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

\[ \text{GAAATTTC} \]

\[ \text{CTTAAAG} \]

digest

\[ \text{GAAATT} \]

\[ \text{AAATTC} \]

Endonuclease
\rightarrow cut DNA

Sites are palindromic

"Sticky" or Blunt ends

\rightarrow insert for ligation
Subtleties of system

IPTG system can isolate issue with Plux-λ

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<td>+/-</td>
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<td>+IPTG</td>
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- Column 3 higher "off" state of Plux-λ due to leaky pumpC (cI) for λD
- Columns 2-4 higher "off" state of Plux-λ due to poor dynamic range of pumpC, lower [cI] when pumpC 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 \( \rightarrow \) lacZ transfer function cultures
  – Anneal oligos

• Move all “completed” DNA up front on ice!