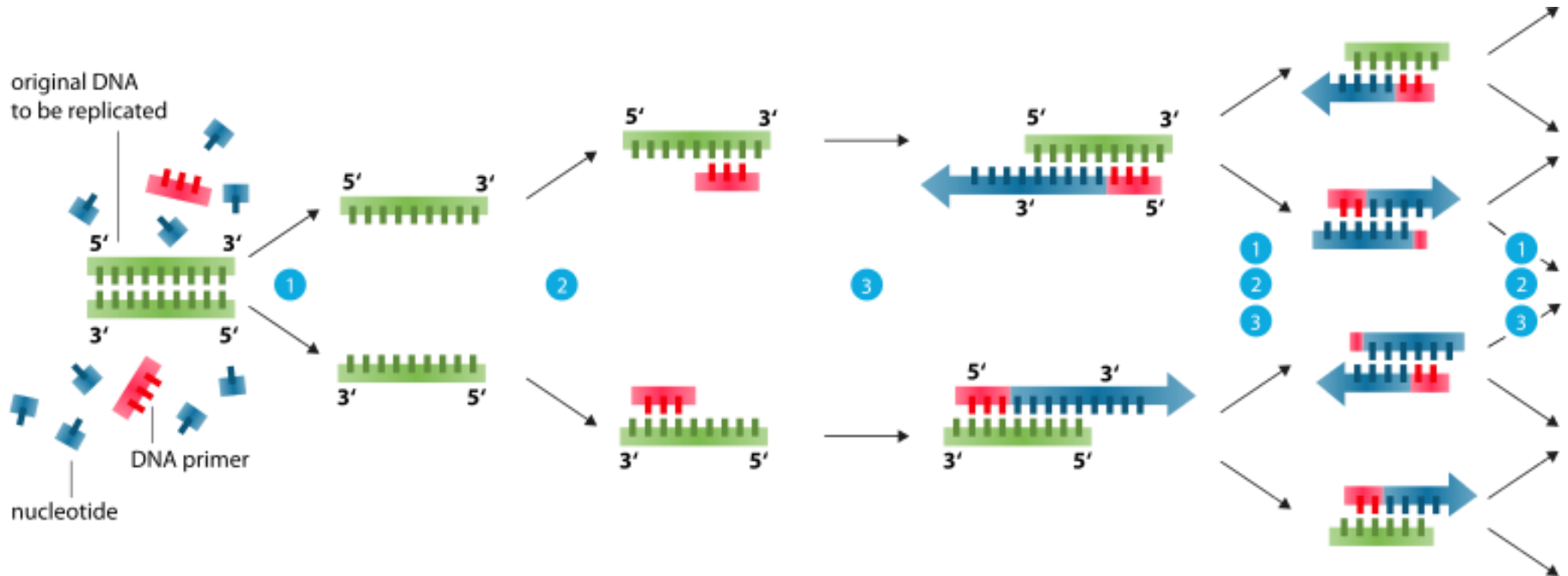


Blotting & PCR

Prof. Dr. Andrei Leitão

Polymerase Chain Reaction (PCR)

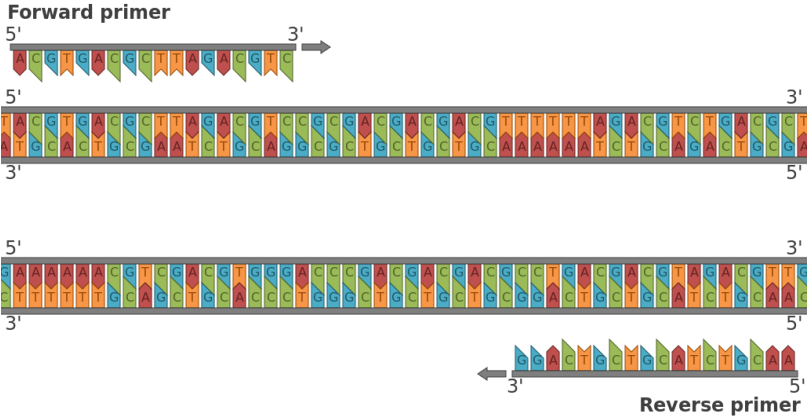
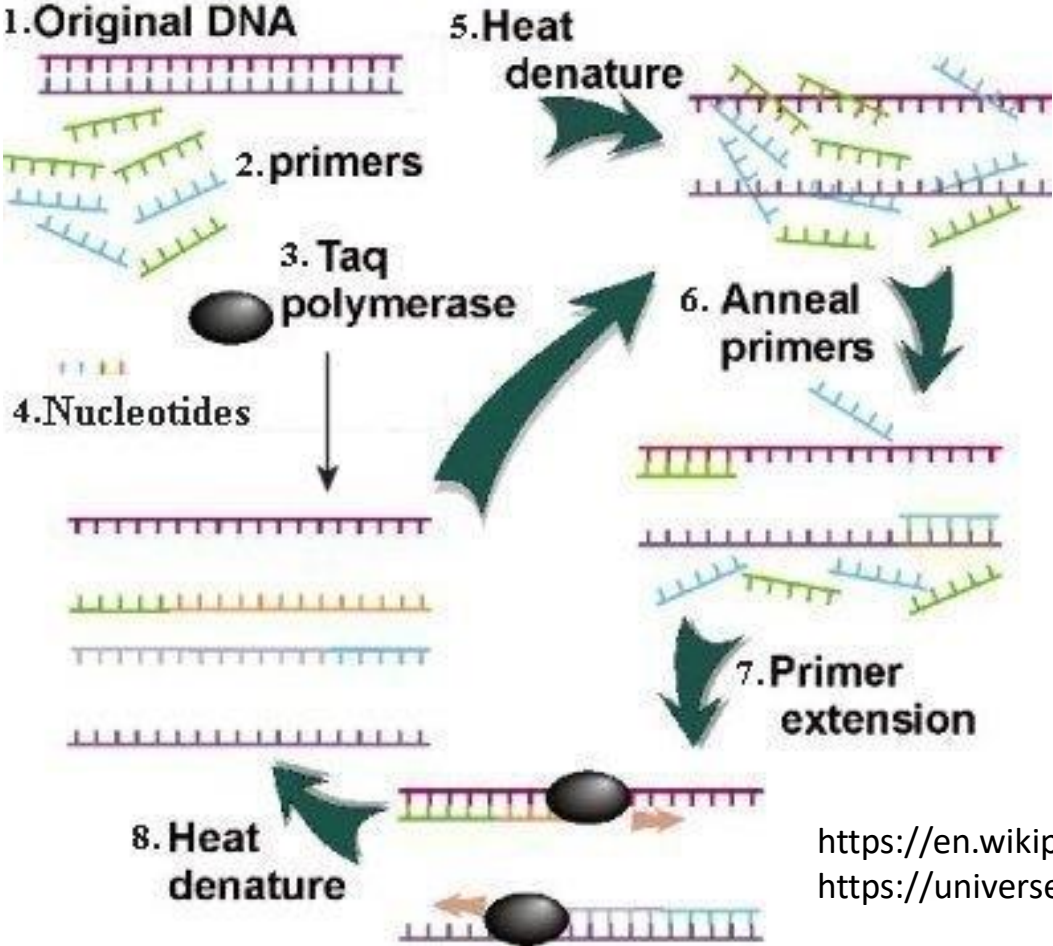


- 1 **Denaturation** at 94-96°C
- 2 **Annealing** at ~68°C
- 3 **Elongation** at ca. 72 °C

https://en.wikipedia.org/wiki/Polymerase_chain_reaction
<https://universe-review.ca/R11-16-DNAsequencing.htm>

Polymerase Chain Reaction (PCR)

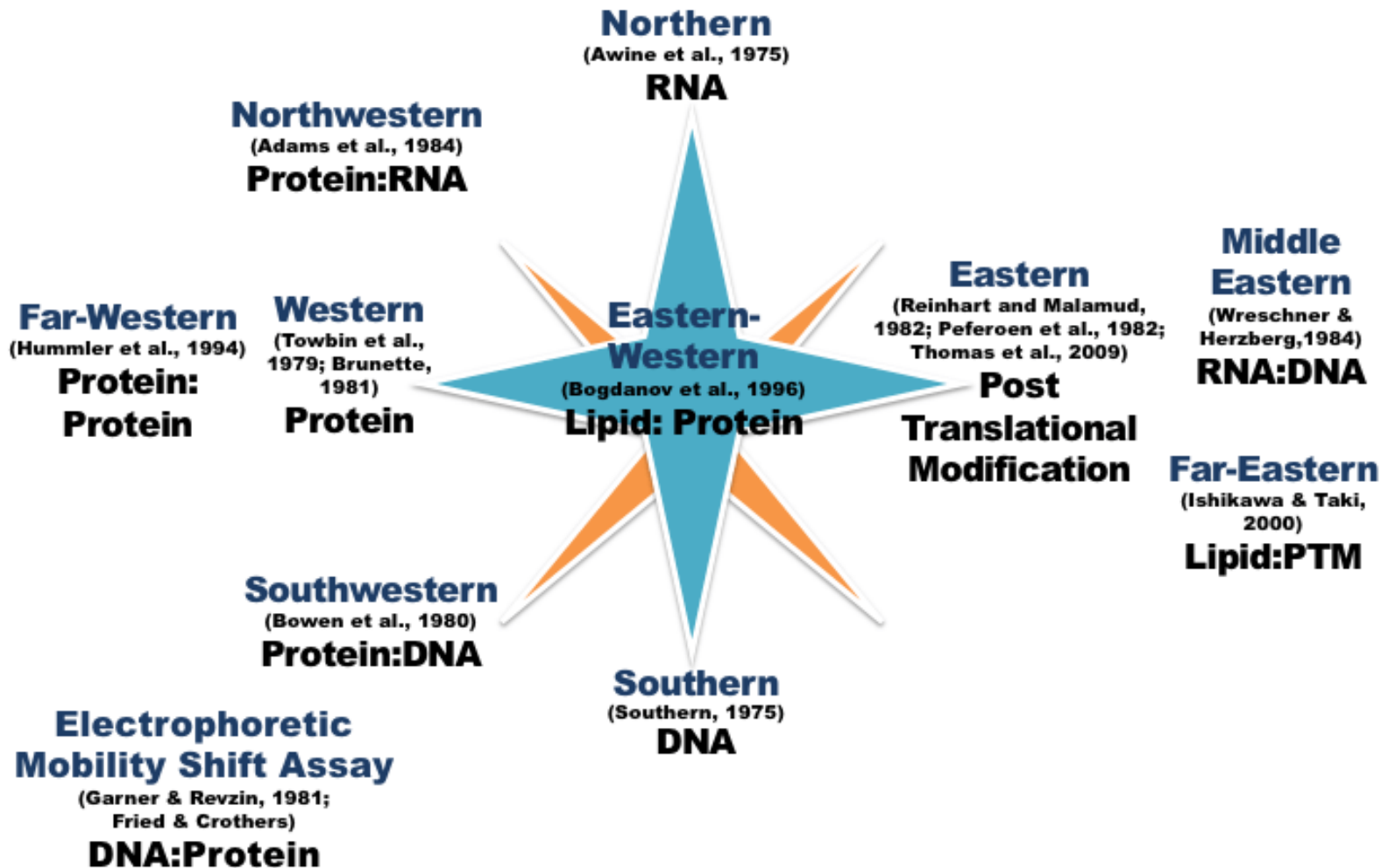
The PCR Process



https://en.wikipedia.org/wiki/Polymerase_chain_reaction
<https://universe-review.ca/R11-16-DNAsequencing.htm>

Blots

Blotting Compass



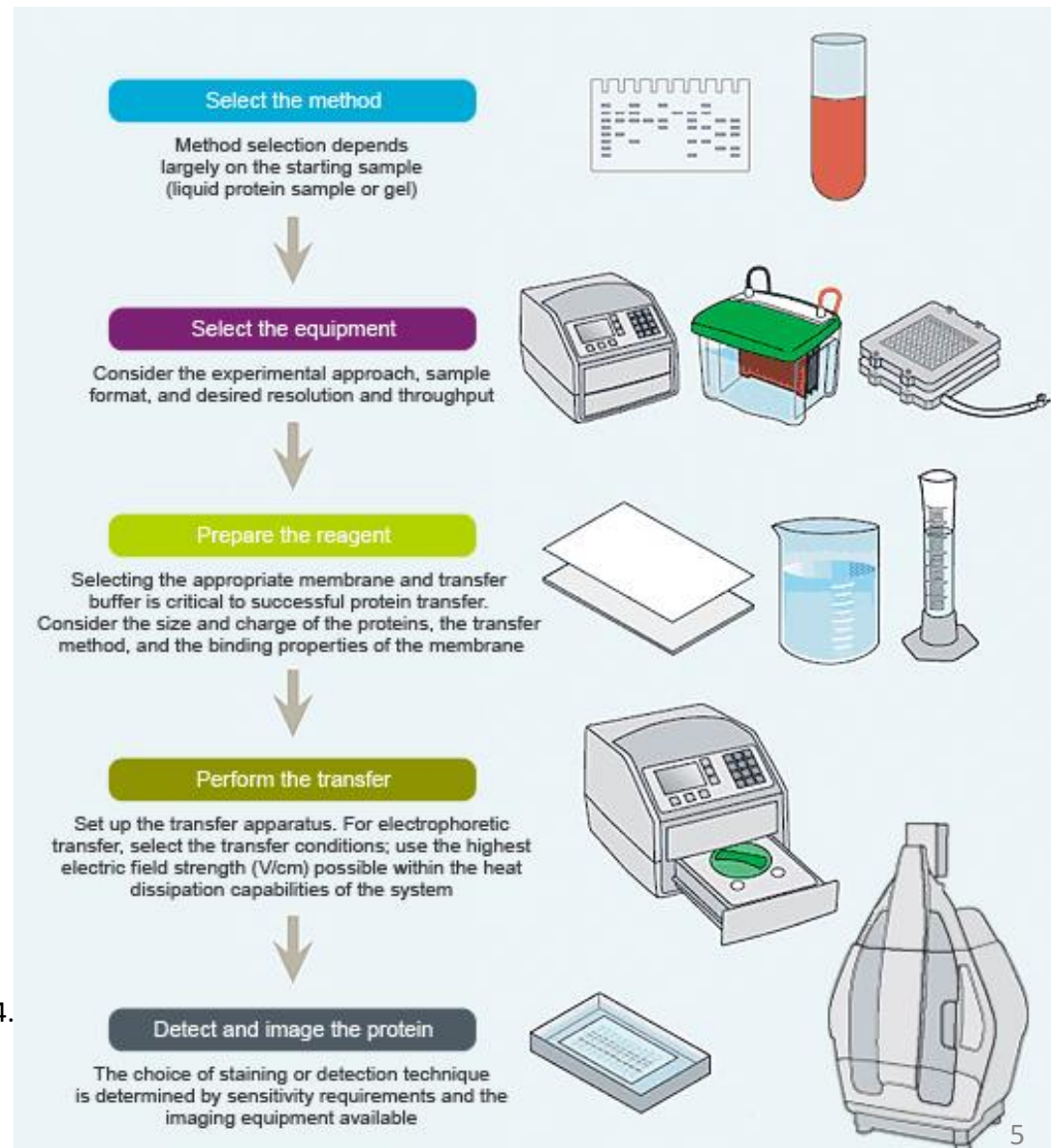
Western blot - basics

The most commonly used protein blotting technique, western blotting (immunoblotting), was developed to probe for proteins that were inaccessible to antibodies while in polyacrylamide gels. Western blotting refers specifically to the immunological detection of proteins that have been separated by gel electrophoresis and transferred onto a membrane.

<http://www.bio-rad.com/pt-br/applications-technologies/introduction-western-blotting>

<http://www.spacesrl.com/wp-content/uploads/2011/03/WesternBlottingBrochure.pdf>

Mahmood T, Yang P. *North Am. J. Med. Sci.* **2012**,4, 429-434.



Western blot - application

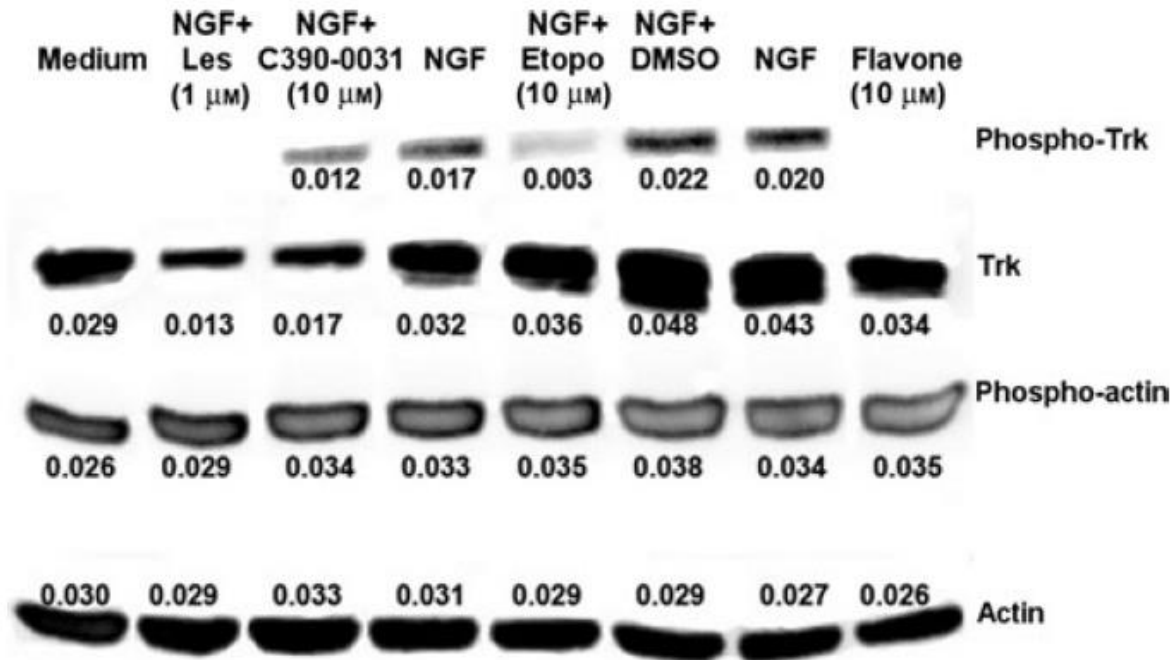
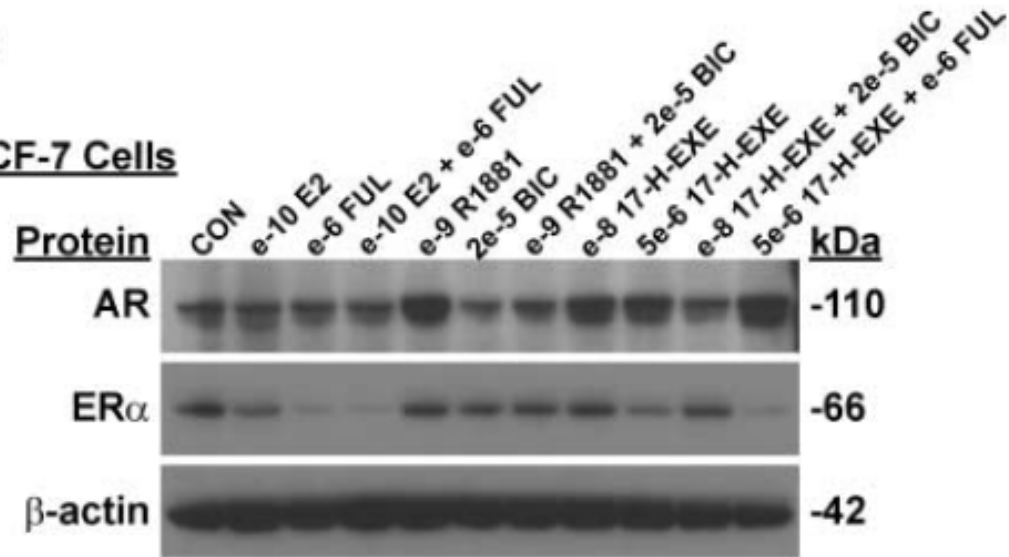


Figure 6: TrkA phosphorylation activity and its inhibition by small chemicals. Normalization and the semiquantitative analyses were established after the depicted numbers. Actin was the control for all experiments.

Western blot - application

A

MCF-7 Cells



B T47D Cells

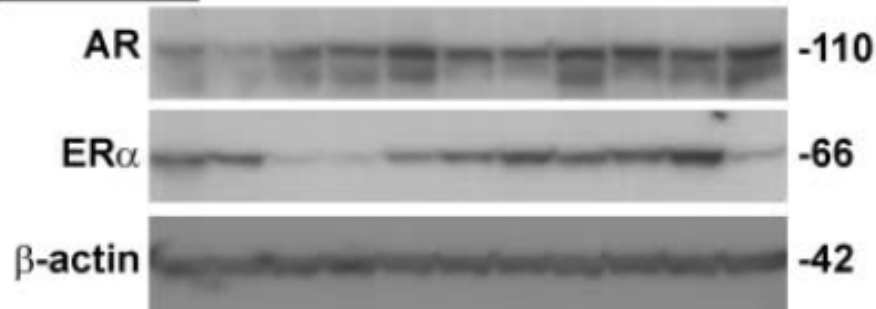


Figure 4. 17-Hydroxemestane modulates AR and ER α protein levels. Immunoblot analysis of AR and ER α in (A) MCF-7 cells and (B) T47D cells. Cells were treated as indicated for 24 h, and 20 μ g of cellular protein were resolved by 4% to 12% SDS-PAGE and then transferred to a nylon membrane. Membranes were probed for AR, ER α , and β -actin, and immunoreactive bands were visualized by chemiluminescence and autoradiography. Cropped blots are shown. 17-hydroxemestane up-regulated AR protein levels at 10^{-8} mol/L in both cell lines and down-regulated ER α in MCF-7 cells at 5×10^{-6} mol/L.

Ariazi, E.A. ; Leitao, A.; et al.
Mol. Cancer Ther. **2007**, *6*, 2817-2827.