

## **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*

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

*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**