

Chromosome & Genome Overview

TECHNIQUES—OVERVIEW

Several techniques, widely used in many experiments, most importantly in genetic mapping experiments are discussed here.

I. Southern hybridization

- A. Cleave DNA with a restriction endonuclease.
 1. Cut at specified points in the genome, related to a recognition site
 2. Site length varies: 4 bp, 6 bp.
 3. Cut in a specific way; e.g. *EcoRI* cuts between the G and A in GAATTC
- B. Separate fragments with agarose gel electrophoresis (0.5-25 kb)
 1. DNA molecule negatively charged
 2. Load digested DNA fragments into electric field--migrate toward anodes.
 3. Smaller fragments move faster through matrix.
- C. Southern blotting
 1. Gel can be stained with ethidium bromide (DNA specific dye).
 2. Transfer denatured DNA from gel to solid support membrane (nylon or cellulose).
 3. Southern blot--saves separated DNA fragments in membrane semi-permanent state at -20°C.
- D. Hybridization
 1. Add a denatured, radiolabeled probe (usually ³²P) to Southern blot.
 2. Incubate overnight, then wash off excess probe.
 3. Probe binds homologous sequence on blot.
 4. Visualize binding by placing blot against X-ray film.
 5. Autoradiogram will show bands where probe binds.

II. Libraries

- A. Random genomic library
 1. Represent random regions in genome.
 2. Usually screened against repetitive sequences (simple sequence repeats, SSR).
 3. Typically short segments, 1-4 kb.
 4. Digested DNA is cloned into a plasmid or phage vector.
 5. The vector is transformed/transfected into bacteria and amplified.
 6. Collectively, all the DNA fragments constitute a library of cloned probes.
- B. cDNA libraries
 1. Represent transcribed regions--i.e., genes.
 2. mRNA is isolated, reverse transcribed to produce complementary DNA (cDNA).
- C. BAC libraries
Large DNA fragments cloned as bacterial artificial chromosomes in *E. coli*.
- D. YAC libraries
Large DNA fragments cloned as yeast artificial chromosomes in yeast.

III. Polymerase Chain Reaction (PCR)

- Amplifies a given DNA sequence millions of times.
 - A. Denaturation: DNA is denatured.
 - B. Annealing: Primer sequences bind to the denatured DNA.
 - C. Extension: DNA is synthesized by extending from the primers
 - D. Repeat cycle 20-40 times depending on protocol

- E Two primers binding opposite strands fairly close together (~4 kb) in the correct orientation will produce an exponential number of copies each cycle visualized as a product (band) in a gel. Copies increase as: 1-2-4-8-16-32-etc.
- G. A single primer with no counterpart will produce only as many copies as cycles. Copies increase as: 1-2-3-4-5-6-etc.

MOLECULAR MARKERS

(Kochert, 1994); (Liu, 1998); (Reiter, 1998)

Common acronyms associated with molecular markers

AFLP	Amplified Fragment Length Polymorphism
AP-PCR	Arbitrarily Primed PCR
AS-PCR	Allele Specific PCR
ASAP	Allele Specific Amplified Polymorphism
BAC	Bacterial Artificial Chromosome
BSA	Bulked Segregant Analysis
CAPS	Cleaved Amplified Polymorphic Sequence
DAF	DNA Amplification Fingerprinting
DGGE	Denaturing Gradient Gel Electrophoresis
EST	Expressed Sequence Tag
NIL	Near Isogenic Lines
PAC	Plant Artificial Chromosome (BAC + transformation competent) P1 Artificial Chromosome
PCR	Polymerase Chain Reaction
PFGE	Pulsed Field Gel Electrophoresis
QTL	Quantitative Trait Locus
RAPD	Randomly Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
RIL	Recombinant Inbred Line
SCAR	Sequence Characterized Amplified Region
SNP	Single Nucleotide Polymorphism
SSCP	Single Strand Conformational Polymorphism
SSR	Simple Sequence Repeat (microsatellite)
STS	Sequence Tagged Site
VNTR	Variable Number Tandem Repeat (minisatellite)
YAC	Yeast Artificial Chromosome.

I. Types of markers

A. Classical genetic markers

1. Simple inheritance
2. Few in any one background
3. Many are deleterious--not in good lines

B. Isozymes

1. Enzyme polymorphism.
2. Easily assayed for many individuals.
3. Limited enzyme systems (e.g. 15-20).

C. Molecular markers

General characteristics

- a. "Unlimited" number in any genetic background.
- b. Neutral markers--no effect on fitness (assumed).
- c. Many types (see acronym list).

1. RFLP markers

- a. Based on nucleic acid hybridization.

Carry out by Southern blot analysis of parents and segregating materials.

DNA filters prepared from restriction enzyme digested DNA samples are hybridized to the DNA marker probe.

- b. Hybridization

- i. Add a denatured, labelled probe (markers -³²P labelled) to Southern blot or DNA blot.
- ii. Autoradiogram will show bands where probe binds to the DNA fragments.

- e. Polymorphism occurs if two plants have differently sized bands.

- i. Plants are monomorphic if no differences in banding pattern.
- ii. If multiple bands are detected, some may be monomorphic, others polymorphic.
- iii. Causes of RFLP:

- (1) Base change in recognition site
- (2) Insertion or deletion

- f. Libraries, sources of probes (clones).

- i. Random genomic library (plasmid, cosmid, lambda phage, BAC, YAC, etc.)
- ii. cDNA libraries

2. PCR-based markers

- a. Random primer methods (random genome locations amplified):

- i. RAPD, AP-PCR, DAF

- (1) Primers for the PCR are random oligomers 5-15 bp (usu. 10 bp).
- (2) Products produced if the random homologous sequence is present on opposite strands within the length of DNA extension.
- (3) Base changes can affect primer binding.
- (4) Insertions/deletions will affect product.
- (5) Generally a presence/absence reaction- dominant marker: a change in binding site or an insertion will typically result in no band (unlike RFLP)
- (6) Very sensitive to reaction condition--reproducibility is often a problem.

- ii. AFLP

- (1) Carries elements of RFLP and RAPD.
- (2) Digest DNA with two enzymes to create two different fragment ends.
- (3) Ligate adapters to ends.

- (4) Add primers that are homologous to adapter sequence with 1-3 extra random bases for selective amplification of fragments
no. frag. amplified is 1 in 4^{2n} , where n is number of random bases
e.g. 1 in 16 with one degenerate base (1/4 one end and 1/4 the other)
(one primer may be labelled, e.g. with ^{32}P).
 - (5) Amplify random restriction fragments with the corresponding bases.
 - (6) Separate products on denaturing polyacrylamide gel.
 - (7) Highly repeatable.
- b. Genome specific methods
- i. SSR/microsatellites
 - (1) Are di- and tri-nucleotide repeat sequences.
 - (2) Repeated throughout genomes of higher plants.
 - (3) Isolate clones containing repeat sequences (search GenBank).
 - (4) Sequence clones and synthesize primers to regions flanking repeat.
 - (5) Polymorphism levels are high--mismatch pairing.
 - (6) Amplify specific genomic regions.
 - (7) Difficult to develop primers, but simple to use afterward.
 - (8) Polymorphisms result from different numbers of repeat units.
 - ii. SCAR/STS [(sequence characterized amplified region (SCAR) or sequence-tagged-site (STS)] markers
 - (1) Sequence RAPD band (SCAR) or RFLP marker (STS).
 - (2) Develop primers from ends of fragment.
 - (3) Highly reproducible, target-specific PCR, friendly marker, easy to use.
 - iii. Allele-specific methods (ASAP)
 - (1) Design primers that only amplify a specific allele.
 - (2) Can stain with ethidium bromide without running gel to make a +/- screen.
3. SNP (single nucleotide polymorphism): Highly sensitive markers. Single nucleotide variation between any two given lines can be converted to a marker.
- a. Isolate DNA fragments from a parent by screening a library
 - b. Sequence the DNA fragments.
 - c. Design primers for the DNA fragments.
 - d. PCR amplify DNA from the other parent.
 - e. Sequence the PCR fragments.
 - f. Compare sequences from two parents for a given DNA fragment for polymorphisms.
 - g. Usually up to ~1 nt variation/kb DNA can be expected.
 - h. More variation in non-coding sequences such as introns, 3'-end, 5'-end (promoter) of genes and also intergenic regions.
 - i. Design primers for SNP detection. Several approaches are available to detect SNPs.
 - j. Amplify PCR products from a segregating population and spot them on a membrane.
 - k. Hybridize to allele-specific radio-labeled primers for scoring.
 - l. Other more sensitive methods include primer extension using the mutant base-specific complementary di-deoxy nucleotide, which is conjugated to a fluorescent dye; e.g. if the variation is from G to A, then extend the primer with fluorescent labeled (say yellow color) di-deoxy T (for mutant base 'A') and with fluorescent labeled (red color) di-deoxy C (for wild-type base 'G').

TERMINOLOGY OVERVIEW**Chromosome**

centromere/kinetochore
 telomere
 heterochromatin--dark staining; gene poor
 euchromatin--stain normally; gene rich

Homologous Chromosomes: Chromosomes which carry the same genetic loci (though may be with alternate alleles) and pair during meiosis.

Homoeologous Chromosomes: Chromosomes which carry some, but not all, genetic information in common and either do not pair or pair poorly/infrequently during meiosis (e.g., the three breadwheat genomes, A, B, and D). Also spelled “homeologous.”

Chromatid: A longitudinal subunit of a replicated chromosome, joined to sister at centromere i.e. a replicated chromosome has two *sister chromatids*.

Genome: All the genetic information (chromosomes, DNA) in an organism
Note--may refer to the nuclear, mitochondrial, or chloroplast genomes.

Locus: Designates a particular location on the chromosomes.
 Could be a gene--i.e., codes for something--e.g., *wx* --waxy locus in corn
 could be a particular DNA sequence that doesn't code for anything
 e.g., UMC187--a corn RFLP marker.
So--all genes are loci, but all loci are not genes.

Allele: Alternate form of a gene, or more generally, a locus
 Designated by:

1. A, a --traditional method, typically A is dominant and a recessive
2. A₁, A₂, . . . , A_n to account for more than two alleles and not show dominance relationships

*Plant breeders generally select alternate alleles, not alternate genes!