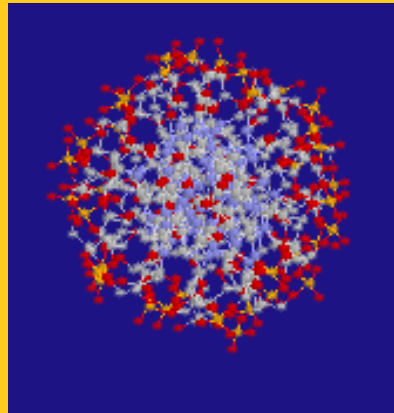


# Gene Cloning



& Creating DNA Libraries

# Gene Cloning

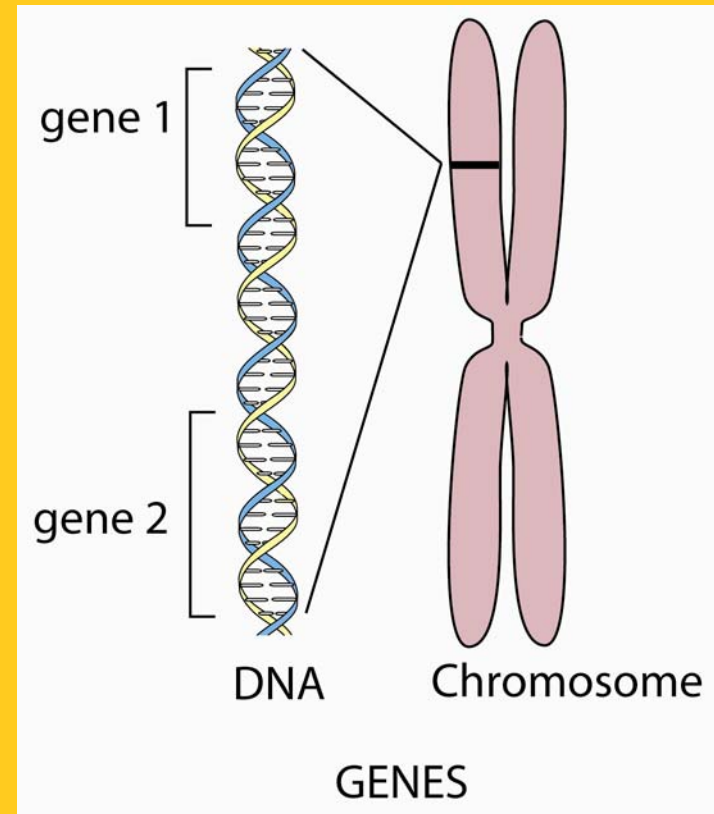
- What does the term cloning mean?
- What is gene cloning? How does it differ from cloning an entire organism?
- Why is gene cloning done?
- How is gene cloning accomplished ?
- What is a DNA 'Library'? A cDNA Library?
- What are some of the ethical considerations regarding gene cloning?

# 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](http://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

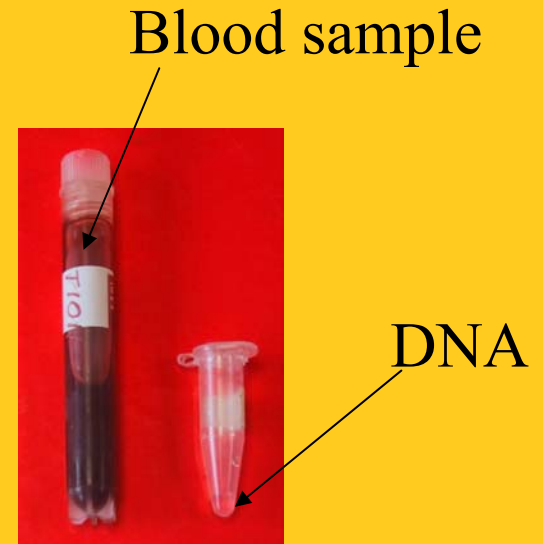


# Why Clone DNA?

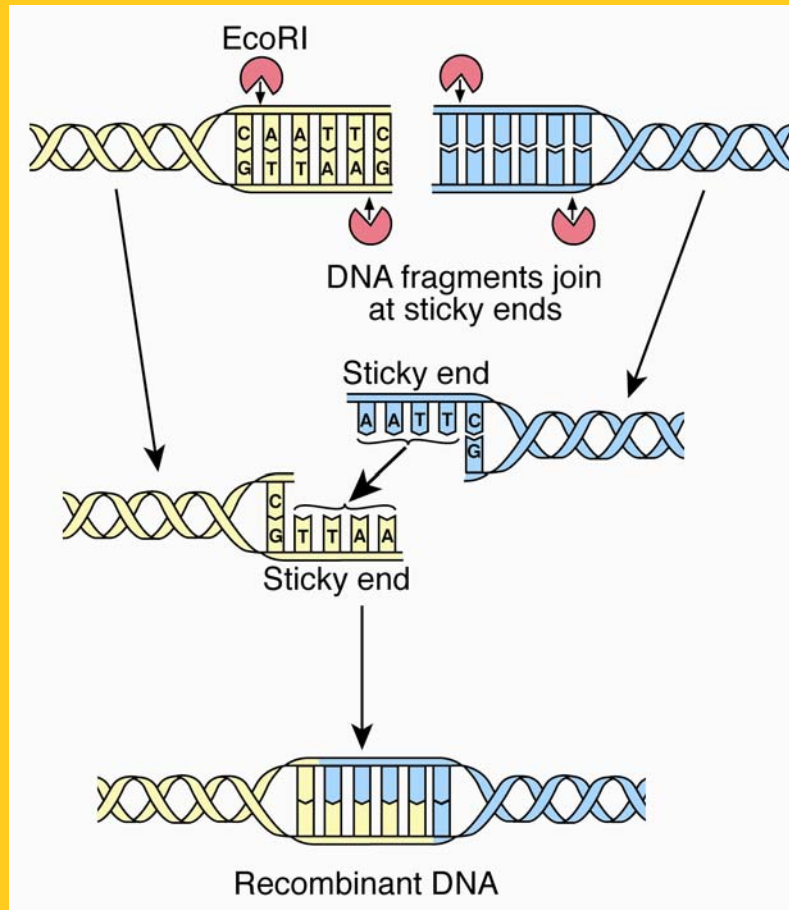
- A particular gene can be isolated and its nucleotide sequence determined
- Control sequences of DNA can be identified & analyzed
- Protein/enzyme/RNA function can be investigated
- Mutations can be identified, e.g. gene defects related to specific diseases
- Organisms can be 'engineered' for specific 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.



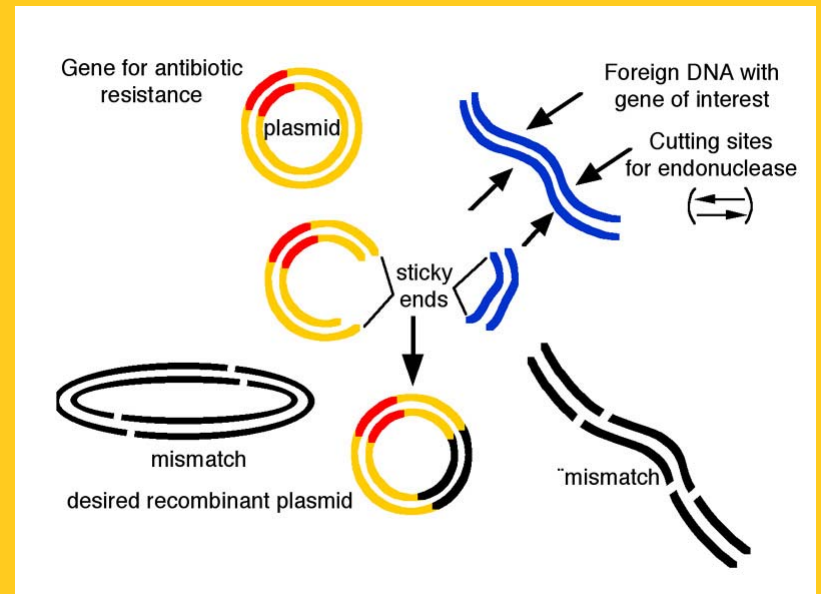
Restriction enzymes



The action of a **restriction enzyme**, *EcoRI*  
*Note: EcoRI gives a 'sticky' end*

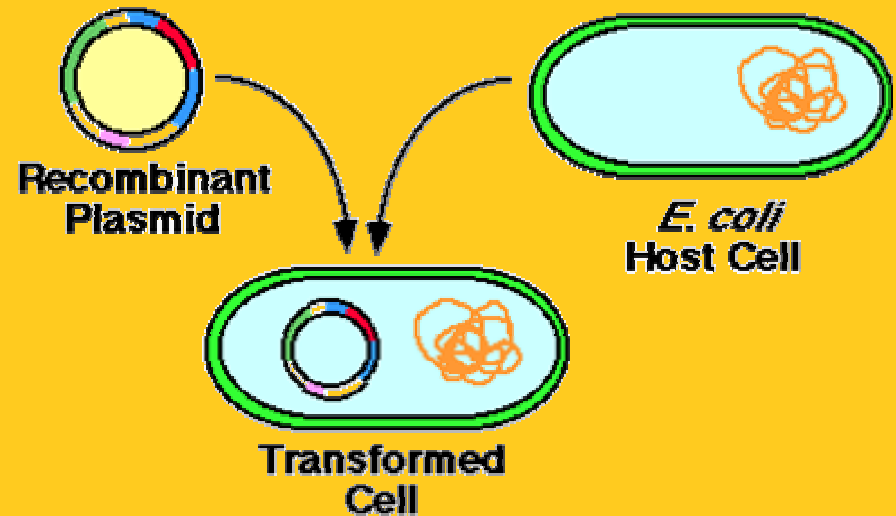
# DNA Cloning, II

- 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"



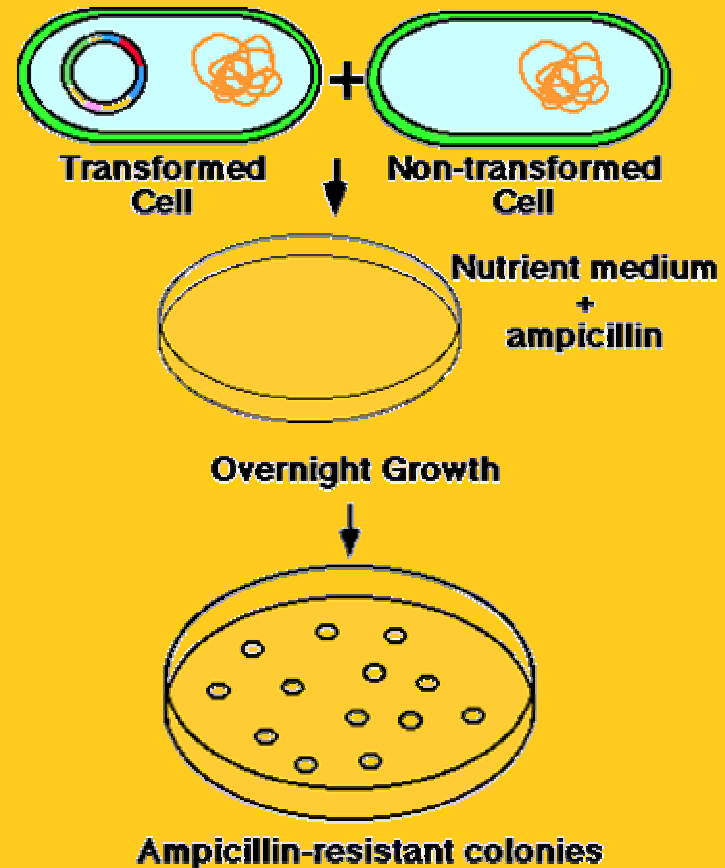
# DNA cloning, III

- 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

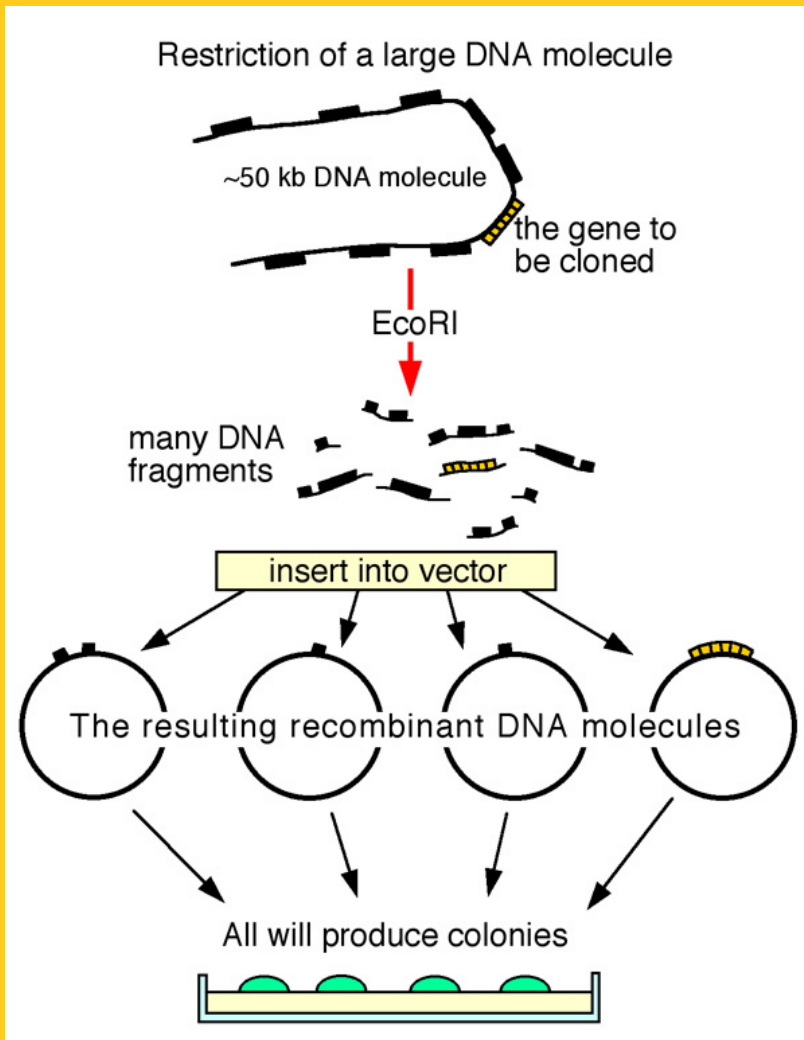


# Antibiotic Resistance

- The medium in this petri dish contains the antibiotic Kanamycin
- The bacteria on the right contain Kan<sup>r</sup>, 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)

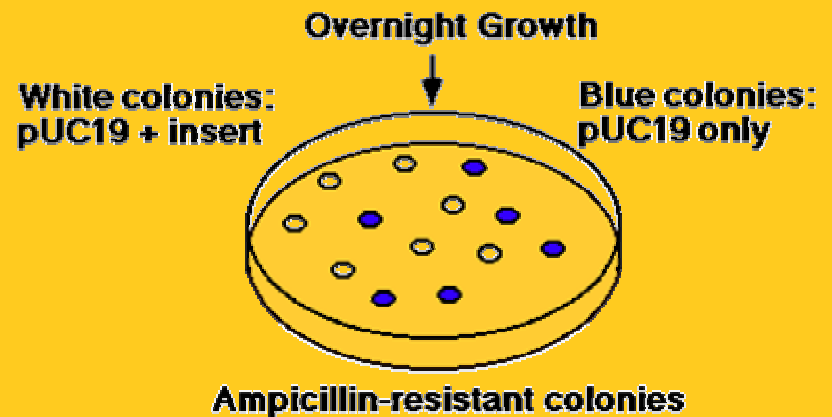
# Gene Libraries

- 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 I

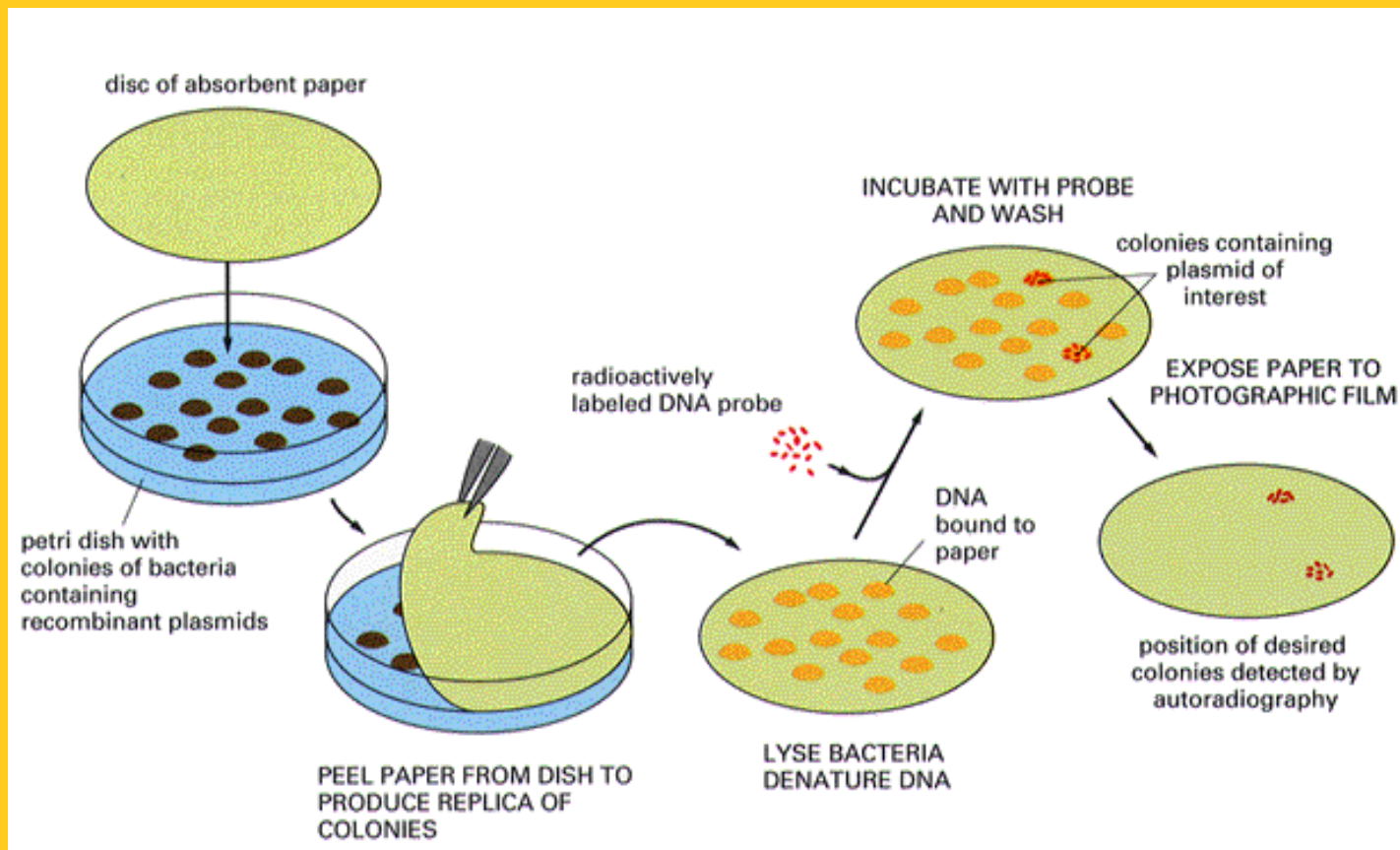
Screening can involve:

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



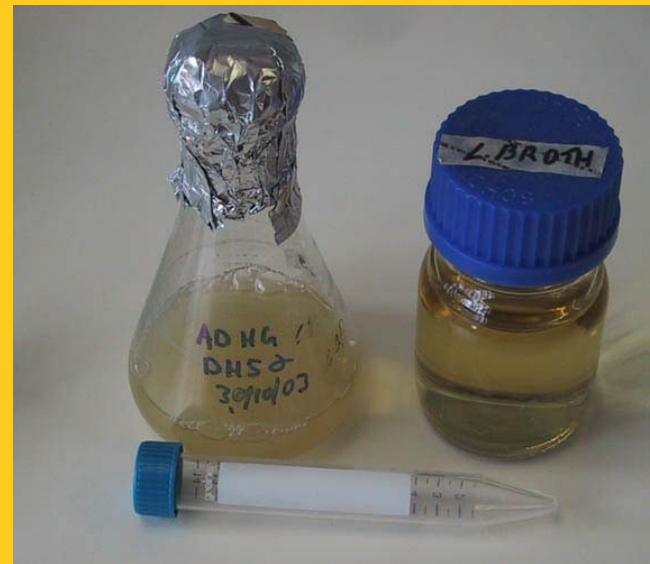
# Screening II

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



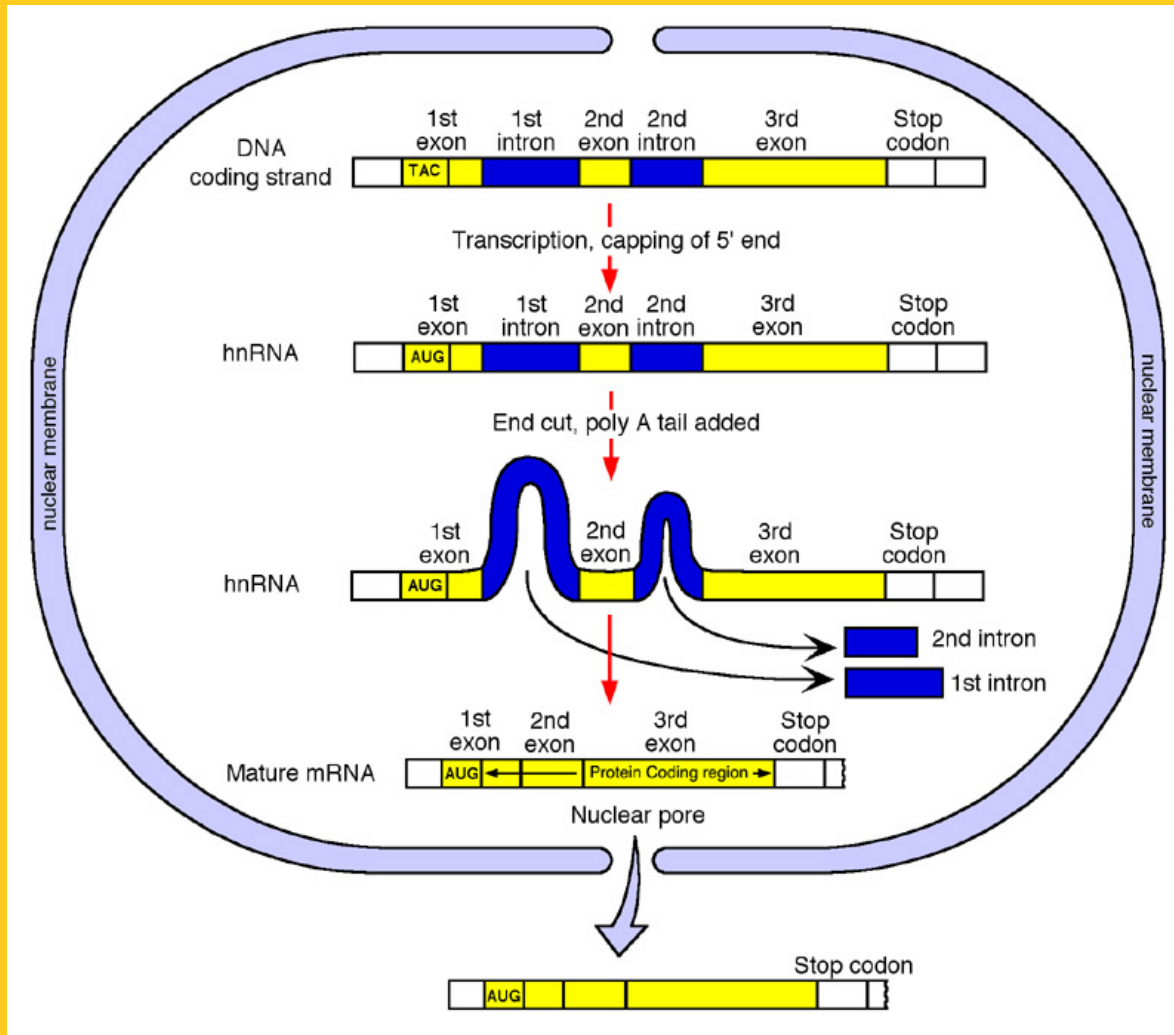
# Screening III

- 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?

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<http://www.biology.arizona.edu>

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