- Announcements, Review HW
- Lab Quiz
- Pre-lab Lecture
  - DNA Extraction (Miniprep)
  - Diagnostic Gel Review
  - Safety + Technical Tips

Announcements

- Your progress report is due in one week
  - OH (room TBA) on: Sun 4-5:30pm (Tue 3-4)
    Natalie Sat. 3-5pm
- Please post your colony counts in *talk* page table before leaving – we'll discuss them next time

• read NCB paper for next time

* use 20109.talk@gmail.com

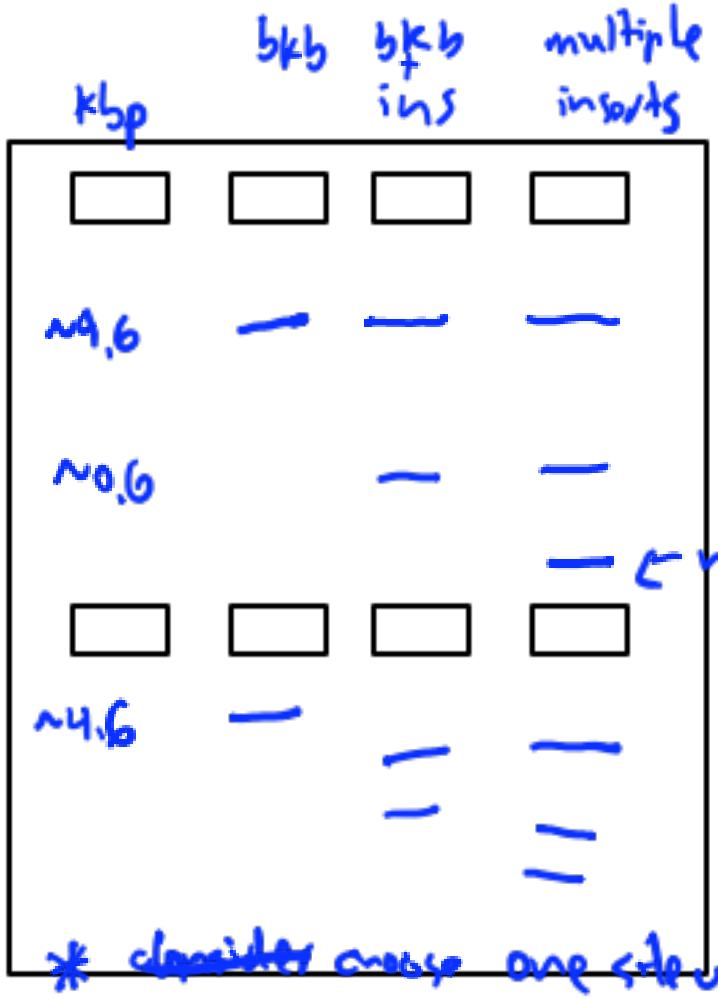
Previous HW comments

- Analyzing error in linearized gel analysis
  - Error measurement? % error, not absolute difference
  - Sources of error? human measurement → band thickness
    gel loading error
- Comments on Figures
  - Titles: Great overall! Hone in on *key* details.
  - Caption:
    - Introduce the players, use consistent naming scheme.
    - Avoid explaining minor methods details.
    - Stick to facts (e.g., expected band sizes), not interpretation.
  - Labeling: often insufficient. Label a few bands on ladder.

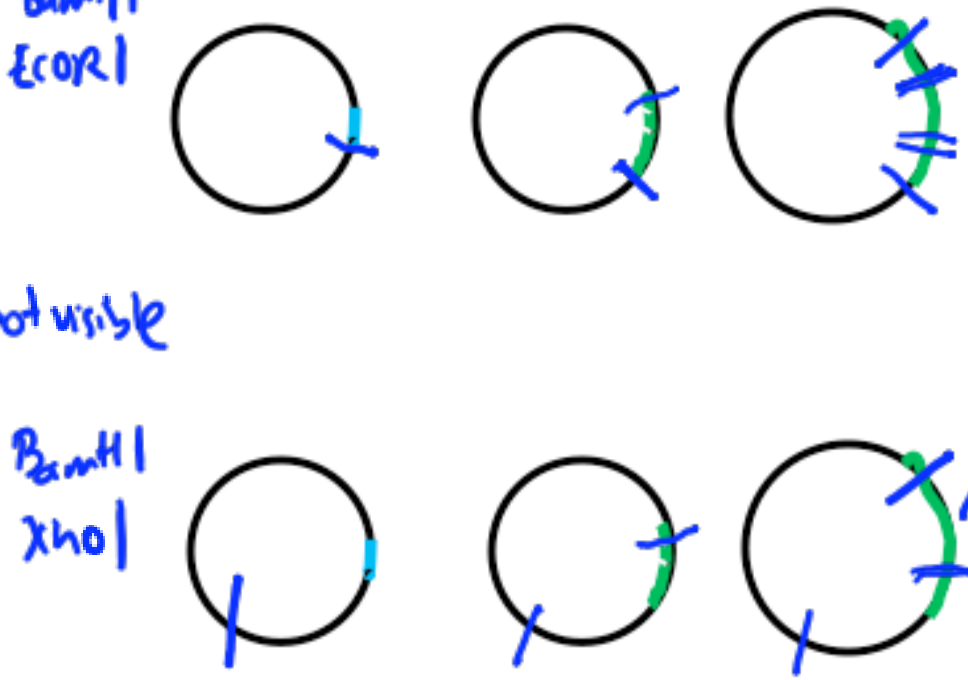
DNA Extraction from Bacteria

Step	Contains	Purpose
Soln. I	EDTA buffer, glucose	→ weakens cell envelope → otherwise stable
Soln. II	SDS ~~O Na+ NaOH	→ disrupt, solubilize lipid membrane (+proteins) → denatures dsDNA → ssDNA
Soln. III	acetic acid, KAc.	neutralize pH, precipitate SDS renature genomic DNA "crashes"
Transfer	N/A	
Final steps	EtOH, H_2O drying	→ precipitates DNA, wash away salt, etc. → EtOH would interfere w/ digest

Diagnostic DNA Gels



Choosing restriction sites for digest



* consider cause one site unique to insert, one on 5kb

* consider results of partial digestion

Today in Lab

- Miniprep three Δ5-EGFP candidates, and bacteria transformed with pCX-NNX

control for your technique

- Count and post colony #s

- Visit from Christine Tafoya, EHS: biosafety talk

4pm

- Set up digests
  - We will add loading dye if lab runs late