Labeling *E. coli* Chromosomes with Antibiotic Markers and/or Fluorescent Proteins

Materials

Target bacterial strain

Constructed DNA cassette

Lambda Red recombinase expression plasmid, pKD46

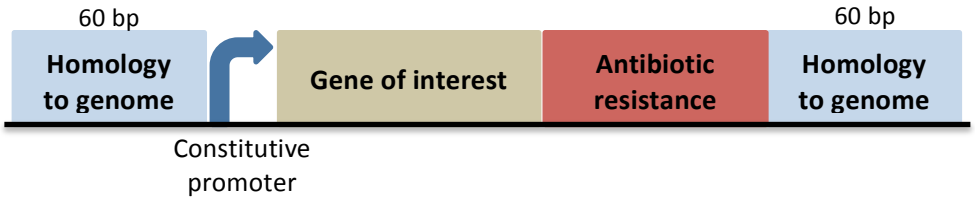
Protocol

1. Make or obtain competent cells of your target strain [comp. cell protocol link] and transform pKD46 (plasmid containing genes necessary for homologous recombination). pKD46 is a temperature sensitive plasmid, so be careful to grow cells at 30º C.

   Ape file containing sequence of pKD46

2. Construct a DNA cassette containing gene intended for insertion, an antibiotic marker, and homology to the genomic DNA.

   The cassette should look like this:



   The sequence that is homologous to the genome is where your cassette will be inserted, so you should pick the beginning and end sequence of a non-vital gene as your homology regions.

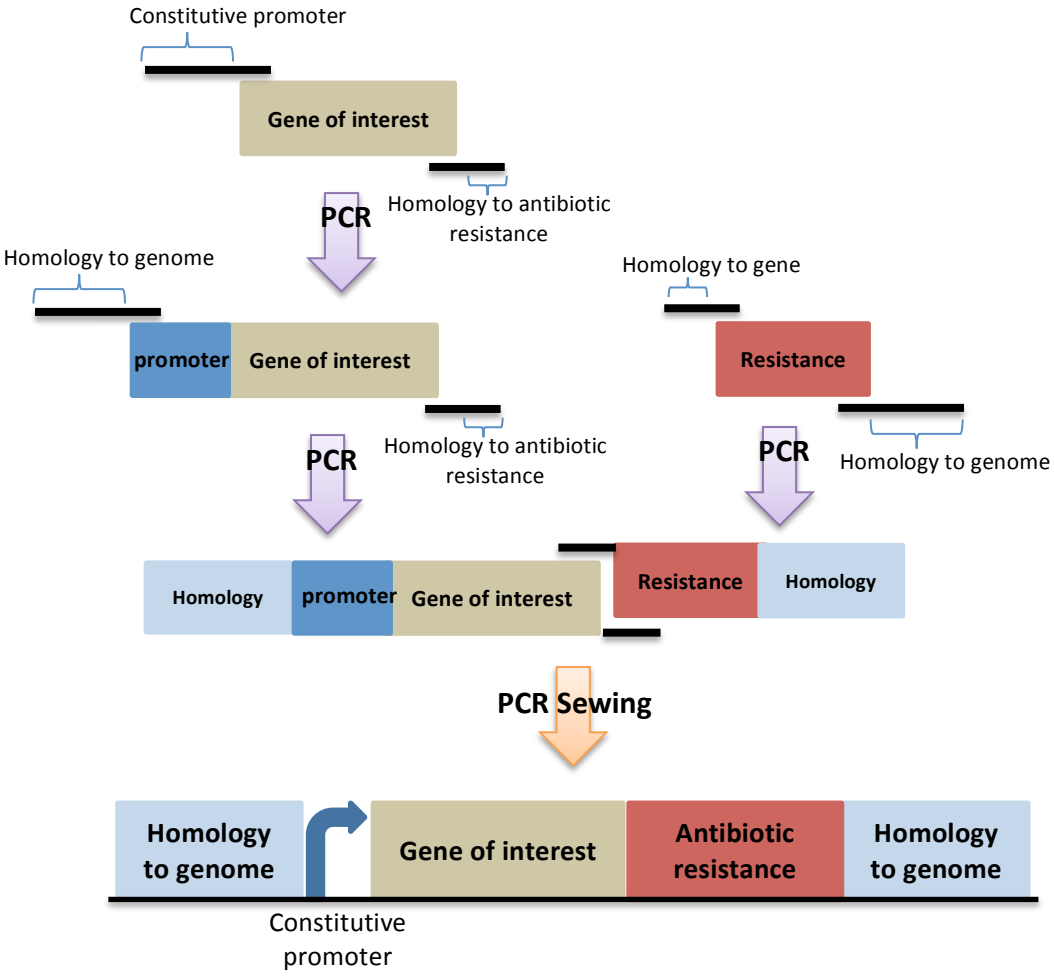
   Suggested regions for cassette insertion:

Gene	Sequence
LacZ	Start: GATTACGGATTCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTAC End: CGGTTTCCATATGGGGATTGGTGGCGACGACTCCTGGAGCCCGTCAGTATCGGCGGAATT
lomR_2	Start: GTGCTGACCGGAAGTGATGACGGTCGCCACAGCAACACGTCTCTGGCGTGGGGAGCTGGC End: CCCGGCAGTGGCGACTGGCGCACTGACGGTTTCATCGTGGGTGTCGGTTATAAGTTCTGA
wbbL_2	Start: ATGCTGGTACGTTTTTCAGATTTTGTGCGTGTAAATGGCTTCGATCAAGGTTACTTTATG End: AGAAAACGTATTTTATCAAATCGCAACTTTGATCGAATTTCATCAGTTTTTCACCCGTAA
yhiS_1	Start: ATGTCAATTGACTTTACCCCAGGTATAATAAATACATATCACGGCGATATTTATAACTGC End: AATTTCACTGACGCAAATTTAGGGAAGGTGCGAATAAGCGGGGAAATTCTTCTCGGCTGA

- Amplify genes [PCR protocol], first adding a constitutive promoter if necessary, then homology sequence and 15+bp overhangs.
  - For fluorescent proteins, the constitutive promoter **BBa_J23100** gives strong expression and **BBa_J23105** gives medium expression. [link to parts?]

- For the antibiotic resistance, you can PCR from the priming sites of lambda red recombinase expression plasmids (pKD3, pKD4, pKD13) which have FRT sites flanking the resistance gene that allow you to knock out the resistance if necessary.
- Provide ape file of an example of our cassette

- Use PCR-sewing [PCR sewing protocol] to sew your PCR products together and obtain a cassette that looks like the diagram above.



- Be sure to gel extract [Gel extraction protocol] your cassette.
- Follow the Lambda Red Recombination protocol [Lambda Red protocol] to insert DNA cassette into the genome of your target strain.