

Customer Developed Protocol

Isolation of single cell suspensions from epidermis

Contributed by:

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CUSTOMER DEVELOPED PROTOCOL

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Contributor Research Profile

The Powell lab develops and applies computational and statistical genomics approaches to investigate the genetic control of genome regulation and its role in contributing to the susceptibility to human disease. Specifically, our research involves the use of large-scale transcriptomic and DNA sequence data from both bulk tissues and single cells, focusing on understanding the genetic mechanisms by which heritable variants contribute to disease susceptibility at a cellular level, and ultimately achieve therapeutic and diagnostic outcomes.

Link to the lab: <https://imb.uq.edu.au/single-cell-and-computational-genomics>

Reference

This protocol was used in:

Lukowski *et al.* Detection of HPV E7 transcription at single-cell resolution in epidermis., *bioRxiv doi:*

<https://doi.org/10.1101/252858>

<https://www.biorxiv.org/content/early/2018/01/24/252858.full.pdf+html>

10x Genomics Products

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

Notices

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Isolation of single cell suspensions from epidermis

Required Buffers and Reagents

1. 2.5 µg/µL Dispase II (Roche)
2. 1 µg/µL Collagenase D (Roche)
3. 0.2 µg/µL DNase (Roche)
4. 0.7 µm filter (BD Falcon)
5. 7-AAD (0.25 µg, eBioscience)
6. Rat anti-mouse CD19/CD32 (5 µg, clone 93, eBioscience)
7. APC-conjugated rat anti-mouse CD45.2 antibodies (0.5 µg, Clone 104, Biolegend)
8. PBS + 2% serum + 2 mM EDTA

Protocol

Tissue collection and dissociation

Skin biopsies were split into dorsal and ventral parts and incubated with Dispase II (Roche) for 1 h at 37°C. Epidermis and dermis were separated with closed forceps. The epidermis was further homogenized and digested with 1 µg/µL of collagenase D (Roche) and 0.2 µg/µL of DNase, (Roche) for 1 h at 37°C. Digested samples were passed through a 0.7 µm filter (BD Falcon) to generate a single cell suspension for staining and sorting.

Cell sorting

Flow cytometry of single cell suspensions of digested epidermis samples were incubated with Fc Block (5 µg, rat anti-mouse CD19/CD32, clone 93, eBioscience) diluted in PBS for 30 min on ice. Samples were subsequently incubated with APC-conjugated rat anti-mouse CD45.2 antibodies (0.5 µg, Clone 104, Biolegend) diluted in PBS + 2% serum + 2 mM EDTA for 30 min on ice. Prior to sorting, cells were labelled with 7-AAD (0.25 µg, eBioscience). Live CD45- cells (7-AAD- and APC- or PE-Cy7-) were sorted using the BD ARIA Fusion sorter at 12 psi with a 100 µm nozzle. ~300,000-500,000 events/cells were collected per sample.

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