Utilization of magnetic affinity cell sorting for the isolation of stable transformants

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Various techniques have been developed for the stable transformation of eukaryotic cells with exogenous DNA which confer drug resistance, including Eco-gpt (1) and neo (2). A critical problem associated with drug resistant markers is that many cell types are very dependent on cell to cell interactions for growth. Thus, if the transformation frequency is low, drug resistant cells may be inhibited from growing due to the low survival density. On the other hand, if the cell density is too high following transfection, nontransformants will escape selection, resulting in high background. A second problem is that the currently available procedures make it difficult to record the number of population doublings the cells have undergone; this becomes a dilemma with non-immortalized cell populations, for e.g., when studying cellular senescence. We have utilized the system of magnetic affinity cell sorting (MACS), which we developed for the study of transient transformants (3,4), to circumvent these problems. Synchronized HeLa S3 cells were transfected by electroporation with pRSV-IL2R, treated with butyrate and sorted (4). The positive cells were plated at a 4 X 10^5 cell/75 cm^2 flask, grown to subconfluence, and resorted at each passage. These cell populations went through a period in which transiently transfected cells lost the ability to express IL2R prior to the stabilization of the culture at 4-6 passages. Thereafter, the percentage of cells expressing IL2R remained stable, both in the presence and absence of sorting, through 20 passages (Figure 1; losses during the sorting procedure prevent the recovery of 100% of the stable population). It was previously reported that in cotransfection experiments with pSV2-neo and pSV2-gpt, when one plasmid was used for selection and the other was nonselected, 25% of the resulting colonies were resistant to both antibiotics (2). We find that following cotransfection of pRSV-IL2R and pRSV-neo by electroporation and selection by MACS, up to 50% of the IL2R population are also resistant to G-418 after 20 passages. This method is unique in that: i) selection is very rapid, allowing for the study of both transiently and stably transfected cells; ii) plating densities as well as cell passage number can be monitored; and iii) non-toxic negative selection is possible, allowing for the isolation of non-transfectants and revertants.

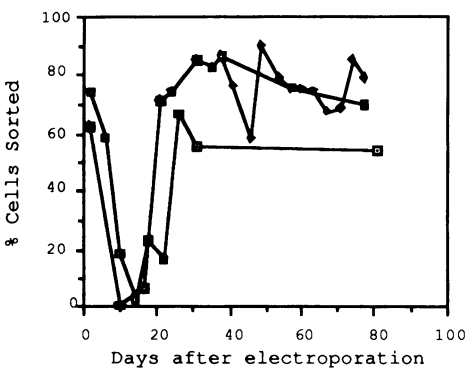

Figure 1:Generation of Stable Populations of HeLa Cells Expressing IL-2 Receptor. MACS was performed as designated by the symbols for each of 3 populations. Closed symbols represent a single population which was split after stabilization, with one fraction sorted at each passage and the other sorted at passages 15 and 20.

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