Melanocytes

From: Duke/UNC/UT/EBI ENCODE group
Date: 12/1/09
Prepared by: Patrick Tompkins in William Kaufmann's lab (UNC)

1. **Source:** Science cell online (www.sciencellonline.com)
   Cat. # 2200,
   NHM22=lot#1002
   NHM23=lot#1014

From Supplier (Science cell online): Epidermal layer was isolated by enzyme digestion. Further enzyme digestion of the epidermal layer was used to obtain single cell suspension. Single cell suspension (mostly melanocytes and keratinocytes) was plated in cell culture flask in melanocyte medium (ScienCell, cat. no. 2201) to enrich melanocytes. The primary melanocyte culture still contains few keratinocytes and need to be further purified by differential trypsinization method. The purified melanocytes were plated in new flask (passage one) and fed with melanocyte medium again. Melanocytes were cryopreserved on passage one culture (ScienCell, cat. no. 2200) when the culture reached around 90% confluency. Commercially available cells were stored at liquid nitrogen and delivered to end user on dry ice.

2. **Lineage of cells:** Primary melanocytes

3. **Donor information:** Unknown

4. **Karyotope:** Unknown

5. **Media for cell lines**
   Invitrogen
   Base Media=Medium 254, cat#M-254CF-500
   HMGS-1 suppplement, cat#S-002-5
   HMGS-2 supplement, cat#S-016-5

6. **Growth conditions:** 5% CO_2, never below 30% confluency

7. **Protocol of cell growth:** After cells were thawed, they were spun down to remove DMSO. Approximately 5x10^5 cells were thawed into a T25 and expanded to T75 flasks. Cells were

split when they reached ~90% confluence by gently washing with PBS then adding 0.5mls of 0.05% trypsin to each T75 flask. Trypsinized cells were diluted with media and never split higher than 1:3 as these cells do not like to be sparse. Media was changed 2-3 times a week, maintaining 15mls of media on the cells. Any cell clumps were broken up when adding the media after trypsinization.

8. **Cell passages:** Cells started from passage 1, and were grown to passage <10