Paper 3 compiled

ICS

Why genes seldom used as markers for genetic testing

- Genes encode a functional polypeptide, sequence of DNA is normally conserved due to its impt biological function. Mutation rate in genes is lower than in non-coding regions, not able to differentiate between individuals.

Restriction enzyme

- Recognises a specific sequence at the restriction site, 4-6 bases long, binds to specific sequence of bases, cleaves DNA through hydrolysis of phosphodiester bonds between nucleotides.
- Natural role: protect bacteria against invading bacteriophages by cutting up viral DNA. Enzyme helps to prevent foreign DNA from replicating in the bacterium, while bacterium's own DNA is protected from digestion by methylation at the restriction sites.

Principles of RFLP

- Different indivs have genetic variations among individuals of a species caused by mutations found within coding or non-coding regions, giving rise to gain or loss of restriction sites. When cut with the same RE, result in different length of DNA fragments.
- Normal and diseased individual have differences in genetic sequences. Upon digestion with the same set of RE, different number and length of DNA fragment will be produced. Gel electrophoresis and hybridisation with labelled probes will result in different unique band patterns for normal and diseased individual

Uses of RFLP

- RFLP as genetic markers in genomic mapping
- DNA fingerprinting for paternity testing/forensic investigation

Target region of probe

- Must recognise same target sequence for alleles A and B. Diseased allele is closely linked with polymorphic RFLP locus. Probe binds to different fragment lengths for normal and diseased allele

Gel electrophoresis

- DNA fragments can be separated based on molecular size using gel electrophoresis. The gel provides a matrix, in which DNA fragments manoeuver through the pores of the gel. Thus in fixed amount of time, the larger DNA will move a shorter distance compared to a small fragment, which will move a longer distance thus further from the well. DNA is negatively charged due to phosphate group, DNA will migrate from negative to positive electrode when subjected to an electric current.

Which marker more suitable

- For DM1, all indivs with BS have only 1 5kb band except II3 who has 2 bands 5kb and 7kb. For DM2, 5 BS indivs have only 1 4kb band while 4BS indivs have 2 bands 3kb and 4kb. Thus the inheritance of the DM1 fragment is more consistent compared to DM2
    - This is because DM1 is closer to the BLM gene compared to DM2, thus higher tendency for the 5kb fragment to be inherited together with the BLM gene, hence lower number of recombinants for DM1 compared to DM2

Lac Z gene

- Clones of bacteria that contain recombinant plasmid will appear white as the gene of interest caused insertional inactivation of lac Z gene, B galactosidase not produced to break down X gal, hence colony remains white.

Advantages of PCR

- Fast and efficient way to amplify DNA with exponential increase in amount of DNA because the process of PCR can be automated
- PCR is highly sensitive, only minute amounts of DNA is required as starting material for amplification.
- PCR is highly specific, only sequence flanked by forward and reverse primers are amplified
- PCR is a cheaper technique as compared to cloning as it can be performed in vitro, hence no need to culture and maintain large quantities of host cells
- PCR is faster than cloning n replication of DNA
    - E.g. many copies of a gene of interest can be amplified for cloning purposes as long as primer sequences are known
    - Clinical diagnosis e.g. in prenatal diagnosis of human genetic disorders. DNA from a single embryonic cell is amplified using PCR. Screening for certain genetic diseases eg cystic fibrosis even before the phenotype is expressed provides assurance to the couple. If child is diagnosed with certain diseases, couple can be prepared and make informed choices
    - Early detection of infection with HIV. PCR can detect presence of t HIV genome at very early stages of the diseases before symptoms appear
    - Forensic science: minute amount of DNA found at crime scene can be amplified to sufficiently large amounts to be analysed
    - Fossil study: PCR is used to amplify fragments of ancient DNA

Limitations of PCR

- Taq polymerase lacks 3’ to 5’ proofreading mechanism. This makes it impossible for the enzyme to check the base it has just inserted and remove it if it is incorrect. Polymerase induced errors may accumulate during PCR
- DNA sequences flanking target sequences to be amplified must be known to enable synthesis of primers
- DNA fragments are limited to 3kb. Further increase in length will decrease the efficiency of amplification
- Possible contamination from non-template DNA if primer sequences are not specific

Luciferin

- Cells that grow on the ampicillin and luciferin plate but do not glow contain recombinant plasmid as gene of interest inserted into luciferin gene led to insertional inactivation hence cells do not glow

Replica plating not necessary

- Bacteria with recombinant plasmids can be differentiated by their ability to glow and they do not die in the presence of ampicillin

Why genetic markers can be used to detect presence of disease causing alleles

- These genetic markers are polymorphic, and can be recognised and cut differently by the same restriction enzyme
- They are tightly linked to the disease-causing alleles on the same chromosome and are likely to be inherited together as one unit with the disease-causing allele

Discrepancy regarding band pattern of CF

- C has both RFLP variant 1 and 2 which suggests that she should have 1 copy of normal CFTR allele and 1 copy of mutant CFTR allele. However C suffers from CF which meant that she should have 2 copies of mutant CFTR recessive alleles which are linked to RFLP variant 2. There could be a mutation in RFLP variant 2 resulting in creation of a restriction site or crossing over occurred at a region between KM19 and CF locus causing RFLP variant 1 to become associated with mutant allele.

In absence of gel electrophoresis and nucleic acid hybridisation, possible method in detecting RFLP

- Design primers that are complementary to the RFLP variant 2 and use PCR to synthesise variant 2 of KM19 and mutant CFTR

Recombinant DNA

- Genes from 2 different sources are combined in vitro into a single plasmid

Gene of interest cannot be taken from chromosomal DNA

- Eukaryotic DNA contains introns and bacterial host cells do not have post-transcriptional modification mechanism to remove introns, hence non-functional protein may be synthesised

Replica plating

- Replica plate the master plate containing the bacterial clones on two separate agar plates containing ampicillin and tetracycline. Bacterial clones that grow on both antibiotic plates are resistant to both antibiotics and are non-recombinant. Select for bacterial clones sensitive to the antibiotic of the gene marker in D but resistant to the other antibiotic from the master plate. Insertional activation occurred when gene was inserted into the plasmid.

Select using X gal

- Culture the bacterial clones on X gal medium with ampicillin. Bacterial clones that appear blue are non-recombinant as LacZ gene is intact. Select for white bacterial clones that are recombinant. Lac Z gene is disrupted due to insertional inactivation.

Can use 2 different RE to isolate gene

- The gene for HGH may insert into the plasmid in more than one orientation, leading to non-functional protein being synthesised if the gene is inserted in the wrong orientation. Having two different restriction enzymes produces 2 different sticky ends to isolate the gene at the ends will ensure uni-directional insertion of the gene into the plasmid.

Steps for RFLP analysis

- Same set of restriction enzymes are used to digest DNA samples. Gel electrophoresis to separate DNA fragments of different length. Southern blotting to transfer DNA fragments to nitrocellulose membrane. Nucleic acid hybridisation with radioactively labelled probes complementary to target sequences. Autoradiography to visualise DNA fragment containing target sequences.

Why DNA construct must include regulatory elements

- Regulatory elements of goat casein gene can be bounded by transcription factors present in cells of mammary gland. To allow expression/transcription and translation of the human AT gene only in the goat mammary gland

Benefits of precision medicine

- Greater drug efficacy, prevents dangerous side effects
- Disadvantage: discrimination/stereotyping of certain ethnic group based on genetic profile, people will be tempted to draw connections between genes and propensity to violence, intelligence, creativity etc

How hTERT expression may help cells regain stem cell properties

- hTERT catalyses the formation of phosphodiester bonds between DNA nucleotides which are added to the 3' end of parental DNA strands via complementary base pairing with telomerase RNA during DNA replication. Increases length of telomeres, prevents telomeres from reaching critical length after multiple rounds of DNA replication. iPS cells are able to self-renew and divide by mitosis indefinitely

Goals of HGP + examples of application

Goal of HGP	Possible benefit to plant cloning
To determine the sequence of the coded information contained in the DNA of the various genomes studied Completing the sequences of several other organisms to be used as models for research → knowledge gained by the study of genomes in other organisms would assist in the analysis of the human genome	Determine sequence of psy gene from daffodil or crtI gene from soil bacterium OR Bt gene from Bacillus thuringiensis In the process of sequencing and mapping of human genome, genetic engineering and techniques of cloning were developed, which could be applied to create genetically modified crops
Studying gene's normal functions	Allow the isolation of genes of interest from other orgs which code for traits which are desirable to have in GM crop e.g. Golden rice: psy gene and crtI gene code for enzymes phytoene synthase and carotene desaturase that convert

	a natural compound in rice to B carotene, a precursor of Vitamin A → improve nutrition value, increase quality of rice OR Bt gene codes for toxin which kills corn borer, increases crop yield without use of chemical insecticides To improve the yield and quality of crop plants to solve world demand for food
Storing all sequence information in databases that are accessible by all	Easy access of shared data enables different groups of researchers to search for information about gene sequences and functions without having to do time-consuming research on their own to create new GM crosp with novel traits
Studying and addressing the ethical, legal and social implications of genome research	
Improving tools for analysis such as sequencing technology, development, developing technology or functional genomics, developing technology in bioinformatics	

Importance of DNA marker

- comprises a collection of DNA fragments of known lengths, used to compare with DNA fragments of sample to estimate size of the DNA fragments

RFLP facilitates construction of a genomic linkage map

- A linkage map depicts the order of genes along a chromosome and their relative distance. RFLPs serve as genetic markers for a locus in the genome
- Linkage map can be constructed based on recombination frequencies between 2 RFLPs and the map units calculated using the formula (no. of recomb offspring/total no. of offspring)x100%
- Two homozygous individuals with different RFLPs at both loci are crossed to produce a heterozygote
- The heterozygote is then crossed with a homozygous individual. The resulting offspring will have more parental RFLP combinations than recombinant RFLP combinations
- The genome are cut by restriction enzymes and undergo gel electrophoresis
- The genotype is detected using 2 probes via nucleic acid hybridisation, one for each RFLP, and analysed using southern blot and visualised using autoradiography.

Why 2 primers are used for PCR

- Forward and reverse primers flank the target sequence to be amplified. Allows the amplification of a specific sequence of DNA in a short period of time. 1 primer anneals to 1 of the separated DNA strands after denaturation, provides free 3'OH group for Taq polymerase to elongate the complementary strands of both templates to produce 2 DNA molecules

Difference in expected RFLP

- Crossing over occurred during gamete formation in the mother between the disease gene and RFLP locus, giving rise to gametes with a X cr with different combination of alleles.

Ethical implication of using RFLP to screen for disease

- Stigmatisation of the parents and child even before the onset of the disease
- Parents may want to terminate the pregnancy if the unborn baby is diagnosed with the disease
- Parents may have to pay a higher premium for child insurance even before the onset of the disease

How RFLP can help in paternity testing

- Each band in the baby's DNA fingerprint will match the band in either the mum's or dad's DNA fingerprint

Replica plating

- Use of sterile velvet to transfer bacteria from master plate of nutrient agar containing ampicillin. Press velvet onto replica plate of nutrient agar containing tetracycline
- Bacteria carrying plasmid with human DNA, tetracycline resistance gene has undergone insertional inactivation, hence will be killed while bacteria with no extra DNA in plasmid will have intact tetracycline resistance gene, bacteria carrying plasmid only will survive on both plates.

Use of antibiotic resistance genes are rare

- Risk spread of resistance to other bacteria via horizontal transfer of genetic material into bacteria in gut.

Gene therapy

Ex vivo vs in vivo

- In ex vivo gene therapy, the therapeutic gene is introduced into target cells extracted from patient, and the altered cells are injected back into the patient.
- In in vivo gene therapy, the therapeutic gene is introduced directly into target cells in the patient's body

Why HSC are ideal for gene therapy

- HSC can undergo indefinite self-renewal, hence they can multiply indefinitely, thus sustaining the gene therapy treatment
- HSC are unspecialised and have the potential to differentiate, thus can be stimulated to differentiate to produce the therapeutic gene product
- HSCs can be manipulated to secrete biotherapeutic molecules such as X directly into the bloodstream
- HSCs are derived from patient, hence lower risk of immune rejection

Why somatic cell nuclear transfer cannot be used to treat patients with haemophilia

- All somatic cells from the patient will contain the defective gene. The resulting pluripotent cells isolated from inner cell mass from such a procedure will contain the same genetic defect, hence remain unable to produce the necessary clotting factors. If a donor cell

nucleus containing a functional gene is used for SCNT, the stem cells may contain antigens that cause them to be rejected by the immune system

Genetic basis of Xlinked SCID

- Interleukin-2-Receptor Gamma gene found on the X chromosome encodes common gamma chain subunit of the interleukin receptor. These receptors reside in the plasma membrane of the immune cells, and allow communication between T and B cells. Mutations of IL2RG gene results in a non-functional version of the common gamma chain, or no protein formation. Without common gamma chain, these interleukin receptors cannot form, hence preventing development and differentiation of T and B cells. This results in near complete failure of the immune system to develop and function due to low number or absence of T and B cells.

Suitable for gene therapy

- Single gene recessive disorder, mutation in 2 copies of IL2RG/ADA gene gives rise to SCID, hence only require one copy of normal functional allele to be delivered to target cell to restore the normal phenotype

Why stem cells used

- Multipotent, differentiate into a limited range of cell types. Unspecialised, no tissue-specific structures that allow specialised functions, capable of dividing by mitosis and long term proliferation, stem cells used belong to the patient hence no risk of immune rejection

Why diff cells are used for diff treatments

- In X-linked SCID, IL2RG gene is mutated which results in absence of functioning interleukin receptors. The patient's T cells cannot be stimulated to proliferate by treating them with interleukin 2 before the infection with viral vector, hence T cells are not used for gene therapy for X linked SCID and stem cells are used instead. In autosomal recessive form of SCID, normal IL2RG gene is present. Interleukin 2 receptors are functional in immune cells and can bind to IL2, therefore both T cells and stem cells can be targeted.

Adenovirus vs retrovirus

- Retroviral vector allowed for integration of recombinant DNA that could be stably propagated hence resulting in long term stable expression. However adenovirus could not integrate into chromosomal DNA hence transferred gene does not segregate equally into daughter cells

Retroviral vector

- RNA copies of normal functional IL2RG gene are inserted into inactivated non-pathogenic retrovirus. Haematopoietic stem cells are infected with retroviruses which introduce the normal functional allele into cells. RNA are reverse transcribed into the DNA of normal functional IL2RG allele which is subsequently integrated into genome of haematopoietic stem cells by integrase
- Retroviral vectors are able to integrate the normal allele into the chromosome. The HSCs are able to undergo long term self renewal by mitotic cell divisions. Hence patient will have the normal gene in his white blood cells since the normal allele would be replicated and passed ot the daughter cells. When the HSCs differentiate into white blood cells, the normal gene can be expressed and produce the normal enzyme.

Ethical and social consideration for use of gene therapy

Social

- Society may be less accepting of people who have genetic diseases, leading to possible discrimination and practice of eugenics
- Attempts at gene therapy are exorbitantly expensive leading to social class divide with the rich having better accessibility to treatments as compared to the poor/ genetic enhancements creating an advantage for those who can afford the treatment.

Ethical

- Safety of gene therapy to humans: long term consequences have not been fully established due to inadequate understanding of biological interactions of vector and host. In the event of undesirable outcomes brought about by the treatment, unclear who should be held responsible/who should participate in human trial
- There are concerns about the protection of privacy and confidentiality of medical information of patients involved in clinical trials or those who have gone for such treatments as this could have implications on their medical insurance coverage/future employability
- Difficulty in determining which condition is normal and which are considered as a disability/disorder
- Gene therapy for non-health related enhancement→ gene therapy may be abused to alter human traits not associated with disease, for genetic enhancement
- Altering one's genes and changing the way one was created goes against many religious and cultural beliefs.
- Germ-line therapy will alter offspring genetics, we may not have the right to alter our children's genes
- Those involved in the research related to germ-line gene therapy regularly create and destroy embryos as part of their research. There are objection to killing embryos used for research as human life is considered to have begun at conception.

Embryonic stem cells	**Adult stem cells**
Pluripotent	multipotent
Undifferentiated unspecialised cell found at inner cell mass of blastocyst	Undifferentiated unspecialised cell found among differentiated cells in a tissue or organ
Have the ability to differentiate into almost any cell type in the body except extra-embryonic tissue. Inner cell mass can give rise to the derivatives of the 3 germ layers: endoderm, mesoderm, and ectoderm which eventually form all the highly specialised cells needed to produce an adult organism. This means that embryonic stem cells have the potential to make almost all cell types in the body	To maintain and repair the specific tissue where they reside by replacing worn out or damaged cells

Effect of SCID

- Adenosine deaminase breaks down adenine. Hence without ADA, amines which accumulate is toxic to T and B cells, causing them to die

- Gamma chain of interleukin receptors allow the differentiation and proliferation of T cells. Hence without the progenitors there will be no generation of T cells.

Problems with retrovirus

- DNA of modified retroviruses integrate into host cell genome, causing insertional mutagenesis. Integration may cause gain of function mutation resulting in proto-oncogene converted to oncogene. Integration may cause loss of function mutation in 2 alleles of the tumour suppressor gene.

Why stem cells preferred

- Gene targeting T cells does not result in long-term expression of the corrected genes, since most of these cells die rather than self-renew. Gene targeting haematopoietic stem cells result in long-term expression of the corrected genes as these cells have the capability to self-renew and differentiate to give rise to more T cells and are multipotent stem cells.

Factors that prevent effective use of iPS cells

- Difficult to ensure that specialised somatic cells become pluripotent completely as it is difficult to determine the growth factors to be used
- Treatment may not be effective as iPS cells obtained are unable to differentiate into blood stem cells in the bone marrow

Limitations of using liposome vector

- Lower probability of liposomes binding to cell surface membrane of iPS cells and membrane fusion occurring to release DNA into target cells, therefore not all target cells receive normal IL2RG allele, some still express non-functional protein
- Normal allele not integrated into cell's DNA, leading to transient expression of normal functional proteins and multiple treatments are required

Arguments for and against use of ES cells

Against	For
Involves removal of inner cell mass of blastocyst, destruction of embryo which has the potential to develop into a human being, akin to killing of a life to treat own disease	Unclear whether ES cells are considered human life, thus should be used to treat and save patients with life-threatening diseases
ES cells are able to divide continuously via mitosis, potential of developing tumours, causing more harm to the patient	ES cells are pluripotent, able to differentiate into any other cell types of the 3 germ layers → potential to treat diseases where harvesting of adult stem cells are difficult

Differentiation of haematopoietic stem cells for SCID

- Haematopoietic stem cells are relatively unspecialised cells that can differentiate into T cells when appropriate molecular signals are present, through differential switching on of genes. H stem cells can proliferate and self-renew indefinitely, thus replenishing T cells population indefinitely

GMOs

Why this GM wheat might be acceptable to people against GMO

- Gene encoding for EBF is already found in various plant species' genomes. EBF is not toxic or harmful to human health, unlike Bt maize, EBF does not kill insects since it is naturally secreted from aphids. Aphids are thus still available for their predators/minimal impact on food chain.

How GE improves yield of crops

- Genetic engineering refers to a special set of technologies that alter the genetic material of animals, plants or bacteria by introducing genes from different species into target organisms
- Bt corn
    - Bt toxin genes from Bacillus thuringiensis is inserted into corn genome. Bt toxin are lethal to insect larvae when they are ingested. The protein toxin produced kills the Larvae of the European corn borer. Larvae which ingest the toxin die because of the damage caused to the gut. Hence these larvae are unable to cause further damage to crops, farmers will have higher crop yield. This allows farmers to concentrate resources on other aspects like fertiliser, improved farming practices that result in further improved yield
    - Concerns: Bt toxin may cause allergies in some human when Bt corn is consumed. Bt toxin may be toxic to close relatives of targeted pests/beneficial insects, leading to reduced numbers or extinction of that organism, hence affecting the ecological balance. Pollen of Bt corn may be carried by wind or by animals and pollinate nearby non-GM crops or wild plants. Mixing of M crops with non-GM products confounds labelling attempts.

Pros and cons of GMOs

- <u>pros:</u>
    - GE can enhance crop yields
    - GE may permit crops to grow outside their usual location/season so that people have more food
    - GM crops can also be enhanced with a certain nutritional content eg Golden rice so that people have better nutrition
    - GM crops can be more pest-resistant, which will lower cost as pesticide usage will be reduced. There will also be less pollution to the environment
    - GM crops can be more drought-resistant, increasing crop yield, and avoid installing costly irrigation systems to ensure enough water is provided
    - Shelf life of crops can be increased e.g. flavor savr PG gene
- <u>Cons</u>
    - Introduced genes may be transferred by pollen to wild relatives whose hybrid offspring will become more invasive superweeds, which may lead to additional cost for removal, and may also reduce plant biodiversity by out-competing natural plants
    - Modified plant may produce toxins which will endanger humans or animals and disrupt ecological systems
    - Toxic residues may also cause unintended damage to the environment by causing pollution
    - Modified plants may produce allergies upon consumption

    - Some companies patent their GE seeds to prevent farmers from re-sowing the seeds, resulting in higher costs for farmers
    - Crossing species barrier and violating the genetic integrity of the orgaism

Suitability of Ti plasmids as vectors

- Contain T-DNA region which can be integrated into plant chromosome, are small, allowing it to be taken up by plant cell

Plant cell better than animal cell

- Plant cells are totipotent, can give rise to a whole plant. Genetic manipulation can be performed on any plant cell to generate crop plants with new traits. To create GM fishes, genes need to be injected into nucleus of fertilised egg

Advantages of GM fish

- Increase the quality of farmed fishes as fishes will be rich in omega 3 oil. Possibility of mass production of GM plant, reduces need for fishing, less disturbance to marine food chain. More sustainable source of omega 3 fatty acids, seeds produced can be used to plant new GM plant

Plant cloning

PGR result in plantlet formation

- Higher auxin to cytokinin ratio stimulates root growth and cell elongation
- Higher cytokinin to auxin ratio stimulates shoot growth and cell division

How plant tissue culture is used to clone plants

- Explant containing meristematic cells is selected from shoot tip/root tip
- Surface sterilise explants with dilute sodium hypochlorite to kill bacterial and fungal pathogens. Explant is aseptically transferred to culture vessels containing aseptic culture medium containing nutrients and plant growth regulators needed for plant growth. Auxin and cytokinin in equal ratio is added to nutrient agar to stimulate the cells of the explant to divide by mitosis to form a callus. Containers holding explants are then sealed and incubated for 1 to 9 weeks.
- All procedures must be sterile to ensure that microorgs do not grow in the cultures as they would grow faster and out-compete the plants in these conditions.
- As callus increases in size, callus is sub-cultured to create many genetically identical clones of the original plant.
- By adjusting concentration of auxin to cytokinin ratio in growth medium, cells in callus can be induced to differentiate into roots and shoots to enable plantlet formation
- Plantlets are then taken out from culture vessels, washed to remove agar, soaked in fungicide and antibiotics and then planted in sterile soil in green house for 4-8 weeks. This is to allow the plants to acclimatise to the outdoor environment.
- After acclimatisation, the plant is transferred to soil for field planting

Advantages of plant cloning

- Less space required to grow the plants in the lab as compared to the field

- Plant cultures are easier and cheaper to transport via air freight compared to adult plants
- Disease-free plants can be produced
- Genetic modification of plant cells is possible during tissue culture
- Production is not affected by environmental conditions
- Plants that are difficult to germinate from seeds can be easily produced via tissue culture

Disadvantages of plant cloning

- Expensive because of labour intensive/equipment/requirement to maintain a sterile environment via the use of laminar flow hoods which are expensive
- An infected stock plant can produce many infected progeny
- Not all plants can be successfully tissue-cultured, often because the growth medium for the plant is not known
- Monocultures are susceptible to new diseases, pests, or changes in environment due to genetic uniformity
- Monoclonal variation may occur, producing undesirable characteristics in plants