2025 Bi H2 Q1

Section: DNA and the Genome

Topic: DNA Replication

Question Summary:

PCR diagram question. Parts (a) to (e) cover: the 3 prime end of DNA, PCR temperatures and polymerase role, why ligase and human polymerase are not used, a percentage calculation from a reaction mix, and copy number after 8 PCR cycles.

Worked Solution:

(a) The 3 prime end of a DNA strand has a free hydroxyl group (OH) on the deoxyribose sugar.
So the part of a nucleotide at the 3 prime end is the deoxyribose sugar (with its 3 prime OH).

(b)(i) Stage 3 is the extension stage. Taq polymerase works best at about 72 C.
So a suitable temperature is 72 C.

(b)(ii) In stage 3 DNA polymerase adds complementary nucleotides to the 3 prime end of each primer.
It extends the new strand in the 5 prime to 3 prime direction to copy the template.

(c)(i) Ligase joins Okazaki fragments during normal DNA replication.
In PCR, each new strand is made continuously from a primer by DNA polymerase,

so there are no fragments to join. Therefore ligase is not required.

(c)(ii) Human DNA polymerase would be denatured at the high temperature used in PCR
(about 95 C during strand separation). It is not heat stable, so it would stop working.
Taq polymerase from thermophilic bacteria is heat stable and survives these temperatures.

(d) Total volume in the tube:
15.8 + 2.4 + 1.7 + 2.1 + 2.0 + 0.4 + 0.6 = 25.0 microlitres.
Percentage that is DNA polymerase:
(0.4 / 25.0) x 100 = 1.6 percent.

(e) Each PCR cycle doubles the number of DNA molecules.
Starting number = 70.
After 8 cycles: 70 x 2^8.
2^8 = 256.
So copies = 70 x 256 = 17920 DNA molecules.

Final Answers:

(a) Deoxyribose sugar (3 prime OH).
(b)(i) 72 C.
(b)(ii) Adds complementary nucleotides to primers to extend new strands.
(c)(i) No Okazaki fragments in PCR, so no joining needed.
(c)(ii) Human polymerase is not heat stable and denatures at 95 C.
(d) 1.6 percent.
(e) 17920 copies.

Revision Tips:

- PCR steps: 95 C denature, about 55 C anneal, 72 C extend.
- DNA polymerase always adds to the 3 prime end, so strands grow 5 prime to 3 prime.
- Ligase is needed only when fragments must be joined.
- Taq polymerase is heat stable.
- PCR doubling rule: N = N0 x 2^cycles.