Gene Cloning

& Creating DNA Libraries
Gene Cloning

• What does the term cloning mean?
• What is gene cloning? How does it differ from cloning an entire organism?
• Why is gene cloning done?
• How is gene cloning accomplished?
• What is a DNA ‘Library’? A cDNA Library?
• What are some of the ethical considerations regarding gene cloning?
Cloning - a definition

• From the Greek - klon, a twig
• An aggregate of the asexually produced progeny of an individual; a group of replicas of all or part of a macromolecule (such as DNA or an antibody)
• An individual grown from a single somatic cell of its parent & genetically identical to it

www.m-w/cgi-bin/dictionary
What is DNA cloning?

- When DNA is extracted from an organism, all its genes are obtained.
- In gene (DNA) cloning a particular gene is copied (cloned).
Whole organisms are cloned too, but differently
Why Clone DNA?

• A particular gene can be isolated and its nucleotide sequence determined
• Control sequences of DNA can be identified & analyzed
• Protein/enzyme/RNA function can be investigated
• Mutations can be identified, e.g. gene defects related to specific diseases
• Organisms can be ‘engineered’ for specific purposes, e.g. insulin production, insect resistance, etc.
How is DNA cloned?

- DNA is extracted- here from blood
- Restriction enzymes, e.g. *EcoRI, HindIII*, etc., cut the DNA into small pieces
- Different DNA pieces cut with the same enzyme can join, or recombine.
The action of a restriction enzyme, EcoRI
Note: EcoRI gives a ‘sticky’ end
DNA Cloning, II

- Bacterial plasmids (small circular DNA additional to a bacteria’s regular DNA) are cut with the same restriction enzyme.
- A chunk of DNA can thus be inserted into the plasmid DNA to form a “recombinant”
DNA cloning, III

- The recombinant plasmids are then mixed with bacteria which have been treated to make them "competent", or capable of taking in the plasmids.

- This insertion is called transformation.
DNA Cloning IV

- The plasmids have naturally occurring genes for antibiotic resistance.
- Bacteria containing plasmids with these genes will grow on a medium containing the antibiotic - the others die, so only transformed bacteria survive.
Antibiotic Resistance

• The medium in this petri dish contains the antibiotic Kanamycin

• The bacteria on the right contain Kan\textsuperscript{r}, a plasmid that is resistant to Kanamycin, while the one on the left has no resistance

• Note the difference in growth
Cloning V

- The transformed bacterial cells form colonies on the medium
- Each cell in a given colony has the same plasmid (& the same DNA)
- Cells in different colonies have different plasmids (& different DNA fragments)
Gene Libraries

• A gene library is defined as “a collection of living bacterial colonies that have been transformed with different pieces of DNA from the organism that is the source of the gene of interest”

• The gene library then must be screened to find the colony with the gene in which the researchers are interested
Screening can involve:

1. Phenotypic screening - the protein encoded by the gene changes the colour of the colony
2. Using antibodies that recognize the protein produced by a particular gene
Screening II

3. Detecting the DNA sequence of a cloned gene with a probe (DNA hybridization)
Screening III

• Once colonies are identified, they are cultured in broth to increase numbers and therefore the amount of DNA

• Samples are also prepared for storage at -80 degrees. They can be kept for many years this way.
cDNA I

• Eukaryotic DNA differs from bacterial (prokaryotic) DNA in that it has introns (intervening sequences) and exons (expressed or translated sequences).

• In order for a eukaryotic gene to be expressed, the introns are ‘edited’ out of mRNA after transcription
A simplified diagram of transcription in eukaryotes

hnRNA = ‘heterogenous nuclear’RNA in the process of being cut and spliced into messenger RNA
cDNA II

• Bacteria can’t deal with introns, so in cases where a product (e.g. insulin) is to be expressed by the bacteria, an uninterrupted coding sequence is needed.

• Also, since introns can account for up to 90% of an eukaryotic gene, and cloning long fragments is difficult, it is sometimes desirable to work only with the expressed sequences (exons)
cDNA III

• To deal with this, special DNA is synthesized using mRNA as a template. This process also requires a primer and an enzyme, reverse transcriptase (a DNA polymerase that synthesizes a DNA strand from the mRNA)
• This complementary DNA is called cDNA
• cDNA may be attached to a vector such as a plasmid and then introduced into bacterial cells.
Considerations

• The plasmids used in gene cloning contain naturally occurring genes for some type of antibiotic resistance—e.g. Ampicillin or Tetracycline. When these genes are used to make a transgenic organism, the resistance gene may be transferred. There is concern that this resistance could be acquired by other organisms, thus creating further problems with antibiotic resistance.
Considerations, ctd.

- There is a reluctance on the part of some cultures and individuals to accept the concept of transgenesis, without which gene cloning could not be accomplished.
- Some cloned genes are used in ‘engineering’ food crops, and food safety has become an issue with the public.
- There has been a move to patent genes of interest – this can raise the cost of research and diagnosis – who ‘owns’ a human gene?
These points and others require research and informed debate- What are Your thoughts?

What legislation has been passed here in New Zealand? Who controls work in genetic manipulation?
References


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