Plasmids are excellent cloning vectors

Figure 5.21
Figure 11.11

Ampicillin resistance

Polylinker

PUC19
2686 base pairs

lacZ'

Origin of DNA replication

Order of restriction enzyme cut sites in polylinker
Apo I - EcoRI
BanII - SacI
Acc651 - KpnI
Aval - BsoBI - Smal - Xmal
BamHI
XbaI
Accl - HincII - SalI
BspMI - BfuAI
SbfI
PstI
Sphi
HindIII
**Figure 11.12**

Vector

Foreign DNA

Digestion with restriction enzyme

Opened vector

Join with DNA ligase

Recyclized vector without insert

Vector plus foreign DNA insert

Transform into *Escherichia coli* and select on ampicillin plates containing Xgal

Transformants blue (β-galactosidase active)

Transformants white (β-galactosidase inactive)

Daniel Nickrent and David Clark
Figure 11.18

Capsid genes

J  att  int  xis  N  QSR  cos

Replaceable region

Wild-type lambda

β-Gal gene

Another substitution

Charon 4A
(replacement vector)

β-Gal gene

Another substitution

Charon 16
(insertional vector)

Wild-type lambda

Charon 4A
(replacement vector)

β-Gal gene

Another substitution

Charon 16
(insertional vector)

Wild-type lambda

Charon 4A
(replacement vector)

β-Gal gene

Another substitution

Charon 16
(insertional vector)

Wild-type lambda

Charon 4A
(replacement vector)

β-Gal gene

Another substitution

Charon 16
(insertional vector)
Figure 11.19

1. **Digestion with restriction enzymes**
2. **Ligation with foreign DNA**
3. **Packaging cloned DNA into phage head**
4. **Phage assembly**
5. **Infective lambda virion**
### Figure 11.13

<table>
<thead>
<tr>
<th><strong>Bacteria</strong></th>
<th><strong>Eukaryote</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td><em>Saccharomyces cerevisiae</em></td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td></td>
</tr>
</tbody>
</table>

#### Advantages

**Bacteria**
- Well-developed genetics
- Many strains available
- Best known bacterium

**Eukaryote**
- Well-developed genetics
- Nonpathogenic
- Naturally secretes proteins
- Can process mRNA and proteins
- Endospore formation simplifies culture
- Easy to grow

**Comparison with E. coli**
- Potentially pathogenic
- Periplasm traps proteins
- Genetics less developed than in *E. coli*

#### Disadvantages

**Bacteria**
- Genetically unstable

**Eukaryote**
- Plasmids unstable
- Will not replicate most bacterial plasmids
Figure 11.15

- Ampicillin resistance
- oriC
- oriY
- CEN
- Promoter
- Polylinker (cloning site)
- ESM
- t/pa
**Figure 11.20**

![Polylinker Diagram](image)

**EcoRI**  **KpnI**  **XbaI**  **SalI**  **PstI**  **HindIII**  **BamHI**

**lacP**  **lacZ'**

M13 genomic DNA

---

Phage in clear plaques have cloned DNA

Phage in blue plaques do not have cloned DNA
X-gal Cloning Results
Three major types of restriction endonucleases

<table>
<thead>
<tr>
<th>Type</th>
<th>Structure</th>
<th>Cofactor(s)</th>
<th>Recognition Sequence</th>
<th>Cleavage</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>$R_2M_2S$</td>
<td>ATP (hydrolysis) AdoMet, Mg$^{2+}$</td>
<td>Asymmetric interrupted -TGA($N_2$)TGCT-</td>
<td>Cut DNA at sites distant from the recognition sequence</td>
</tr>
<tr>
<td></td>
<td>EcoB</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>$R_2$</td>
<td>Mg$^{2+}$</td>
<td>Palindrome 4–8 bp</td>
<td>Within the recognition sequence to produce blunt or staggered ends -GATATC-</td>
</tr>
<tr>
<td></td>
<td>EcoRV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>RM</td>
<td>ATP (no hydrolysis) Mg$^{2+}$, AdoMet</td>
<td>Asymmetric -AGACC-</td>
<td>Cut DNA close to the recognition sequence</td>
</tr>
<tr>
<td></td>
<td>EcoPl</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Abbreviations:** R, restriction endonuclease subunit; M, modification subunit; S, specificity subunit; AdoMet, S-adenosylmethionine.

Restriction endonucleases produce blunt or sticky ends

Type II enzymes cut at palindromic sequences
Restriction endonucleases cleave DNA at specific sites

<table>
<thead>
<tr>
<th>Organism of Origin</th>
<th>Restriction Endonuclease</th>
<th>Recognition Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Arthrobacter luteus</em></td>
<td>Alul</td>
<td>5’...A G↓CT ... 3’</td>
</tr>
<tr>
<td><em>Anabaena variabilis</em></td>
<td>Ava I</td>
<td>5’...C↓PyCGPuG...3’</td>
</tr>
<tr>
<td><em>Bacillus amylobifaciens H</em></td>
<td>Bam HI</td>
<td>5’...G↓GATCC...3’</td>
</tr>
<tr>
<td><em>Bacillus globigii</em></td>
<td>Bgl II</td>
<td>5’...A↓GATCT...3’</td>
</tr>
<tr>
<td><em>Escherichia coli RY13</em></td>
<td>Eco RI</td>
<td>5’...G↓AATTc...3’</td>
</tr>
<tr>
<td><em>Escherichia coli J62 pLG74</em></td>
<td>Eco RV</td>
<td>5’...GAT↓ATC...3’</td>
</tr>
<tr>
<td><em>Haemophilus aegyptius</em></td>
<td>Hae II</td>
<td>5’...PuGCGC↓Py...3’</td>
</tr>
<tr>
<td><em>Haemophilus aegyptius</em></td>
<td>Hae III</td>
<td>5’...GCG↓CC...3’</td>
</tr>
<tr>
<td><em>Haemophilus haemolyticus</em></td>
<td>Hha I</td>
<td>5’...GCG↓C...3’</td>
</tr>
<tr>
<td><em>Haemophilus influenzae Rd</em></td>
<td>Hind II</td>
<td>5’...GTPy↓PuAC...3’</td>
</tr>
<tr>
<td><em>Haemophilus influenzae Rd</em></td>
<td>Hind III</td>
<td>5’...A↓AGCTT...3’</td>
</tr>
<tr>
<td><em>Haemophilus parainfluenzae</em></td>
<td>Hpa I</td>
<td>5’...GTT↓AAC...3’</td>
</tr>
<tr>
<td><em>Haemophilus parainfluenzae</em></td>
<td>Hpa II</td>
<td>5’...C↓CGG...3’</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>Kpn I</td>
<td>5’...GTTAC↓C...3’</td>
</tr>
<tr>
<td><em>Moraxella bovis</em></td>
<td>Mbo I</td>
<td>5’...↓GATC...3’</td>
</tr>
<tr>
<td><em>Nocardia otitidis-caviarum</em></td>
<td>Not I</td>
<td>5’...GC↓GGCCGC...3’</td>
</tr>
<tr>
<td><em>Providencia stuartii</em></td>
<td>Pst I</td>
<td>5’...CTGCA↓G...3’</td>
</tr>
<tr>
<td><em>Serratia marcescens</em></td>
<td>Sma I</td>
<td>5’...CCC↓GGG...3’</td>
</tr>
<tr>
<td><em>Streptomyces stanford</em></td>
<td>Sst I</td>
<td>5’...GAGCT↓C...3’</td>
</tr>
<tr>
<td><em>Xanthomonas malvacearum</em></td>
<td>Xmal</td>
<td>5’...C↓CCGGG...3’</td>
</tr>
</tbody>
</table>

*aPy, pyrimidine; Pu, purine.*
Restriction maps

Figure 5.18ab

Photo courtesy of FOTODYNE Incorporated.
Constructing a restriction map

Figure 5.19ab
Figure 11.5

Introduction of recombinant vector into a host

Cut with restriction enzyme

Add vector cut with same restriction enzyme

Add DNA ligase to form recombinant molecules

Foreign DNA

Sticky ends

Vector

Cloned DNA

Introduction of recombinant vector into a host
cDNA synthesis by reverse transcriptase

1. Oligo-(dT) primer annealed
2. Reverse transcriptase + dATP, dGTP, dCTP, dTTP
3. Terminal transferase + dCTP (terminal transferase does not require a template to add deoxyribonucleotides to the 3'-ends of a linear duplex.
4. Alkaline digestion of RNA
5. Oligo-(dG) primer annealed
6. DNA polymerase I + dATP, dGTP, dCTP, dTTP

Figure 5.45
Figure 11.14

Before gas release

- Plunger
- Helium gas
- Gas vent
- Disc
- Microprojectiles with transfecting nucleic acid
- Fine screen
- Rough screen
- Target tissue

After gas release

- Target tissue
Figure 11.6

Transformant colonies growing on agar surface

Replica-plate onto membrane filter

Partially lyse cells; add specific antibody; add agent to detect bound antibody in radiolabeled form

Lyse bacteria and denature DNA; add RNA or DNA probe (radioactive); wash out unbound radioactivity

Autoradiograph to detect radioactivity

X-ray film

Positive colonies

(a)
Polymerase chain reaction (PCR) to amplify DNA

Figure 5.31
Southern blotting is used to detect specific DNA fragments

Figure 5.22