Bacteria

Binary Fission	<u>Describe the process of binary fission.</u> Binary fission is the process by which bacteria replicate **asexually**. DNA replication begins at the **origin of replication** which is made up of a **specific sequence** of nucleotide bases. The DNA double helix **separates** and forms a **replication bubble** that is made up of two single stranded DNA. Replication takes place outwards from the origin in both directions with two replication forks. **DNA polymerase** adds free nucleotides via **complementary base pairing** between the **template** strand and **free deoxyribonucleoside triphosphates** where **adenine** forms **two hydrogen bonds** with **thymine** and **guanine** forms **three hydrogen bonds** with **cytosine**. The new DNA strand is synthesized in the **5' to 3**. One of the daughter strands known as the **leading strand** is synthesized **continuously** towards replication fork in the 5' to 3' direction. The other strand known as the **lagging strand** is synthesized **discontinuously** away from the replication fork, giving rise to **Okazaki fragments**. . **DNA ligase** catalyzes the formation of **phosphodiester bonds** between the **Okazaki fragments**, **sealing the nicks**. As the chromosome replicates, the two newly formed **origins of replication** move to **opposite poles** of the cell and **attach** to the **plasma membrane**. The cell also **elongates** to prepare for division. With the completion of replication, an **interlocking structure** is made up of the two daughter DNA molecules as the bacterial chromosome is **circular** with no free ends. **Topoisomerase cuts**, **separates** and **reseals** the two DNA molecules. **Invagination** of the **plasma membrane** and the deposition of new cell wall divide the parent cell into **two genetically identical daughter cells**.

DNA Transfer	Transformation		Fragments of **foreign naked DNA** from **lysed** bacterial cells in the surrounding medium are **taken up** by a **competent** bacterial cell via **surface proteins**. The foreign DNA is **incorporated** into the **bacterial chromosome**, **replacing the homologous region** via **homologous recombination**, resulting in a **recombinant** cell and possibly a **different allele** expressed in the bacterial cell.
	Transduction	Generalized Transduction	A **phage** infects a **bacterium**, injecting its viral genome into the host cell and undergoes the **lytic cycle**. The bacterial chromosome is **hydrolyzed** into small fragments, one of which may be **randomly packaged** into a **capsid head** during the **assembly** of new viruses. Upon cell **lysis**, the defective phage will infect **another bacterium** and inject **bacterial DNA** from the previous host cell into the new bacterium. The foreign bacterial DNA can **replace the homologous region** in the recipient cell's chromosome if **homologous recombination** takes place, possibly allowing the expression of a **different allele** from the previous host.
		Specialized Transduction	A **temperate phage** infects a **bacterium**, injecting its viral genome into the host cell. The **viral DNA** is **integrated** into bacterial chromosome forming a **prophage** which may be **improperly excised** to include **adjacent segments** of **bacterial DNA** during an **induction** event. The bacterial DNA may be packaged into a **capsid head** during the **assembly** of new viruses. Upon cell **lysis**, the defective phage will infect **another bacterium** and inject **bacterial DNA** from the previous host cell into the new bacterium. The foreign bacterial DNA can **replace the homologous region** in the recipient cell's chromosome if **homologous recombination** takes place, possibly allowing the expression of a **different allele** from the previous host.
	Conjugation		The **sex pilus** of a **F^+ bacterial cell** makes contact with a F^- cell and **retracts** to bring the F^- cell closer so a **mating bridge** is formed between the two cells. One of the two strands of the **F plasmid** DNA in the F^+ cell is **nicked** and transferred from the F^+ cell to the F- cell through the **mating bridge** via the **rolling circle mechanism** as the other DNA strand is used as a **template** for elongation. The **single stranded** F plasmid DNA **circularizes** in the F^- cell and is used as a **template** to **synthesize** a **complementary strand**, producing a **double-stranded** F plasmid, resulting in the recipient cell becoming a **F^+ cell**.

Regulation of Gene Expression Lac Operon	Inducible Operon	An **inducible operon** catalyzes **catabolic processes** which involve the breaking down of substances. The lac operon is an inducible operon since when the **inducer allolactose** binds to the **repressor**, the repressor is **inactivated** and **does not bind** to the **operator**, allowing **transcription** of the **structural genes** to take place.
	Structure	The **lacI** gene is the **regulatory gene** located **upstream** of the operon with its own promoter and terminator sequences. It codes for the **production** of **lac repressor protein**. The operator sits between the promoter and structural genes to control the transcription of the structural genes. It is the **binding site** of the **lac repressor protein**. The promoter is the **binding site** for **RNA polymerase** in order to initiate transcription. The **structural genes lacZ**, **lacY** and **lacA** code for **β-galactosidase**, **permease** and **transacetylase** respectively. β-galactosidase catalyzes the **hydrolysis** of **lactose** to allolactose, while **permease** is a membrane **transport protein** that enables cells to take up **lactose**.
	Function	In the **absence of lactose**, the **regulatory gene lacI** is **constitutively transcribed**, resulting in the continued production of the **active lac repressor protein** which **binds** to the lac **operator** sequence via its **DNA-binding site**. Hence, **RNA polymerase cannot bind** to the promoter to initiate translation, and hence the lac operon is switched **off** and the structural genes are **not transcribed**. However, a **basal level** of **β-galactosidase** and **permease** is present within the cell because repression of the lac operon by the repressor is **leaky**. In the **presence of lactose**, the small number of **permease** present can **transport lactose** from the surrounding medium into the cell. Some lactose will be **converted** to **allolactose** by **β-galactosidase**. Allolactose then acts as an **inducer** molecule which **binds to the repressor protein** at its **allosteric site**. This alters the **conformation** of the **DNA-binding site** of the repressor such that the repressor is **inactivated** and is **no longer complementary in shape and charge** to the operator and thus **cannot bind** to the operator. This allows **RNA polymerase** to bind to the **promoter** and **transcribe** the **structural genes** to form a **polycistronic mRNA**. In the **absence of glucose**, high **cAMP levels** result cAMP binding to the **catabolite activator protein** (CAP) at its allosteric site, activating it and forming a **cAMP-CAP complex** which binds to the **CAP-binding site** within the promoter **strengthening the affinity** of the promoter for **RNA polymerase**. This **increases the rate of transcription**, turning on the operon, increasing the synthesis of β-galactosidase, permease and transacetylase for the metabolism of lactose.

Regulation of Gene Expression	Lac Operon	Significance	Glucose used in **preference** to lactose as a **respiratory substrate** since there is considerable **energy expenditure** required to synthesize additional lactose-metabolizing enzymes such as β-galactosidase. The lac operon is under dual control: **negative regulation** by the lac repressor and **positive regulation** by the catabolite activator protein in order to ensure that lactose-metabolizing enzymes are only produced when **lactose is present** and **glucose is absent**.
	Trp Operon	Repressible Operon	A **repressible operon** catalyzes **anabolic processes** which involve the synthesis of molecules. It avoids the devotion of resources to unnecessary synthetic activities once the end product is present in sufficient levels.
		Structure	The trpR gene is the regulatory gene located **upstream** of the operon with its own promoter and terminator sequences. It codes for the **production** of **trp repressor protein**. The operator is within the promoter and controls the transcription of the structural genes. It is the **binding site** of the **lac repressor protein**. The promoter is the **binding site** for **RNA polymerase** in order to initiate transcription. The **structural genes trpE**, **trpD**, **trpC**, **trpB** and **trpA** code for products involved in the synthesis of the amino acid **tryptophan**.
		Function	The trp repressor is synthesized in its **inactive form** with **little affinity** for the trp operator. Hence, **RNA polymerase** is able to **bind** to the promoter and initiate transcription. In the presence of the **corepressor** tryptophan, tryptophan binds to **trp repressor** at its **allosteric site** to change the repressor to its **active form** which is **complementary in shape and charge** to the trp repressor binding site on the **operator**. The active repressor **binds** to the operator, preventing the binding of **RNA polymerase** to the promoter to initiate transcription, hence preventing the expression of the trp operon
	Importance		<u>Explain the importance of regulation of gene expression.</u> Regulation of gene expression allows the bacteria to make economical use of energy and resources as the gene is expressed and the protein produced only when necessary. It also enables the bacteria to respond appropriately and rapidly to changes in the environment. This convers a selective advantage to bacteria who are able to regulate gene expression.