Multiple *E.coli* transformations on a single bacterial plate

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We have found that routine transformations can be scaled down so that 36 independent transformations can be selected on a single bacterial plate. The method blends two established techniques; plating transformed bacteria in top agar, and spot titrations beloved of phage geneticists. When combined with the use of 96 well microtitre trays this method offers considerable savings in time and materials over conventional transformations.

10 μ l aliquots of competent cells are placed in a sterile 96 well microtitre tray, complete with lid, on ice. Following the addition of plasmid DNA and incubation, on ice for 10-30 minutes, heatshock is performed by floating the microtitre tray (wrapped in plastic film) in a 42°C water bath for 2 minutes. After returning to ice, 5 μ l aliquots of each transformation are spotted onto selective plates over which a 3ml layer of antibiotic free LB soft-agar has just (5 minutes earlier) been poured and allowed to set. The plates are immediately incubated, uninverted at 37°C. Leaving the lid ajar for 5 minutes aids in the rapid absorption of the 5 μ l spots into the top agar layer. The bacteria begin to grow on the drug free surface and have a short time to express resistance genes before the antibiotic diffuses from the plate below. By adjusting either the amount of DNA or by making dilutions of bacteria before plating, it is possible to generate single colonies within the area of a 5 μ l spot. Alternatively, purifying single clones on fresh selective plates by streaking from a confluent 5 μ l patch is equivalent to the initial selection of an isolated colony. Figure 1 demonstrates the technique. Various amounts of the colE1 based vector pRSVBgal were transformed into competent XAC cells selecting for ampicillin resistance. The mean efficiency of transformation was $1.95 \times 10^6 \mu\text{g}^{-1}$.