

Pharmaceutical Protein Production (II)

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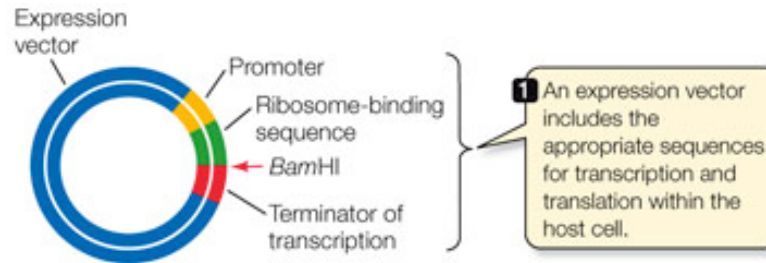
Objectives of this lecture

By the end of this lecture you will be able to:

1. Recognize different expression systems
2. Choose proper vehicle for recombinant protein production
3. Identify considerations for small-scale production

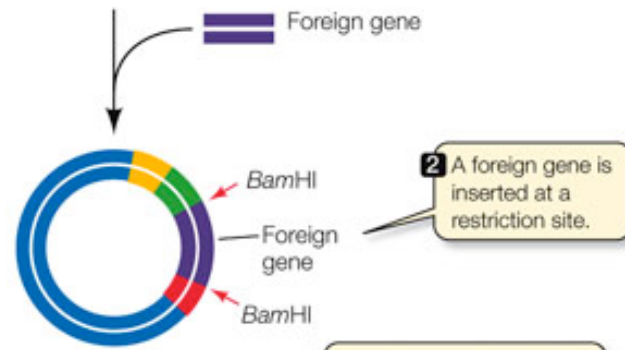
Requirements for recombinant protein production

Vector



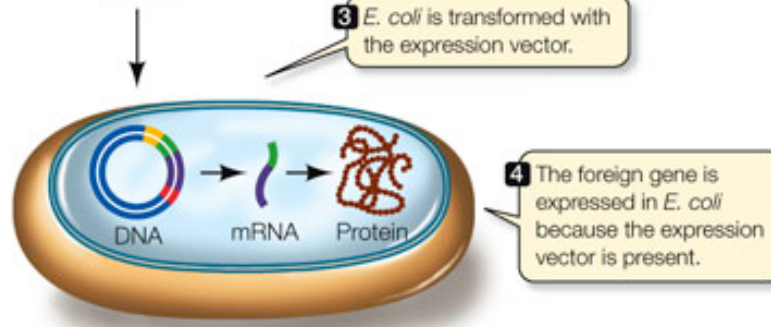
Plasmid

Gene

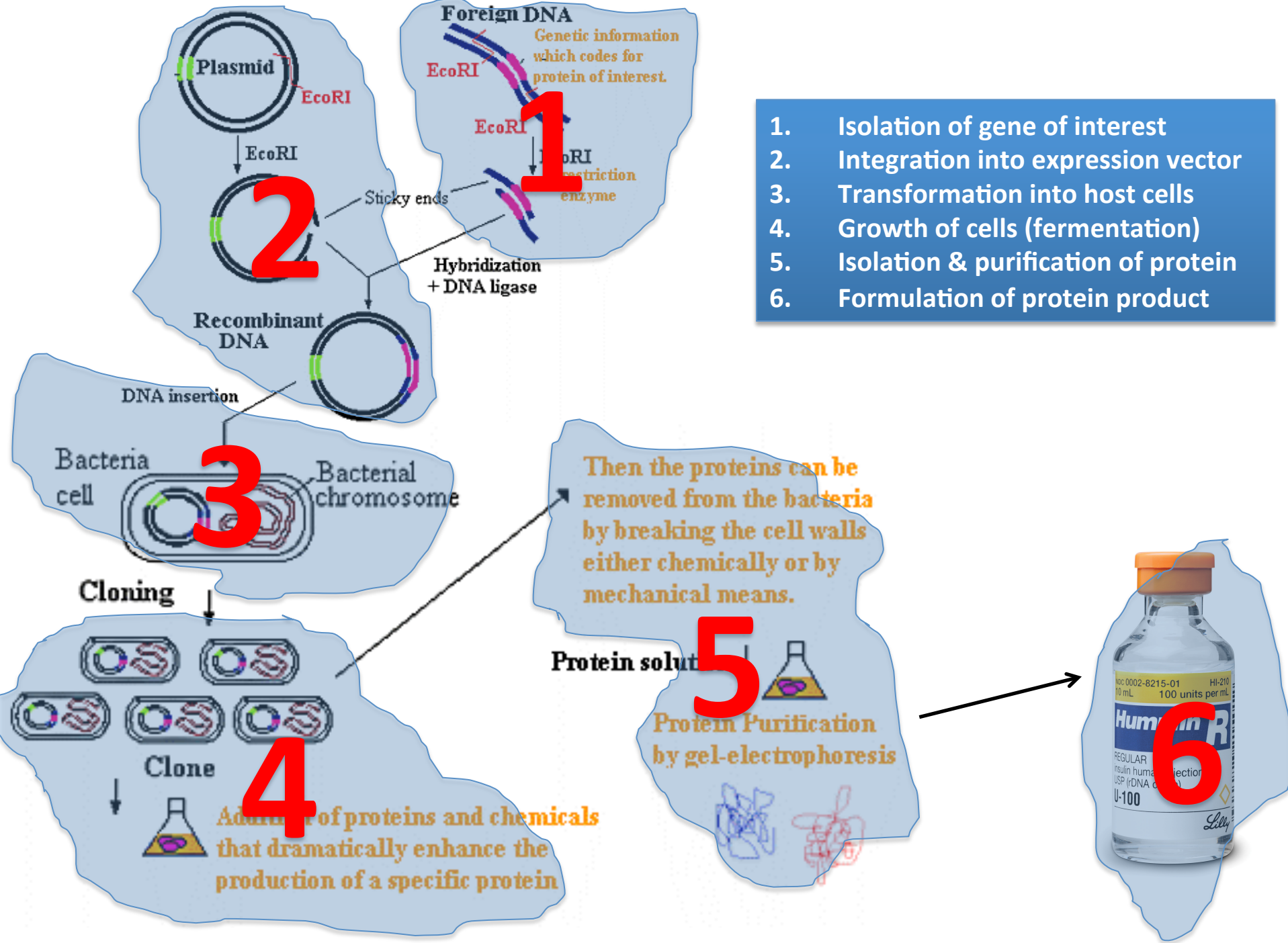


Insulin

Host

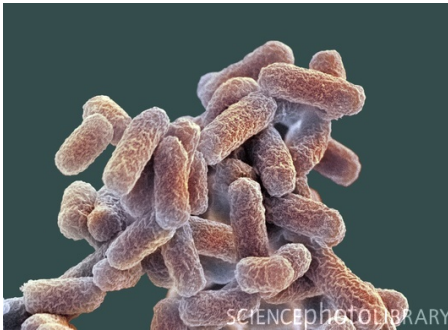


E. coli



Choice of production vehicle

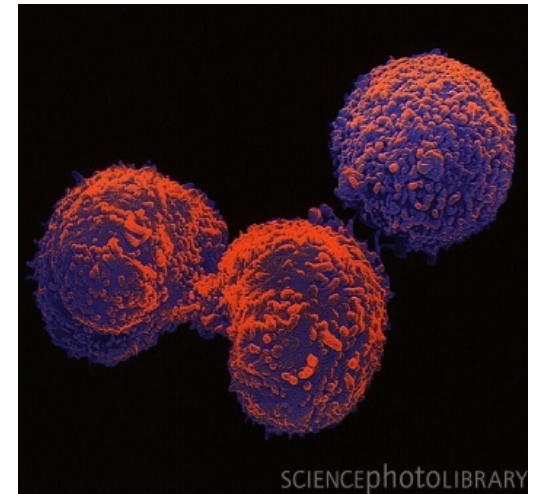
- In principle, any protein can be produced using any genetically engineered organism



Bacteria



Yeast



Mammalian

Recombinant Protein Expression Systems

- *Escherichia coli* and other bacteria
- *Pichia pastoris* and other yeasts
- Baculovirus
- Mammalian cell culture
- Plants
- Animals
- Cell free

Choice of Expression System

- Choice depends on size and character of protein
 - Large proteins (>100 kD)? Choose **eukaryote**
 - Small proteins (<30 kD)? Choose **prokaryote**
 - Glycosylation essential? Choose **baculovirus** or **mammalian cell culture**
 - High yields, low cost? Choose ***E. coli***
 - Post-translational modifications essential? Choose **yeast, baculovirus** or other **eukaryote**

Choice of Expression System

- Not every type of protein can be produced by any cell type

Protein Feature	Prokaryotic	Eukaryotic	Eukaryotic
	Bacteria	Yeast	Mammalian
Concentration	High	High	Low
Molecular weight	Low	High	High
Secretion	No	Yes/No	Yes
Folding	Incorrect	Correct	Correct
Glycosylation	No	Incorrect	Correct
Retrovirus	No	No	Possible
Contamination	Endotoxin	Low risk	Viruses
Production Quality	Low	Moderate	High
Scale-Up Capacity	High	High	Low

Cultivation Method

- The culture method will determine the separation and purification methods.
- For research (small scale):
 - Cell culture flasks
- For productions (large scale)
 - Fermentors and Bioreactors



Growth of Microorganisms

- Culture medium
- Oxygen
- Temperature control



Culture Media

- It is important to provide nutritional conditions that exist in bacterial natural habitat.
- **Common components:**
 - Water
 - Source of carbon and energy
 - Source of nitrogen
 - Trace elements
 - Growth factors
 - Buffer



Terrific Broth

- **Formulation per one liter:**

- 12 g Peptone (peptic digest of casein) or Tryptone (tryptic digest of casein)
- 24 g Yeast Extract
- 9.4 g dipotassium hydrogen phosphate
- 2.2 g potassium dihydrogen phosphate
- 4 g Glycerol
- Water
- Phenol Red



Common Media

- **Commonly used bacterial *E.coli* culture media:**
- **LB (Luria Bertani) Miller broth (1%NaCl):** 1% peptone, 0.5% yeast extract, and 1% NaCl
- **LB (Luria Bertani) Lennox Broth (0.5% NaCl):** 1% peptone, 0.5% yeast extract, and 0.5% NaCl
- **SOB medium (Super Optimal Broth):** 2% peptone, 0.5% Yeast extract, 10mM NaCl, 2.5mM KCl, 10mM MgCl₂ , 10mM MgSO₄
- **SOC medium (Super Optimal broth with Catabolic repressor):** SOB + 20mM glucose
- **2x YT broth (2x Yeast extract and Tryptone):** 1.6% peptone, 1% yeast extract, and 0.5% NaCl
- **TB (Terrific Broth) medium:** 1.2% peptone, 2.4% yeast extract, 72 mM K₂HPO₄, 17 mM KH₂PO₄ and 0.4% glycerol
- **SB (Super Broth) medium:** 3.2% peptone, 2% yeast extract, and 0.5% NaCl

Choice of Medium

Medium Name	Applications
LB Miller Broth	<i>E.coli</i> growth, culture and propagation; plasmid DNA and protein production
LB Lennox Broth	<i>E.coli</i> growth, culture and propagation; plasmid DNA and protein production
SOB Medium	Prepare high efficiency competent cells; plasmid DNA and protein production
SOC Medium	Plasmid transformation and growth of competent cells
2x YT Medium	Phage DNA production
TB	High yield protein and plasmid DNA production
SB	High yield plasmid DNA and protein production

Fetal Bovine Serum (Fetal Calf Serum)

- Fetal bovine serum comes from the blood drawn from a bovine fetus via a closed system of collection at the slaughterhouse.



Fetal Bovine Serum (Fetal Calf Serum)

- **Advantage:**

- Nutrition

- **Disadvantages:**

- Contaminating proteins
- Variability among animals
- Microbial contamination (viruses / bacteria / mycoplasma / fungi)
- Endotoxin (even with sterile serum)

- **Serum-free media are preferable in large scale production**

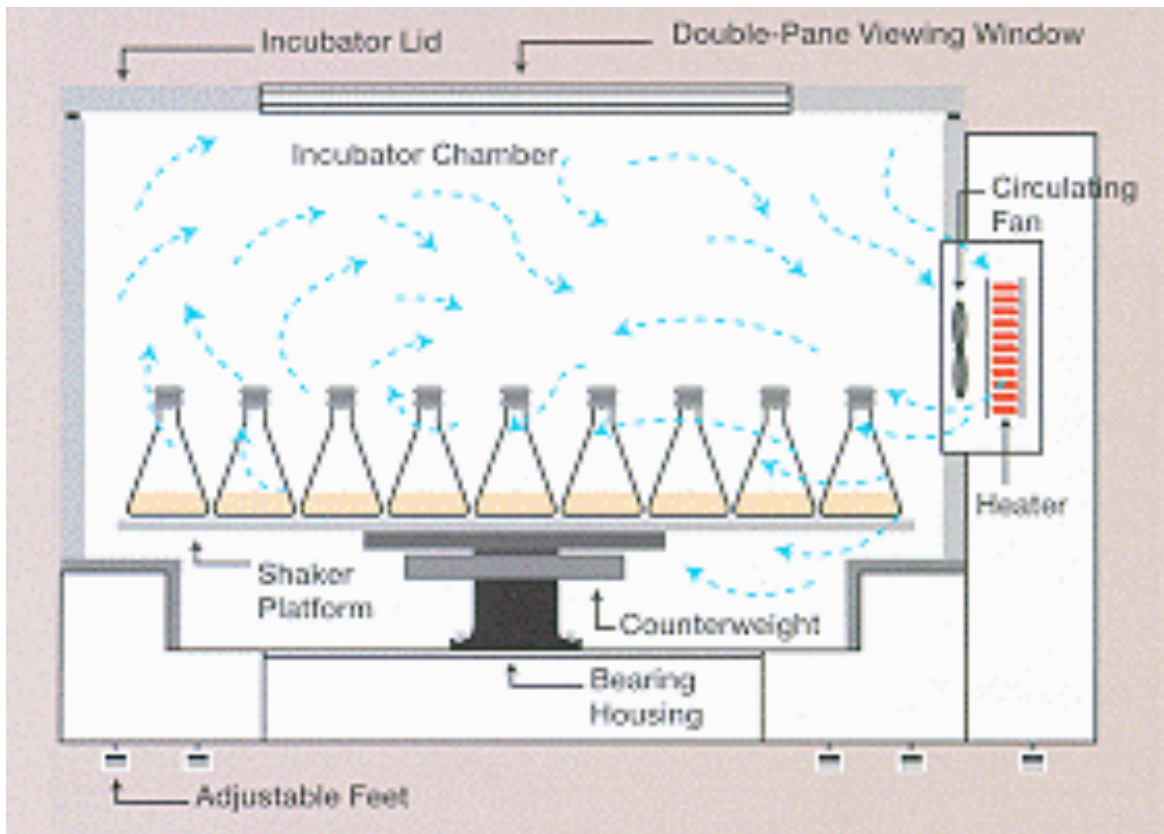
Contaminants

- Contamination does not always mean “dirtiness” but it may refer to the presence of unwanted materials
- **Contaminants can be:**
 - Host related
 - Product related
 - Process related

Contaminants

Host Related	Product Related	Process Related
Viruses, Bacteria	Aminoacid substitution or deletion	Growth medium components
Host-derived proteins and DNA	Denatured protein	Purification reagents
Glycosylation variants	Conformational isomer	Metals
N- and C-terminal variants	Dimers and aggregates	Column materials
Endotoxin	Disulfied pairing variants	
	Deamidated species	
	Protein fragments	

Shake Flask Incubator



Shaking increases oxygen transfer

Growth of Cells

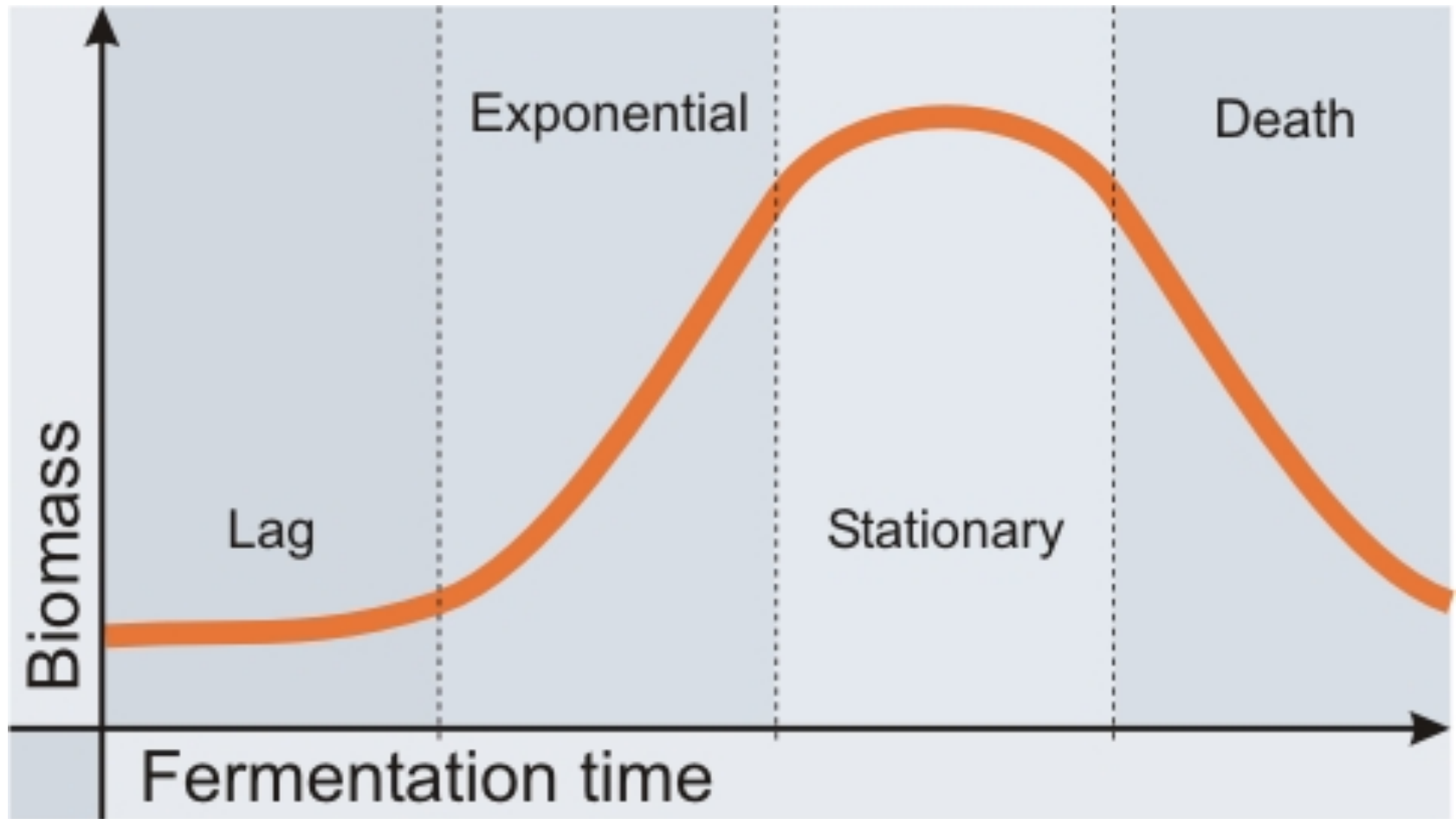


Shake Flask Incubator

Shake Flask Incubator

- Heavily insulated, heated with thermoregulation to keep temperature within 0.5 °C
- Rotatable platform to spin up to 500 rpm to facilitate aeration (dissolves N₂ and O₂ needed for growth)
- Designed for small-scale growth

Microorganisms Growth Curve



Now you are able to:

- ✓ Recognize different expression systems
- ✓ Choose proper vehicle for recombinant protein production
- ✓ Identify considerations for small-scale production