Customer Developed Protocol

Tissue dissociation and single cell preparation of breast cancer patient-derived xenografts

Contributed by:
Ioannis Ragoussis, Ph. D. and Morag Park, Ph. D.

Contributor Research Profile (Ioannis Ragoussis)

The Ragoussis lab works on Technical Developments including the Single Cell Genomics Lab of MUGQIC and Mutation Detection Technologies with focus on the genomic and transcriptomic analysis of single cells. Specifically, his group focuses on 4 main areas: 1) Single cell genomics to study tumor heterogeneity, tumor microenvironment, and circulating tumor cells, 2) Methodologies for the highly sensitive detection of both somatic as well as germline mutations and different cancer types, 3) The development of long read single molecule sequencing based methodologies and diagnostic tests, and 4) High throughput whole genome genotyping using Affymetrix Axiom technology as part of Canada's Longitudinal Study of Aging.

Link to the lab: http://www.mcgillgenomecentre.org/ioannis-ragoussis/

Contributor Research Profile (Morag Park)

The Park lab aims to identify new signal transduction pathways that are important for the development of human cancers and how these can be targeted with drug therapies. This is a complex question that requires a full understanding of how signals are integrated in normal cells and how these signals become altered in tumor cells, in the context of other genetic alterations. Specifically, Park's research focuses on 1) Studying mechanisms of activation and cell transformation by receptor tyrosine kinases, 2) Investigating the role of Gab Family Signal Amplifiers in Epithelial Morphogenesis and tumorigenesis, 3) Identifying signals regulating epithelial cell motility and invasion in breast cancer

Link to the lab: https://www.mcgill.ca/biochemistry/about-us/department/faculty-members/park

Reference

This protocol was used in:

Savage *et al*. A Targetable EGFR-Dependent Tumor-Initiating Program in Breast Cancer, *Cell Reports*, 2017, 21(5):1140-49
https://www.ncbi.nlm.nih.gov/pubmed/29091754

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Required Buffers and Reagents

1. MACS Octo Dissociator with Heater (Miltenyi)
2. Mouse Cell Depletion Kit (Miltenyi)
3. PBS with 0.04% BSA

Protocol

Single-cell suspensions were generated by mechanical and enzymatic dissociation using the gentle MACS Octo dissociator with heaters (Miltenyi), according to manufacturer's protocol. Murine stromal cells were removed using a Mouse Cell Depletion Kit (Miltenyi), according to manufacturer's protocol.

Mouse-depleted single-cell suspensions were washed three times in PBS with 0.04% BSA. An aliquot of cells was used for LIVE/DEAD viability testing (Thermo Fisher Scientific). Single-cell libraries were generated using the GemCode Single-Cell Instrument, the Chromium™ Single Cell 3' Library & Gel Bead Kit v2 and Chromium™ Single Cell A Chip Kit (10x Genomics), according to the manufacturer's protocol.