Module Questions

1. Which of the following steps is NOT typically involved in Next Generation Sequencing protocols?
    a) Isolating DNA or RNA from tissue
    b) Checking the quality and quantity of the fragment library
    c) Aligning reads to a reference genome
    d) Excluding all homopolymer regions from analysis
    e) Comparing variant allele frequencies to normal population data

Rationale: Homopolymer regions are typically not excluded from NGS data analysis because the read length and coverage depth, coupled with the common use of paired-end reads, permits homopolymers to be sequenced with reasonable accuracy in the consensus sequence derived from the NGS coverage. Furthermore, homopolymers may be utilized in important clinical data analyses such as the evaluation of microsatellite loci for MSI status. Reference: PMID 28782984.

2. Each of the following are types of reads generated by NGS EXCEPT?
    a) Paired-end reads
    b) Single-end reads
    c) Reverse-end reads
    d) RNA-derived reads
    e) DNA-derived reads

Rationale: There is no such term in NGS as reverse-end reads

3. Why do many laboratories continue to offer Sanger sequencing?
    a) It is the cheapest sequencing technology available
    b) It is the fastest sequencing technology available
    c) It does not involve dideoxy nucleotides
    d) It is used for clinical confirmation when needed
    e) It is actually not used anymore

Rationale: By using Sanger sequencing, laboratories can easily and affordably design custom confirmation assays for variants detected by other genomic testing methods. Sanger sequencing is particularly useful to confirm variants found by in the germline by other sequencing methods as the variants are typically present with a VAF of 50% or 100%, which is well within the sensitivity of Sanger sequencing. In somatic (oncology) testing, Sanger sequencing may be more challenging as it has a limited sensitivity (10-15% VAF) and may not detect variants identified in samples with a low neoplastic cell content. Reference: PMID 28362156.

4. In the context of DNA sequencing data analysis, what is a contig?
    a) A set of overlapping aligned NGS read data that define a continuous stretch of the original genome
    b) The number of nucleotides per fragment
    c) The complete set of RNA transcripts in a sample at a certain point of time
    d) The complete set of exons in an organism's genome
    e) A computational process to reconstruct a longer sequence from short sequences

Rationale: The term "contig" is an abbreviated form of "contiguous", meaning aligned reads along a continuous stretch of chromosomal sequence in a reference genome that provide complete coverage depth and breadth appropriate to call variants and in some cases, to provide haplotyping information.

5. NGS read data obtained from both ends of the library fragments is referred to as which of the following?
    a) Double-ended
    b) Duplicate reads
    c) Aligned reads
    d) Paired-end reads
    e) Indel reads

Rationale: PMID 28782984.

6. Single molecule sequencing approaches differ from NGS in which of the following ways?
    a) No library construction step is needed for single molecule sequencing
    b) No instrumentation is needed for single molecule sequencing
    c) Significantly longer, more error-prone reads are produced from single molecule sequencing
    d) Much less DNA input is needed for single molecule sequencing
    e) DNA polymerase for nucleotide incorporation is required for single molecule sequencing

Rationale: PMID 30478097.

7. The incorporation of a nucleotide into a primed DNA strand by DNA polymerase results in which of the following?
    a) Release of a hydrogen ion
    b) Release of a hydroxide group
    c) Emission of light
    d) Release of energy
    e) Termination of the elongation reaction

Rationale: DNA polymerase adds nucleotides to the 3′ end of a polynucleotide chain during DNA synthesis. The polymerase catalyzes the nucleophilic attack of the 3′-hydroxyl group terminus of the polynucleotide chain on the α-phosphate group of the nucleoside triphosphate to be added, causing the release of a hydrogen ion and phosphate. This release of hydrogen ions is used as the detection method for specific nucleotide incorporation in some NGS platforms.