Halo (Killer) Assay

## BMB-YPD Plates

1. Prepare **phosphate-citrate buffer** stock.
   1. Prepare citrate solution.
      1. Add 42 g of citrate to 200 mL H2O.
   2. Prepare potassium phosphate solution.
      1. Add 8.7 g K2HPO4 to 50 mL H2O.
   3. Combine citrate solution and potassium phosphate solution.
   4. Add NaOH until pH reaches 3.1.
   5. Filter sterilize.
2. Prepare 0.3% **methylene blue** stock.
   1. Add 0.75 g methylene blue powder to 250 mL H20.
   2. Filter sterilize.
3. Add 10 mL **phosphate-citrate buffer** and 10 mL 0.3% **methylene blue** to 500 mL of melted YPD agar. Mix well by inversion, rolling, etc. Pour plates.

## To Assay Killer Phenotype

1. Dry BMB-YPD plates in advance so that liquid is readily absorbed.
2. Prepare overnight cultures (>1 mL) of all query and control strains.
3. Dilute “lawn strain” 1:20 and spread 100 uL onto BMB-YPD plate. Allow plate to dry before proceeding.
4. Concentrate “spot strain” 100:1 and spot 5 uL onto lawn. Allow plate to dry before inverting.
5. Incubate plates at room temperature for 2-3 days. Record observations, particularly at the interface between the lawn and spot.