Corrections

1. PCR = Polymerase Chain Reaction = laboratory technique used to amplify a specific segment of DNA, creating millions to billions of copies of a particular DNA sequence in a short time. Applications: genetic testing, forensic analysis, medical diagnostics.

PCR involves repeated cycles of heating and cooling. Each cycle consists of 3 main steps:
(1) Denaturation (94–98°C): The double-stranded DNA is heated to a high temperature to separate the DNA into two single strands.
(2) Annealing (50–65°C): The temperature is lowered, allowing short synthetic DNA sequences called primers to bind (anneal) to their complementary sequences on the single-stranded DNA. Primers are designed to flank the target DNA sequence to be amplified.
(3) Extension (72°C): The temperature is raised to the optimal working temperature of Taq DNA polymerase (a heat-stable enzyme derived from Thermus aquaticus). The enzyme extends the primers by adding complementary nucleotides to the DNA strand, synthesizing new DNA strands.

Key Components of PCR:
Template DNA: The DNA that contains the target sequence to be amplified.
Primers: Short single-stranded DNA sequences designed to bind specifically to the target region.
DNA polymerase: A heat-stable enzyme (e.g., Taq polymerase) that synthesizes new DNA strands.
Deoxynucleotide triphosphates (dNTPs): The nucleotides (A, T, G, C) that the enzyme incorporates into the new DNA strands.
Buffer solution: Maintains the optimal conditions for enzyme activity.

Cycle Repetition:
The three steps (denaturation, annealing, extension) are repeated about 30 times in a thermal cycler. Each cycle doubles the number of DNA molecules, resulting in exponential amplification.

2. Sanger sequencing = technique developed by Sanger to determine the nucleotide sequence of DNA.
Principle: relies on the selective incorporation of modified nucleotides called dideoxynucleotides (ddNTPs) into a growing DNA strand during DNA synthesis. These ddNTPs lack the 3'-hydroxyl group (-OH) required for forming the next bond, leading to chain termination.
The reaction mixture contains:
Template DNA: The sequence to be determined.
Primer: A short single-stranded DNA that binds to the template near the region to be sequenced.
DNA polymerase: An enzyme that synthesizes the complementary DNA strand.
Deoxynucleotides (dNTPs): Normal A, T, G, and C nucleotides.
Dideoxynucleotides (ddNTPs): Modified nucleotides labeled with different fluorescent dyes, each specific to A, T, G, or C.

Key feature: Only a small fraction of nucleotides in the reaction are ddNTPs, so most strands are extended normally, but occasionally a ddNTP is incorporated, terminating the strand.

DNA Synthesis and Chain Termination:

During synthesis, the DNA polymerase randomly incorporates either a dNTP (normal nucleotide) or a ddNTP (terminating nucleotide).

Incorporation of a ddNTP stops further extension of the DNA strand.

This creates fragments of various lengths, each ending at a specific nucleotide.

Fragment Separation (Electrophoresis):

The DNA fragments are separated by size. Shorter fragments move faster through the gel or capillary than longer ones.

Detection and Data Interpretation:

A laser detects the fluorescent labels on the ddNTPs.

Each fluorescent signal corresponds to a specific nucleotide (A, T, G, or C).

The sequence of the original DNA is reconstructed based on the order of detected signals. The output is a chromatogram.

2. Looking at the chromatogram, we can see that the *BRCA1* mutation is the insertion of AGGTTTGCA at the position of the read arrows, together with a deletion of the 2 nucleotides GC. It will lead to a shift in the peaks of the second mutant allele.

3. Yes, double peaks should be found **all the way** to the end of the chromatogram. NB: By chance, there are 3 peaks with just A at the end of Fig. 2 due to the A stretch.

4. The c311_312delinsAGGTTTGCA mutation is an insertion of 9 bp (this deletion alone will not lead to a frameshift), and a deletion of 2 nucleotides (not a multiple of 3), so this will lead to a frameshift and thus disruption of the full amino acid sequence after the mutation.
No need to use the genetic table here to infer the effect of the mutation.