Biotechnology and Energy Conservation

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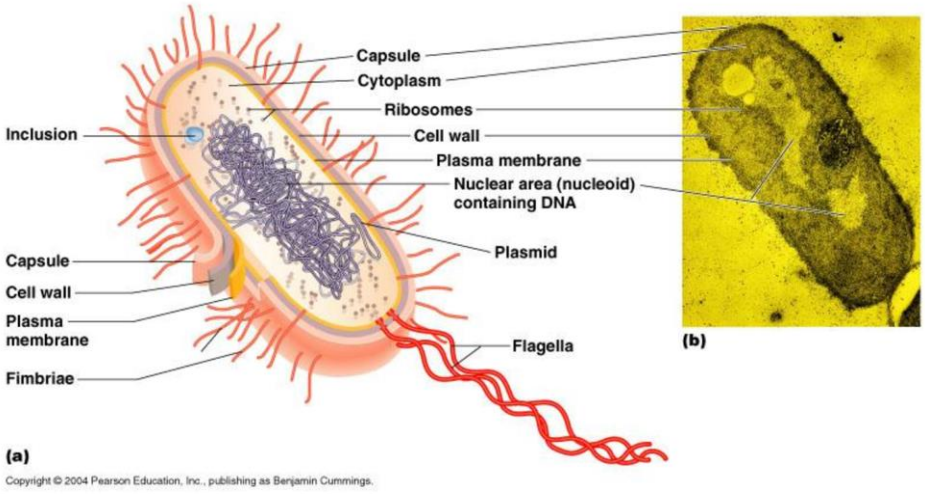
12th Lecture
Genetic Engineering

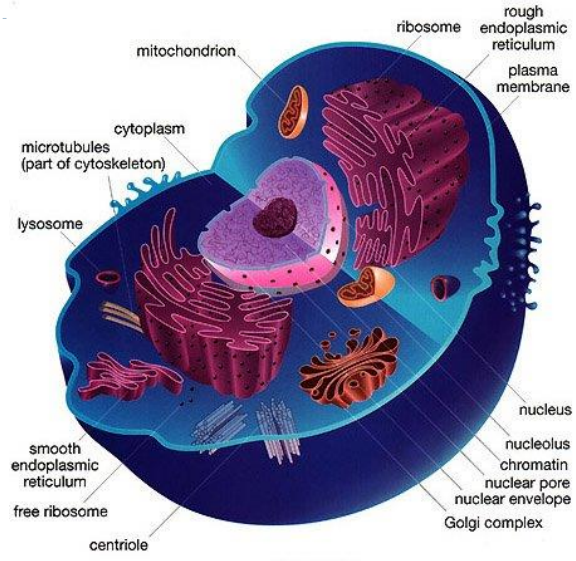
The Aim:

- Students can explain the use of genetic engineering technology in Bio-industry
- Students can describe a simple method in improving the genetic characteristics by genetic engineering in bacteria

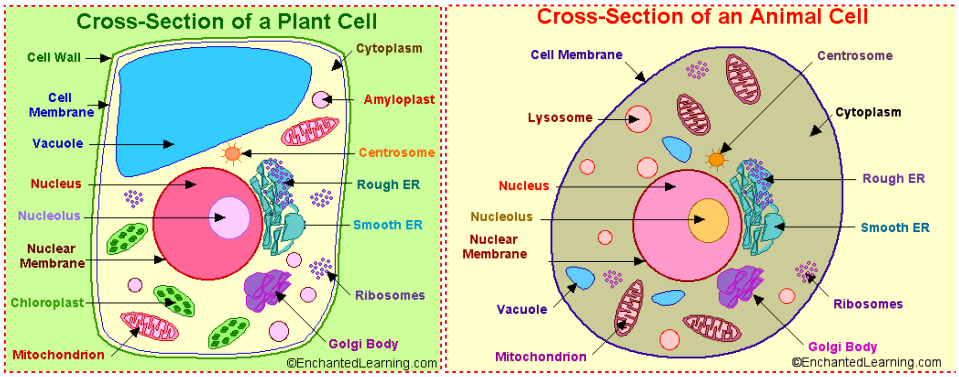
Cell structure of procaryotic cells



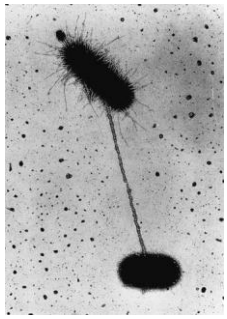
Cell structure of eucaryotic cells

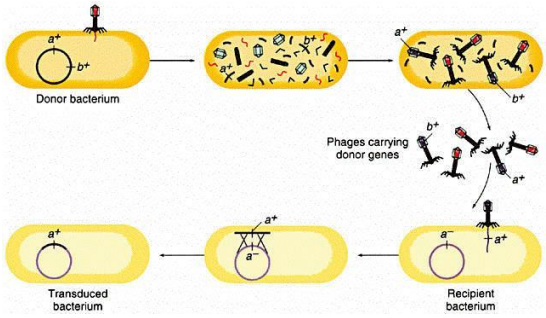


Cross section of cell



Genetic recombination

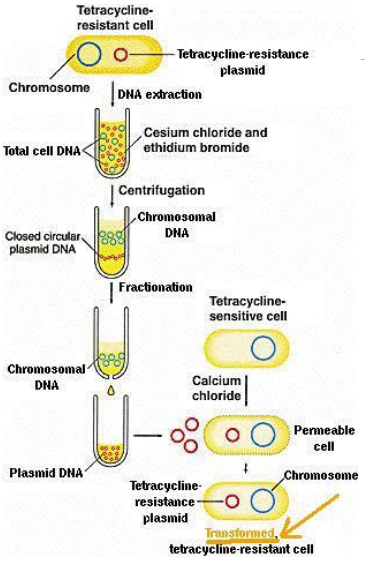




Conjugation *Transduction*

http://www.cbs.dtu.dk/staff/dave/roanoke/genetics980309.html

Rekayasa Genetika (transformasi)



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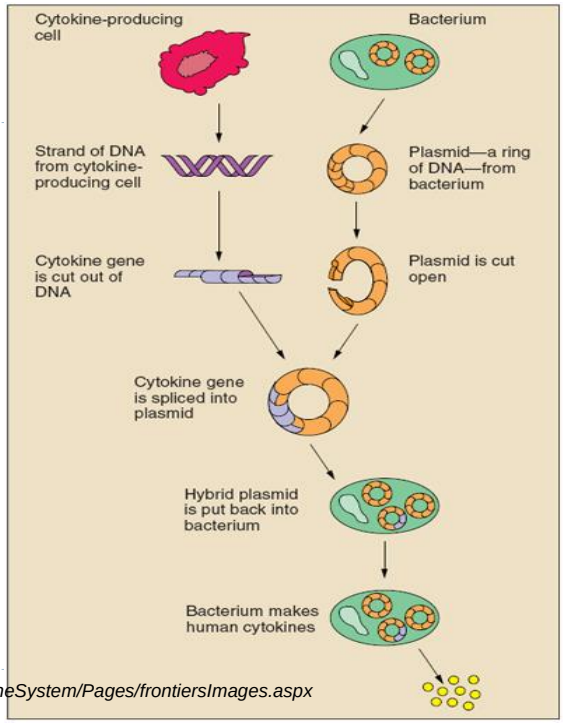


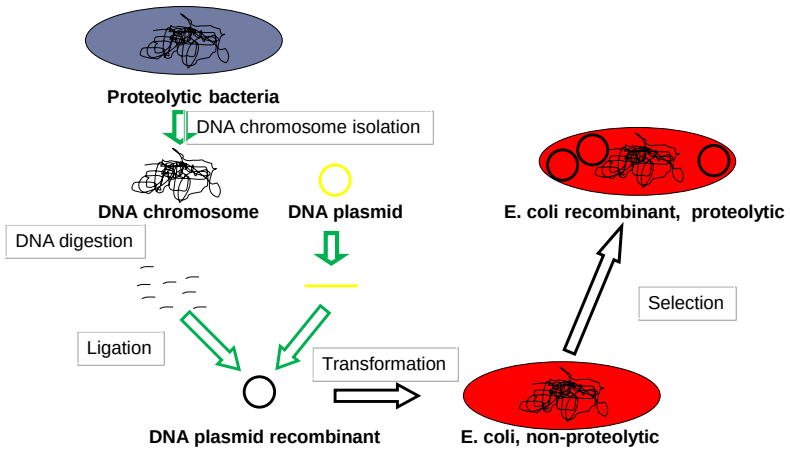
- *http://www.niaid.nih.gov/topics/immuneSystem/Pages/frontiersImages.aspx*

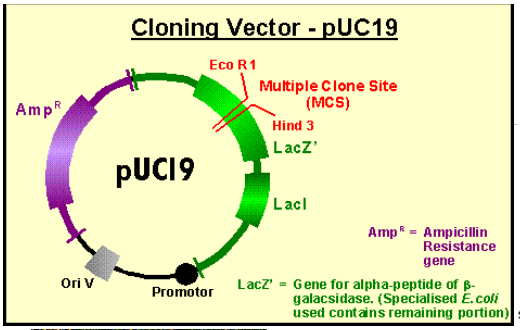
Agents and enzyme involve in in-vitro genetic recombination

- Agents
  - DNA donor (fragment DNA, usually DNA chromosome)
  - DNA Plasmid (vector)
  - Host bacteria
- Enzymes
  - DNA restriction (endonuclease)
  - DNA Ligase

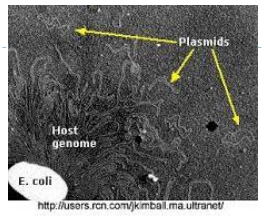
Shotgun cloning

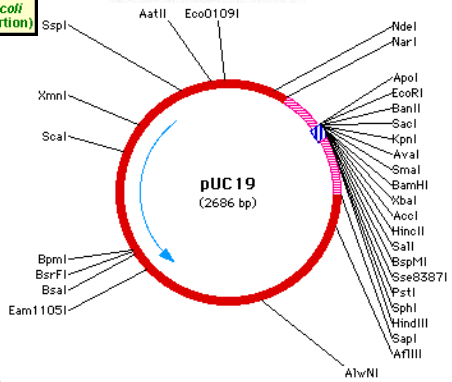
- To improve the productivity of agents (microbes), genetic engineering can be introduced





Plasmid



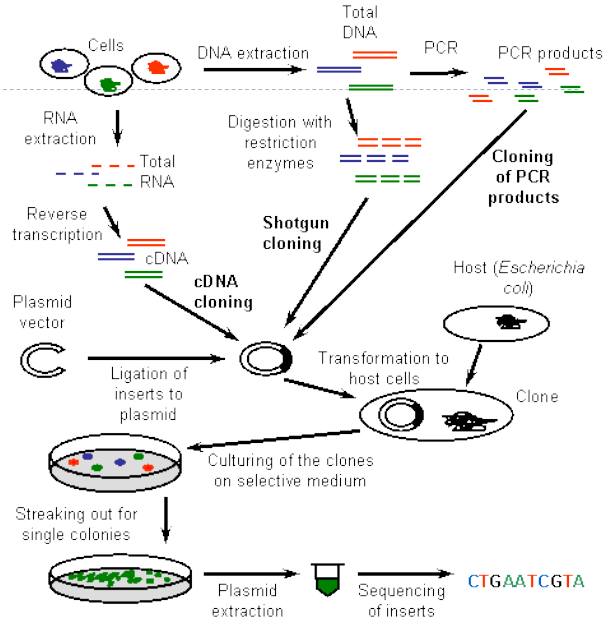


DNA cloning

cDNA Cloning steps: RNA extraction, cDNA construction, ligation to plasmid, transformation, recombinant selection.

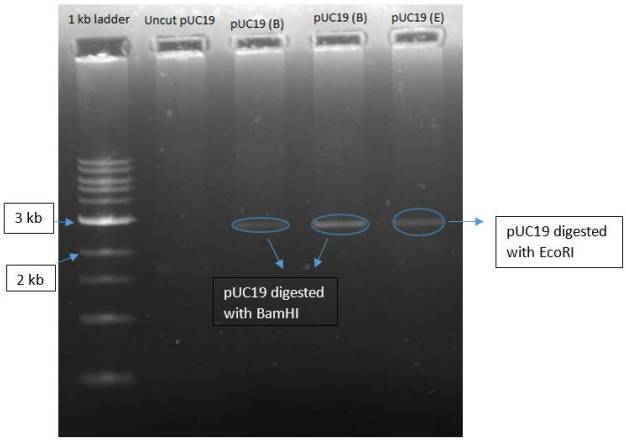
Shotgun Cloning steps: Total DNA extraction, DNA digestion by restriction endonuclease, ligation to plasmid, transformation, recombinant selection

Cloning of PCR product steps: Total DNA extraction, PCR product construction, ligation to plasmid, transformation, recombinant selection



DNA electrophoresis

DNA Electrophoresis: to determine purity of plasmid, PCR product, cDNA, or recombinant plasmid



Selection of Recombinant Cell

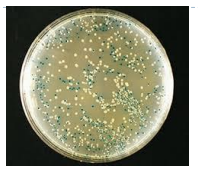
Blue and white selection

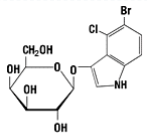
- Blue and white selection is applied using lac transformation system (β-galactosidase system)
  - Host is E. coli DH5α, genotype of F- 80dlacZ M15 (lacZYA-argF) U169 recA1 endA1hsdR17(rk-, mk+) phoAsupE44 -thi-1 gyrA96 relA1 (having gen lac X dan lac Y)
  - Plasmid used is lac plasmid (pUC19) (having ampicilin resistant and lac Z genes)
  - E. Coli DH5α and pUC showing a complementary of lac gene (lac X, lac Y, and lac Z)

Selection of Recombinant Cell

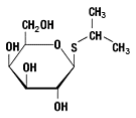
Blue and white selection

- In ligation process, 3 possibilities occur:
  - Self-ligation of plasmid
  - Self-ligation of DNA fragment
  - Ligation between plasmid and DNA fragment
- In transformation process the 3 kinds of ligation product will going into the cell
- In selection process (using media containing antibiotic and X-gal and IPTG):
  - Cells owing the self-ligation plasmid will grow and have blue color because they have antibiotic resistant gene (from the plasmid) and a complete lac gene.
  - Cells owing the self-ligation of DNA fragment will not grow or death because they do not have antibiotic resistant gene .
  - Cells owing the ligation between plasmid and fragment product will grow and have white color. They resistant to antibiotic but can not express the lac gene because they have insertion DNA in MCS (multiple cloning sites) of lac Z in the plasmid.
- The white cells is the recombinant cell





X-Gal
Formula: $C_{14}H_{15}BrClNO_6$
Molecular weight: 408.6



IPTG
Formula: $C_9H_{18}O_5S$
Molecular weight: 238.3

- X-gal (5-bromo-4-chloro-3-indolyl-beta-D-galacto-pyranoside) is substrate analog of lactose. X give blue color when separated.
- IPTG (isopropylthio-β-galactoside) is an inducer of β-galactosidase in bacteria