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Cloning in bacteria

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Abstract
In a conventional molecular cloning experiment, the DNA to be cloned is obtained from an organism of interest, then treated with enzymes in the test tube to generate smaller DNA fragments. Subsequently, these fragments are then combined with vector DNA to generate recombinant DNA molecules. The recombinant DNA is then introduced into a host organism. This will generate a population of organisms in which recombinant DNA molecules are replicated along with the host DNA. Because they contain foreign DNA fragments, these are transgenic or genetically modified microorganisms. Strictly speaking, recombinant DNA refers to DNA molecules, while molecular cloning refers to the experimental methods used to assemble them. Different DNA sequences could be inserted into a plasmid and that these foreign sequences would be carried into bacteria and digested as part of the plasmid. That is, these plasmids could serve as cloning vectors to carry genes.

Keywords: Molecular cloning, recombinant DNA, plasmids, cloning vectors, host DNA, transgenic or genetically modified microorganisms

Introduction
Cloning refers to the fact that the method involves the replication of one molecule to produce a population of cells with identical DNA molecules (Pasin *et al.,* 2019) [8]. Molecular cloning generally uses DNA sequences from two different organisms. The species that is the source of the DNA to be cloned, and the species that will serve as the living host for replication of the recombinant DNA. A single bacterial cell can be induced to take up and replicate a single recombinant DNA molecule. This single cell can then be expanded exponentially to generate a large amount of bacteria, each of which contain copies of the original recombinant molecule. Thus, both the resulting bacterial population, and the recombinant DNA molecule, are commonly referred to as clones (Singh *et al.,* 2018) [10].

Steps in Cloning
Choice of host organism and cloning vector Bacterium *E. coli* (*Escherichia coli*) and a plasmid cloning vector. Specialized applications may call for specialized host-vector systems. If the experimentalists wish to harvest a particular protein from the recombinant organism, then an expression vector is chosen that contains appropriate signals for transcription and translation in the desired host organism. If replication of the DNA in different species is desired (for example transfer of DNA from bacteria to plants), then a multiple host range vector (shuttle vector) may be selected (Millan, 2018) [6]. Whatever combination of host and vector are used, the vector always contains four DNA segments that are critically important to its function and experimental utility. a) An origin of DNA replication to replicate inside the host organism b) One or more unique restriction endonuclease recognition sites that serves as sites where foreign DNA may be introduced c) A selectable genetic marker gene that can be used to enable the survival of cells that have taken up vector sequences d) An additional gene that can be used for screening which cells contain foreign DNA (Burdette *et al.,* 2018) [2].

Preparation of vector DNA
Most modern vectors contain a variety of convenient cleavage sites that are unique within the vector molecule and is located within a gene (beta- galactosidase) whose inactivation can be used to distinguish recombinant from non-recombinant organisms. The cleaved vector may be treated with an enzyme (alkaline phosphatase) that dephosphorylates the vector ends. Vector molecules with dephosphorylated ends are unable to replicate, and replication can only be restored if foreign DNA is integrated into the cleavage site (Mamishi *et al.,* 2019) [6].

Preparation of DNA to be cloned

DNA to be cloned is extracted from the organism of interest. Virtually any tissue source can be used (even tissues from extinct animals), as long as the DNA is not extensively degraded. DNA is then purified using simple methods to remove contaminating proteins (extraction with phenol), RNA (ribonuclease) and smaller molecules (precipitation and/or chromatography). Polymerase chain reaction (PCR) methods are often used for amplification of specific DNA or RNA (RT-PCR) sequences prior to molecular cloning (Nicoloff *et al.*, 2019) [7].

DNA for cloning experiments may also be obtained from RNA using reverse transcriptase (complementary DNA or cDNA cloning), or in the form of synthetic DNA (artificial gene synthesis). cDNA cloning is usually used to obtain clones representative of the mRNA population of the cells of interest. Synthetic DNA is used to obtain any precise sequence defined by the designer (Huo *et al.*, 2019) [4].

Creation of recombinant DNA

DNA prepared from the vector and foreign source are simply mixed together at appropriate concentrations and exposed to an enzyme (DNA ligase) that covalently links the ends together. This joining reaction is often termed ligation. The resulting DNA mixture containing randomly joined ends is then ready for introduction into the host organism (Anderson *et al.*, 2019) [1].

Introduction of recombinant DNA into host organism

When bacteria are able to take up and replicate DNA from their local environment, the process is termed transformation. In mammalian cell culture, the analogous process of introducing DNA into cells is commonly termed transfection. Electroporation uses high voltage electrical pulses to translocate DNA across the cell membrane. Transduction is injection of recombinant DNA by a bacteriophage virus into the host bacterium (Richardson *et al.*, 2018) [9].

Selection of organisms containing recombinant DNA

When bacterial cells are used as host organisms, the selectable marker is usually a gene that confers resistance to an antibiotic that would otherwise kill the cells. Typically ampicillin is used as marker. Cells harboring the plasmid will survive when exposed to the antibiotic, while those that have failed to take up plasmid sequences will die (Gorlenko *et al.*, 2020) [3].

Screening for clones with desired DNA inserts and biological properties

Modern bacterial cloning vectors (e.g. pUC19 and later derivatives including the pGEM vectors) use the blue-white screening system to distinguish colonies (clones) of transgenic cells from those that contain the parental vector (i.e. vector DNA with no recombinant sequence inserted). In these vectors, foreign DNA is inserted into a sequence that encodes an essential part of beta-galactosidase, an enzyme whose activity results in formation of a blue-colored colony on the culture medium that is used for this work. Colonies containing transformed DNA remain colourless (white). Therefore, experimentalists are easily able to identify and conduct further studies on transgenic bacterial clones, while ignoring those that do not contain recombinant DNA (Vo *et al.*, 2021) [11].

Conclusion

Molecular cloning has led directly to the elucidation of the complete DNA sequence of the genomes of a very large number of species and to an exploration of genetic diversity within individual species, work that has been done mostly by determining the DNA sequence of large numbers of randomly cloned fragments of the genome, and assembling the overlapping sequences. Obtaining the molecular clone of a gene can lead to the development of organisms that produce the protein product of the cloned genes, termed a recombinant protein. Once characterized and manipulated to provide signals for appropriate expression, cloned genes may be inserted into organisms, generating transgenic organisms, also termed genetically modified organisms. Gene therapy involves supplying a functional gene to cells lacking that function, with the aim of correcting a genetic disorder or acquired disease.

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