- Announcements
- Quiz
- Pre-lab Lecture
  - Closer look at plasmids
  - Site-directed mutagenesis
  - Restriction enzymes recap
  - Today in Lab: M2D2

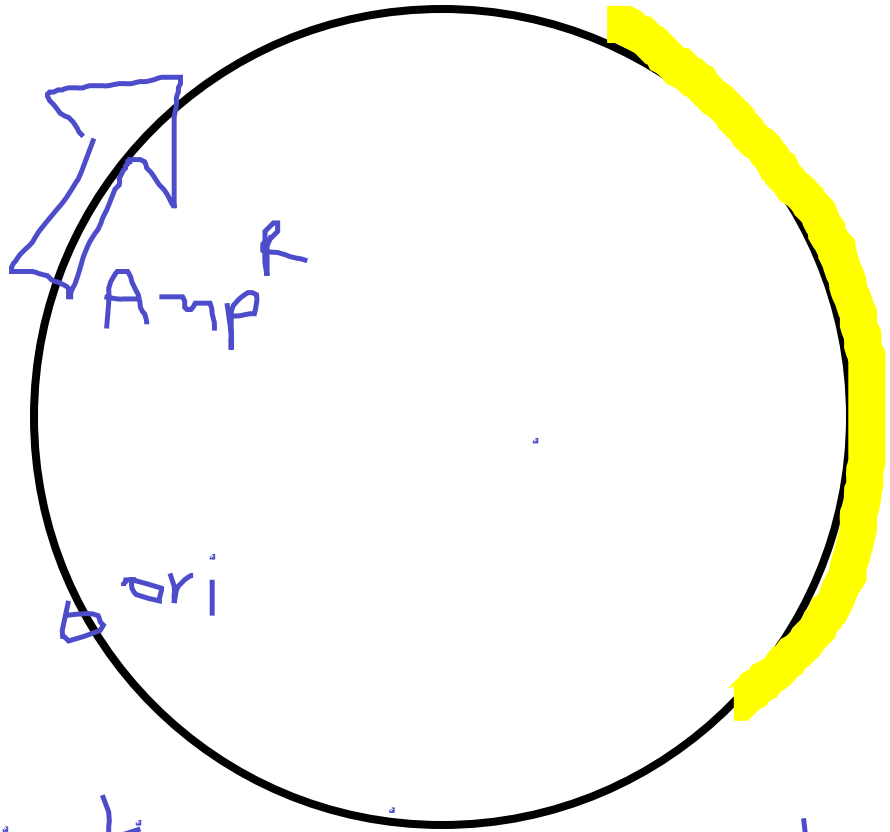
Announcements

- Troubleshooting Watcut
  - name your sequence something *unique*
  - be sure “all enzymes” is checked
  - overall, careful to limit interference from old data…
  - … should you use it again someday
- Reflections clarification
  - If you choose not to revise the M1 report, you can write the relevant reflection with reference to FNT drafts only.

printer issues

Plasmid Overview

antibiotic resistance



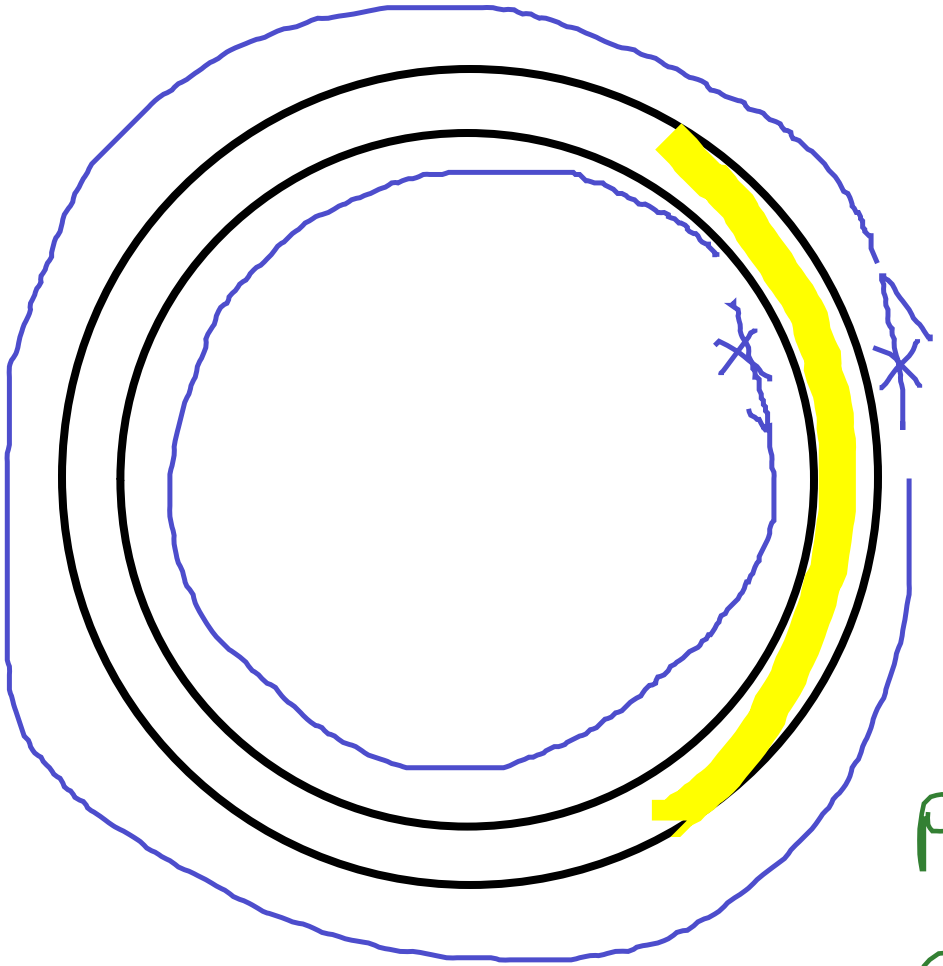
— ds, circular, extrachromosal

why? vector to introduce foreign gene in cells

Ampicillin resistance → select bacteria that have plasmid on Amp plates

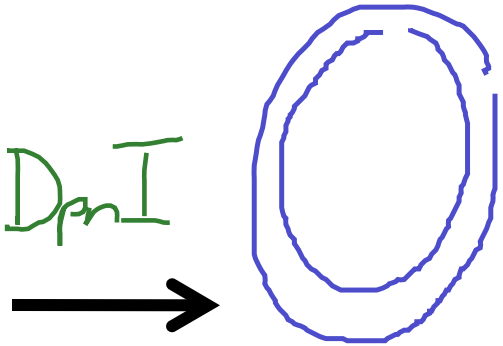
replication origin
- organism-dependent
- affects copy #

SDM Process



mutant

parent



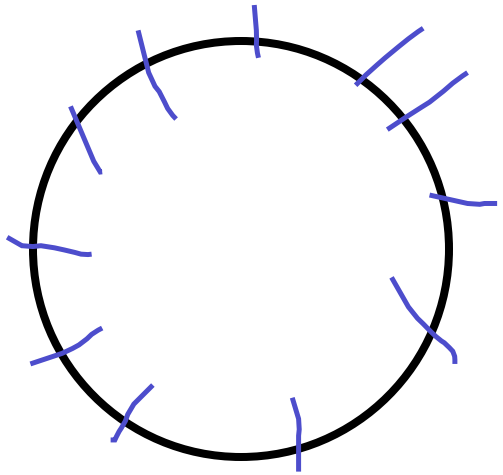
DpnI

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
- Some are less selective and 6-8 bp



GATC

CH3

XcmI CCAN9TGG

N = A, C, T, C

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.

- For next time:
  - Short answer questions