

1. In addition, healthy cells growing in log phase are critical for optimal transformation efficiency in gene targeting experiments. The criteria used in our laboratory to qualify an ES cell clone for making chimeras is that at least 50% of the chromosome spreads analyzed must be 40 XY. In our experience, our DBA/1LacJ ES cells (12) meet or exceed that criterion Murine Embryonic Stem Cells 1 at least 86% of the time, whereas our 129 strain of ES cells meet or exceed the criteria 45% of the time. Smithies and colleagues later demonstrated that ES cells, modified by gene targeting when reintroduced into blastocysts, could transmit the genetic modifications through the germline (7). ES cells have been reported for other mammalian species (i.e., hamster, rat, mink, pig, and cow), however, only murine ES cells have successfully transmitted the ES cell genome through the germline. ES cells are isolated from the inner cell mass (ICM) of the blastocyst stage embryo and, if maintained in optimal conditions, will continue to grow indefinitely in an undifferentiated diploid state. ES cells that are not cared for properly will spontaneously differentiate, even in the presence of feeder layers and leukemia inhibitory factor (LIF). Today, genetic modification of the murine genome by ES cell technology is a seminal approach to understanding the function of mammalian genes in vivo. (1–4).