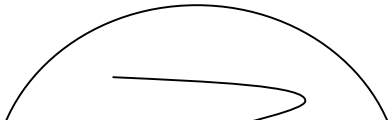
Replating Cells for a Mutation accumulation Experiment

Materials Needed:

SM plates
Ka
Loop

1. Label new, blank plates with the transfer number, date, line, and a dot placed randomly on the plate (ex: T70, 5/6/04, Line 4).

   Note: Plates should be allowed to age for at least one day, preferably two days.
2. Sort plates into groups of ten (1-10, 11-20, etc).
3. Sort plates from previous transfer into groups of ten, and find the plaque that is closest to the dot and also is obviously derived from a single cell (perfectly circular morphology UNLESS all other plaques on that plate are also non-circular) and can be transferred without touching another plaque. Circle this plaque.
4. Wash hands up to elbows.
5. Plate out 300 microliters of Ka onto the new plate and spread.
6. In the hood, scrape up the plaque with a sterilized and **cooled** loop (try to get as little bacteria as possible) and plate out like this:



NOTE: If the plaque is large, take only the growing edge of cells, not the entire plaque.

7. Keeping the plate level, carefully move to the back of the hood to dry without the lid.
8. Once plate has dried, cover and store lid up in a drawer.
9. Repeat with all lines.