### Techniques Described in 7.28--Spring 2005

<table>
<thead>
<tr>
<th>Lecture</th>
<th>Technique</th>
<th>Assay For</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lecture 1</td>
<td>dNTP Incorporation Assay</td>
<td>DNA Synthesis (Fast; Quantitative for Amount of Synth.).</td>
</tr>
<tr>
<td></td>
<td>Filter Binding Assay</td>
<td>Method to separate incorporated from unincorporated dNTPs.</td>
</tr>
<tr>
<td></td>
<td>Gel Electrophoresis</td>
<td>Method to separate DNA on the basis of length.</td>
</tr>
<tr>
<td></td>
<td>Primer Extension Assay</td>
<td>DNA Synthesis (Slow; Quantitative for Length of Synth.).</td>
</tr>
<tr>
<td></td>
<td>Template Challenge Assay</td>
<td>Assay for Processivity.</td>
</tr>
<tr>
<td>Lecture 2</td>
<td>DNA Helicase Assay</td>
<td><em>In vitro</em> DNA Helicase Activity.</td>
</tr>
<tr>
<td></td>
<td>Helicase Polarity Assay</td>
<td>Assay for directionality of helicase movement; used to study function of replication termination sequences.</td>
</tr>
<tr>
<td></td>
<td>Topoisomerase assay</td>
<td>Assay for changes in DNA topology; distinguish supercoiled, relaxed, nicked, linear, forms of DNA and measure catenation/decatenation.</td>
</tr>
<tr>
<td>Lecture 3</td>
<td>2-D Agarose Gels</td>
<td>Assay for Origin of Replication (Must have an idea of where the origin is to test).</td>
</tr>
<tr>
<td></td>
<td>Southern Blotting</td>
<td>Identifies specific DNA molecules transferred to membrane.</td>
</tr>
<tr>
<td></td>
<td>DNA Microarray Assay</td>
<td>Assays the relative amount of replicated DNA sequences during a synchronized round of DNA replication to identify sites of replication initiation.</td>
</tr>
<tr>
<td></td>
<td>Plasmid Replicator Assay</td>
<td>Identifies region of DNA sufficient for Replicator activity.</td>
</tr>
<tr>
<td></td>
<td>Mutational Mapping Assay</td>
<td>Identifies regions of DNA necessary for Replicator activity.</td>
</tr>
<tr>
<td></td>
<td>Biochemical Fractionation</td>
<td>Mechanism to purify biochemical activity that can be assayed <em>in vitro</em>.</td>
</tr>
<tr>
<td></td>
<td>Biochemical Complementation</td>
<td>Combines mutant extracts with biochemical fractionation to identify proteins required for assay (in this case DNA replication).</td>
</tr>
<tr>
<td>Lecture 4</td>
<td>Genetic Screen for DNA Replication Mutants (self explanatory)</td>
<td>Assay for Sequence Specific DNA Binding (Slower and not quantitative; Gives Information about bound DNA sequence).</td>
</tr>
<tr>
<td></td>
<td>DNase I Protection Assay</td>
<td>Assay for Sequence Specific DNA Binding (Fast and Quantitative but less information about bound sequence).</td>
</tr>
<tr>
<td></td>
<td>Gel Mobility Shift Assay</td>
<td>Assay for Sequence Specific DNA Binding (Slow and Quantitative for Length of Synth.).</td>
</tr>
</tbody>
</table>
DNA Unwinding Assay

Assays for formation of ssDNA through the use of a ssDNA specific nuclease.

**Lecture 5**

Template Association Assay

Uses gel filtration to separate molecules bound to a plasmid from molecules that are not bound to a plasmid.

**Lecture 6**

Heteroduplex DNA analysis

Assay for DNA repair *in vivo*.

Restriction Analysis

Assay for methylation state of DNA.

Restriction site repair

Assay of DNA repair *in vitro*; also used incorporation assay.

**Lecture 7**

Ames Test

Assay for chemicals and other factors that increase the frequency of mutations.

Nicking of circular DNA

Assay for excision-type mechanism of DNA repair.

Lesion bypass DNA synthesis

Assay for ability of replication/repair proteins to synthesize DNA off of a damaged template.

**Lecture 8**

Strand Exchange Assay

Assay for homologous DNA pairing & branch migration.

**Lecture 9**

ATPγS

Used in determining whether ATP binding or ATP-hydrolysis is required for a reaction.

DMS footprinting

Assay for protection of the bases in DNA, rather than the phosphodiester backbone.

Cooperative DNA binding

Gel-shift assay to measure cooperative vs. non-cooperative DNA binding.

**Lecture 10**

Concensus sequences

Identification of important elements by conservation.

Native-Denaturing 2D Gel

Detection of nicks vs DSB’s in DNA.

**Lecture 11**

DNA bending assay

Assay for whether a protein bends DNA upon binding and position of bend.

**Lecture 12**

Genomic southern

Identifies specific DNA molecules transferred to membrane (here used for transposition).

Plasmid mating

Assay for transposition in vivo by determining movement of drug resistance to a new plasmid.
**Lecture 13**

- Incorporation Assay: Assay for Transcription \textit{in vitro}.
- S1 Protection Assay: Assay for Transcription. Maps start site of transcription.
- Northern Blot: Assay for Transcription. Maps steady state RNA \textit{in vivo}.
- Pulse Labeling of \textit{in vivo} RNA: Assay for actively transcribing genes.
- Promoter Fusion Assay: Assay to indirectly measure promoter activity.

**Lecture 14**

- DNA Unwinding Assay: Assay for open complex formation.
- Indirect end-labeling: Method to extend a labeled DNA primer to measure breaks or modification of DNA.

**Lecture 15**


**Lecture 16**

- Transcription Factor DNA Binding Domain Mapping
- Transcriptional Factor Activation Domain Mapping
- Chromatin Immunoprecipitation: Assay to determine the \textit{in vivo} binding site of proteins.

**Lecture 17**

- R-loop formation: Assay for regions of mRNA that anneal to different DNA fragments.
- Gel electrophoresis assay: For intermediates and products formed during splicing \textit{in vitro} (done using labeled RNA).

**Lecture 18**

- Calculation of “Consensus Fold” and Rnase digestion: Determination of RNA secondary structures.

**Lecture 19**

- cDNA cloning: Determine mRNA sequence and structure, protein expression.
- RT-PCR: Generate segments of amplified DNA starting with specific RNA template.
- Exon trapping: Find exons in DNA fragments of regions thought to contain genes.

**Lecture 20**

- none

**Lecture 21**

- none
Lecture 22
none

Lecture 23
Incorporation Assay for Translation
Identifies mRNAs that are being translated
in vivo.
Polysome Assay
Measures Binding of Small Molecules to
Larger Molecules (e.g., Binding to
Ribosome).
Gel Filtration Analysis of
Protein Binding
Filter Binding Assay
Measures Binding of RNA to Protein.