

Molecular biotechnology

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Learning outcomes

- Overview of cell biology
- Gene expression
- Recombinant deoxyribonucleic acid (DNA) technology
- Recombinant DNA (rDNA)

The procaryotic cell

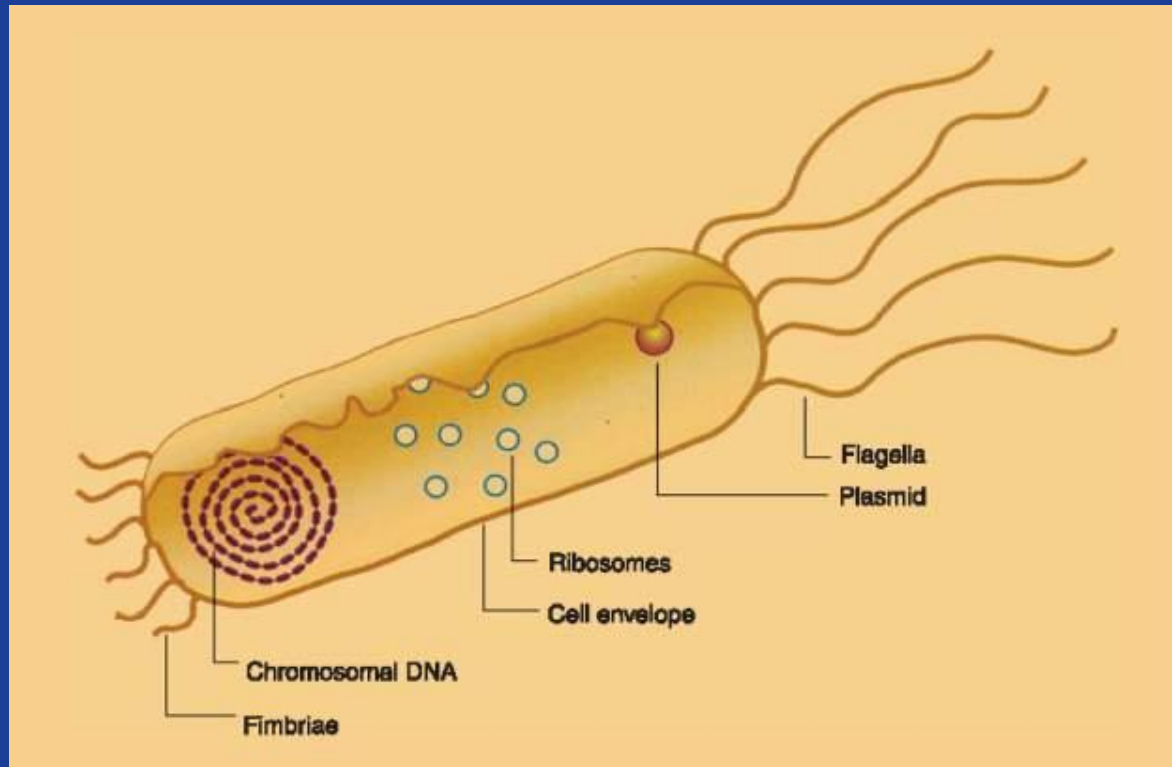
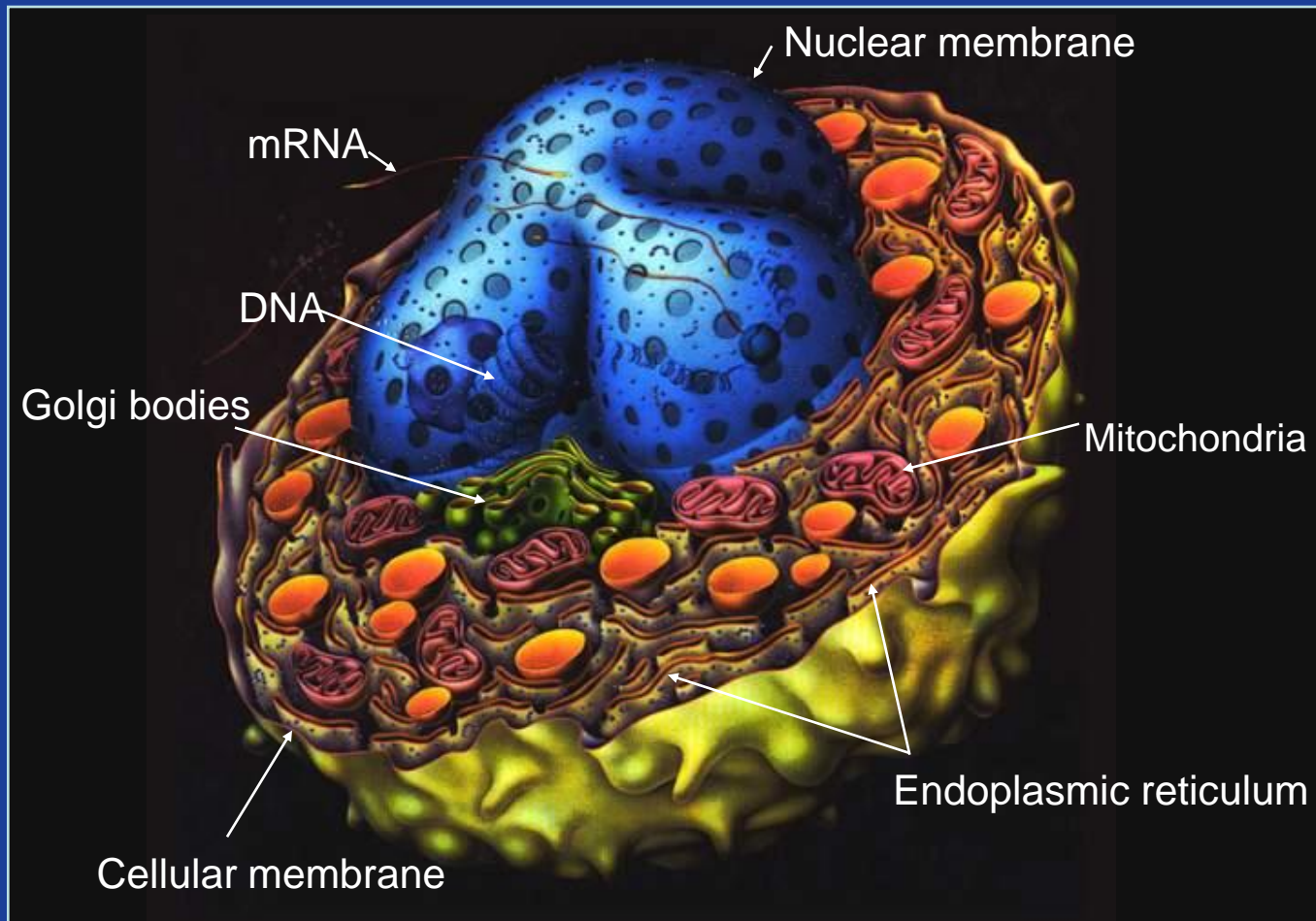


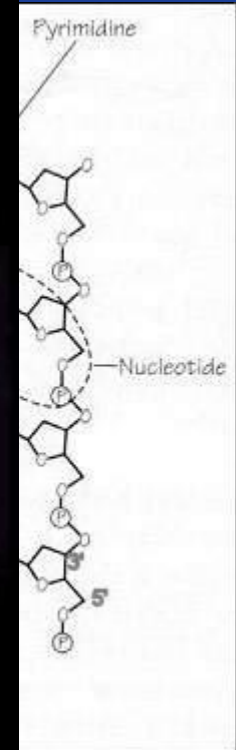
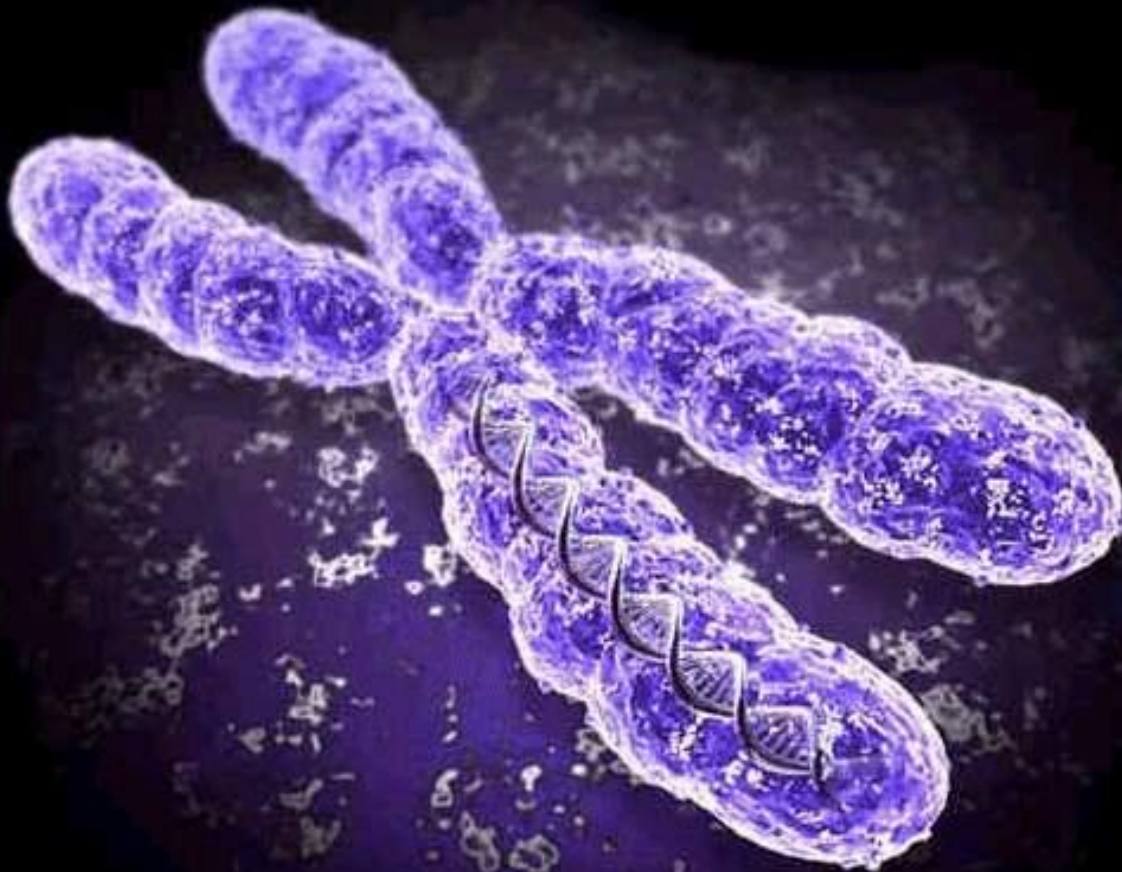
Figure 1. Cross section (*artificial*) through a bacterial cell. The surface structures (*fimbriae and flagella*) are not essential structures but allow the cells to adhere and to move

The eucaryotic cell



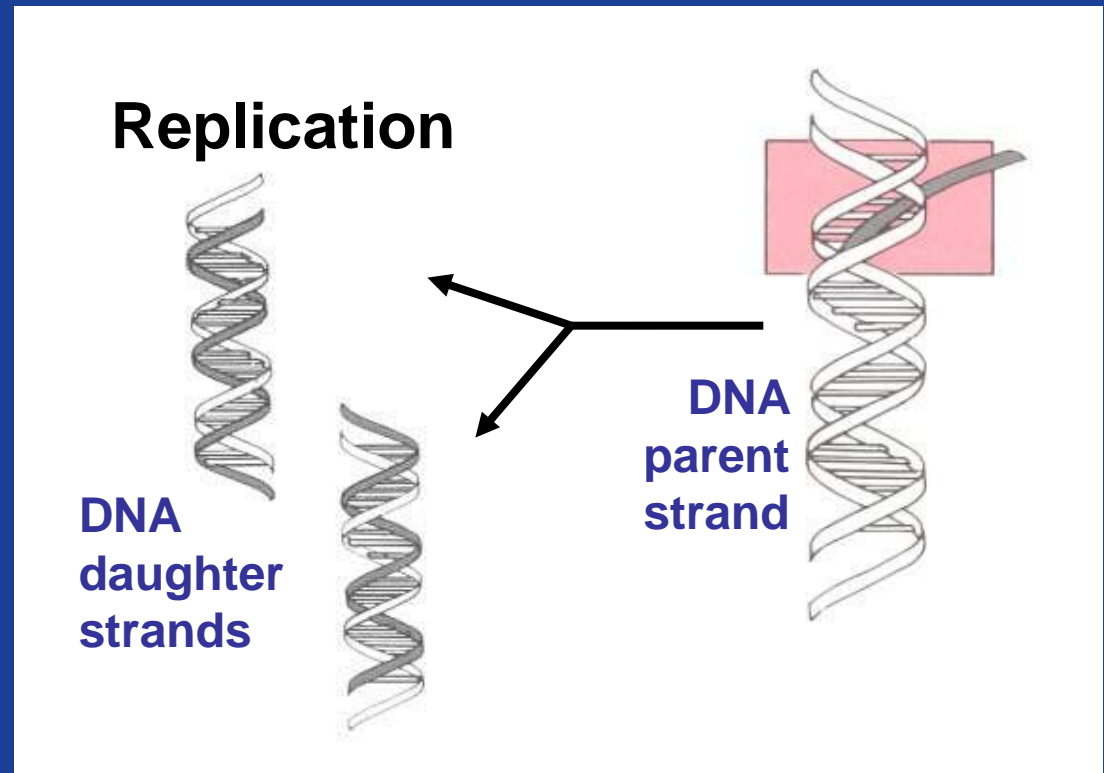
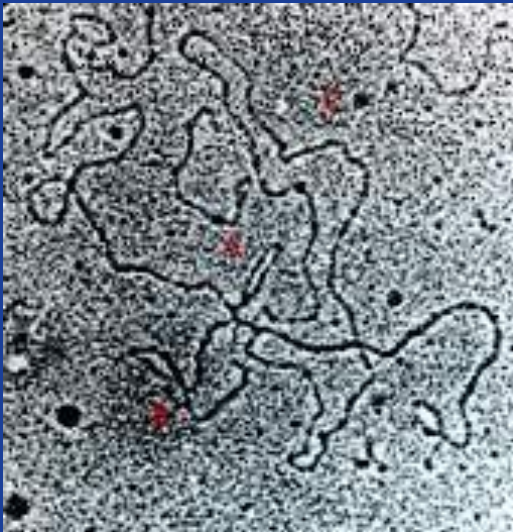
DN

es

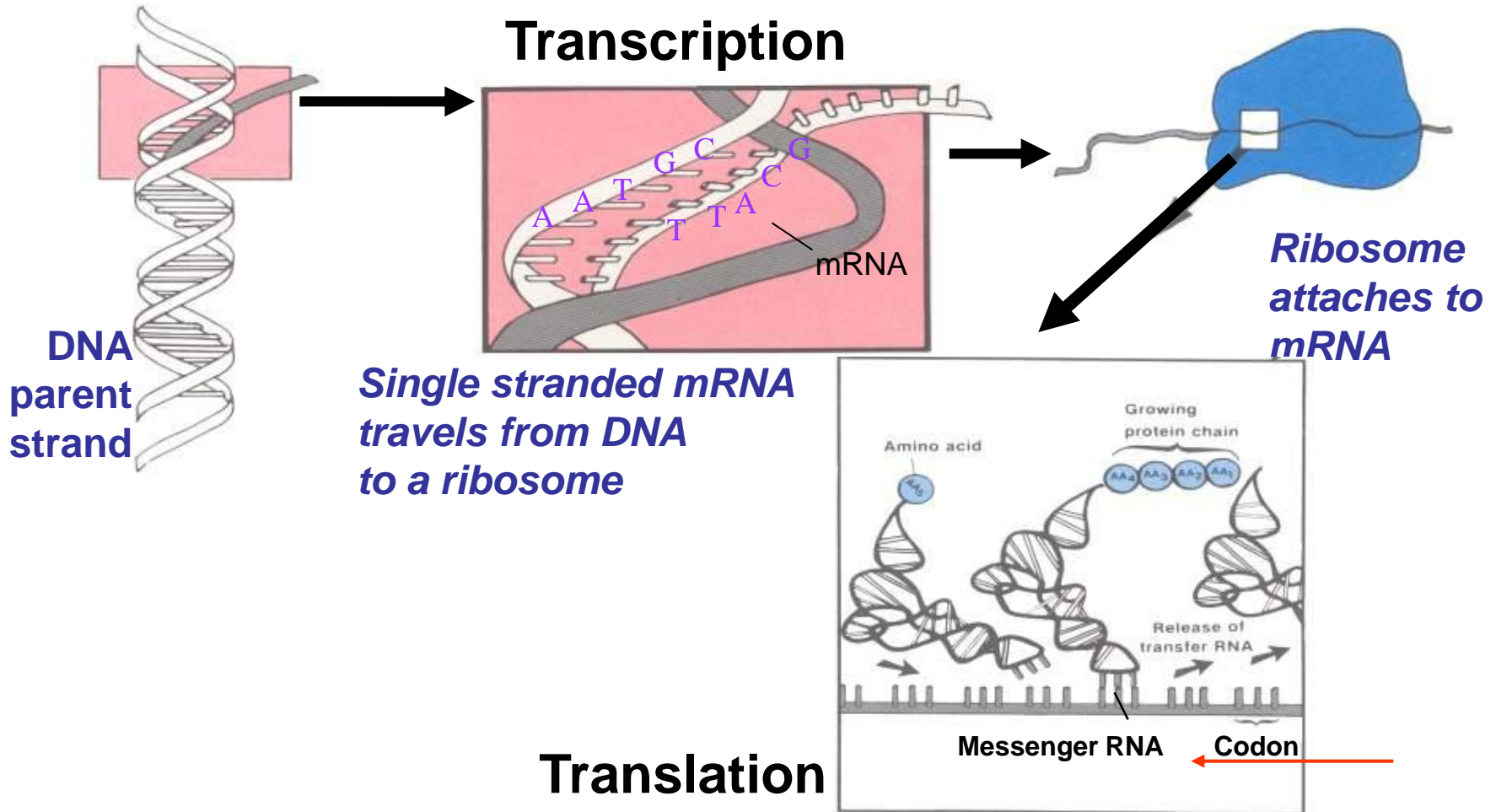


http://www.virtualsciencefair.org/2005/anna5m0/public_html/images/chromosome.gif

The cell cycle

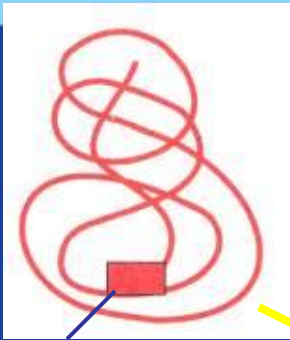


The cell cycle



Recombinant DNA technology

Source DNA



Target DNA

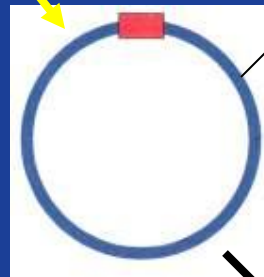
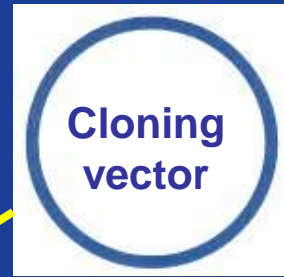


Enzymatic fragmentation

Vector DNA



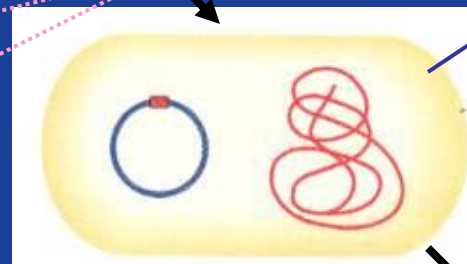
Enzymatically linearize



DNA construct

Join target DNA and cloning vector

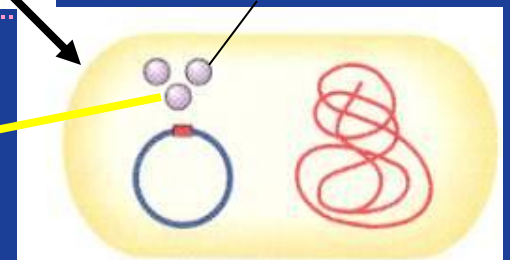
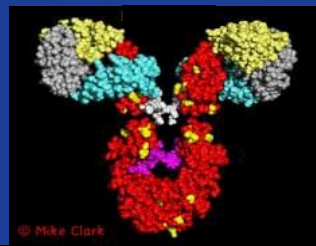
*Introduce DNA into host cell
Isolate cells with cloned gene*



Host cell

Produce protein from cloned gene

Protein encoded by cloned gene

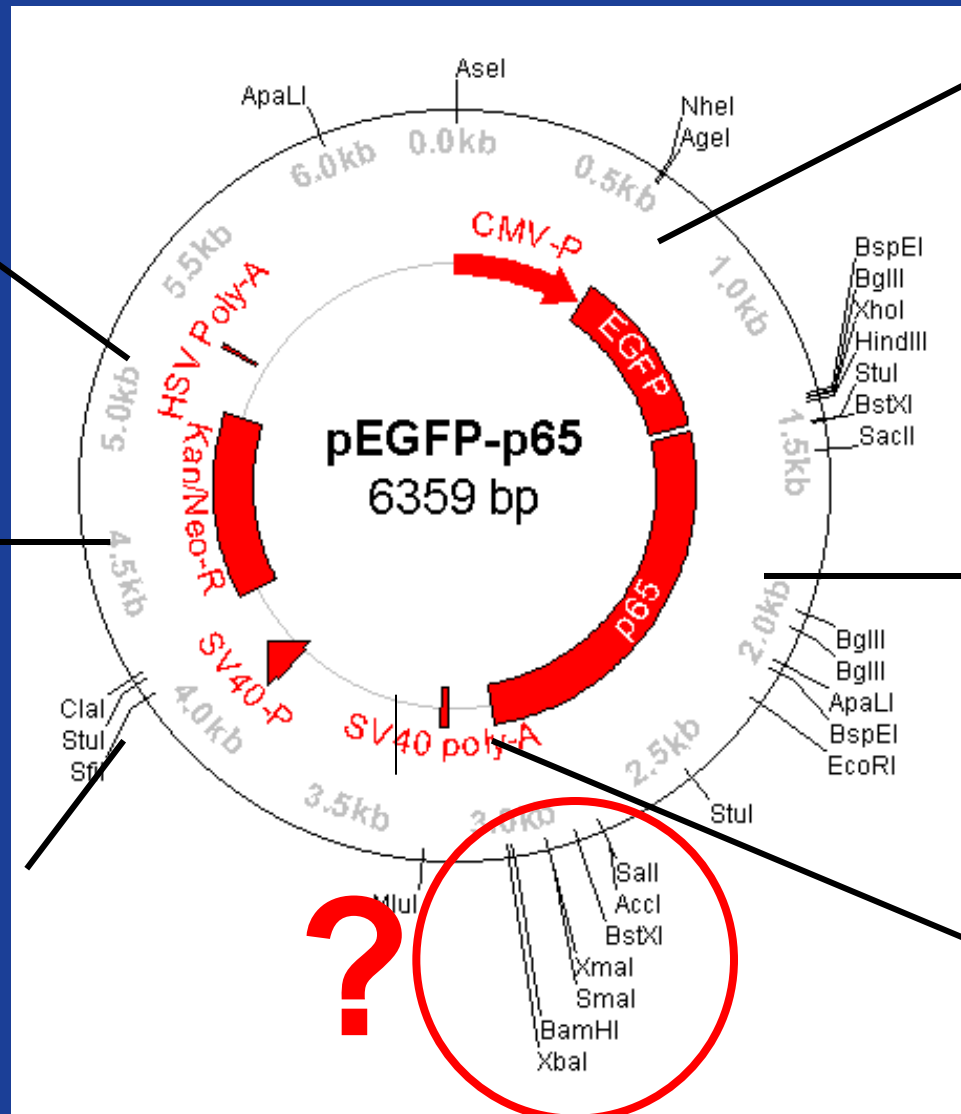


Expression plasmid: eg for a eucaryotic cell

Poly-adenylation
signal

Gene for
selection
(bacterial
selection with
kanamycin,
mammalian
cell selection
with geneticin)

SV40-
promoter



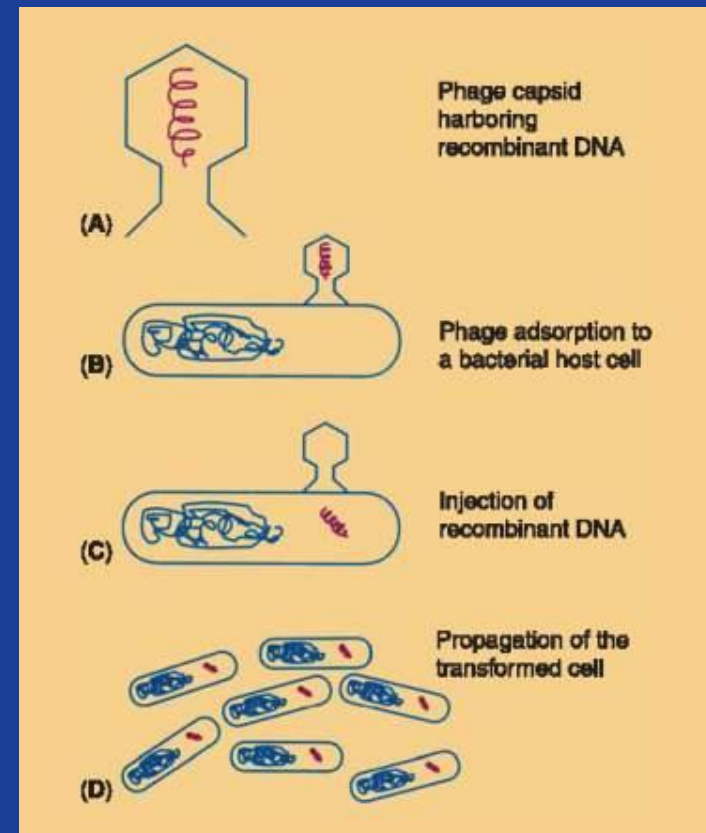
CMV-promoter

Multiple
cloning
site

Target gene

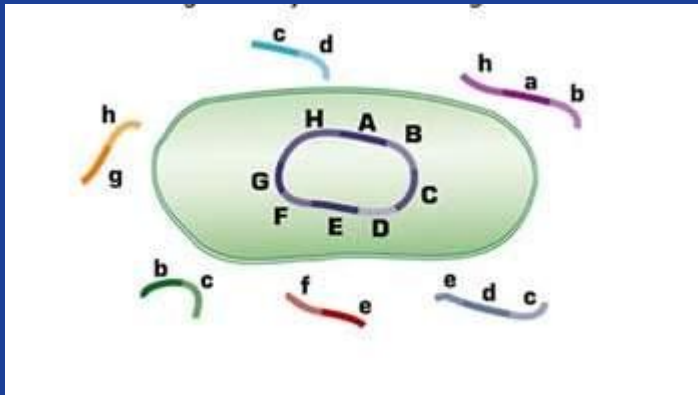
Poly-adenylation
signal

Phage introduction

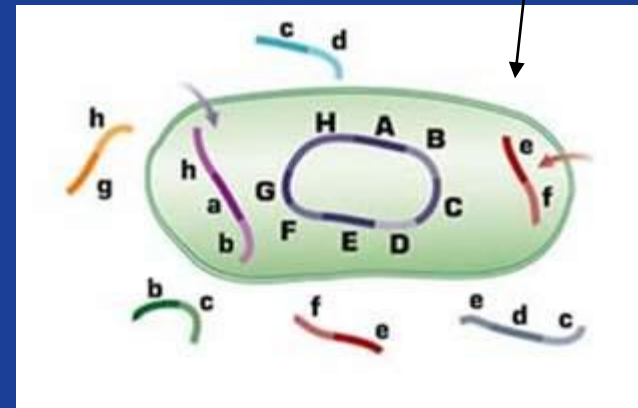


Bacterial transformation

1. Naked DNA fragments from disintegrated cells in the area of a potential recipient cell. This cell must be of correct genus and be in a state of competence, allowing the entry of the DNA fragments

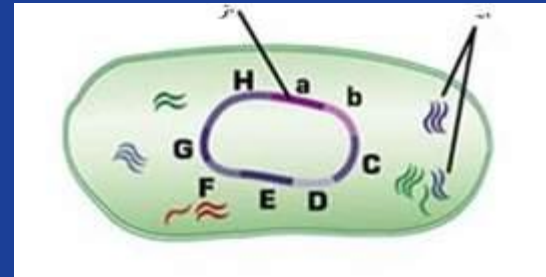


2. Entry of naked DNA into competent cell



3. Some DNA fragments replace (recombine with) original host cell DNA. The resultant recombinant DNA will now express the foreign genes it has received and pass them on to all its offspring

DNA that has not recombined is broken down by enzymes



Non-bacterial transformation

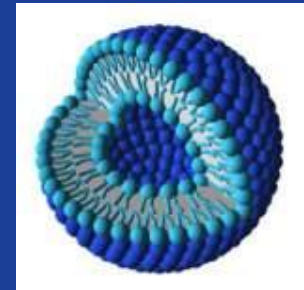
- Chemical methods:



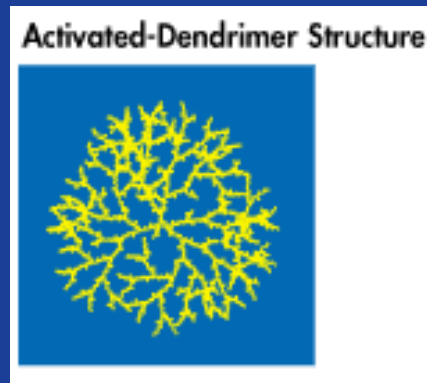
- CaPO₄-precipitation method

Ca²⁺

- Lipofection



- Dendrimers



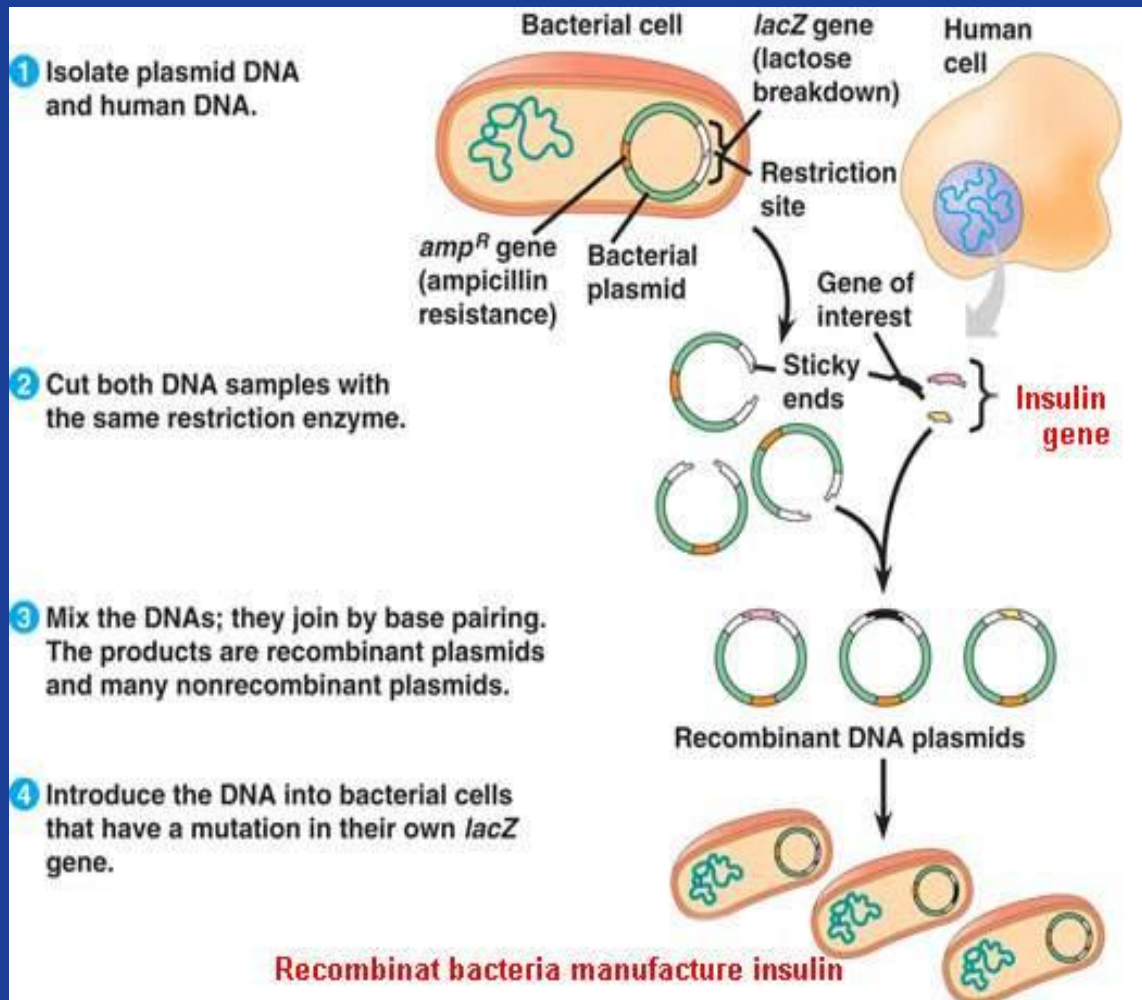
- DEAE-Dextran

Non-bacterial transformation

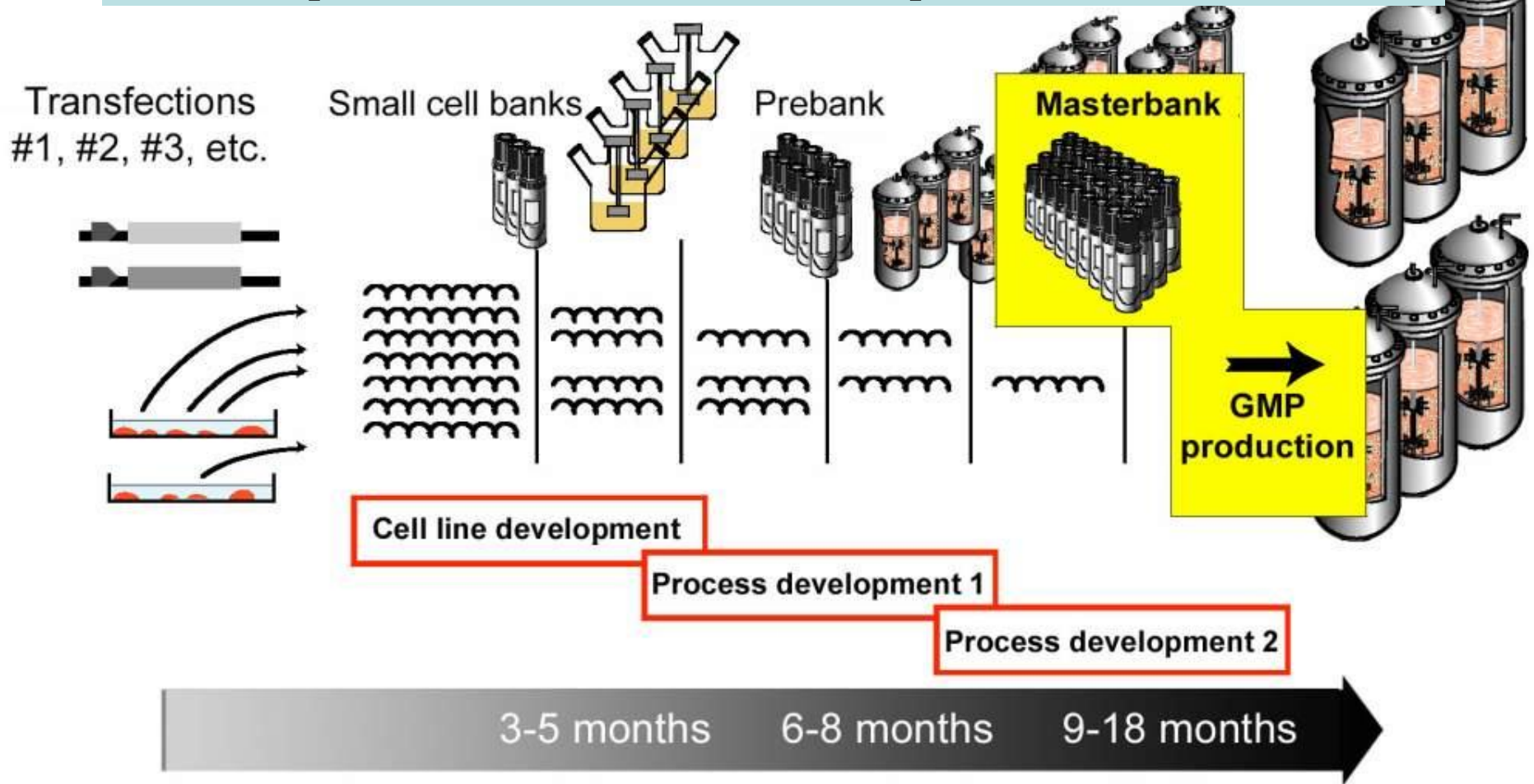
- Physical methods:
- Gene-gun
- Electroporation
- Micro-injection



New techniques towards drug development



Mass production of pharmaceutical products



Possible cure of genetic disease

- Sickle cell anaemia
- Cystic fibrosis

Conclusion slide

- Overview of cell biology
 - *Different cells can be used*
- Gene expression
 - *Cell cycle and DNA*
- Recombinant DNA technology
 - *Getting the cells do what you want*
- Recombinant deoxyribonucleic acid (rDNA)
 - *The basis for development of novel drugs*