Customer Developed Protocol

Generation of single cell suspension from E8.25 mouse embryos

Contributed by:
Bertie Gottgens, Ph.D.

Contributor Research Profile

The Göttgens group uses a combination of experimental and computational approaches to study how transcription factor networks control the function of blood stem cells and how mutations that perturb such networks cause leukaemia. This integrated approach has resulted in the discovery of new combinatorial interactions between key blood stem cell regulators, as well as experimentally validated computational models for blood stem cells. Current research focuses on (i) single cell genomics of early blood development, (ii) computer models to chart the transcriptional landscape of blood stem and progenitor cell differentiation, (iii) transcriptional consequences of leukaemogenic mutations in leukaemia stem/progenitor cells, and (iv) molecular characterization of human blood stem/progenitor cell populations used in cell and gene therapy protocols.

Link to the lab: https://www.stemcells.cam.ac.uk/research/pis/gottgens

Reference

This protocol was used in:

Ibarra-Soria *et al.* Defining murine organogenesis at single-cell resolution reveals a role for the leukotriene pathway in regulating blood progenitor formation., *Nat Cell Biol.* 2018 Feb;20(2):127-134
https://www.ncbi.nlm.nih.gov/pubmed/29311656

Publication Press Release: https://www.stemcells.cam.ac.uk/news/new-2018cell-map2019-reveals-intricate-details-of-mammalian-development

10x Genomics Products

10x Chromium Single Cell Gene Expression Solution - https://www.10xgenomics.com/single-cell/

Notices

THIS PROTOCOL IS NOT SUPPORTED BY 10X GENOMICS

This Customer Developed Protocol is provided for general information only and is not directly supported, endorsed, or certified by 10x Genomics. 10x Genomics gives no warranties and makes no claims about the provided protocol.

For questions or to share protocol adjustments, please visit the 10x Community at community.10xgenomics.com

Required Buffers and Reagents

1. PBS with 3% FBS
2. TrypLE
3. 0.04% BSA
4. Flowmi Cell Strainer (40 micrometer)

Protocol

Uteri were harvested from time-mated dams and embryos micro-dissected in ice-cold 3% FBS in PBS. Embryos were pooled together by developmental stage and larger later stage embryos fragmented using dissecting forceps for more efficient dissociation. Embryo fragments were transferred to ice-cold 3% FBS/PBS in 1.5mL Eppendorf tubes. The embryo fragments were centrifuged at 400G for 5 minutes, the supernatant aspirated and re-suspended in 50-100 µL of TrypLE ensuring samples were adequately submerged. Samples were incubated at 37°C in a shaker at 1400rpm for 2-5 minutes depending on developmental stage. The resulting suspensions were visually inspected to ensure adequate dissociation prior to quenching with FBS and wash (400G, 5 minutes) with 3% FBS/PBS. Cell suspensions were centrifuged and resuspended in 70 - 100 µL of 0.04% BSA. Intermediate cell counts were taken at the quenching stage and in combination with target cell recovery used to inform final resuspension volume. The cells suspended in 0.04% BSA were filtered through a 40 micrometer Flowmi cell strainer into a new 1.5mL Eppendorf and the final cell count performed.

THIS PROTOCOL IS NOT SUPPORTED BY 10X GENOMICS

This 10x Community Customer Developed Protocol is provided for general information only and is not directly supported, endorsed, or certified by 10x Genomics. 10x Genomics gives no warranties and makes no claims about the provided protocol.

For questions or to share protocol adjustments, please visit the 10x Community at community.10xgenomics.com