Key terms

Tandem repeats: a short DNA piece repeated one copy after another; **STRs**: repeats of 2–6 bases (GATA GATA…).

DNA fingerprinting (DNA profiling, DNA test): identifying a person, or how people are related, from the DNA pattern, mainly lengths of repeated pieces at chosen places.

PCR: polymerase chain reaction; copies repeat regions millions of times.

Clone: organism(s) with exactly the same genes as the single parent, by asexual reproduction. **Cloning**: producing such copies, naturally or artificially.

SCNT: somatic cell nuclear transfer; a body cell's nucleus put into an egg without a nucleus.

Genetic engineering (genetic modification, recombinant DNA technology): direct change of DNA in the laboratory: add, remove or change a gene.

Recombinant DNA: DNA joined from two sources. **GMO / transgenic**: an organism carrying it.

Ethics: what is right and wrong.

Bio-weapons: microorganisms, or toxins made by living things, used deliberately to cause disease or death in people, animals or crops. **Biological warfare**: their use in war. **Bioterrorism**: use to frighten or harm for a political aim.

Organ transplantation: moving a healthy organ or tissue from **donor** to **recipient**; the part moved = **graft**.

HLA: marker proteins on cells, almost as different between people as DNA fingerprints.

Rejection: recipient's white blood cells and antibodies attack the graft.

Crossmatch: recipient's serum + donor's cells; checks for antibodies ready to attack. Positive → donor can't be used.

Immunosuppressants: daily medicines that calm the immune system.

Brain death: brain dead completely, heart kept beating by machines.

Numbers

- places on different chromosomes: independent, so **chances multiply**
- $1/10 \times 1/20 \times 1/8 = 1$ in 1,600; 20 places at 1 in 10 → 1 in 10^{20}
- bacteria: after n doublings, 2^n from one; doubling about every 20 min or more
- dialysis: 4 h × 3 a week; with 1 h travel each way: $6 \times 3 \times 52 = 936$ h a year ≈ 39 days

5.1 DNA fingerprinting

- 99.9% of DNA same in any two people; the 0.1% differs
- repeat numbers vary by person (8 and 11; 9 and 14), one chromosome of each pair from each parent → inherited
- like a fingerprint, unique; but readable from a few cells, and shows relatives

Steps

1. **Sample**: any cells with a nucleus:
   - blood (white cells)
   - saliva
   - cheek swab
   - hair root
   - skin
   - semen
   - bone
   - teeth

   Red blood cells: no nucleus; cut hair: very little DNA.
2. **Extract** DNA: cells broken with chemicals; proteins, fats separated.
3. **Cut or copy**: restriction enzymes (Jeffreys, 1984) or, today, PCR.
4. **Gel electrophoresis**: wells at one end of agarose gel (from seaweed) in salt solution, voltage across. DNA **negative** (phosphate groups) → moves to **positive** electrode. **Small pieces farthest**, big held back.
5. **Make visible**: dye glowing under UV, or radioactive or fluorescent labels → **bands**.
6. **Compare** lane by lane: every band matches → very probably same person.

- 15–24 places on different chromosomes (US 20, Britain 17)
- a shared band ≠ a match; one band different → different people
- better at proving innocence than guilt
- identical twins: same DNA, different finger ridges; non-identical: like any brother and sister

Importance:

- crimes (and freeing the innocent)
- paternity and maternity
- identifying the dead
- reuniting families
- wildlife (seized horns, skins; counting tigers, rhinos, snow leopards in Nepal from droppings)
- history and ancestry
- medicine and food (inherited diseases, germs, food sold under a wrong name)

Limits:

- contamination
- old, burnt, tiny or mixed samples
- identical twins
- shows whose DNA was there, not who did the crime
- privacy of DNA databases

5.2 Cloning

- **Natural**:
  - bacteria, amoebas (binary fission)
  - potato tubers
  - onion and garlic bulbs
  - runners (strawberry, doob grass)
  - banana suckers
  - rhizomes (ginger, turmeric, bamboo)
  - identical twins (of each other, not of a parent)
- **Plants, artificial**:
  - cuttings (sugarcane, roses, mint)
  - grafting (mango, apple, citrus)
  - layering
  - **tissue culture**: shoot-tip cells on nutrient and hormone jelly, sterile bottle → thousands of disease-free plantlets in months (bananas, potatoes, orchids, strawberries, in Nepal too)

- **Animals**: **embryo splitting** (copies of one another, not of an adult; cattle) and **SCNT** (copy of the adult)
- **Reproductive**: a whole new animal. **Therapeutic**: cloned embryo only for stem cells → tissues without rejection. Human reproductive cloning banned by law in many countries.

Dolly (Roslin Institute, Scotland; Wilmut, Campbell)

1. Udder cells of 6-year-old **Finn Dorset** (white face) grown, starved → stop dividing
2. Unfertilized egg of **Scottish Blackface** (black face): nucleus sucked out with a glass needle
3. Electric pulse: cells fuse, division starts
4. Mitosis: ball of cells, about a week
5. Into womb of another Scottish Blackface
6. Born 5 July 1996, white-faced; announced 22 February 1997

- three mothers (nucleus, egg, womb); no father
- 277 fused → 29 embryos → 1 lamb; nucleus must be **reprogrammed**, most fail
- showed an adult cell holds all genes for a whole animal
- Gurdon 1962 (frog); shared 2012 Nobel with Yamanaka
- six lambs; put to sleep at 6½ (lung disease)

Characteristics of clones

- same nuclear genes; same sex, blood group, inherited features
- not perfect copies: **mitochondria from the egg**; genes switched on and off differently (CC the cat ≠ Rainbow)
- born a baby, no memories, different surroundings
- all equally at risk from one disease
- early clones often unhealthy; 13 cloned sheep (2016) aging normally

5.2 Genetic engineering

- possible because DNA and the genetic code are the same in all living things
- **Restriction enzymes**: "scissors"; cut at one short sequence, leave **sticky ends**
- **DNA ligase**: "glue"; joins backbones
- **Vector**: **plasmid** (small DNA ring in a bacterium, apart from its chromosome) or virus
- **Host**: *E. coli* or yeast

Insulin:

1. gene obtained
2. plasmid taken out
3. **both cut with the same enzyme** (matching sticky ends)
4. ends pair, **ligase seals**
5. recombinant plasmid into bacteria
6. grown in fermenters
7. insulin purified

Genentech 1978; approved 1982, the first medicine made by genetic engineering. Before: from pancreases of cattle and pigs; some patients allergic.

Other uses:

- growth hormone, hepatitis B vaccine (yeast), clotting factors, antibodies
- Bt cotton, Bt brinjal, Golden Rice (beta-carotene)
- goats with medicines in milk, faster salmon
- gene therapy, **CRISPR** (Charpentier, Doudna, 2020 Nobel)
- research mice and bacteria

Ethics: cloning:

- animal suffering
- risk to a cloned child, whose child, spare parts
- identity and dignity
- less variety
- "playing God"

Ethics: genetic engineering:

- food safety (reviews: approved GM foods as safe as ordinary; each new crop tested)
- genes spread to wild relatives, resistant pests and weeds, other insects harmed
- patents, buying seed yearly
- embryo editing passes to descendants without consent (China, 2018: condemned, prison)
- labeling

Nepal has not approved GM crops for farming.

5.4 Cloning minimizes variation

- sexual: meiosis, crossing over, fertilization → new mixtures
- clone: **mitosis alone** → same genes; only rare mutations

Effect	Good	Bad
Qualities	kept exactly (mango; seedless bananas, grapes)	harmful genes kept too
Crop	uniform; fast; disease-free stock	one disease can wipe out all
Future	rare plants, animals, best farm animals copied	can't adapt
Diversity	—	local varieties lost

- **Gros Michel** banana: fungus (Panama disease), replaced by Cavendish by the 1960s; new strain now attacks Cavendish
- **Ireland, 1840s**: potato blight, about a million died
- **Reduce harm**:
  - several varieties
  - seed and gene banks (Khumaltar, Lalitpur)
  - new breeding by sexual reproduction, then clone the best
  - watch for disease, stop infected planting material

5.5 Bio-weapons

- counted with nuclear and chemical weapons as weapons of mass destruction

History

- old reports (Caffa, 1346); harm not known
- **1925 Geneva Protocol**: bans **use**, not making or keeping
- **1930s–1945 Unit 731** (Japan's army, Pingfang near Harbin): infected thousands of prisoners, most Chinese civilians; many thousands more died in towns and villages
- 1940s–1990s: large programs, e.g. US (ended 1969), Soviet Union (to at least 1992)
- **1972 Biological Weapons Convention**, in force 1975
- **1979 Sverdlovsk**: accidental escape from a secret laboratory; over 60 died; admitted only in 1992
- **2001 letters** (US): 5 died, 17 more fell ill

The convention (BWC): bans

- developing
- producing
- stockpiling
- acquiring
- transferring

Requires destroying. First treaty to ban a whole kind of weapon of mass destruction. Nepal: signed 1972, ratified 2016. Peaceful research (vaccines, medicines, tests) allowed.

Claimed "advantages" (write as claims):

- thought cheap compared with nuclear weapons
- hard to detect at first
- hard to tell who did it
- harm people, not buildings

Disadvantages:

- can't be controlled, can strike one's own side
- strike civilians
- unpredictable
- banned, a war crime
- accidents
- poison trust in science
- destroy food and farms

They outweigh the claims.

5.6 Organ transplantation

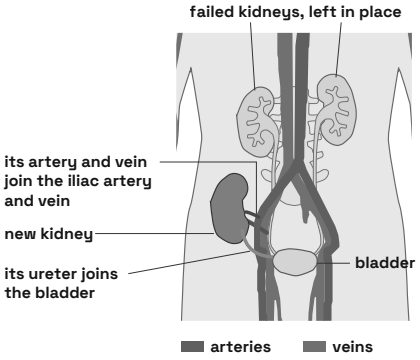
- organs:
  - kidneys
  - liver (part; grows back)
  - heart
  - lungs
  - pancreas
  - intestines
- tissues:
  - cornea
  - skin
  - bone
  - heart valves
  - bone marrow

Kind	From	Rejected?
Autograft	same person (skin, thigh → burn)	no
Isograft	identical twin (kidney, 1954)	no
Allograft	another person	yes, unless medicines
Xenograft	another species (pig valves; pig kidney, 2024)	strongly

- **living donor**: one kidney or part of the liver; **deceased (brain dead)**: up to eight lives
- reduce rejection:
  - matching blood groups
  - HLA close (relatives)
  - crossmatch
  - immunosuppressants as long as the organ works (infections, some cancers more likely)
- **Importance**:
  - saves lives
  - better life
  - work again
  - costs less over the years
  - a death gives life
  - moves medicine forward
- **Nepal's law** 2055 BS (1998): living donor only to close family, to stop organ sales; 2073 BS (2016): brain-dead donors too; first such transplant May 2017

5.7 Kidney transplantation

- failure: most often **diabetes, high blood pressure**, or long inflammation; wastes and water build up
- treatment: **dialysis** (hemodialysis, or peritoneal, fluid changed several times a day), or a **transplant**



Process: assess patient → find donor → match (blood group, HLA, crossmatch) → remove donor's kidney with artery, vein, ureter → place in **iliac fossa** → immunosuppressants for life, creatinine tests.

- renal artery → **external iliac artery**; renal vein → **external iliac vein**; ureter → **bladder**
- failed kidneys **left in place** (removal adds risk, they do no harm)
- donor lives normally; other kidney grows a little
- **Importance**:
  - longer life
  - better life
  - less cost after the first year
  - frees dialysis places
  - a living donor can give safely
- **1954, Boston**: Murray; Ronald → twin Richard Herrick; same genes; lived 8 more years; 1990 Nobel
- **Nepal**: first in 2008; Shahid Dharmabhakta National Transplant Centre, Bhaktapur (2012): over 1,500 by 2025; also livers; dialysis free at government hospitals

COMMON MISTAKES

✗ DNA moves to the negative electrode
✓ to the **positive**: DNA is negative.

✗ big pieces travel farthest
✓ **small** pieces farthest.

✗ a shared band proves a match
✓ **every** band, at many places.

✗ a match proves who did the crime
✓ only whose DNA was there.

✗ a clone is an exact copy
✓ same nuclear genes; mitochondria, gene switching, surroundings differ.

✗ Dolly's genes came from all three mothers
✓ from the **nucleus donor** only.

✗ gene and plasmid cut with different enzymes
✓ the **same** enzyme, so ends match.

✗ Geneva Protocol banned keeping bio-weapons
✓ only **use**; BWC bans keeping.

✗ "bio-weapons are cheap" as fact
✓ "were **considered** cheap", then why banned.

✗ new kidney placed where the old one was
✓ low, in the iliac fossa; old ones stay.