Culture of MEL murine cell lines

Created by: Jin Lian (Sherman Weissman lab-Yale) **Modified by:** Zhihai Ma and Stephen Landt (Michael Snyder lab- Stanford)

Obtaining cell lines:

Weissman lab- Yale University sherman.weissman@yale.edu

Mouse erythroleukemia (MEL), derived from a B-cell lymphoma, are rapidly dividing murine cell lines that are maintained in suspension cultures and grow as loose clumps. Cells should be maintained at 37° C with 5% CO₂ at a density between 1×10^{5} and 1×10^{6} cells/mL. Cultures should be split 1:8-10 ~every two days to maintain this concentration.

MEL growth medium: 10% HI-FBS(heat-inactivated), 1% Pen-strep(Penicillin and Streptomycin), RPMI 1640 with L-Glutamine;

MEL freezing medium: 90% HI-FBS, 10% fresh DMSO, freeze at a concentration of 1X10⁷ cells/ml.

Starting cultures from frozen stocks:

- 1. Thaw cell vials at 37°C.
- 2. Add to 15mL tube with 10 ml fresh growth medium, centrifuge 1500 rpm/ 3min., and discard supernatant.
- 3. Resuspend cells in 10ml fresh growth medium, add to small flask and incubate in 5% CO₂/37°C.
- 4. Cells can normally be split ~1:8 after 2 days and maintained as described above.

DMSO-induction of MEL cells: To induce erythroid differentiation, MEL cells are treated with 2% DMSO. DMSO-induction of differentiation slows growth so that cells only need to be split 1:2 at day 2 or 3 after induction. Differentiating cells will become noticeably red when pelleted by 2-3 days after induction and color will increase in following days.