

Solutions

Each answer shows the steps, then the **result**.

2.1

(a)

- restriction endonuclease

(b)

- DNA ligase

2.2

- to check (confirm) that the gene has been taken up and is expressed

2.3

- Restriction endonuclease; DNA ligase

2.4

- Each restriction enzyme cuts at one specific sequence and leaves a particular sticky end; using the same enzyme on both means their overhangs are complementary, so the gene and the plasmid pair together specifically

2.5

- It allows the cells that have taken up the plasmid to be identified; for example an antibiotic-resistance gene lets only transformed cells grow on a medium containing that antibiotic

3.1 B

- A plasmid is a vector carrying the gene into the cell

3.2 B

- Shorter fragments move further through the gel

3.3

(a)

- the promoter is the switch that starts transcription; without it the transferred gene would not be transcribed / stay silent

(b)

- heat to ~95 °C to separate the strands; cool to ~55 °C so primers attach; 72 °C lets Taq polymerase build new strands — repeating the cycle doubles the DNA each time

3.4

- the restriction enzyme cuts the gene and the plasmid, leaving matching sticky ends; DNA ligase joins the gene into the plasmid to make recombinant DNA

3.5

(a)

- Extract the mRNA for the protein from a cell that expresses it; use reverse transcriptase to make complementary DNA from that mRNA template; this cDNA is the gene, and it has no introns. (Cutting the gene from the genome with a restriction endonuclease is also accepted.)

(b)

- Sticky ends are short single-stranded overhangs left when the restriction enzyme cuts; the gene and the plasmid are cut with the same enzyme, so their overhangs are complementary; the bases pair by hydrogen bonding, holding the gene in place in the plasmid

(c)

- DNA ligase; the product is recombinant DNA

(d)

- The plasmid also carries a marker gene, for example antibiotic resistance; the bacteria are grown on a medium containing that antibiotic; only the cells that took up the plasmid survive and form colonies, so those are the transformed cells. (A fluorescent marker viewed under UV is equally acceptable.)

(e)

- Any two of: the protein is identical to the human one, so it is less likely to cause an immune response; it can be produced in unlimited quantity by culturing bacteria; production does not depend on a supply of animal tissue; it avoids religious or ethical objections to an animal product

(f)

- Cut the plasmid with the same restriction enzyme to release the insert; run the fragments through a gel under an electric field, where smaller fragments travel further; compare the distance travelled with a set of fragments of known length, and a band at the expected position shows the insert is the right size