

- **Announcements** → sign up for WAC mtg  
before you leave today
- **Lab Quiz**
- **Pre-lab Lecture**
  - ❖ Restriction enzymes
  - ❖ Subtleties of M2 systems
  - ❖ Today in Lab: M2D3

# Intro to Restriction Enzymes

EcoRI



↓ digest



endonuclease  
→ cut DNA

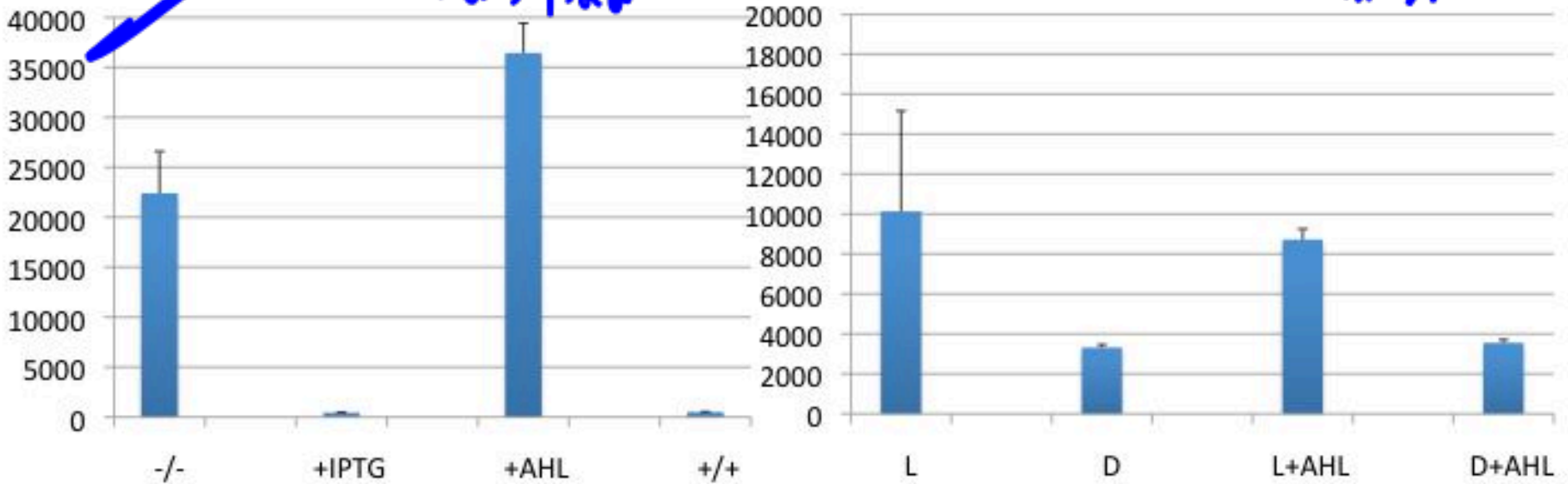
sites are ~ palindromic

"sticky" or blunt ends  
→ insert for ligation

# Subtleties of system

1PTG system  $\rightarrow$  can isolate issue w/  $P_{lux-\lambda}$

Milner units



column 3  $\frac{\text{high}}{\text{lower}}$  "on" state <sup>of  $P_{lux-\lambda}$</sup>  due to leaky  $p_{amp}(cI)$  for  $\lambda$

columns 2+4 higher "off" state <sup>of  $P_{lux-\lambda}$</sup>  due to poor dynamic range of  $p_{amp}$ , lower  $[cI]$  when  $p_{amp}$  on

# Today in Lab (M2D3)

- Start gel for backbone purification (45' run)
- Prepare control backbone digest (1h at 37 °C)
- Purify backbone (some 10-20' incubations)
- During down-time
  - Prepare calculations for remaining parts
  - Set up IPTG → lacZ transfer function cultures
  - Anneal oligos
- Move all “completed” DNA up front on ice!