WESTERN BLOTTING FOR QUANTIFYING PROTEIN SIGNAL TRANSDUCTION

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“Housekeeping” reference proteins (e.g., Hsp90, Gapdh, tubulin)

Signaling pathway enzymes (e.g., ERK)

Source: Hanahan & Weinberg, Cell (2000)
Growth factor stimuli (e.g., EGF)

Kinase enzymes are phosphorylated ("P") in a cascade

Goals:
Develop quantitative protein assays for:
• phospho-ERK
• 3 housekeeping proteins

WESTERN BLOT

- Separates proteins based on mass and charge
- C2C12 (mice myoblast) cell line used
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- **Cells are thawed and incubated in growth media**
- **Gel prepared with 10% APS**
- **BCA Assay run with lysed C2C12’s**
WESTERN BLOT

- Gel loaded with 30 uL/lane
- The gel is run between 1-2hrs at 100V
WESTERN BLOT

- Protein from the gel is transferred to a membrane
- The membrane is blocked with a 5% milk solution to reduce non-specific antibody bonding
- Primary antibody (diluted in the milk mixture at 1:1,000 or 1:5,000 typ.) added to membrane
- Membrane rinsed with TBS-T
- Secondary antibody added
WESTERN BLOT
WESTERN BLOT
BLOTS

- Alpha Tubulin (1:400)
- 50 kDa
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GAPDH (1:5,000)
37 kDa
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- HSP90 (1:1,000)
- 90kDa
- GAPDH 37 kDa
CONCLUSION

- Western blots can determine the abundance of specific proteins in a cell
- Abundance of certain proteins provides information about signal transduction
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