Gene cloning

A **clone** is an exact copy of an organism, organ, single cell, organelle or macromolecule. Gene cloning is the act of making copies of a single gene. **Molecular cloning** refers to the procedure of isolating a defined DNA sequence and obtaining multiple copies of it *in vivo*. Cloning is frequently employed to amplify DNA fragments containing genes, but it can be used to amplify any DNA sequence such as promoters, non-coding sequences, chemically synthesised oligonucleotides and randomly fragmented DNA. Cloning is used in a wide array of biological experiments and technological applications such as large scale protein production. It is used in many areas of research and for medical applications such as gene therapy. Selective amplification of genes depends on the ability to perform the following essential procedures.

1. Amplification of a specific gene

The discovery of thermostable DNA polymerases, such as Taq Polymerase, made it possible to manipulate DNA replication in the laboratory and was essential to the development of the polymerase chain reaction (PCR). Primers specific to a particular region of DNA, on either side of the gene of interest, are used, and replication is stopped and started repetitively, generating millions of copies of that gene. These copies can then be separated and purified using gel electrophoresis.

2. Cutting DNA at precise locations

The discovery of enzymes known as restriction endonucleases has been essential to protein engineering. These enzymes cut DNA at specific locations based on the nucleotide sequence. Hundreds of different restriction enzymes, capable of cutting DNA at a distinct site, have been isolated from many different strains of bacteria. DNA cut with a restriction enzyme produces many smaller fragments, of varying sizes. These can be separated using gel electrophoresis or chromatography.

3. Join two pieces of DNA

In genetic research it is often necessary to link two or more individual strands of DNA, to create a longer strand, or close a circular strand that has been cut with restriction enzymes. Enzymes called DNA ligases can create covalent bonds between nucleotide

chains. The enzymes DNA polymerase I and polynucleotide kinase are also important in this process, for filling in gaps, or phosphorylating the 5' ends, respectively.

4. Selection of small self-replicating DNA

Small circular pieces of DNA that are not part of a bacterial genome, but are capable of self-replication, are known as plasmids. Plasmids are often used as "vectors" to transport genes between microorganisms. In biotechnology, once the gene of interest has been amplified and both the gene and plasmid are cut by restriction enzymes, they are ligated together generating what is known as a recombinant DNA. Viral (bacteriophage) DNA can also be used as a vector, as can cosmids, recombinant plasmids containing bacteriophage genes.

5. Method to move a vector into a host cell

The process of transferring plasmids into new host cells is called transformation. This technique requires that the host cells are exposed to a heat-shock, which makes them "competent" or permeable to the plasmid DNA. The larger the plasmid, the lower the efficiency with which it is taken up by cells. Larger DNA segments are more easily cloned using bacteriophage vectors or cosmids.

6. Method to select hosts expressing recombinant DNA

Not all cells will take up DNA during transformation. It is essential that there be a method of detecting the ones that do. Generally, plasmids carry genes for antibiotic resistance and transformed cells can be selected based on expression of those genes and their ability to grow on media containing that antibiotic. Alternative methods of selection depend on the presence of other reporter proteins such as the x-gal/ *lacZ* system, or green fluorescence protein, which allow selection based on color and fluorescence, respectively.

Questions:

1. A clone is an exact copy of …….

   a). An organism
   b). Organ
   c). Single cell
   d). All the above

2. Molecular cloning refers to …….

   a). Isolating a defined DNA sequence
   b). Obtaining multiple copies of defined DNA sequence *in vivo*
   c). Both a and b
   d). None of the above

3. Cloning is frequently employed to amplify …….

   a). DNA fragments containing genes
   b). DNA sequence such as promoters, non-coding sequences
   c).Chemically synthesised oligonucleotides
   d). All the above

4. Cloning is mainly used for …….

   a). Large scale protein production
   b). Gene therapy
   c).Both a and b
   d). None of the above

5. Taq polymerase is …….

   a). Thermostable
   b). Thermounstable
   c). Thermoliable
   d). None of the above

6. ……. enzymes cut DNA at specific locations based on the nucleotide sequence.

   a). Restriction enzymes
   b). DNA ligase
   c). Nuclease
   d). None of the above

7. ……. enzymes unite DNA segments

   a). Restriction enzymes
   b). DNA ligase
   c). Nuclease
   d). None of the above

8. DNA ligases create …… bonds between nucleotide chains.

   a). Covalent
   b). Hydrogen
   c). Ionic
   d). None of the above

9. The process of transferring plasmids into new host cells is called …….

   a). Transformation
   b). Transduction
   c).
   d). None of the above

10. The larger the plasmid, ……is the efficiency with which it is taken up by cells.

a). Lower b). Higher

c). Medium d). None of the above

Additional readings..

http://highered.mcgraw-hill.com/olcweb/cgi/pluginpop.cgi?it=swf::535::535::/sites/dl/free/0072437316/120078/micro10.swf::Steps%20in%20Cloning%20a%20Gene

http://biotech.about.com/od/cloning/tp/DNAcloning.htm

http://highered.mcgraw-hill.com/olcweb/cgi/pluginpop.cgi?it=swf::535::535::/sites/dl/free/0072437316/120078/micro10.swf::Steps%20in%20Cloning%20a%20Gene