Biology A level - Manipulating genomes Cheat Sheet

by Anais (Anais_Pe) via cheatography.com/151793/cs/43584/

Human genome - introns

Minisatellites	20-50 base pairs (bp) repeated 50-100 times. *Also called VNTRs (Variable Number Tandem Repeats).*
Microsatellites	2-4 bp repeated 5-15 times. *Also called STRs (Short Tandem Repeats) or stutters*

Can repeat a varying amount of times on each homologous chromosome. The number of repeats is inherited from parents.

Satellite DNA is a repeating sequence of DNA found in introns (non-coding DNA).

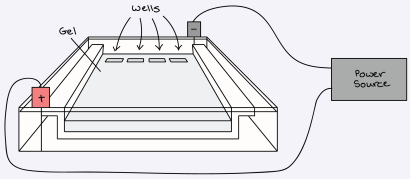
Producing a DNA profile

1. Extract DNA	Tissue sample is mixed with phenol solution. PCR can be carried out to multiply sample (more detail later).
2. DNA fragmentation	Restriction endonucleases - cut DNA at specific base sequence (restriction site).
3. Gel electrophoresis	Used to separate DNA fragments using charge and Mass of DNA fragments (more detail later).
4. Denaturation	Hello soaked with alkali to separate strands.
5. Southern blotting	Membrane that single strands of DNA are transferred on to.

Producing a DNA profile (cont)

6. Hybridisation	Complementary radioactive or fluorescent probes are added. Their base sequence is known and so they tag complementary regions so specific genes can be pinpointed.
7. Disclosure	X-rays or UV lights are used depending on what type of tag was used. The result will show lines where specific sequences were tagged and a DNA profile is created.

Gel electrophoresis



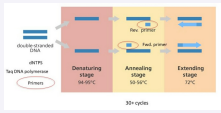
Gel electrophoresis

DNA cut up by restriction enzymes and mixed with loading dye is placed in wells in agarose gel. Buffer solution is added to maintain a constant pH and a current is passed through with the anode at the opposite end to the DNA.
Because DNA is negatively charged, the fragments will be pulled towards the anode. Smaller fragments travel faster and further. The loading dye allows us to make sure the DNA doesn't fall off the other end.

Gel electrophoresis (cont)

An alkaline solution is then added to denature the DNA and the other steps of DNA profiling take place (Southern blotting and disclosure).

PCR



Polymerase chain reaction (PCR)

PCRis used when only a small sample of DNA is available, for example at a crime scene. The DNA sample along with excess bases, primers and DNA polymerase are mixed and placed in the PCR machine

1. Separating the strands (90-95C)	30 seconds, this denatures the DNA - breaks hydrogen bonds between bases.
2. Annealing the strands (55-60C)	Primers bind to the ends of the DNA strands.
3. DNA synthesis (72-75C)	At least 1 minute, DNA polymerase (Taq polymerase) adds bases to the primer, complementary strand is made.

The cycle is repeated as many times as necessary to produce enough DNA for the necessary usage.

DNA sequencing (Sanger)

DNA mixed with primers, DNA polymerase, excess nucleotides and terminator bases (4 separate containers, one for each base).

PCR performed to synthesise DNA.

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DNA sequencing (Sanger) (cont)

Terminator nucleotides cause transcription to stop. This therefore produces different lengths of DNA fragments.

Process similar to gel electrophoresis started. Terminator nucleotides contain fluorescent markers, so base which ends sequence can be identified.

Shortest fragment travels furthest so order of bases can be determined.

Next-generation sequencing or massively parallel sequencing is more commonly used than the Sanger method. This is where DNA fragments are put through a plastic slide (flow cell) instead of gel electrophoresis. PCR is then carried out in situ.

Bioinformatics and computational biology

Bioinformatics	Stores and organises data.
Computational biology	Uses data to form theoretical models.

Uses of genome-wide comparisons

Human genome	10,000 Genomes Project UK10K.
Genomes of pathogens	Find source of infection.
	Identify antibiotic-resistant bacteria.
	Track progress of an outbreak.
	Identify target areas of pathogen genome.

Sequencing for classification

Identifying species (DNA barcoding)	Identify sections of the genome that remain the same within species. Conserved regions in animals are in mtDNA and in plants chloroplast DNA.
Evolutionary relationships	Can calculate rate of mutations --> See how long ago two species had same DNA in common ancestor.

Genomics and proteomics

Genomics is the study of genome.

Proteomics is the study and amino sequencing of organisms' entire protein complement. More proteins exist than genes.

Spliceosomes	Enzyme complexes which cut out introns and some exons out of pre-mRNA. Exons can be rearranged differently, therefore one section of DNA can code for many proteins.
Protein modification	Proteins modified by other proteins. Can be lengthened or shortened.

Spliceosomes and protein modification are some of the reasons why existing proteins do not reflect the genome of an organism.

Synthetic biology

Design and construction of novel artificial pathways, organisms...

Genetic engineering.

Industrial contexts - fixed/immobilised enzymes and drug production.

Synthesis of new genes e.g. treat cystic fibrosis.

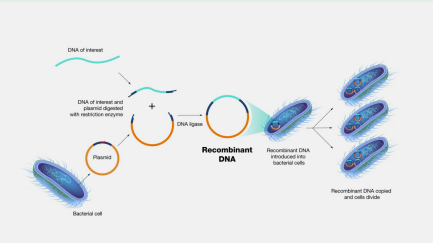
Synthesis of new organism. New nucleotides developed (other than ACTG) incorporated in DNA introduced in bacteria.

Isolating desired gene

mRNA is isolated from target cell using restriction endonucleases and treated with reverse transcriptase to create complementary DNA (cDNA).
Plasmid genetically engineered to have markers, cut by restriction endonucleases (same ones used for DNA for complementary sticky ends).Second marker is added to show plasmid contains recombinant gene. This marker should be corrupted when DNA is added.
DNA ligase fuses cDNA and the plasmid by forming phosphodiester bonds. The recombinant plasmid is placed in the host cell. The bacteria multiply in a fermenter.

Could also use electrofusion: merge two cells + their DNA to form polyploid cells. More used in plants, animal polyploids usually don't survive.

Isolating genes



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Genetic engineering in different organisms

Prokaryotes	Easily genetically modified for hormones, antibiotics...
Plants	*Agrobaterium tumefaciens* which usually forms tumours. Desired gene inserted in plasmid, then in plant DNA. Forms a callus of GM plant cells
	Can also use **electrofusion** - Tiny electric shocks used to fuse the cells and nuclear membranes of two different cells together. Also used for producing monoclonal antibodies.
Animals	Cell membranes are harder to manipulate than plant cell membranes. Engineering used for medically important proteins and curing human genetic diseases.

Ethics for GM plants

Pest resistance	+ Less pesticide spraying
	- Non-pest insects are also affected
Disease resistance	+ Less crop loss
	- Superweeds (if genes spread).
Herbicide resistance	+ Less competition, higher yield
	- Lower biodiversity, superweeds
Shelf-life extended	+ Less food waste

Ethics for GM plants (cont)

	- Lower commercial value and demand
Growing conditions	+ Can grow in a wider range of conditions e.g. flood resistant
	- N/A
Nutritional value	+ Higher nut. val.
	- Allergies can develop due to new proteins
Medical uses	+ Medicines and vaccines
	- N/A
Patenting	+ N/A
	- Companies charge for seeds, can't harvest seeds, people who may need it the most cannot afford it

Ethics for GM animals

GM pathogens used for research - modify virus to insert new genes in cells e.g. swine fever-resistant pigs, faster-growing salmon...

Pharming	Using animals to produce human medicines and research subjects.
	Medicine - From GM fertilised animals, human protein collected from milk...
	Research - Knock-out mice engineered to develop cancer for research.
Issues	- Human genes in animals
	- Reduce animals to commodities
	- Welfare compromised

Gene therapy in humans

Germ line cell gene therapy	**Germ cells** - Sex cells / embryo post-fertilisation.
	Insert healthy gene in germ cell.
	Illegal for human embryos - violation of human rights of unborn child, concerns of long term impacts...
Somatic cell gene therapy	Replace mutant allele with healthy one using viral vector.
	Higher rates of success, but still issues to fix e.g. mutant alleles passed on rather than healthy ones.

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