



Dr Elizabeth Glennon

Module:
Techniques in Neuroscience

Week 5:
Molecular biology: Going inside the cell

Topic 1:
**An introduction to molecular
biology methods**
Part 3 of 3

Part 3

Lecture outline



In this section, we will study the western blotting process:

1 Western blotting

2 Limitations of western blotting

3 Examples of western blotting

Click **Next** to continue

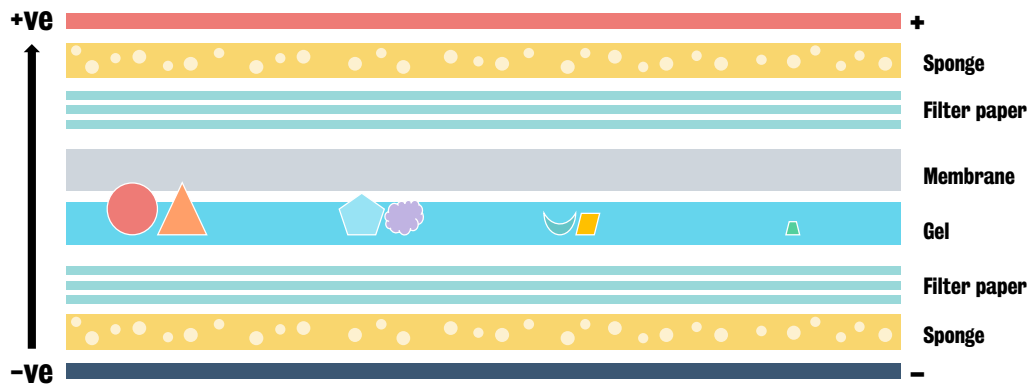
Introduction to western blotting



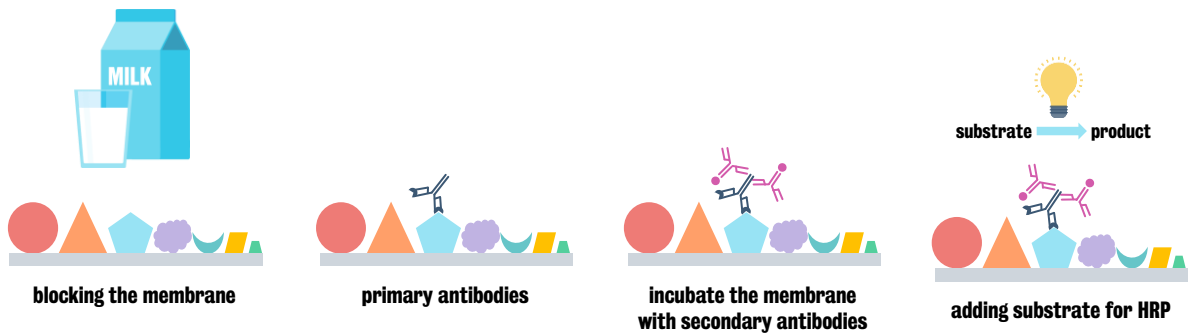
Introduction

- western blotting is used to detect specific proteins from a mixture of proteins
- during western blotting, we are processing further the polyacrylamide gel
- the technique is used to transfer the proteins from the gel onto a membrane

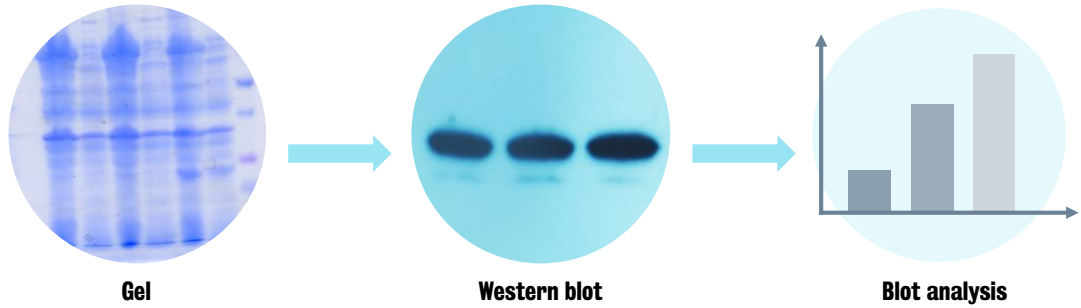
Introduction to western blotting



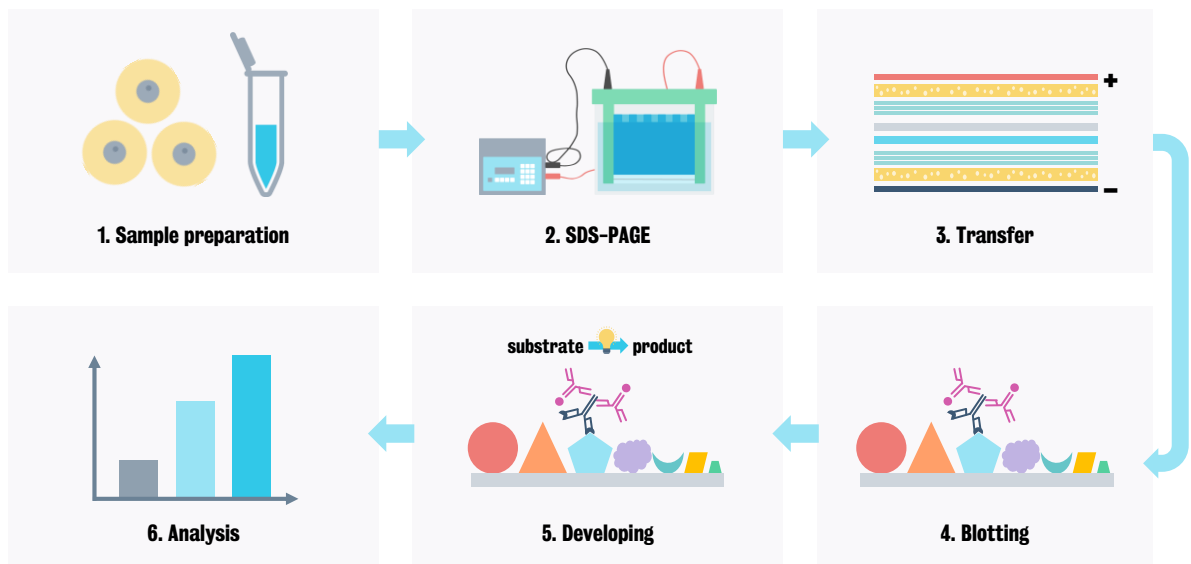
Introduction to western blotting



Introduction to western blotting



Introduction to western blotting



Why use western blotting?



Advantages:

- takes 1 1/2 days (can be faster)
- does not require a lot of specialist equipment
- will work for a wide range of proteins
- can be sensitive and specific with good antibodies
- can be applied to many cells/tissues/experimental procedures
- semi-quantitative



Disadvantages:

- only as good as your antibody
- antibodies are expensive
- post-translational modifications may alter binding or mobility
- does not work as well if your proteins are very large or very small
- reasonably sensitive, but does not work well if protein levels are low
- semi-quantitative

Real life examples of western blotting: BIN1

Example 1: BIN1



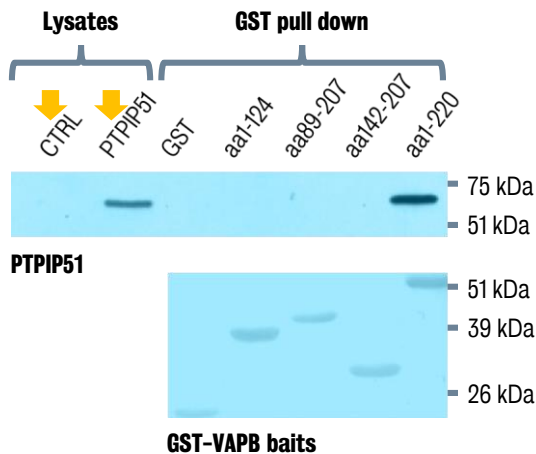
- BIN1 levels in people with AD, compared to age-matched controls
- BIN1 has an unclear role in the development of AD
- the authors determined how BIN1 is altered in AD

Western blotting showed a signal reduction in the AD samples compared to control, thus indicating a reduction of the protein.

Glennon et al. (2013)

Real life examples of western blotting: PTPIP51 and VAPB

Example 2: PTPIP51 and VAPB



- the aim was to investigate the interaction of motor neuron disease-linked proteins PTPIP51 and VAPB
- cells were transfected with an empty vector or the vector which codes for PTPIP51 and incubated with fragments of VAPB
- VAPB fragments were immobilised on beads
- cell lysate was incubated with the beads, and everything that bound to them could be determined by western blotting
- the blot showed that PTPIP51 only bound to the full length VAPB protein
- PTPIP51 binds to a 3d epitope that's formed when the entire VAPB protein folds

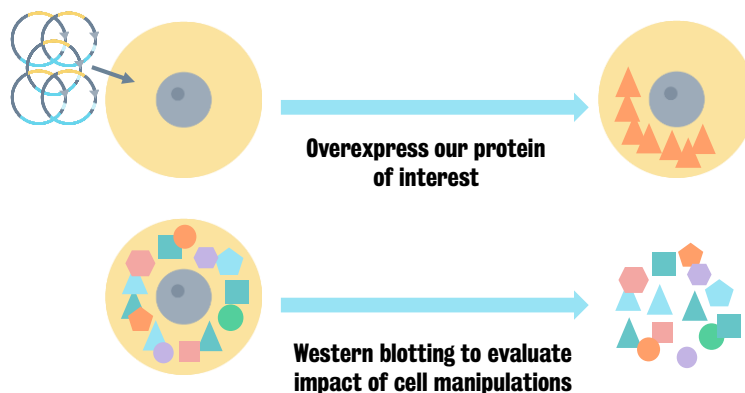
Stoica R et al. (2014)

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Concluding remarks



- we are dealing with an artificial system which can be affected by a range of factors
- important to use a variety of techniques to understand molecular events inside the cell

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References

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Glennon, E. B., Whitehouse, I. J., Miners, J. S., Kehoe, P. G., Love, S., Kellett, K. A., & Hooper, N. M. (2013). BIN1 is decreased in sporadic but not familial Alzheimer's disease or in aging. *PLoS One*, 8(10), e78806.

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End of topic