

## 22126 NGS analysis recap test

Q1. What are the commonalities and differences between how Sanger sequencing operates and the second generation of next-generation sequencing machines (Illumina, Ion Torrent)?

Q2. What are the primary differences between how the second generation of next-generation sequencing machines (Illumina, Ion Torrent) operate and third generation NGS platforms?

Q3. What is the main type of sequencing error seen with Illumina data? Why?

Q4. After 10 cycles (a cycle of 1 type of dNTP are bound, pH measure, unattached dNTP molecules are washed out) of Ion torrent, for which kind of sequence are you guaranteed to have exactly resolved 10 bases for a read.

Q5. How many lines is one read in fastq format? What are the lines?

Q6. What does it mean that a base in a read has a base quality of Q20?

Q7. A sequence has a length of 200 bases. An Illumina sequencer is used with 75 cycles. How many bases will have been **unsequenced** if used in single-end mode? In paired-end mode?

Q8. A sequence has a length of 100 bases. An Illumina sequencer is used with 75 cycles. How many bases will have been **sequenced twice** if used in single-end mode? In paired-end mode?

Q9. What does it mean to have sequenced a genome to 50X?

Q10. Briefly describe the principle of the Seed and Extend algorithm.

Q11. Why are longer reads better for aligning or assembly?

Q12. Create the Burrows-Wheeler Transformation of this sequence "TAGC".

Q13. Create the de Bruijn graph of this sequence using k=3: ACGTTGGTCGTG

Q14. How do we create contigs and scaffolds from a de Bruijn graph?

Q15. Why is de novo assembly much harder for metagenomic data compared to single genome data?

Q16. How would you analyze if your metagenomic sequencing has sampled enough? Both for 16s rRNA amplicon data and for shotgun-metagenomic sequencing.

Q17. Someone says: "We have observed a read aligning at that position and there was an 'A', therefore, the sample is homozygous A". I can think of half-dozen reasons why this is not true. List 3.