

SOP: MLL-AF9 Quaternary Transplant Model
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Description of Cells

We introduced the human MLL-AF9 oncogene via retroviral transduction into flow-sorted GMPs from Actin-dsRed transgenic mice (C57BL/6 background). Transduced GMPs were then transplanted to a lethally irradiated recipient mouse with leukemia generation after about 100 days. The spleens of these primary mice, which are densely packed with leukemic cells, were harvested and then serially transplanted through sublethally irradiated secondary, tertiary, and quaternary mice, forming leukemias with decreased latency at each round. The long bones of quaternary transplant mice, which develop leukemia in roughly two weeks, may be harvested, crushed, and filtered to isolate leukemic cells. The cells are then flow-sorted (dsRed+, c-kit high) to purify cells in the leukemia stem cell compartment.

Flow sorted quaternary transplant MLL-AF9 cells were resuspended in 90% FBS + 10% DMSO (freezing medium), dispensed into cryovials, and frozen at -80°C in a Nalgene Cryo freezing container overnight. Cryovials were transferred the next day to liquid nitrogen freezer for long-term storage.

A full description of the MLL-AF9 quaternary transplant model is available (Hartwell et al., manuscript in preparation) (Miller et al., manuscript in preparation).

Procedure

Receipt of Frozen Cells

- 1) Immediately place frozen flow-sorted quaternary transplant MLL-AF9 cell aliquots in liquid nitrogen storage until ready to thaw.
- 2) When ready, quickly thaw in a 37°C water bath.
- 3) As soon as ice crystals disappear, swab outside surface of the ampoule with 70% ethanol, then dispense contents of ampoule into a 15mL Corning centrifuge tube containing 9mL RPMI + 10% FBS + Pen/Strep/Glut
- 4) Pellet cells at 1500 RPM for 5 minutes (4°C).
- 5) Resuspend cell pellet in 10mL 1X PBS + 10% FBS + Pen/Strep/Glut.
- 6) Pellet cells at 1500 RPM for 5 minutes (4°C).
- 7) Proceed with desired use of cells.