

**1. Effect of overexpressing carotenoid pathway genes in *Rhodococcus***

First goals:

- Prepare reagents for preparation of genomic DNA
  - Prepare genomic DNA from *Rhodococcus erythropolis* AN12
  - Design primers for PCR of target ORF
  - Prepare AN12 competent cells
1. Obtain plates of *R. erythropolis* AN12
  2. Inoculate small (2-3 ml) LB cultures to prepare your own frozen stocks; grow 1-2 days at 30°C
  3. Pour LB Gent10 plates
  4. Pour LB Kan100 plates
  5. Inoculate 10 ml LB 1% sucrose culture with a colony of AN12; grow at 30°C for 1-2 days; to be used for genomic miniprep
  6. Inoculate small (10 ml) culture of LB 1% sucrose with AN12 to be used for competent cells
  7. Grow cultures for 1-2 days at 30°C
  8. Chill a bottle of distilled sterile water for making competent cells
  9. Prepare sterile 10% glycerol for making competent cells
  10. Find genomic miniprep protocol on web site; prepare all necessary reagents
  11. Design primers to amplify target ORF from AN12