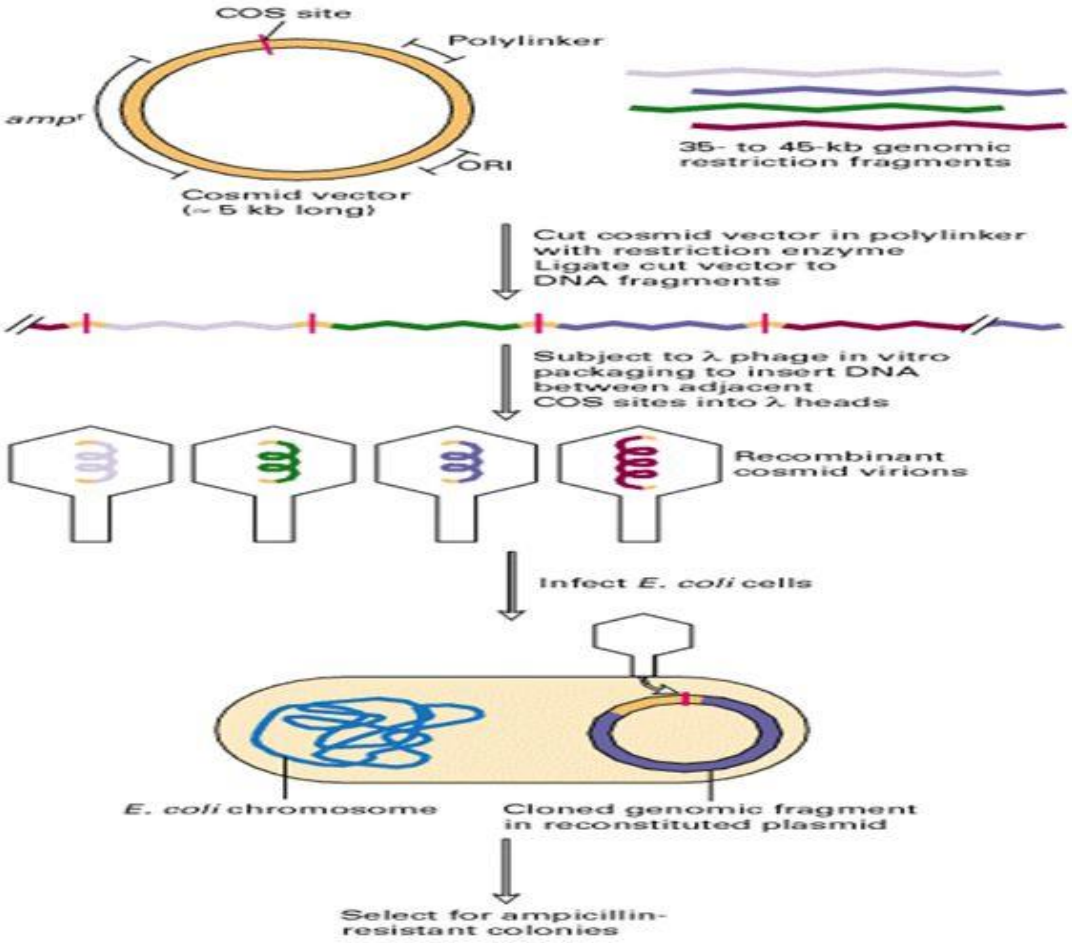
COSMIDS

- Cosmids are hybrids between a phage DNA molecule and a bacterial plasmid, and their design centers on the fact that the enzymes that package the λ DNA molecule into the phage protein coat need only the cos sites in order to function.
- The in vitro packaging reaction works not only with λ genomes, but also with any molecule that carries *cos* sites separated by 37–52 kb of DNA.
- A cosmid is basically a plasmid that carries a *cos* site .
- It also needs a *selectable marker*, such as the ampicillin resistance gene, and a plasmid origin of replication, as cosmids lack all the λ genes and so do not produce plaques.
- Instead colonies are formed on selective media, just as with a plasmid vector.




λ phage DNA concatenate
Cohesive ends (cos)
Clone cos DNA into plasmid.
Genomic DNA
tetR
cos sites
BglII
ori
Partially cleave with Sau3A. Isolate 35–45-kb fragments.
Cosmid vector
Linearize at BglII site.
tetR
cos sites
Ligate
tetR
Vector DNA
cos sites
Genomic DNA
In vitro packaging recognizes two cos sites that are 35–45 kb apart.
Infect E. coli. Select for tet resistance.
Recombinant cosmid replicates as a plasmid.
cos
tetR
E. coli cell
Genomic DNA

Cosmids


COS site
Polylinker
ampr
ORI
Cosmid vector
(~ 5 kb long)
35- to 45-kb genomic
restriction fragments
Cut cosmid vector in polylinker
with restriction enzyme
Ligate cut vector to
DNA fragments
Subject to λ phage in vitro
packaging to insert DNA
between adjacent
COS sites into λ heads
Recombinant
cosmid virions
Infect E. coli cells
E. coli chromosome
Cloned genomic fragment
in reconstituted plasmid
Select for ampicillin-
resistant colonies

Problems associated with lambda and cosmid cloning

-Since repeats occur in eukaryotic DNA rearrangements can occur via recombination of the repeats present on the DNA inserted into lambda or cosmid.

-Cosmids are difficult to maintain in a bacterial cell because they are somewhat unstable.

VIRUSES

- They are complex molecules with unsafe genome, hence they are with limited application in cloning.

-Viral vectors are generally genetically - engineered viruses carrying modified viral DNA or RNA that has been rendered noninfectious, but still contain viral promoters and also the transgene, thus allowing for translation of the transgene through a viral promoter.

-because viral vectors frequently are - lacking infectious sequences, they require helper viruses or packaging lines for large-scale transfection.

-Viral vectors are often designed for permanent incorporation of the insert into the host genome.

Examples: CamV, SV40, BPV

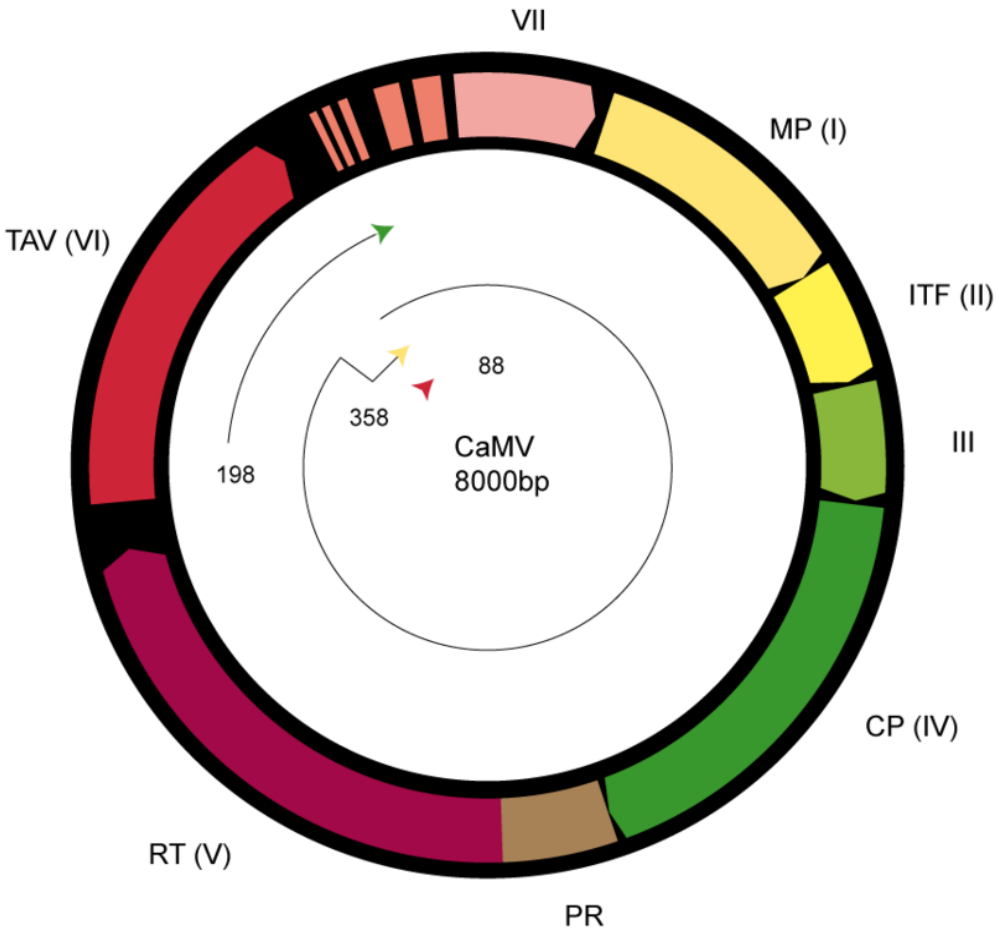
Caulimo viruse – Cauliflower mosaic virus-CamV

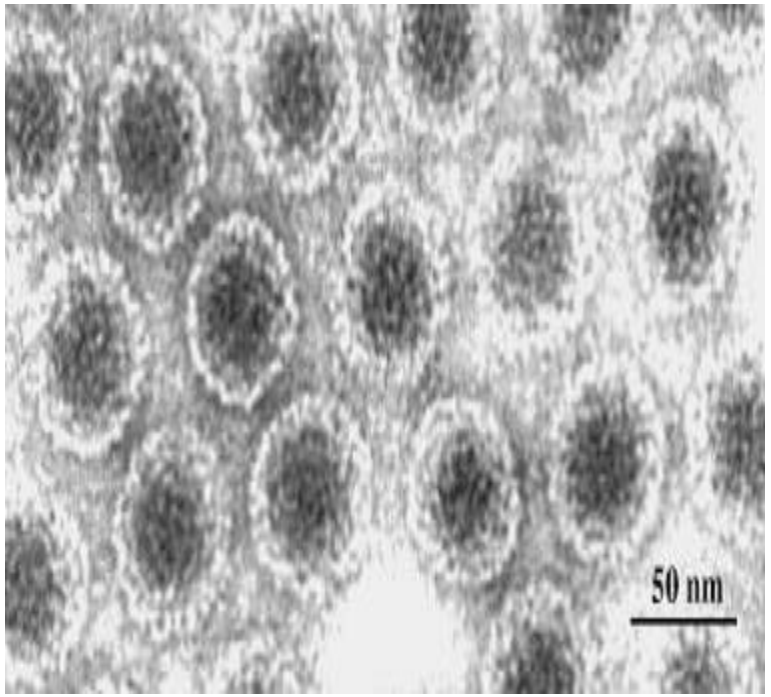
-a member of the genus *Caulimovirus*, that infect plants.

-Pararetroviruses replicate through reverse transcription just like retroviruses, but the viral particles contain DNA instead of RNA.

CaMV infects mostly plants of - the *Brassicaceae* family (such as cauliflower and turnip) but some CaMV strains (D4 and W260) are also able to infect *Solanaceae* species of the genera *Datura* and *Nicotiana*.

-CaMV induces a variety of systemic symptoms such as - mosaic, necrotic lesions on leaf surfaces, stunted growth, and deformation of the overall plant structure. -
- CaMV contains a circular double-stranded DNA - molecule of about 8.0 kb with 6 genes plus intergenic region.
-After entering the host cell, these single stranded - "nicks" in the viral DNA are repaired, forming a supercoiled molecule that binds to histones. This DNA is transcribed into a full length.
-packaged as phage with 300bp capacity-

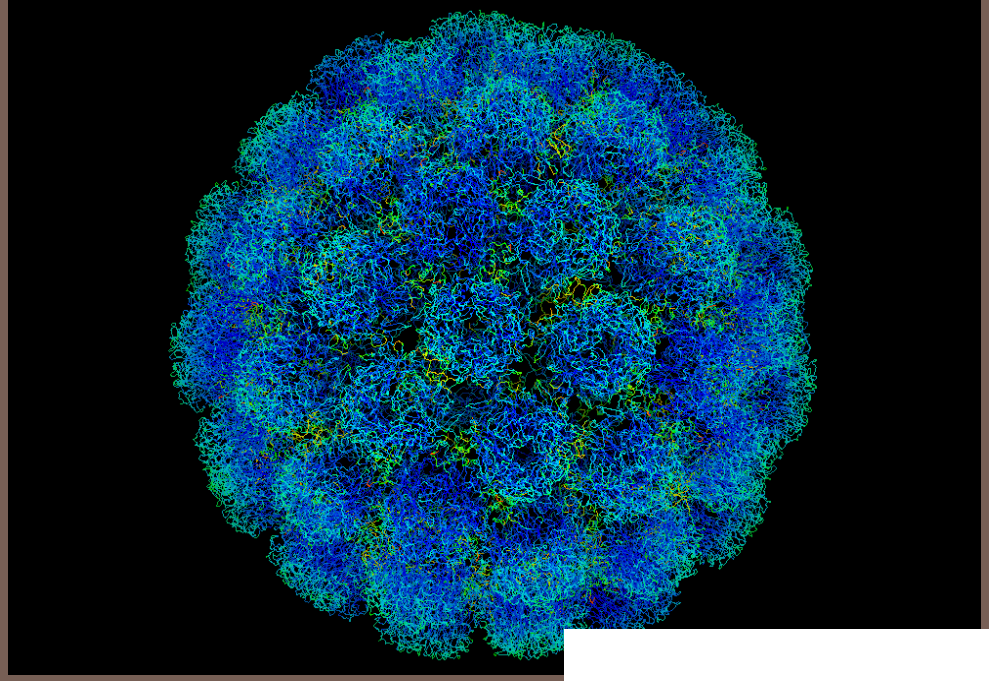

VII
MP (I)
ITF (II)
III
CP (IV)
PR
RT (V)
TAV (VI)
88
358
198
CaMV
8000bp

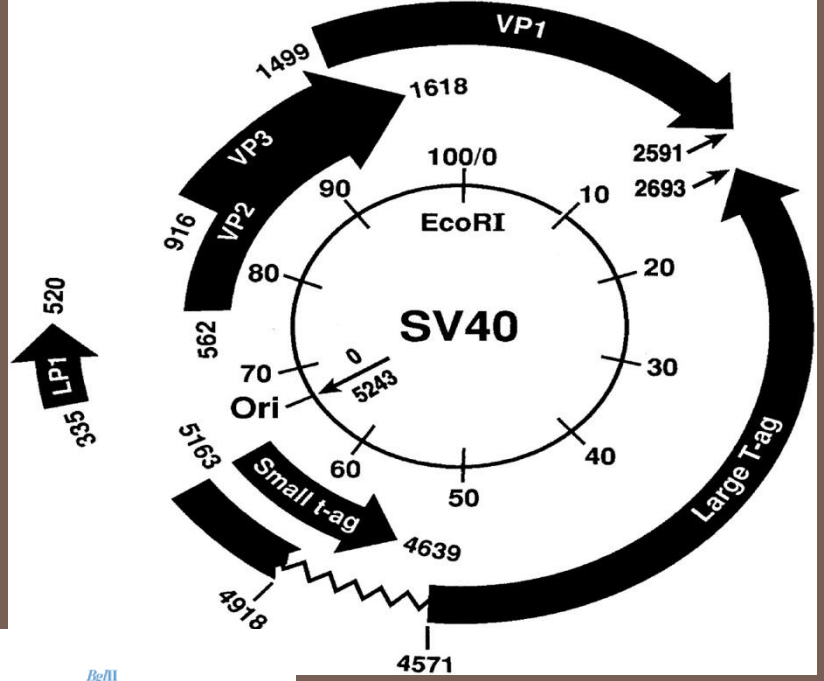

50 nm

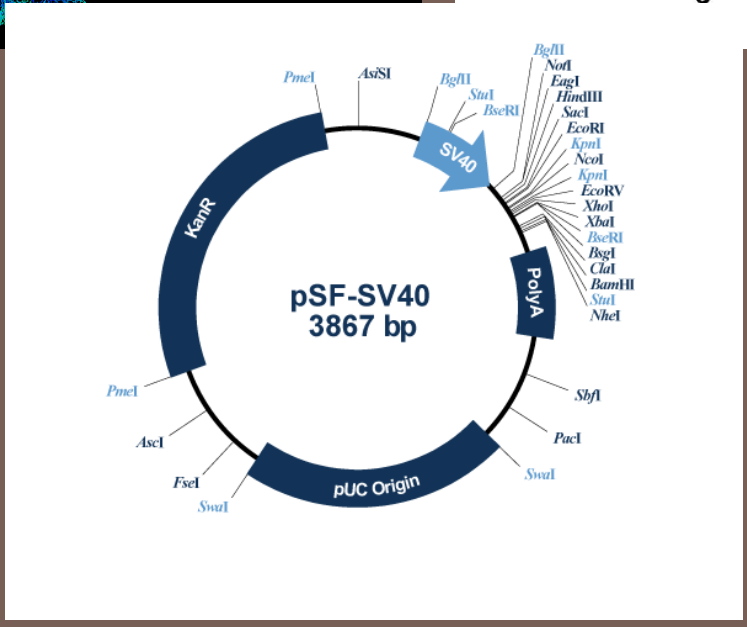

-3-

Simian 40 virus- SV40

-Mammalian virus
-5.2 Kb with 200-300bp capacity.
-need other virus for replication.
-a polyomavirus that is found in both monkeys and humans.
-It was named for the effect it produced on infected green monkey cells, which developed an unusual number of vacuoles.
-Like other polyomaviruses, SV40 is a DNA virus that has the potential to cause tumors in animals, but most often persists as a latent infection.




VP1
1499
1618
2591
2693
VP3
VP2
916
562
100/0
EcoRI
90
10
80
20
70
30
40
60
50
SV40
0
5243
Ori
520
LP1
335
5163
Small t-ag
4639
Large T-ag
4918
4571


pSF-SV40
3867 bp
KanR
SV40
PolyA
pUC Origin
PmeI
AsiSI
BglII
StuI
BseRI
NotI
EagI
HindIII
SacI
EcoRI
KpnI
NcoI
KpnI
EcoRV
XhoI
XbaI
BseRI
BsgI
ClaI
BamHI
StuI
NheI
SbfI
PacI
SwaI
PmeI
AscI
FseI
SwaI

Bovine Papillom virus

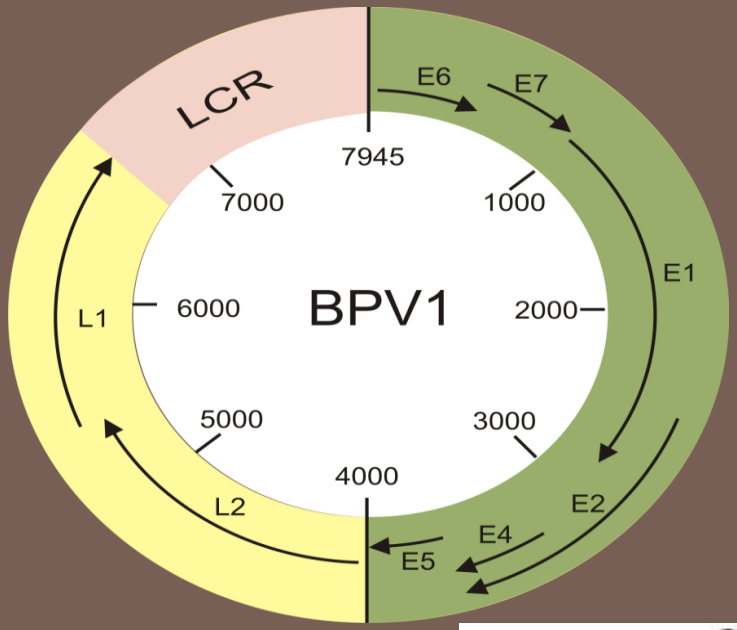
-A double circular DNA with 8 kb.-

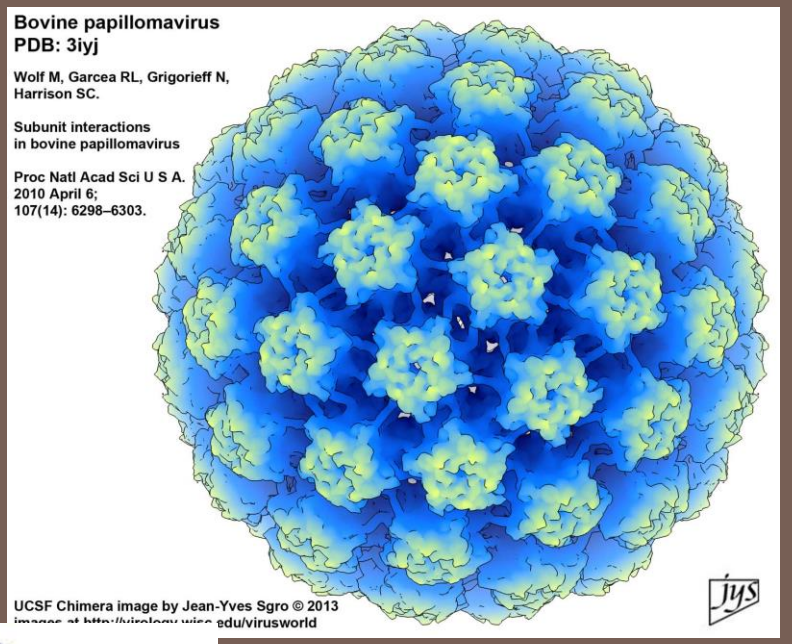
-Behave as plasmid inside cells with many copies.

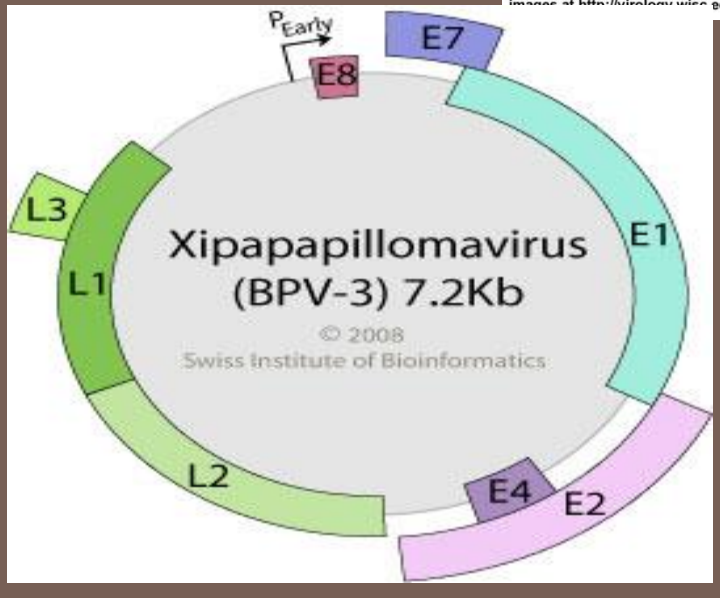
-Transmit from cells to their progeny.

-Need immuno compatibility with host .

-Modified with PBR 322 plasmid to increase capacity to 10 kb and to infect different types of human cells and bacteria.


LCR
E6
E7
7945
7000
1000
E1
L1
6000
BPV1
2000
5000
3000
4000
L2
E2
E4
E5


Bovine papillomavirus
PDB: 3iyj
Wolf M, Garcea RL, Grigorieff N, Harrison SC.
Subunit interactions in bovine papillomavirus
Proc Natl Acad Sci U S A. 2010 April 6; 107(14): 6298–6303.
UCSF Chimera image by Jean-Yves Sgro © 2013
jys


P Early
E8
E7
L3
E1
Xipapapillomavirus
(BPV-3) 7.2Kb
L1
© 2008
Swiss Institute of Bioinformatics
L2
E4
E2

Human cytomegalovirus

Next Week…Students Talk

Expression vectors

Vectors with suitable strong promoter and gene expression control sequences to have maximum gene expression.

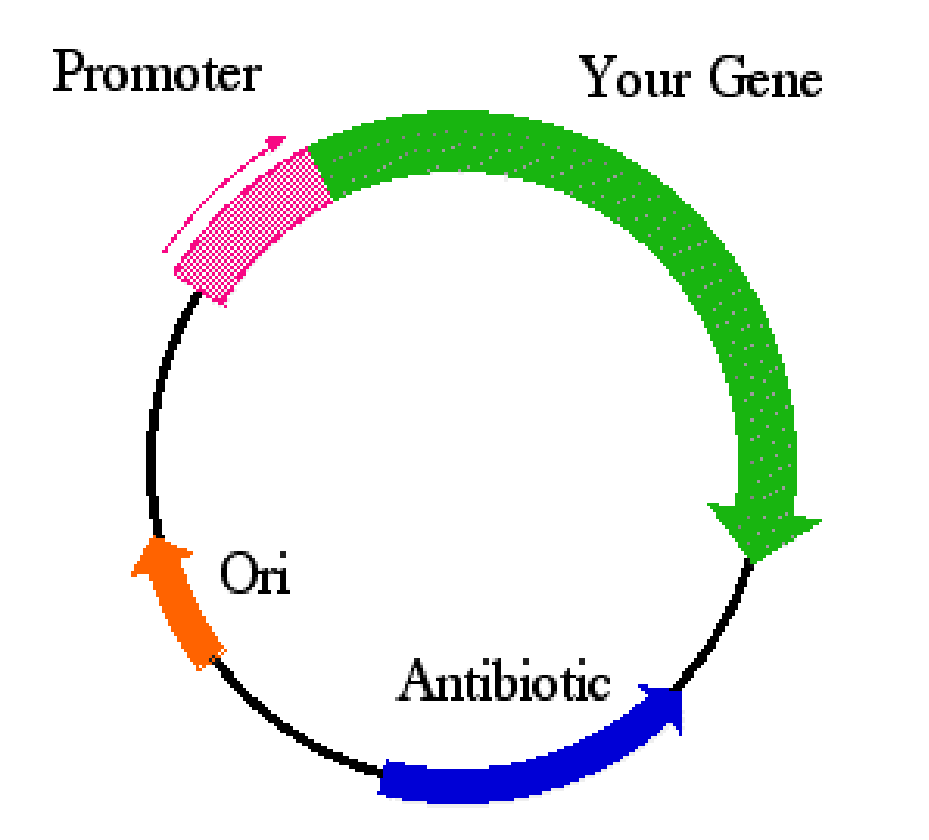
1.Insertion of a strong promoter.

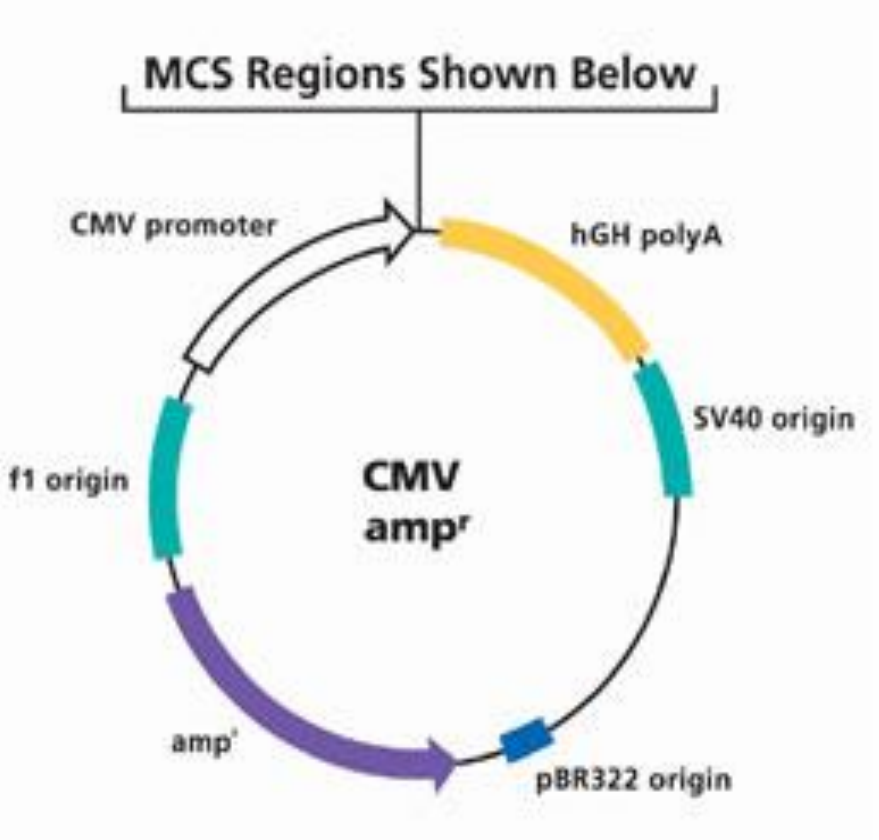
2.Insertion of a strong termination codon.

3.Adjustment of distance between promoter and cloned gene.

4.Insertion of transcription termination sequences.

5.Insertion of a strong translation initiation sequence.


Promoter
Your Gene
Ori
Antibiotic


MCS Regions Shown Below
CMV promoter
hGH polyA
SV40 origin
f1 origin
CMV
amp^r
amp'
pBR322 origin

Promoter	Expression Level	Applications
CMV	High	Commonly used in most cell lines (HeLa, HEK293, HT1080)
MCSV	High	Hematopoietic and Stem cells
EF1	Medium	Robust in most cell types, primary cells and Stem cells
PGK	Medium	Robust in most cell types, primary cells and Stem cells
UbC	Low	Low and steady in most cell types, primary cells and Stem cells

Types of expression vector

1. Simple EV
2. Cassette EV
3. Prokaryotes EV
4. Eukaryotes EV

Simple Expression vectors

1. With low or medium or strong promoter plus cloning site.

   e.g. Trp, tac, lac,lambda PL, rec A promoters.

2. Gene expression controlled by adding or omitting media materials.

e.g.

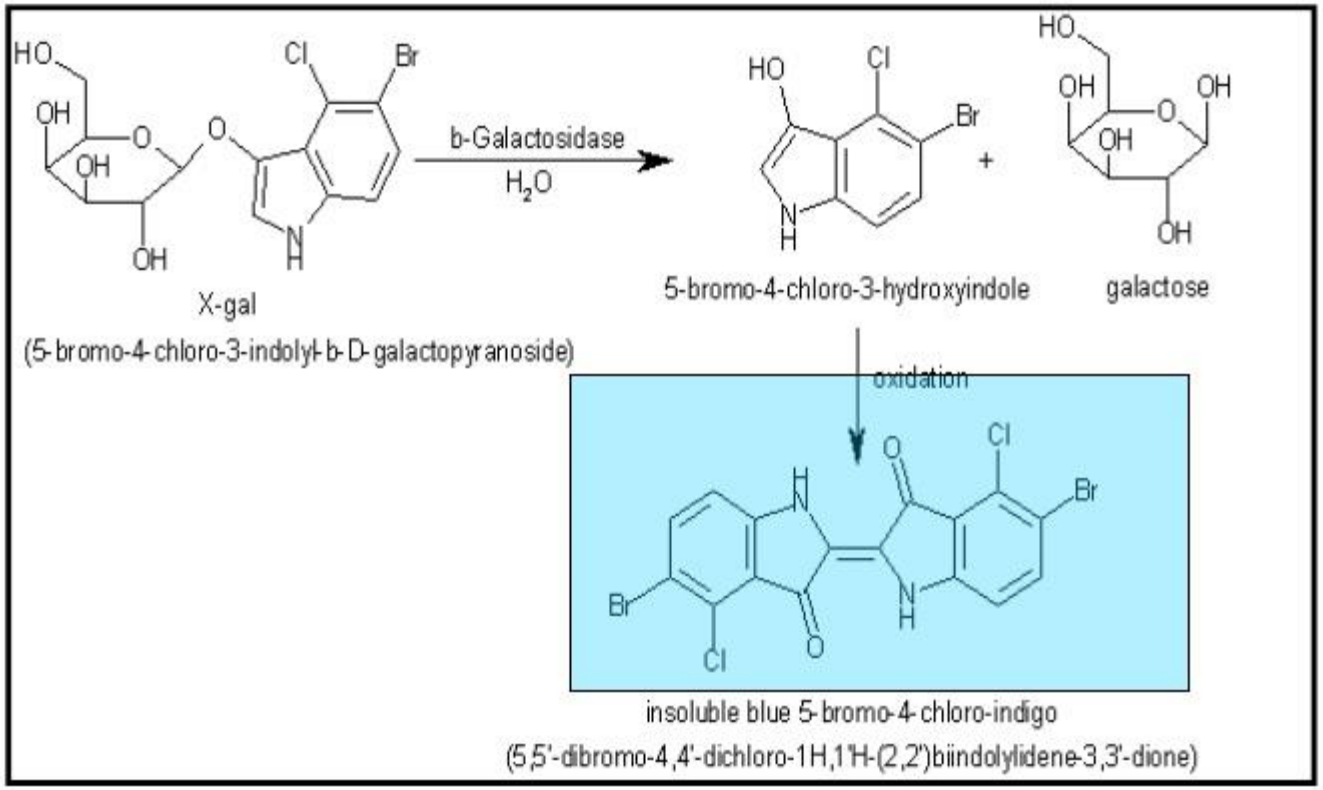
--Add tryptophan to stop expression and remove tryptophan for inducing expression.

--3-indoly acetic acid for trp

--isopropyl-B-D-thioglactoside-IPTG for Lac and tac

Problems?????

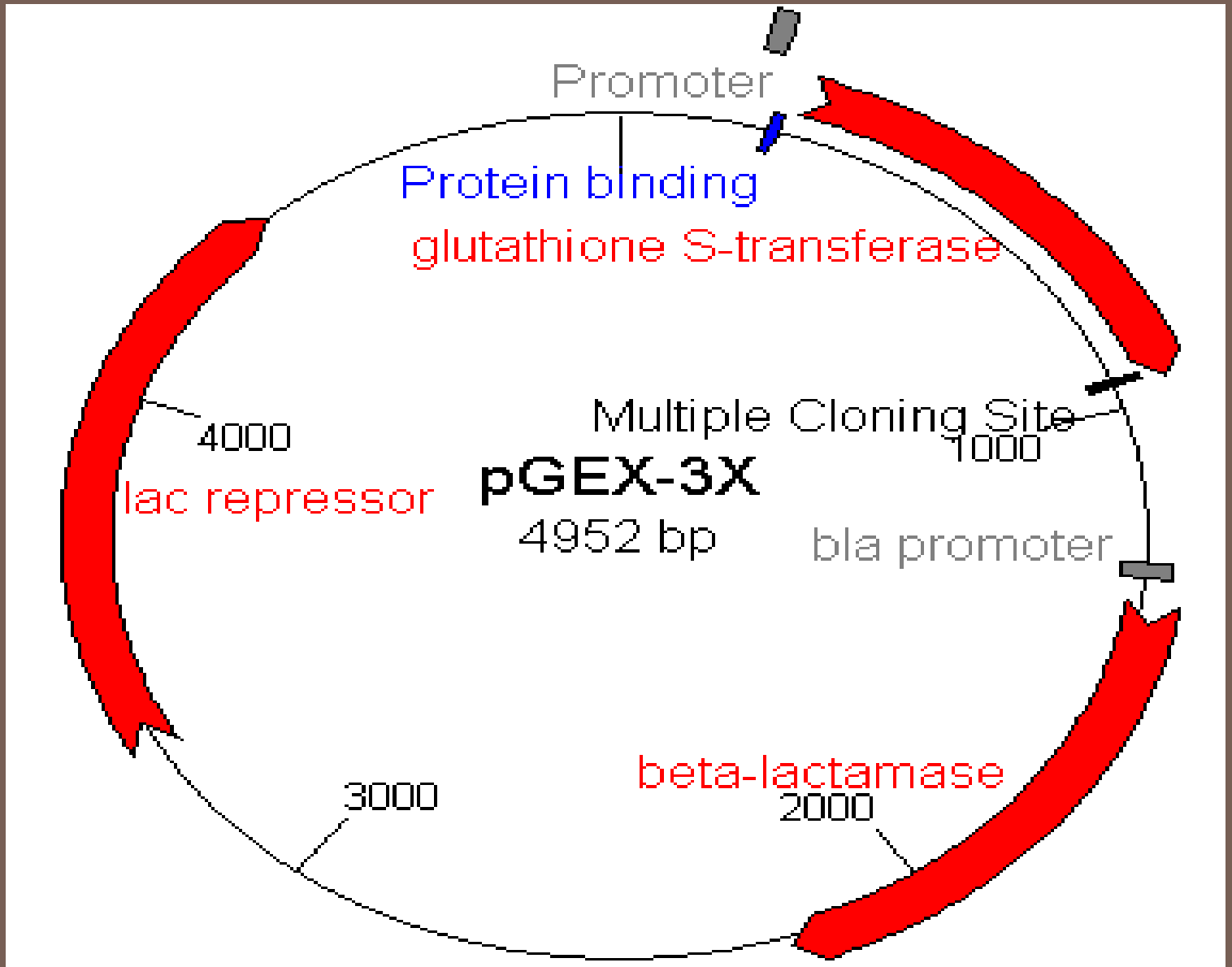
Based on the enzymatic reaction of β-galactosidase



Cassette Expression Vectors

1.with strong promoter.
2. With all gene expression parts.
3. With many restriction enzyme sites.
4. With ribosomal association site.
5. With terminator .

Problems????


Promoter
Protein binding
glutathione S-transferase
Multiple Cloning Site
1000
4000
lac repressor
pGEX-3X
4952 bp
bla promoter
beta-lactamase
2000
3000

Artificial chromosome

1. Constructed from cloned structural elements and centromere.
2. The best characterized human DNA associated with the centromeric region of the chromosomes is the human a-satellite present at the centromeres of all normal human chromosomes.

yeast artificial chromosomes (YAC)

The basis of a YAC are the vector arms, which contain all functions necessary for mitotic segregation in yeast, including a centromeric sequence (CEN), an autonomous replication sequence (ARS), and telomere sequences (TEL). In addition, the vector arms harbor selectable markers (*TRP1 and URA3, mediating* tryptophan and uracil autotrophy in suitable yeast hosts) and an interruptible marker containing the *EcoRI cloning site (SUP4-o, mediating a red/white* color selection for DNA insertion events). The most frequently used YAC vector is pYAC4 *(1), which, besides the elements previously mentioned, contains* the *Col E1 replication origin and the ampicillin selectable marker for propagation in Escherichia coli.*

YAC map and schematic of YAC cloning

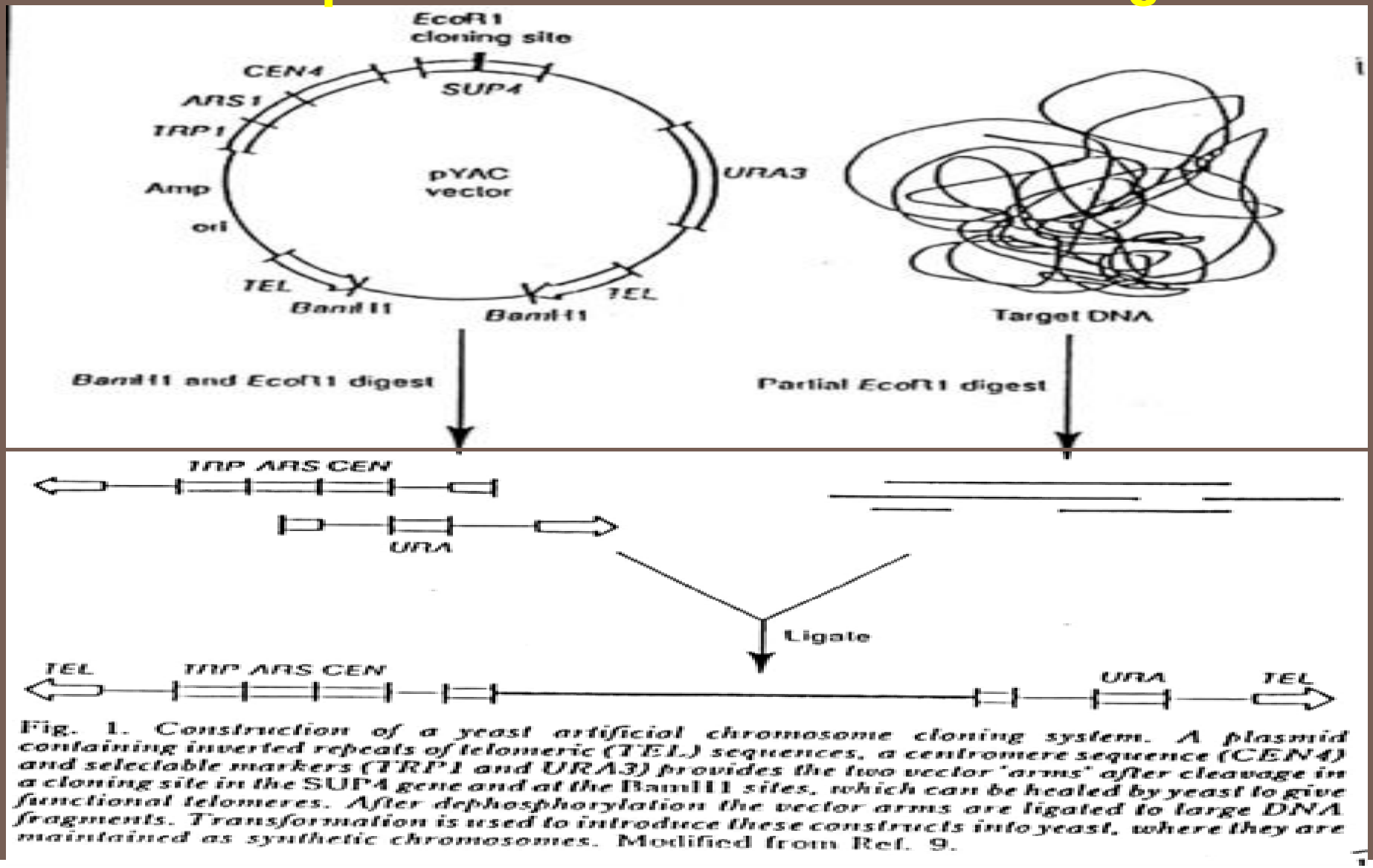


Fig. 1. Construction of a yeast artificial chromosome cloning system. A plasmid containing inverted repeats of telomeric (*TEL*) sequences, a centromere sequence (*CEN4*) and selectable markers (*TRP1* and *URA3*) provides the two vector "arms" after cleavage in a cloning site in the *SUP4* gene and at the BamH1 sites, which can be healed by yeast to give functional telomeres. After dephosphorylation the vector arms are ligated to large DNA fragments. Transformation is used to introduce these constructs into yeast, where they are maintained as synthetic chromosomes. Modified from Ref. 9.

Next week

you have talks about mammalian, bacterial and yeast artificial chromosomes