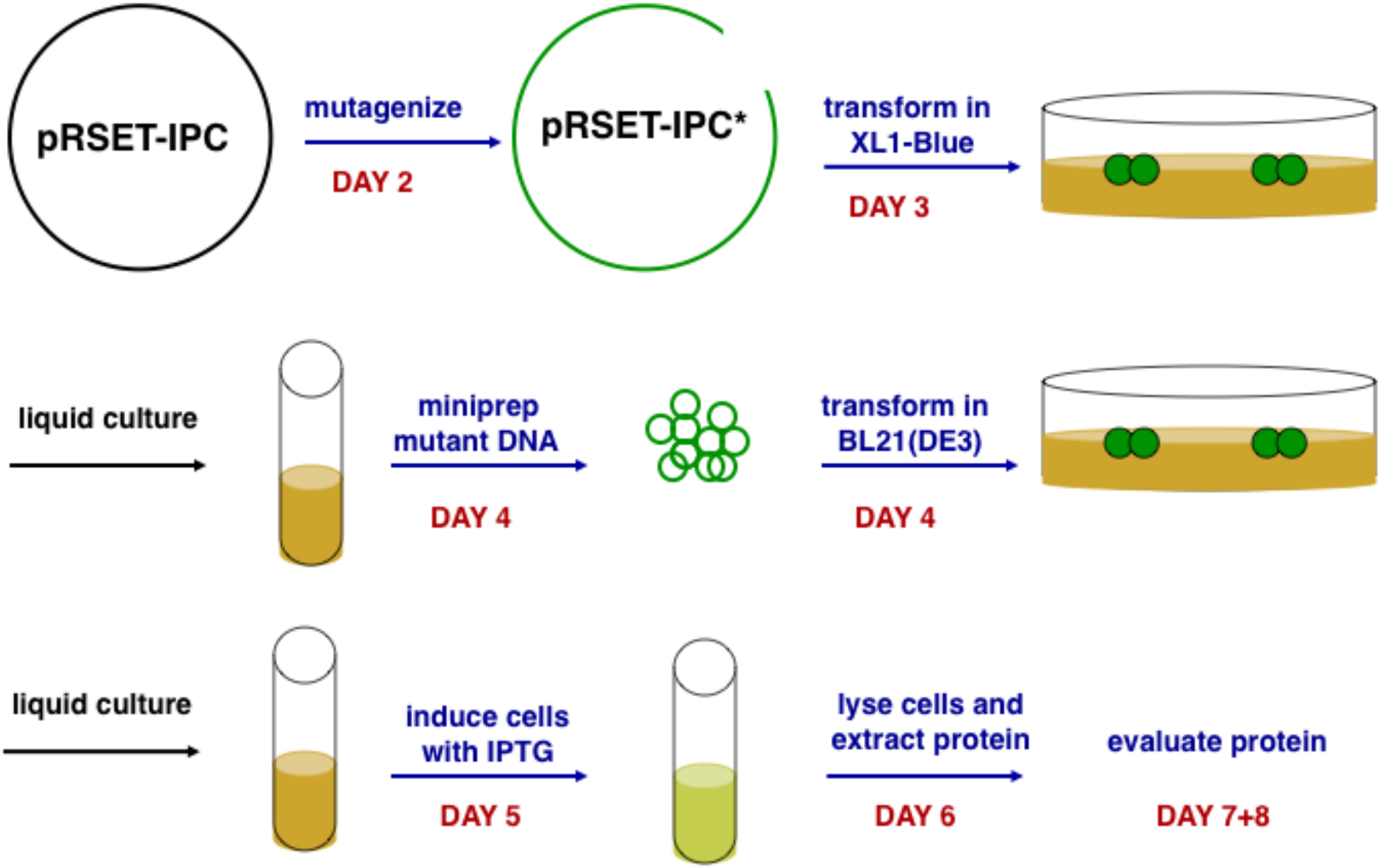
- Announcements
- Pre-lab Lecture
  - Mod 2 lab steps overview
  - Site-directed mutagenesis
  - Restriction enzymes recap
  - Today in Lab: M2D2

Announcements

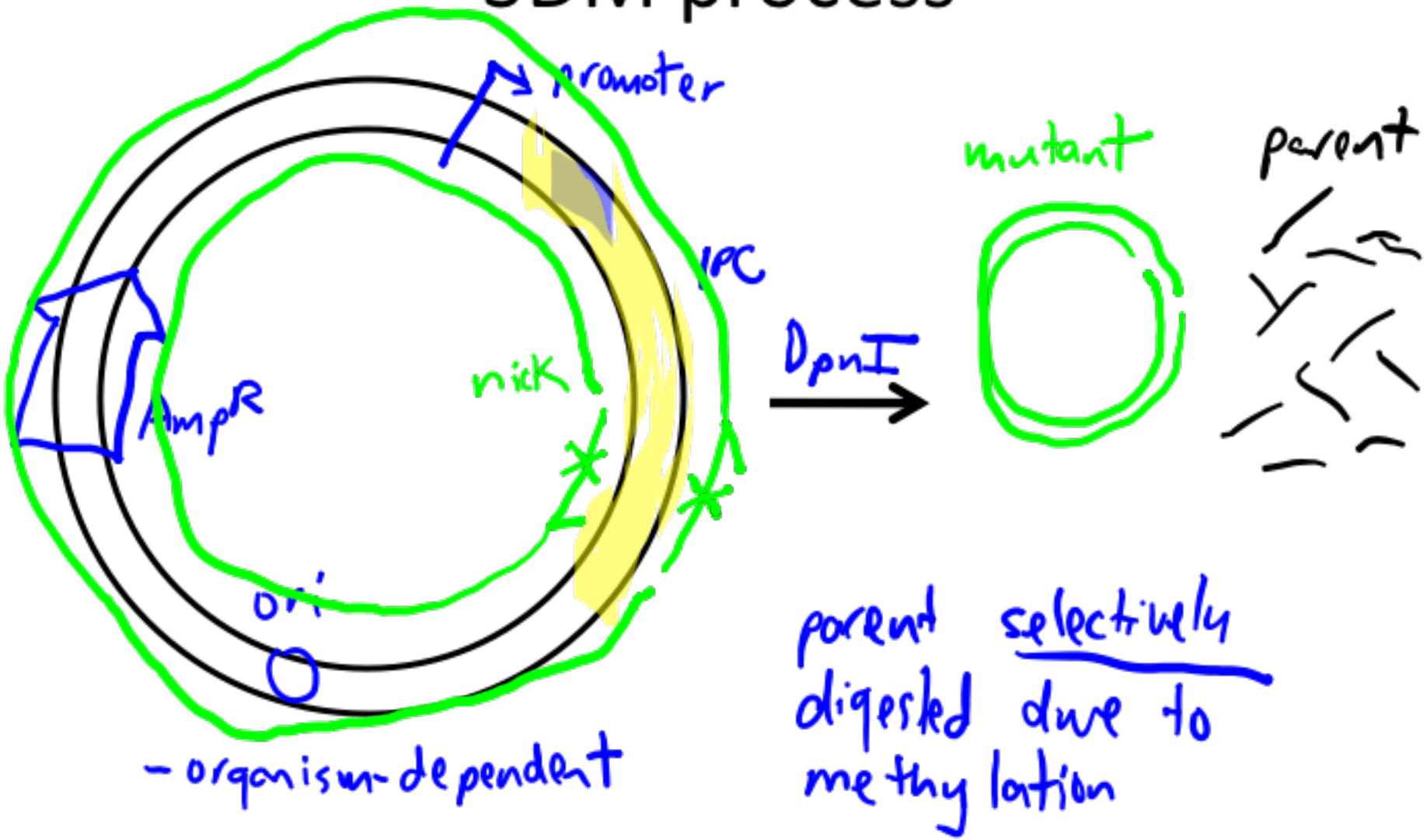
- First M2 quiz next time!
- Plus a short-answer FNT due
- Primer design summary due Tuesday by 11 AM
  - again, to 20109.submit@gmail.com
  - individual, and *not* subject to revision
  - expectations vs observations? reasoning?
  - consider both size and intensity of each band

DNA stain ∝ bp # (mass, not moles)

——— brighter than —


pRSET-IPC
mutagenize
DAY 2
pRSET-IPC*
transform in
XL1-Blue
DAY 3
liquid culture
miniprep
mutant DNA
DAY 4
transform in
BL21(DE3)
DAY 4
liquid culture
induce cells
with IPTG
DAY 5
lyse cells and
extract protein
DAY 6
evaluate protein
DAY 7+8

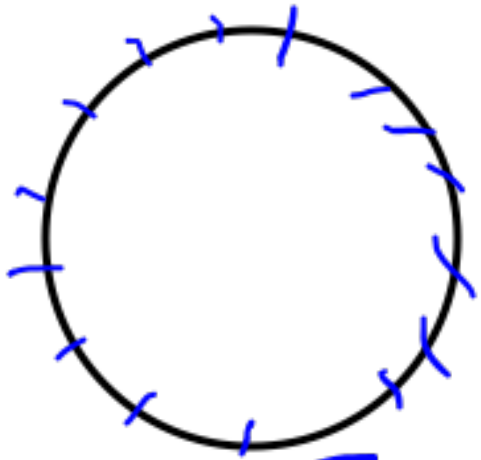
SDM process


promoter
IPC
AmpR
nick
ori
DpnI
mutant
parent
- organism-dependent
parent selectively
digested due to
methylation

Restriction enzyme recap

- Allow selective cutting of DNA
- Some are more common than others

  e.g., DpnI v. common
- Often, but not always palindromic and 6-8bp
- Some are less selective



XcmI CCAN$_9$TGG N = A,T,C,G

PpuMI RGGWCCY R = A/G Y = C/T

W = A/T

Today in Lab: M2D2

- Prepare primer stock and dilution.
  - Read part 1, step 3 carefully to prepare your primers in the appropriate volume at the appropriate concentration.
- SDM rxn: careful not to contaminate shared stocks!
- Paper reading period and discussion.
- Start digestion of SDM rxn.

Nagai - required

Heim - recommended

- For next time:
  - Short answer questions