Chapter 15 Lecture Notes: Applications of Recombinant DNA Technology

I. In Vitro Mutagenesis: It is possible (and relatively easy) to make specific mutations in a gene using a variety of methods which are collectively called site directed mutagenesis

II. Gene synthesis: It is possible to synthesize small segments of DNA with a particular nucleotide sequence. These segments are called oligonucleotides.

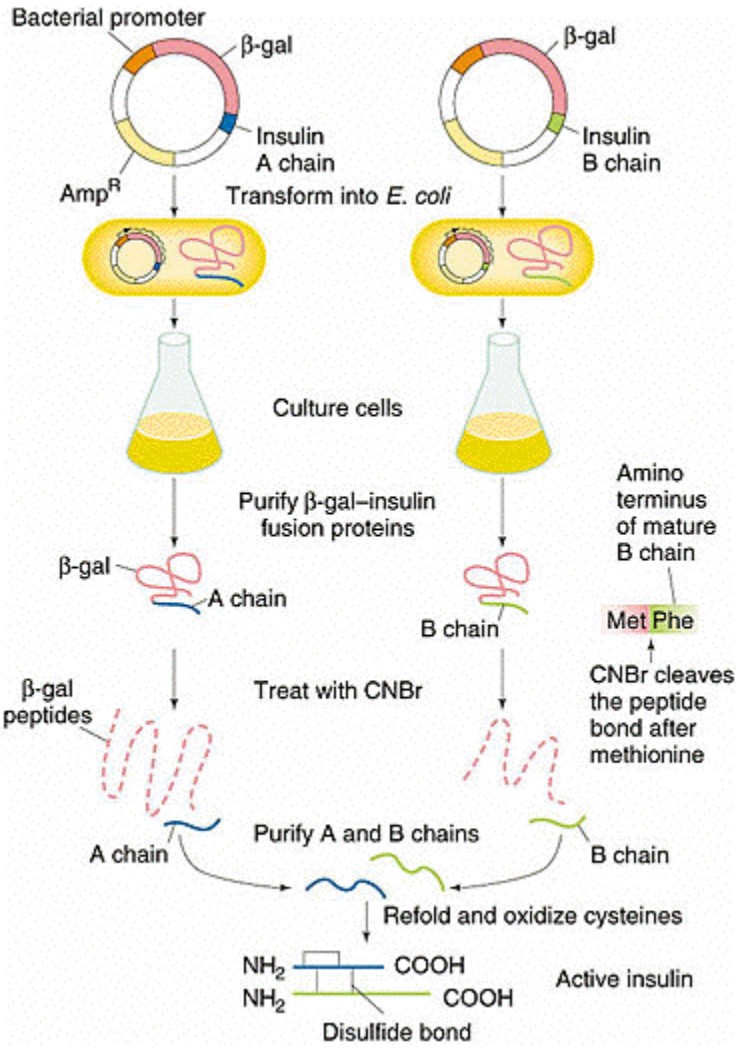
III. Expressing eukaryotic genes in bacteria

A. Requirements

1. Bacterial transcriptional signals (promoter)
2. Bacterial translational signals (Shine-Delgarno sequence and ATG)
3. If secreted, bacterial secretion signal

B. Examples

1. Insulin – 1st recombinant product to be licensed for therapeutic use in 1982



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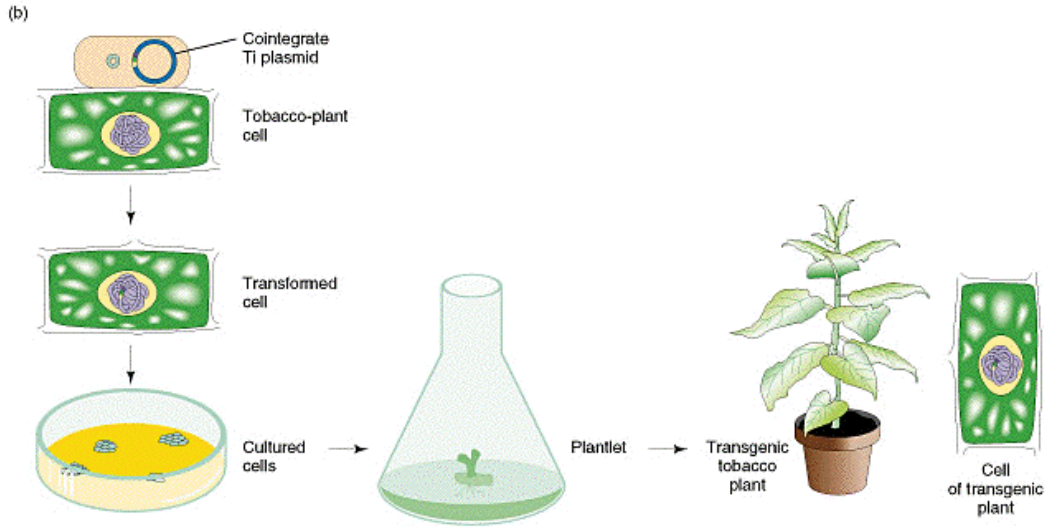
2. Other examples
    a) Human growth hormone
    b) Factor VIII
    c) Other pharmaceuticals

IV. Genetic Engineering in Yeast – " *Saccharomyces cerevisiae* as the *E. coli* of eukaryotes" – really neat genetic tricks can be done; useful and simple model organism

V. Genetic Engineering in Plants

A. Transgenic plants are plants that carry a foreign gene

B. Use Ti plasmid from *Agrobacterium tumefaciens* as a vector: The Ti plasmid is a plasmid carried by the bacterium *A. tumefacien*. This bacterium causes crown gall disease in plants due to the insertion of part of the Ti plasmid into the plant genome. Ti has been engineered so that it can deliver genes to a plant without causing disease.



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C. Examples of uses for transgenic plants

1. Reporter genes for basic research
2. Flavrsavr tomatoes (Calgene Inc.)
    a) Normally tomatoes are picked when the are unripe so that they will not bruise during transit. Prior to marketing ethylene is provided which initiates the ripening process; however, although the tomatoes appear to ripen, the flavor is poorer than vine-ripened tomatoes. Thus, researchers at Calgene tried to block only the enzyme that causes softening of the fruit (polygalacturonase or PG) so that the tomatoes could remain on the vine longer. They did this by constructing an antisense RNA that binds to the normal sense PG mRNA to block translation. The gene encoding the antisense RNA was inserted into the plant chromosome along with a kanamycin resistance marker gene. Their approach worked: the tomatoes can remain on the vines longer and are less susceptible to bruising in transit.

b) Concerns

(1) Kn resistance gene in the tomatoes may be picked up by soil bacteria.

(2) Toxic compounds from genetic manipulation

(3) Unnatural method of food production

c) Depending upon the source you get your info from, the FlavrSavr tomato was/was not a financial success.

3. Other examples of transgenic plants

a) Herbicide resistant plants

b) Cotton plants that are resistant to pests due to incorporation of a bacterial gene that is toxic to insects

c) Soybeans that produce more healthful combinations of fatty acids

d) Flowers that stay fresher longer by inhibition of genes that are involved in senescence.

4. Ultimately the success/failure of genetic engineered crops will depend on the consumer.

VI. Genetic Engineering in Animals

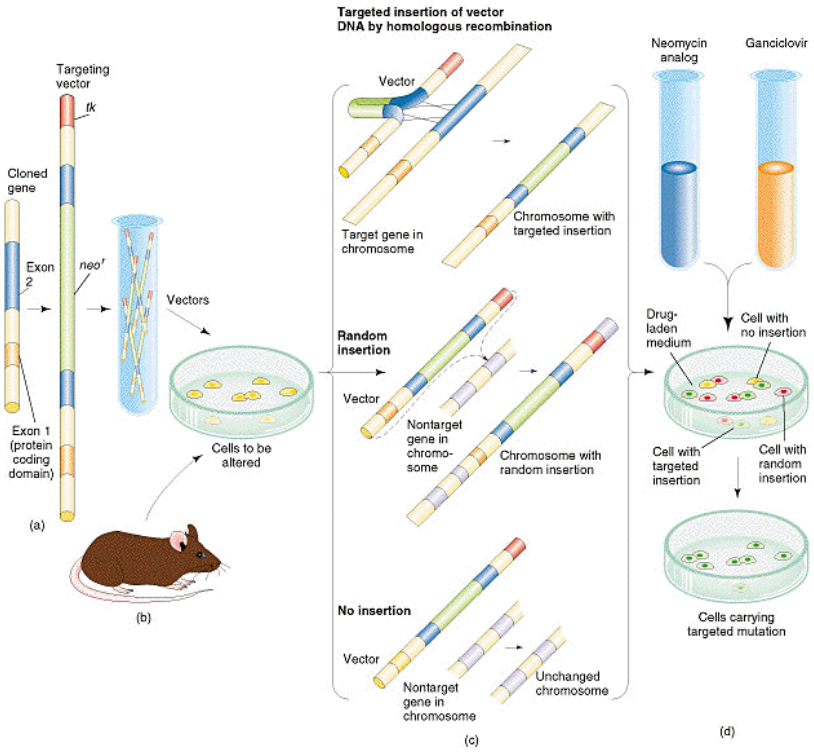
A. Method for constructing transgenic animals (2 main steps):

1. Construction of gene disruptions in a cell

clone the gene on a cloning vector that contains the *tk* gene which encoded SENSITIVITY to the chemical ganciclovir → insert the *neo* resistance gene into your cloned gene of interest (gene X) → introduce construct into cultured cells from a mouse embryo → there are three fates of the tranfected construct:

- Homologous recombination with the wild type gene on the chromosome via a double crossover (top in figure) → cells will have a chromosomal copy of gene X which has been disrupted by the *neo* gene
- Ectopic (Random insertion into the chromosome (middle in figure) → cells will have a chromosomal copy of gene X which has been disrupted by the *neo* gene as well as a wild type copy; also the *tk* gene and any other vector sequences will be present on the chromosome
- No insertion (bottom in figure) → cells will have only wild type gene X and therefore no Neo resistance

The cells in which homologous recombination with wild type gene X on the chromosome occurred will be SELECTED FOR because the are resistant to Neo. The insertion at an ectopic site will be SELECTED AGAINST because they have the *tk* gene which renders them sensitive to the chemical ganciclovir. No insertion at all will be SELECTED AGAINST because the cells are Neo sensitive.



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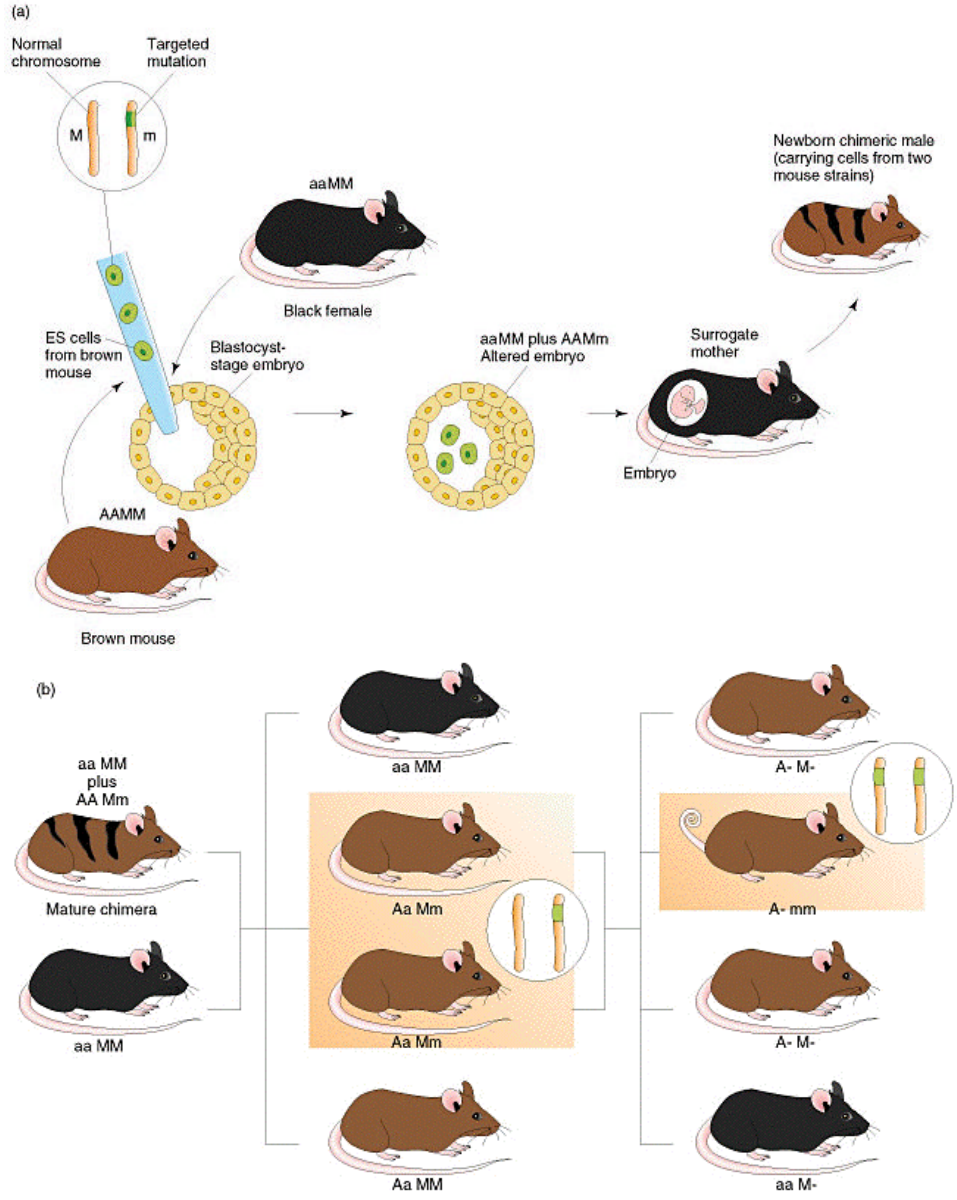
2. Construction of transgenic mouse carrying the mutated gene from A
After disrupting a gene in the manner described in (1), the disrupted gene (m) can be put back into an animal as follows: Note that the disruption above was done in cells that came from an agouti (AA) mouse. Inject the cells (embryonic stem cells) from above into a blastocyst stage embryo from a non agouti mouse (aa) in vitro (now the embryo is a mosaic of two different cell types – AAMm and aaMM→ transplant embryo into a non-agouti pregnant mouse → examine progeny for agouti patches indicating that some of the AAMm cells survived → breed agouti mice to homozygocity for the m locus, screening for mm in candidate mice by PCR



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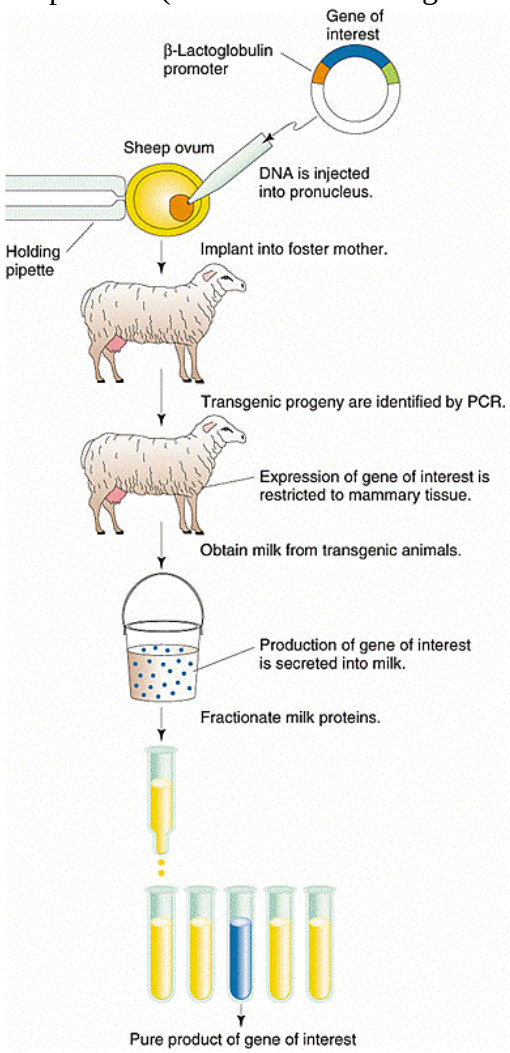
B. Uses of transgenic animals

1. Basic research
    a) Knockout mice for determining the function of a gene
    b) Knockout mice for genetic disease models
2. Production of useful proteins (factor IX for treating hemophilia B)



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3. Transgenic animals paved the way for gene therapy

VII. Gene Therapy

A. Introduction

The correction of a genetic deficiency in a cell by the addition of new DNA to the cell. This definition has been expanded to include treatments of acquired diseases by the addition of new DNA.

B. Somatic gene therapy (introduction of transgene into somatic tissues)

1. Some diseases that have gene therapy studies in clinical trials (cystic fibrosis, muscular dystrophy, adenosine deaminase deficiency, familial hypercholesterolemia, cancer, AIDS)
2. Delivery techniques for getting gene into cells of interest
    a) Ex vivo (remove cells from selected tissue → expose to gene transfer vectors → return corrected cells to patient)
    b) In vivo (deliver gene transfer vectors to body via injection or inhalation → vector is targeted to correct tissue to deliver corrected gene)
3. Delivery vectors (safety, efficacy, and specific maintenance needs are issues)
    a) Viruses – depending on the virus, will either integrate into the chromosome, simply be maintained in the nucleus, or remain only transiently
    b) Nonviral
        (1) Liposomes (small, lip spheres) and lipoplexes (more complex, cationic liposomes) harboring DNA
            (a) Pros: nonviral therefore no infectious risks, no immune response
            (b) Cons: delivery is not as efficient (alterations in chemical composition of the lipids to address this problem)
        (2) Naked DNA – surprisingly naked DNA is as effective as lipoplexes
4. What to deliver depends on the disease being treated – Delivered gene can take the place of flawed ones, instill an entirely new property (anticancer), or prevent a destructive gene from being produced
5. Cancer approaches using gene therapy
    a) Delivery of genes encoding toxic molecules to cancer cells to kill them
    b) Delivery of genes encoding chemokines to cancer cells to activate the immune response to recognize and kill them
    c) Antibody therapies: DNA vaccines with genes that encode antibodies to cancer specific proteins in tumor cells
    d) Insertion of normal tumor suppressor genes into cells
    e) Antisense therapy: DNA that blocks synthesis of proteins encoded by deleterious genes
    f) Suicide gene therapy: Renders cells that contain the gene sensitive to certain drugs)
6. Other considerations
    a) Level/regulation of expression
    b) Circumvention of host “defenses”

C. Germinal gene therapy (introduction of transgene into the germ line)

1. Technically more difficult
2. Ethical considerations such as do infants have the right to inherit an unmanipulated genome

VIII. Screening for Genetic Diseases

A. Over 500 recessive diseases that have been identified

B. Amniocentesis

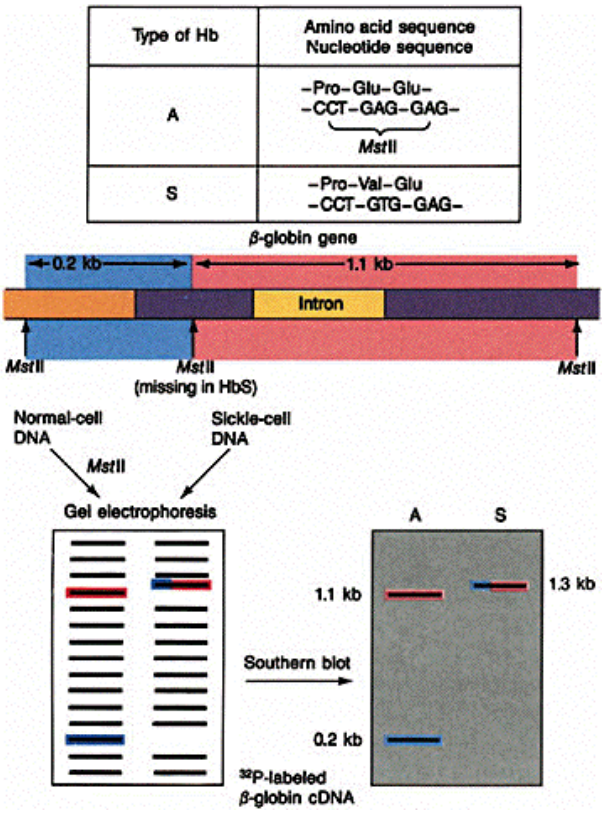
1. Fetal cells are taken from the amnionic cavity via syringe and sampled for chromosome patterns, proteins, biochemical reactions
2. Can pinpoint many disorders this way (ex. Table 15-1)

C. Chorionic villus sampling

Fetal cells are taken from the placenta – can be done earlier than an amniocentesis

D. Recombinant DNA technologies have improved the ability to screen for genetic diseases in utero

a) Alterations in RE sites accounts for 5-10% of all point mutations (isolate DNA → RE digest → Southern blot with gene probe)



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b) Probing for altered sequences

(isolate DNA → RE digest → Southern blot with oligonucleotide gene probe) – used when a mutation does not affect a RE site

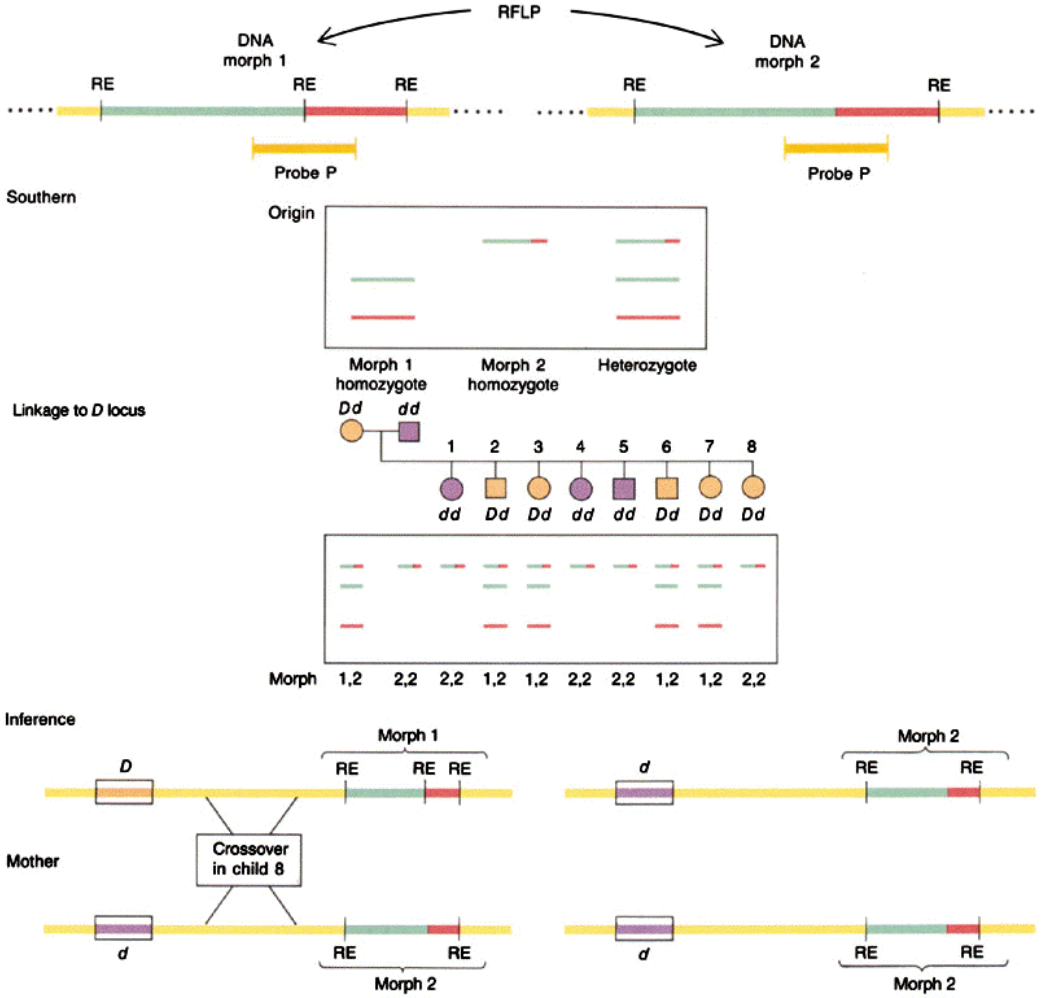
c) PCR

(use primers flanking the putative mutation site to PCR fragment → sequence to compare to the wild type)

d) Linkage to restriction sites

(a) The coexistence of two or more RE patterns in a population is a restriction fragment length polymorphism (RFLP)

(b) RFLP can be linked to genes



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IX. Forensic analysis

A. Can use RFLP in forensic testing

B. Results of test are analyzed based on statistics, probability, and population genetics