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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.

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