

Utilization / Analysis of Cloned DNAs

1. *Determining Sizes of DNA Inserts*

Isolate chimeric (recombinant) vector
Cleave with original endonuclease
Separate DNA fragments with electrophoresis
Determine sizes using known standards

2. *Mapping Locations of Internal Endonuclease Sites*

Two approaches:

- A. Examine known sequence and look for sites*
- B. Create restriction map from experimental data*

Distances in Kb, not percent recombination
Useful in future manipulation of cloned DNA

Example: Analysis of cloned 5.7 Kb PstI fragment

Add EcoRI: 2 fragments: 1.1 and 4.6 Kb
Add HindIII: 2 fragments: 2.5 and 3.2 Kb
Add both: 3 fragments: 1.1, 1.4, 3.2 Kb

4.6 EcoRI fragment cut into 1.4 and 3.2 kb fragments

Restriction map of fragment:

3. **Southern Blot Hybridization:** *Identifies DNA fragments complementary to probe*

*Isolate DNA; cleave with endonuclease
Separate fragments - gel electrophoresis
Denature DNA - transfer to nylon filter
Incubate filter with labeled DNA probe
Detect hybridization with autoradiography*

4. **Northern Blot Hybridization:** *Used to study gene expression*

Similar to Southern EXCEPT:

*Separate mRNAs rather than DNA fragments
Endonucleases not required*

Example: Transgenic soybean plants with herbicide resistance gene

Southern: Confirm presence and organization of foreign gene in maize genome

Northern: Study influence of light or temperature on expression of foreign gene

5. **in situ Hybridization:** *Reveals chromosomal location(s) of cloned sequence*

*Partially denature chromosome on slide
Add 32-P ss DNA probe; remove unbound probe
Autoradiograph reveals location(s) of hybridization
Best results with giant polytene chromosomes*

Example: Used to localize repetitive DNA sequences to telomeric and centromeric regions

6. **DNA Sequencing**

Determine nucleotide sequence of cloned DNA fragment

A. *Maxam and Gilbert method (chemical cleavage)*

B. *Sanger Method (enzymatic synthesis)*

* *Cloned DNA fragments with label attached
Chemical/enzymatic treatment*

* *Small fragments, variable in length
Separate by gel electrophoresis*

* *Banding pattern visualized / analyzed
Manual (old) vs automated (new)*

* *Pattern translated into sequence data*

* *Sequencing machines (ABI 377 and 3700)*

* *Sequencing centers (Genome projects)*

7. Computer Analysis of Sequence Data

* *Assembly of sequenced clones into larger contigs
Critical step in sequencing large DNA molecules*

* *Gene prediction programs
Identify likely genes and intron/exon boundaries*

* *Sequence comparison programs*

Example: BLAST search for related sequences

Compares your favorite nucleotide or AA sequence with every other sequence stored in GenBank

Estimates probability that similar sequences reflect chance or relationship preserved through evolution

*Principle: Proteins with related functions have similar AA sequences
Genes for these related proteins have similar nucleotide sequences*

Application: Allows you to determine likely function of sequenced gene from any organism

Alternative Cloning Methods

Polymerase Chain Reaction (PCR)

Rapid, efficient, inexpensive, simple method

Does not involve vector or host cells

Does not require large amounts of DNA

Often used in criminal investigations

*Requires: Oligonucleotide primers (short ss DNAs)
Heat-resistant DNA polymerase
“Taq” from *Thermus aquaticus*
Thermal cycler machine (hot / cold)*

1. *Denature DNA that you want to amplify*

2. *Hybridize target DNA with oligonucleotides*

3. *Extend DNA with Taq polymerase*
4. *Repeat steps 1-3 many times*
Step 1 = High T; Steps 2-3 = Low T
5. *Result = Multiple copies of target DNA sequence*

Applications: Molecular Biology, Field Biology, Agriculture, Forensics, Evolution

Insertional Mutagenesis

Create mutation using known DNA sequence

Recover unknown sequence flanking insertion site

This sequence corresponds to part of the disrupted gene

Examples of Insertion Agents:

Transposable elements (many organisms)

*T-DNA from *Agrobacterium tumefaciens* (plants)*

Agrobacterium: Soil bacterium that infects many dicotyledonous plants

Transfers segment of plasmid DNA molecule into genome of infected plant cell

Random T-DNA insertion into genome may cause mutation in specific gene of interest

Tagged mutant allele more easily cloned than alleles derived from chemical mutagenesis

*Widely used to clone mutant genes of *Arabidopsis**