

MOLECULAR CLONING: IS IT PERMITTED IN ISLAM?Siti Rubaini Matⁱ, Asmat Ahmadⁱⁱ & Mohamed Akhiruddin Ibrahimⁱⁱⁱⁱ (Corresponding author). Language Teacher, Tamhidi Centre, Universiti Sains Islam Malaysia.
sitirubaini@usim.edu.myⁱⁱ (Ph.D) Associate Professor, School of Biosciences and Biotechnology, Universiti Kebangsaan Malaysia.
asmat@ukm.myⁱⁱⁱ (Ph.D) Senior Lecturer, Faculty of Quranic and Sunnah Studies, Universiti Sains Islam Malaysia.
akhiruddin@usim.edu.my**Abstract**

*Cloning has a broad definition. For Islamic scholars, human and animals cloning is mostly discussed. Despite conclusion made saying that human cloning is a contradiction to the nature of the creation by Allah SWT, molecular cloning on the other hand is beneficial for improvement in medicinal sciences and biotechnology. Research in the field of biochemistry, genetics and bioinformatics gives rise to developments of antibiotics and vaccination. In order to do so, genetic material is cloned and employed for humanity as expansion of knowledge. In this study, a simple experiment was carried out to understand the process of cloning for the simplest living organism; the bacteria. Then, authors would like to explore and list the benefits of molecular cloning from the perspective of a muslim scientist. A genome from UKM's soil; *Aeromonas hydrophilla* is selected for isolation and extraction; in order to get genetic material for a chitin-binding protein, CBP. It is a protein which is non-catalytic and essential for chitin degradation. Polymerase chain reaction (PCR) was performed on the particular gene once extracted, and it was successfully cloned. It was found that the optimum annealing temperature is at 69°C. Having millions copies of the gene, the protein can be expressed and degradation of chitin can be studied. Cloning at a molecular level is an important tool to understand genetic engineering. These developments pose many new and challenging questions for scholars of Islamic Law, questions that need to be dealt with on the basis of the immutable principles set forth in the Quran and Hadith.*

Keywords: Cloning, molecular, biochemistry, antibiotics, vaccination.

INTRODUCTION

The term clone is derived from the Greek word *clonos* that means twig (Diamandopoulos, 2000). A clone is produced when a living thing is copied, having the same genetic information. Different types of cloning include gene cloning, molecular cloning, cell cloning, recombinant DNA technology or DNA cloning, reproductive cloning and therapeutic cloning. Generally, discussions on cloning are separated into partial cloning, non-human cloning and human cloning. But, the most talked topic among Islamic scholars is the human reproductive cloning and question whether this type of cloning is permitted or not has been debated. Muslim scholars (fuqaha), view human cloning as haram (forbidden by religion), summarized by Damad (2011).

In general, cloning is an asexual biological process in which identical copies or exact replica of a living thing is produced (Gogarty, 2002). Cloning of a certain part of DNA, is also known as gene cloning or molecular cloning. Molecular cloning is a set of experimental methods in molecular biology that are used to assemble recombinant DNA molecules and to direct their replication within host organisms. An experiment is carried out to understand the principles and techniques involved in molecular cloning. A gene that is responsible for the production of a protein, which involves in the metabolism of chitin in bacteria is chosen for cloning.

In this paper, a specific fragment of DNA is copied. The DNA fragment of interest is a chitin binding protein. It is placed within a cloning vector which is a bacterial plasmid (pET28b) to produce copies of that DNA. In this way sufficient amounts of a given fragment of DNA can be generated for study. Then, the DNA is transferred to a foreign host cell, a bacteria cell, *E.coli* DH5 α . This form of cloning has been around since the 1970s and is widely used in molecular biology research (Harvey et. al., 2000). This experiment is carried out to understand the process of cloning for the simplest living organism; the bacteria.

Two types of bacteria, *Serratia marcescens* AY665558 and *Aeromonas hydrophilla* UKMCC_CTT6 was grown in Luria-Bertani (LB) medium. Wizard Genomic Purification Kit was used to extract the genome for both species. Primers were designed manually and then analysed using Primer Premier 5.0, which is then ordered from First Base Lab S/B. Polymerase chain reaction (PCR) results for CBP21 of *S. marcescens* and CBD459 of *A. hydrophilla* is obtained. CBP21 and CBD is a chitin binding protein which is non-catalytic and essential for chitin degradation. The annealing temperature for CBP21 and CBD459 is 69°C. *Escherichia coli* (DH5- α) were used as host organism harbouring plasmids.

RESULTS

Serratia marcescens is a bacteria once used to track infection because of its pink colourisation. Now that it is known to cause human infection and many strains are resistant to multiple antibiotics, studies were carried out to investigate how it can grow well in the environment. One of the factor is because this bacteria has the ability to feed on chitin. Chitin is the most abundant organic compound exists naturally after cellulose, so bacteria having chitinase can survive better. *Serratia marcescens* shown in Figure 1(a) is isolated from mussel whereas *Aeromonas hydrophilla* in Figure 1(b) is from UKM soil. Then, both were grown in LB broth before genome extraction.

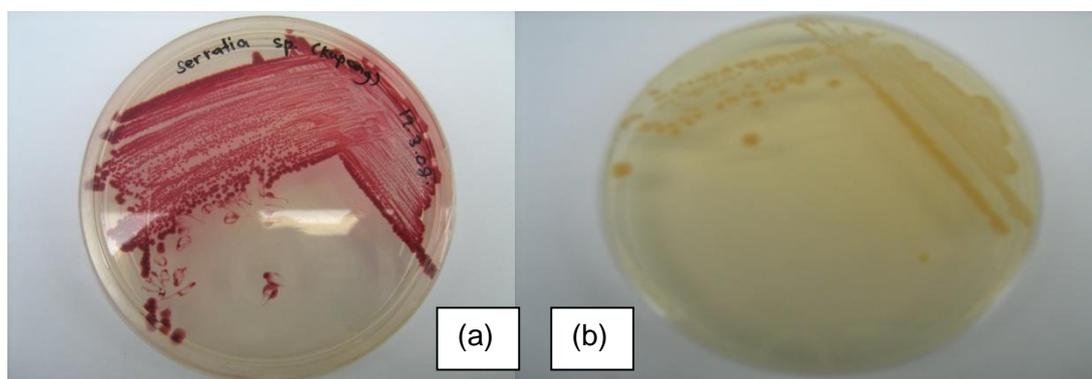


Figure 1: (a) Bacteria culture of *Serratia marcescens* AY665558 and (b) *Aeromonas hydrophilla* UKMCC_CTT6.

A genome is an organism's complete set of DNA. Each genome contains all of the information needed to build and maintain that organism. In humans, a copy of the entire genome, more than 3 billion DNA base pairs (bp) is contained in all cells that have a nucleus. It is possible to isolate a specific region of a genome to produce unlimited number of copies of it, and to determine the sequence of its nucleotides overnight. In order to do this, genetic material of the bacteria has to be extracted. Figure 2 shown the image taken of the agarose gel 1% of the electrophoresis done when the genome extracted were run.

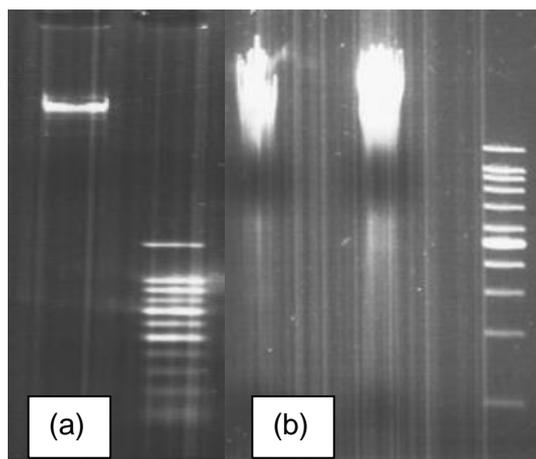


Figure 2: (a) 1 microliter of the genome extracted from *Serratia marcescens* AY665558 compared to a 100 bp DNA ladder; (b) 2 and 3 mikroliter of the genome extracted from *Aeromonas hydrophilla* UKMCC_CTT6 compared to a 1 kb DNA ladder.

Table 1: The primers sequences used in this study.

Name of protein	Primer sequence
CBP21	Forward 5' CCACCATATGAACAAAACCTTCCCGTACCCT 3' Reverse 5' CTCGAGTTATTTGCTCAGGTTGACGTCGAT 3'
CBD459	Forward 5' CATATGCCAAGACAAAACAAGGAAGATAAC 3' Reverse 3' GTCGACTTAGGCCGCCGCGCAGCCGGCGAGCT 5'

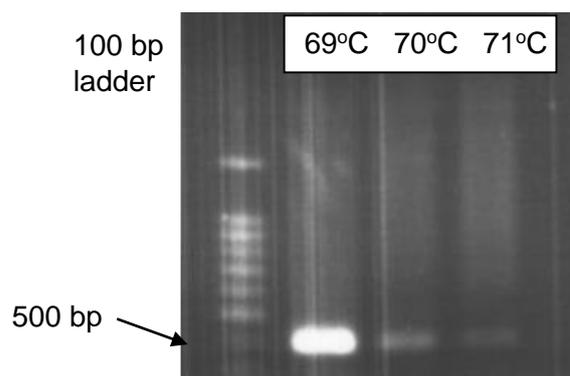


Figure 3: Gel electrophoresis showed gene CBD459 is cloned at the annealing temperature of 69, 70 and 71°C.

In molecular cloning, millions of identical copies of genes of molecular size are produced in order to have sufficient material for testing. This is done by using the polymerase chain reaction (PCR), a laboratory technique used to make multiple copies of a segment of DNA. PCR is very precise and can be used to copy a specific DNA target from a mixture of DNA molecules (genome).

In this experiment the two genes were cloned using PCR machine. The CBP21 clone has been studied and was expressed previously by Rafedah (2008). PCR results run by the electrophoresis gel shown that CBP21 and CBD459 has been successfully cloned. The agarose gel in Figure 3 shows a clear and thick band at the temperature 69°C. The length and purity of DNA molecules can be accurately determined by the same types of gel electrophoresis methods that have been proven so useful in the analysis of proteins.

The band shown in Figure 3 is the product of the experiment which is going to be used further for expression of the chitin binding protein. Once cut and purified, the gene is then stored in the -80°C freezer. This method produced identical copies of the gene responsible for the production of chitin binding protein in the chitinase. Gene cloned were

sent for sequencing and it came back positive. Molecular cloning is therefore has been carried out, proven to be easily done with the help of technology.

DISCUSSION

The era of biotechnology starts beginning with the discovery of the DNA structure in 1953, a revolution in biology understanding. Our ancestors would never imagine that one day via micro manipulation at early stages, single cell can duplicate extensively given the right condition. These created issues on human reproductive cloning, therapeutic cloning, embryo research, organ transplant, genetic engineering (Nordin, 2006). This is due of the rapid progress made in scientific knowledge in the past century.

As a complete way of life, the teachings of Islam includes all fields of human endeavours, spiritual and material, individual and societal, economics and politics, national and international. And Islam does not place any restrictions on the freedom of scientific research, or place any obstacles in its way, because it is a mean of understanding the laws of Allah in His creation, as long as it is not against the principles of Sharia (Samarqandi, 1996).

Cloning is interpreted as a bad thing. The reason is it focuses on human reproductive cloning. Reproductive cloning is a process whereby the genetic material from the nucleus of an adult donor cell is transferred to a female reproductive cell of the same species from which the original genetic material of the nucleus has been removed. Cell division is then stimulated by electrical or chemical means and a new embryo is formed. This results in a new organism that is genetically almost identical to the donor (The National Academies Press, 2002).

Some scientists still believe that it may be possible to clone a human being from the cells of one adult. For a long time this has been thought of as science fiction. Many countries have put bans on cloning or other experiments that produce cloned human embryos. As to this date, no human clone has ever been born. This is due to the Declaration on Human Cloning in 2005, whereby the United Nations General Assembly prohibits all forms of human cloning (Human Cloning Foundation, 2016).

Ban On Human Cloning

There is a lot of interest expressed regarding human cloning. Although recent advances in cloning offering new hope for curing diseases such as diabetes, Parkinson's, neurologic degeneration, Alzheimer's, and osteoporosis, the technology has been accompanied by social, political, economic, legal, religious and ethical questions worldwide (Damad, 2007). Even the Islamic world concerned about the recent developments in genetic engineering because it interferes with the process of creation and lead unethical and immoral practices.

According to the Quran, human beings are created through a family system, through a male and female: al-Nisa' 4:1; al-A'raf 7:189; al-Hujurat 49:13. While modern jurists allow a woman to carry the fertilized ovum of a person to whom she not married, Sharia prohibits the production of single-cell to allow a woman to carry the embryo produced from a single cell of even her husband or herself. Cloning technology is termed haram because it may cause problems such as danger to human personality, dignity, honour and family system as well to society (Aida, 2010). Human reproductive cloning is expensive and highly inefficient. For example, Dolly, the first mammal successfully cloned after 227 attempts cost a great deal with a failure rate exceeding 98% and died due to premature rheumatism.

However, in terms of reproduction aid, cloning is implemented using stem cells from embryos that were formed from a process called in-vitro fertilization (IVF) (Ang Kean Hua, 2016). In Islam, IVF is only allowed for married couples those have difficulties in pregnancy in a normal way as long as the fertilised ovum is placed in the womb of the woman from whom the egg was taken. Any therapeutic (related to healing) research which is not violent against the Sharia as long it not to promote destructive purposes is permitted. The Islamic World League has declared that stem cell research is permissible if its source is legitimate (Batchelor, 2012).

Use Of Molecular Cloning

Cloning is not a completely evil technology. It is not prohibited directly, as we cannot provide a verse or prophetic saying that opposes it. Anything that is cloned is also a creature of Allah. The cloners did not provide the materials used for cloning. Hence, scientists are not creators (Damad, 2011).

The experiment done described in this paper proves that molecular cloning is very simple that an experiment as simple as this should benefit us in lots of things. In this case, the cloned gene is going to be used to study a type of protein which is important is the metabolism of microorganism depending on chitin as their energy source.

These advances are also called metagenomics technology which involves extracting DNA directly from an environmental sample (seawater, soil, or insect guts, for instance), cutting it with restriction enzymes, and cloning it into a culturable host, such as *Escherichia coli* (Tringe & Rubin, 2005).

Among the benefits of gene cloning is to discover different types of antibiotics. Most of the antibiotics used today are erythromycin and vancomycin. They come from cultured soil bacteria. But, these organisms represent only the tip (0.1%) of a microbiological iceberg; the vast majority of soil denizens are unculturable by standard methods. Given the many antibiotics cultured soil bacteria have already provided us, the rest of the population is certainly worth exploring (Handelsman, 2005).

Some cloning is often performed for medical reasons. Experiments are carried out on animals that carry a disease which causes mutations in their genes. Farm animals are cloned to produce drugs and other substances that are useful in medicine. Cloning is a way to produce large numbers of genetically engineered animals and plants (Kumar, 2012). In therapeutic cloning stem cells are reproduced in order to create new organs and tissue. New, healthy tissue can help a person get a new heart or a new liver to replace his ill one. Such a method could also create stem cells that you could use for patients who suffer Alzheimer's or Parkinson's disease (Smith, 2016).

As for all these beneficial usage, there is no reason to prohibit this type of cloning. If DNA cloning is carried out for lawful purposes that can benefit humanity and expand the horizons of knowledge, then it is permitted. If it is employed for evil, then it is prohibited (Sachadina, 2008). As mention in al-^oAlaq 96: 5; "*Allah teaches mankind what they did not know*", these advances pose many new and challenging questions for scholars of Islamic Law (The Question Of Cloning, 2003). Applying the rule, preventing harm is preferable to procuring benefits to cloning, the real challenge is to find a balance between the need to preserve human dignity and the need for continued improvement in the quality of human life through research and development.

Cloning In Nature

Naturally, we got our genes from our parents. Many people think that cloning only can be done in laboratories, but in fact they also occurs in nature. Clones occur naturally when a mother gives birth to two identical twins. They come from the same fertilized eggs and therefore they should have the same identical genes combining chromosomes from the mother and father. However, it has been found that in some cases, one twin's DNA differed from the others at certain point on their genomes. At this sites of genetic divergence, one bore a different number of copies of the same gene, a genetic state named copy number variants (Bruder, 2008). Normally people carry two copies of every gene, one inherited from each parent. For twins, regions in the genome that deviate from that two-copy rule would make you have copy number variants.

Nature has been cloning organism for billions of years. Bacteria and animals with one cell can reproduce themselves. Plants can also produce clones. For example, when a farmer is marcotting a rambutan tree; this breeding techniques involves vegetative propagation. By cutting-off a little bit of a skin from the tree ranch, a new plant will grow when he wraps some soil onto the cut skin. That new plant is a clone. Similar clones occur in planting durian trees, oranges, mangos and figs. When a leaf is taken from a plant and it is grown into a new plant, the original plant is being cloned because the new plant has the

same genetic makeup as the donor plant. These two examples of human twins and plants, are both reproductive clonings. It is time for people to accept the word cloning as a positive term.

CONCLUSION

Cloning is a relatively new knowledge. People should know that the word clone does not reflect 'a photocopy' of a human. Cloning has good applications, for the benefits of the society in whole. A brief and direct experiment has been carried out to illustrate that cloning is something very normal and applicable to a lot of advances. As a complete religion, Islam acknowledges the need to accommodate its teachings to life's realities and necessities and to human well-being (Mirza, 2004).

Muslim scientists should be encouraged to play a leading role in biotechnology research. If they take the lead, many evils that exist now will certainly vanish and humanity will receive the real reward of biotechnology researches. In Muslim countries, therefore, biotechnology research should be made a priority area and for fostering it all kinds of incentives should be provided.

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