

NATIONAL SENIOR CERTIFICATE EXAMINATION MAY 2023

LIFE SCIENCES: PAPER II

SOURCE MATERIAL BOOKLET FOR QUESTIONS 1, 2 AND 3

SECTION A

QUESTION 1

IDENTIFICATION OF THE CHARACTERISTICS OF A 5 700-YEAR-OLD GIRL

1. DNA and archaeology

Archaeology is the study of the ancient and recent human past through material remains. The goal of archaeology is to analyse the physical remains of the past in order to understand past human cultures.

Even the smallest archaeological site may contain a wealth of important information. Artifacts are objects made, modified, or used by humans. Archaeologists analyse artifacts to learn about the people who made and used them. Many archaeological sites are prehistoric – in other words they date from before people developed writing. These sites are more difficult to study as there are no written records for reference.



Figure 1.1 – Working at an archaeological site in Israel.

[<https://static.timesofisrael.com>]

However, DNA has come to the rescue for archaeologists. Identifying events in human history using evidence from both ancient DNA and archaeology has been happening since the first DNA-sequence was recovered from human remains.

2. Entry of humans into Europe

One of the uses of DNA in archaeological studies involves tracking the prehistoric migration of humans around the world. It is known that *Homo sapiens* originated in Africa and likely reached Europe via central Asia and Turkey about 42 000 years ago. These people were hunter-gatherers. The lifestyle of hunter-gatherers relies on moving from place to place, hunting and fishing for food and foraging for wild vegetation and other nutrients like honey.

The climate in Europe at that time was much colder than it is now – the continent was undergoing one of many Ice Ages. Ice covered large parts of northern Europe up until about 10 000 years ago. Once the ice melted, some of these people moved into northern Europe, including the modern-day country of Denmark.



[Adapted: <https://www.latinamericanstudies.org>]

3. The Syltholm woman – Lola

In 2019, an archaeological site was being investigated near the town of Syltholm in Denmark. One of the artifacts that was found at the site was a small black object called birch pitch.

Birch pitch is a black-brown substance obtained by heating the bark of birch trees. It has been used as a glue for many thousands of years to attach bone, metal, or stone to a handle or strap. Tooth imprints that have been found in pieces of birch pitch suggest that ancient people may have chewed the substance to keep it soft. Chewing pitch traps the chewer's DNA in the pitch. The antiseptic nature of the birch pitch helped to keep bacteria out and prevent decay.



Figure 1.3 – Birch trees

[<https://www.gardeningknowhow.com>]

Researchers attempted to find any DNA that might be trapped in the pitch and analyse it to determine the characteristics of the chewer. DNA from many species, besides humans, were discovered.



Figure 1.4 – The chewed piece of birch pitch from Syltholm, Denmark.

4. Analysis of DNA samples



Researchers then tested for the presence of a certain gene that is only present on the Y chromosome. They failed to find this gene in the DNA and therefore concluded that the person who chewed the birch pitch was female. Due to the fact that Syltholm is on the island of Lolland in Denmark, the woman was named Lola.



Figure 1.6 – Process of DNA hybridisation

[Adapted: Lewis,R. 2019. 5 700-Year-Old Lola, her genome sequenced from gum, joins other named forebears. *PLOS ONE*]

Table 1.1 below shows the results of mixing hair colour, eye colour and skin colour alleles with Lola's DNA and measuring the temperature required to separate the hybridised DNA.

Table 1.1 Temperature required to separate DNA that has been hybridised with alleles for various characteristics

Allele name	Temperature required to separate DNA strands (°C)
Skin colour – dark	99,2
Skin colour – light	87,4
Eye colour – brown	76,2
Eye colour – blue	97,2
Hair colour – black	70,2
Hair colour – brown	88,2
Hair colour – blonde	64,2

DNA fragments from several bacteria and viruses were also found in the birch pitch, including Epstein-Barr virus, as well as DNA from various plants and animals, which may have derived from a recent meal. It shows that Lola most likely had recently eaten hazelnuts and duckmeat before chewing the birch pitch.

5. Menkes syndrome

Researchers have also found evidence of an allele for the genetic condition known as **Menkes** syndrome in Lola's DNA.

Menkes syndrome occurs on the X chromosome. It is caused by a mutation in the gene coding for a protein called ATP7A, which is responsible for transporting copper into cells. Certain enzymes require copper to function and therefore the lack of copper means that various chemical reactions in the body cannot occur.

Symptoms include easily broken hair, a loss of early developmental skills, weak muscle tone, sagging facial features, seizures and nervous system deterioration. Onset occurs during infancy. Degeneration of nervous tissue eventually occurs in the brain. Arteries in the brain can also break or become blocked. Affected infants often do not live past the age of three years.

Once diagnosed, Menkes syndrome can be treated with copper supplements.

The following pedigree (Figure 1.7) shows the presence of Menkes syndrome in a family.





[Adapted: <https://www.coursehero.com>]

[Adapted: Günther, T., Malmström H., Svensson, E. M., Omrak, A. Sánchez-Quinto, S, Kılınç, G. M., Krzewińska, M., Eriksson, G., Fraser, M., Edlund, H., Munters, A. R., Coutinho, A., Simões, L., G & Jakobsson, M. 2018. Population genomics of Mesolithic Scandinavia: Investigating early postglacial migration routes and high-latitude adaptation. *PLOS ONE*]

[Adapted: Jensen, T. Z. T. et al. 2019. A 5 700 year-old human genome and oral microbiome from chewed birch pitch. *Nature Communications* 10: 5520]

[Adapted: Skoglund, P., Storå, J., Götherström, A. & Jakobsson, M. 2013. Accurate sex

identification of ancient human remains using DNA shotgun sequencing. J. Archaeol. Sci. 40, 4477– 4482.]

[Adapted: <https://www.nationwidechildrens.org>]

[Adapted: <https://www.saa.org>]

[Adapted: <https://www.smithsonianmag.com>]

QUESTION 2

CAN A TRANSGENIC CHESTNUT TREE SAVE A SPECIES?

1. The American chestnut tree (Castanea dentata)

American chestnut trees, towering 30 meters or more, once dominated the forests throughout the Appalachian Mountains in the east of the USA. Up to 40% of the trees in the forests in these areas were American chestnuts. This adds up to approximately four billion American chestnut trees.



2. Chestnut blight fungus

Unfortunately, only a very small number of American chestnut trees grow in these forests today. In the early 1900s a fungal infection appeared on the trees at the Bronx Zoo in New York City, and then spread rapidly. This fungus is called 'blight' (*Cryphonectria parasitica*). It was brought to the USA by accident when some Chinese chestnuts were introduced into the USA as garden plants and planted in the Bronx Zoo.

The blight colonises a wound in the bark of a tree and releases a toxin called oxalic acid. The oxalic acid causes the tissue in the tree trunk to die, eventually killing the tree. By the mid 20th century, over 3 billion large American chestnuts had all but disappeared. The death of the trees significantly altered forest ecosystems and has had a severe economic impact on the nut and wood industries.



[Adapted: <https://www.natlands.org>] [Adapted: <https://cdn.theconversation.com>]

3. Solutions to the problem

A. Breeding for blight resistance

The American Chestnut Foundation has been breeding American chestnut trees with Chinese chestnut trees to try to produce offspring that are resistant to blight. (Even though the Chinese chestnut and American chestnut are considered to be different species, they can produce fertile offspring when cross-bred.)

As the Chinese chestnut and the blight fungus are both from East Asia, modern-day Chinese chestnut trees are mostly resistant to the blight (as any susceptible individuals have been killed by the fungus).

Breeding these two plants produce many different hybrids. Those plants that combine the blight resistance of the Chinese chestnut with desirable qualities of the American chestnut, such as wood quality, are selected and grown, ready to be planted in the forests. However, these plants often look too much like Chinese chestnuts. So the hybrids are then crossed with American chestnuts over the next few generations to dilute out genes from Chinese chestnut, except those that provide blight resistance. Only those plants with the correct characteristics are selected to be grown.

After nearly 30 years and three generations of breeding, a range of hybrid trees have been produced. These hybrids have inherited between 60% and 90% of their genome from the American chestnut and exhibit a level of disease resistance that is intermediate between the American chestnut and the Chinese chestnut.

B. Genetic modification

In 1990, geneticists at the State University of New York attempted to create resistant chestnuts with the then-new technology of genetic engineering. They inserted a gene from wheat called O1 into the tree's genome. This gene codes for an enzyme called oxalate oxidase, or OxO, that breaks down the oxalic acid that the pathogen releases.



4. Is it safe to grow these trees?

The key now is getting these genetically modified (GM) trees into the forests, where they can breed with wild American chestnuts to help them gain better resistance to the disease. As the GM trees carry only **one** O1 gene in each cell, half of the gametes they produce will carry this gene. When a GM tree is planted near a wild tree and they cross, half the resulting nuts (seeds) will carry the O1 gene.

Though the restoration effort has won tremendous public support, researchers say regulators now need to hear from all those who want American chestnut trees to thrive again in the forest. If the regulators approve the request, it would be the first use of a GM tree to try to restore a native species in North America. However, deciding whether to unleash a GM tree into the wild could take years.

The Food and Drug Administration will study whether the tree's fruit is safe to eat, and the Environmental Protection Agency will consider whether the tree's blight-blocking enzyme should be regulated as a fungicide.

Perhaps squirrels could once again travel from tree to tree through the branches of restored chestnut forests by the end of this century.



GM chestnuts are genetically almost identical to natural American chestnuts – they retain 100 percent of their natural genes; no genes have been removed or replaced, and expression of nearby genes is not affected.

The modified American chestnuts are owned by the company who made them, therefore they can decide to charge for the use of these plants.

The *01* genes could be passed to other species of plants



No significant ecological effects from inserting the oxalate oxidase gene – pollen, flowers, and decaying leaves from the GM trees don't harm bees or beneficial soil fungi.

Experiments are underway to determine whether the tree could become a weed or otherwise threaten existing plants.



Figure 2.5 – Costs and benefits of planting GM American chestnut trees

[Adapted: <https://www.honorsociety.org>]

[Adapted: Corrow, J. 2020. USDA to decide fate of American chestnut restoration] [Adapted: Kurzenko, N. The American Chestnut Foundation https://www.acf.orgs] [Adapted: Popkin, G. 2018. Can a transgenic chestnut save a forest icon? *Science* 361(6405): 830–831] [Adapted: Zhang, B., Oakes, A. D., Newhouse, A. E., Baier, K. M., Maynard, C. A. & Powel, W. A. 2013. A threshold level of oxalate oxidase transgene expression reduces *Cryphonectria parasitica*-induced necrosis in a transgenic American chestnut (*Castanea dentata*) leaf bioassay. *Transgenic Res.* 22(5): 973–982] [<https://www.britannica.com/science/chestnut-blight>] [Adapted: https://www.allianceforscience.cornell.edu]

SECTION B

QUESTION 3

SOURCE A

WHAT IS GERMLINE MODIFICATION?

Somatic cell modification

The alteration of the DNA in any body cell that cannot contribute to gamete formation and thus cannot be passed on from the individual to offspring.

Germline modification

The alteration of the DNA in a germ cell (another word for a gamete), or any cell that divides by meiosis to produce gametes (e.g. germinal epithelium). It results in changes that are present in all cells of the embryo and therefore is also passed from the modified individual to offspring.

Germline modification is now possible in our species, raising a host of ethical, social, and legal issues that need careful consideration and deliberation. At the moment there are no procedures that allow humans to be born from embryos that have been modified in this way. Is it time that we legalise this process and allow people to be born from modified embryos?

Due to the fact that human germline modification has potential effects on both the treated individual and future generations of persons, it requires more ethical considerations than those of somatic cell modification.

[Adapted: Ormond, K. E., Mortlock, D. P., Scholes, D. T., Bombard, Y., Brody, L. C., Faucett, W. A., Nanibaa, N., Garrison, A., Hercher, L., Isasi, R., Middleton, A., Musunuru, K., Shriner, D., Virani, A. & Young, C. E. 2017. Human germline genome editing. *Am. J. Hum. Genet.* 101(2):167–176.]

SOMATIC GENE EDITING VS. GERMLINE GENE EDITING					
EDIT		Somatic therapies target genes in specific types of cells (blood cells, for example).	SPERM, OVUM, OR EARLY	Germline editing is done so early in development that any change is copied into all of the new cells.	
СОРҮ		The edited gene is contained only in the target cell type. No other types of cells are affected.		The edited gene is copied in every cell, including sperm or ova.	
RISKS NEXT GENERATION		Any changes, including potential off-target effects, are limited to the treated individual. The edited gene is not passed down to future generations		If the person has children, the edited gene is passed on to future generations.	
CONCENSUS		Somatic cell therapies have been researched and tested for more than 20 years and are highly regulated.		Human germline editing is new. Heritability of germline changes presents new legal and societal considerations.	

[Adapted: <https://www.news.harvard.edu>]

PLEASE TURN OVER

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SOURCE B – Professional opinions

'Given the nature and number of unanswered scientific and ethical questions, it is inappropriate to perform germline modification that results in human pregnancy.'

'At present, clinical application of human germline modification should not proceed.' 'Currently, there is no reason to prohibit **research** into germline modification on human embryos and gametes in order to conduct research into the possible future clinical applications of gene editing.'

... SIGNED: American Society of Human Genetics, UK Association of Genetic Nurses and Counsellors, Canadian Association of Genetic Counsellors, International Genetic Epidemiology Society, US National Society of Genetic Counsellors, American Society for Reproductive Medicine, Asia Pacific Society of Human Genetics, British Society for Genetic Medicine, Human Genetics Society of Australasia, Professional Society of Genetic Counsellors in Asia, Southern African Society for Human Genetics

Scientists have the tools - but how should they use them, and who should decide?



[Adapted: Licholai, G. 2018. Is CRISPR worth the risk? Lecturer, Yale School of Management; Co-director, Yale Center for Digital Health; Chief Medical Information Officer, PRA Health Sciences
 [Adapted: Ormond, K. E., Mortlock, D. P., Scholes, D. T., Bombard, Y., Brody, L. C., Faucett, W. A., Nanibaa, N., Garrison, A., Hercher, L., Isasi, R., Middleton, A., Musunuru, K., Shriner, D., Virani, A. & Young, C. E. 2017. Human germline genome editing. *Am. J. Hum. Genet.* 101(2):167–176.]
 [The American Journal of Genetics is a scientific journal publishing papers dealing with genetics.]

In late 2015, a group of scientists held the first international conference on Human Gene Editing. Led by Caltech's Prof David Baltimore and Robert Andrews Millikan, Professor of Biology, the group concluded that gene-editing technology was far too underdeveloped to be used on humans.

They did encourage further research to perfect the methods involved in germline modification. The second conference, in 2018, was meant to take stock of the advances of the previous three years and to decide how perspectives had changed. New, safer methods of germline modification had been developed, but the moral and practical uncertainties remained to be resolved, and it would be irresponsible to initiate trials in humans.

There may be situations in which the benefit–risk ratio is very much in favor of the benefit. At that point, there is a moral argument to be made to use germline modification.

When is a gene alteration a way of improving an individual's health and when is it an aesthetic preference or a socially desirable characteristic? And how about genes that people would just like to see in their children? Blue eyes, or intelligence, or the like. The general feeling is that we shouldn't be doing that, but there is a concern that once we perfect the methods for improving health, the same methods could be used for other purposes. People are even saying we should not use the methods for dealing with serious diseases because it opens up an ethical slippery slope.

Predicting all the consequences of a gene alteration is difficult. For instance, in the U.S., sickle cell disease is clearly something we would want to avoid if possible – but in Africa, the sickle cell trait protects an individual against malaria and therefore has a positive consequence as well as a negative one.

[Adapted: Lessons on Human Genome Editing: A Conversation with David Baltimore. 2018. https://www.caltech.edu]

[[]David Baltimore is currently President Emeritus and Distinguished Professor of Biology at the California Institute of Technology (Caltech) and he has joined many other researchers in warning people about the use of germline modification as a genetic technique.]

SOURCE C CRISPR/Cas9 problems

CRISPR/Cas9 is highly efficient in many cell types, but it is seldom 100% effective at introducing alterations at a target site. More concerning is that the desired 'editing' event often results in unwanted mutations at the target site.

The safety requirements for any clinical human genome modification are very strict. New methods are being used to better estimate the risk that off-target mutations will occur and their potential effects on the patient. Rapid strides are being made to reduce the off-target effects of CRISPR/Cas9.

As with any new technology, there could be scientific bumps in the road. The safety concern is that this field is moving so quickly and some researchers want to get into human clinical trials right away. Editing one nucleotide could actually result in multiple nucleotides being altered. The long-term danger is unintended changes to the genome of an organism that go on and get carried through to the next generation.

[Adapted: Ormond, K. E., Mortlock, D. P., Scholes, D. T., Bombard, Y., Brody, L. C., Faucett, W. A., Nanibaa, N., Garrison, A., Hercher, L., Isasi, R., Middleton, A., Musunuru, K., Shriner, D., Virani, A. & Young, C. E. 2017. Human germline genome editing. *Am. J. Hum. Genet.* 101(2):167–176.]

Researchers at Sun Yat-sen University in Guangzhou, China, attempted to modify the gene responsible for β -thalassaemia, a potentially fatal blood disorder. The team injected 86 embryos and then waited 48 hours, enough time for the CRISPR/Cas9 system and the molecules that replace the missing DNA to act — and for the embryos to grow to about eight cells each. Of the 71 embryos that survived, 54 were genetically tested. This revealed that just 28 were successfully spliced, and that only a fraction of those contained the replacement genetic material. 'If you want to do it in normal embryos, you need to be close to 100%,' Huang says. 'That's why we stopped. We think the science is too immature.'

The team also found many 'off-target' mutations assumed to be introduced by CRISPR/Cas9 acting on other parts of the genome. This effect is one of the main safety concerns surrounding germline gene editing because these unintended mutations could be harmful.



Graph showing success rate* of germline modification experiments

*success rate = when more than 50% of cells in a study actually contain the inserted genetic material

[Adapted: Cyranoski, D. & Reardon, S. 2015. Chinese scientists genetically modify human embryos. *Nature* 17378]

SOURCE D Are we ready for the problems?

Impact on the individual and family:

- Genes are modified with children's consent.
- Clinical ethics accepts that parents are the most appropriate medical decision-makers for their children until the children develop their own decision-making capacity. However, many children feel strongly that they would not wish to change or remove their own medical condition (e.g. surgical decisions around sex assignment for disorders of sexual differentiation).
- The ability to 'easily' request interventions intended to reduce medical risks and costs should make parents less tolerant of perceived imperfections or differences within their families.

Impact on society:

- Eugenics refers to both the selection of positive traits and the removal of diseases or traits viewed negatively.
- Used to reinforce prejudice and narrow definitions of what is normal in our societies.
- Historically, eugenics has also been associated with ideas of genetic determination and pseudoscience and genocide.
- Allowing parents the choice to control aspects of their child's genetic future could create an obligation to 'create the best children'.
- Sends a message about the 'fitness' of certain traits
 or conditions, reflecting on the worth and value of people who have that trait or condition.

Not ethical to expose individuals to unsafe techniques, unless benefits outweigh the risks. Magnitude of the potential risks of off-target or unintended consequences are yet to be determined.



Cost and access:

- Increases the inequities in societies as human germline genome editing is likely to be expensive and not covered by all health schemes. Access is likely to be limited geographically.
- Unequal access could create large differences in the relative incidence of given conditions by region, ethnic group, or socioeconomic status.
- Genetic disease could therefore become something only present in certain classes, in certain geographic locations or cultures.
- Reduced incidence of disease could affect resources available to individuals and families dealing with genetic conditions.

[Adapted: <https://www.boldbusiness.com>]

[Adapted: Ormond, K. E., Mortlock, D. P., Scholes, D. T., Bombard, Y., Brody, L. C., Faucett, W. A., Nanibaa, N., Garrison, A., Hercher, L., Isasi, R., Middleton, A., Musunuru, K., Shriner, D., Virani, A. & Young, C. E. 2017. Human germline genome editing. *Am. J. Hum. Genet.* 101(2):167–176.]

SOURCE E

On 26 November 2018, a Chinese researcher named He Jiankui announced that his team had disabled a receptor protein called CCR5 in the embryos of twin baby girls. This modification can be passed on to their descendants. The CCR5 protein occurs on white blood cells and is used by the HI-virus to enter cells. By disabling the CCR5 gene in the embryos, Jankui's team said that they aimed to provide resistance to HIV infection in the children later in life.



Although Jankui reportedly consulted with ethics specialists, he received a lot of negative feedback. Many scientists don't rule out making such heritable changes, but the United States's National Academies of Sciences, Engineering, and Medicine and the United Kingdom's Nuffield Council on Bioethics agreed they should only be undertaken to address a serious medical need that cannot be treated in any other way.

Criticisms of Jankui's work:

- Many scientists say that an independent body should confirm Jankui's results. They faulted Jankui for a lack of transparency and the seemingly careless nature in which he embarked on such a potentially risky project.
- 'I was really horrified and stunned when he described the process he used,' says Jennifer Doudna, a pioneer of the CRISPR–Cas-9 gene-editing technique that he used.
- Alta Charo, a bioethicist at the University of Wisconsin stated that: 'I can only conclude that this was misguided, premature, unnecessary and largely useless.'

Many scientists have criticised Jankui's choice to alter this gene, in part because there are other ways to stop people from contracting HIV. Critics also say that other diseases would make more obvious targets for elimination through editing embryonic genomes.

'Do you see your friends or relatives who may have a disease? They need help,' Jankui said. 'For millions of families with an inherited or infectious disease – if we have this technology, we can help them.'

Jankui's talk leaves a host of other questions unanswered, including whether the prospective parents were properly informed of the risks; why Jankui selected CCR5 modification when there are other methods for HIV prevention and whether the CCR5 gene could have necessary but still unknown functions.

Researchers at the University of California, Berkeley, have since found that people who naturally had two copies of the mutated gene were found to be 20 percent more likely to die by age 76 than those with either one copy or none. This means that these girls may have a shorter lifespan due to the fact that their DNA has been altered.

[[]Adapted: Cyranoski, D. 2018. CRISPR-baby scientist fails to satify critics. *Nature* 564, 13–14.] [*Nature* is a British journal for publication of a wide variety of science papers. It is one of the most prestigious journals in which to publish a paper.]

'There are so many ways to adequately, efficiently, and definitively protect yourself against HIV that the thought of editing the genes of an embryo ... in my mind is unethical,' says Anthony Fauci, who heads the U.S. National Institute of Allergy and Infectious Diseases in Bethesda, Maryland. Jankui has now been jailed for three years.



[<https://i.guim.co.uk>]

How can we not do it?

There's no question that gene-editing technologies are potentially transformative and are the ultimate precision medicine. If you could precisely correct or delete genes that are causing problems – mutating genes – that is the ultimate in precision. It would be so transformative for people with diseases caused by a single gene mutation, like sickle cell anemia and cystic fibrosis. Developing safe, effective ways to use gene editing to treat people with serious diseases with no known cures has so much potential to relieve suffering that it is hard to see how anyone could be against it.

SOURCE F



With *in vitro* fertilisation (IVF), 'test tube babies' was an intentionally scary term. But after Louise Brown, the first IVF baby, was born healthy 40 years ago, attitudes changed radically. Ethics flipped 180 degrees: from it being a horrifying idea to being unacceptable to prevent parents from having children by this new method.

Germline genome editing is less offensive than other approaches (such as prenatal testing and abortion) because it involves altering genes rather than selecting against individuals with inherited conditions.

[Adapted: Bergman, M. T. 2018. Perspectives on gene editing. https://www.news.harvard.edu]

Does germline gene modification consider an individual's human rights?

No unborn child, whether conceived naturally or artifically through IVF and germline gene modification, is able to choose their genetics and whether they are born with or without a particular condition. Individuals produced through germline gene therapy cannot give their consent for their genetic material to be modified.

Is germline gene modification ethically acceptable?

YES

In the UK, the Gene Therapy Advisory Committee (GTAC) was set up in 1993 to regulate the use of gene therapy.

This regulation prevents gene therapy being used to select characteristics for non-medical purposes to 'design' babies.

Is germline gene therapy affordable? YES

If germline gene therapy has the potential to completely remove a disease from the population it will reduce/ remove the long-term healthcare costs of treating the disease.

NO

Theoretically, germline modification could be used to select for particular characteristics regardless of whether they are important for the health of the individual.

On a large scale, germline gene therapy could result in the selection of characteristics to 'improve' the genetics of a population.