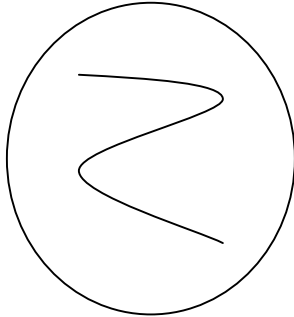


Protocol for Measuring Cell Growth from MA Lines  
23/10/04 S. Middlemist

1. Streak out lines from frozen with 300 microliters Ka. Mark a dot on the plate.



2. When plaques appear, replate clonally with 300 microliters Ka. Select plaque closest to the dot.
3. Repeat once, marking 5 dots on the plate at random.
4. In 48 hours, collect the five individual plaques that are closest to the dots. Suspend each plaque in 50 microliters of KK2. Using the hemocytometer, count the number of cells in 25 squares. Do two counts per plaque.
5. Calculate the number of cells/ml using the following formula:  
$$10,000 \times (25 / \# \text{ of squares}) \times \# \text{ cells} \times \text{dilution factor}$$
6. Take the number of cells/ml and divide by 1000 to get the number of cells/microliter, then multiply that number by the number of microliters of KK2 you collected the cells into.  
Ex:  $3.60 \times 10^6 \text{ cells/ml} \times 1 \text{ ml} / 1000 \text{ microliters} = 3.60 \times 10^3 \text{ cells/microliter} \times 50 \text{ microliters} = 1.80 \times 10^5 \text{ cells/50 microliters}$