Molecular Biology Techniques

USABO

Basics

- PCR
- Sanger method for sequencing
- Microscopy techniques
  - Light
  - Fluorescence
    - XFP tagging, bleaching, toxicity
  - Electron
  - (Dis)advantages of each type
  - Basic preparation steps
- Northern, Southern, Western blots

Protein Gel Electrophoresis/Western

- Native vs. denaturing (SDS-PAGE)
- Specialized: 2D gels, pH gradients (pI)
- Phosphate-state specific antibodies
- Monoclonal vs. polyclonal

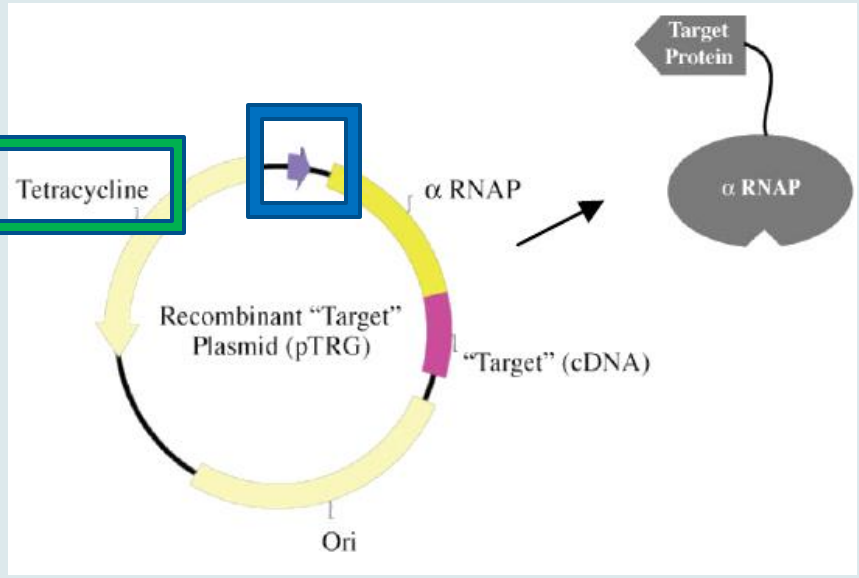
Western Blot

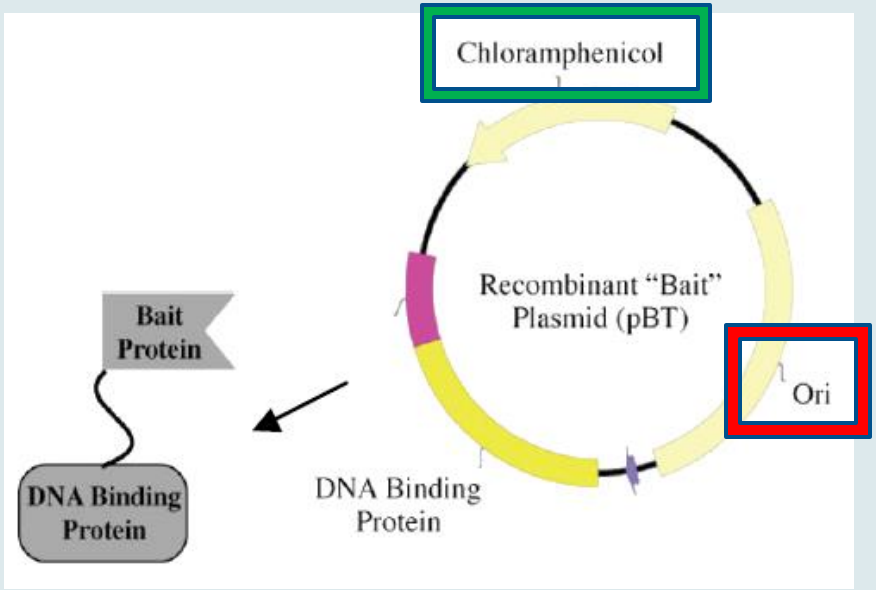
- Phosphate-state specific antibodies
- Monoclonal vs. polyclonal

Protein-Protein Interactions: Two-Hybrid

Bait fusion: DNA binding protein + bait protein

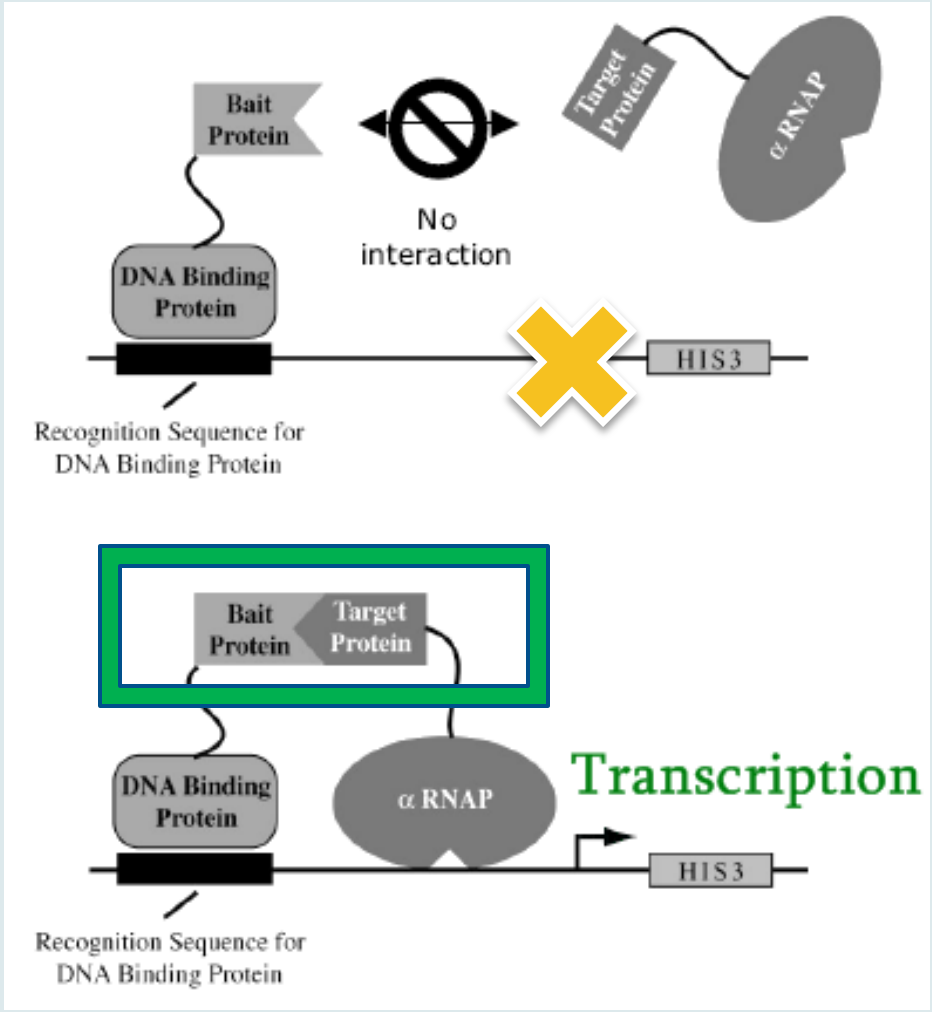
Target fusion: RNA polymerase + target protein





Protein-Protein Interactions: Two-Hybrid

- Bait fusion protein binds sequence upstream of reporter and recruits polymerase iff bait and target interact
- Use with screens
- Usually in yeast
- Sometimes in bacteria
- What are the (dis)advantages of each?

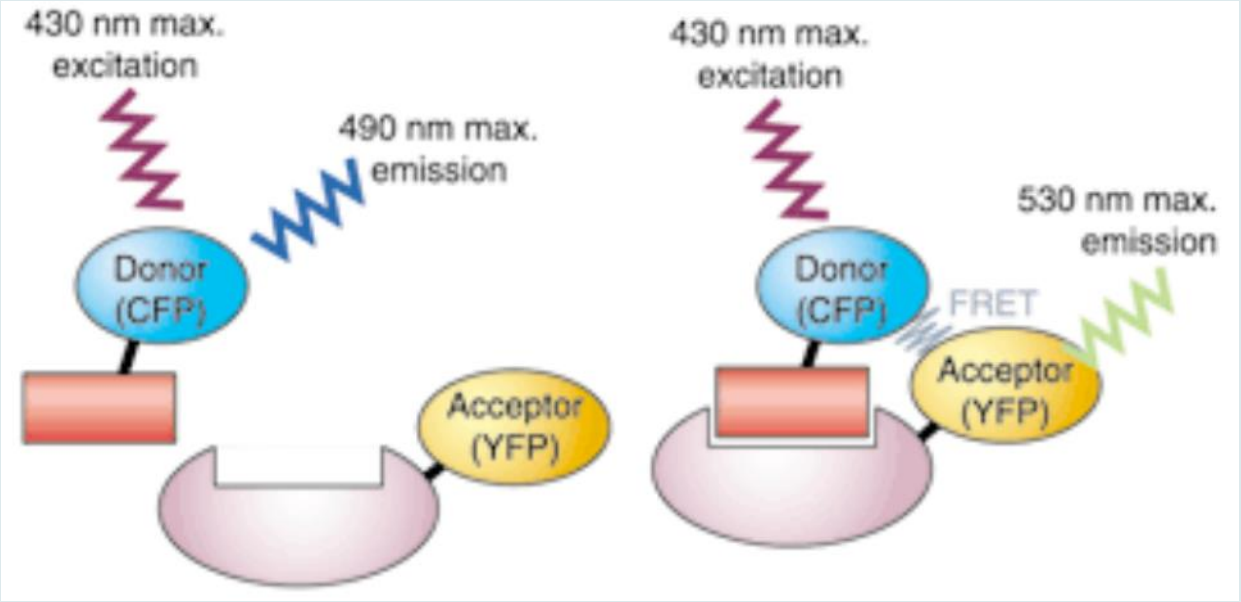


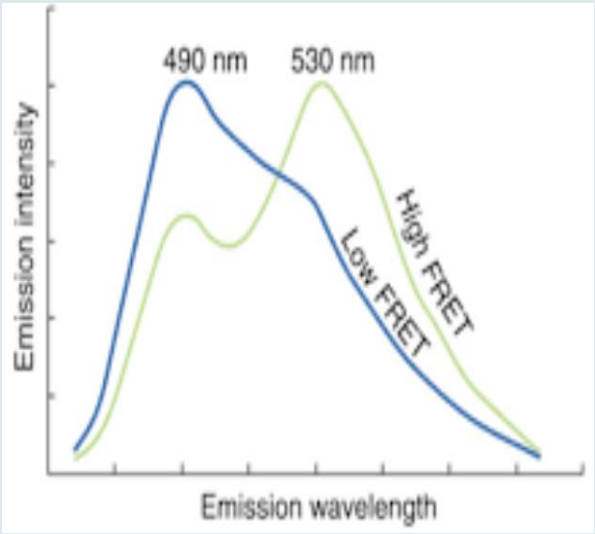
Protein-Protein Interactions: EMSA

- Electrophoretic mobility shift assay
- What if there are multiple shifts?

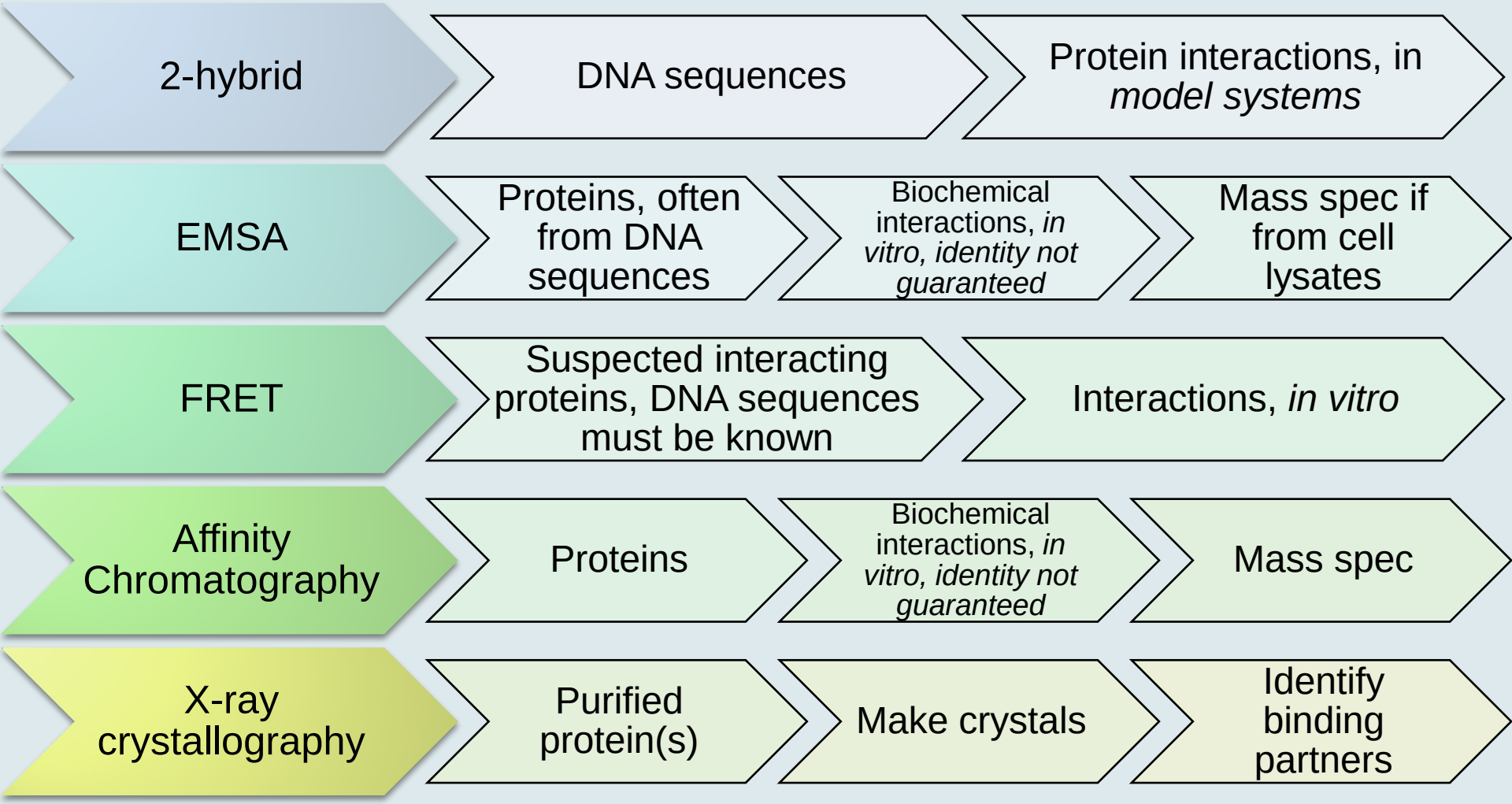
Protein-Protein Interactions: FRET

- Fluorescence resonance energy transfer
  - Two proteins, each fused to a different XFP variant
  - Use laser @ excitation wavelength of one fusion
  - Examine emission wavelength of other fusion





Protein-Protein Interactions



DNA Cloning

- Restriction enzyme use requires sequence knowledge
- Plasmids designed for cloning often have restriction sites that occur uniquely on plasmid for inserting several fragments in succession
- Retroviruses can allow for genomic integration> cannot replicate as rapidly as a high copy number plasmid
- Introduction of DNA: electroporation, calcium chloride, liposomes, viral constructs
- Replica plating

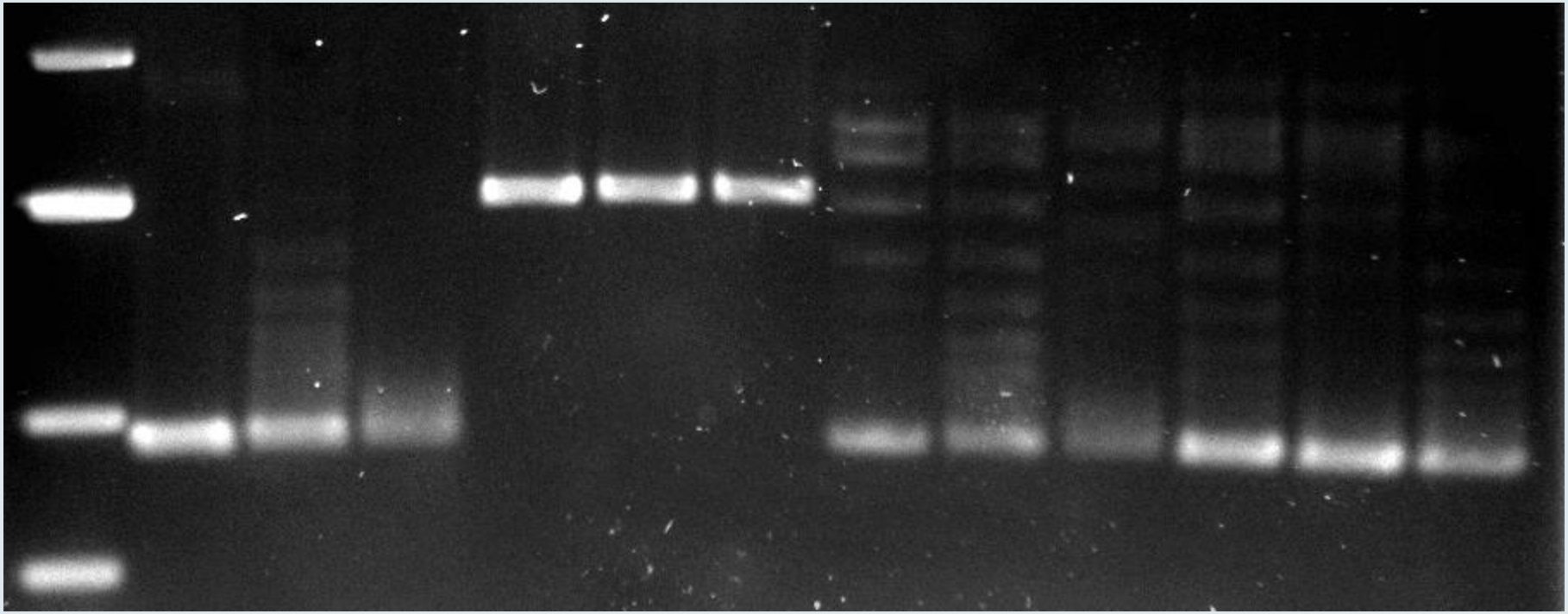
DNA extraction/miniprep

- Break cell membranes/wall
- Remove organelles /fragments
- Remove proteins, RNA, genomic DNA (if isolating plasmid)

Prep	Lyse	Precipitate genomic DNA	Remove RNA	Remove proteins
Overnight culture (7+ hrs)	Alkali lysis (NaOH/ SDS)	$KC_2H_3O_2$ (also for neutralizing NaOH)	Isopropanol to precipitate nucleic acids	Phenol/ chloroform/ isoamyl alcohol to denature proteins
Pellet	Lysozyme	Keep supernatant	Pellet	Centrifuge organic phase to bottom
Resuspend			RNAse treatment	Proteins @ bottom/interface
				Keep supernatant

DNA gel electrophoresis

- Agarose vs polyacrylamide gels
- Circular vs linear
- Supercoiling and branching
- Footprinting (DNAse I)



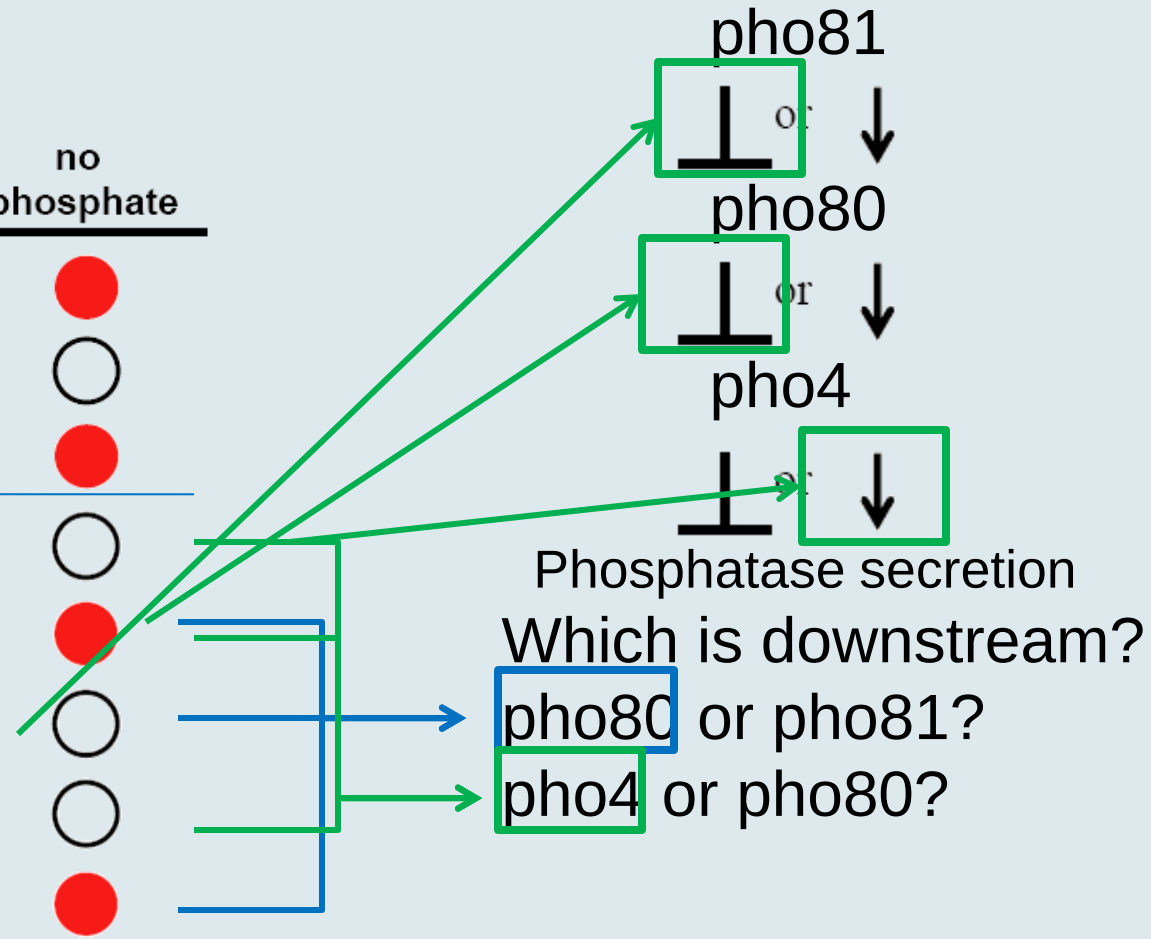
Analysis of Function

- RNAi
  - siRNA effect (exact match) → mRNA degradation
  - miRNA (close match) → transcription and translation block
  - Inject RNA, soak in RNA, viral vector, expression of shRNA
- Antibodies may knock down *some* protein activity
- Complementation analysis

Deduce the Phosphatase Secretion Pathway

Assay secreted phosphatase - dye that turns red when phosphatase is present

	high phosphate	no phosphate
wild-type	○	●
uninducible	○	○
constitutive	●	●
pho4	○	○
pho80	●	●
pho81	○	○
pho80 pho4	○	○
pho80 pho81	●	●



Which is downstream?

pho80 or pho81?

pho4 or pho80?