

Practical 3
SDS-PAGE and Western blot
(Immunoblot)

**Cell culture, protein isolation,
protein electrophoresis, protein
transfer**

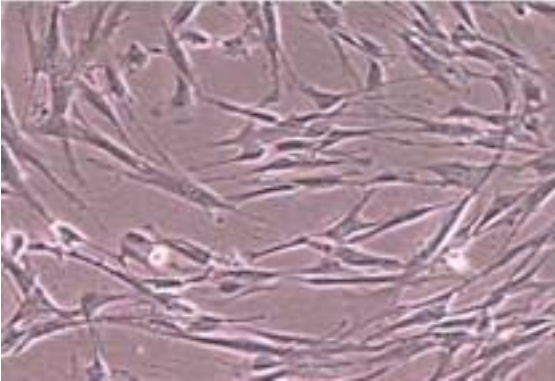
Cell (Tissue) Culture



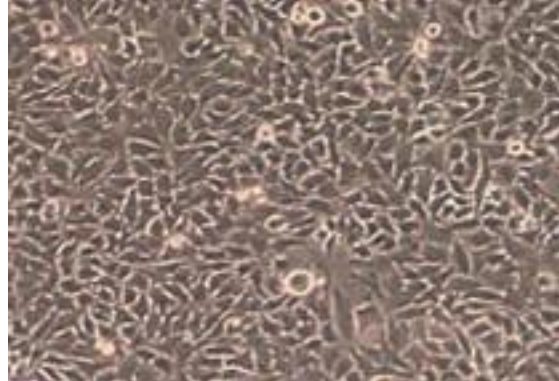
Cell culture - classification

- **Primary cultures** – cells isolated from tissues and grown in the culture. Can only grow for a short period of time.
- **Cell lines** – immortal cells, transformed (HeLa, U2OS, HEK293). Often derived from tumors.
- **Cell strain** - a subpopulation of a cell line positively selected from the culture by cloning or some other method
- **Hybrid cell lines** – fusion of two different cell types

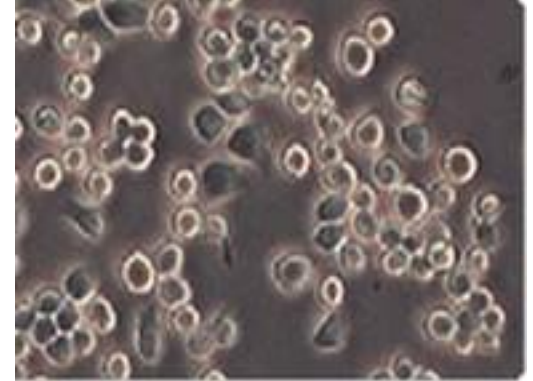
Cell morphology



Fibroblasts



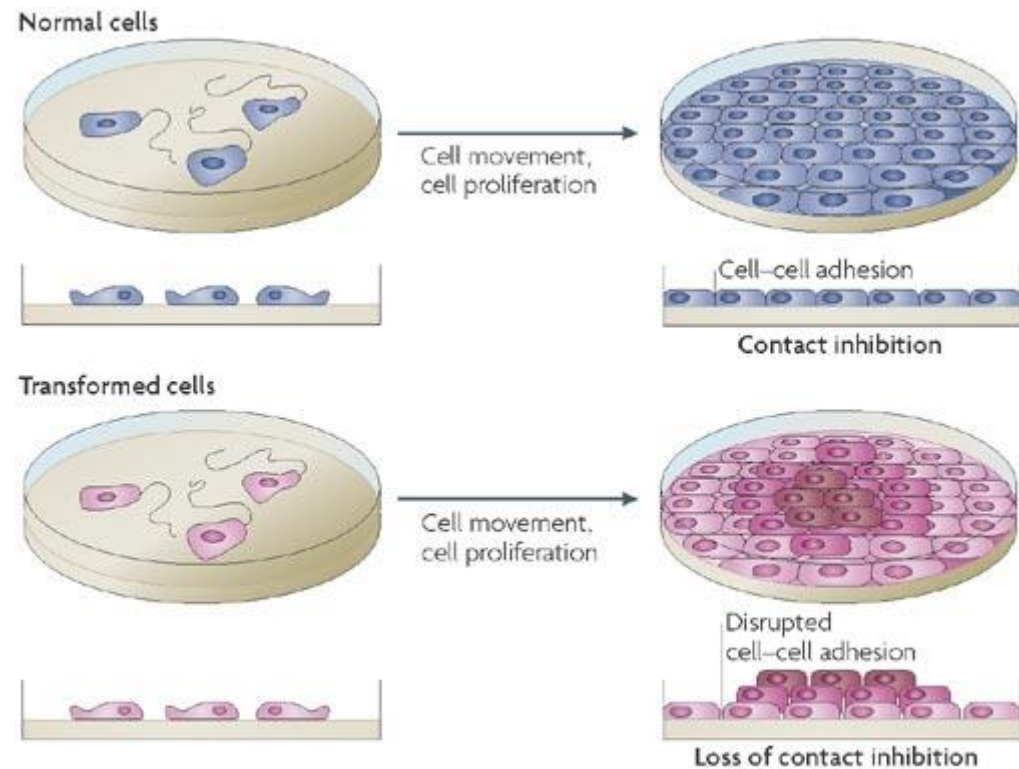
Epithelial cells



Lymphoblasts

Contact inhibition

- Property of normal adherent cells to stop dividing and moving when they reach **full confluence**
- Normal cells grow in a single layer on a plate
- The transformed cells grow in 3D, grow over each other and have lost contact inhibition property

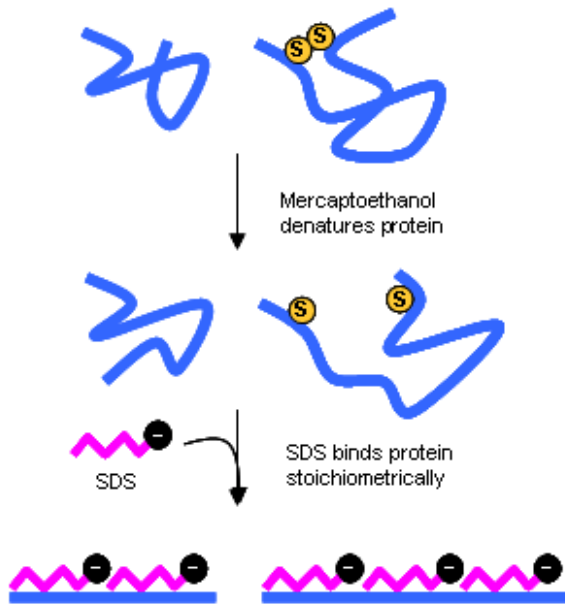


Culture conditions

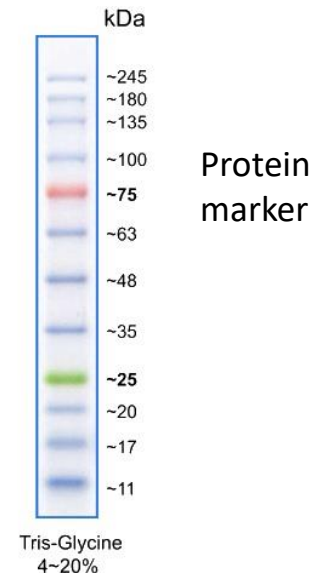
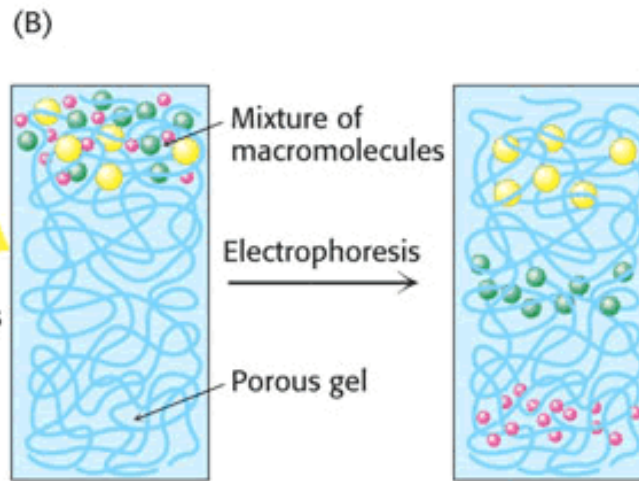
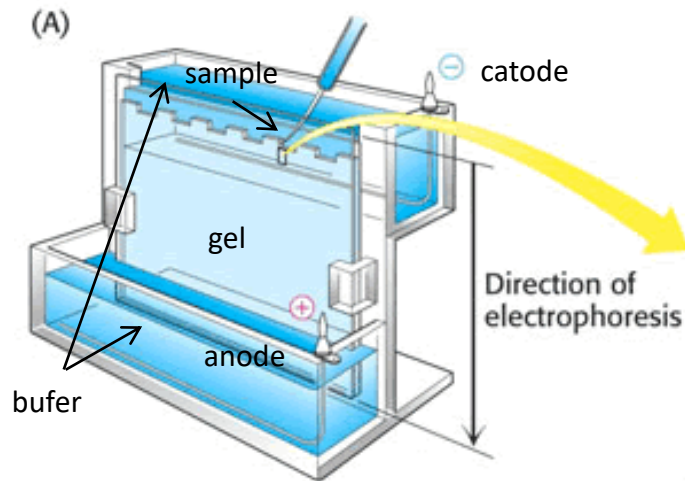
- Cells are growing in medium (Dulbecco's modified Eagle's medium, **DMEM**) supplemented with 10% **fetal bovine serum** (FBS) and antibiotics (100 U/ml penicillin/streptomycin) at 37°C, 5% CO₂.
- **Trypsin** – enzyme used for passaging adherent cells (detaching adherent cells from substratum)
- **Inverted** microscope
- **Transfection** – protocol for introducing foreign DNA into eukaryotic cells

SDS-PAGE

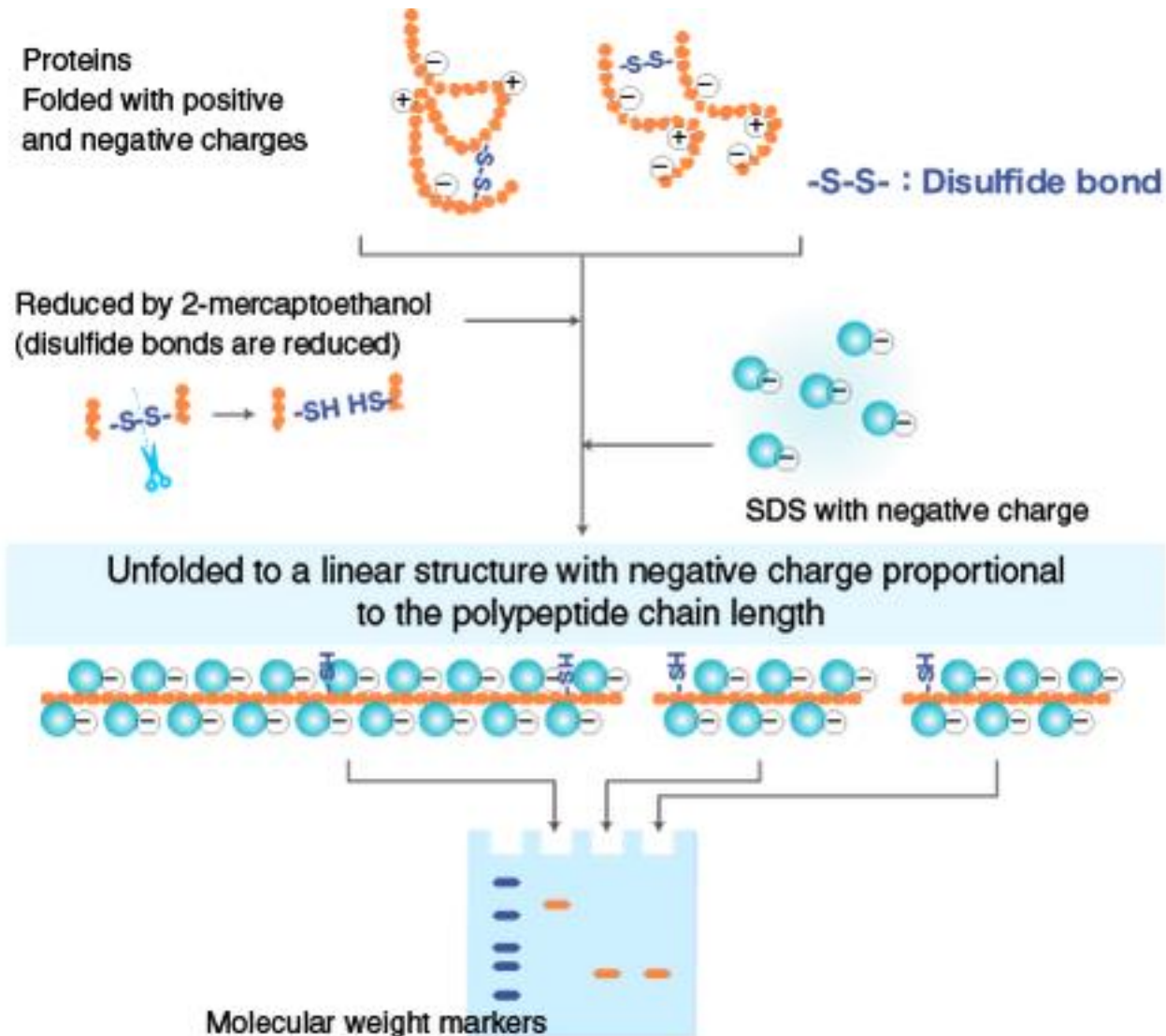
Sodium Dodecyl Sulphate-PolyAcrylamide Gel Electrophoresis



All proteins will have the same charge to mass ratio
aminoacid:SDS = 2:1
Smaller proteins travel faster through the gel

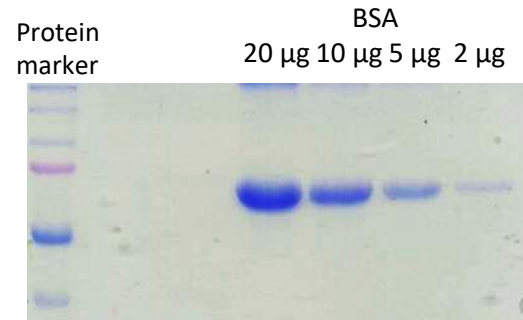


Western Blot



“In gel” protein visualization

- Coomassie brilliant blue



- Silver staining



Transfer of proteins to the membrane

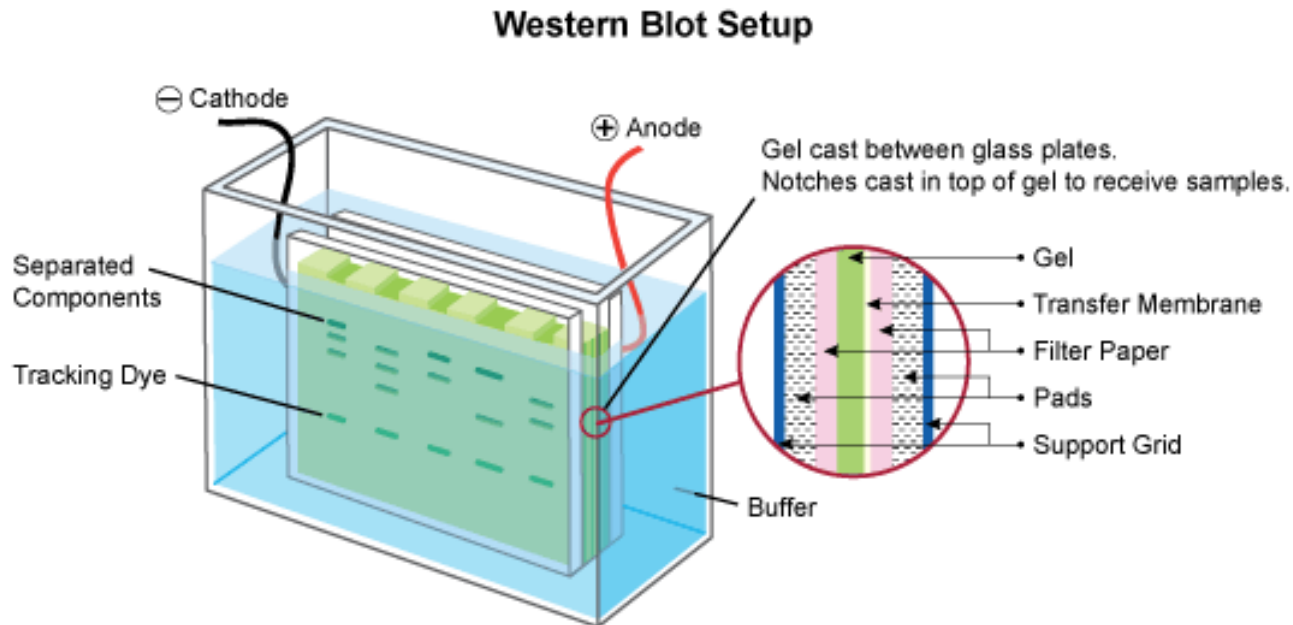
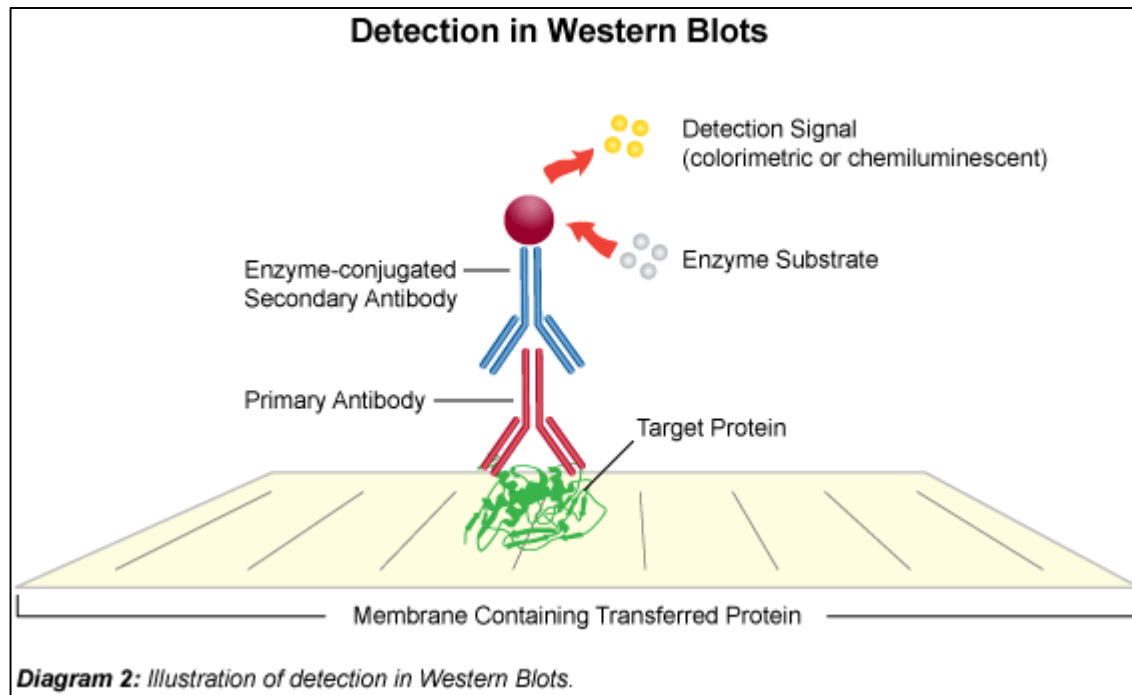
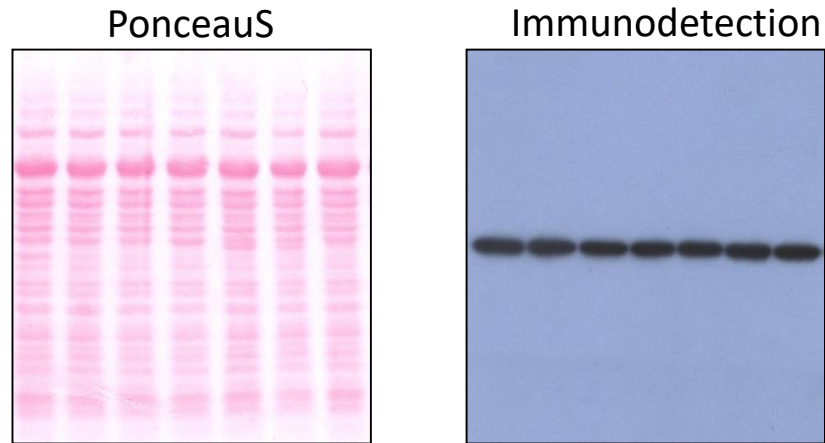


Diagram 1: Illustration of Western Blot Setup.

- Nitrocellulose membrane
- PVDF (Polivinilidenfluorid) membrane

WESTERN BLOT

1. **PonceauS** - reversible staining of the membrane
2. **BLOCKING** of the membrane in **5%BSA** (*Bovine serum albumin*)
3. **PRIMARY ANTIBODY** staining overnight, or 1-2h room temp.
4. **WASH** in TBS Tween-20 buffer (*Tris Buffered Saline*)
5. **SECONDARY ANTIBODY** staining
6. **WASH** in TBS Tween-20 buffer (*Tris Buffered Saline*)
7. **ILUMINOGEN** staining HRP-conjugate
8. **VISUALIZATION**

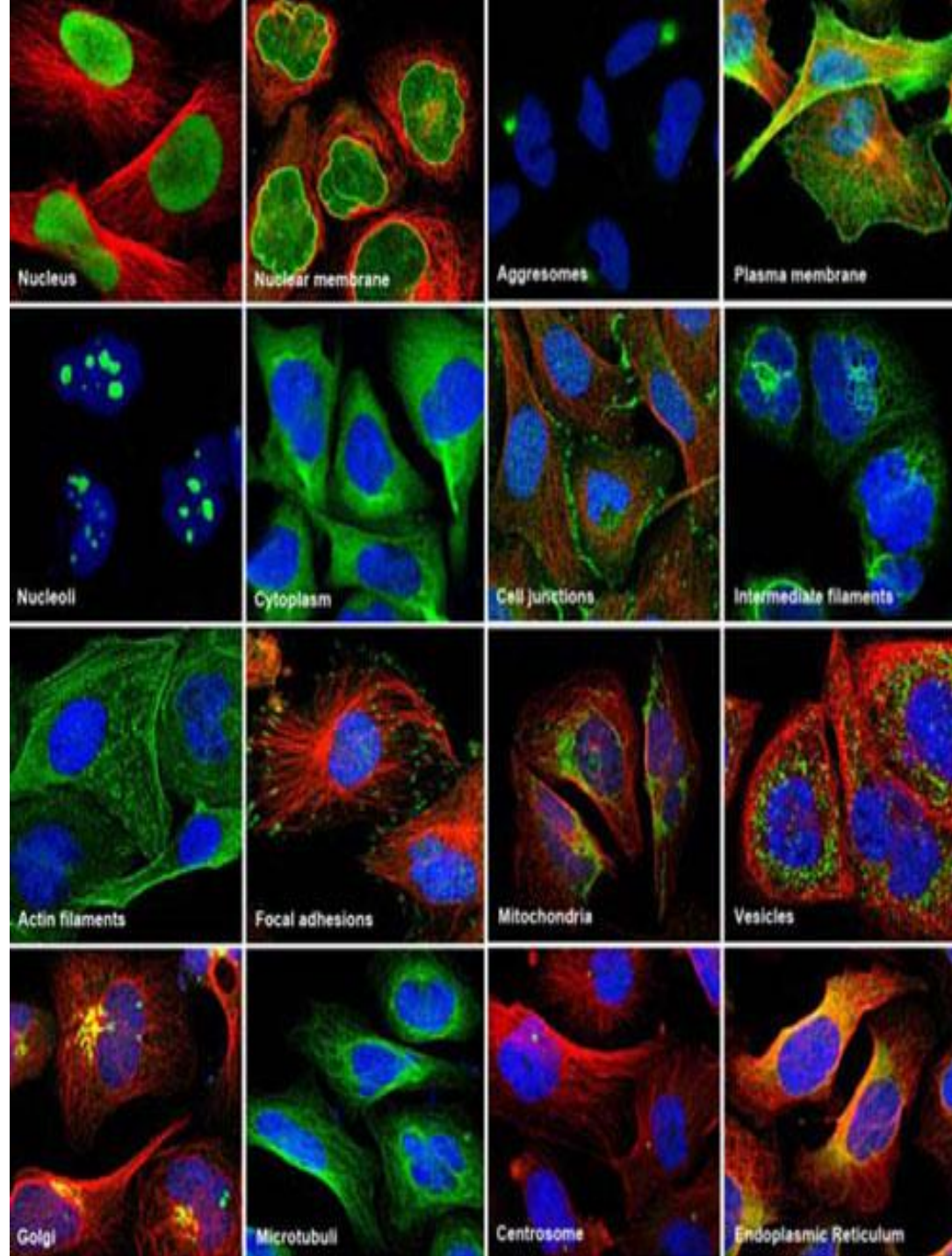


SDS PAGE <http://www.youtube.com/watch?v=toPpdoBYPWo>

WB https://www.youtube.com/watch?v=loVzpL_heFo

Immunofluorescence

<http://www.youtube.com/watch?v=pteO6FRWo3g>



Immunohistochemistry

