(2) Detection of disease causing alleles through linkage analysis

Linkage analysis is used to map chromosomal locus of a genetic disorder – finding out which chromosome and which region on the chromosome the unknown disease-causing gene is situated, with respect to genetic markers

An RFLP locus is almost always inherited together or co-inherited with the diseases – hence they are tightly linked, allowing identification of individuals with the disease-causing allele

- Allows one to establish linkage between an RFLP genetic marker and a disease-causing gene, hence the phenotype
- However, it is important to note that linkage will not be absolute unless the marker is located in the gene itself
- Thus there will always be instances of crossing over

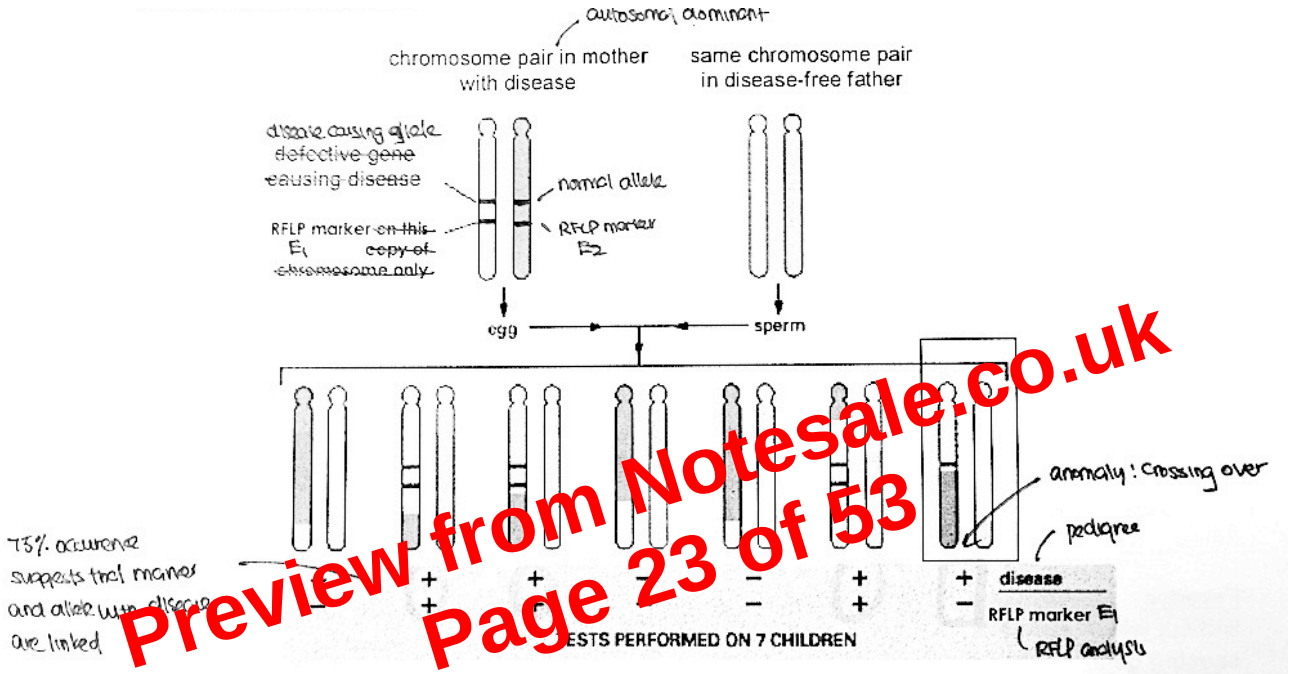


Figure 13b. Using RFLP analysis to map the chromosomal locus of an unknown disease gene - Linkage analysis of the disease gene and a known RFLP marker.

- An example is shown below where there is a genetic disorder

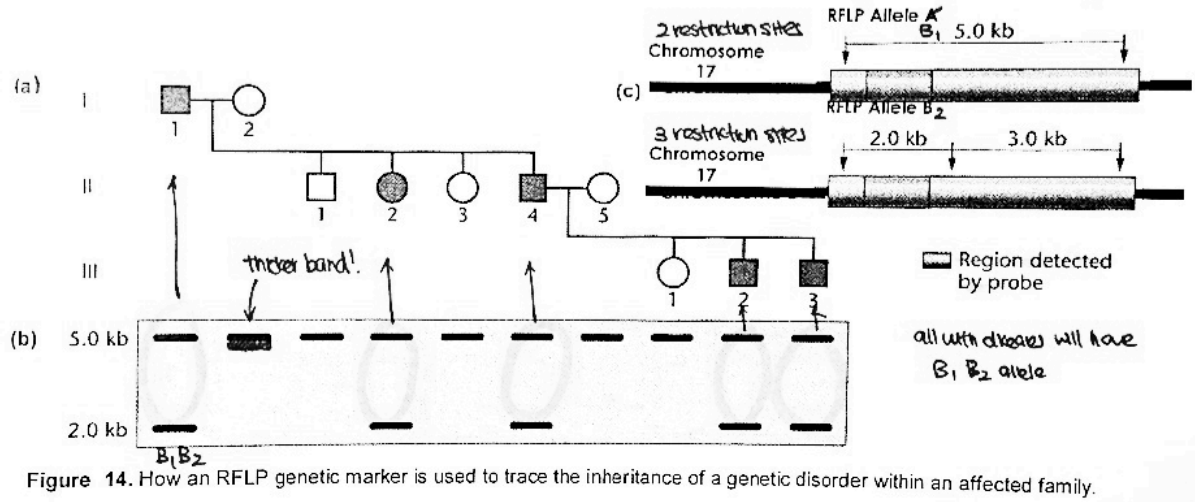


Figure 14. How an RFLP genetic marker is used to trace the inheritance of a genetic disorder within an affected family.

HUMAN GENOME PROJECT

It was launched in 1990, with the aim of sequencing the entire human genome – all 3 billion nucleotide pairs

Goals of the Human Genome Project:

It aims to sequence all DNA in the 24 human chromosomes & establish functions of all genes

- Identify all of the approximate 20,000 – 25,000 human genes
- Construct detailed physical map of the entire human genome
- Determine the nucleotide sequences of 3 billion chemical base pairs of all 24 chromosomes
- Store this information in databases
- Improve tools for data analysis
- Transfer related technologies to the private sector
- Address the ethical, legal and social issues that might arise from the project

The Ethical, Legal and Social Implications (ELSI) program was established as an adjuct to the HGP to safeguard genetic information and ensure that it would not be used in discriminatory ways. The HGP also worked on sequencing genomes of important model organisms like bacterium (*E. coli*)

Benefits of HGP:

1. Pharmacogenomics

- Genetic differences makes us unique in our physiology and biochemistry, affecting the way we react to drugs
- Some are dangerous, some are ineffective
- Knowledge about which genes affect a person's response to a drug, called pharmacogenomics, may allow doctors to prescribe the right drugs for the right individuals at the right dosage

2. Risk assessment for certain diseases

- Genetic differences may put an individual at high risk of developing cancer, heart disease, depression or alcoholism
- Knowledge of these genetic variations can help prevent or treat these diseases
- Diseases resulting from defects in more than one gene (diabetes) can also be addressed

3. Insights through comparative genomics

- It directly compares genetic information between organisms to identify genomic similarities and differences that enable inferences to be made about genome evolution
- This is applied in gene discovery, development of animal model of human diseases and elucidation of evolutionary history and relationship between genes, genomes and species
- Further comparative studies will help determine the yet unknown function of thousands of other genes

4. Gene therapy

- Gene sequences are now available and we understand which genes are associated to which disease
- Allows us to use gene therapy to treat diseases

2. **Inaccurate / improper gene targeting**

- Targeting a gene to correct cells is important – delivering the transgene to the wrong tissue would be inefficient and can cause health problems
- Improper targeting could incorporate into a patient's germline or reproductive cells, which could produce sperm and eggs

3. **Immune response**

- Anytime a foreign body is introduced into human tissues, the immune system is design to attack the invader and it might reduce the gene therapy effectiveness or leads to rejection
- The human body tends to suppress gene expression, essentially turning new genes off or suppressing introduced genes and low levels of gene expression affect gene therapy efforts
- Gene delivery vectors need to be able to avoid the body's natural surveillance system
- The immune system's enhanced response to invaders it has encountered also make it difficult to repeated in patients

4. **Problems with viral vectors**

- Viruses while the carrier choice in most gene therapy studies, present a variety of potential problems to patients including
- Toxicity and other safety concerns
- Immune and inflammatory responses
- Gene targeting issues – there is no control of where the foreign DNA is inserted within the host DNA
- There is always the fear that viral vector, once inside the patient, may recover its virulence (ability to cause disease)

5. **Multigene disorders**

- Conditions or disorders that arise from mutations in a single gene are the best candidates for gene therapy
- However many diseases arise from the combined effects of mutations in many genes – and are difficult to treat effectively using gene therapy

CONSIDERATIONS FOR USE OF GENE THERAPY

Social considerations:

1. **Cost and commercialization**

- Patients – concerns regarding access to gene therapy when such expensive therapies are only accessible to the wealthy and not to those who cannot afford it, thus the concern is how to ensure gene therapy can be brought to all who need it
- Cost of development – gene therapy can be life saving, but the high cost of developing a treatment, including taking it through clinical trials for government approval
- Commercialization – the biopharmaceutical industry has to handle socially sensitive issues of patents, regulatory affairs and optimum business model, in the course of delivering gene therapy to the public

2. **Safety concerns**

- Existing risks of using gene therapeutic techniques

Definitions:

Micropropagation: Cloning of identical cells or small pieces of plant tissue (extracts) of usually meristematic cells in vitro in a special culture medium (tissue culture)

Tissue culture: Ability to establish and maintain plant organs and plant tissues in aseptic culture and grown in vitro

- Medium used is usually in the form of a solution or agar gel containing nutrients and growth regulators (auxins and cytokinins)
- Tool allowing for rapid production of many genetically-identical plants using relatively small amounts of space, supplies and time

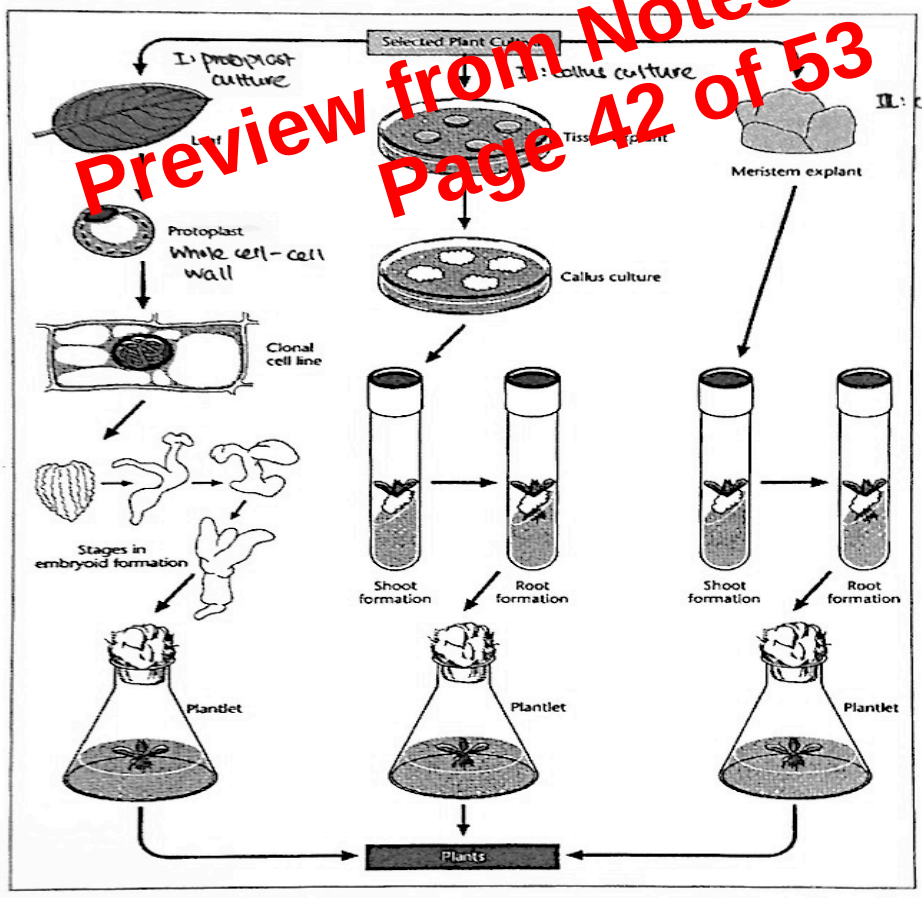
Explant: Piece of plant used to initiate micropropagation or tissue culture process – can be a portion of shoot, leaf, roots, buds or some cells of a plant

Callus: Mass of disorganized and unspecialized cells that can be induced to form roots or shoots

Types of tissue culture systems:

Structures that can be formed in tissue culture include the formation of

1. Plantlets
2. Seedlings
3. Callus
4. Somatic embryos



Chapter 6: Genetic Engineering and GMOs

INTRODUCTION
Biotechnology refers to the use of genetically modified organisms in agriculture and industry

Definition:

Genetic engineering: Covers a wide range of special molecular biological techniques that alter the genetic makeup of living organisms including plants animals and microorganisms

Genetically modified organism: An organism that has acquired, by artificial means, one or more genes from the same or different species

Significance:
They generally include the enhancement of an effect already natural to the organism; increase resistance to the disease and improving yield and thus efficiency of land use

Crop plants
- Enhanced taste, texture and quality – tomato
- Increased nutritional value – **Golden Rice** that contains high beta-carotene content
- Increased resistance to diseases / pests / weeds / herbicides – ***Bt* corn** producing natural insecticides
- Longer shelf life
- Creation of pharmaceutical value – development of edible vaccines in tomatoes and potatoes

Animals
- Increased growth rate, feed efficiency – fast growing **GM Salmon**
- Enhanced reproductivity
- Boosted nutritional value
- Creation of medicinal or commercial value – transgenic livestock that can secrete foreign proteins in their milk

Purpose of genetic engineering:
Genetic engineering aids in reducing world hunger and malnutrition – eight hundred million people on Earth suffer from nutritional deficiencies

Causes of hunger include
- Inequities in distribution
- Food shortages due to overpopulation of the world

Genetic engineering serves to increase yields on available land

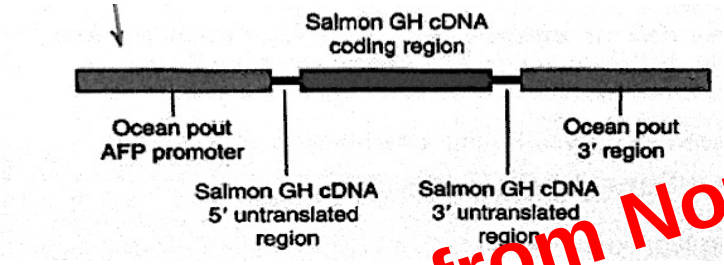
Growth: GM (Transgenic) Salmon
Salmon has been genetically modified to improve growth rates, disease resistance, cold resistance, tolerance to low oxygen levels and genetically superior broodstock

GM Salmon
Cloning the gene for growth hormone, somatotrophin, has borough about development of transgenic species of salmon
- They demonstrate greatly accelerated growth rates compared to native strains

Antifreeze proteins protect living organisms from freezing
- Binding to surface of ice crystals to modify or block ice crystal formation
- Lower freezing temperature of biological fluids
- Protect cell membranes from cold damage

Thus, AFPs are used to create transgenic fish and plants with enhanced resistance to cold temperatures and freezing

1. A recombinant DNA plasmid construct with an AFP gene promoter from ocean pout attached to cDNA for salmon growth hormone is used to produce rapidly growing fish



2. Transcription from AFP promoter sequence is stimulated by cold temperatures
3. AFP promoter can be used to stimulate transcription of "downstream" genes under cold water conditions
4. Hence transgenic fish synthesizes large amounts of growth hormones when raised in cool water

Benefits of GM Salmon
- Faster growth rates means farmers can market their fish sooner, thus increased overall production and profits
- Shortened growing periods reduced risk of disease and feed requirements – lowers cost
- Reduced pressure on wild fisheries due to overfishing and habitat alteration by humans
- Less pollution from fish farms (fish excrement and uneaten food)

Concerns associated with GM Salmon
- If GM salmon were to escape and release their added growth hormone genes through interbreeding with wild populations, resulting hybrids could show reduced survival
- GM salmon mature earlier, larger and more attractive to females and outpace wild salmon in the mating game
- Yet they have a higher mortality rate in the wild, could ultimately lead to demise and extinction of the species – thus GM salmon in the US is sterile
- They grow faster and are more aggressive, could displace wild salmon out of their native habitats by competing with them for resources

IMPLICATIONS OF GENETICALLY MODIFIED ORGANISMS

There are generally major social and ethical implications

Social implications:

Threat to human safety

1. **Genetic markers**

- Vectors in genetic engineering contain antibiotic resistance marker genes and enter the transformed crop
- Concerns that antibiotic resistance gene, if not properly broken down by digestive system when consumed, may be passed to potentially harmful bacteria and make them resistant
- Flavr Savr tomatoes: The first GM food with kanamycin resistance with longer shelf life

2. **Allergenicity**

- Transgenic food may be a potential cause of allergens
- Peanuts: may cause potentially allergic reactions in susceptible individuals

3. **Possibility of creation of hazardous new pathogens**

- If cancer cell genes were transferred into bacteria or viruses, "rogue microbes" can be created

4. **Other unknown effects on human health**

- There is no certainty what, if any, health risks GMOs might pose for humans
- In general, there is lack of research and information of potential health risks when consuming GMOs, thus they should be introduced with caution

Threat to safety of the environment

5. **GM crops might establish themselves as weeds**

- Seeds from GM crops modified to withstand unfavourable environmental conditions (herbicide resistant) may be carried to other places
- May establish themselves as weeds
- GM oilseed rape: contains genes for resistance to herbicide Basta

6. **Spread of resistance from GM crops to weeds through unintended gene transfer**

- Pollen grains from GM plants can be carried in the wind by insects and cross-breed with wild relatives and weeds
- Lead to "superweeds" – resistant to herbicide and become more invasive and compete with natural resources

7. **Reduced effectiveness of pesticides**

- Concern that insects may become resistant to GM crops that can produce their own pesticides
- *Bt* corn: produces their own toxin *Bt* toxin

8. **Upsetting ecological balance**

- When GMOs are introduced into the ecosystem, they may affect the food chains in undesirable ways
- GM salmon: grow rapidly to big size and are more aggressive – upset balance of wild salmon populations and result in decrease in biodiversity

Ethical implications:

1. **Issues on acceptability of genetic manipulation of animals and plants**
- Whether humans have the right to "play God" and tamper with nature by mixing genes among species
- Unnatural violation of natural organisms' intrinsic values
- Whether living organisms have rights in the case of "human rights"

2. **Issues on GMOs to be used as food for human consumption**
- Seems to be little concern on whether GM animals are biologically designed to withstand additional burden / stress
- Or whether there are other side-effects of increased milk or egg or meat production
- Use of BST (Bovine Somatotrophin) in dairy cattle to allow them to produce more milk increases their risk of mastitis

3. **Religious concerns or dietary restrictions**
- Plants that have been modified to carry animal genes face objections from vegetarian groups
- Objections to genes from some species for religious groups (pig genes for Muslim)

4. **Issues with patency of a GM plant or animal**
- Many research companies have sought to patent transgenic plants and animals
- Some have argued that doing so is unethical as it reduces them to levels of objects but there is a need for companies to protect the results of their lengthy and expensive research

5. **Impact of GE on crop production in developed countries vs 3rd world countries**
- Whether richer countries are benefitting at the expense of poorer countries
- World food production may be dominated by a small number of large companies with the technical know-how
- Terminator technology: genetically switch off a plant's ability to germinate a second time, thus forcing farmers to buy a fresh supply of seeds each time

6. **Issues with inadequate risk assessment for use of GMOs**
- No one can anticipate all the potential risks and possible consequences when GMOs are released into the biosphere
- Whether it is morally right for companies to market their products for human use without knowing for use all the potential health risks that their products may bring

7. **Issues with consumer rights**
- Consumers have a legitimate interest in and right to information with regard to GMO
- Rules to transparent sharing of relevant information and communication of associated risks ensure protection of consumer rights
- Labeling measures might not be adequate as it is not mandatory, unjust to those who object to GMOs
- Regulations against mixing of GM foods with non-GM products are also not strictly enforced

~ END ~