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Epstein-Barr Virus Transformation of Cryopreserved Lymphocytes: Prolonged Experience with Technique

To the Editor:

We wish to support Louie and King's (1991) comment, published in the March 1991 issue of the *Journal,* that one can successfully transform frozen lymphocytes to long-term cell lines by using Epstein-Barr virus (EBV). With slight modification, the basic method described in their letter has been used successfully in our laboratory since 1987. We have established nearly 2,900 lymphoblastoid cell lines by EBV transformation, about 600 of these from frozen lymphocytes. The success rates using freshly isolated versus previously frozen lymphocytes are similar, both approximating 97% in our hands.

We feel that freezing has two advantages—it can be accomplished at sites distant from the transformation laboratory, and it allows the preservation of potentially important material with a lesser commitment of resources. However, once we are committed to a specific project, we generally prefer to go to direct transformation—because we will always freeze aliquots at the end, and this avoids one freezing step. We have excellent success in developing cell lines from live cells that have been sent to us from as far away as Israel and that have been as long as 3 d in transit.

The method we use is based on those of Miller and Lipman (1973) and Anderson and Gusella (1984) and is adapted to suit our laboratory's purposes. We can identify the following as ways in which our method differs from that described by Louie and King: (1) direct separation on a density gradient without first isolating the buffy coat, to save time; (2) washing the lymphocytes only twice and at lower speeds, to be less harsh on the cells; (3) using cyclosporin A in the transformation step, rather than PHA-P; and (4) using a controlled-rate cell freezer (Cryomed model 700A) for a more consistent and accurate rate of freezing, giving a better rate of recovery of the frozen cells.

SHEILA PRESSMAN AND JEROME I. ROTTER
Medical Genetics Birth Defects Center
Cedars-Sinai Medical Center
Los Angeles

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