19. Genetic Technology

19.1 Principles of Genetic Technology

Paper 4

Marking Scheme

Q1.

(b)	1 only small amount of DNA available ; **A** gene 2 amplify, DNA / (*BRCA1*) gene ;	**2**

Q2.

(b)(i)	**1** (heat to) 90–98 °C so DNA, denatures / strands separate ; **2** (cool to) 50–65 °C so primers, bind / base pair / anneal (to DNA) ; **3** (heat to) 68–75 °C so, *Taq* / DNA, polymerase, makes DNA / (new) strand ; **4** repeat cycles to amplify DNA ;	**4**
(b)(ii)	used, a (named) database / bioinformatics ;	**1**

(c)(i)	**A** ;	**1**
(c)(ii)	$(2^3) = 8$;	**1**
(d)(i)	(named) plasmid / vector (used) ; (containing) gene / DNA, for (green) fluorescent protein ; (containing) a (prokaryotic) promoter ;	**3**
(d)(ii)	to act as a, marker / reporter, gene **or** to identify, recombinant / GM / transgenic, bacteria / organisms **or** to show expression of another transferred gene ;	**1**

Q3.

(a)	*any **three** from:* 1 manipulation of, genetic material / gene / DNA **or** described ; 2 *ref. to* gene / allele, transfer (into, cell / organism) **or** described ; 3 expression of gene ; 4 (named) phenotype modified / (named) protein produced ; 5 AVP ; e.g. *ref. to* gene editing / produce GMO	**3**

(c)(ii)	*any **three** from:* 1 **mutated** DNA is, deleted / removed / cut out **or** DNA replaced with **normal** DNA ; **Ignore** exon X is deleted as this is mRNA not DNA **Reject** gene deleted / cut out 2 specific (locations) / between exon 26 and exon 27, (in the genome) ; 3 functional protein / CEP290, now made **OR** correct functioning of photoreceptors ; 4 acts on person's own DNA ; 5 AVP ; e.g. STOP codon no longer present	**3**

Q4.

(a)	*any **three** from*: **1** make cDNA from, messenger RNA / mRNA ; **2** using reverse transcriptase ; **3** synthesise (gene chemically) from nucleotides / join nucleotides ; **4** (using known) amino acid / nucleotide, sequence from database ;	**3**
(b)	<table><tr><th>role in genetic engineering</th><th>DNA ligase</th><th>DNA polymerase</th><th></th></tr><tr><td>joins two sections of sugar phosphate backbone in DNA</td><td>✓</td><td>×</td><td>;</td></tr><tr><td>adds a gene to a plasmid</td><td>✓</td><td>×</td><td>;</td></tr><tr><td>adds free activated DNA nucleotides to a polynucleotide</td><td>×</td><td>✓</td><td>;</td></tr></table>	**3**
(c)	**1** 90–98°C – DNA, denatures / strands separate ; **2** 50–65°C – primers, bind / base pair / anneal (to DNA) ; **3** 68–75°C – Taq / DNA, polymerase, makes, DNA / (new) strand ;	**3**

Q5.

(b)	*any **four** from:* 1 obtain (specific restriction endonuclease) gene, qualified ; e.g. gene extracted from the, DNA / genome, of E. coli / bacteria gene synthesised from the mRNA of E. coli / bacteria gene synthesised from nucleotides 2 PCR to make more copies of, gene / DNA ; 3 cut a plasmid, with a restriction enzyme / to give sticky ends ; 4 combine / mix / join, gene with plasmid using ligase ; 5 add plasmid to, E. coli / bacteria ; **A** transformation of E. coli 6 use marker genes to, select / identify, bacteria / E. coli, with recombinant plasmids ; 7 *ref.* culture bacteria / use fermenter ; 8 *ref.* extraction / purification (of enzyme) ; 9 AVP ; e.g.heat shock / calcium ions / electroporation (detail mp5) complementary sticky ends anneal / make phosphodiester bonds (detail mp4)	**4**

(c)	*any **three** from:* 1 (data stored is) a large amount of, amino acid / nucleotide / base / DNA, sequences ; 2 share data / access data quickly ; 3 compare (amino acid / nucleotide / base / DNA) sequences ; 4 use to, predict / visualise / model, the structure of the enzyme ; 5 identify restriction endonuclease to use **or** find / discover, new restriction endonucleases ;	**3**

(d) *any **four** from:* **4**

	feature	electrophoresis	chromatography	
1	separation role	both – are separating techniques **or** both – involve movement of substances		;
2	molecule separated	DNA / RNA / proteins	pigments / named pigment / amino acids	;
3	medium	gel / named gel	paper / glass / silica gel / named medium	;
4	separated by	(electric) current / electric field / electricity	solvent / named solvent	;
5	separated due to	charges / polarity / lengths / mass	solubilities / mass	
		both – separate by mass		;
6	visualisation using	dyes / ethidium bromide / UV light	different colours of pigments / dyes not added / UV light not used	
		both - dyes can be added (if TLC)		;
7	identification by	use, DNA ladder / known length of DNA **or** use of DNA probe	use R_f value	
		both - use of, references / known values		;

Q6.

(a)(i)	DNA, combined / joined, from, two / different, organisms / sources ;	1

(b)(ii)	*any **three** from:* **1** obtain (normal human), gene / DNA, from (m)RNA by reverse transcriptase / using restriction enzyme ; **2** use gene editing (to alter human gene) ; **3** obtain (nucleotide) sequence for (normal human) insulin gene ; **4** synthesise, new / analogue, gene / nucleotide sequence ; **5** put, new / analogue, gene in, bacteria / yeast ; **6** add promoter for, gene expression / protein synthesis ; **7** AVP ;	3
(c)	*any **three** from:* **1** can, share / search / access, data(base) / information / sequences ; **2** from anywhere / online ; **3** can, compare / align / analyse, (multiple) sequences ; **4** rapid / fast(er) (process) ; **5** can, quantify / count, nucleotide differences ; **6** closely-related species have few, differences / mutations ;	3

Q7.

(a)	*any **four** from:* 1 marker gene added with gene of interest (GOI) ; 2 use same promoter ; 3 (so) both genes, transcribed / expressed (together) ; 4 (so) both proteins are produced ; 5 use UV light (to detect fluorescence) ; 6 fluorescence shows, transcription / expression, of gene of interest (GOI) ; 7 AVP ; e.g. fluorescent proteins not harmful / have no effect on plant / do not affect quantity of desired protein produced	4

(c)(i)	complementary (base pairing between DNA strands) / described ;	1
(c)(ii)	*any **two** from:* 1 use mRNA to make (ss)cDNA ; 2 fluorescent, tag / dye / substance / label, attached to cDNA ; 3 hybridisation / binding, of cDNA to probe ; 4 use, lasers / UV light, to produce fluorescence ; *plus* 5 idea of intensity of, fluorescence / light, indicates level of (gene) expression ;	3

Q8.

(a)(i)	1 DNA, joined / combined / made, from two / different, sources / species ; 2 using (named), enzymes / vectors **or** to create a, GMO / transgenic organism ;	2

Q9.

(b)(i)	reverse transcriptase ;	1

Q10.

(a)	*any **four** from:* 1 add DNA to give a new (named), characteristic / protein ; 2 restriction, enzyme / endonuclease, to obtain, gene / allele / section of DNA **or** restriction, enzyme / endonuclease, to cut plasmid ; 3 combine gene with / insert gene into, vector / plasmid ; 4 introduce, (recombinant) vector / plasmid, to, bacterium / (named host) cell ; 5 clone / multiply, (named, recombinant / GM) organism ; 6 AVP ;	4

(b)(ii)	*any **two** from:* 1 to switch gene on / so gene is expressed / allow transcription ; 2 at right time / all the time / in sufficient quantities ; 3 to allow binding of, RNA polymerase / transcription factors ;	2

(c)	*any **three** from:* 1 HD is caused by a dominant allele ; 2 adding a 'normal' recessive allele would not work ; 3 gene editing can / we need to, delete / remove / inactivate, HD / mutant, allele / DNA ; 4 ref. (surplus CAG) repeats / triplets / stutter ;	3

Q11.

(a)

statement	genetic engineering using a plasmid	gene editing
It can produce a transgenic organism.	✓	✓ ;
It can modify the characteristics of an organism.	✓	✓ ;
It can delete unwanted DNA.		✓ ;
It uses an enzyme that cuts DNA.	✓	✓ ;
It can use RNA to precisely locate the target gene.		✓ ;

5

(b)(i)	**A** ;	1

Q12.

(a)

statement	genetic engineering using a plasmid	gene editing
It may cause the organism to produce a different protein.	✔	✔ ;
It may cause a single base pair in a gene to be changed.		✔ ;
The success of the technique can be evaluated using marker genes.	✔	;
It may use the CRISPR system.		✔ ;
It uses DNA ligase.	✔	;

5

Q13.

(d) *any **four** from:*

1 obtain / use, blood / cells / tissue / DNA ;

2 from, both species / cattle and European bison ;

3 use PCR to, replicate / amplify, DNA ;

4 use, electrophoresis (described) / DNA profiling / DNA fingerprinting, to see genetic differences / compare the banding (patterns) ;

5 sequence DNA and, count / identify, (percentage) difference / similarity
or
sequence DNA and compare the, nucleotide / base, sequence ;

6 use, genome / DNA, microarray ; **R** for gene expression

7 *ref. to* bioinformatics / database / (computer) software ;

8 similarity indicates, close relationship / more recent split ;

9 if they can breed to produce (viable) offspring (they are closely related) ;

4

Q14.

(a)	*any **five** from:* **1** (named) restriction, enzyme / endonuclease ; **2** cut / fragment, DNA ; **3** produced by / present in, bacteria / archaea / prokaryotes **or** to defend against, viruses / (bacterio)phages ; **4** (DNA) ligase ; **5** joins DNA / forms phosphodiester bonds / seals sugar-phosphate backbone ; **6** during DNA, repair / replication **or** joins Okazaki fragments ; **7** reverse transcriptase ; **8** forms / makes, (c)DNA from mRNA (template) ; **9** produced by / present in, retroviruses ; **10** DNA / *Taq*, polymerase ; **11** replicates / copies, DNA (at high temperatures) ; **12** produced by / present in, *Thermus aquaticus* ;	**5**

Q15.

(a)(i)	*any **four** from:* 1 obtain single-stranded DNA ; 2 label DNA with fluorescent, dye / tag / label ; 3 probes on, chip / microarray ; 4 each probe is unique to a particular, SNP / gene / allele ; 5 DNA (added), binds / hybridises, to probes ; 6 wash off excess DNA (after hybridisation) ; 7 (view under) UV light / laser scanner / high resolution digital camera ; 8 fluorescence indicates presence of, SNP / gene ; **R** gene expressed	**4**
(a)(ii)	*any **two** from:* 1 large database ; 2 use database to find, SNPs / probes ; 3 fast / accurate / efficient ; 4 *ref. to* (computer) software / algorithms ; 5 *ref. to* statistics / statistical analysis ; 6 *idea of*, 17 000 × 500 000 / 8.5×10^9, data points ;	**2**
(b)(i)	1 and 6 ;	**1**

(b)(ii)	*any **two** from:* 1 all diseases, are autosomal / not associated with X chromosome / not sex-linked ; 2 all diseases are associated with SNP on chromosome 6 ; 3 type 1 and type 2 diabetes SNPs are associated with chromosomes 12 and 16 (but not rheumatoid arthritis) ; 4 type 2 diabetes SNP associated with chromosome 10 (but not type 1 diabetes and rheumatoid arthritis) ; 5 arthritis and type 1 diabetes have higher level of association (with each other) than with type 2 diabetes ; 6 rheumatoid arthritis has fewest number of SNPs **or** type 1 and type 2 diabetes has more SNPs (than rheumatoid arthritis) ;	**2**

(c)	*any **three** from:* 1 DNA analysis not available for everyone ; 2 lifestyle change ; **A** e.g. lose weight / stop smoking / take exercise 3 early treatment ; 4 allows people to plan ; e.g. organise care / appoint power of attorney / take out medical insurance / make will / gift property to children / retire early 5 decide whether to have children / ref. (therapeutic) termination ; 6 results may cause, anxiety / stress / panic / depression (if positive) **or** reduce worry (if negative) ; 7 results may affect ability to get, insurance / credit / jobs ; 8 *idea of* predictions may not be accurate ;	**3**

Q16.

(b)	*any **two** from:* 1 bind to complementary base sequences in mtDNA ; 2 (so) only amplify specific mtDNA sections / AW ; 3 mtDNA section, differences / similarities, used to assess how closely related the subspecies ;	**2**

Q17.

(b)(i)	1 (because) sequences already known ; 2 (because) large, number of base pairs / quantity of data, to search ;	**2**
(b)(ii)	chromosome **D** as it contains the most (possible) Mig1-binding promoter sites ;	**1**
(b)(iii)	1 Mig 1 (also) regulates genes involved in other pathways ; 2 there could be several copies of (one or more of) these five genes ;	**2**

Q18.

(a)(i)	1 crime scene DNA may be present in, small amount / minute quantity ; 2 PCR, amplifies / replicates / copies / multiplies, DNA ;	**2**
(a)(ii)	1 (negative) DNA moves to, positive electrode / anode ; 2 smaller / lighter, fragments move, faster / further / longer distance ;	**2**

(b)(i)	*any **two** from:* 1 (GEDmatch) database is large(r than police database) ; 2 police database only covers people, convicted of / suspected of, crime ; 3 GEDmatch has data for, innocent people / people with no reason to hide their DNA ; 4 provides multiple leads to guilty people who are hiding their DNA ;	**2**
(b)(ii)	*any **two** from:* 1 store of / large quantity of / contains, biological / DNA / genome, data / information ; 2 for, analysis / processing ; 3 by, computer / software / algorithm / statistics ;	**2**

Q19.

(b)(i)	*Freedom II because:* 1 contains, viral / foreign, DNA / genes *or* has DNA from 2 different, sources / organisms ; 2 *ref. to* genetic engineering / GMO / genetic modification / transgenic ;	**2**

Q20.

(b)(i)	*any two from mp1-4 and one for mp5* *any **two** from:* 1 DNA sample placed in well (at end of gel) ; 2 *ref. to* electric field / current ; 3 (negatively charged) DNA attracted to, anode / positive electrode ; 4 shorter fragments move further in unit time / ora ; ***one** from:* 5 compare band position to identify cat species ;	**3**

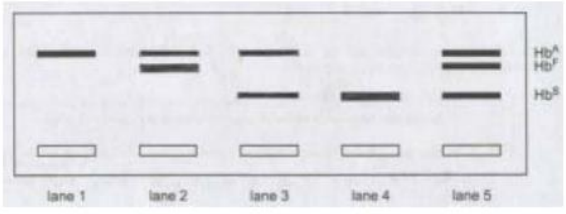
(b)(ii)	*any **two** from:* 1 quicker test ; 2 cheaper / good for low income countries ; 3 portable / no special lab needed / no need to send off sample to be tested ; 4 many sequences can be stored / *ref. to* bioinformatics ; 5 AVP ; e.g. more successful prosecutions deters others form illegal trading / new enforcement measures available	**2**

Q21.

(b)(ii)	*any **three** from:* *why* **1** (primers) anneal / bind, to DNA ; **A** hybridises **2** so that, DNA / *Taq*, polymerase, can bind to DNA **or** so that, DNA / *Taq*, polymerase, can replicate or amplify DNA ; *how* **3** (if) normal primers bind then, normal (genotype) / Hb^AHb^A ; **4** (if) mutant primers bind then, sickle cell anaemia (genotype) / Hb^SHb^S ; **5** (if) both primers bind then, SCT (genotype) / heterozygote / $Hb^A Hb^S$;	**3**

(b)(iii)	**1** correct genotypes for **A** and **B**: **A** – $Hb^A Hb^S$ **accept** $Hb^N Hb^S$ **and** **B** – $Hb^S Hb^S$; **2** correct phenotypes for **A** and **B**: **A** – SCT **and** **B** – sickle cell anaemia ;	**2**

Q22.

(c)(i)	*any **four** from:* **1** current / potential difference / electric field (across gel) ; **2** (protein / Hb) moves / attracted, to, anode / positive electrode ; **3** HbS (more positive so) moves, more slowly **4** HbS moves, shorter distance / less (far) from negative end ; **5** compare band positions to, known haemoglobins / reference bands ; **6** if single band seen at HbS position person has sickle cell anaemia ;	**4**
(c)(ii)	 lanes 2, 3 and 4 correct = 2 marks ;; two correct = 1 mark one or none correct = 0 marks	**2**

Q23.

(b)(i)	$\frac{16}{32} \times 100$ **or** $\frac{100}{32} \times 16$; 50 ; **A** ecf to two or three significant figures	**2**
(b)(ii)	*any **two** from:* 1 (bioinformatics is) a store / database, of, base / DNA / protein / amino acid / primary, sequence data ; 2 for comparison (of, database / base / DNA / protein / amino acid / primary, sequence data) ; 3 (to search for base / DNA / protein / amino acid / primary, sequences) similar to human calcitonin ; 4 *idea of* modelling / predicting, tertiary / 3D / protein, structure ; 5 AVP ; e.g. sequences data pooled from all over the world	**2**

(c)(i)	restriction, enzyme / endonuclease ; (DNA) ligase ;	**2**
(c)(ii)	*any **two** from:* *one mark if only two features stated without explanation* 1 small **so** can be inserted (into cells) ; 2 replicate, independently / fast, **so**, high copy number / large number of plasmids **or** have origin of replication **so** high copy number / large number of plasmids ; 3 has restriction site(s) / can be cut by restriction enzymes, **so** (new) gene can be added ; 4 have, multiple cloning site / polylinker, **so** can be cut by different restriction enzymes ; 5 have marker genes **so**, recombinants / transformed bacteria , can be recognised / AW ; 6 circular **so** stable ;	**2**

(d)	*any **two** from:* 1 eukaryote and prokaryote promoter sequences are different ; 2 eukaryote and prokaryote RNA polymerase enzymes are different ; 3 prokaryotic RNA polymerase does not, recognise / bind to, eukaryotic promoter **or** prokaryotic RNA polymerase only binds to prokaryotic promoter ; 4 so no, transcription / mRNA made / gene expression ; **ora** 5 eukaryotic promoter requires binding of (many) transcription factors that are not present in prokaryotic cell ;	**2**

Q24.

(c)(i)	*any **five** from:* 1 obtain mRNA from wild and captive bred fish / AW ; 2 reverse transcription of mRNA to produce cDNA ; 3 add fluorescent label to (c)DNA ; **I** colour **A** dyes / markers / tags 4 (microarray has) ssDNA probes ; 5 each from a, different / known, gene ; 6 (c)DNA hybridises / AW, to, probes / ssDNA (on microarray) ; 7 fluorescence shows the expressed genes ; 8 intensity of fluorescence (shows level of gene expression) ; 9 compare, fluorescence / gene expression (between fish groups) ; 10 AVP ; e.g. *ref. to* washing off excess (c)DNA (only), after hybridisation UV light / laser scanning (to record fluorescence pattern)	**5**

Q25.

(a)	*any **four** from:* 1 gene (for herbicide resistance) from, another species / variety / (soil) bacterium ; **A** allele 2 *ref. to* restriction enzyme ; 3 *ref. to* (Ti) plasmid / vector / *Agrobacterium* / gene gun ; 4 (DNA) ligase ; 5 *ref. to* recombinant, DNA / plasmid ; 6 introduced into soybean, cells / genome / DNA ; **I** embryos 7 (new) gene, expressed / transcribed ; 8 (to produce) protein / enzyme, resistant to, glyphosate / herbicide ; 9 AVP ; e.g. *ref. to* tissue culture / produce callus / detail such as grow cells on growth medium / marker genes / insert promoter	**4**

Q26.

(a)	*any **four** from:* 1 gene(s) from, another species / (soil) bacterium / *B.(acillus) thuringiensis* ; 2 restriction, enzyme / endonuclease ; 3 (Ti) plasmid / *Agrobacterium (tumefaciens)* / vector / virus / liposome / gene gun ; 4 (DNA) ligase ; 5 (forms) recombinant, DNA / plasmid / vector ; 6 new gene / rDNA / plasmid, enters, maize / MON810, embryos / cells / genome / DNA ; 7 gene, expressed / transcribed (and translated), to make, protein / toxin ; 8 *ref. to* marker genes / insert promoter ;	**4**

Q27.

(c)(i)	*any two from:* success rate (in altering gene) is, greater / 100% (instead of 1%) ; (only) specific gene is altered / targets gene more precisely ; (unwanted) gene is, removed / deleted / disabled / knocked out ;	**2**
(c)(ii)	*one or two arguments about statement's truth to question max 3:* 1 not selective breeding as, DNA / genes, manipulated / altered / changed / removed ; 2 not selective breeding as IVF is used ; 3 not selective breeding as no, crossing and, selection (of offspring) / repetition ; 4 not genetic engineering as no new gene is put into, organism / zygote ; *one or two reasons why more acceptable to question max 3:* 5 no foreign gene inserted / no cross-species gene transfer ; 6 (only) one / single, gene, altered / silenced ; 7 (application) reduces, suffering of / (viral) disease in, pigs ;	**3**