Preparing Mouse Organs for Flow Cytometry

AUTHORS

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TYPE OF PROTOCOL
Real Methods

ABSTRACT
This protocol is a description of the Real Methods used to disaggregate tissues into single cell suspensions. In order to perform flow cytometric analysis on single cells from mice, it is necessary to mechanically mince the tissue and chemically digest connective components while leaving cells alive and intact. This protocol provides information to create cell suspensions from pancreas, liver, breast, intestine, spleen, brain, lung tissues.

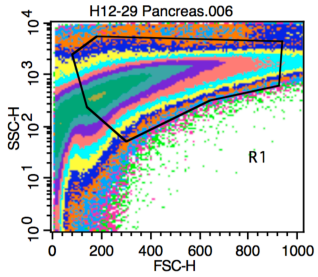
KEYWORDS
Disaggregate tissue, flow cytometry, mouse organ, cell suspension

PUBLICATIONS CITING RESEARCH USING THE PROTOCOL
Kiraly O, Gong G, Olipitz W, Muthupalani S, Engelward BP (2015) Inflammation-Induced Cell Proliferation Potentiates DNA Damage-Induced Mutations *In Vivo*.PLoS Genet 11(2): e1004901. doi: 10.1371/journal.pgen.1004901

Sukup-Jackson MR, Kiraly O, Kay JE, Na L, Rowland EA, et al. (2014) Rosa26-GFP Direct Repeat (RaDR-GFP) Mice Reveal Tissue- and Age-Dependence of Homologous Recombination in Mammals *In Vivo*. PLoS Genet 10(6): e1004299. doi: 10.1371/journal.pgen.1004299

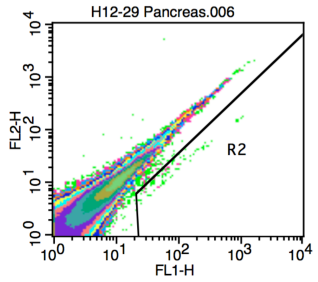
EXAMPLE DATA

RaDR mouse – mutated cells express EGFP



File: H12-29 Pancreas.006 Sample ID: H12-29 Pancreas cells
Acquisition Date: 17-Oct-12 Gate: No Gate
Gated Events: 2411907 Total Events: 2411907

Region	Events	% Gated	% Total	X Mean	Y Geo Mean
R1	1428136	59.21	59.21	339.50	638.62
R2	117	0.00	0.00	387.84	554.87



File: H12-29 Pancreas.006 Sample ID: H12-29 Pancreas cells
Acquisition Date: 17-Oct-12 Gate: G1
Gated Events: 1428136 Total Events: 2411907

Region	Events	% Gated	% Total	X Geo Mean	Y Geo Mean
R1	1428136	100.00	59.21	6.47	6.68
R2	72	0.01	0.00	79.98	18.35

P:

PROCEDURES

Overview
Tissues harvested from mice are mechanically minced, digested with collagenase, and strained into single cell suspensions.

Materials
Mouse organs of interest
gentleMACS Dissociator (Miltenyi Biotec 130-093-235)*
gentleMACS C Tubes (Miltenyi Biotec 130-093-237)*
2mg/mL Collagenase V (Sigma Aldrich C9263) in HBSS (Life Technologies 14025)
37C Incubator with shaker
DMEM (Life Technologies 11965) with 10% FBS
Pipetman
Serological pipets (10 mL)
70 um Cell strainers (VWR 21008-952)
50 mL Falcon tubes
Bucket of ice
Centrifuge
OptiMEM (Life Technologies 31985)
1000 uL micropipette with tips
5 mL flow tubes with cell strainer caps (VWR 21008-948)

Duration: ~2 hours

Process
1. Harvest organ(s) from mouse and place each in separate gentleMACS C tube with 5 mL of collagenase (2 mg/ml Collagenase V in HBSS). Screw on lid tightly,

invert, and insert in gentleMACS dissociator. Mince organ on appropriate gentleMACS program.*

a. Liver = Liver settings (all)
b. Pancreas = Liver settings (all)
c. Breast = Liver settings (1, 2, then 2 again)
d. Colon and intestines = Intestine settings (all)
e. Spleen = Spleen settings (all)
f. Brain = Brain settings (all)
g. Lung = Lung settings (all)

**Note*: If your lab does not have a gentleMACS dissociator, this step can be performed by finely mincing the tissue with scalpels or razors in a plastic dish. The minced tissue should then be added to a tube containing 5 mL of collagenase, and the procedure continued as below.

2. Place gentleMACS tubes with tissue and collagenase in 37C incubator on shaker. Set timer for 40 minutes.
    a. If processing many tissues, set multiple timers as sets of organs go into incubator.
3. While tissues are digesting, prepare 50 mL Falcon tubes with 10 mL media (10% FBS in DMEM). Place cell strainer on top of each tube.
4. After 40 minutes, remove tubes from incubator. Triterate collagenase/tissue mixture ~10-20 times with 10 mL serological pipet, avoiding bubbles. Pipet all contents into the cell strainer. (Most of the liquid should flow through easily, separating out visible chunks of tissue). Place tubes on ice.
5. Centrifuge tubes at 1000 rpm in centrifuge at 4C for 10 minutes.
6. Decant or aspirate supernatant from tubes, being careful not to disrupt pellet.
7. Resuspend cells in ~350 uL of OptiMem or other low-serum media and transfer to flow cytometry tubes through strainer caps. This may take some force. If the strainer cap breaks, transfer the whole sample through a new strainer cap into a new tube.
8. Hold tubes on ice until ready to process.