Microbiology

Unit 4 · Lesson 4 · Gene transfer and mutation

The notes for Lesson 4 of Unit 4: chapters 4.7 to 4.8 of the unit book, with their figures, worked examples, exam traps and quizzes, in one file. The full unit book, with every chapter, the exam question bank and the glossary, is linked at the end.

`Lesson 4 of 4` `2 chapters` `Chapters 4.7 to 4.8`

About these notes

These are the notes for **Lesson 4 · Gene transfer and mutation** of the University free n easy video lessons on Microbiology Unit 4, Microbial Nutrition, Growth and Genetics. They are chapters 4.7 to 4.8 of the unit book, unchanged, so a page here is the same page in the full book.

4.7 Bacterial recombination: transformation, transduction, conjugation

Bacteria reproduce asexually, yet they swap genes all the time. In **horizontal (lateral) gene transfer**, DNA moves from a donor cell to a recipient cell of the same generation, not from parent to daughter. There are three routes: naked DNA taken up from outside (**transformation**), DNA carried by a bacteriophage (**transduction**), and DNA passed through direct cell contact (**conjugation**). Incoming DNA survives only if it is a self-replicating plasmid or if it is joined into the chromosome by **homologous recombination**.

You need: bacteriophage lytic and lysogenic cycles from Unit 3, *Bacteriophages: structure and lytic and lysogenic cycles*.

Homologous recombination

Donor DNA that enters a cell is usually a fragment. The RecA protein coats a single strand of it and pairs it with the matching (homologous) stretch of the recipient chromosome. Two crossovers swap the donor segment in, and the replaced recipient piece is degraded. A recipient that has taken in a donor gene this way is a **recombinant**. A *recA* mutant cannot recombine, so it is used to keep cloned DNA stable.

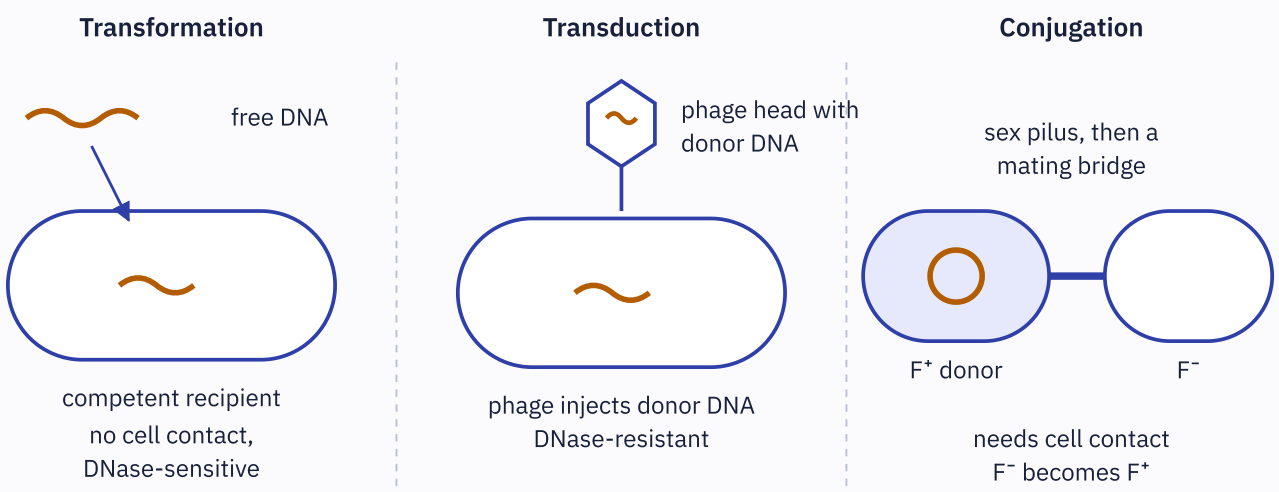


Figure 4.7.1 The three routes of gene transfer. The two tests that tell them apart: adding DNase to the medium blocks only transformation, and separating cells by a filter that lets phage through (the U-tube test) blocks only conjugation.

Transformation

In 1928 Frederick Griffith injected mice with live harmless (rough, R) *Streptococcus pneumoniae* plus heat-killed virulent (smooth, S) cells. The mice died, and live S bacteria were recovered: something from the dead cells had "transformed" the living ones. In 1944 Oswald Avery, Colin MacLeod and Maclyn McCarty showed the transforming principle was DNA, since only DNase destroyed it.

A cell able to take up DNA is **competent**. Natural competence is found in S. pneumoniae, Bacillus subtilis, Haemophilus influenzae and Neisseria. Usually one strand of the fragment enters and is recombined in. E. coli is not naturally competent, so labs make it so **artificially**: cold $CaCl_2$ treatment followed by a brief heat shock (about 42 °C), or **electroporation**, a short high-voltage pulse that opens pores. This is how plasmids are put into bacteria in genetic engineering.

Transduction

Zinder and Lederberg discovered transduction in Salmonella with phage P22 in 1952.

- **Generalized transduction.** During the lytic cycle, a phage such as P22 or P1 sometimes packs a piece of host DNA instead of its own. This "transducing particle" injects it into the next cell. Any gene can be carried.
- **Specialized transduction.** A temperate phage such as λ (lambda) leaves the chromosome imprecisely when induced, taking genes beside its attachment site (gal or bio for λ). Only those neighbouring genes are carried.

Two genes carried in the same particle are **cotransduced**; the more often it happens, the closer they lie. If the donor DNA is neither recombined nor degraded, it persists in one cell line without replicating: **abortive transduction**.

Conjugation

Joshua Lederberg and Edward Tatum showed gene exchange between E. coli strains in 1946; Bernard Davis's U-tube showed it needs contact. The donor carries the **F (fertility) plasmid**, which codes for the sex pilus and the transfer machinery.

1. **$F^+ \times F^-$.** The pilus contacts the F^- cell and pulls the cells together. One strand of F is nicked and passed across while the donor makes a new copy (rolling-circle replication). The recipient makes the complementary strand and becomes F^+. No chromosomal genes move.
2. **Hfr × F^-.** In an Hfr (high frequency of recombination) cell, F is integrated into the chromosome. Transfer starts inside F and drags chromosomal genes behind it in a fixed order. The bridge usually breaks before all of it crosses (the whole E. coli chromosome needs about 100 minutes), so the recipient gets donor genes but stays F^-.
3. **F′ × F^-.** When F leaves the chromosome imprecisely it takes nearby genes, giving an F′ plasmid. The recipient becomes F′ and partly diploid (a merodiploid) for those genes.

Interrupted mating (Wollman and Jacob) maps genes: Hfr and F^- cells are mixed, samples are shaken apart in a blender at set times, and each gene is recorded when its recombinants first appear. Map distance is given in minutes.

Implications

Recombination spreads antibiotic resistance: R plasmids carrying several resistance genes move by conjugation, even between species, which is why a resistant gut bacterium can hand resistance to a pathogen (Unit 5, *Antimicrobial resistance*). Pathogenicity islands and toxin genes (the cholera toxin gene arrived by phage) also move this way. The same tools let us map genes, build strains and clone DNA.

WORKED EXAMPLE 1: TRANSFORMATION EFFICIENCY

Question. 2 µL of plasmid at 50 ng/µL is added to competent E. coli and the final volume after recovery is 1 mL. 100 µL is spread on ampicillin agar and 180 colonies grow. Find the transformation efficiency in transformants per µg. (4 marks)

1. DNA added = 2 µL × 50 ng/µL = 100 ng = 0.1 µg.
2. Fraction plated = 100 µL ÷ 1000 µL = 0.1, so DNA on the plate = 0.01 µg.
3. Efficiency = 180 ÷ 0.01 µg = 1.8×10^4 transformants per µg.

WORKED EXAMPLE 2: INTERRUPTED MATING

Question. In an Hfr × F^- cross, recombinants for genes a^+, b^+, c^+ and d^+ first appear at 25, 9, 18 and 11 minutes. Give the gene order and the distance between a and c. (3 marks)

1. Order by entry time: b (9), d (11), c (18), a (25). Gene order from the origin: b–d–c–a.
2. Distance a to c = 25 − 18 = 7 minutes.

EXAM TRAPS

- $F^+ \times F^-$ turns the recipient F^+; Hfr × F^- usually does not, because the F sequence enters last.
- Generalized carries any gene; specialized only genes next to the prophage site.
- DNase blocks transformation only; the U-tube blocks conjugation only.
- A chromosomal fragment must be recombined into the chromosome or it is lost; only a plasmid can replicate on its own.

CHECK YOURSELF

– REMEMBER **Who showed that the transforming principle is DNA, and when?** 1 mark

Avery, MacLeod and McCarty, 1944.

– REMEMBER **What does Hfr stand for?** 1 mark

High frequency of recombination: a strain with F integrated in the chromosome.

– UNDERSTAND **Why does specialized transduction carry only certain genes?** 2 marks

The phage DNA is integrated at one site; faulty excision takes only the host genes next to that site.

– UNDERSTAND **What makes a cell competent?** 2 marks

A physiological state with DNA-binding and uptake proteins on the surface; natural in some species, induced artificially by $CaCl_2$ and heat shock or electroporation.

– APPLY **0.5 µg of plasmid, all plated, gives 400 colonies. Efficiency?** 2 marks

400 ÷ 0.5 = 800 transformants per µg (8×10^2 /µg).

– APPLY **Gene transfer occurs between two strains in a U-tube with a filter, and DNase has no effect. Which mechanism?** 2 marks

Transduction: no contact needed and DNA is protected inside phage.

– ANALYZE **Why do genes near the Hfr origin appear in more recombinants than distant ones?** 3 marks

Transfer is linear from the origin and the bridge breaks at random times; early genes cross in most matings, late genes only in long ones.

BEYOND THE SYLLABUS

Integrative conjugative elements (ICEs) behave like plasmids that live in the chromosome. CRISPR–Cas systems are bacteria's defence against incoming phage and plasmid DNA, later turned into gene-editing tools. Search "resistome" and "integrative conjugative element". This is not for the exam.

4.8 Genetics of viruses and bacteria

Microbial genetics asks how a microbe's genes are organised and how they change. Bacteria have small genomes, one copy of each gene (they are haploid) and a new generation every half hour, so a mutation shows up at once and can be selected from billions of cells. Viruses change even faster. That speed made E. coli and its phages the organisms in which much of molecular biology was worked out. It is tagged advanced because few syllabi give it a unit of its own.

The bacterial genome

Most bacteria have one circular chromosome, packed in the **nucleoid** without a nuclear membrane; E. coli K-12's is about 4.6 million base pairs. Genes are close together with little non-coding DNA, and related genes are often grouped in **operons** under one promoter (the lac operon is the classic case, covered in Biochemistry and Molecular Biology). Extra genes sit on **plasmids**: small, usually circular DNA that replicates on its own and carries optional traits such as antibiotic resistance (R plasmids), toxins or the F factor (see 4.7). **Transposons** ("jumping genes") move from place to place in the genome, and insertion sequences are the simplest of them.

Mutation

A **mutation** is a heritable change in DNA sequence. **Spontaneous** mutations come from replication errors; **induced** ones from mutagens such as UV light (thymine dimers), base analogues (5-bromouracil) and alkylating agents. Point mutations can be silent, missense (one amino acid changed) or nonsense (a stop codon); insertions or deletions that are not a multiple of three cause **frameshifts**. A **prototroph** (wild type) grows on minimal medium; an **auxotroph** needs a supplement because it has lost a biosynthetic gene. Conditional mutants such as temperature-sensitive ones work only at the permissive temperature.

Two classic experiments showed that mutations arise at random, before selection, not in response to it. The **Luria–Delbrück fluctuation test** (1943) found that the number of phage-resistant E. coli varied hugely between parallel cultures, which fits random early mutations. **Replica plating** (Joshua and Esther Lederberg, 1952) showed resistant colonies were already present on a plate never exposed to the agent.

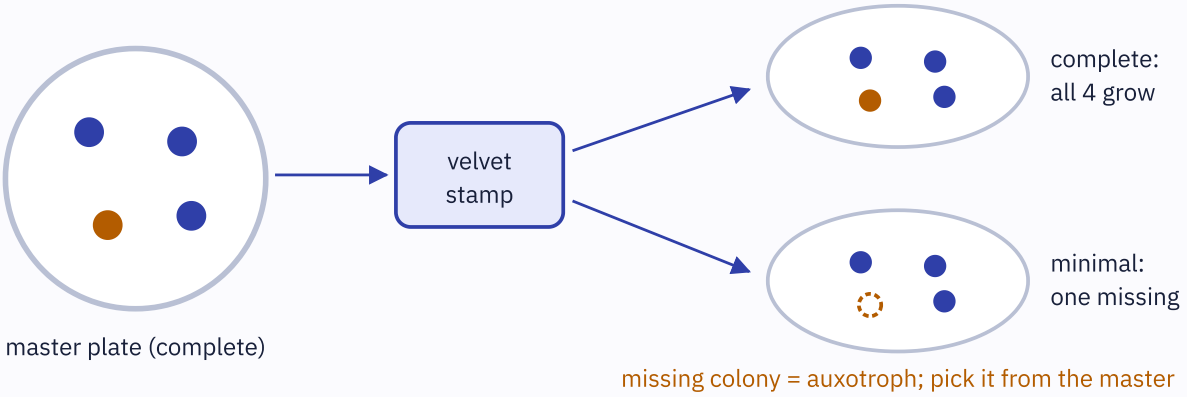


Figure 4.8.1 Replica plating. One velvet press copies every colony, in the same positions, onto two plates. The colony that grows on complete medium but not minimal medium (orange) is an auxotroph, and it is recovered from the master plate.

The **Ames test** uses histidine-requiring Salmonella mutants: a chemical that raises the rate of reversion to his^+ is a mutagen and a possible carcinogen. Liver enzyme extract is added because some chemicals become mutagenic only after the body processes them.

Viral genetics

Viral genomes are DNA or RNA, single- or double-stranded, and the Baltimore scheme sorts them by how they make mRNA (Unit 3 covers structure and classification). Key genetic features:

- **High mutation rates** in RNA viruses, because RNA polymerases do not proofread. Small changes in surface proteins (antigenic drift) are why flu vaccines are updated.
- **Reassortment**: viruses with segmented genomes, such as influenza, swap whole segments when two strains infect one cell, giving antigenic shift and pandemic strains.
- **Recombination and complementation** in phages: when two mutant phages infect one cell, recombination can give wild-type progeny, and the frequency maps the mutations. If two mutants help each other grow without recombining, they are in different genes (complementation). Seymour Benzer used phage T4 rII mutants this way to map a gene at fine scale.

WORKED EXAMPLE

Question. In an Ames test, a plate without the chemical shows 25 his^+ revertant colonies; with the chemical and liver extract it shows 300; with the chemical but no liver extract it shows 28. Interpret. (4 marks)

1. 25 colonies is the spontaneous reversion rate (background).
2. With liver extract the count is 300 ÷ 25 = 12 times background: the chemical is mutagenic.
3. Without extract, 28 is about background, so the chemical itself is not mutagenic; a product made by liver enzymes is.
4. Conclusion: a pro-mutagen, and a suspected carcinogen needing further animal testing.

EXAM TRAPS

- Mutations are random; selection only reveals them (replica plating, fluctuation test).
- Auxotroph needs a supplement; prototroph does not. Do not swap them.
- Antigenic drift (point mutation) differs from antigenic shift (reassortment).
- Plasmids are optional; the chromosome carries the essential genes.

CHECK YOURSELF

- REMEMBER **What is an auxotroph?** 1 mark

  A mutant that cannot make a needed nutrient and must be given it.

- REMEMBER **Name two mutagens and their effect.** 2 marks

  UV light, thymine dimers; 5-bromouracil, a base analogue that mispairs.

- UNDERSTAND **Why do RNA viruses evolve faster than DNA viruses?** 2 marks

  Their RNA polymerases lack proofreading, so many more errors occur per copy.

- APPLY **How would you find a tryptophan auxotroph among 200 colonies?** 3 marks

  Replica-plate onto minimal medium and minimal plus tryptophan; a colony growing only with tryptophan is the auxotroph.

- ANALYZE **What did the wide spread of resistant counts in the fluctuation test prove?** 3 marks

  If phage induced resistance, every culture would show similar counts. Large jackpots mean mutations arose at random times before phage exposure, early ones giving many resistant descendants.

BEYOND THE SYLLABUS

Whole-genome sequencing now tracks outbreaks mutation by mutation, as was done for SARS-CoV-2 variants. Tn-seq floods a genome with transposon insertions to find which genes are essential. Search "genomic epidemiology" and "Tn-seq". This is not for the exam.

Key terms of this lesson

TERM	MEANING OR FORMULA	CHAPTER
Transfer routes	transformation (free DNA), transduction (phage), conjugation (contact, F plasmid)	4.7
Transformation efficiency	`colonies ÷ µg DNA plated`	4.7
Random mutation	fluctuation test (1943), replica plating (1952)	4.8

Glossary (terms used in this lesson)

Agar Seaweed polysaccharide used at about 1.5% to solidify media; few microbes digest it.

Auxotroph Mutant that needs a nutrient the wild type can make.

Competence The state in which a bacterium can take up free DNA.

Conjugation DNA transfer through direct contact, driven by a conjugative plasmid such as F.

Hfr Strain with the F plasmid integrated into the chromosome.

Plasmid Small, self-replicating DNA separate from the chromosome.

Prototroph Strain that grows on minimal medium, like the wild type.

Replica plating Copying colonies by velvet onto several media to find mutants.

Transduction Transfer of bacterial DNA by a bacteriophage.

Transformation Uptake of free DNA from the surroundings.

The full unit book

Every chapter of Unit 4, the unit summary, the exam question bank with model answers and the full glossary: Microbiology · Unit 4 · Microbial Nutrition, Growth and Genetics (one file, works offline).

Sources and further reading

Textbooks

- Willey, Sandman and Wood, *Prescott's Microbiology*, McGraw-Hill: microbial nutrition, growth, metabolism and genetics chapters (from memory; check).
- Madigan and others, *Brock Biology of Microorganisms*, Pearson: reduction potentials, anaerobic respiration and phototrophy (from memory; check).
- Pelczar, Chan and Krieg, *Microbiology*, McGraw-Hill (Indian edition widely used) (from memory; check).
- Tortora, Funke and Case, *Microbiology: An Introduction*, Pearson (from memory; check).
- Shuler and Kargi, *Bioprocess Engineering: Basic Concepts*, for Monod and chemostat kinetics (from memory; check).

Open resources

- OpenStax, *Microbiology*, section 9.1 "How Microbes Grow": growth phases, 30–300 plate rule, direct and indirect counts. openstax.org/books/microbiology/pages/9-1-how-microbes-grow
- OpenStax, *Microbiology*, section 11.6 "How Asexual Prokaryotes Achieve Genetic Diversity": Griffith and Avery, Hfr and F′ cells, 100-minute E. coli transfer. openstax.org/books/microbiology/pages/11-6-how-asexual-prokaryotes-achieve-genetic-diversity

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