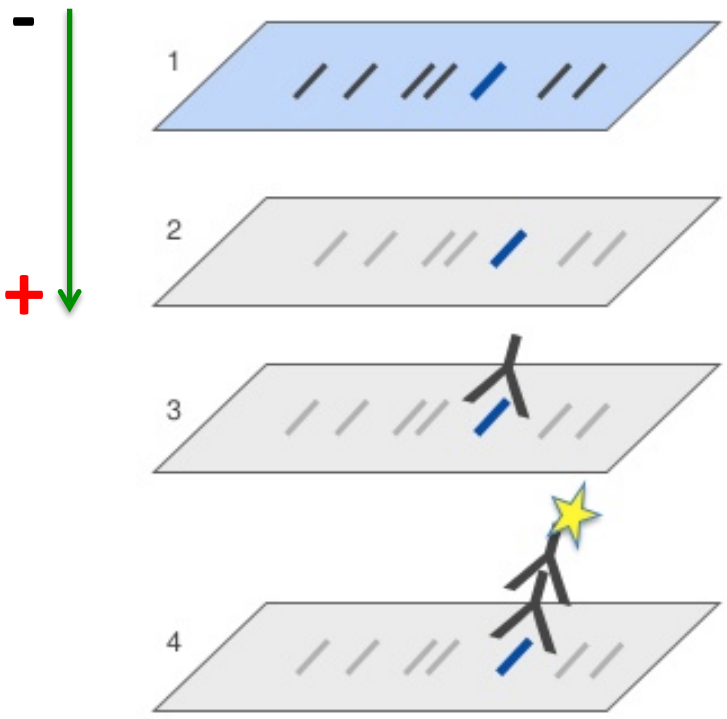
M2D3: Western Blot & Prepare Damaged DNA

03/15/16

1. Pre-lab discussion
2. Set-up restriction enzyme digest
3. Complete Western blot and image
4. Gel purify restriction enzyme digest

- Submit Mini Presentation by 10pm tonight to bioeng20.109@gmail.com
- Journal Club Presentations are in 16-336 Thursday and **class starts at 1:05pm!**
- Submit slides via Stellar by 1pm; Choice of presentation order determined by submission order

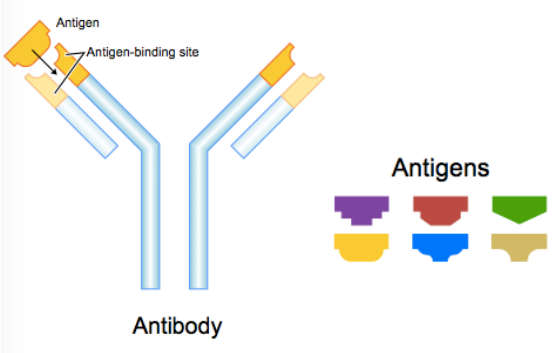
Western blot workflow:

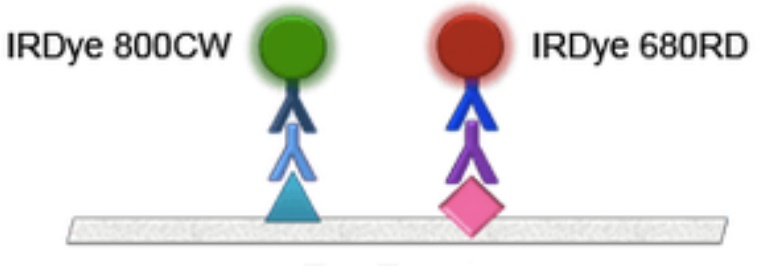


1. Protein separation by SDS-PAGE
   - HiMark stained ladder
2. Protein transfer to nitrocellulose membrane
   - immobilizes proteins once bound
   - high affinity for protein
3. Block membrane
   - reduce non specific interactions
4. Probe with primary antibodies specific to
   - DNA-Pk cs
   - tubullin
5. Wash with TBST Tris buffered saline with 0.1% tween
   - reduce non specific interactions
   - wash away excess primary antibody
6. Probe with labeled secondary antibodies specific to primary antibodies
   - secondary antibody dye labeled
7. TBST Wash
8. Image fluorescence signal on LiCOR imager

from GE Healthcare

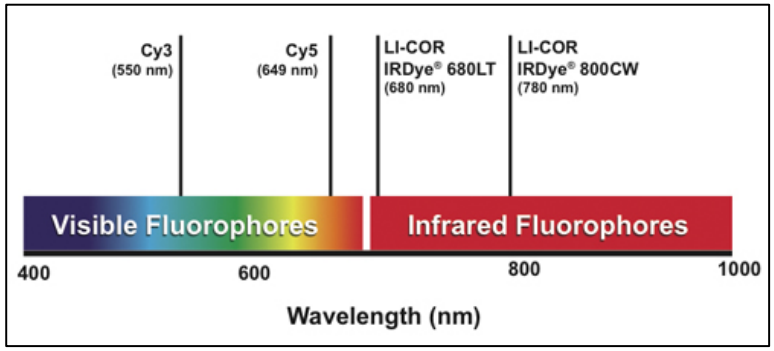
Suite of antibodies for *LI-COR* Western blot

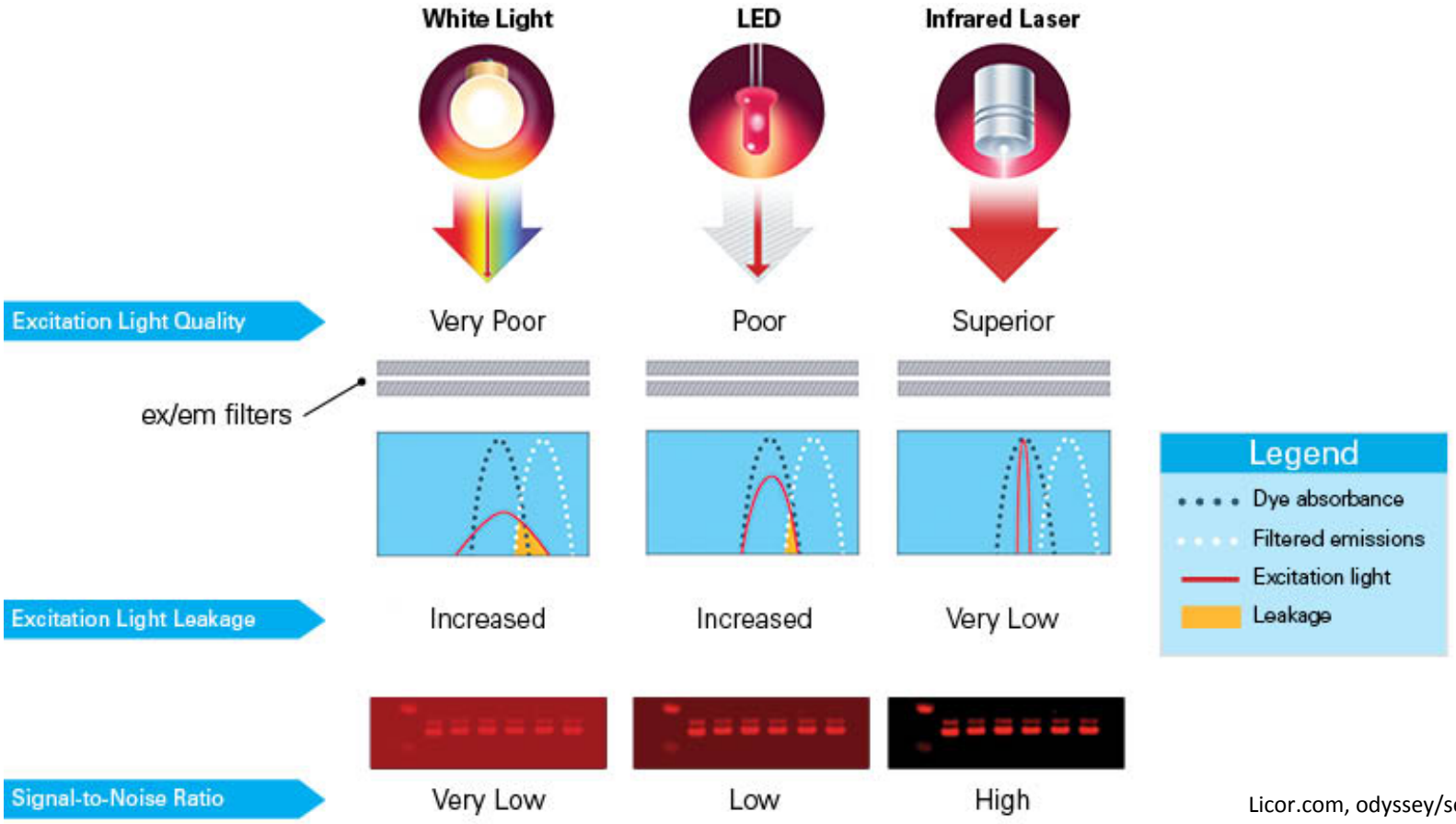




protein of interest	DNA-PKcs	tubulin
primary antibody	mouse anti-human anti-DNA-PK	rabbit anti-human anti-tubulin
secondary antibody	goat anti-mouse	donkey anti-rabbit
fluorescent dye IR wavelength	800 nm	680 nm
pseudo-color	green	red
molecular weight	~ 465 kDa	~ 50 kDa

Near-infrared fluorescence increases sensitivity

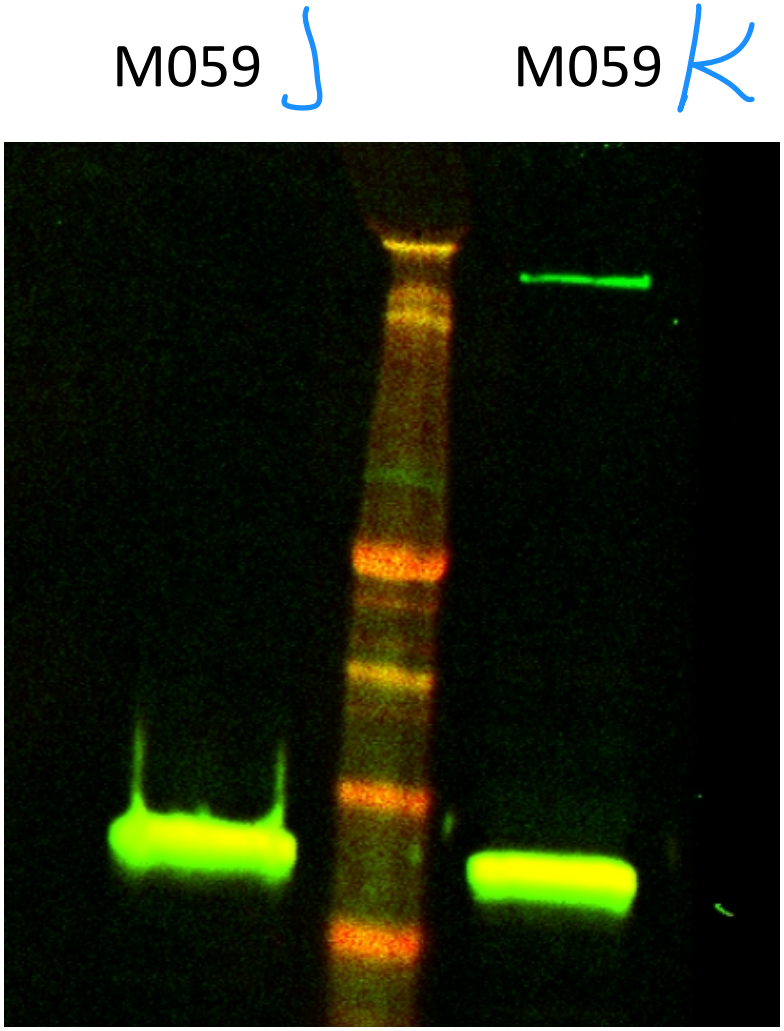




Licor.com, odyssey/sensitivity

Verify M059J is missing DNA-PKcs by *LI-COR* Western blot:

Lysed



800 = DNApk

Signal bleed through

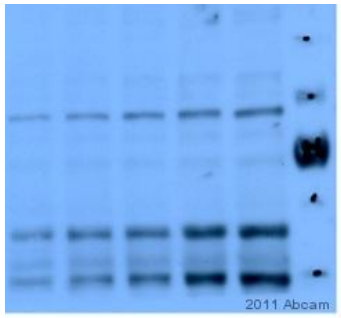
680 ~50 KDa tubulin

Western blot detection systems:

Colorimetric

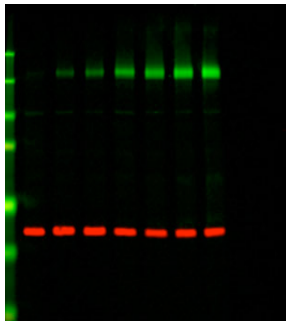
Pro: inexpensive, easy,
no equipment required
Con: medium sensitivity

Chemiluminescent

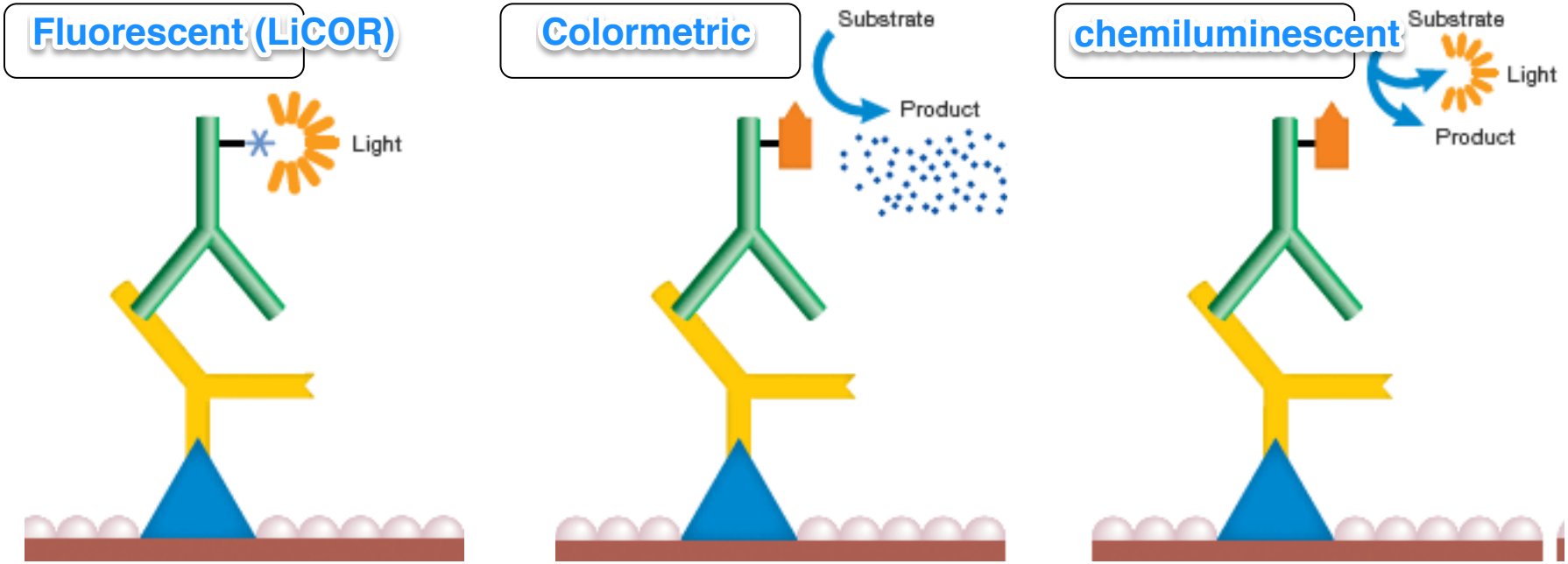


Pro: sensitive, fast,
film developer common
Con: requires trial and error

Fluorescent

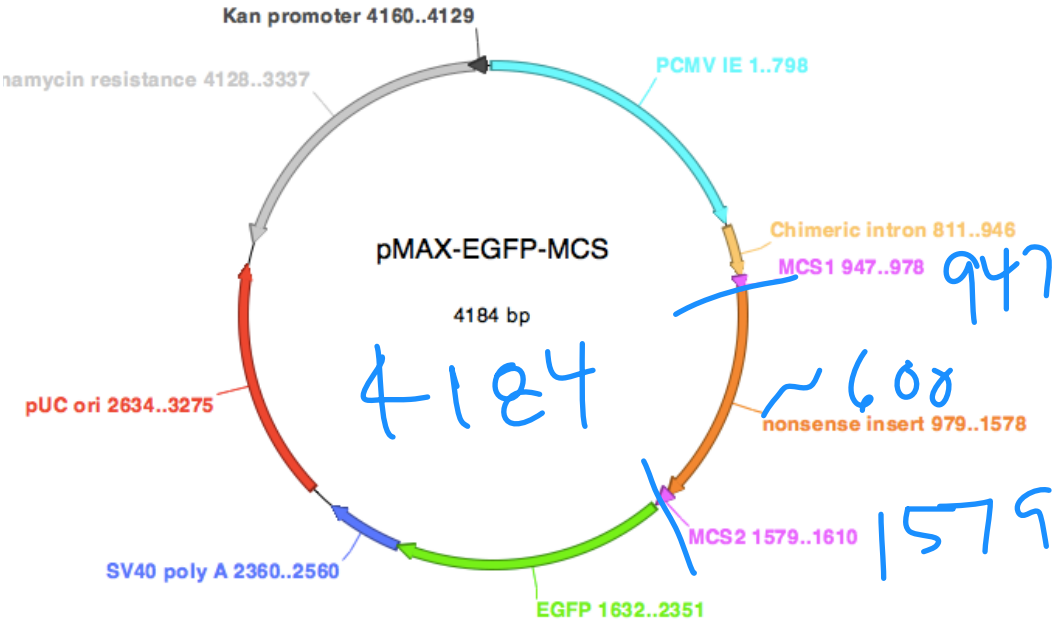


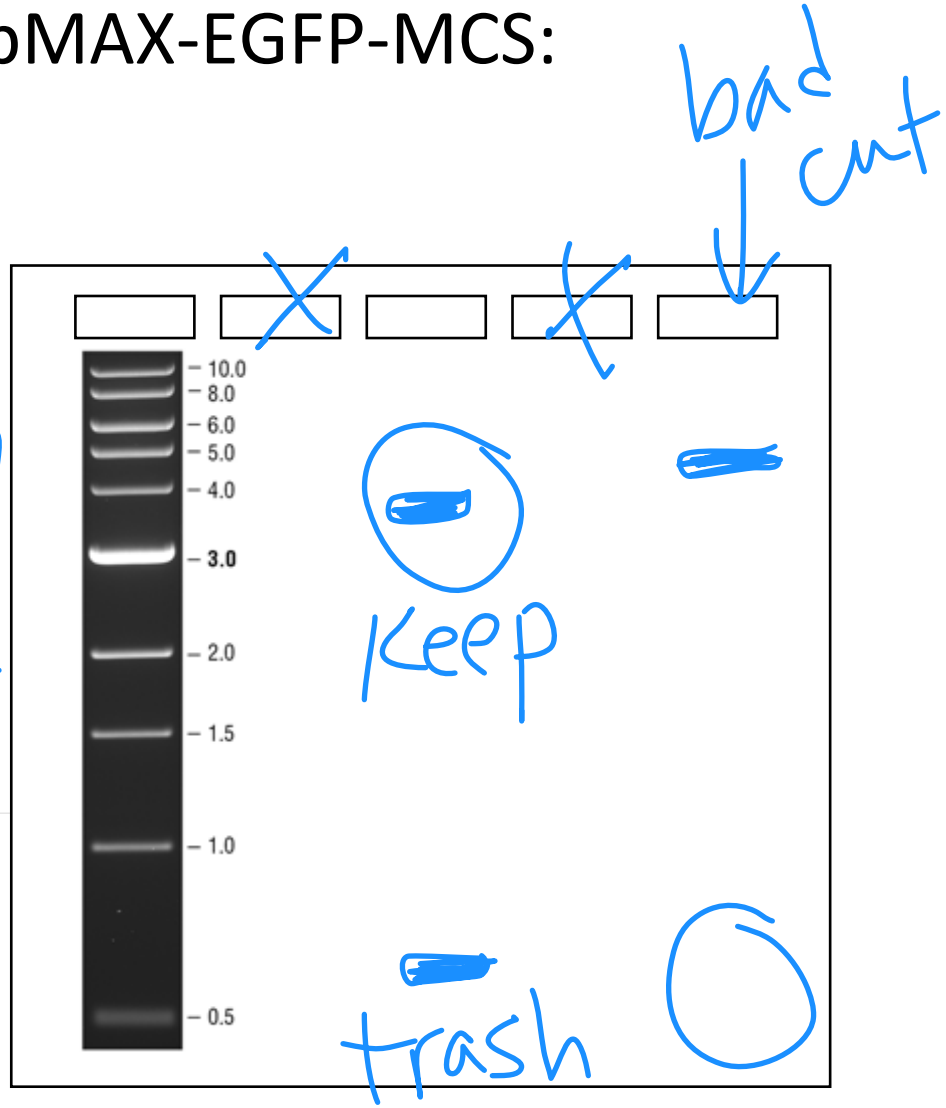
Pro: Sensitive, stable,
ability to multiplex
Con: expensive



Biorad.com, Detection Methods

Preparation and purification of pMAX-EGFP-MCS:





Restriction enzyme choices (buffer):

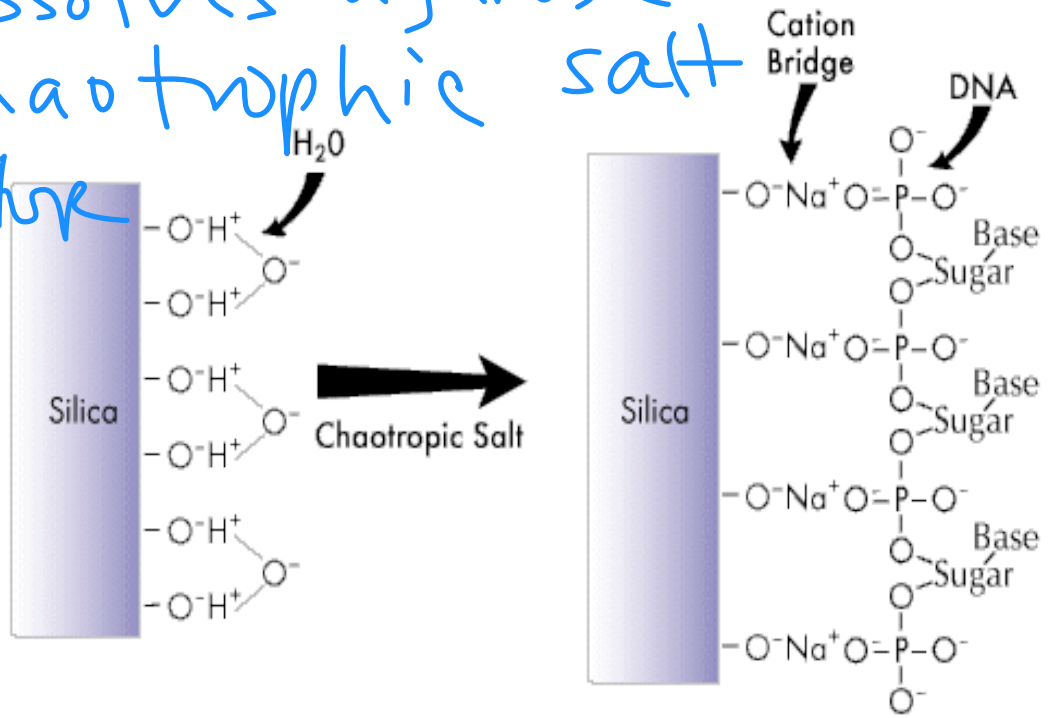
- *PmeI*
- *BglII* and *EcoRI*
- *BglII* and *PstI-HF*

Always add enzyme to buffered solution!

Gel Purification:

Review of mini-prep, Bind DNA to column

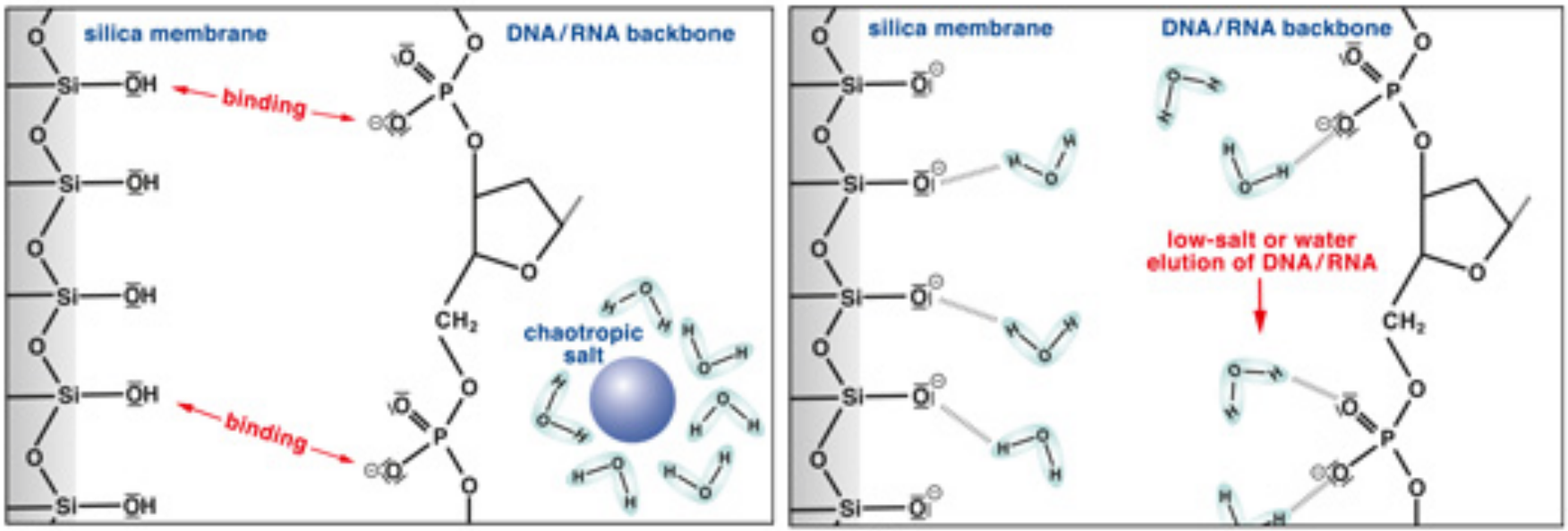
QG. dissolves agarose
chaotrophic salt
pH indicator



- Washes with PE
  - remove residual contaminants (eluent)
  - maintain DNA onto column

Gel Purification:
Review of mini-prep, Elution with water

- Water competes DNA off of column



Wait 5min

Today in lab:

- Set-up restriction enzyme digest (prepare damaged DNA)
- Complete your Western blot
- Gel purify digest product
- Image your Western blot on LiCOR imager

- *Mod2 report requires compiling class data, consider variables to compare*