Intro to Bacterial Genetics & Microbial Biotechnology

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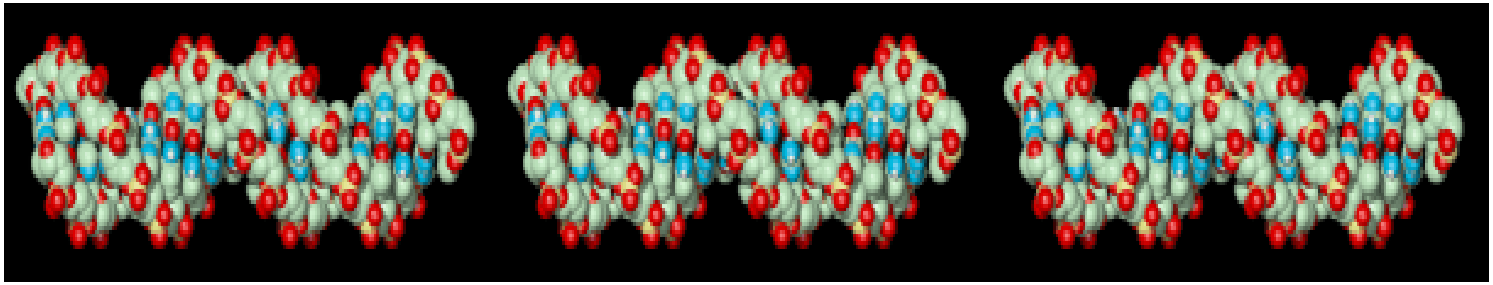
Assumed knowledge

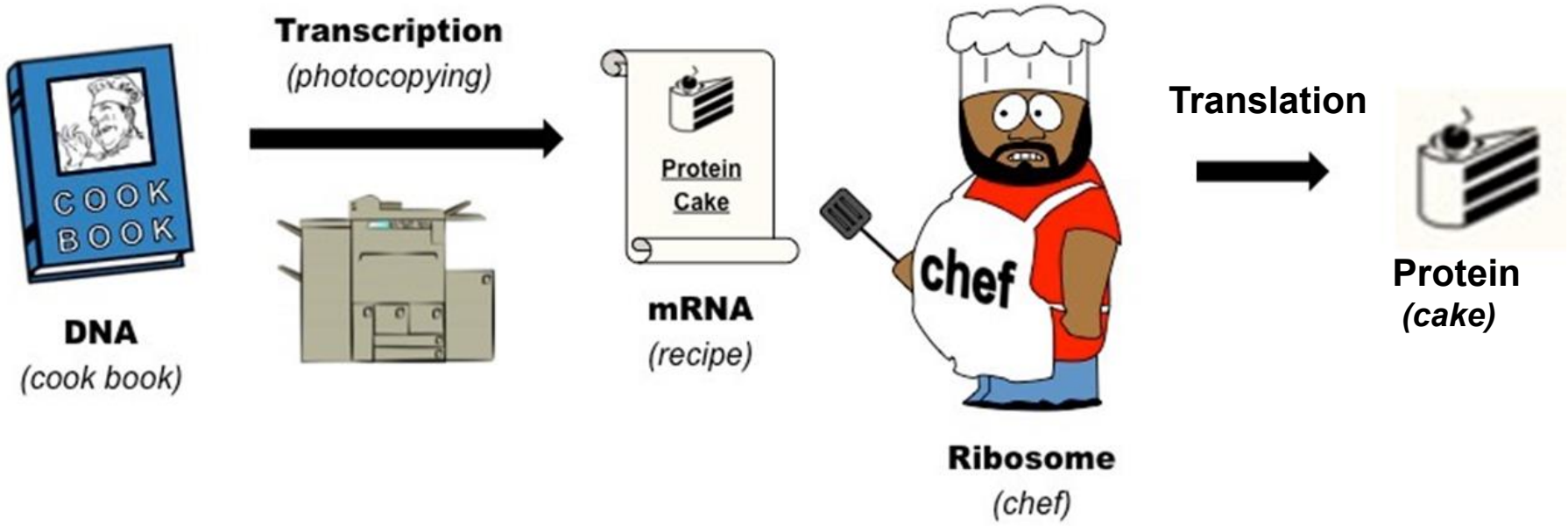
- **Basic structure and functions of DNA, RNA, protein**
- **“Central dogma” of mol. biol: DNA → RNA → protein**
- **Basics of transcription – RNA polymerase, mRNA**
- **Basics of translation– ribosomes, proteins**
- **Use of plasmids to ‘clone’ genes**



What is a gene? How does it work?

- A gene is a length of DNA that codes for a protein. Typical length approx 500-1000 base pairs (bp)

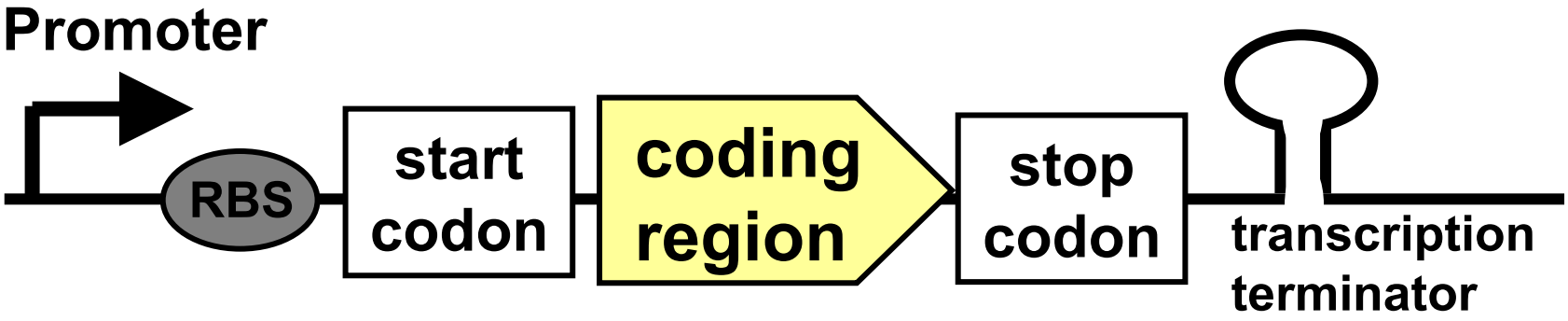
- Bacterial genes do not usually have introns, but do require non-coding DNA sequences at 5' and 3' ends



What is a gene? How does it work?

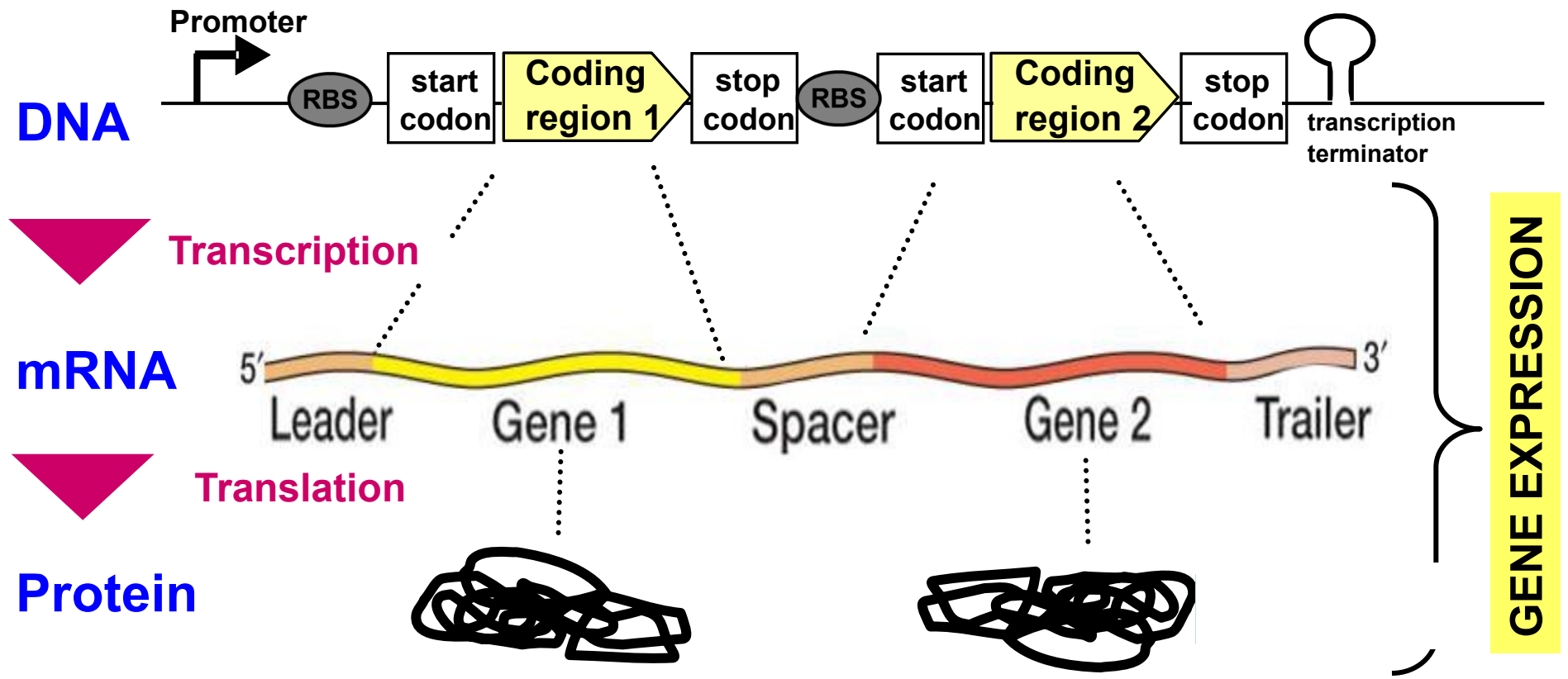
- Transcription of gene into mRNA requires promoter to bind RNA polymerase and terminator to stop RNA pol.

- Translation of mRNA to protein requires a ribosome binding site (RBS), a start codon, and a stop codon. A "gene" usually means start-coding region-stop



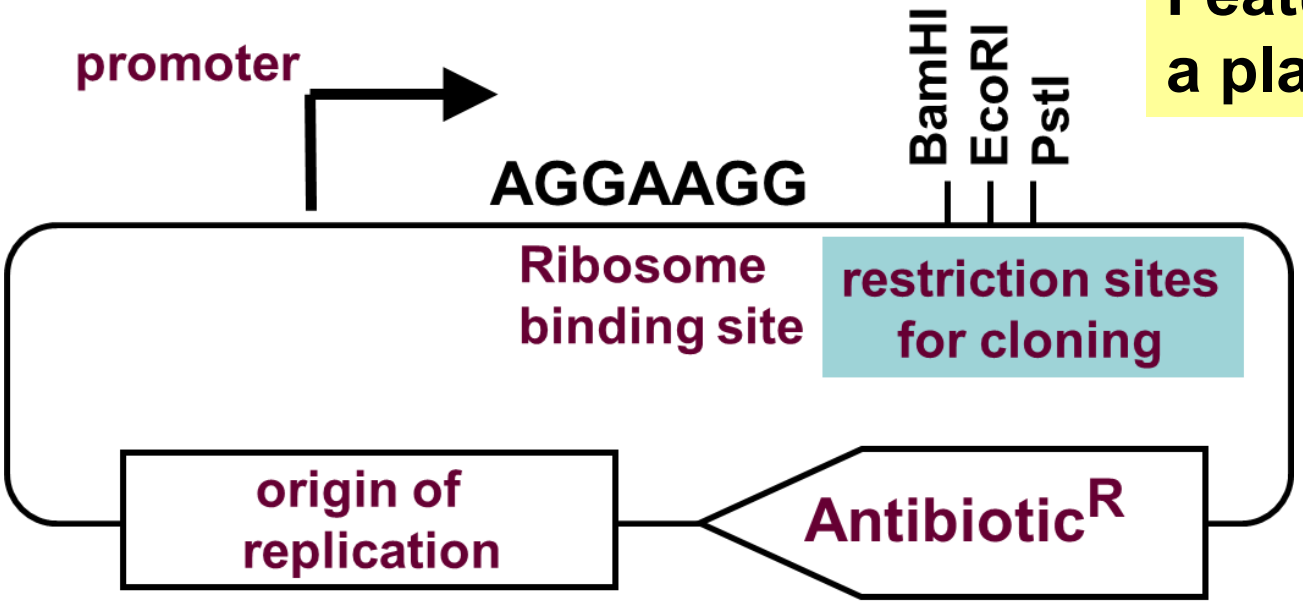
Bacterial genes are organised in operons

- An operon is a cluster of genes that share a promoter
- Genes in an operon are co-transcribed on one mRNA
- Operons usually encode functionally-related proteins



Cloning vectors

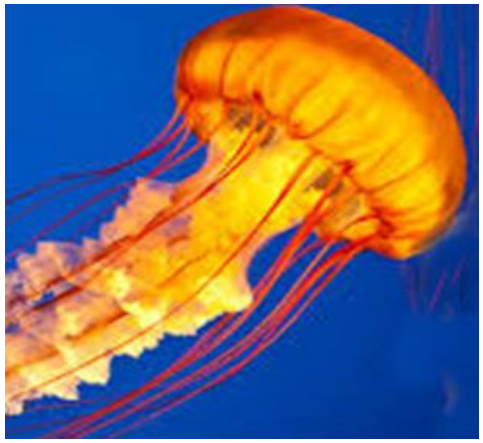
- **Cloning vector**: A DNA or RNA element that carries other genes eg. a plasmid or virus
- **Marker gene**: allows <u>selection</u> or <u>detection</u>
  - **selection**: eg. resistance to antibiotics
  - **detection**: eg. green fluorescence



Features of a plasmid vector

More on this topic in next lecture

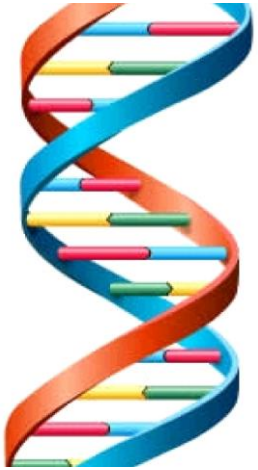
DNA Cloning… digestion and ligation



Organism of interest

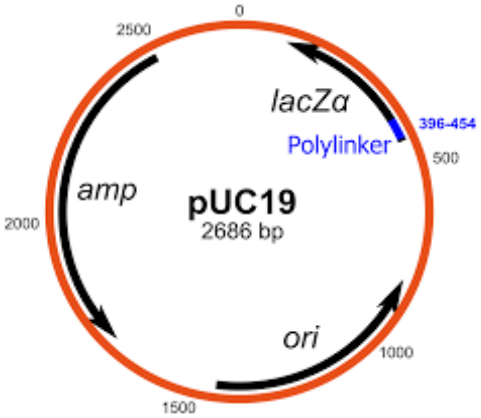
Extract DNA

Or copy DNA



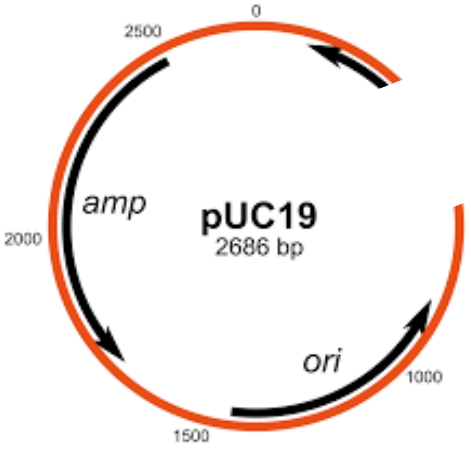
Cut DNA into pieces

‘digestion’



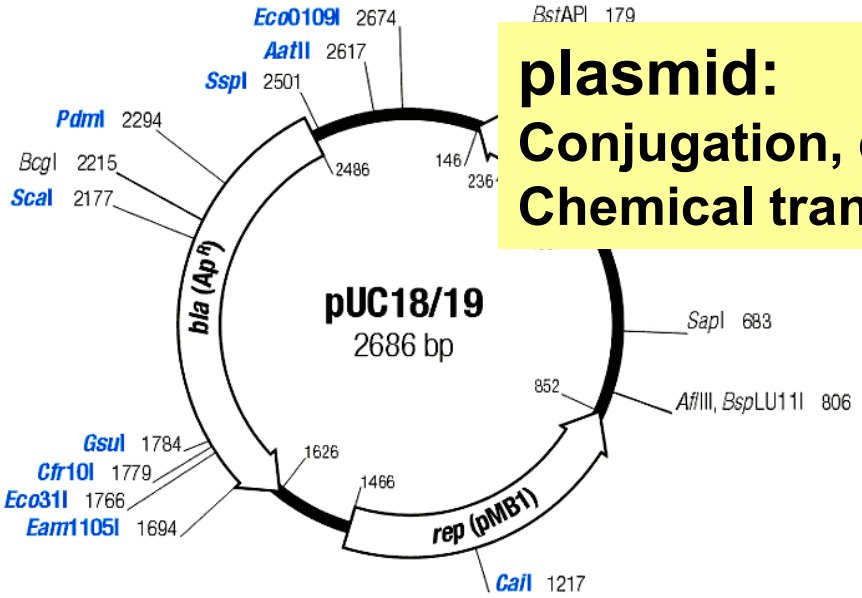
Plasmid

Cut

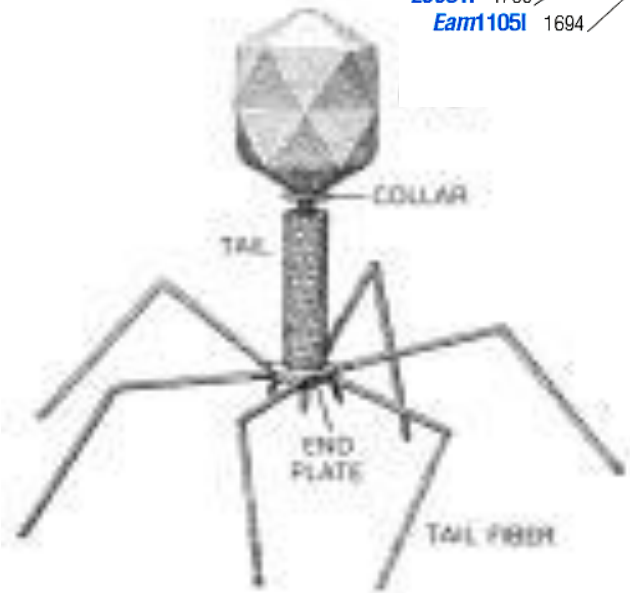


‘ligation’

Types of vectors and methods of DNA transfer



plasmid:
Conjugation, electroporation, Chemical transformation



phage: transduction

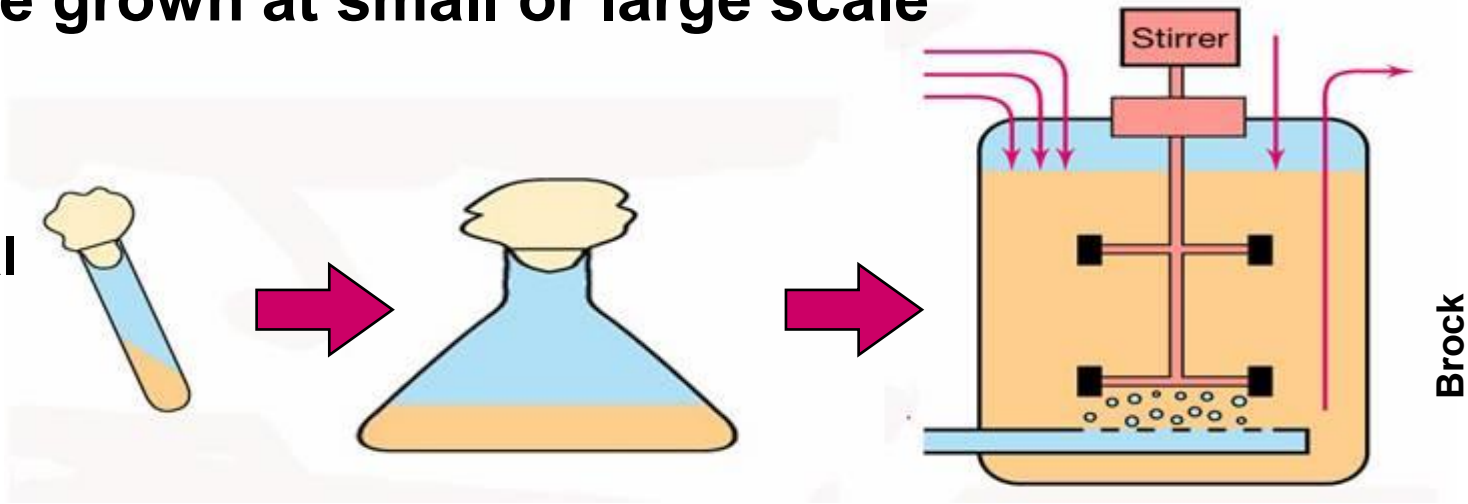
naked DNA (no vector):
natural transformation

Microbes for biotechnology

Microbes have some advantages over macroorganisms:

- **Easy to grow**
  - **simple nutrient requirements**
  - **very rapid growth (exponential!)**
  - **can be grown at small or large scale**

Microbial protein factory:



Brock

Animal protein factory:

Microbes for biotechnology

More advantages of microbes :

Easy to genetically manipulate

- **prokaryotes are haploid, no introns***
- **many host/vector systems available**
- **easy to get DNA in and out of cells**

Electroporation machine

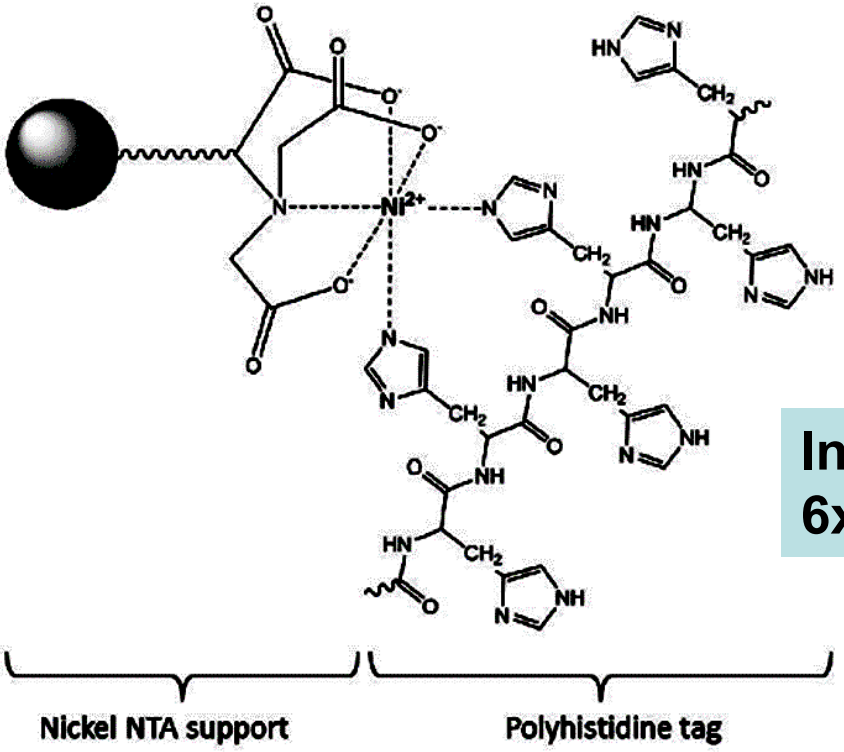
Electroporation cuvettes

Microbes for biotechnology

More advantages of microbes:

Many heterologous expression systems available

- Can control protein expression level
- Can add tags to aid function or purification



In MICR3052 prac we will use a 6xHis tag to purify a protein