Mini-lecture: Ligation and Transformation

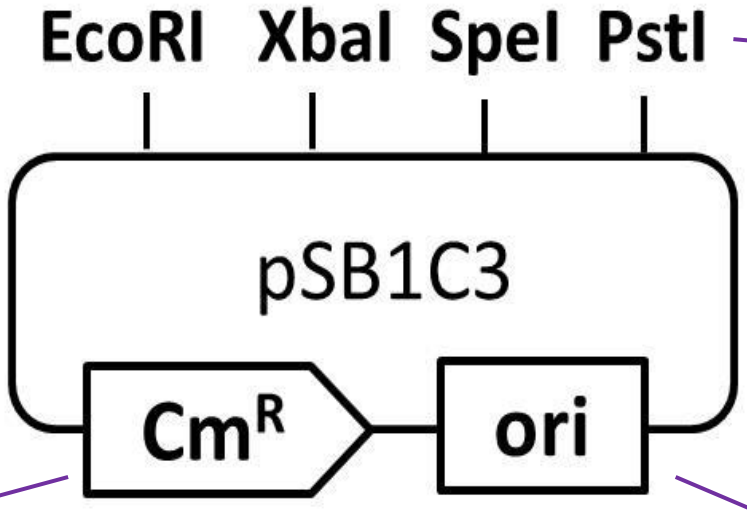
Dr. Nick Coleman

Learning outcomes

- **Know the role of these elements in synthetic biology:**
  - **Plasmid**
  - **Restriction enzyme**
  - **Ligase enzyme**
- **Understand the theory behind these procedures:**
  - **Restriction digestion**
  - **Ligation**
  - **Transformation**
  - **Selection (of plasmid-containing cells)**
  - **Screening (for cells containing recombinant plasmid)**
- **Be able to explain the different controls in a ligation and transformation experiment and why we need them**

Plasmids

- Circular pieces of DNA found in bacteria
- Replicate independently of the chromosome
- Found naturally in many bacteria
- Useful tools for cloning genes and expressing proteins



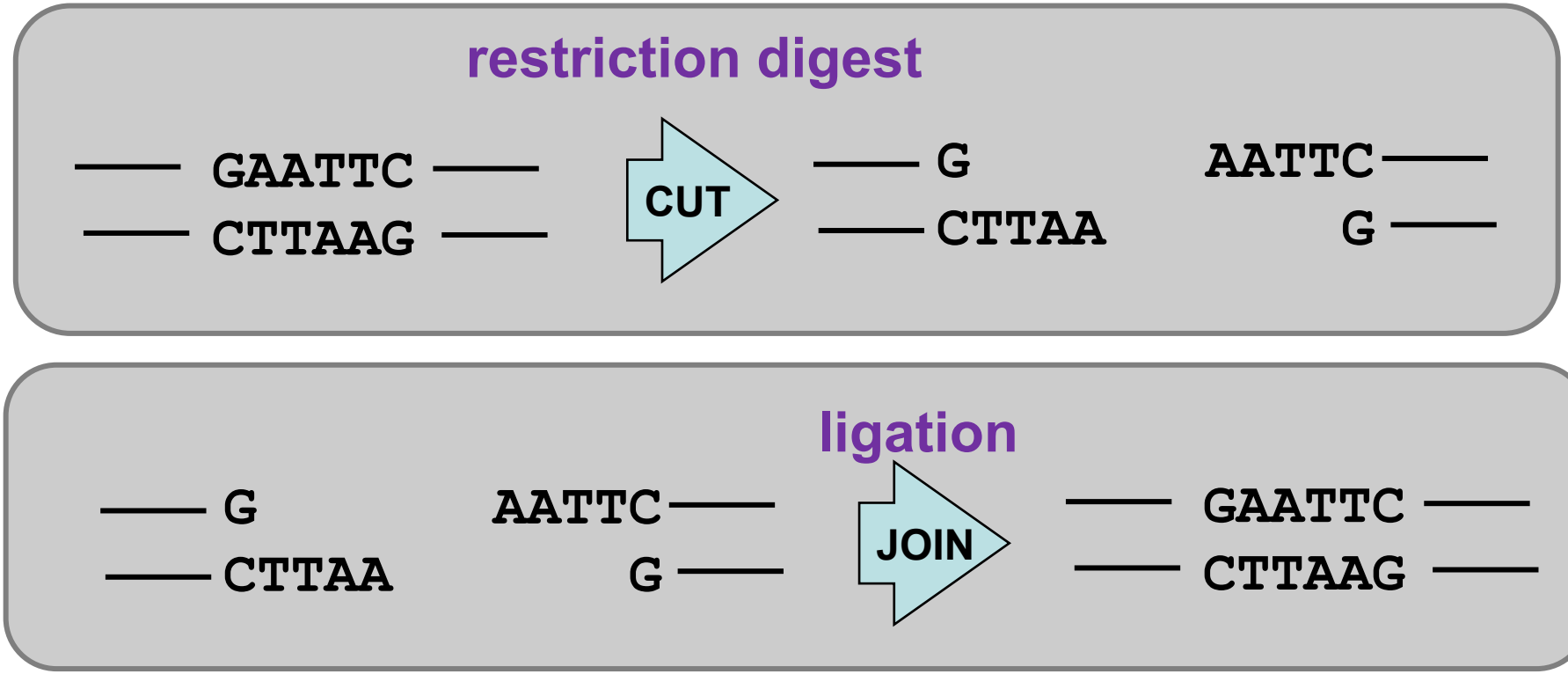
Restriction sites:
enable cutting plasmid
at specific locations

Selective marker
e.g. chloramphenicol resistance

Origin of replication

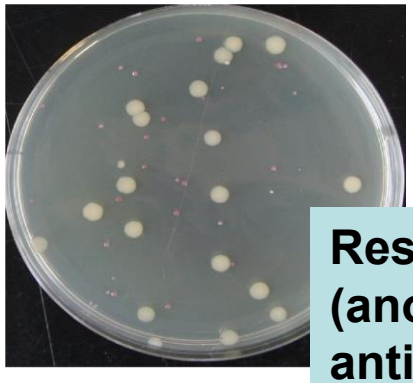
Restriction enzymes (RE) and ligase enzyme

- RE cuts DNA at a specific location : a restriction site
- DNA cut with RE is left with "sticky ends"
- Two DNAs with the same type of sticky ends can be joined using DNA ligase enzyme

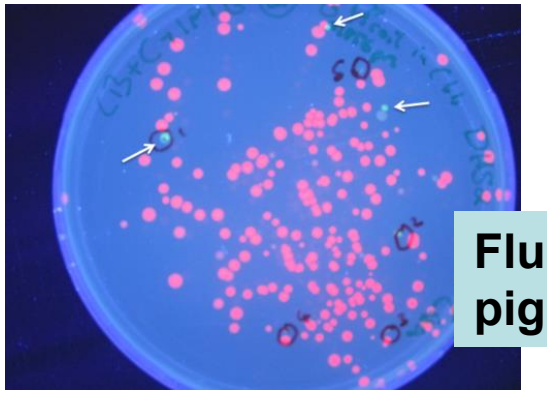


Gene to be cloned = “insert DNA”

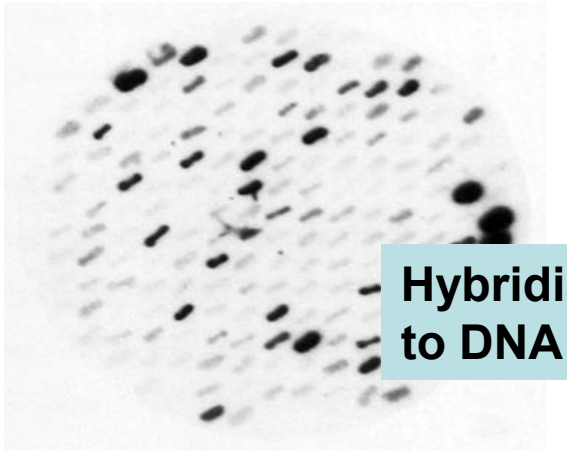
- Can be PCR product, genome fragment, or synthetic DNA
- Both insert and plasmid need to be cut with same RE(s)
- Need a way to screen for insertion of insert into plasmid:



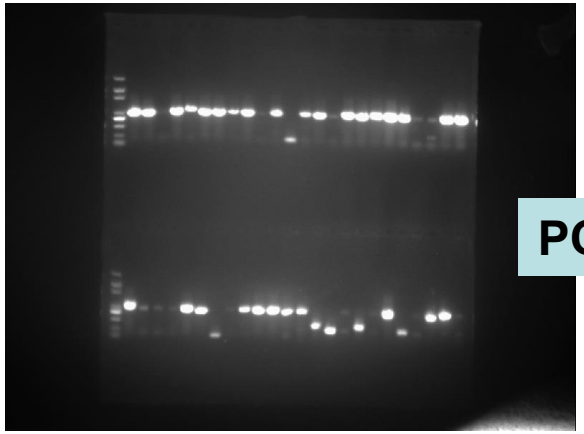
Resistance to (another) antibiotic



Fluorescence or pigment production



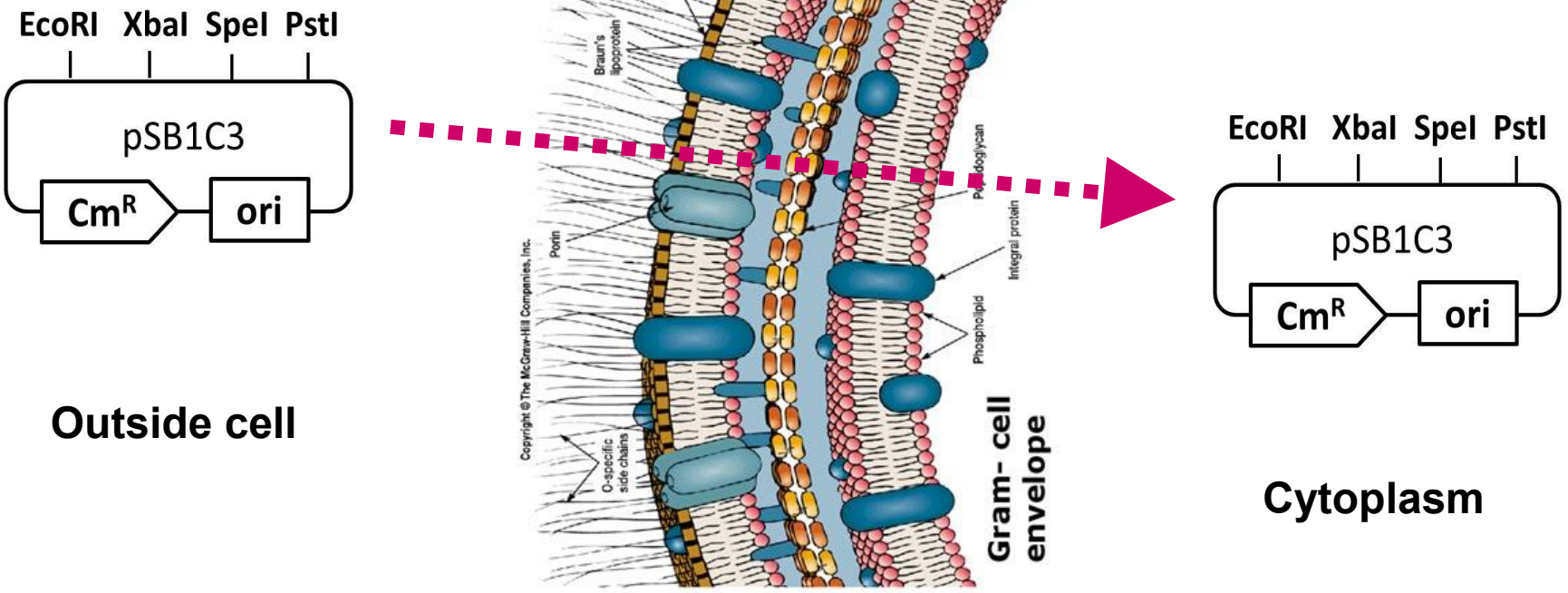
Hybridisation to DNA probe



PCR screen

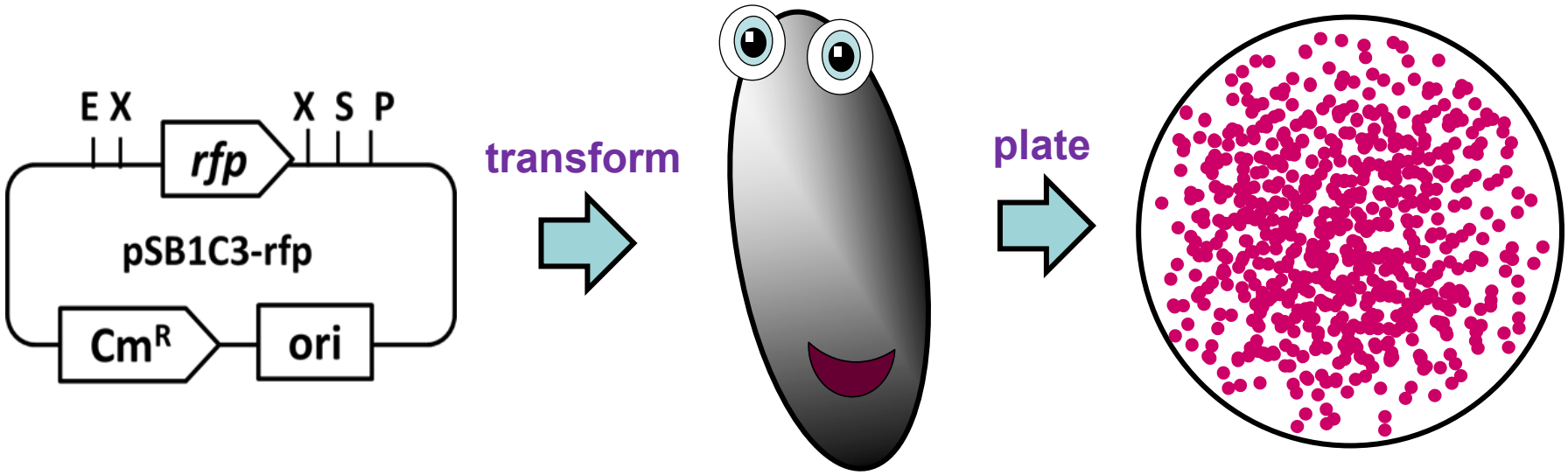
Transformation

- Introduction of plasmid DNA into bacteria
- Most bacteria need to be made competent first
- Measured as efficiency or frequency of transformation: number of colonies per microgram of plasmid DNA



Ligation & Transformation control #1

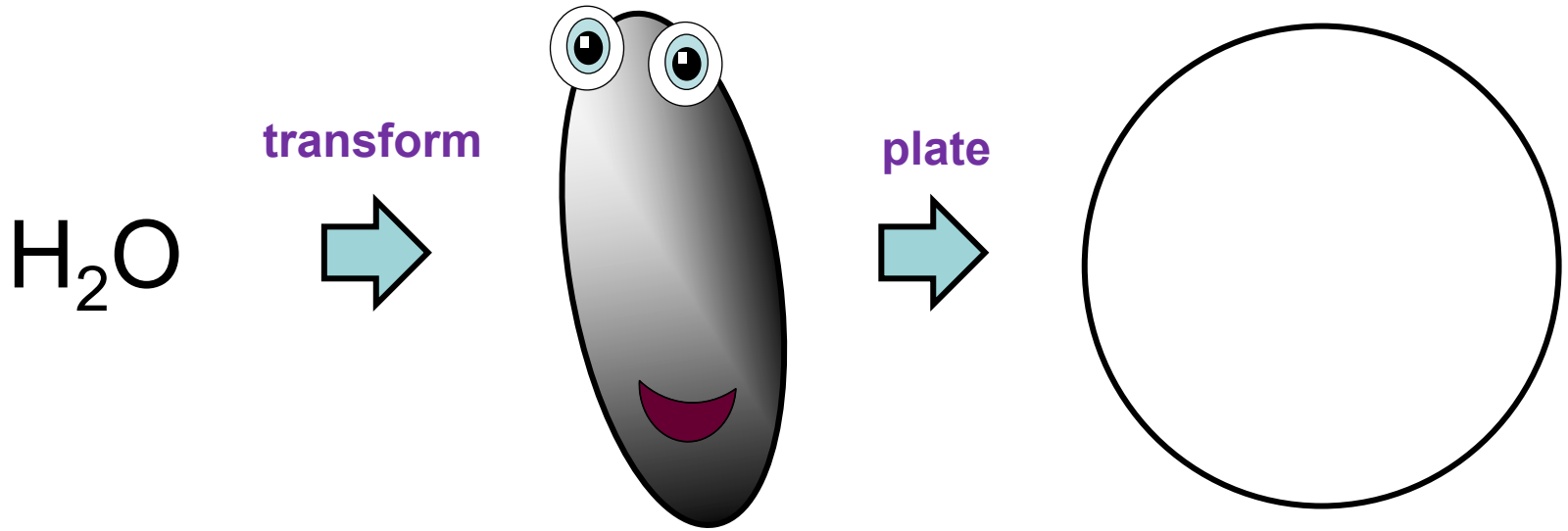
- **UNCUT PLASMID**
  - **Is the plasmid used for cloning in good condition?**
  - **Are the bacterial cells competent?**
- **Expect many colonies (>1000), with original genotype**



Ligation & Transformation control #2

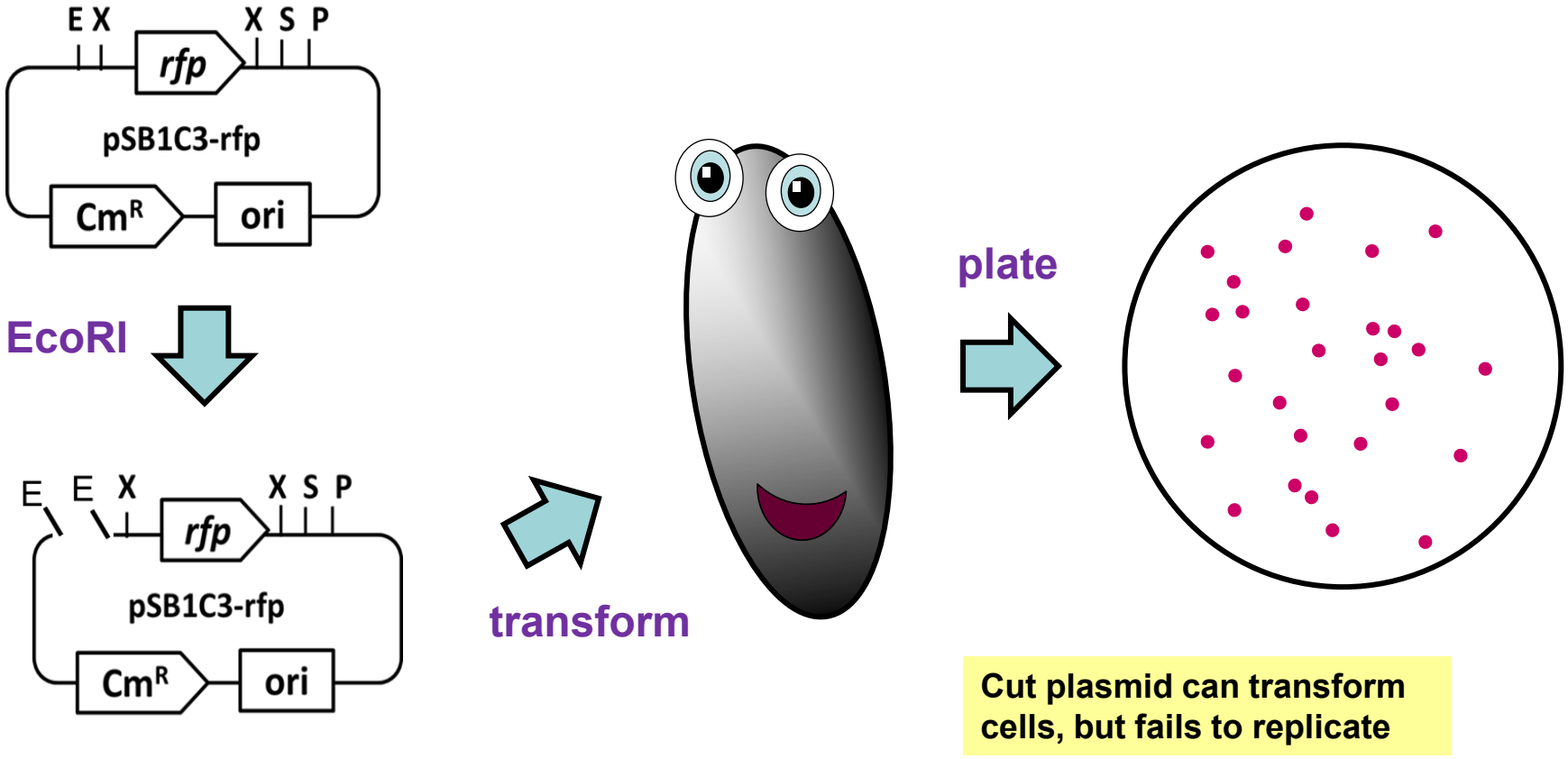
- **NO PLASMID**
  - **Are the agar plates OK? (is antibiotic working?)**
  - **Is aseptic technique OK?**

- **Expect no colonies**



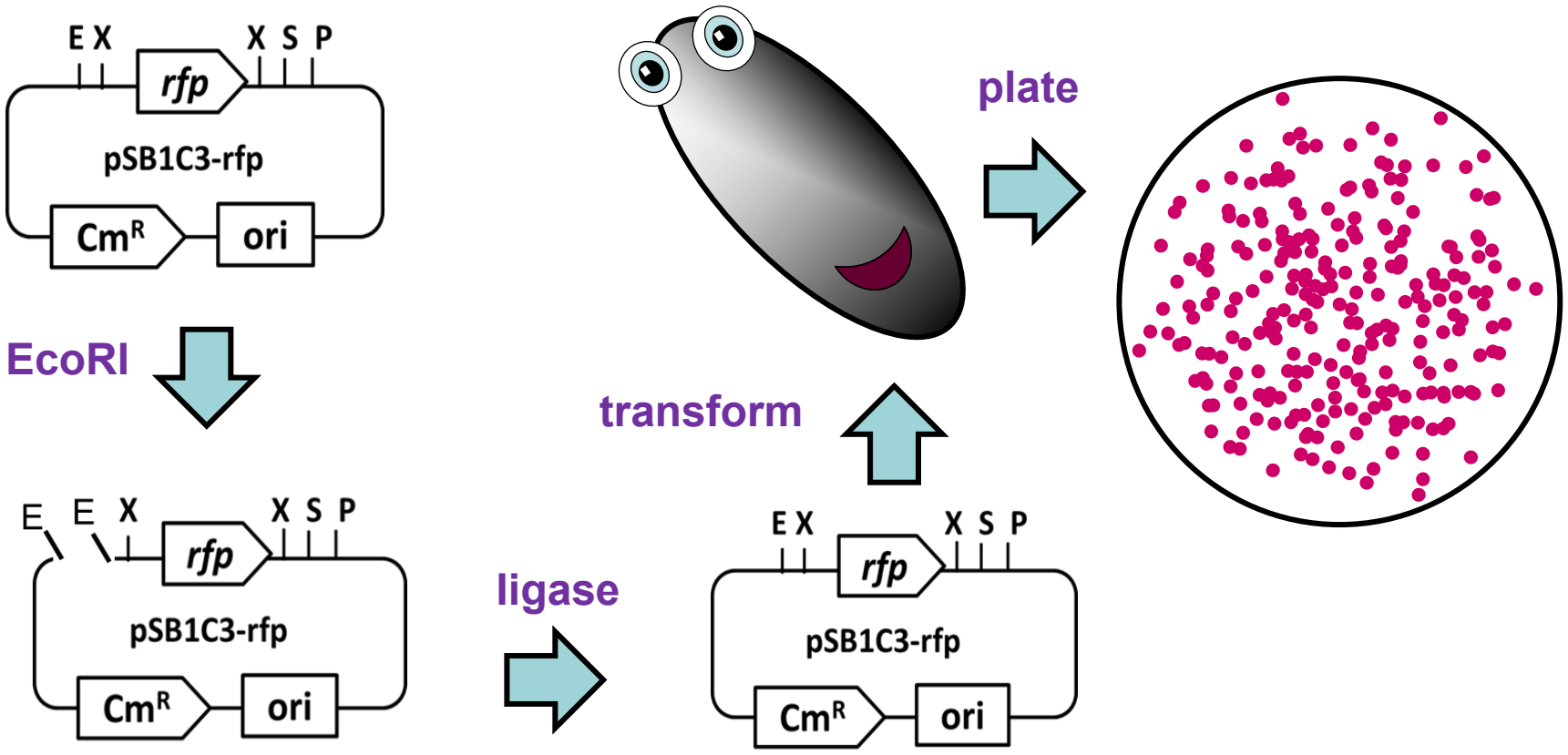
Ligation & Transformation control #3

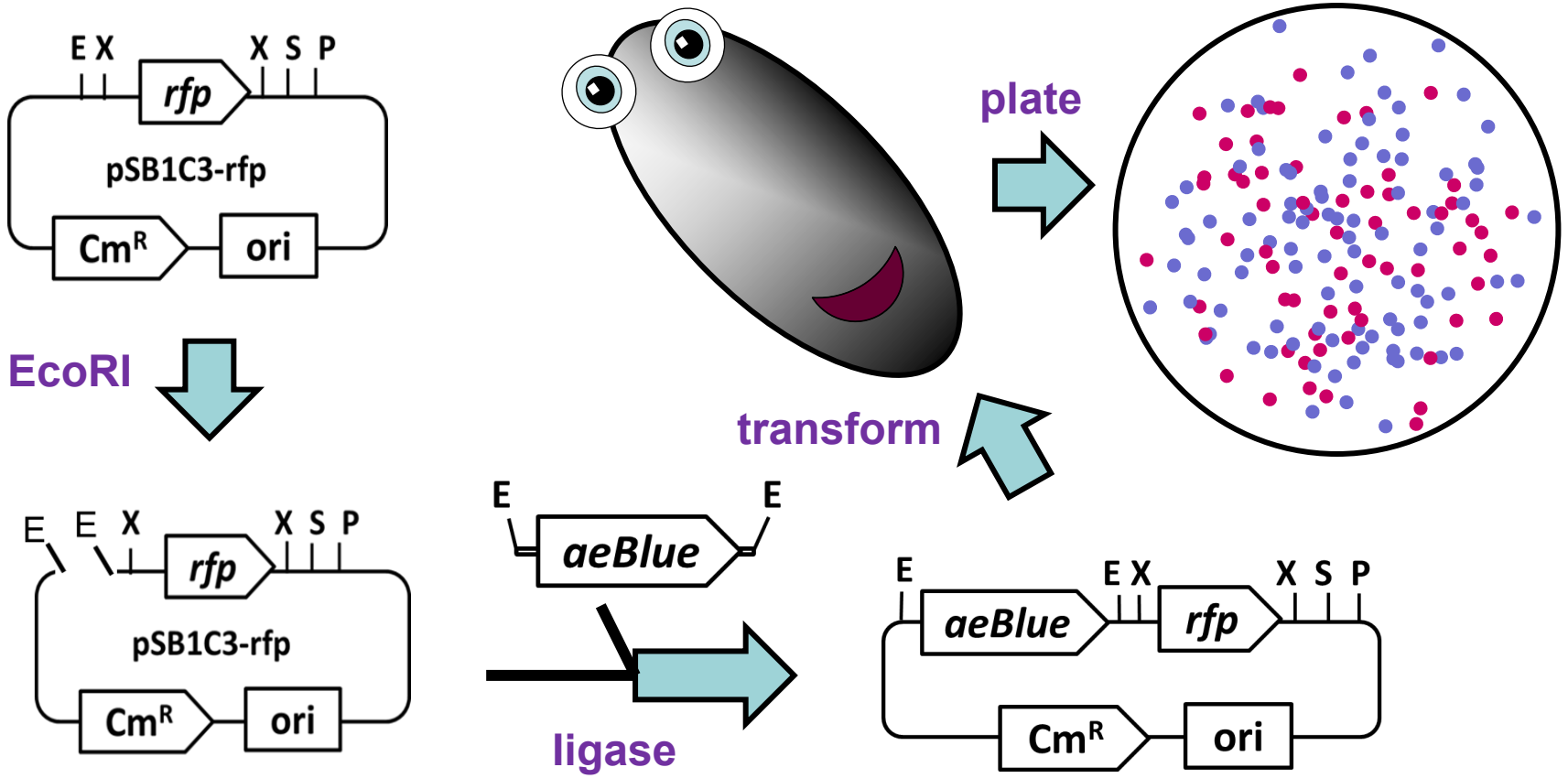
- **CUT PLASMID**
  - **Is the restriction enzyme working?**
- **Expect only a few colonies (<100), with original genotype**



Ligation & Transformation control #4

- **CUT PLASMID, LIGATED TO ITSELF**
  - **Is the ligase working?**
- **Expect many colonies (>100), with original genotype**



Ligation & Transformation – actual experiment

- **CUT PLASMID, LIGATED TO aeBlue**
  - **Has insert DNA been cloned into the plasmid?**
- **Expect mix of colonies with old + new genotypes**