Techniques Described in 7.28--Spring 2005

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

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 *in vitro*.
S1 Protection Assay	Assay for Transcription. Maps start site of transcription.
Northern Blot	Assay for Transcription. Maps steady state RNA *in vivo*.
Microarray Analysis of RNA	Assay for relative levels of gene expression. Can assay many genes at once.
Pulse Labeling of *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

Antibody Supershift Assay	Assay to determine the components of protein-DNA complex in a gel shift assay.

Lecture 16

Transcription Factor DNA Binding Domain Mapping
Transcriptional Factor Activation Domain Mapping

Chromatin Immunoprecipitation	Assay to determine the *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 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 Polysome Assay	Identifies mRNAs that are being translated *in vivo*.
Gel Filtration Analysis of Protein Binding	Measures Binding of Small Molecules to Larger Molecules (e.g., Binding to Ribosome).
Filter Binding Assay	Measures Binding of RNA to Protein.