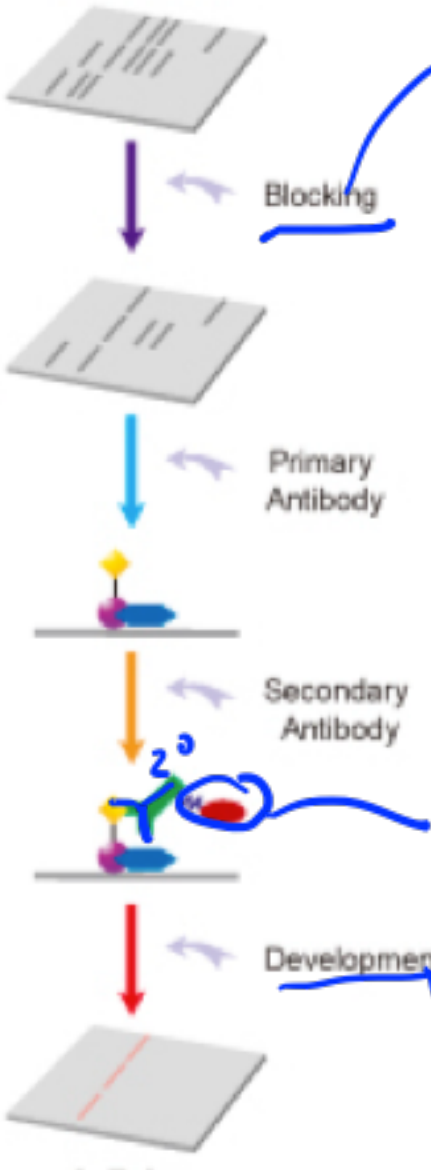
- Announcements
- Pre-lab Lecture
  - Western visualization
  - Damaged DNA prep
  - Today in Lab (M2D4)

Announcements

- Quiz next time
- FNT
  - Western figure + results text
  - Cell doubling calculation
  - Look ahead at transfection protocol/calculations
  - Microbiota summary revision (due Friday)

Steps of a Western



milk / BSA / fancy non-mammalian solution
→ block non-specific binding sites for Ab

1° = α-Ku80 raised in rabbits (4°C, o/N)

★ washes : TBS-T T = Tween 20 detergent
reduce low affinity Ab binding + dilute Ab away

2° = α-rabbit raised in goats
(against constant regions)

alkaline phosphatase label / conjugate / tag...

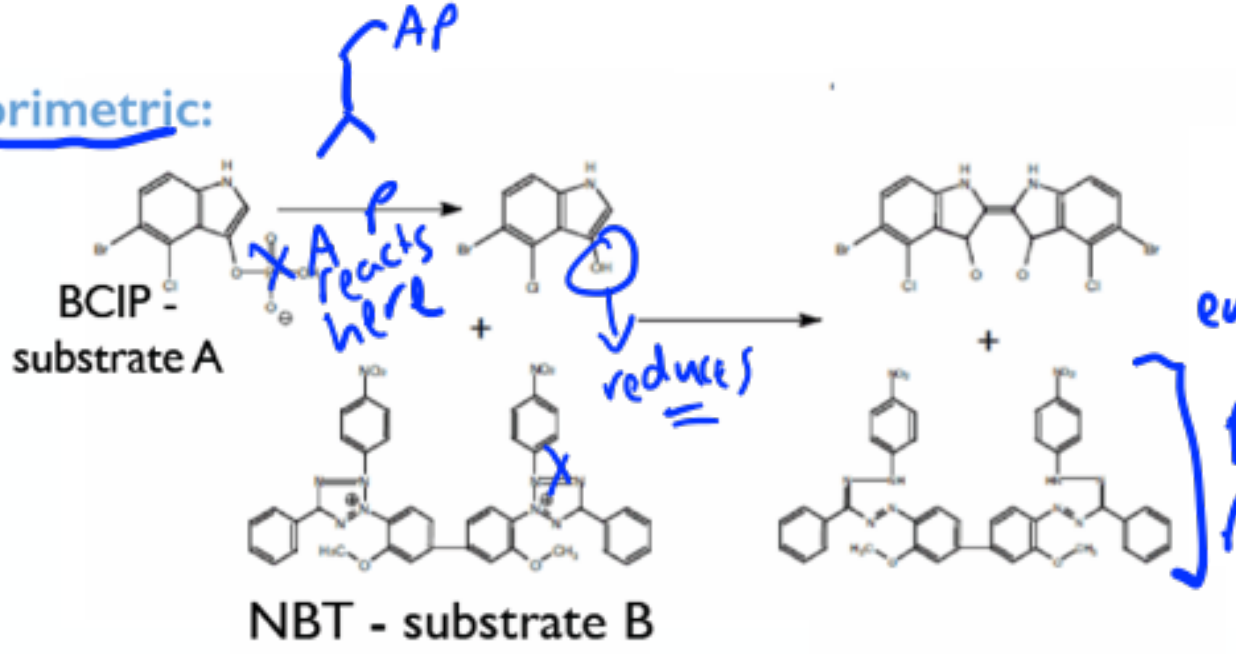
★ washes

develop by adding substrate

Image from genscript.com

Western development options

Colorimetric:

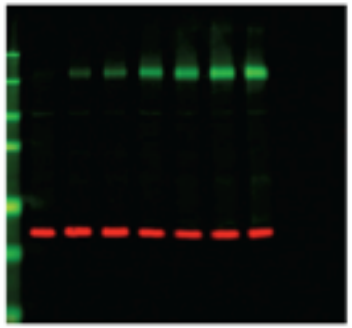


Chemiluminescent:

x-ray film

HRP

Fluorescent:

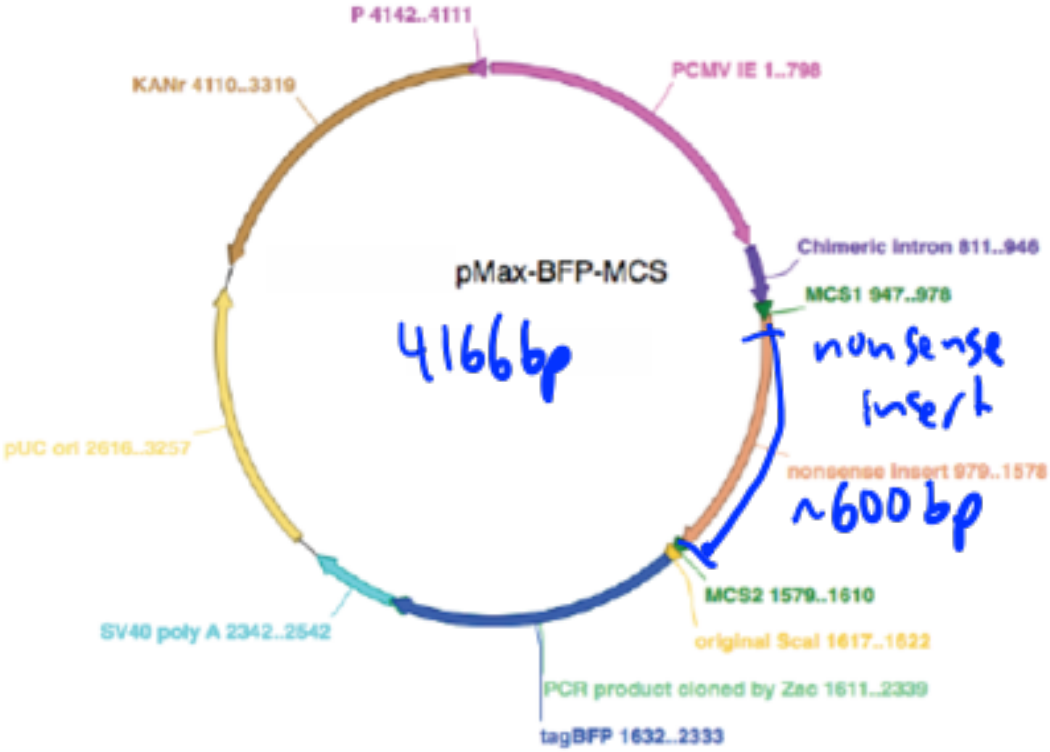


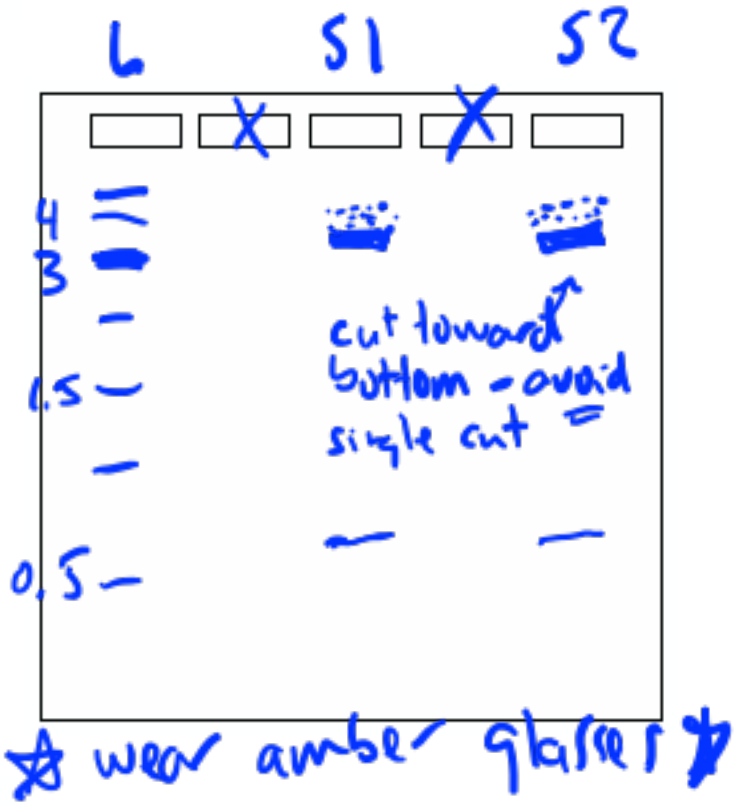
IR dye

quantify multiple protein sequence

Chemical reaction pathway from sigma.com: http://www.sigmaaldrich.com/technical-documents/articles/biofiles/colorimetric-alkaline.html

Prepare and validate damaged DNA





Restriction digest calculation

option A

	1x (mL)	2x
DNA	5	X
enzyme	0.5	1
buffer	2.5	5
H_2O	17	34

→ take 20 mL
add 5 mL DNA

option B (see also SKH slides)

- dilute enzyme 1:10 in buffer
  → 1 mL + 9 mL 1x buffer
  at 1x rxn, now using 5 mL enzyme
- need to reduce amount of 10x buffer added!
  essentially have added 0.5 mL of 10x buffer
  ∴ only add 2 mL more of 10x buffer

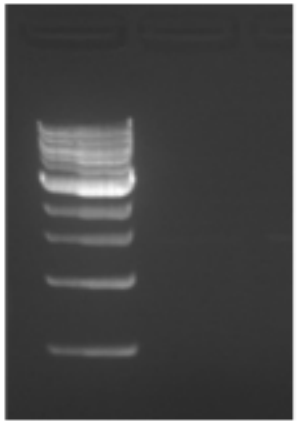
Today in Lab (M2D4)

Digest DNA

1 hour → @ 37°C + parafilm

Load and run gel

>30 min →



Purify DNA

30ish min →

final elution in EB w/filter tips

Wash, probe with 2° Ab

45 min →

Wash, develop

10's of min →