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10 Genomics, Proteomics, and Genetic Engineering

10.1 Genome Sequencing

- In most methods, short, doubled stranded **adapter molecules**, which are complementary to oligonucleotide primers allowing for PCR amplification.
- **Sequencing by synthesis**: shear DNA, spread out over a flat surface, and subjected to PCR amplification. Result is many small clusters of PCR products, that are analyzed with reversible terinators, with 3' end blocked, forcing addition of one base as well as a floresent tags.
- **Ion torrent sequencing**: a method of DNA sequencing based on the detection of hydrogen ions that are released during the polymerization of DNA. This technology differs from other sequencing-by-synthesis technologies in that no modified nucleotides or optics are used.
- **Single-molecule sequencing**: A single DNA polymerase enzyme is affixed at the bottom of a ZMW with a single molecule of DNA as a template. The ZMW is a structure that creates an illuminated observation volume that is small enough to observe only a single nucleotide of DNA being incorporated by DNA polymerase.
- **Nanopore sequencing**: a single molecule of DNA or RNA can be sequenced without the need for PCR amplification or chemical labeling of the sample. Has higher rate of error, up to 5-10%.
- **Comparative genomics**: a field of research where organisms are compared, mostly by alignment of genome sequences and checking extent of conservation.
- Comparative genomics can also help target regulatory motifs, which are hard to find due to their relatively short sequences and ability to change position.

10.2 Genomics and Proteomics

- **Functional genomics**: a dynamic focus on genome-wide paterns of gene expression and coordination instead of just DNA structures.
- **DNA mircoarray**: a collection of microscopic DNA spots attached to a solid surface that allow for the measurement of the expression levels of large numbers of genes simultaneously or to genotype multiple regions of a genome.

10.3 Recombinant DNA

- **Recombinant DNA**: isolated DNA is cut into fragments by one or more restircion enzyme, joined back together in a new combination, and then reintroduced into a cell or organism.
- Restriction enzymes have the same sticky ends regardless of organism as log as they were produced by same enzyme.
- Most useful vectors are easily introduced, contain a replication origin, and allow the growth of a cell on a solid selective medium.
- Some vectors can accept large DNA fragments, up to 100-200 kb, and are called *artificial chromosomes*.
- Most common are bacterial artificial chromosomes (BACs).
- The sticky ends are joined together using DNA ligase.
- Reverse transcriptase can synthesize a complementary strand of DNA called cDNA.
- The 3’ end of cDNA can fold back on itself, creating a hairpin primer for second-strand synthesis.
- *Polylinker*, or multiple cloning site (MCS), in a vectors makes directional cloning possible.

13 Molecular Genetids of the Cell Cycle and Cancer

13.1 The Cell Cycle is Under Genetic Control

- **Centrosome**: a small region of clear cytoplasm near the interphase nucleus, typically (in most eukaryotes) made up of a pair of **centrioles**.
- The function of both are microtubule-organizing centers and a regulators of the cell cycle.
- Genes encoding proteins that are needed in the cell cycle are typically transcribed right before they are needed.
- **cyclin-CDK complexes**: regulator of progression in the early stages of the cell cycle.
- Protein degradation also helps regulate the cell cycle.

13.2 Cell Checkpoints

- Checkpoints help maintain the correct steps as the cell cycle progresses, causing the cycle to pause until correction is done.
- Three principal checkpoints: DNA damage, centrosome duplication, and spindle checkpoints.
- Failure to stop may lead to aneuploidy(spindle), polyploidy(centrosome), of increased number of mutations (DNA; translocation,deletion, of amplification).
- **p53 transcription factor**: key proteins that come in slightly different forms that respond to stress and DNA damage.
- In norman cells, p53 is low and is removed by Mdm2 for degradation. Damaged DNA results in phosphorylation and and inability of Mdm2 to export it.
- Increased p53 turns on/off transcription of other genes that halt the cell cycle and other cellular properties.

- **Oncogenes**: genes associated with cancers, which can interfer with apoptosis.
- Shortage of oxygen, DNA damage, or shortage of nucleoside triphosphates can increase p53 activity.
- Apopotsis, angiogenesis/metastasis, or arrest/repair pathways may all be activated by activated/deactivated genes.
- Centrosome duplication checkpoint is one that monitors the formation of the spindle and coordinate entry into mitosis.
- The spindle assembly checkpoint may work with the centrosome checkpoint, but it also monitors spindle attachment of kinetochores (spindle fiber attachment site on the chromosome).
- Improper spindle assembly blocks the separation of sister chromatides, preventing anaphase.

13.3 Cancer Cell Mutations

- **Familial**: clear evidence for segregagion of a gene that prediposes cells to progress to the cancerous state.
- **Sporadic**: cancer resulting in genetic changes in somatic cells that is not the result of a familial case.
- Six attributes of cancer cells:
  - loss of growth-factor dependence.
  - Insensitivity to anti-growth signals.
  - Evasion of apoptosis.
  - No cell senescence.
  - Ability to metastasize and invade other tissues.
  - Sustained angiogenesis (formation of blood vessels)
- *proto-oncogenes*: promote cell division of prevent apoptosis.
- *tumor-suppressor*: prevent dell division or promote apoptosis.

14 Molecular Evolution and Population Genetics

14.1 Evolutionary Relationships Among Species

- **Molecular evolution**: study of how the sequences of macromolecules change through time.
- **Molecular phylogenetics**: study of evolutionary relationships among species.
- a mutant allele is **fixed** if it replaces all other allels in the population.
- **Gene tree**: a methoad to estimate the pattern of evolutionary relationships in different species based on a single gene.
- Rates of evolution can differ dramatically from one protein to another.
- **Synonymous substitution**: a modification in which the amino acid is not affected.
- **Nonsynonymous substitution**: a modification that does result in an amino acid replacement.
- Nonsynonymous sites occurs primarily at the first and second codon positions.
- **Pseudogenes**: duplicate genes that have lost their function due to mutations.
- *fourfold degenerate site*: a synonymous nucleotide site that produces same amino acid regardless of nucleotide.
- *Twofold degenerate site*: encoded amino acid only is affected depending on if it is a pyrimidine or purine.
- **Subfunctionalization**: specialization of paralogs accompanying loss of functional capabilities.

14.2 Genotypes Frequency in Populations

- **Genotype frequency**: proportion of a population that have a particular genotype e.g., amounts of AA.

- **Allele frequency**: proportion of of all alleles that of specified type e.g., amount of A.

14.3 Random Mating

- One important implication ot the Hardy-Weinberg principle is that the allele frequencies remain constant from generation to generation.
- Hardy-Weinberg depends on several assumptions:
  - Mating is random.
  - Allele frequencies are the same in males and females.
  - All genotypes have equal survivability and fertility.
  - Mutation does not occur.
  - Migration is absent.
  - The population is sufficiently large to avoid chance of sudden major allele frequency changes.
- For a rare allele, the frequency of heterozygotes far exceeds the frequency of the rare homozygote.
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14.4 Highly Polymorphic Sequences and DNA Typing

- **Polymorphic**: two or more allele are common in the population.
- **DNA typing**: the use of polymorphisms to identify an individuals DNA characteristics.
- **Simple tandem repeat (STR)**: a polymorphism that contains units of DNA repeated in tandem.
- Most people are heterozygous for STR alleles that produce restriction fragments of different sizes.
- The restriction fragments from different people cover a wide range of sizes– indicating the population as a whole contains many STR allels.

14.5 Inbreeding

- Inbreeding reduces frequency of heterozygous genotypes by half for each generation.
- **Inbreeding coefficient**: or F, is defined ase the proportionate reduction in H_1 compared with the value of 2pq expected in random mating.
- Inbreeding is so frequent in plants that additional inbreeding often has no effect.

15 The Genetic Basis of Complex Traits

15.1 Complex Traits

- **Multifactorial traits**: when multiple genetic and environmental factors implicated in their causation.
- **Genetic architecture**: a description of all genetic and environmental factors
- **Quantitative traits**: another name for complex traits, since they often varyin in quantity rather than type.
- **Continuous traits**: variation in one phenotypic extreme to another with no clear breaks.
- **Categorical traits**: traits in which phenotype corresponds to any one of a number of discrete catetories.
- **Threshold traits**: traits that have only few, typically two, phenotypic classes, but inheritance is determined by multiple genes and the environment.
- **Distribution**: a description of a population in terms ot the proportion of individuals that have each possible phenotype.
- Distribution implies nothing about a trait's inheritance.

15.2 Genetic and Environmental Components of Variation

- Four sources contribute to phenotypic variation: genotypic, environmental, genotype-by-environment interaction, and genotype-by-environment association.
- Selective breeding can create an improved population in which every individual greatly exceeds that of the best individuals from the original population.
- Variation in a trait can be entirely genetic or environmental, or a combination of both.
- When genetic and environment effects contribute independently, then the total variance equals the sum of genotypic and environmental variance.

- **Genotype-by-environment interaction**: when environmental effects on phenotype differ according to genotype.
- **Genotype-by-environment association**: when certain genotypes are preferentially associated with certain environments.
- **Broad-sense heritability (H^2)**: a ratio of variances, specifically between the genotypic variance and the total phenotypic variance.

15.3 Artificial Selection

- **Truncation point**: the selection practiced by choosing some arbitrary level of phenotype that determines which individuals with will be saved for breeding purposes.
- **Narrow-sense heritability (h^2)**: includes only the additive effects of alleles, or genetic effects that are transmissible from parent to offspring in order to measure how similar offspring are to their parents.
- Narrow-sense is used to predict changes in the population mean with individual selection.
- Selection limits are reached when natural selection and artificial selection balance out. Unless population is large enough to introduce new mutations, then the limit is continuously pushed back.
- **Heterosis, or hybrid vigor**: when the F_1 generation of a cross between two inbred populations is superior, due to canclation of recessive allels in the cross, but retention of superior dominant allels.