(c) DNA grows only from the 3' end
(d) Bases pair up according to complementary base-pairing rules – A to T and G to C

(4) Tools
(a) DNA fragment to be copied
(b) Heat-stable DNA polymerase
(i) Thermophilic – tolerant to heat and not denatured by the extreme temperatures used during the process
(ii) Derived from Taq (a thermophilic bacterium) that grows and lives in hot springs where temperature regularly reach up to 90°C
(iii) Capable of joining tens of thousands of nucleotides together in minutes
(c) Primers – oligonucleotides with complementary bases to those at one end of each of the two DNA strands to be copied
(i) Oligonucleotides – short, single-stranded nucleotide sequences
(ii) Used in sequencing reactions and polymerase chain reactions
(iii) Around 10-20 bases in length
(iv) Needed to bind to a section of DNA because the DNA polymerase enzyme cannot bind directly to single-stranded DNA fragments
(d) Free-floating nucleotides that contain all four bases in DNA
(e) Thermocycle – computer controlled machine that varies the temperature of the environment precisely over a period of time
(i) Eg. Cools so that primer hybridises to the DNA

(5) Stage 1 – separation of DNA
(a) Place all DNA fragments to be copied, free-floating DNA nucleotides and DNA polymerase into the thermocycler
(b) Heat to 95°C – heat does the job of DNA helicase enzymes in nature
(c) Heat breaks hydrogen bonding holding the complementary strands together
(d) Strands of DNA separated and made single-stranded

(6) Stage 2 – annealing (joining)
(a) Add primers to the thermocycler
(b) Cool mixture to 55°C
(c) Primers allowed to anneal to the complementary bases at the end of the DNA fragments via hydrogen bonding
(d) Small sections of double-stranded DNA at either end of the DNA sample formed
(e) Primers provide double-stranded sections for DNA polymerase to bind and work

(7) Stage 3 – synthesis of DNA
(a) Increase temperature to 72°C
(b) Optimum temperature for DNA polymerase
(c) DNA polymerase starts to add free, complementary DNA nucleotides along each of the separated DNA strands
(d) Double stranded sections of DNA provided by the primers are extended
(e) This will continue until the DNA polymerase reaches the end of the chain

*ix) Explain how isolated DNA fragments can be placed in plasmids, with reference to the role of ligase*

(1) DNA ligase enzyme – catalyses a condensation reaction which joins the sugar-phosphate backbones of the DNA double helix together

(2) Used in natural DNA replication to seal DNA nucleotides together to form new DNA

(3) Both DNA fragments need to have originally been cut with the same restriction enzyme to be joined together by DNA ligase

(a) Ensures nucleotide bases of sticky ends are complementary to one another

(b) Bases of sticky ends can anneal – pair up and hydrogen bond together

(c) DNA ligase can seal the sugar-phosphate backbone to form recombinant DNA

*x) State other vectors into which fragments of DNA may be incorporated*

(1) Choice of cloning vector depends upon the nature of the experiment undertaken

(2) Naturally occurring vectors include…

(a) Bacteriophages – viruses which act as parasites, infecting and replicating inside a bacteria, requiring much preparation before being used as cloning vehicles

(b) Plasmids – small (relative to major chromosome) double-stranded circular pieces of DNA found in many bacteria, separate from the main bacterial chromosome

(i) Capable of self-replication independent of the host cell chromosome – more than one can be found in a single bacterial cell

(ii) Carry genes needed only under special circumstances eg. those genes coded for antibiotic resistance

(iii) Gene sealed into a bacterial plasmid using DNA ligase

(c) Virus genomes

(d) Yeast cell chromosomes



*xi) Explain how plasmids may be taken up by bacterial cells in order to produce a transgenic microorganism that can express a desired gene product*

(1) Recombinant plasmid – plasmid containing a gene from another source

(a) Cut plasmid with the same restriction enzyme that is used to cut the donor DNA

(b) Sticky ends on the plasmid are now complementary to the sticky ends on the DNA

(c) Mix quantities of plasmid and DNA fragments with DNA ligase enzyme

(d) Sticky ends on the DNA fragment anneal to the sticky ends on the plasmid

(e) Blunt end ligations are less successful than sticky end ligations – no sticky ends to hold a fragment of DNA colliding with a cut plasmid in place during ligation

(f) DNA ligase catalyses the condensation reaction for that forms phosphodiester bonds between the phosphate on one DNA nucleotide and the sugar on another

(g) Plasmid and DNA segment sealed together

(h) Some (not all) plasmid will thus combine with the gene

(i) Plasmid will be sealed up to form a recombinant plasmid

(2) Transformation – vector acts as a vehicle to transport the gene into the host cell

(iii) DNA ligase enzyme used to seal up the plasmid

(i) Bacteria transformed with plasmid

(i) Mix plasmids with bacteria

(ii) Some of recombinant plasmids taken up by the bacteria

(j) Bacteria colonies grown on an agar plate – clones of bacteria that have arisen from a single bacterial cell that has grown and divided

(k) Transformed bacteria identified on an agar plate using marker genes

(l) Host bacteria cells containing the desired gene are grown

(i) Identified, transformed/transgenic bacteria picked off the agar plate

(ii) Transformed bacteria transferred to a fermenter with nutrient broth

(iii) Transgenic bacteria cultured on a large-scale

(iv) Many copies of the desired gene generated

(v) Bacteria expressed desired gene, secreting large quantities of human insulin

(m) Insulin extracted from fermenter and purified for use

*xv) Outline the process involved in the genetic engineering of 'Golden Rice$^{TM}$' (HSW6a)*

(1) Vitamin A (retinol)

(a) Only comes directly from animal sources in the diet

(b) Beta-carotene aka. pro vitamin A – precursor molecule which is converted to active vitamin A in the human gut

(c) Fat-soluble – lipids needed in diet if vitamin A is to be absorbed properly

(d) Deficiency is significant in poorer populations where rice is the staple food – vitamin and other food supplements to target groups has made little impacts on the devastating effects of malnutrition on these populations

(2) Functions of vitamin A – central role in maintain integrity of the immune system

(a) Eyesight – forms part of the visual pigment rhodopsin

(b) Cell growth and development – involved in synthesis of many glycoproteins

(c) Epithelial tissue – needed for maintenance and differentiation of epithelial cells, helps reduce the risk of infection

(d) Bones – essential for bone growth

(3) Rice plant (Oryza sativa) – contain genes that code for production of beta carotene

(a) Green tissues – inedible part of the plant that produces beta carotene

(i) Photosynthetic pigment molecule codes for the production of beta carotene

(b) Endosperm – grain, edible part of the seed that does not produce beta carotene

(i) All required genes to produce beta carotene are present in the grain

(ii) But some of these genes are turned off during development

(c) Outer coat of dehusked grains – contains valuable nutrients but no beta carotene

(i) Eg. Vitamin B and nutritious fats

(ii) Not eaten – lost with bran fraction in the processes of milling and polishing

(iii) Not apt for long term storage – fatty components of outer coat affected by oxidative processes that make the grain turn rancid when exposed to air

(f) Cells from to patient become genetically altered
(g) Altered cells injected into the patient
(h) Genetically altered cells transcribe and translate functional gene
(i) Desired protein is produced inside the body
(j) Patient may no longer have the symptoms associated with the genetic disorder

(2) Interference RNA – could silence genes by binding to mRNA
(a) Used to treat infections in AIDS patients
(b) Cytomegalovirus replication is blocked

*xviii) Explain the differences between somatic cell gene therapy and germ line cell gene therapy*

(1) Germline cell gene therapy – functional allele placed in the cells that produce the gametes (the sperm or the egg), a fertilized egg (zygote), or an early embryo
(a) Germline cell – cell of an early embryo with the potential to become a new being
(i) Stem cells – can divide and specialise to become any cell type within the body
(ii) Form when a sperm cell fertilises an egg cell to form a zygote
(b) Germline cells treated with the functioning allele
(c) Straightforward delivery techniques but not being considered at present
(d) Long-lived treatment – all cells derived from the germline cell will contain the functioning allele which will be expressed in those requiring the gene product
(e) Cells containing the functioning allele will pass the allele to their offspring
(f) Genetic manipulations could be passed on to the patient's children – could eliminate disease in future generations as original genetic defect is repaired

(2) Somatic cell gene therapy – functional allele placed in the cells of the affected tissues
(a) Specialised cells still have a full genome but have only certain genes switched on and active, whilst the rest (the majority) are switched off
(b) Augmentation – gene therapy by adding genes
(i) Some conditions are caused by inheritance of faulty alleles
(ii) Inheritance of faulty alleles leads to loss of a gene product and therefore function
(iii) Engineer functioning copy of the gene into the relevant specialised cells
(iv) Polypeptide is now synthesised
(v) Cells can function normally
(c) Gene therapy by killing specific cells
(i) Targeted treatment of cancers by eliminating certain populations of cells
(ii) Make cancerous cells express genes to produce proteins
(iii) Eg. Cell surface antigens produced will make the cells vulnerable to attack by the immune system
(d) Targeted, somatic cells treated with the functioning allele
(e) Difficulties in getting the allele into the genome in a functioning state
(i) Use of ex vivo therapy – specific, somatic cells removed, treated and replaced

(a) Research into genetic engineering is harming current living organisms
    (i) Trials using inactivated viruses in germline cell gene therapy to carry healthy genes into the patient's cells is not as safe as researchers had once thought
    (ii) Inadvertent modification of DNA in germline cell – can't tell whether allele has been successfully introduced without unintentional changes to the embryo
(b) Lack of long-term knowledge – unknown level of risk on future generations
    (i) Expression of a gene influenced by presence of other genes and environment
    (ii) Risk means genetically manipulating animals for any reason is unethical
(c) Reduction in genetic variation
    (i) GE crop plant passes on genes to wild relatives
    (ii) GE organism competes with the natural species which is then lost
(d) Widespread resistance to antibiotics – genetic engineering often uses antibiotic resistance genes as markers which could be passed to other microorganisms
    (i) E.coli is used to produce insulin
    (ii) Antibiotic resistant forms of E.coli (a bacteria which forms part of the natural fauna in the human gut) could enter humans
(e) Mutated genes transferred to pathogenic microorganisms – GE microorganism producing useful products may escape from containment and transfer mutations
(f) Hybrid crops produced are less useful – produced as GE crop plant and wild relatives share genes
(g) Super-weeds – herbicide resistance could be passed to weeds so stronger chemicals would need to be developed to remove the weed
(h) Super-pests – pesticide resistance could be passed to pests so stronger chemicals would need to be developed to remove the pest
(i) More rapid evolution of attack mechanisms in pathogens – to counteract more plants becoming resistant to the pathogen
(j) Stability of ecosystems could be affected – pesticide resistance could be passed to pests, affecting many other organisms in the associated food chains
(k) GM plants may be toxic to other organisms
(l) GM plants may lead to allergic responses in humans
(m) Drugs produced by GE animals could contaminate milk/meat supplies
(n) Large companies get patents for GE organisms and exploit farmers in the 3rd world eg. research on transgenic mouse, seeds from GM crops have to be bought
(o) Wrong to genetically engineer animals or harvest their organs for human benefit as it leads (or at least has the potential to lead) to animal suffering
(p) Religious beliefs – orthodox Jewish and Muslim faiths prohibit eating pork, cows are sacred to Hindus
(q) Individuals resulting from germline cell gene therapy would have no say in whether their genetic material should have been modified
(r) Eugenics could interfere with human evolution – germline cell gene therapy taken advantage of to enhance favourable characteristics