PIPADOR REVISION

MRCOG Part 1 Revision Notes

Recombinant DNA Technology

MOLECULAR BIOLOGY • LECTURE 97

VISUAL EXAMINATION REVISION NOTES

2026 Edition

MRCOG Part 1 Revision Notes

Lecture 97: Recombinant DNA Technology

Molecular biology • Visual revision edition

COLOUR CODE USED THROUGHOUT THESE NOTES

VECTOR / MAMMALIAN	INSERT / CUT / RISK	ENZYME / mAb NAMES	HOST / E. COLI	YEAST / PRODUCTS

LEARNING OBJECTIVES — tick off as you go

- ☐ Explain restriction enzymes, ligase and vectors.
- ☐ Describe the cloning workflow and selection.
- ☐ Compare expression systems and their products.
- ☐ Explain monoclonal antibody production, naming and use in pregnancy.
- ☐ List recombinant products used in O&G; vaccines, biosimilars and regulation.

1 The Toolkit

IN ONE LINE: *Cut DNA with restriction enzymes, paste it into a vector with ligase, put it in a host cell, select the clones that took it up — then let the host make the protein.*

Molecular scissors and glue

EcoRI — STICKY ends (5' overhang)



palindrome: GAATTC reads the same 5'→3' on both strands

SmaI — BLUNT ends



Restriction enzymes

bacterial defence vs phages; cut at 4–8 bp palindromes (Arber, Smith, Nathans — Nobel 1978)

Names

EcoRI = E. coli strain R, 1st enzyme • BamHI = Bacillus amyloliquefaciens H • HindIII = Haemophilus influenzae Rd

Sticky ends

matching overhangs base-pair → easy, directional joining

DNA ligase

seals the backbone (phosphodiester bond; T4 ligase uses ATP; joins sticky or blunt ends)

Also

Southern blot digests (L93); RFLPs; Golden Gate uses type IIS enzymes

Figure 1. Restriction enzymes and ligase.

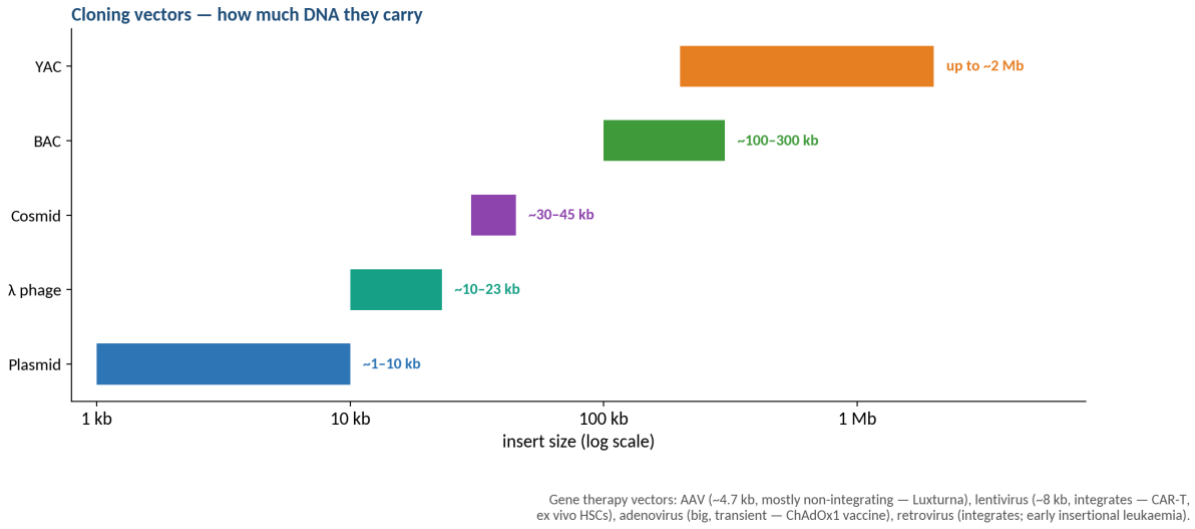


Figure 2. Vector capacity.

2 Cloning

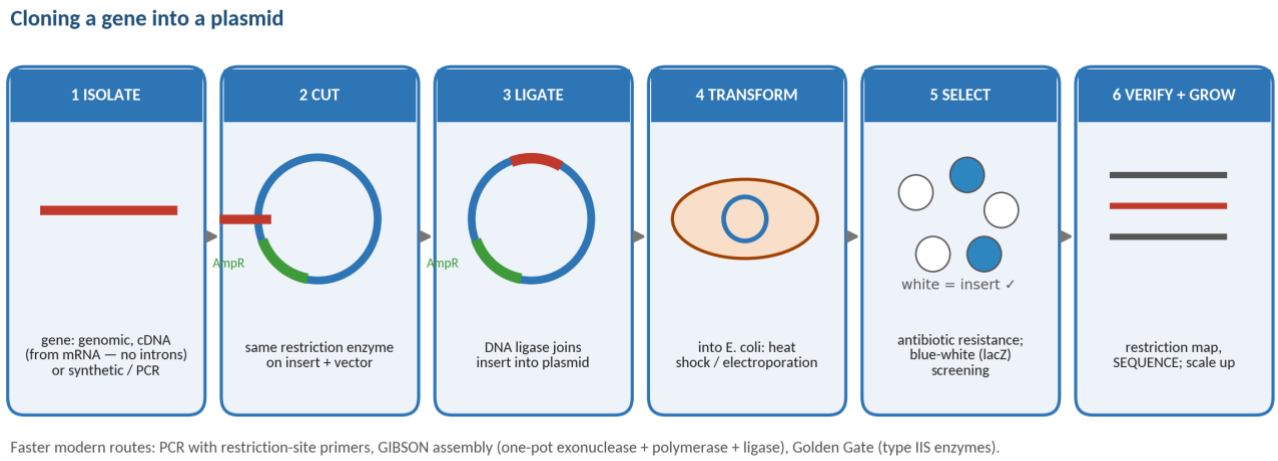


Figure 3. Cloning workflow.

MNEMONIC / MEMORY HOOK

'Cut, Paste, Transform, Select' — the four verbs of cloning.

- **cDNA** (reverse-transcribed mRNA) has **no introns**, so bacteria — which can't splice — can express human proteins from it.

3 Expression Systems and Biologics

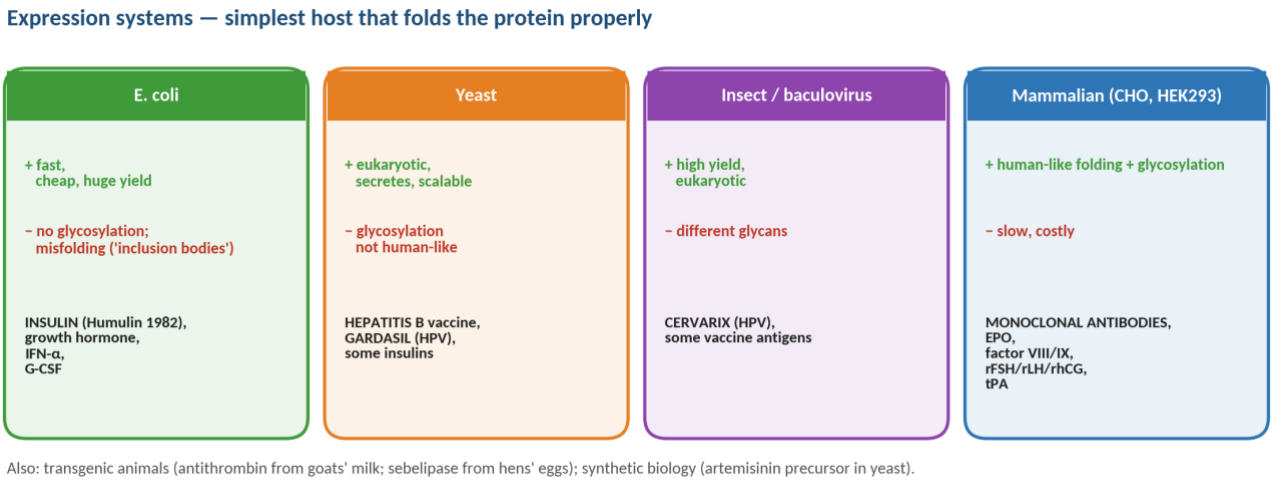


Figure 4. Expression systems.

Product	Key points
Human insulin	First recombinant medicine (Humulin, 1982); analogues alter absorption (lispro/aspart fast; detemir/glargine long)
Growth hormone	Replaced cadaveric pituitary GH (iatrogenic CJD)
Epoetin, G-CSF, tPA	Anaemia (CKD, chemotherapy); neutropenia/stem-cell mobilisation; thrombolysis
Factor VIII/IX	No plasma-borne infection risk; emicizumab (bispecific mAb) for haemophilia A prophylaxis
rFSH, rLH, rhCG	IVF/ovulation induction — purer, consistent batches
Biosimilars	Close copies of off-patent biologics (adalimumab, trastuzumab, epoetin) — similar, not identical; strict MHRA/EMA approval

4 Monoclonal Antibodies

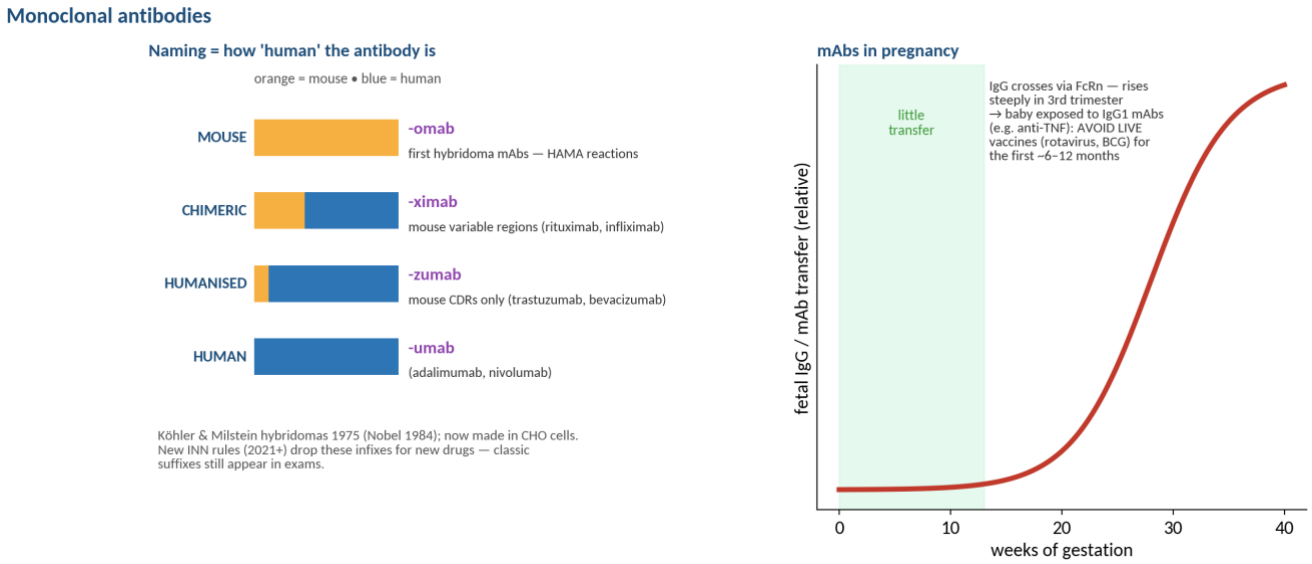


Figure 5. Naming and placental transfer.

mAb	Use / pregnancy point
Trastuzumab (anti-HER2)	Breast cancer — avoid in pregnancy (oligohydramnios)
Bevacizumab (anti-VEGF)	Ovarian, cervical cancer — impairs angiogenesis/wound healing
Pembrolizumab / dostarlimab (anti-PD-1)	Cervical cancer; dMMR endometrial cancer
Anti-TNF (adalimumab, infliximab)	IBD/RA — most data in pregnancy; plan 3rd-trimester dosing; no live vaccines for the baby early on
Rituximab, eculizumab, belimumab, tocilizumab	Lymphoma/autoimmune; PNH/aHUS; SLE; IL-6 — individual risk–benefit

5 Vaccines and O&G Applications

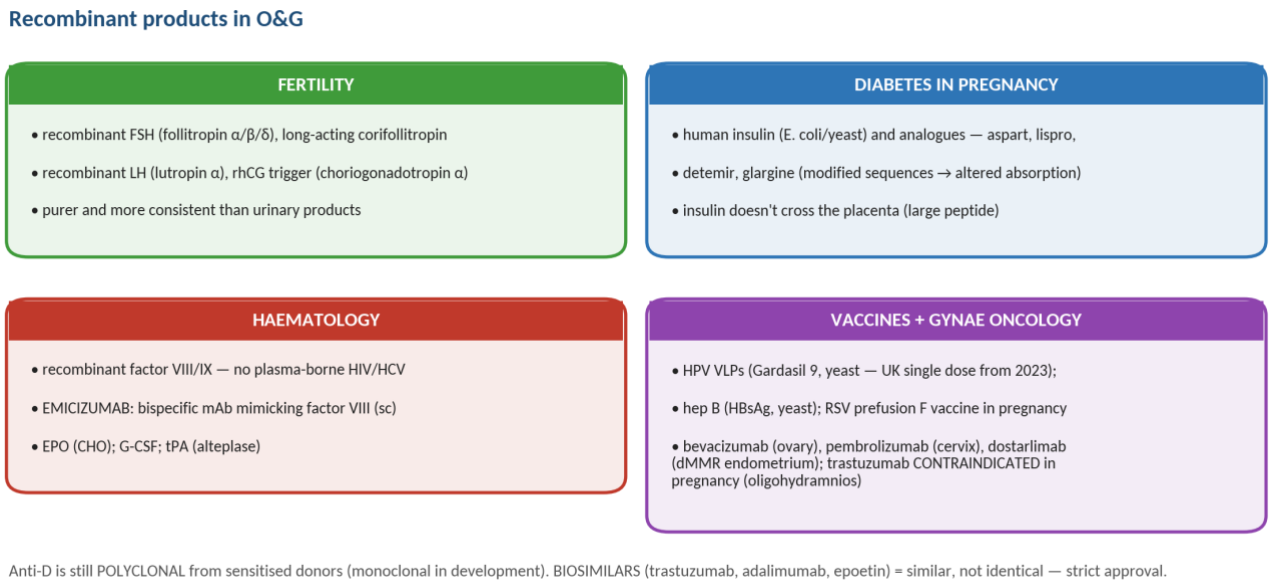


Figure 6. Recombinant products in O&G.

- **Vaccines**: subunit/VLP (hepatitis B, HPV, acellular pertussis), viral vector (ChAdOx1), mRNA (Lecture 88). **Pregnancy**: pertussis (16–32 wk), RSV (from 28 wk), influenza and COVID-19 are recommended — all non-live.

6 Safety and Regulation

Area	Key points
Contained use	GMO (Contained Use) Regulations 2014 — containment levels CL1–CL4 by risk
ATMPs	Advanced therapy medicinal products (gene/cell therapies) — MHRA/EMA: Strimvelis, Luxturna, Casgevy, CAR-T
Embryos	HFEA (Lectures 86, 96)
Transgenics / GM crops	Antithrombin from goats' milk; Golden Rice (β-carotene), Bt crops

★ PEARL

EcoRI → sticky ends; SmaI → blunt. Ligase seals. Plasmid < phage < cosmid < BAC < YAC.

IgG mAbs cross the placenta mainly in the 3rd trimester — exposed babies avoid live vaccines (rotavirus, BCG) early on.

7 MRCOG Exam Pearls — Last-Minute Page

Numbers to know

4–8 bp Restriction site	**1978** Nobel: restriction enzymes	**1975** Hybridomas	**1982** Humulin (1st)
~10 kb Plasmid insert	**~2 Mb** YAC insert	**~4.7 kb** AAV capacity	**CL1–4** Containment levels

HIGH-YIELD CHECKLIST

- ☐ Restriction enzymes: palindromes; sticky vs blunt; bacterial defence.
- ☐ Ligase: phosphodiester bonds (T4, ATP).
- ☐ Vectors: plasmid, phage, cosmid, BAC, YAC; viral vectors for gene therapy.
- ☐ Cloning: cut, ligate, transform, select (antibiotic, blue-white), verify.
- ☐ cDNA = no introns → bacterial expression.
- ☐ E. coli (insulin), yeast (hep B, Gardasil), insect (Cervarix), CHO (mAbs, EPO, rFSH).
- ☐ mAb suffixes: -omab, -ximab, -zumab, -umab.
- ☐ mAbs cross placenta in 3rd trimester; trastuzumab contraindicated.
- ☐ rFSH/rLH/rhCG, insulin, factor VIII, emicizumab, anti-D (polyclonal).
- ☐ Biosimilars; GMO contained use; ATMPs.

Self-assessment — cover the right-hand column

Question	Answer
What sequence does EcoRI cut?	GAATTC (palindrome) → sticky ends
Which enzyme seals the vector and insert?	DNA ligase
Vector with the largest insert capacity?	YAC (up to ~2 Mb)
Why use cDNA for bacterial expression?	No introns — bacteria can't splice
White colonies in blue-white screening mean…	The insert disrupted lacZ (recombinant)
Host for monoclonal antibodies?	Mammalian cells (e.g. CHO)
Meaning of '-zumab'?	Humanised monoclonal antibody
First recombinant medicine?	Human insulin (1982)
Which mAb is avoided in pregnancy due to oligohydramnios?	Trastuzumab
Is anti-D currently recombinant?	No — polyclonal from donors

8 References

- Strachan T, Read A. Human Molecular Genetics. 5th ed. 2018.
- UKHSA. The Green Book — immunisation in pregnancy (pertussis, RSV, influenza, COVID-19); HPV programme (single dose, 2023).
- RCOG / BSR guidance on biologics in pregnancy and breastfeeding.