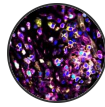MAR 21, 2024

🌐 HuBMAP | Digestion and scRNA Analysis of Skin

Arivarasan Karunamurthy[1], HJ Park[1]

[1]University of Pittsburgh Medical Center

GE Research

Liz McDonough
GE HealthCare

ABSTRACT

This method details digestion and scRNA analysis of the HuBMAP tissue skin specimens.

Protocol status: Working
We use this protocol and it's
working

Created: Mar 21, 2024

1   The fresh skin sample is digested enzymatically using Miltenyi Biotec Whole Skin Dissociation Kit, human for 2 hours and further dispersed using the Miltenyi gentleMACS Octo Dissociator.

2   The resulting cell suspensions are filtered through 70 μm cell strainers twice and resuspended in phosphate-buffered saline (PBS) containing 0.04% BSA.

3   Resulting cell suspensions are then loaded into 10× Genomics Chromium instrument (Pleasanton, CA) for library preparation. V1 and V2 single-cell chemistries were used per manufacturer's protocol.

4   The resultant libraries are sequenced (~200 million reads/sample), using the Illumina NextSeq-500 platform.

5   The output sequencing reads were then examined by quality metrics, transcripts mapped to reference human genome (GRCh38) and assigned to individual cells according to cell barcodes, using Cell Ranger (10× Genomics).