PIPADOR REVISION

MRCOG Part 1 Revision Notes

DNA Sequencing

MOLECULAR BIOLOGY • LECTURE 92

VISUAL EXAMINATION REVISION NOTES

2026 Edition

MRCOG Part 1 Revision Notes

Lecture 92: DNA Sequencing

Molecular biology • Visual revision edition

COLOUR CODE USED THROUGHOUT THESE NOTES

SANGER / EXOME	NGS / GENOME	LONG-READ	BIOINFORMATICS	ETHICS / INTERPRETATION

LEARNING OBJECTIVES — tick off as you go

- ☐ Explain Sanger chain-termination sequencing and its role today.
- ☐ Describe the NGS workflow, panels vs exome vs genome, and depth.
- ☐ Know the uses of long-read sequencing.
- ☐ Outline variant annotation and classification.
- ☐ Apply to rare disease, pregnancy, cancer — and the ethical issues.

1 Sanger Sequencing

IN ONE LINE: *Sanger (1977): a polymerase copies the template until it adds a fluorescent dideoxynucleotide (no 3'-OH) and stops; the fragments, sorted by size, spell out the sequence.*

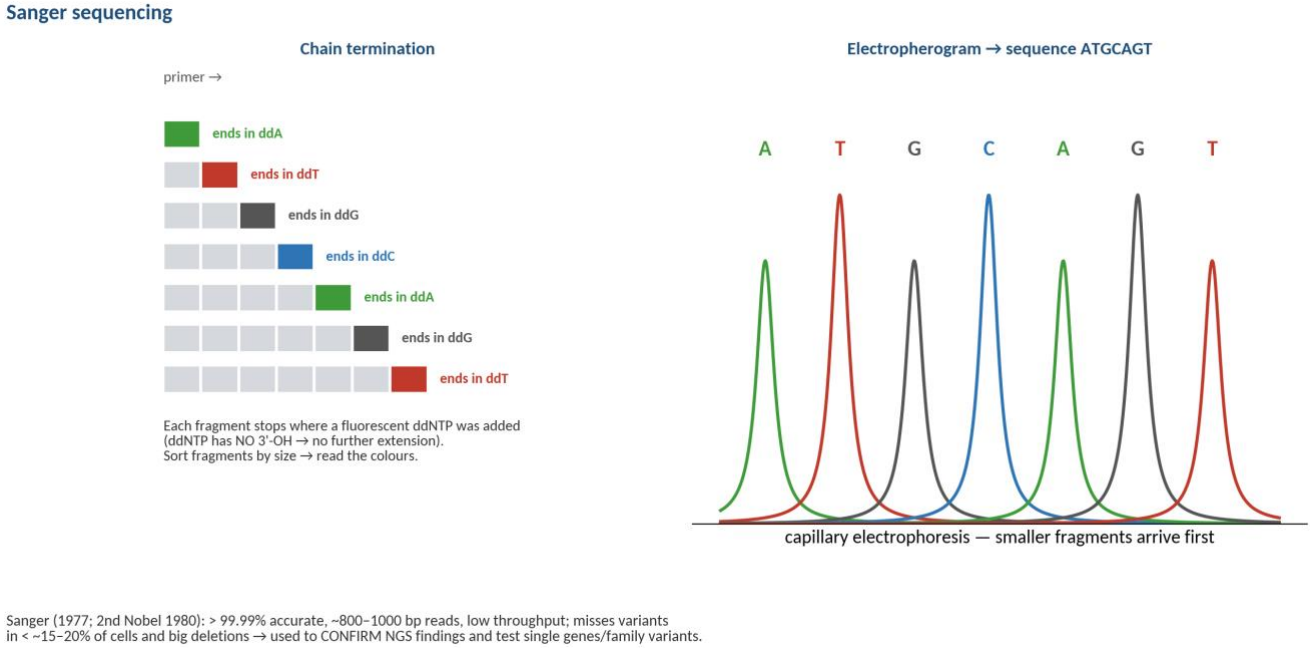


Figure 1. Chain termination and the electropherogram.

MNEMONIC / MEMORY HOOK

'ddNTP = Dead-end' — no 3'-OH, nothing can be added, the chain stops.

2 Next-Generation Sequencing

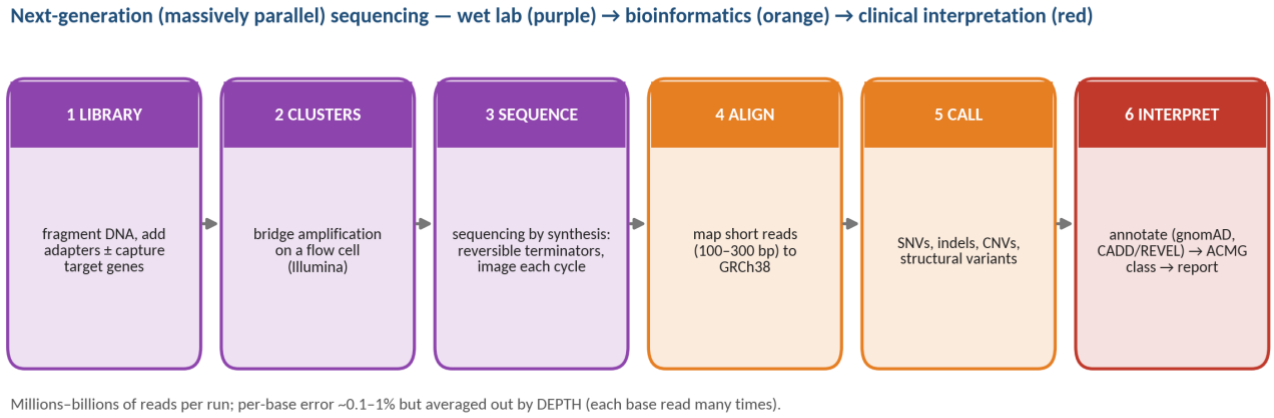


Figure 2. NGS workflow.

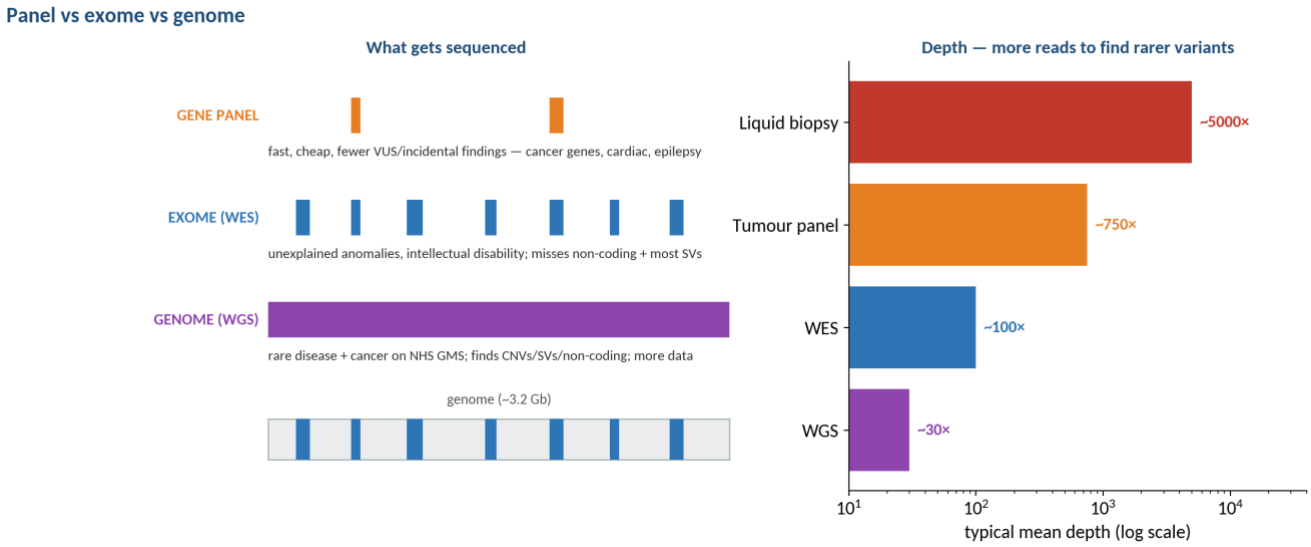


Figure 3. Panel vs exome vs genome; depth.

- **RNA-seq** reads the transcriptome (expression, abnormal splicing). **Amplicon panels** = PCR-enriched hotspots (tumours, pathogens). Short reads struggle with repeats, GC-rich regions, pseudogenes and large structural variants — and **sequencing alone misses large deletions** (add MLPA or use read-depth CNV calling).

3 Long-Read Sequencing

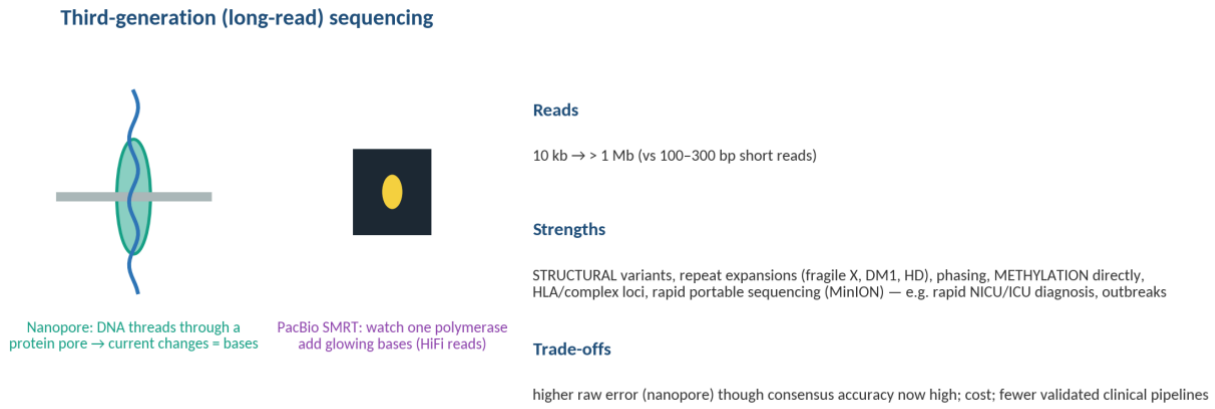


Figure 4. Nanopore and PacBio.

4 From Reads to a Report

Step	Key points
Alignment	Map reads to the reference genome (GRCh38; GRCh37 legacy)
Variant calling	SNVs, indels, CNVs, SVs — results depend on the pipeline
Annotation	Effect on protein/splicing; population frequency (gnomAD); conservation and predictors (CADD, REVEL, SpliceAI)
Classification	ACMG/AMP (UK ACGS) 5 classes — pathogenic, likely pathogenic, VUS, likely benign, benign (Lecture 90)
Confirmation + re-analysis	Sanger/orthogonal confirmation where needed; re-analyse unsolved cases as knowledge grows

5 Clinical Applications

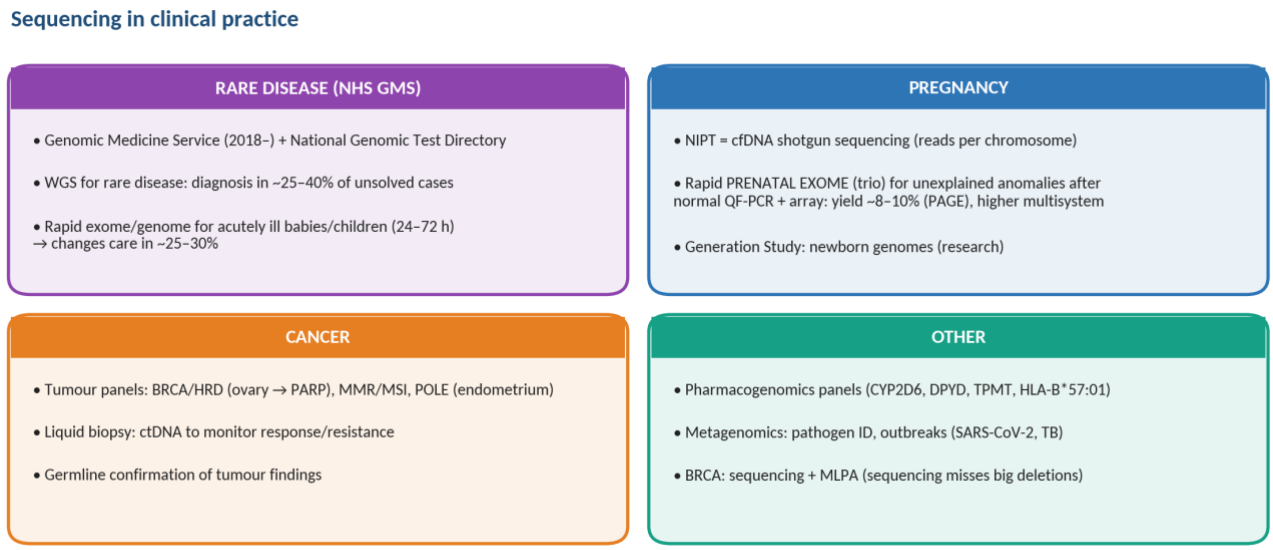


Figure 5. Sequencing in practice.

6 Ethics and Limits

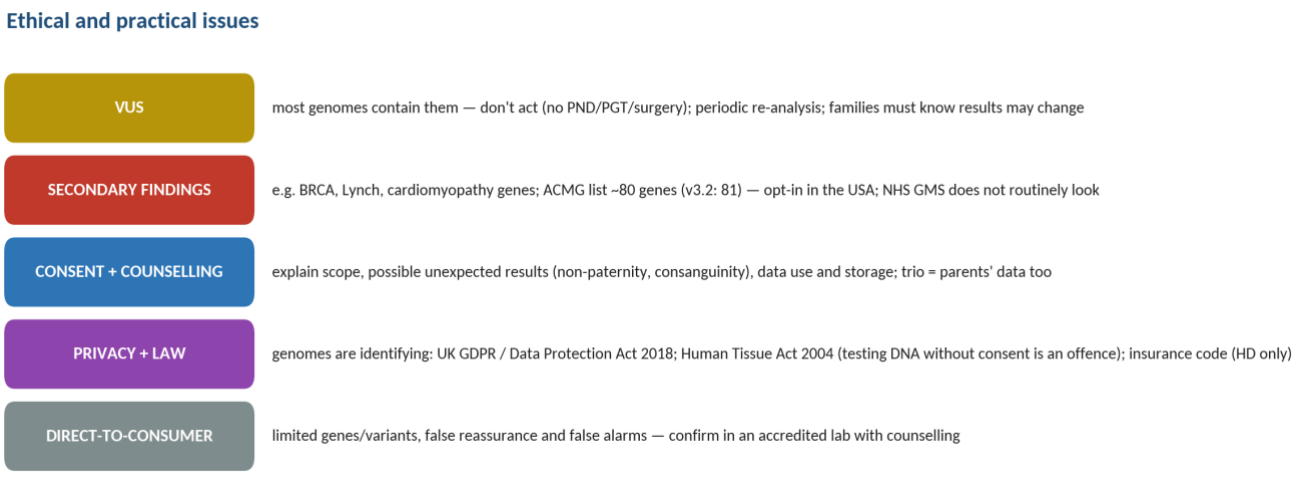


Figure 6. Ethical and practical issues.

★ PEARL

Sanger confirms; NGS discovers. Exome = ~1.5% of the genome; WGS ~30×, WES ~100×, tumour ~500–1000×.

Prenatal exome is for unexplained anomalies AFTER normal QF-PCR + microarray — and never act on a VUS.

7 MRCOG Exam Pearls — Last-Minute Page

Numbers to know

1977 Sanger method	**~800–1000 bp** Sanger read	**100–300 bp** Short reads (NGS)	**> 10 kb** Long reads
~30× / ~100× WGS / WES depth	**~1.5%** Exome share of genome	**~25–40%** WGS rare-disease yield	**~8–10%** Prenatal exome yield (PAGE)

HIGH-YIELD CHECKLIST

- ☐ Sanger: ddNTP chain termination, capillary electrophoresis, > 99.99%, confirms NGS.
- ☐ Sanger misses low-level mosaicism (< 15–20%) and big deletions.
- ☐ NGS: library → clusters → sequencing by synthesis → align → call → interpret.
- ☐ Panel vs WES (~1.5%) vs WGS; depth 30× / 100× / 500–1000×.
- ☐ Long reads: SVs, repeats, methylation, phasing (Nanopore, PacBio).
- ☐ GRCh38; gnomAD; ACMG/ACGS 5 classes; re-analysis.
- ☐ NHS GMS WGS for rare disease/cancer; rapid exome/genome in sick children.
- ☐ NIPT = cfDNA sequencing; prenatal exome after normal QF-PCR + array.
- ☐ Tumour: BRCA/HRD, MMR/MSI, POLE; liquid biopsy.
- ☐ VUS, secondary findings, consent, privacy, DTC tests.

Self-assessment — cover the right-hand column

Question	Answer
Why does a ddNTP stop the chain?	It lacks the 3'-OH needed for the next bond
Main role of Sanger sequencing today?	Confirming NGS variants; single genes/family variants
What does 'depth' mean?	Average number of reads covering each base
Typical WGS depth?	~30×
What fraction of the genome does WES cover?	~1.5% (the exons)
Best technology for repeat expansions and SVs?	Long-read sequencing
Population database used to filter variants?	gnomAD
When is prenatal exome offered?	Unexplained anomalies after normal QF-PCR + microarray
Why add MLPA to BRCA sequencing?	Sequencing misses large deletions/duplications

Question	Answer
Should a VUS guide prenatal decisions?	No

8 References

- Lord J et al. Prenatal exome sequencing analysis in fetal structural anomalies (PAGE). Lancet 2019.
- Miller DT et al. ACMG SF v3.2 list for reporting of secondary findings. Genet Med 2023.
- NHS England. National Genomic Test Directory; Genomic Medicine Service.