PIPADOR REVISION

MRCOG Part 1 Revision Notes

Polymerase Chain Reaction

MOLECULAR BIOLOGY • LECTURE 91

VISUAL EXAMINATION REVISION NOTES

2026 Edition

MRCOG Part 1 Revision Notes

Lecture 91: Polymerase Chain Reaction (PCR)

Molecular biology • Visual revision edition

COLOUR CODE USED THROUGHOUT THESE NOTES

DENATURE / INFECTION	ANNEAL / qPCR	EXTEND / CONTROLS	QF-PCR / GENETICS	PRIMERS / ONCOLOGY

LEARNING OBJECTIVES — tick off as you go

- ☐ List PCR components and the three steps of each cycle.
- ☐ Explain exponential amplification, qPCR and the Ct value.
- ☐ Describe RT-PCR, QF-PCR, MLPA, nested, allele-specific, digital and isothermal methods.
- ☐ Know quality controls and the causes of false results.
- ☐ Apply PCR to O&G: infections, prenatal diagnosis, RhD, oncology.

1 How PCR Works

IN ONE LINE: *Heat to separate, cool to let primers bind, warm to copy — repeat. Each cycle doubles the target: ~2ⁿ copies after n cycles (Kary Mullis, 1983; Nobel 1993).*

One PCR cycle (Mullis, 1983) — doubles the target each time

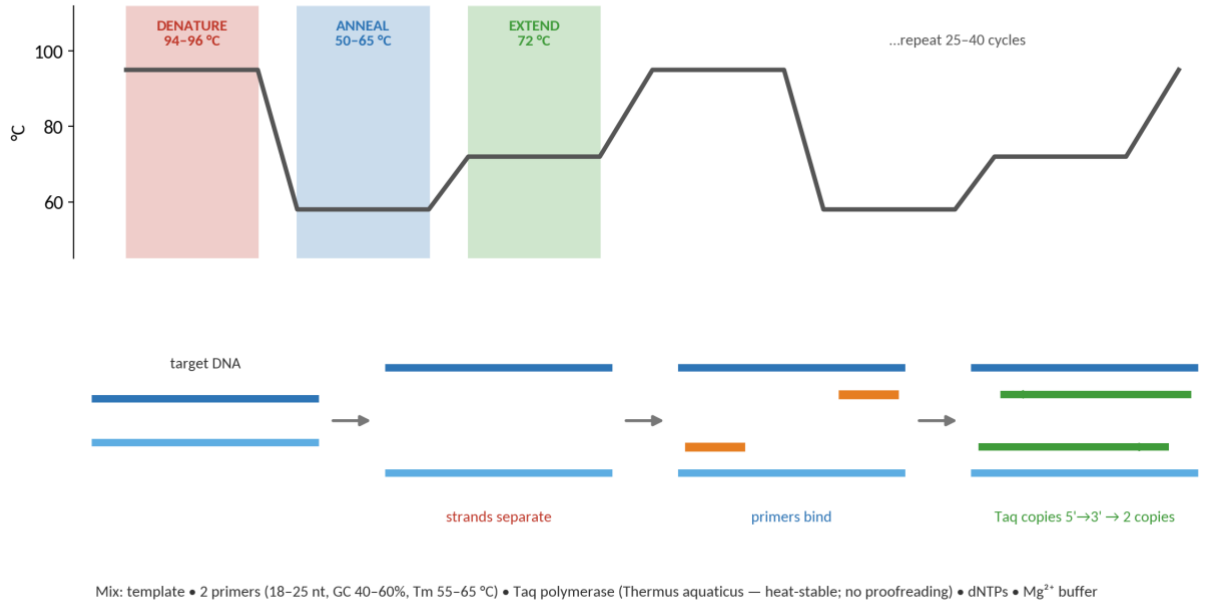


Figure 1. One PCR cycle.

MNEMONIC / MEMORY HOOK

'Dance, Arrive, Extend' — 95, 55, 72 (Denature ~95 °C, Anneal ~55 °C, Extend 72 °C).

- **Taq** is heat-stable but lacks 3'→5' proofreading (error rate ~1 in 10^4–10^5) — **high-fidelity** enzymes (e.g. Pfu) are used when the exact sequence matters.

2 Real-Time Quantification

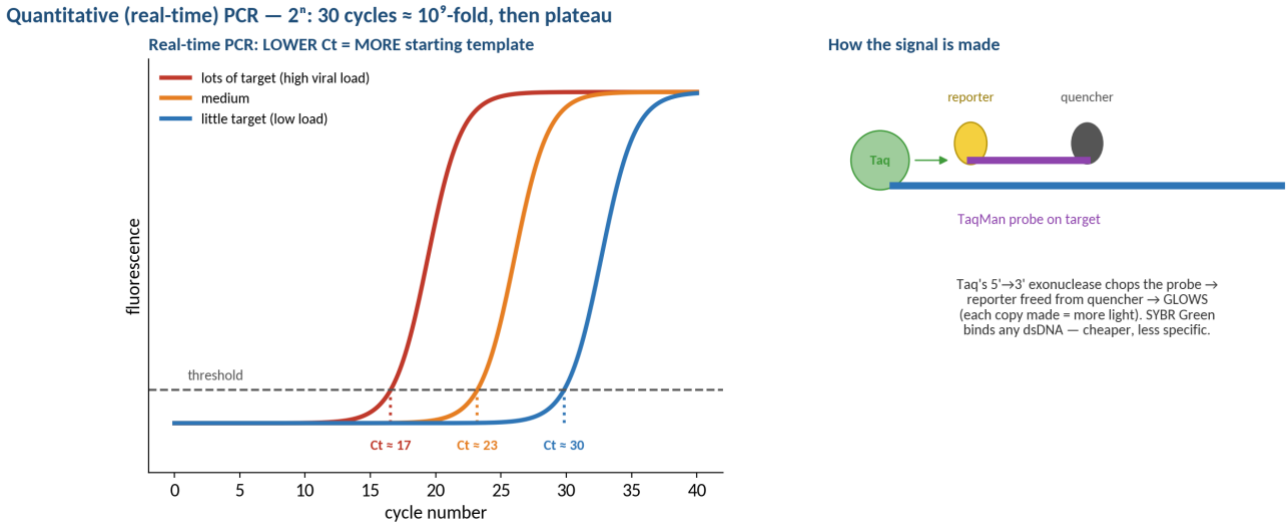


Figure 2. Amplification curves and TaqMan probes.

- **Ct** (cycle threshold) is inversely related to log starting quantity: each ~3.3 cycles earlier = 10× more template. Used for viral loads (HIV, HBV, HCV, CMV), BCR-ABL, SARS-CoV-2. **Digital PCR** counts molecules directly — no standard curve.

3 The PCR Family

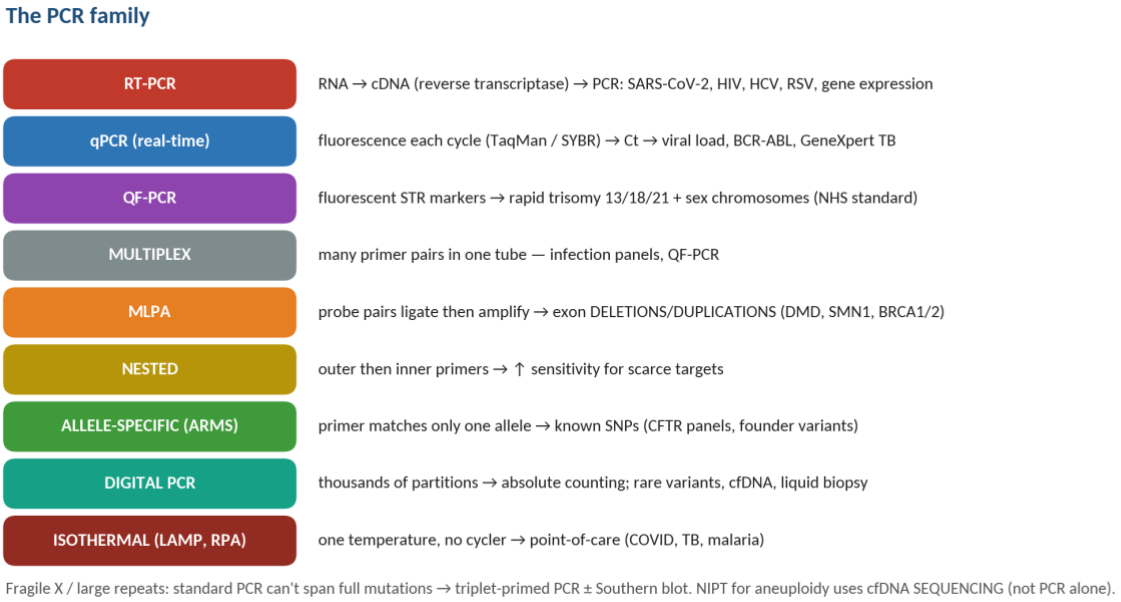


Figure 3. PCR variants.

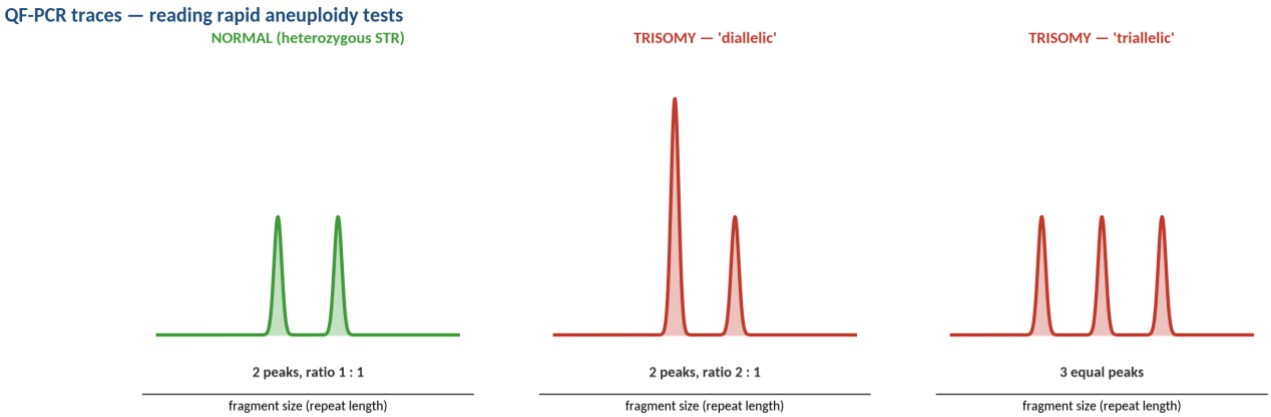


Figure 4. QF-PCR traces.

4 Quality and Pitfalls

Issue	Key points
Controls	Positive (known template), NEGATIVE / no-template (contamination), INTERNAL (housekeeping gene or spiked control — proves the sample could amplify)
False positive	Carry-over contamination (separate pre/post-PCR areas, UNG enzyme), non-specific products, primer dimers
False negative	Inhibitors (haem, heparin, EDTA, polysaccharides), poor sample, variant under primer (allele drop-out), low load below limit of detection
Detection	Agarose gel (end-point), real-time fluorescence (qPCR), capillary electrophoresis (QF-PCR, STR profiling, Sanger sequencing)
Primer design	18–25 nt, GC 40–60%, Tm 55–65 °C (matched within ~5 °C), unique, no self-complementarity

5 Clinical Applications in O&G

PCR in O&G practice

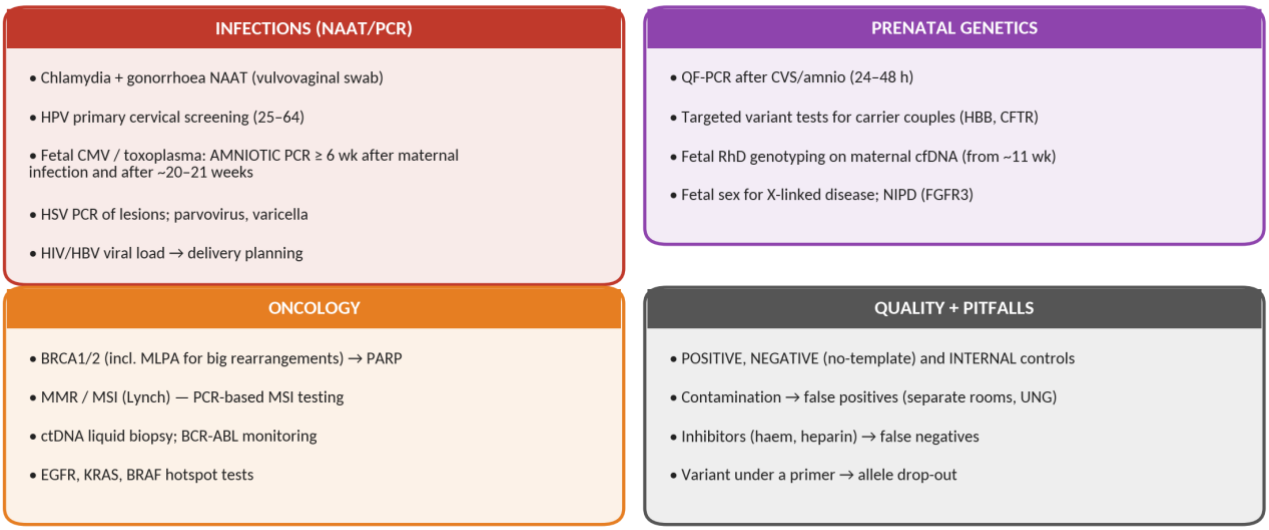


Figure 5. PCR in O&G practice.

FETAL RhD GENOTYPING

- Cell-free fetal DNA in plasma of RhD-negative women — offered from ~**11 weeks** (often 11–16)
- Fetus RhD-negative → no routine antenatal anti-D (NICE DG25)
- Still give anti-D if the result is inconclusive; cord blood check at birth per local policy

FETAL INFECTION

- CMV, toxoplasma: amniotic fluid PCR ≥ **6 weeks** after maternal infection AND after ~**20–21 weeks** (fetal urine production) — earlier tests risk false negatives
- Parvovirus: maternal IgM/PCR; fetal MCA Doppler for anaemia

★ PEARL

Lower Ct = more target. RT-PCR is for RNA. QF-PCR = rapid trisomy check; MLPA = exon deletions/duplications.

A negative no-template control is what proves the positives aren't contamination.

6 MRCOG Exam Pearls — Last-Minute Page

Numbers to know

~95 °C Denature	**~50–65 °C** Anneal	**72 °C** Extend	**2^n** Copies after n cycles
30 cycles ~10^9-fold	**~3.3** Ct shift per 10× template	**24–48 h** QF-PCR result	**~11 wk** Fetal RhD cfDNA from

HIGH-YIELD CHECKLIST

- ☐ Components: template, 2 primers, Taq, dNTPs, Mg^{2+}.
- ☐ Denature 95, anneal 55, extend 72; 2^n amplification.
- ☐ Taq: heat-stable, no proofreading.
- ☐ RT-PCR for RNA; qPCR Ct inversely related to load; TaqMan vs SYBR.
- ☐ QF-PCR: STRs on 13, 18, 21, X, Y; 2:1 or 3-peak pattern.
- ☐ MLPA: exon deletions/duplications (DMD, SMN1, BRCA).
- ☐ Nested, ARMS, multiplex, digital PCR, LAMP.
- ☐ Controls: positive, no-template, internal.
- ☐ O&G: chlamydia/gonorrhoea NAAT, HPV, amniotic CMV/toxo PCR, RhD cfDNA.
- ☐ NIPT aneuploidy = cfDNA sequencing; fragile X needs TP-PCR/Southern.

Self-assessment — cover the right-hand column

Question	Answer
Temperatures of the three PCR steps?	~95 °C denature, ~55 °C anneal, 72 °C extend
Why Taq polymerase?	Survives denaturation temperatures
How is RNA detected by PCR?	Reverse transcription to cDNA first (RT-PCR)
High viral load — high or low Ct?	Low Ct
Rapid trisomy test after amniocentesis?	QF-PCR
QF-PCR pattern in a diallelic trisomy?	Two peaks in a 2:1 ratio
Test for exon deletions in DMD?	MLPA
Which control detects contamination?	No-template (negative) control
When is amniotic CMV PCR reliable?	≥ 6 weeks after infection and after ~20–21 weeks
Use of fetal RhD genotyping?	Target antenatal anti-D to RhD-positive fetuses only

7 References

- NICE DG25. High-throughput non-invasive prenatal testing for fetal RHD genotype. 2016.
- RCOG Scientific Impact Paper No. 56. Congenital cytomegalovirus infection. 2017.
- Strachan T, Read A. Human Molecular Genetics. 5th ed. 2018.