# 7.13 Experimental Microbial Genetics

Fall 2008

**Bootcamp Day 1 – Start a culture of *E. coli* + pMQ64 and streak *Pseudomonas aeruginosa* PA14 for single colonies**

1. Start a culture of *E. coli* + pMQ64 for tomorrow's maxi prep

Dilute the starter culture of *E. coli* DH5α + pMQ64 you have been given 1/1000 into LB + 30 ug/ml gentamicin medium. For high-copy plasmids like pMQ64, inoculate ● 150 ml medium. For low-copy plasmids, inoculate ● 250 ml medium. Grow at 37°C for 12–16 h with vigorous shaking (approx. 300 rpm).

*Use a flask or vessel with a volume of at least 4 times the volume of the culture. The culture should reach a cell density of approximately 3–4 x $10^9$ cells per milliliter, which typically corresponds to a pellet wet weight of approximately 3 g/liter.

2. Streak the cryostock of *Pseudomonas aeruginosa* PA14 you have been given for single colonies to LB.

- Obtain an LB agar plate (with antibiotic if appropriate).
- Using a sterile wooden stick, touch the bacteria growing within the punctured area of the stab culture.
- Run the stick lightly over a section of the plate, as shown in the figure, to create streak #1.
- Using another sterile wooden stick, pass through streak #1 and spread the bacteria over a second section of the plate, to create streak #2.
- Using a third sterile wooden stick, pass through streak #2 and spread the bacteria over the last section of the plate, to create streak #3.
- For routine growth of *Pseudomonas aeruginosa* and *E. coli*, wrap the outer edges of the plate with parafilm and incubate the plate at 37°C overnight.
- In the morning, single colonies should be visible. If the bacterial growth is too dense, re-streak onto a new agar plate to obtain single colonies.