



Title	AN ASSESSMENT OF THE PRODUCTION OF MOUSE GERM-LINE CHIMERAS DERIVED FROM EMBRYONIC STEM CELLS INTRODUCED INTO 8-CELL-STAGE HOST EMBRYOS BY CO-CULTURE AND MICROINJECTION METHODS
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AN ASSESSMENT OF THE PRODUCTION OF MOUSE GERM-LINE
CHIMERAS DERIVED FROM EMBRYONIC STEM CELLS INTRODUCED
INTO 8-CELL-STAGE HOST EMBRYOS BY CO-CULTURE AND
MICROINJECTION METHODS

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For assessment of the efficiency of germ-line chimera production techniques, embryonic stem (ES) cells derived from a male blastocyst of the 129/Sv strain were introduced into 8-cell-stage embryos of the C57BL/6 strain by co-culture and microinjection methods.

For the integration of ES cells by co-culture, zona-free 8-cell embryos were settled on the lawn of ES cells (0.5×10^6 cell/ml) in Whitten's medium containing 10% fetal calf serum (FCS) with or without phytohemagglutinin-p (PHA: 10 μ g/ml). Although ES cells initially attached to the surface of the embryos with or without PHA, they were detached from the embryos during overnight culture. The embryos that developed to morphologically normal blastocysts were transferred to the uterus of the recipient on the third day of pseudopregnancy. No coat-color chimeric mice were obtained.

Three to five or ten to fifteen ES cells were microinjected into 8-cell embryos. After the injection, embryos were cultured for 24–28 hrs in M16 medium supplemented with 10% FCS. The embryos developing to the blastocyst stage were transferred to the uterus of the pseudopregnant recipient and allowed to develop to term. Of 129 embryos injected with ten to fifteen ES cells, only 3 (2.3%) were born alive. These offspring did not show coat-color chimerism. In contrast, as many as 28 out of 111 (25.2%) embryos injected with three to five ES cells developed to term. However, for some unknown reason, half of the newborns died at birth. Six of those which survived were overt coat-color chimeras. To test the germ-line transmission of the ES cell genome, 5 chimeric males were mated with C57BL/6 females and 3 fertile males were proved to be germ-line chimeras.

The rate of implantation and subsequent development of embryos injected with ten to fifteen ES cells were significantly hindered. Embryos recovered on day 10 of pregnancy showed growth retardation by more than 24 hrs. Electrophoretic analysis of glucose-phosphate-isomerase isozymes showed that 14 of 21 embryos (66.7%) were chimeric. Apparently, these embryos were destined to be lost before birth.

In conclusion, germ-line chimeras were not obtained efficiently using the two methods tested. However, injection of three to five ES cells into 8-cell stage

embryos was more effective in the production of germ-line chimeras. Injection of ten to fifteen ES cells was effective in producing chimeric embryos, but they failed to thrive until birth. These results suggest that the success of producing germ-line chimeric mouse may be dependent on the proportion of ES cells injected into the host embryo.