Modern Tools of Molecular Biology

Restriction Enzymes, DNA Vectors, Molecular Cloning, High Throughput Sequencing and Protein/DNA Mapping
<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Source Microorganism</th>
<th>Recognition Site*</th>
<th>Ends Produced</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Sac</em>I</td>
<td><em>Streptomyces achromogenes</em></td>
<td>↓ G-A-G-C-T-C- ↑ C-T-C-G-A-G-</td>
<td>Sticky</td>
</tr>
<tr>
<td><em>Sma</em>I</td>
<td><em>Serratia marcescens</em></td>
<td>↓ C-C-C-G-G-G- ↑ G-G-G-C-C-C-</td>
<td>Blunt</td>
</tr>
<tr>
<td><em>Sph</em>I</td>
<td><em>Streptomyces phaeochromogenes</em></td>
<td>↓ G-C-A-T-G-C- ↑ C-G-T-A-C-G-</td>
<td>Sticky</td>
</tr>
</tbody>
</table>

*These recognition sequences are included in a common polylinker sequence (see Figure 9-12).*
Vector DNA

(a')

5' OH
3' TTAAP

Genomic DNA fragments

(a)
P-AATT-  3'
OH-  5'

(b)
P-CG-  3'
HO-  5'

(c)
P-AGCT-  3'
HO-  5'

Complementary ends base-pair

5' (a') OH P
3' TTTAA

(a) 3'

Unpaired genomic fragments (b) and (c)
T4 DNA ligase catalyzes the ligation of DNA fragments that have complementary ends. The reaction requires two molecules of ATP and yields two molecules of AMP and two molecules of PPi.

Unpaired genomic fragments (b) and (c) are also present in the diagram.
HindIII
Sphl
PstI
SalI
XbaI
BamHI
SmaI
KpnI
SacI
EcoRI

Polylinker

Region into which exogenous DNA can be inserted

ori

ampr

Plasmid cloning vector
Plasmid vector + DNA fragment to be cloned

Enzymatically insert DNA into plasmid vector

Recombinant plasmid
Recombinant plasmid

Mix *E. coli* with plasmids in presence of CaCl₂; heat pulse

Culture on nutrient agar plates containing ampicillin

*E. coli* chromosome

Transformed cell survives

Cells that do not take up plasmid die on ampicillin plates
(b) $\lambda$ Phage assembly

Preassembled $\lambda$ head

Preassembled $\lambda$ tail

COS (49 kb) COS

Concatomer of $\lambda$ DNA

Nu1 and A proteins promote filling of $\lambda$ head with DNA between COS sites

$\lambda$ genome (1 copy)

$\lambda$ tail attaches only to filled head

Complete $\lambda$ virion
mRNA 5’ A A A ....Aₙ 3’

3’ poly(A) tail

Oligo-dT primer T T T T 5’

1. Hybridize mRNA with oligo-dT primer

2. Transcribe RNA into cDNA
1. **Sticky end**

2. **Cut with EcoRI**
   - Remove replaceable region

3. **Ligate to λ arms**

4. **Package in vitro**

5. **Recombinant λ virions**
Recombinant $\lambda$ virions

Individual $\lambda$ clones

Infect *E. coli*
Individual phage plaques

Master plate of λ phage plaques on E. coli lawn

Place nitrocellulose filter on plate to pick up phages from each plaque

Nitrocellulose filter

Incubate filter in alkaline solution to lyse phages and denature released phage DNA

Single-stranded phage DNA bound to filter

Hybridize with labeled probe; perform autoradiography

Signal appears over phage DNA that is complementary to probe
454: High Through-put DNA Sequencing
Bead based technology for rapid parallel sequencing
Multi-well Reactions

DNA Library Preparation and Titration
- 4.5 hours

emPCR
- 10.5 hours

Sequencing
- 8 hours
- 4.5 hours

- Well diameter: average of 44µm
- 200,000 reads obtained in parallel
- A single cloned amplified ssDNA bead is deposited per well

Amplified ssDNA library beads

Quality filtered bases
Chemi-luminescent sequencing
Micro Array Technology

- Raw data image with >6,500,000 different complementary probes
- Hybridized probe cell
- RNA/DNA target
- Oligonucleotide probe
- Thousands of copies of a specific oligonucleotide probe in each feature

1.28 cm
5-11 µm
High Probe Densities Enable New Applications

[Table]

<table>
<thead>
<tr>
<th>Feature Size</th>
<th>18um</th>
<th>11um</th>
<th>8um</th>
<th>5um</th>
<th>1um</th>
</tr>
</thead>
<tbody>
<tr>
<td>Features</td>
<td>500,000</td>
<td>1,300,000</td>
<td>2,600,000</td>
<td>6,500,000</td>
<td>163,000,000</td>
</tr>
</tbody>
</table>

Commercial expression arrays
Commercial genotyping arrays
Exon, tiling and 500K Arrays
Future development

*Advances in Genechip technology enable applications requiring high data content*
Tiling Array Applications

- Mapping regions of transcription
- ChIP on chip experiments (Chromatin IP)
- Chromosomal origins of replication
- DNA methylation
- Copy number analysis
- SNP discovery

Genome reference arrays for multiple applications
Chromatin Immunoprecipitation

1. Target protein
2. DNA + formaldehyde
3. Cell lysis
4. Sonicate ~ 1kb
5. Add antibody *
6. Add protein A beads
7. Wash/Elute DNA-Protein complexes
8. Reverse X-links
9. Isolate DNA
10. Amplify DNA, Label, & Hybridize to arrays
Chromatin IP Assay

• Any nucleotide fraction that can be immunoprecipitated can be interrogated on tiling arrays
• DNA binding proteins
  – Transcription factors
  – Modified histones
  – Structural proteins
• Assay is more complex than typical transcription mapping assay
  – Chromatin-protein immunoprecipitation
  – Purify, amplify and label DNA fragments
• Data analysis more comprehensive than typical transcription assay