PIPADOR REVISION

MRCOG Part 1 Revision Notes

Blotting Techniques

MOLECULAR BIOLOGY • LECTURE 93

VISUAL EXAMINATION REVISION NOTES

2026 Edition

MRCOG Part 1 Revision Notes

Lecture 93: Blotting Techniques

Molecular biology • Visual revision edition

COLOUR CODE USED THROUGHOUT THESE NOTES

SOUTHERN / DNA	NORTHERN / RNA	WESTERN / PROTEIN	PROBES / HYBRIDISATION	CLINICAL

LEARNING OBJECTIVES — tick off as you go

- ☐ Describe the steps of Southern, Northern and Western blotting.
- ☐ Match each blot to its target, separation, probe and use (SNoW DRoP).
- ☐ Explain probes, labels and stringency.
- ☐ Know where blots are still used (fragile X, dystrophin, Lyme) and what replaced them.
- ☐ Link hybridisation to FISH and microarray.

1 The Blotting Family

IN ONE LINE: *Separate molecules on a gel → transfer ('blot') them to a membrane → find the one you want with a labelled probe (nucleic acid) or antibody (protein).*

SNoW DRoP — Southern = DNA, Northern = RNA, Western = Protein

SOUTHERN (DNA)	NORTHERN (RNA)	WESTERN (PROTEIN)
Separation: restriction digest → agarose gel	Separation: DENATURING gel (formaldehyde)	Separation: SDS-PAGE (by molecular weight)
Probe: labelled DNA probe	Probe: labelled DNA/RNA probe	Probe: ANTIBODIES (primary + secondary)
Use: fragile X full mutation + methylation; RFLP (historic)	Use: mRNA size + amount — now RT-qPCR / RNA-seq	Use: dystrophin in muscle; Lyme immunoblot

Spin-offs: SOUTHWESTERN (DNA-binding proteins, probed with DNA) • FAR-WESTERN (protein–protein, probed with protein) • EASTERN (post-translational modifications) — research only.

Figure 1. Southern, Northern, Western.

MNEMONIC / MEMORY HOOK

'SNoW DRoP' — Southern = DNA, Northern = RNA, Western = Protein (named as a joke after Edwin Southern).

2 Southern Blot

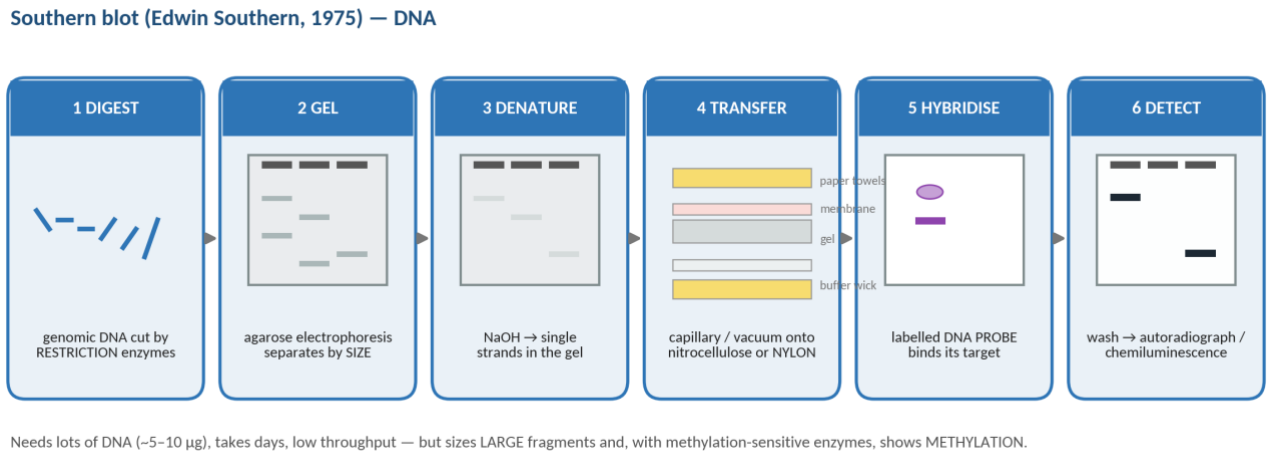


Figure 2. Southern blot steps.

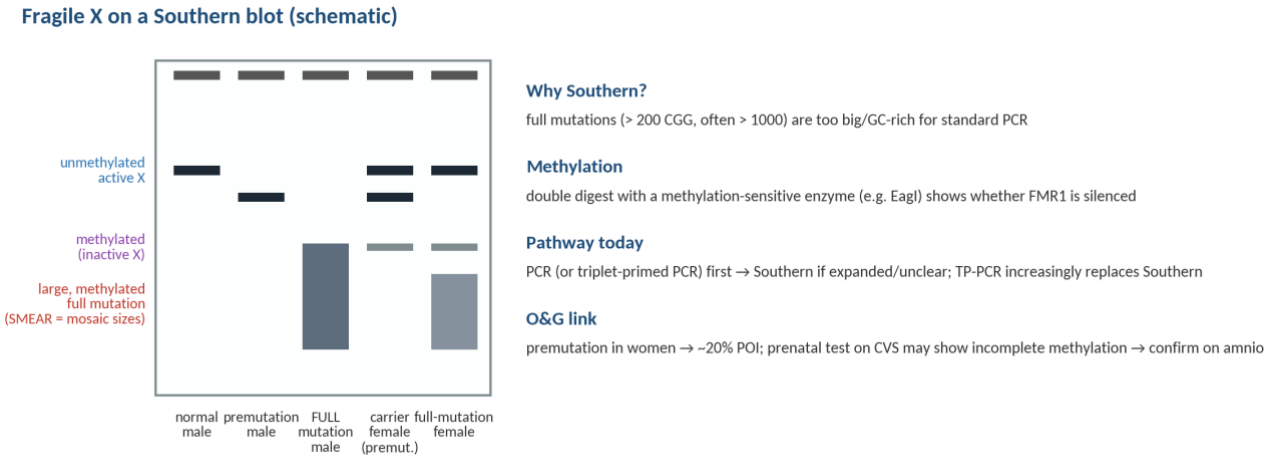


Figure 3. Fragile X on a Southern blot.

3 Northern Blot

- RNA run on a **denaturing** gel (formaldehyde/glyoxal keeps it unfolded) → transfer → labelled probe. Shows transcript **size** (splice isoforms) and amount. Fragile (RNases everywhere) and less sensitive than **RT-qPCR** or **RNA-seq**, which have replaced it clinically.

4 Western Blot

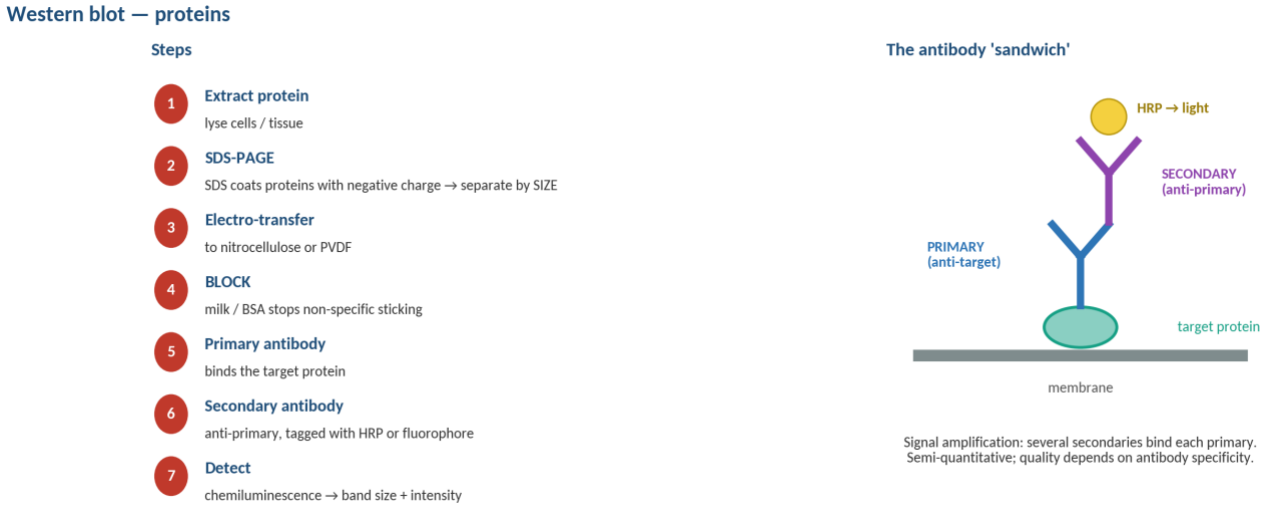


Figure 4. Western blot.

- **Uses**: dystrophin in muscle biopsy (absent = Duchenne; reduced/smaller = Becker), Lyme immunoblot, research. Historically HIV and hepatitis C confirmation. **Limits**: semi-quantitative, antibody-dependent, slow.

5 Probes and Hybridisation

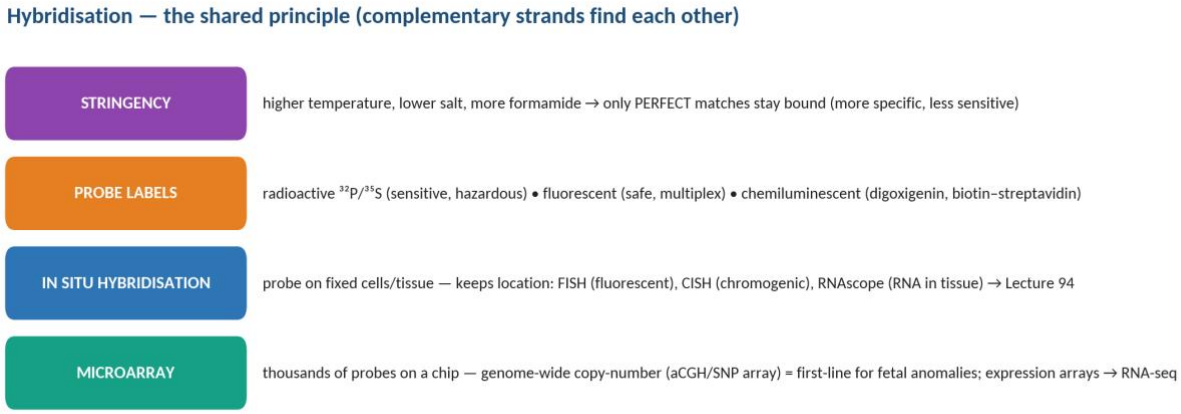


Figure 5. Hybridisation principles.

6 Clinical Correlates

Blots in clinical practice — then and now

Indication	THEN	NOW
HIV	ELISA → WESTERN BLOT confirmation	4th-generation Ag/Ab test; confirm with different assays ± HIV RNA (antenatal screening — opt-out)
LYME	ELISA → Western (two-tier)	UK (NICE NG95): ELISA; if positive/equivocal → IMMUNOBLOT; immunoblot also if ELISA −ve but symptoms > 12 wk
FRAGILE X	Southern blot for sizing + methylation	PCR / triplet-primed PCR first; Southern for full mutations / methylation when needed
DMD	muscle biopsy Western for dystrophin	MLPA + sequencing first; biopsy Western/immunostaining if genetics unclear or to separate DMD vs BMD
PATERNITY / FORENSIC	RFLP Southern	STR PCR (capillary electrophoresis)
GENE EXPRESSION	Northern blot	RT-qPCR, RNA-seq

Figure 6. Then and now.

STILL USED

- **Southern**: fragile X full mutation size + methylation (when PCR/TP-PCR can't resolve); some large rearrangements
- **Western**: dystrophin protein; Lyme immunoblot (second step)
- Reference/research methods

WHY REPLACED

- PCR, qPCR, MLPA, sequencing are faster, more sensitive, need far less sample, automate easily
- Blots: days, µg of DNA, often radioactivity, low throughput
- Microarray = hybridisation scaled to thousands of probes

★ **PEARL**

SNoW DRoP. Southern blot still sizes fragile X full mutations and shows methylation; Western blot still checks dystrophin.

UK Lyme testing (NICE NG95): ELISA first → immunoblot if positive/equivocal (or if negative but symptoms > 12 weeks).

7 MRCOG Exam Pearls — Last-Minute Page

Numbers to know

1975 Southern blot	**~5–10 µg** DNA for a Southern	**Days** Southern turnaround	**> 200** CGG = full mutation
~20% FXPOI in premutation	**> 12 wk** Lyme symptoms → immunoblot	**3** Main blots (S, N, W)	**2** Antibodies in a Western

HIGH-YIELD CHECKLIST

- ☐ SNoW DRoP: Southern DNA, Northern RNA, Western protein.
- ☐ Southern: digest → gel → NaOH → transfer → probe → detect.
- ☐ Northern: denaturing gel; replaced by RT-qPCR/RNA-seq.
- ☐ Western: SDS-PAGE → transfer → block → primary → secondary (HRP).
- ☐ Southwestern, Far-Western, Eastern — research.
- ☐ Stringency: ↑ temperature, ↓ salt → more specific.
- ☐ Labels: radioactive, fluorescent, chemiluminescent.
- ☐ Fragile X: PCR/TP-PCR first, Southern for full mutation + methylation.
- ☐ DMD: genetics first; Western on biopsy for dystrophin.
- ☐ HIV: 4th-gen test (no Western); Lyme: ELISA → immunoblot.

Self-assessment — cover the right-hand column

Question	Answer
What does a Northern blot detect?	RNA
First step of a Southern blot?	Restriction enzyme digestion of genomic DNA
Why is NaOH used in a Southern?	To denature DNA to single strands for probing
What separates proteins in a Western?	SDS-PAGE (by molecular weight)
Why block the membrane?	To prevent non-specific antibody binding
Clinical role of Southern blot today?	Fragile X full mutation size + methylation
Western blot finding in Duchenne?	Absent dystrophin
What does high stringency favour?	Perfectly matched probe binding
UK second-line Lyme test?	Immunoblot (Western)
What replaced the Northern blot?	RT-qPCR and RNA sequencing

8 References

- Southern EM. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J Mol Biol 1975.
- NICE NG95. Lyme disease. 2018.
- Strachan T, Read A. Human Molecular Genetics. 5th ed. 2018.