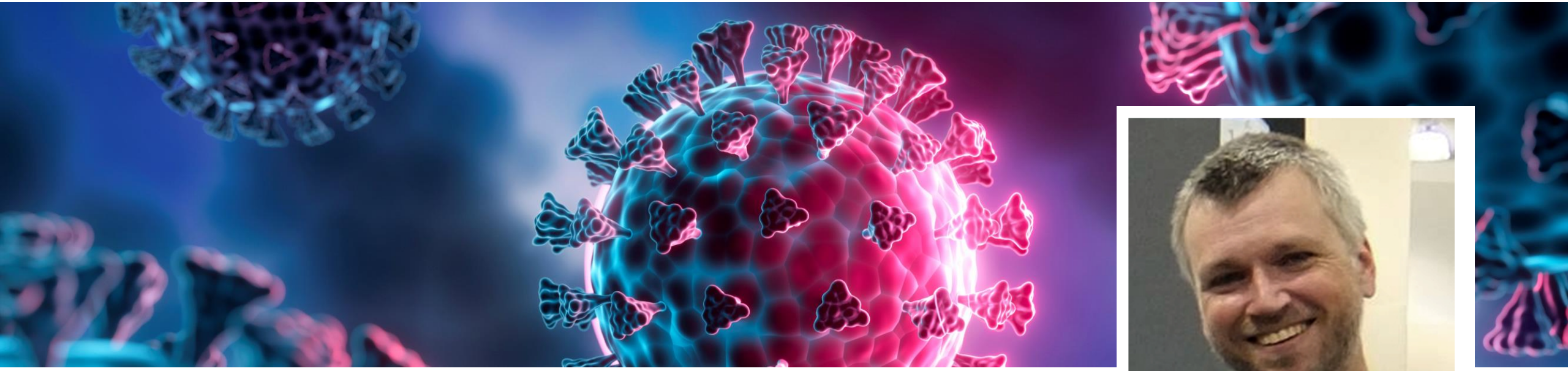
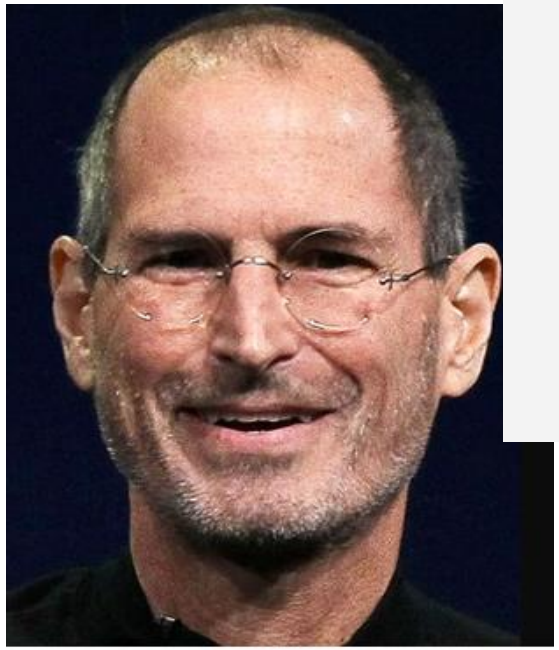
Cell factories and Biotechnology



Dr. Nick Coleman

Why should we care about biotechnology?

Why should we care about biotechnology?



I think the biggest innovations of the 21st century will be at the intersection of biology and technology. A new era is beginning.

— Steve Jobs —

Biotechnology is creating a new industrial revolution based on biology instead of petroleum.

Brent Erickson

Bio Biotechnology Innovation Organization

Traditional biotechnology - Fermentation

- "Biotechnology" has been done by humans for >9,000 years
  i.e. fermentation to preserve foods, or make alcohol

- Early fermentations used
  mixed cultures of
  naturally-occurring
  bacteria & fungi

Scandinavian fermented fish storage unit, circa ~ 7000 BC

(1) http://www.sciencedirect.com/science/article/pii/S0305440316000170

Traditional biotechnology - Fermentation

- Earliest firm evidence for alcoholic fermentation also comes from around 7000 BC, but on the other side of the globe…
- Analysis of jars from Henan province, China, revealed traces of alcohol and the plants used for fermentation (2)

Our relationship with fermenting yeasts is ancient, and remains very strong

http://www.pnas.org/content/101/51/17593

Cellular vs. molecular biotechnology

- Fermentation could be defined as "cellular biotechnology"

→ need some biology skills (esp. microbiology)
→ don't need understanding of DNA, RNA, proteins

Cellular biotechnology…

Cellular vs. molecular biotechnology

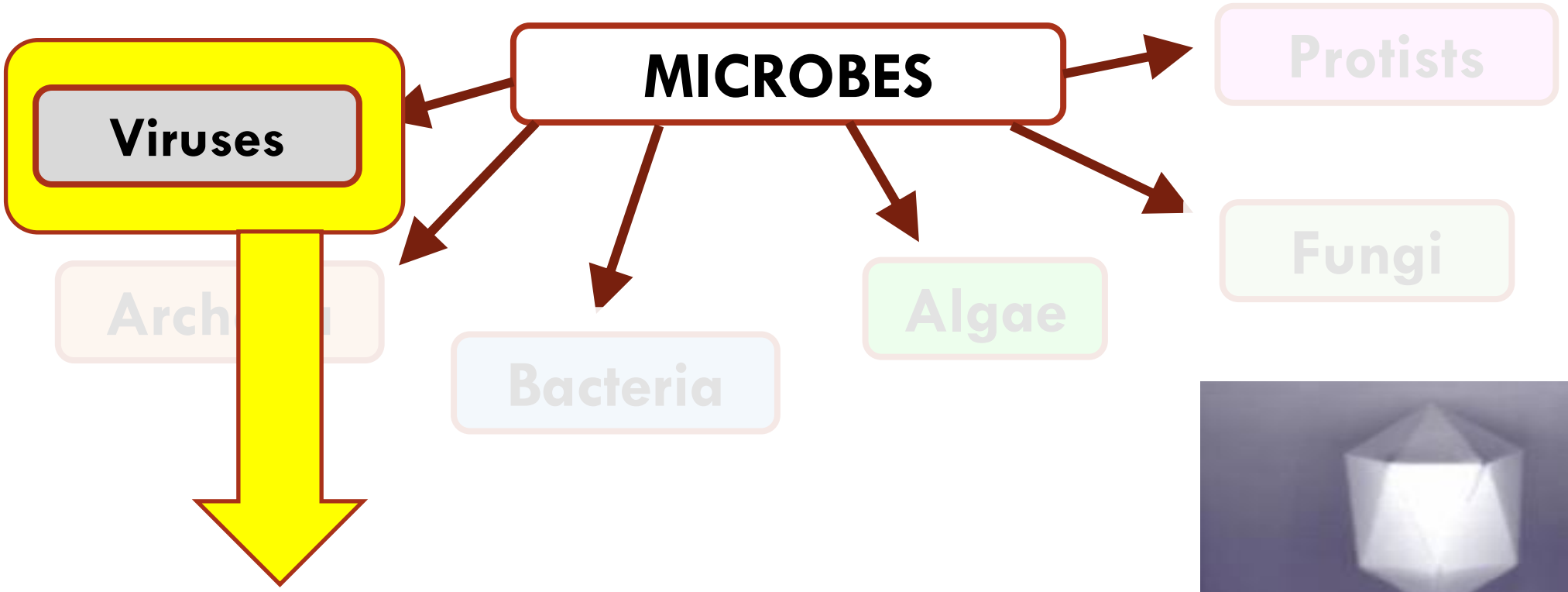
- Modern methods are "molecular biotechnology"

→ need high-level biology skills (esp. microbiology)
→ AND need understanding of DNA, RNA, proteins

…Molecular biotechnology

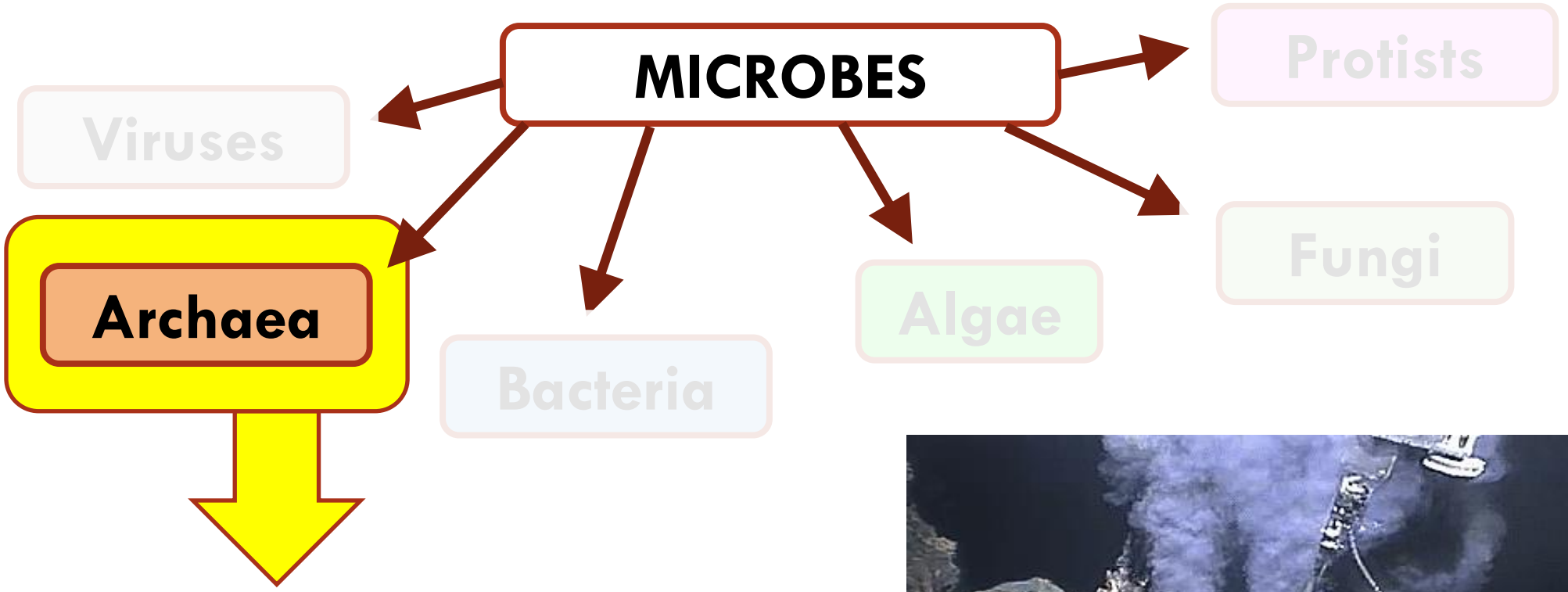
The rest of the lecture will focus on molecular biotechnology

Diverse microbes are useful for biotechnology

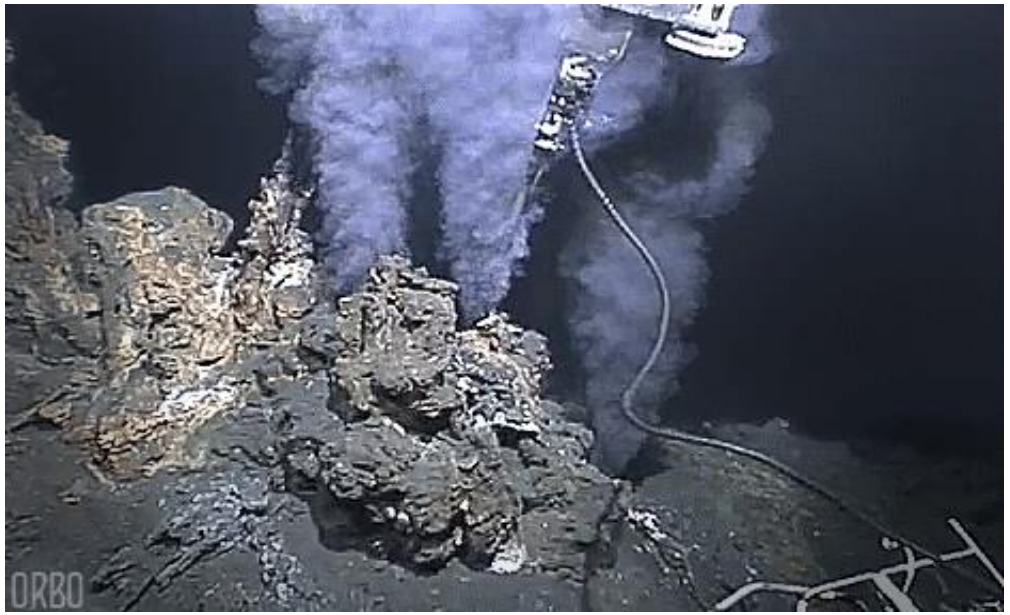


- Vectors to carry genes into new hosts
- Source of enzymes eg. T4 ligase

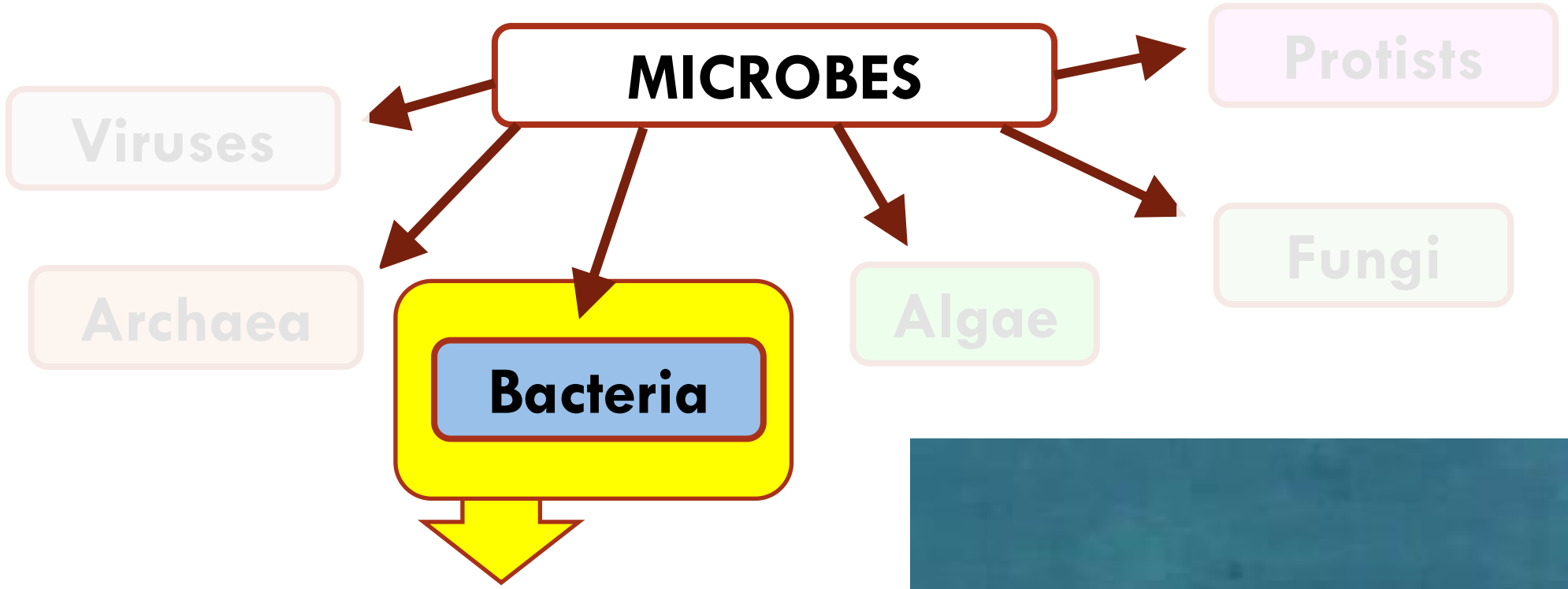
Diverse microbes are useful for biotechnology



- Source of thermostable polymerase enzymes for copying DNA sequences

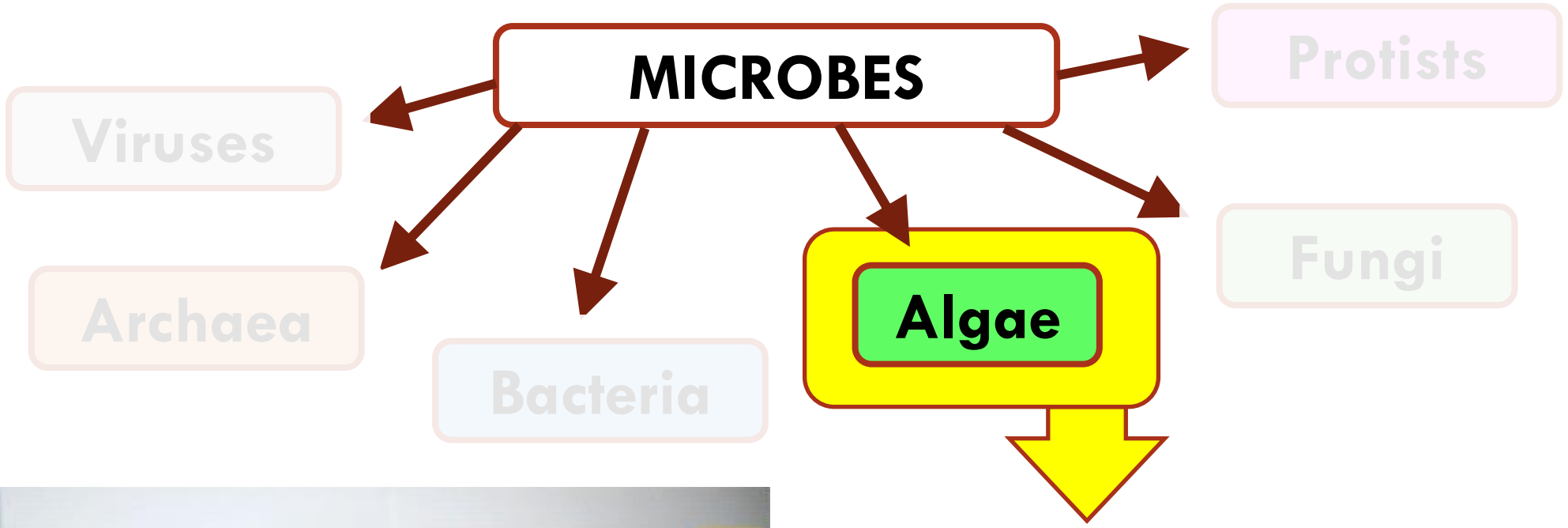


Diverse microbes are useful for biotechnology



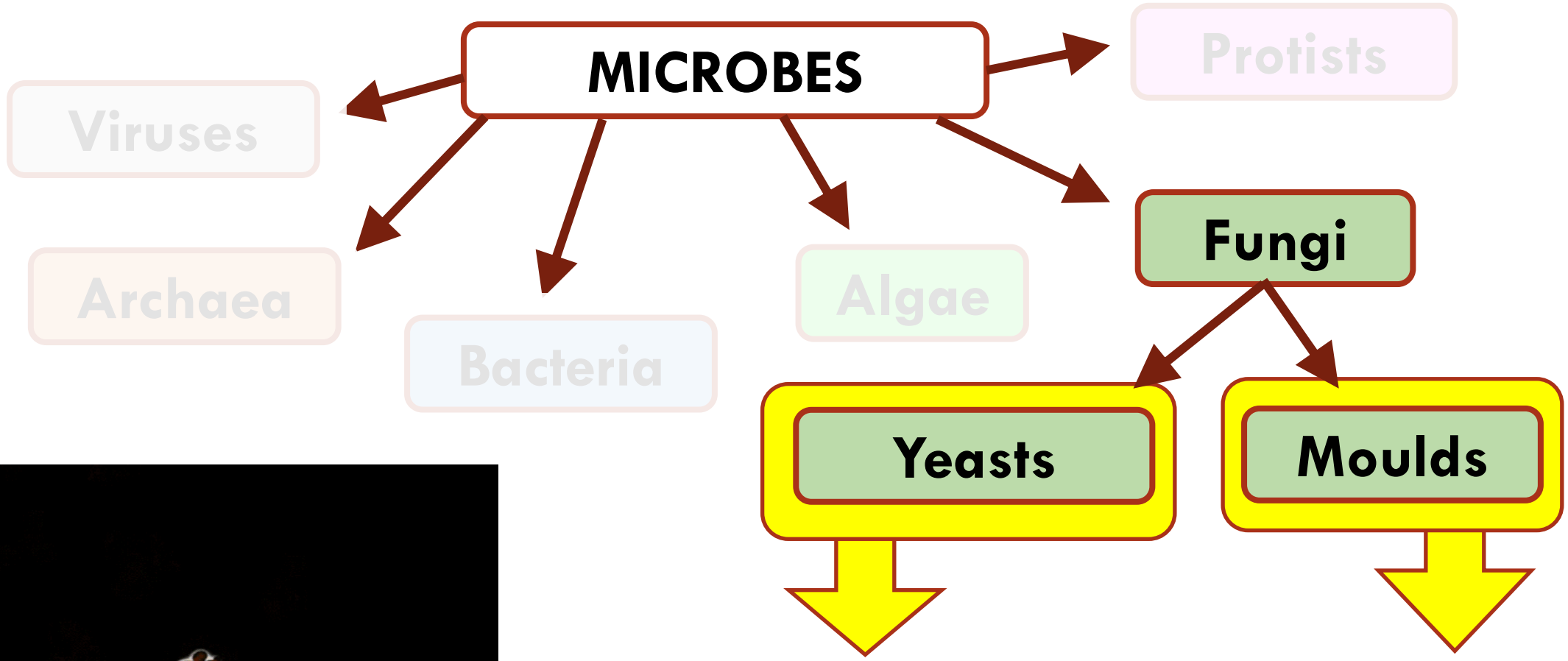
- Excellent hosts for <u>cloning</u> DNA and <u>expressing</u> proteins

Diverse microbes are useful for biotechnology



- Conversion of CO_2 + light into <u>biofuels</u> (ethanol, H_2)

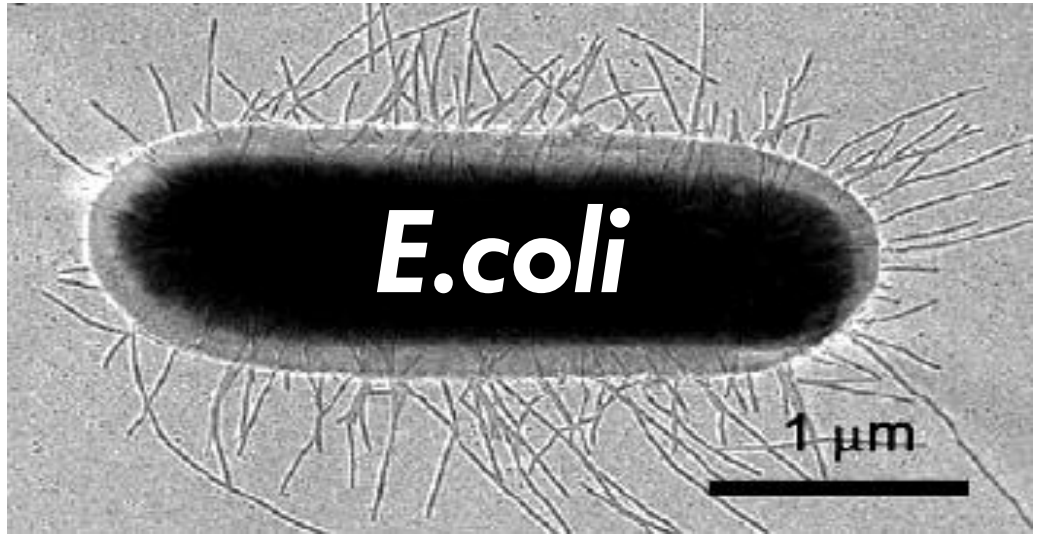
Diverse microbes are useful for biotechnology



- Excellent cloning and expression hosts
- Antibiotic synthesis

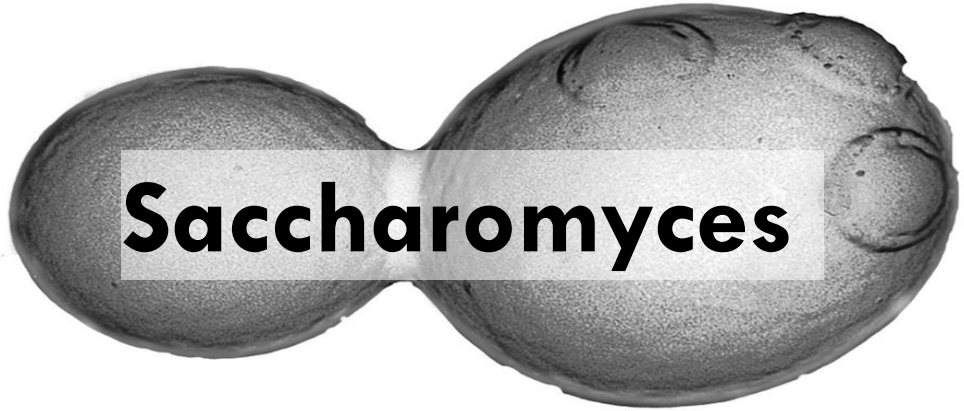
Host cells for biotechnology - the 'workhorses'

Bacteria



- Fastest growth
- Very easy to extract or add plasmid DNA

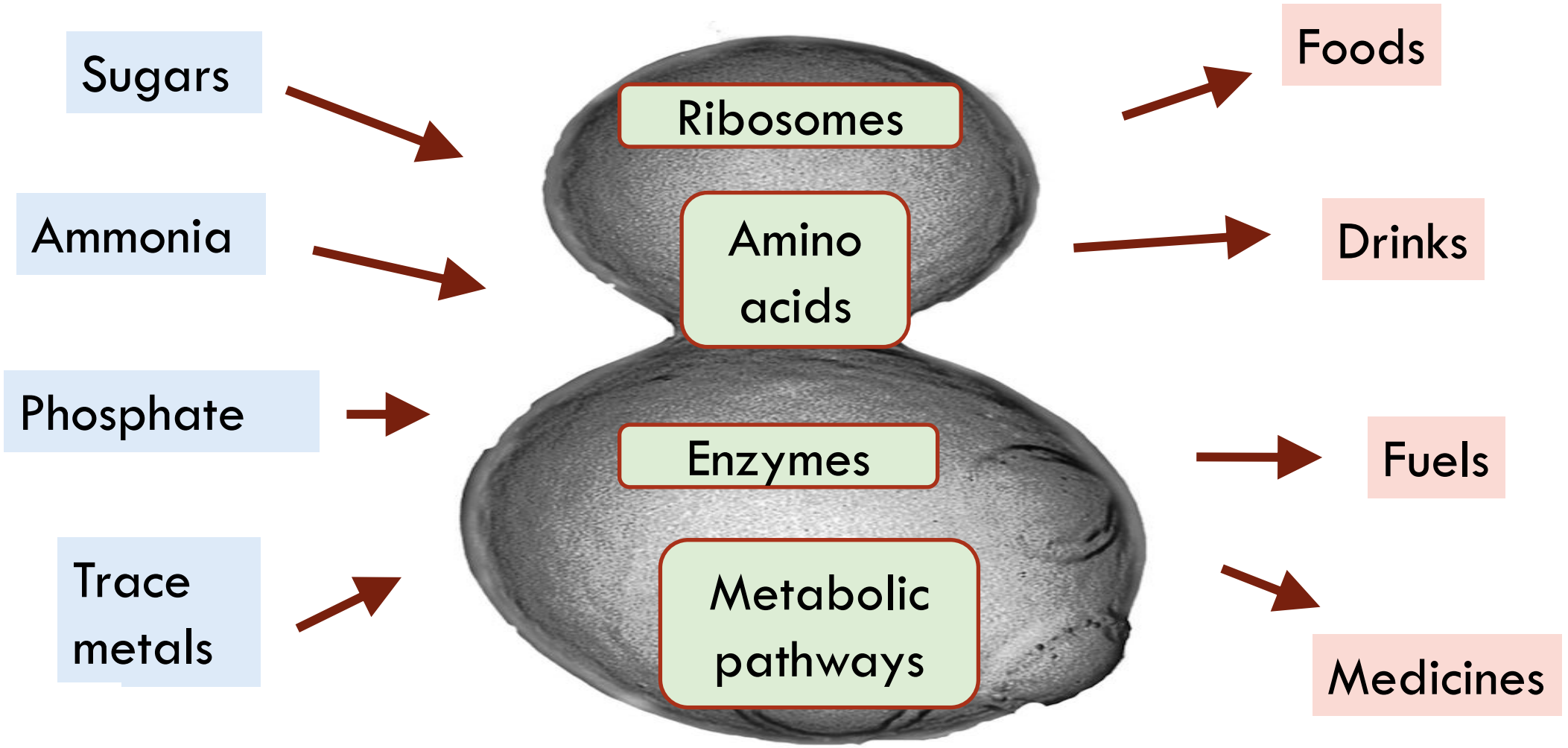
Yeast



- Better for expressing eukaryote genes
- Generally recognized as safe (GRAS)

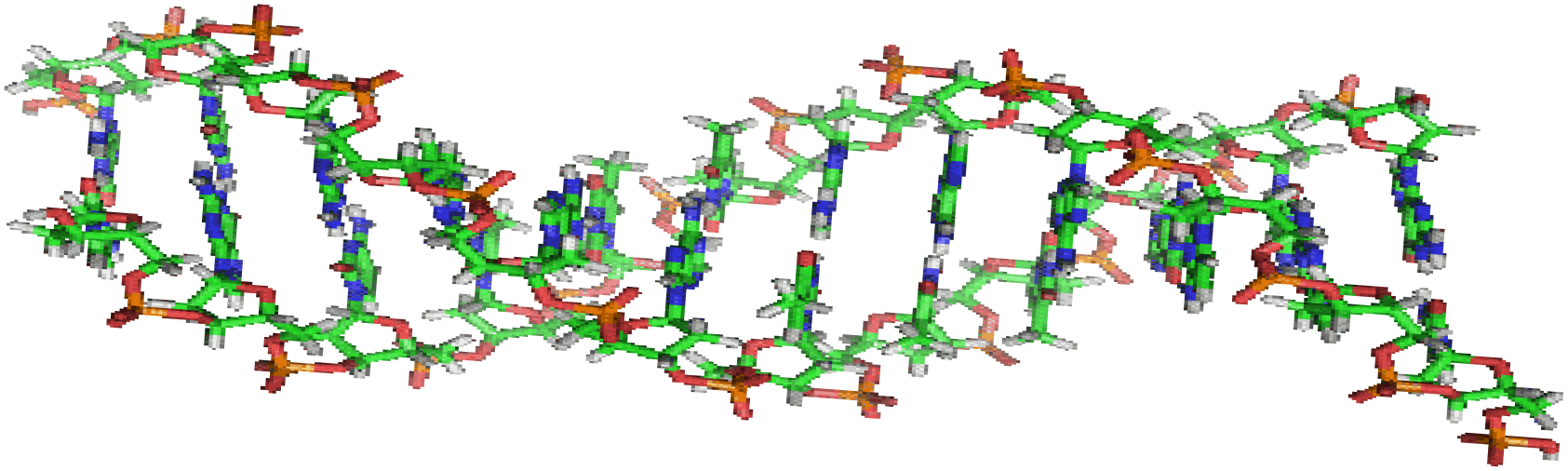
Host cells for biotechnology - what do they provide?

- The host cell contains machinery for biosynthesis of high-value products from simple raw materials



Biotech processes - what do we need to provide?

- The host cell factory needs instructions or a blueprint to tell it which products to make



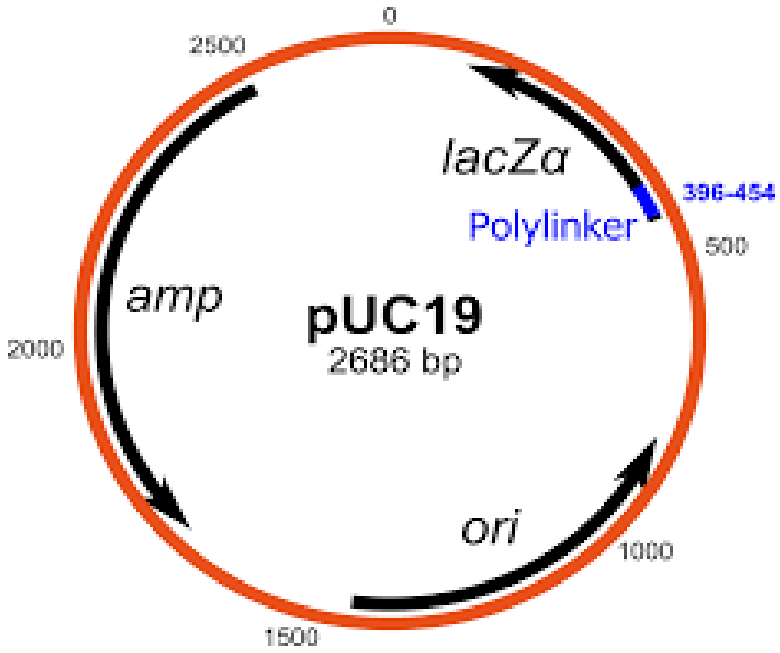
Instructions = add DNA !

How to deliver the instructions? … plasmids

- Plasmids: circular DNA elements found in microbes; replicate independently of the chromosome(s)

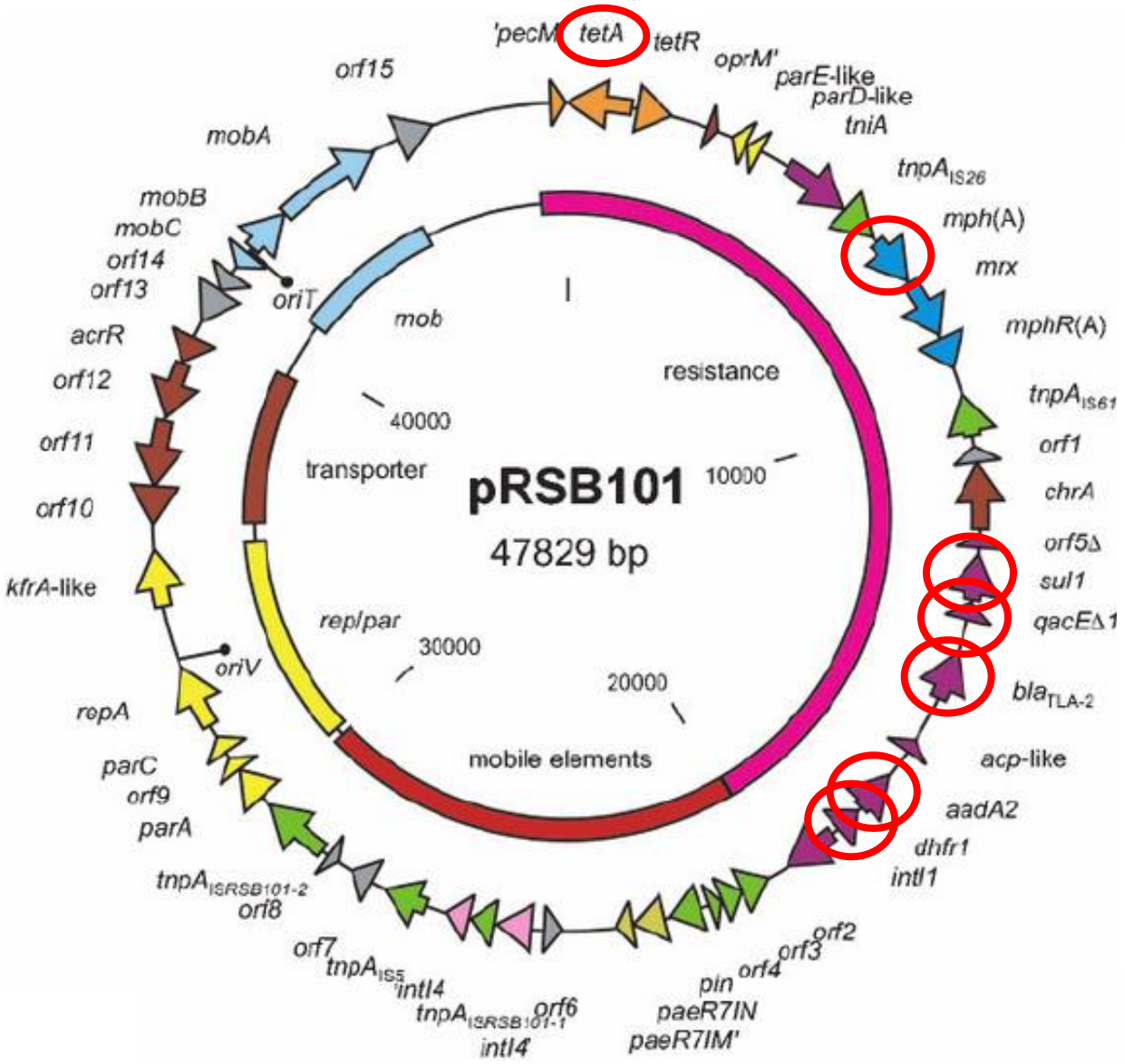
- Plasmids are the most commonly used vector for delivery of foreign DNA into a target host cell

- Viruses can also be used as vectors



Example of a 'tame' lab plasmid

Where do plasmids come from?

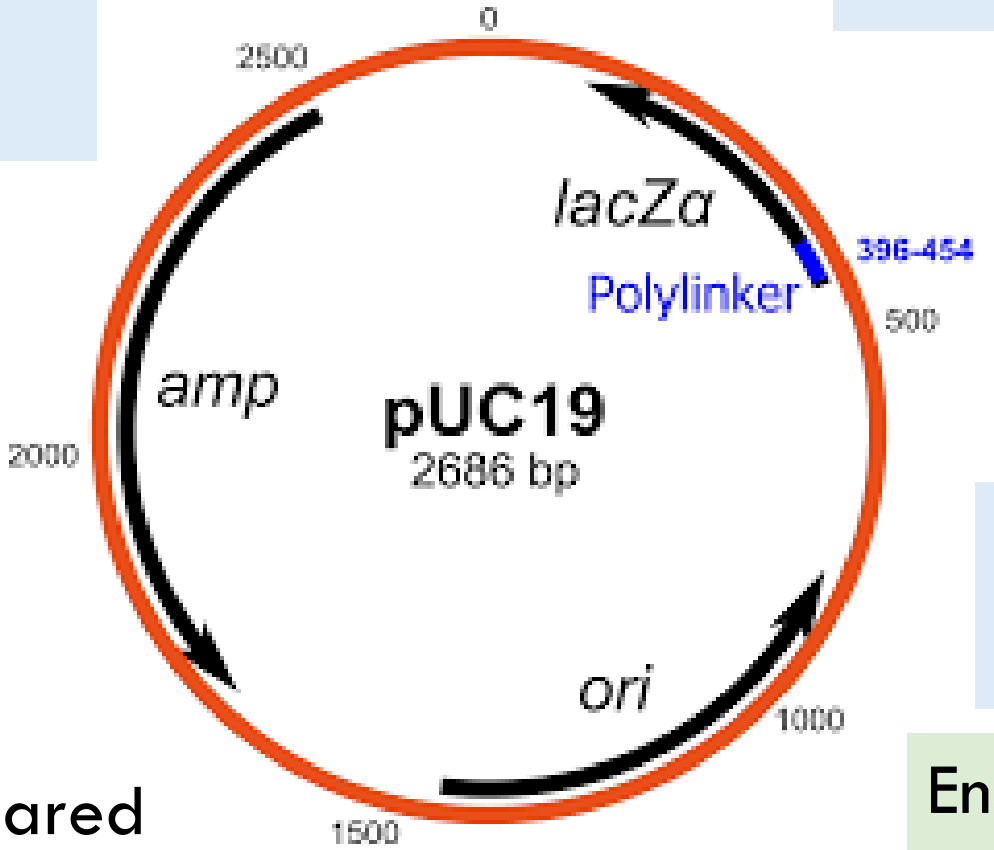


- 'Wild' plasmids found in nature allow microbes to swap useful genes
- Plasmid-borne genes are not essential; but are useful under some specific conditions
- e.g. <u>antibiotic resistance</u> genes !

Key features of plasmids used for biotech

Selectable marker

Enables us to force cells to take up plasmid



Cloning site

Add foreign genes here

Replication functions

Ensures persistence in host

- Note: simple structure compared to ‘wild’ plasmid

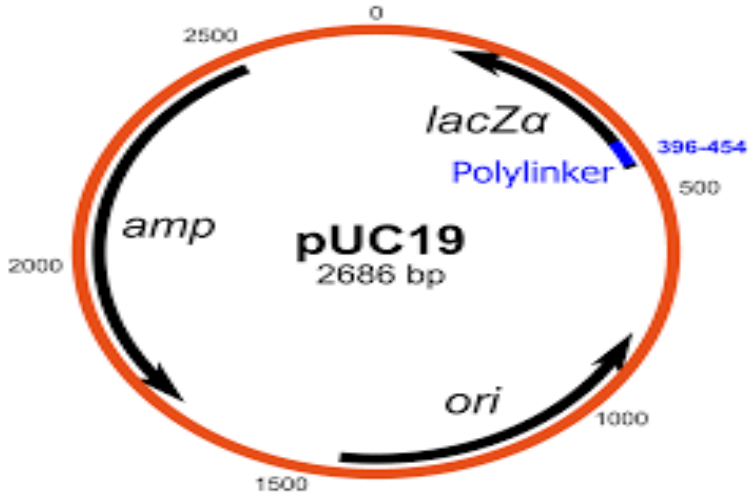
Some important definitions … "Cloning"

- Cloning: to make many copies of a biological entity

EITHER: Creating identical organisms

OR: Making copies of a piece of DNA by adding it into a plasmid, then replicating the plasmid

vs.



Tools needed for DNA cloning – Enzymes !

Copying DNA

Thermostable polymerase

Cutting DNA

Restriction enzyme

Joining DNA

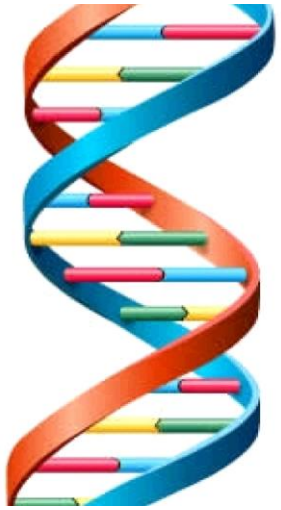
T4 ligase

DNA Cloning - Part 1. Digestion and ligation

Organism of interest

Extract DNA

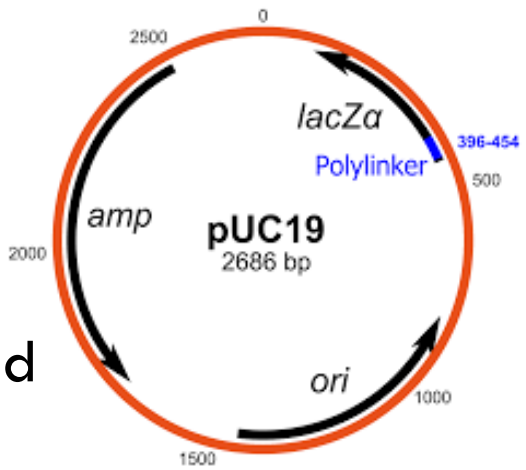
Or copy DNA



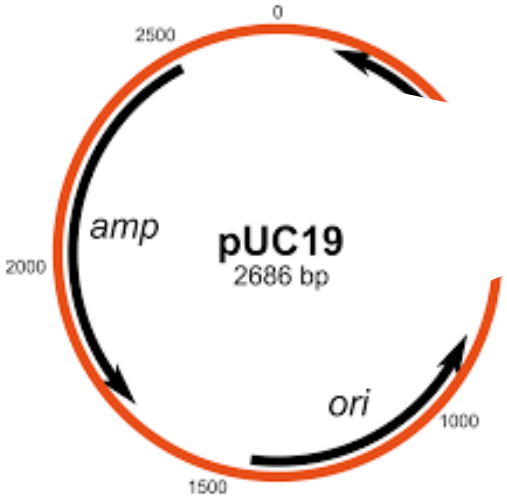
Cut DNA into pieces

'digestion'

Plasmid



Cut

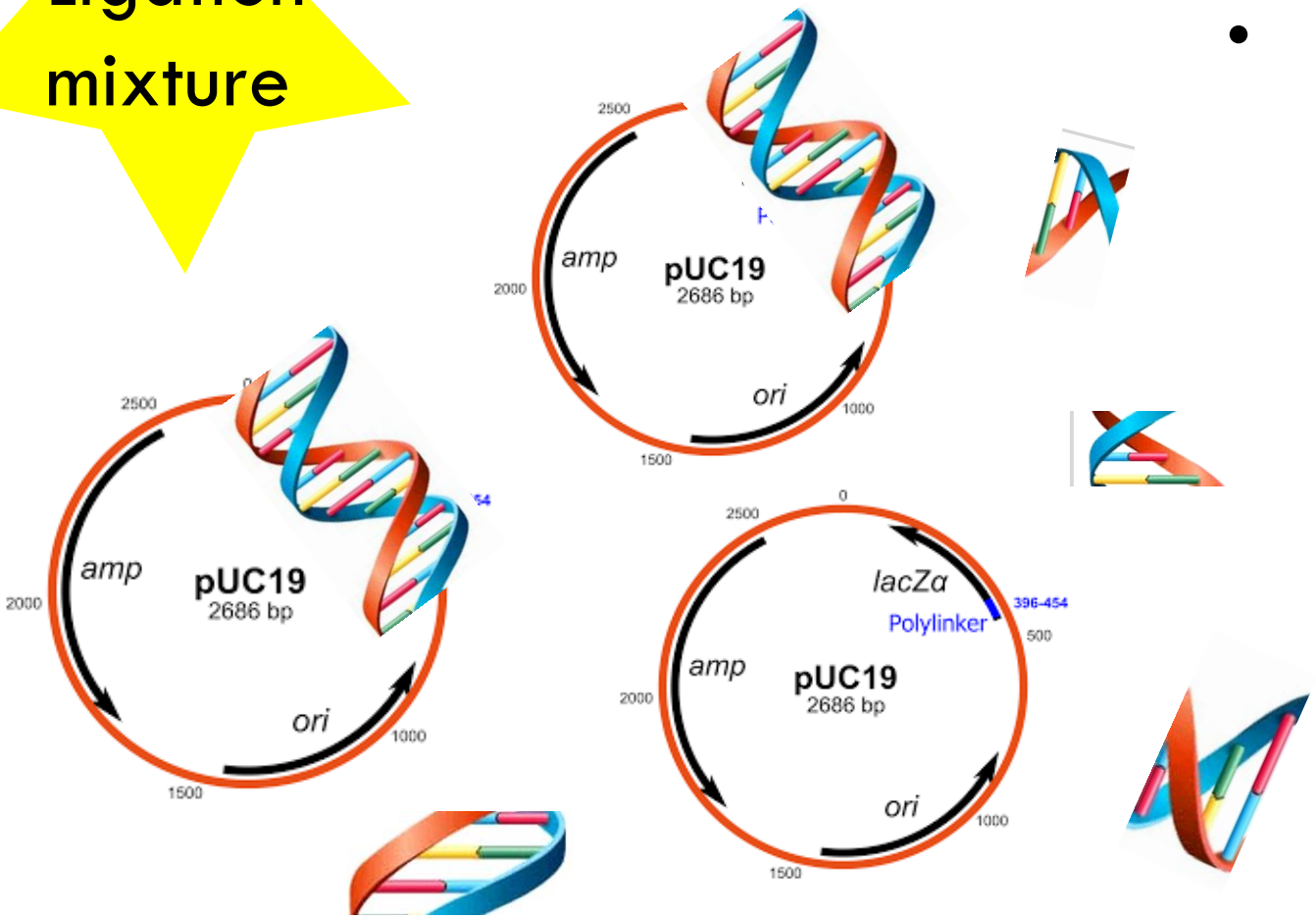


'ligation'

DNA Cloning - Part 1. Digestion and ligation

Ligation mixture

- What is actually in the ligation mixture ?



- Some plasmids that are <u>RECOMBINANT</u> (contain bits of foreign DNA)
  - The starting plasmid (non-recombinant)
  - Non-ligated bits of foreign DNA

DNA Cloning - Part 2. Transformation & Screening

Ligation mixture

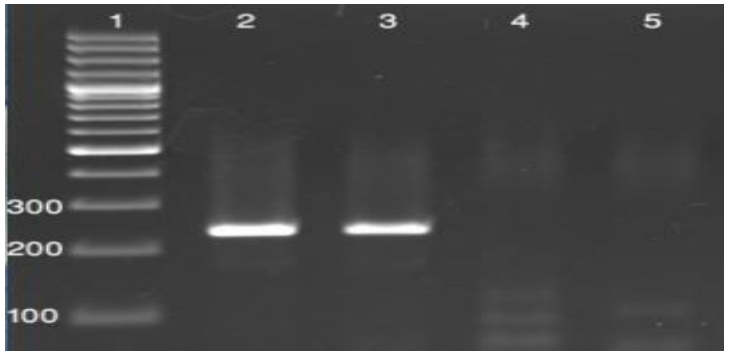
Add mix into cloning host

'Transformation' (=uptake of DNA)

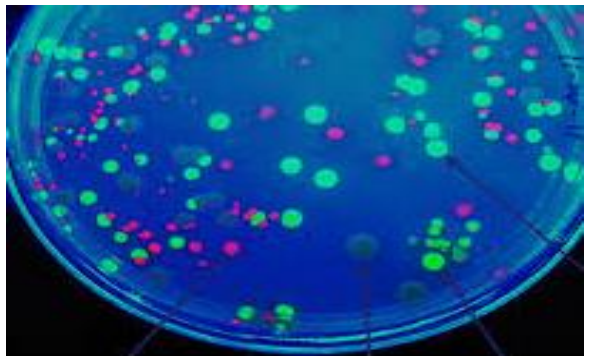
Select plasmid-containing cells

e.g. Plate on antibiotic agar

SCREENING
To find gene of interest

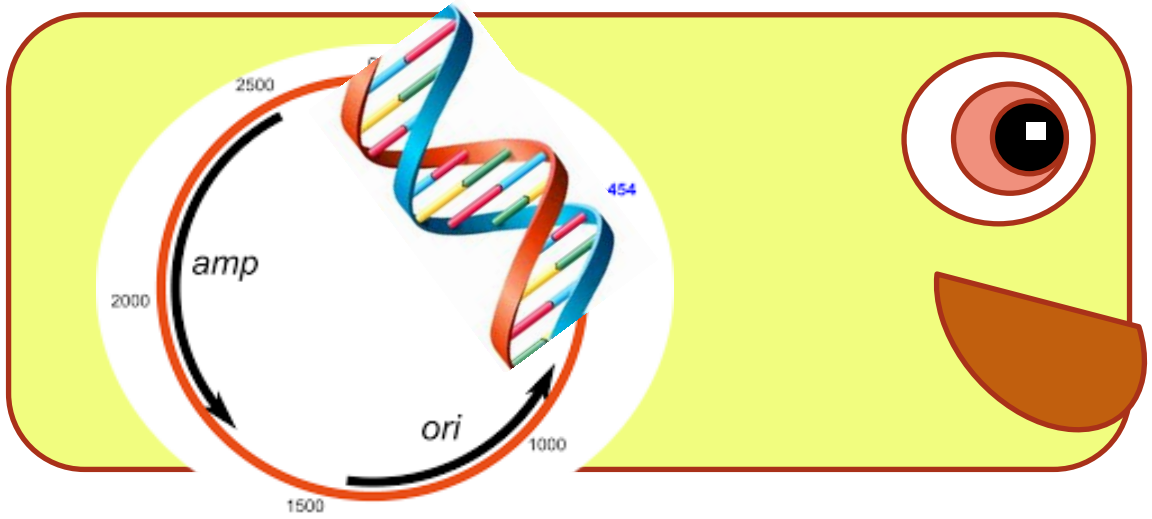


Sequence-based screen (look for DNA directly)



Phenotypic screen (look for effect of gene on host)

DNA Cloning - Part 3. The final product… a GMO

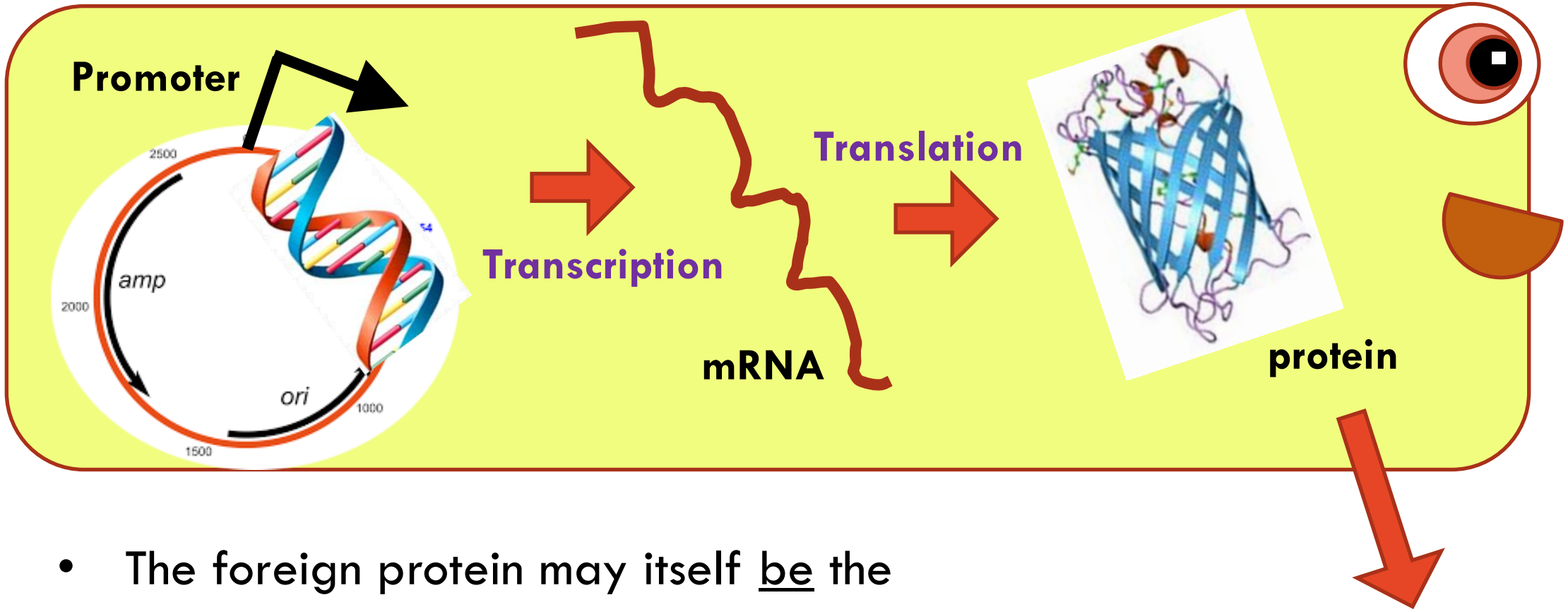


- Recombinant microbe carrying gene of interest
- This is now a GMO: a genetically-modified organism

What risks arise from GMO's ?

- Depends on what foreign genes were added
- Antibiotic resistance gene transfer into pathogens?
- Commercial risks: legal constraints, public perceptions

DNA Cloning Part 4. Expression of gene(s)



- The foreign protein may itself be the end-product (e.g. HepB vaccine) or it could make the end-product (e.g. metabolic enzymes)

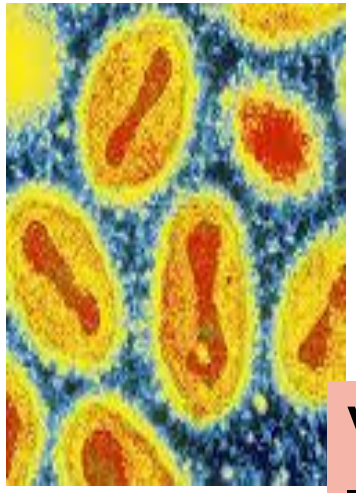
Examples of recombinant products - vaccines

- Vaccines: a primary defence against infectious disease; save approx. 3 million lives every year

- Protect against: smallpox, polio, rabies, diphtheria, pertussis, tetanus, hepatitis, measles, mumps, rubella, influenza, pneumonia, rotavirus, meningitis, papillomavirus (and more)

- Some diseases have no cure… prevention is the only option

- Vaccines lead to 'herd immunity'



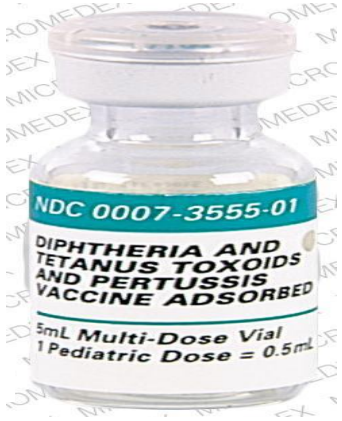
Variola virus (smallpox)
- Eradicated by vaccination

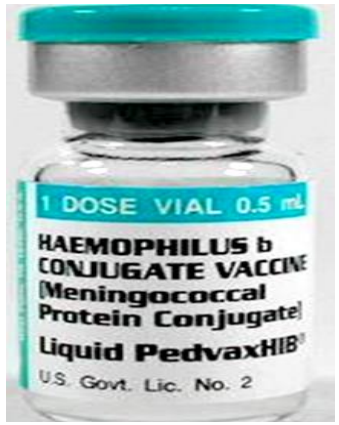
http://www.who.int/mediacentre/factsheets/fs378/en/

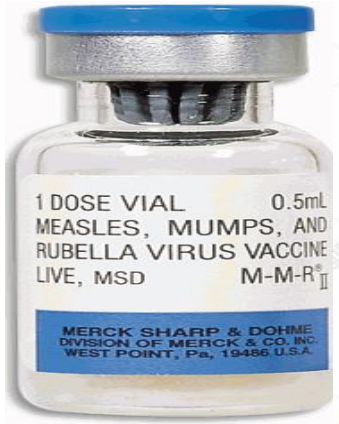
Examples of recombinant products - vaccines

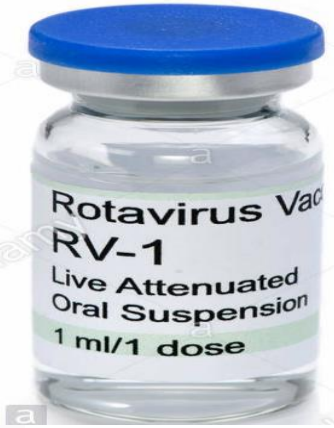
- Vaccines work by 'training' the immune system to recognise antigens associated with an invader. They may consist of:

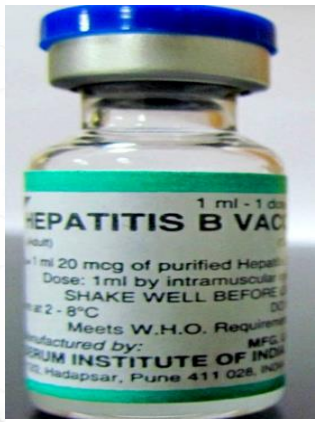
1. live attenuated microbes
2. killed microbes
3. antigens (proteins) produced in a GMO host
4. mRNA coding for antigens



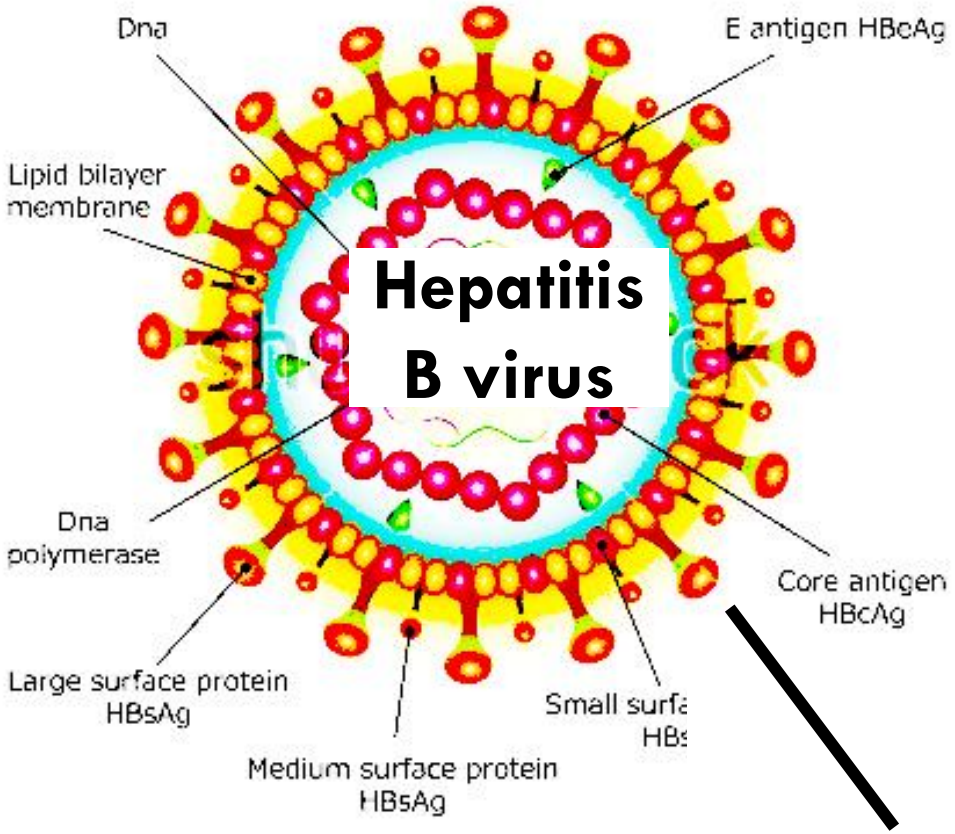








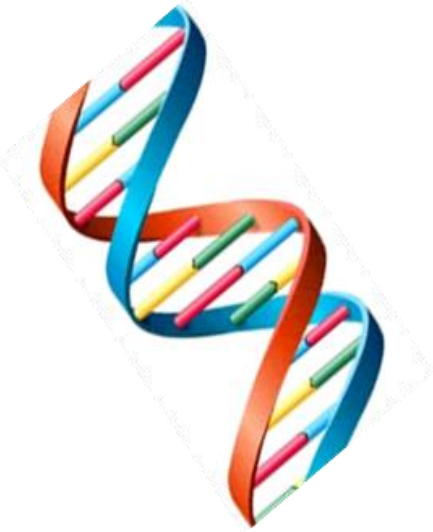
Hepatitis B vaccine 1. Isolate gene coding for antigen



Surface protein antigen HBsAg

Selectively copy DNA

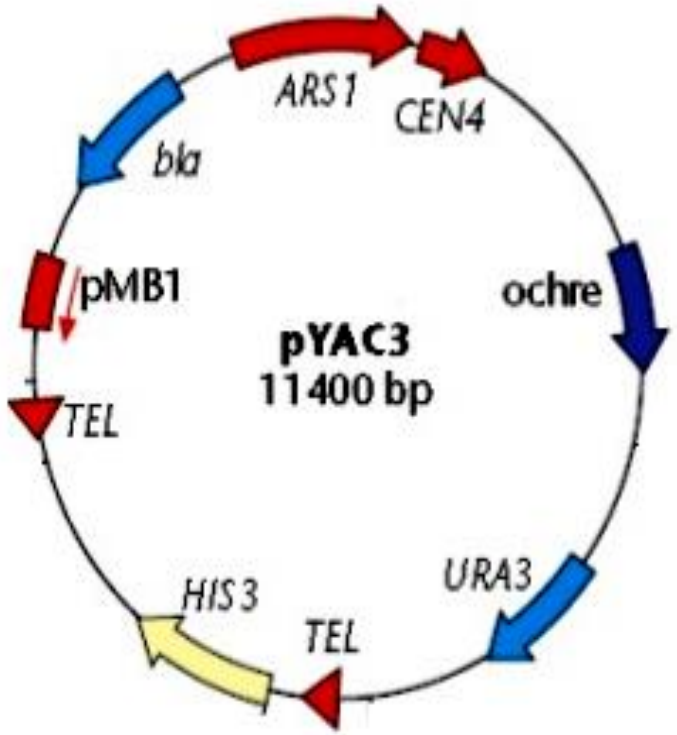
thermostable polymerase



HBsAg gene

Hepatitis B vaccine 2. Cloning antigen gene

Yeast plasmid



HBsAg gene

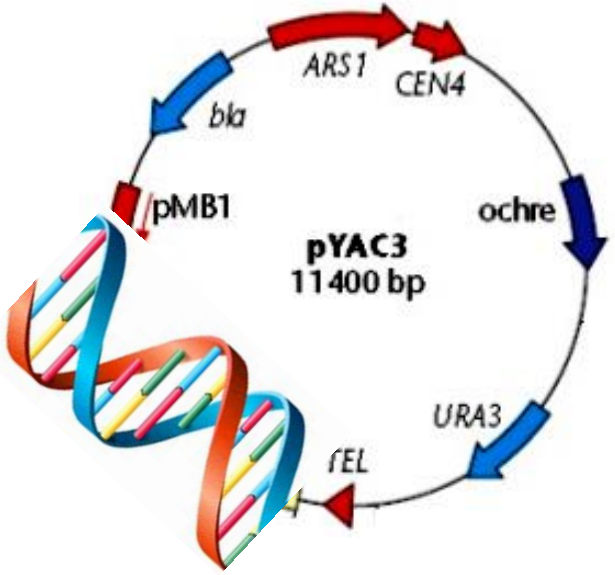
digestion

digestion

ligation

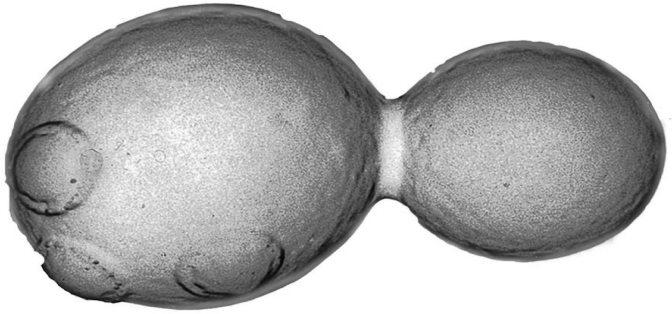
Recombinant plasmid

Hepatitis B vaccine 3. Transformation into yeast

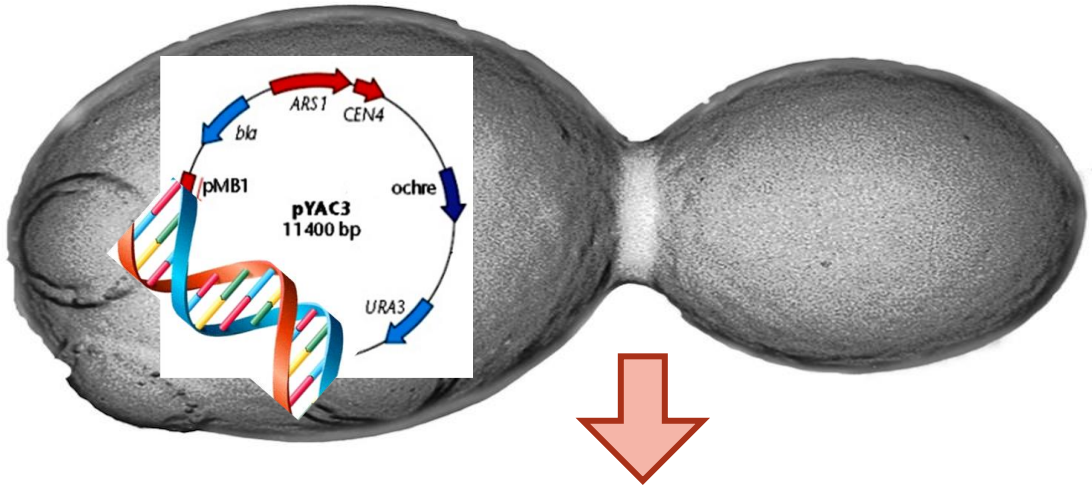


Recombinant yeast plasmid

Transformation



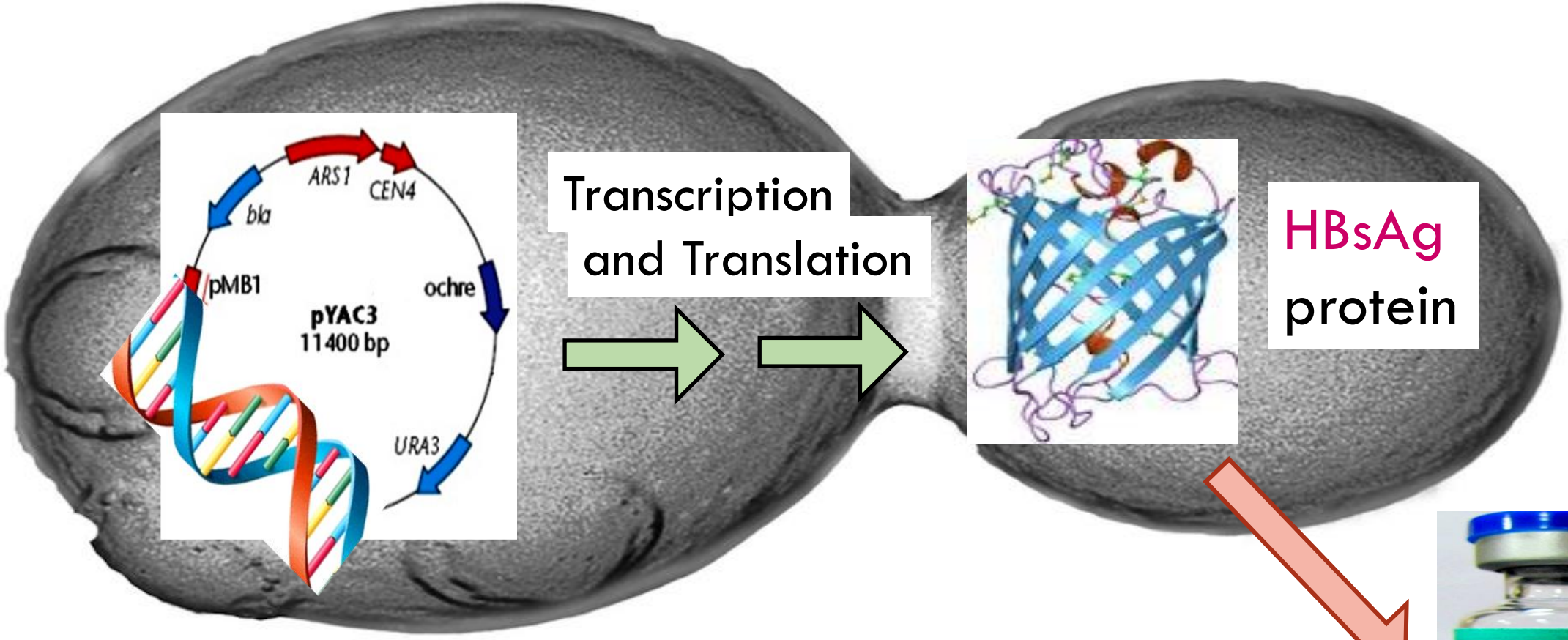
Yeast cloning host

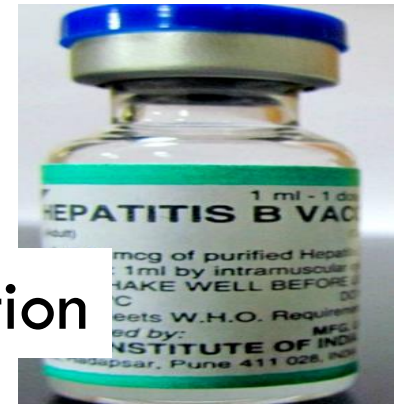


GMO yeast

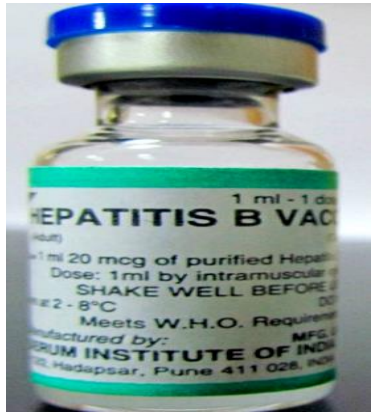
Hepatitis B vaccine

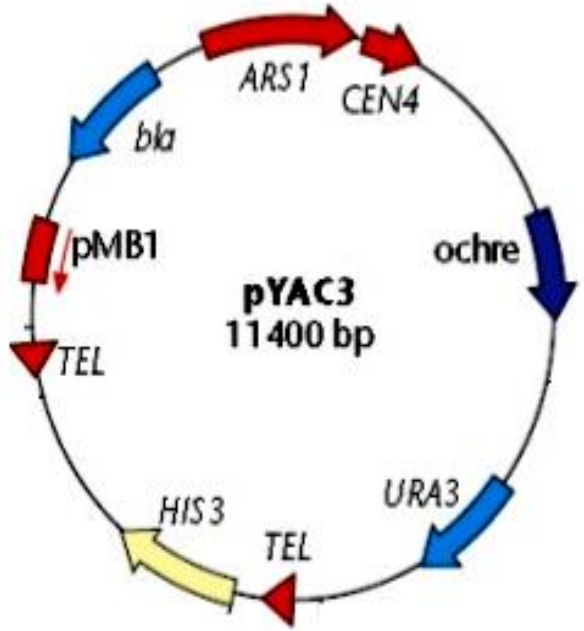
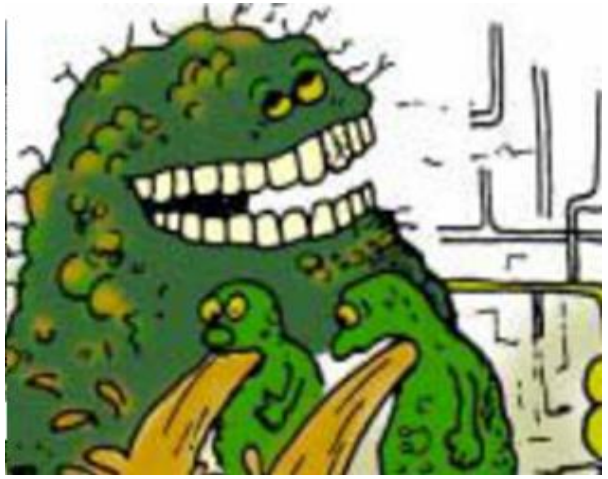
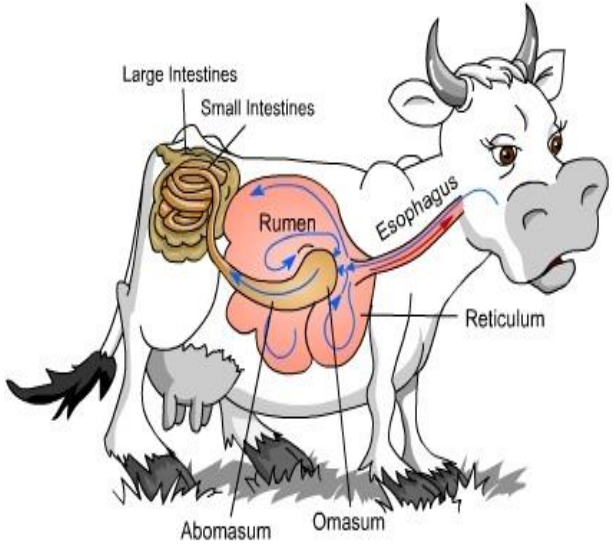
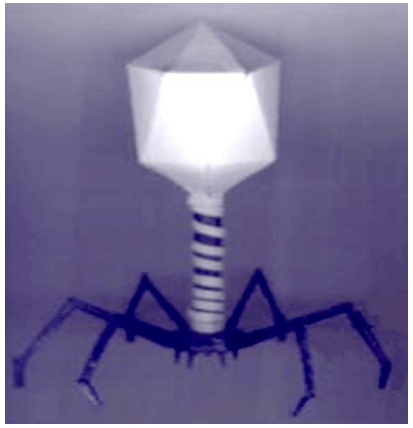
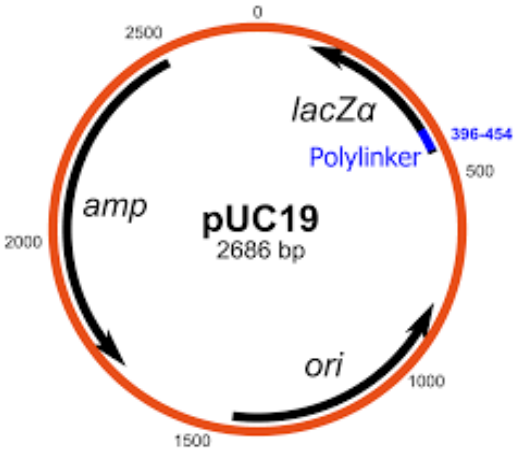
4. Gene Expression, Protein purification





Yeast! Nature's gift that keeps on giving…





Microbiology – small things make a big difference !