

Sugden Lab.

Measure establishment efficiency of OriP, DS, FR-neomycin plasmids in 3  
293/EBNA-1 clones that express different amounts of EBNA-1

1. Measure cloning efficiency of 293 cells:
  - a. dilute 293 cells to 5 cells /ml
  - b. add 200ul to each well of 4 96well plates. So I need  $0.2 \times 3 \times 96 \times 4 = 76.8$  ml of 5cell/ml 293.
  - c. After two weeks, count the number of wells with no cells growing— $P(0) = \# \text{ of empty wells} / 96 \times 4$ — $e^{-m} = p(0)$ , then  $m = \text{cloning efficiency}$

Note: It is 31% from Liz's data.

2. Measure establishment efficiency :

- a. Plasmids:
  - 1591: oriP-neo
  - 1683: FR-neo
  - 1685: DS-neo
  - 1590: neo with no ori.David Mackey made the above plasmids. But his descriptions of these plasmids tell nothing about how he made it!

2510: CMV-RFP

- b. experimental procedure:
  1. grow up 8 dishes for each of the 3 cell lines to 20-50% confluency.
  2. Transfect equalmolar of each of the plasmids plus 2510 into the each of the 3 cell lines. Each transfection is done in duplicate.
  3. 2 days later, count the transfection efficiency. Plate out  $2 \times 10^5$ ,  $10^4$  and  $10^3$  cells per 15 cm dish under 200ug/ml G418. Thus there are total of 72 15cm dishes.