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**Knowledge Rich Curriculum Plan**

GCE Biology- Unit 3.8 Genetic Technologies



| **Lesson/Learning Sequence** | **Intended Knowledge:**  *Students will know that…* | **Prior Knowledge:**  *In order to know this, students need to already know that…* | **Tiered Vocabulary and Reading Activity** |
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| **Lesson 1:**  **Review of prior knowledge** | The impact of genetic mutations on protein synthesis. *(Y12 work: unit 3.4)* | Gene mutations might arise during DNA replication. They include addition, deletion, substitution, inversion, duplication and translocation of bases. Gene mutations occur spontaneously. The mutation rate is increased by mutagenic agents. Mutations can result in a different amino acid sequence in the encoded polypeptide.  Some gene mutations change only one triplet code. Due to the degenerate nature of the genetic code, not all such mutations result in a change to the encoded amino acid.  Some gene mutations change the nature of all base triplets downstream from the mutation, ie result in a frame shift.  Relate the nature of a gene mutation to its effects: specifically, outline how in DNA affect mRNA, tRNA and eventually the encoded polypeptide. | *Mutation*  *(deletion, substitution, inversion, duplication, translocation, frame-shift)*  *Mutagenic agents*  *Degenerate* |
| **Lesson 2:**  **Regulation of transcription and translation** | In eukaryotes, transcription of target genes can be stimulated or inhibited when specific transcriptional factors move from the cytoplasm into the nucleus. The role of the steroid hormone, oestrogen, in initiating transcription.  In eukaryotes and some prokaryotes, translation of the mRNA produced from target genes can be inhibited by RNA interference (RNAi). | Structure of eukaryote and prokaryote cells *(Y12, topic 2)* | *Transcription factors*  *Steroid*  *Oestrogen*  *interference* |

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| **Lesson 3:**  **Stem cells and gene expression** | Most of a cell’s DNA is not translated. During development, totipotent cells translate only part of their DNA, resulting in cell specialisation. Totipotent cells occur only for a limited time in mammalian embryos. Pluripotent, multipotent and unipotent cells are found in mature mammals. They can divide to form a limited number of different cell types.   * Pluripotent stem cells can divide in unlimited numbers and can be used in treating human disorders * Unipotent cells, exemplified by cardiomyocytes.   Induced pluripotent stem cells (IPS cells) can be produced from unipotent cells using appropriate protein transcription factors. | *Stem cells are undifferentiated.* | *Totipotent* |
| **Lesson 4:**  **Gene expression – transcription factors** | A gene is expressed or ‘switched on’ when it is transcribed into mRNA and then translated into a protein. Only a few genes in a cell will be expressed at any one time – this is how cells differentiate and become specialised. Before transcription can begin a gene needs to be stimulated by a regulatory protein, called **transcription factor**. Most eukaryotic genes are controlled by over 20 or 30 transcriptional factors interacting together. Transcription factors are proteins which travel into the nucleus and control the rate of transcription by either activating or repressing RNA polymerase. Each transcriptional factor binds to a specific regionof the DNA before the gene known as the promoter region. The promoter region is the binding site of RNA polymerase and therefore is the starting point for transcription of that gene. Transcription factors can turn genes on and off as they can either activate or block the functioning of RNA polymerase, repressors will bind to the promoter region and prevent the RNA polymerase from binding therefore stopping transcription of that gene.  Regulation of the transcription of these factors adds another layer of complexity to this system. Transcription factors can be switched off by an inhibitor molecule. This can bind to the transcriptional factor, preventing it from attaching to the promoter region. Without the transcriptional factor the gene may not be transcribed (if the TF is an activator) or the gene may be expressed (if the TF is a repressor). | In the previous lesson: different cells are expressing only part of the full genome. Differentiation is the point at which some genes are ‘switched on/off’.  Genes are expressed through protein synthesis. In Y12, students learn the process involving transcription followed by translation. |  |
| **Lesson 5:**  **Oestrogen as a transcription factor** | There are other extracellular molecules that can act as transcriptions factors. A major class of these is the steroid hormones such as oestrogen. A hormone response element (HRE) is a short sequence of DNA within the promoter of a gene, that is able to bind to a specific hormone receptor complex and therefore regulate transcription.  1 – 2 To affect transcription oestrogen has to bind to a transcription factor called an oestrogen receptor forming an oestrogen-oestrogen receptor complex. (not all cells have these receptors so not all cells are affected by oestrogen)  3 The oestrogen-oestrogen receptor complex moves from the cytoplasm into the nucleus  4 The complex binds to the HRE in the promoter of the gene, activating or repressor transcription. | Transcription factors interact with the promotor region of a gene to enable/prevent transcription.  Complementary base-pairs align to allow recognition of the binding region.  Protein molecules have tertiary structure.  Transcription involves the enzyme polymerase binding new nucleotides using phosphodiester bonds. | *Hormone*  *Steroid*  *Oestrogen*  *Oestrogen-oestrogen receptor complex*  *Hormone response element*  *Repressor*  *promotor* |

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| **Lesson 6:**  **iRNA** | RNAi molecules are small lengths of non-coding RNA (they do not code for proteins). They regulate gene expression by affecting translation instead of transcription. They are double stranded (unlike other RNAs!) and stop mRNA that has been transcribed already from being translated into a protein. There are two types: short interfering RNA (siRNA) – in animals only; micro RNA (miRNA) – in plants and animals.  **How they siRNAs and miRNAs (in plants) work:**  Double stranded siRNA associates with proteins in the cytoplasm and unwinds  One of the siRNA strands is chosen and the other degrades (breaks down)  Single strand of siRNA binds to the target mRNA (as it is complimentary to the base sequence in a section)  The proteins associated with the siRNA cut the mRNA into small fragments so it can no longer be translated. The mRNA fragments then move into a processing body which degrades them.  This has the potential use for “gene silencing” of inherited genes which cause disorders. Patients could be treated with siRNA molecules that are complimentary to mRNA transcribed from faulty genes to prevent faulty proteins from being expressed.  How do miRNAs work in mammals:  miRNAs are not fully complimentary to the target mRNA so they are often less specific and can target more than one mRNA molecule. miRNA has to go through processing stages in the cytoplasm to get to be a single strand like siRNAs: from a folded strand to a double strand then two single strands, one of these is degraded as with siRNA.  Once the miRNA is bound to the mRNA it does not cause degradation instead it simply blocks the translation by preventing ribosome from binding. The mRNA is then moved to a processing body where it can be stored (to be returned and translated another time) or degraded. | *RNA is a single-stranded polynucleotide molecule. It is shorter than a DNA molecule as it is only part of the genome for a specific polypeptide.*  *Complementary bases involved in RNA do not have T but rather U.* | *Interfering RNA*  *siRNA*  *miRNA*  *target mRNA*  *gene silencing* |
| **Lesson 7:**  **Gene mutations and cancer** | Control of gene expression therefore also plays an important role in cancer. The rate of cell division is controlled by genes, if mutations occur in these genes then uncontrolled cell division can occur. Uncontrollable cell division causes the cells to keep on dividing and they layer on top of each other forming a tumour – a mass of abnormal cells. Tumours that invade and destroy surrounding tissues are called cancers.  There are two types of genes that control cell division:  1) Proto-oncogenes are genes that normally help cells grow. When they mutate they can become permanently activated. When this happens, the cell grows out of control, which can lead to cancer. A mutated proto-oncogene is known as an oncogene.  2) Tumour suppressor genes are genes that normally help cells slow down cell division, repair DNA mistakes, or tell cells when to die (a process known as apoptosis or programmed cell death). When tumour suppressor genes don't work properly, cells can grow out of control, which can lead to cancer.  Mutations are changes to the base sequence of DNA, these can be caused by mutagens such as ionising radiation from UV rays or carcinogenic chemicals like those found in cigarette smoke. Some people can also inherit mutations in genes that make them more likely to suffer with some cancers.  **Example:** About 5-10% of breast cancer cases are thought to be hereditary (they are the result of gene changes passed on from a parent). The most common cause of hereditary breast cancer is an inherited mutation in the *BRCA1* or *BRCA2* gene. In normal cells, these genes help make proteins that repair damaged DNA. Mutated versions of these genes can lead to abnormal cell growth, which can lead to cancer.  If you have inherited a mutated copy of either gene from a parent, you have a higher risk of breast cancer.  Women with one of these mutations are more likely to be diagnosed with breast cancer at a younger age, as well as to have cancer in both breasts. | Y12 work (unit 4): the genetic code involves combinations of 4 nitrogenous bases. The sequence is specific for the amino acids that are incorporated into polypeptide chains.  In eukaryotes DNA is held in the nucleus surrounding proteins called histones. They package and order the DNA into structural units called nucleosomes. The histones associate (bind to) the DNA and help the DNA to condense into chromatin. Condensed DNA (chromatin) that is tightly bound to histones cannot be transcribed, the unwinding allows access for transcription. | *Cancer*  *Oncology*  *Oncogene*  *Histone*  *Proto-oncogene*  *Mutation*  *Tumour-suppressor gene*  *BRCA1/BRCA2* |
| **Lesson 8:**  **Tumours** | Cancer cells are different to normal cells in their structure and function, they will either die through apoptosis or they can be destroyed by the immune system which recognises them as ‘foreign’ or different to normal body cells because they:  a) Have large dark nuclei sometimes more than one  b) Irregular shape  c) Do not produce proteins needed to function properly  d) Different antigens on their surface membrane  e) Do not respond to the usual growth regulation process  Tumours need their own blood supply but often they suffer from low oxygen as the blood supply cannot satisfy the tumours oxygen demand as the rate of cell division is so high. There are two types of tumour: benign and malignant.  Methylation means adding a methyl group to a chemical. In the case of DNA, methylation regulates gene expression. The presence of the methyl group can either prevent or enhance the transcription of a gene. Too much/too little can cause tumours. Specifically tumours are caused if TSG are hypermethylated; proto-oncogenes are hypomethylated.  **Treating Cancer**  Cancer treatments can control the rate of cell division in cancer cells by targeting the cell cycle to try and stop it. This will kill the tumour cells and prevent them from dividing further. However, the treatments are not able to distinguish between cancer cells and other normal body cells that divide a lot e.g hair cells, stem cells in bone marrow and cells that line the small intestine (this explains side effects of cancer treatment e.g hair loss!). Cancer cells have a faster rate of division, so these cells are more likely to be affected by the cancer treatment. Examples of cancer treatments include:  Surgery is normally conducted where possible to remove a specific tumour if it hasn’t spread but this can lead to breakage and spreading of cells if not done carefully.  Immunotherapy and targeted therapy can help direct treatments to cancerous cells to reduce side effects and damage to the body.  Chemotherapy which prevents the synthesis of enzymes required for DNA replication. This stops the cell cycle before the S phase as it cannot take place. If DNA cannot be replicated cells will not be able to divide.  Radiotherapy and some chemotherapy drugs can damage DNA, if DNA is damaged the cell will not pass the check points in S phase. This will force the cell to kill itself (apoptosis). Cells will not divide/mitosis is prevented  Drugs which prevent spindle fibres from forming will prevent mitosis stage as one cell will contain double the DNA and one cell will contain no DNA. This prevents the cells from functioning or dividing again. | Immune cells recognise cells that need destroying by the protein molecules on their surface known as antigens.  Cell cycle involves different stages: PMATI/in particular, S phase within interphase when DNA replication occurs | *Apoptosis*  *Radiotherapy*  *Chemotherapy*  *Benign*  *Malignant*  *Immortal*  *Hyper/hypo-methylation*  *Tumour-suppressor gene*  *Proto-oncogene.* |
| **Lesson 9:**  **Epigenetic control of gene expression** | Histones are covered in chemicals: “tags”. These tags are known as the epigenome – ‘epi’ comes from the Greek and means “on”. These tags determine the shape of the histone-DNA complex and they help to control the transcription of genes. Epigenetics is the study of inheritable changes in gene function that have not been caused by changes to the base sequence of DNA. Epigenetic changes are reversible which means targeted drug therapy is possible. Examples of drugs: reverse methylation: azacytidine; decreased acetylation of histones causes genes to be switched off. Romidepsin will inhibit enzymes that deacetylate histones – this prevents the gene being transcribed. Issues: drugs will affect normal cells too; therefore target drugs to rapidly dividing cells to minimise the issues.  Fragile X is a genetic disorder. Learning, behavioural and physical differences occur. Caused by heritable duplication mutation (FMR1 gene). CGG sequence repeated too many times. Increased CpG sites causes increased methylation – the gene is switched off. The associated protein is not produced which results in the symptoms above.  Evaluating epigenetics: dopamine is used to regulate eating, but in patients with a faulty allele, this feedback mechanisms is not operated. Studies using twins can be used to evaluate epigenetic factors: genetics will prevail in both but environment would only affect those traits that are environmental. | Histones are proteins that allow for organisation of DNA when it is supercoiled.  Abnormal methylation causes TSG and oncogenes to be expressed differently. | *Histone*  *Epigenome*  *Tag*  *Fragile X*  *Angelman’s syndrome*  *Prada-Willi syndrome* |
| **Lesson 10:**  **Making DNA fragments** | Gene sequencing methods only work on fragments (not the entire genome) That means that the genome is fragmented, sequenced and then reconstituted to give the full genomic sequence. HGP completed in2003.  Proteome is the entire sequence needed to make polypeptides.NB bacteria do not have much non-coding DNA and hence it easier to determine the proteome of a bacterial cell. Use of bacteria in medicine design is made easy by this fact eg., antigens are easily sequenced.  In eukaryotic cells, the non-coding regions have regulatory genes that determine when/if a gene is switched on/off. This makes t very difficult to identify the proteome. Deciding which sequences are regulatory vs which are non-coding.  Process  a. automated, large-scale, cheap process  b. pyrosequencing determines upto 400 million bases in 10 hours  Recombinant DNA technology involves transferring fragments of DNA from one organisms to another.  Making fragments using  a. reverse transcriptase: use mRNA because the number of copies in the cytoplasm of a cell expressing that gene will be high. Enzyme reverse transcriptase | Genetic code is universal so DNA can be transferred from one organism to another.  DNA found in nucleus – two copies of each gene.  RNA found in cytoplasm – lots of copies if the cell is expressing that gene. | *Genome*  *HGP*  *Proteome*  *Reverse transcriptase*  *cDNA*  *Hind111*  *Palindrome*  *Oligonucleotide*  *Pyrosequencing*  *transgenic* |
| **Lesson 11:**  **In vivo amplification** | Small quantities of isolated DNA/genetic material need to be scaled-up to make any use viable. This is known as amplification. The first approach involves the use of cells to copy/clone the genetic material. This is known as in vivo amplification. The host cell is described as transformed. A vector is needed to carry the target sequence into the host cell. The two alternatives are bacteriophage or plasmids. Vector DNA is cut using restriction endonuclease enzymes to create sticky ends that are complementary to the sequence that will be at the insertion site. A second enzyme (DNA ligase) is used to join the ends of the target sequence to the vector sequence. The new sequence is described as transformed. The vector then carries the new sequence into the host cells. If it is a plasmid vector, cells need to be encouraged to take up the plasmid: conditions include ice cold CaCl2 to make cll walls more permeable followed by heat shock (42oC for a couple of minutes). None of this is needed with a bacteriophage approach.  Not all cells (approx. 5%) will be transformed so it is necessary to identify those that have accepted the new DNA sequence. Marker genes are used at the point of inserting target gene. If the marker gene is chosen as resistance to antibiotics, the cells that did not take up the gene can easily be destroyed and then the transformed clone colony established. Alternatively, the code could introduce fluorescence but this is not easy to separate ie it is useful to establish that transformation has occurred.  \*\* if the host cell is required to express the transformed gene, the sequence that is added will also need to include the promotor and terminator regions for that gene. | How to carry out isolation of gene fragments.  Structure and properties of bacterial cells: specifically replication rate and the fact that the genome has non-essential DNA that can easily be altered without impact on the cell eg., plasmids.  Restriction enzymes are used to cut DNA: they may create either sticky/blunt ends. Ligase enzymes are used to join DNA via complementary base-pairing. | *Isolation*  *Amplification*  *In vivo*  *Transformed*  *Vector*  *Plasmid*  *Bacteriophage*  *Restriction endonuclease enzymes*  *Ligase*  *Marker genes*  *Promotor*  *Terminator* |
| **Lesson 12:**  **In vitro amplification/PCR technique** | Polymerase Chain Reaction is an in vitro approach to amplifying DNA fragments. It is automated, fast and efficient. Millions of copies can be made in a few hours.  Reaction mixture = nucleotides, DNA sample, primers, DNA polymerase. Primers are short sequences of DNA complementary to the start of a DNA sequence to be built. Polymerase is the enzyme that makes the phosphodiester bonds that form the backbone of the new DNA strand. Heat (95oC) is used to break H-bonds between double-helix. Cool to 50-65oC to allow primers to anneal. Re-heat to 72oC to allow Polymerase to function (thermophilic source: Taq polymerase) Two new copies are made in each cycle. In the second cycle, 4 copies are available as templates. Critical idea: each cycle doubles the amount of DNA | Amplification is a necessary process to secure sufficient quantities of DNA to work with. In vivo has its benefits but also drawbacks. | *Primer*  *Polymerase*  *PCR*  *In vitro*  *Anneal*  *Thermophilic*  *Taq polymerase* |
| **Lesson 13:**  **Genetic engineering/ Recombinant DNA Technology** | All kinds of organisms can be genetically transformed as a consequence of the universal genetic code. (Microorganisms, plants and animals.) Microorganisms are transformed by in vivo processes eg., foreign DNA inserted to allow the microorganisms to express the proteins for insulin. Plants are transformed using plasmids as vectors too: the bacterium is the vector when it carries the transformed plasmid containing the promotor region, into the plant cells. Animal cells can be transformed by inserting the sequence into embryos/egg cells. All subsequent cells have the new gene sequence.  \*\* in human cells, if we use a promotor sequence that is only activated in certain conditions, the control of gene expression in specific cells can be established. (Producing protein in the wrong cells would not be desirable outcome!)  Benefits of recombinant technology:  Agriculture – crops give higher yields and pest resistance. Eg., golden rice has genes from maize plants and soil bacteria, produces beta carotene used by bodies to make vitamin A and this averts childhood blindness where dietary deficiency occurs in the population.  Industry – enzymes produced from transformed organisms can be produced faster/cheaper. Eg., rennin used to come from cows and would inevitable involve their death. Cheese making now is vegan friendly.  Medicine- drugs and vaccines can be produced using transformed organisms – insulin.  Concerns:  Agriculture – monoculture impact on biodiversity. Interbreeding between weeds and transformed crops could lead to pesticide resistant weeds! Contamination in neighbour fields if pollen blows out of the field.  Industry – smaller companies wiped out. Labelling to allow consumer choice. Economic loss if countries refuse to buy GM food eg., EU.  Medicine – companies who own the materials may restrict sales/increase prices/withhold vital medicines. Unethical use of recombinant technology eg., designer babies.  Ownership issues: who owns the genetic material? Donor or researcher? Patent issues eg., can a company own a seed? Can a farmer be sued for breaking patent after contamination breaches?  Humanitarianism: reduce famine risk, pharmaceuticals available more widely, low cost of medicine, gene therapy possibilities. |  | *Genetic engineering*  *Pharmaceutical*  *Economic*  *Patent*  *Breach* |
| **Lesson 14:**  **Gene probes, gene therapy and medical diagnosis** | Manipulating DNA is useful in diagnostic science.  DNA probes are short strands of DNA with a specific sequence of nucleotides that is complementary to an allele of interest.  DNA probes will hybridize to the sequence that it matches (eg target allele) using complementary base-pairing. To aid with visibility the probes are labelled with radioactive isotopes or fluorescent tags.  The process:  a. digest DNA sample using restriction enzymes  b. separate fragments with gel electrophoresis  c. transfer the separated fragments onto a nylon membrane and incubate with a labelled probe.  d. if the allele is present, the probe will attach.  e. use of UV lamp allows the fragments to be seen.  \* if lots of alleles are to be studies at the same time, a microarray is used. (This is a galss slide with microscopic spots of different probes used in rows. The DNA sample is washed over the microarray. If the sampe matches any of the probes, they will bind. The array is washed before viewing to remove unbound material.  Uses  a. to identify inherited diseases eg., Huntingdons  b. to determine if a patient will respond to certain drugs  c. to identify health risks  d. genetic counselling.  e. personalised medicine: tailor medicine to individual’s DNA  Gene therapy involves altering defective genes inside cells to overcome effects of mutations/cancer. The approach depends on whether the gene fault is dominant / recessive. If dominant allele: silence the expression by inserting a disruptive fragment to prevent it functioning properly. If recessive, add a working dominant allele to supplement the missing impact of that sequence. Both processes involve insering fragments into the patient’s DNA.  Vectors are either plasmids, viruses or liposomes. Somatic cell therapy involves insetting fragments into adult cells within the body: CF affects epithelial cells in the lungs/digestive system. Only that patient will be transformed ie offspring will not benefit.  Germ line therapy involves transforming the sex cells and would benefit offspring: currently illegal! | Structure of DNA  Isotopes are radioactive atoms that give out energy or particles that can be detected. | *Probe*  *Hybrid*  *Microarray*  *Somatic*  *Germ line*  *liposomes* |
| **Lesson 15:**  **Genetic fingerprinting** | Genomes can contain large numbers of non-coding variable number tandem repeats (VNTR).  Not all of the genome codes for proteins. VNTR are non-coding sequences. They have sequences that repeat upto a thousand times eg., CATG. The number of repeats varies in each individual which means that they can be used to identify us. The chance of two people having the same length of VNTR is very low.  A genetic fingerprint compares the VNTR in different individuals.  The process:  a. get the sequence to be studied.  b. amplify the number of copies using PCR and primers  c. add a fluorescent tag to allow them to be viewed  d. use gel electrophoresis to separate the fragments by size.  e. view the separated fragments using a UV lamp. (The bands are known as the fingerprint.)  Uses of genetic fingerprints:  a) determining genetic relationships: VNTR are inherited. Similarities between parent and offspring indicate match.  b) determining genetic variability in a population: more bands that do not match indicate greater diversity.  c) forensic science  d) medical diagnosis: screening embryos made in IVF before implantation; diagnosing tumours is better than observing abnormal cells.  e) animal husbandry/plant propagation: prevents inbreeding | Genetic bases and complementary base pairing.  Proteome and genome.  PCR = polymerase chain reaction  Gel electrophoresis is a process that separates dna fragments that have different charges/sizes. | *VNTR*  *Primer*  *Buffer*  *electrophoresis* |