High-Throughput 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. Pipette 50 mL per single-well plate.

## 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” 1:10 and spread 150 uL per BMB-YPD plate. Allow plate to dry before proceeding.
   1. Alternative: If testing query strain for immunity/sensitivity, run the “Lawn spotting” BioMek protocol on query plate.
      1. Spots 10 uL of 1:32 dilution. Allow plate to dry before proceeding.
4. If testing query strain for killing ability, run “HT Killer Assay” BioMek protocol on query plate. Allow plate to dry before inverting.
   1. Removes supernatant, resuspends in residual media, and spots 2 uL concentrated culture. 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.