Human islet culture and processing for RNA and chromatin studies

Updated protocol, Irene Escala Miguel 2015

I. Islet cell culture

After islet isolation or transportation, islets are cultured for 3 days in RPMI1640 supplemented with 10% heat-inactivated FCS and penicillin/streptomycin 100 units/ml.

1. Upon arrival, transfer islets to 50 ml Falcon tubes, spin at 500g for 1-3 min in a benchtop centrifuge (Eppendorf 5804 R) at room temperature. Alternatively, let islets sediment at RT. All liquids are handled in the primary tissue culture hood.

2. Discard supernatant. For every ~2,000 islets (based on quantitation after islet isolation) add 10 ml of medium. Resuspend and transfer to 100 mm bacterial culture dishes, place in culture at 37ºC.

If there are less than 5000 islets in total, transfer only ~1,000 islets to one of the dishes to be employed for RNA analysis. Ensure that there is always 10 ml medium in each culture dish.

II. Estimation of purity by dithizone staining.

1. Dilute 200 μl dithizone solution with 800 μl Hanks, centrifuge at maximum speed at RT for 5 min.

2. Move dish softly with the intention of dispersing islets uniformly. Use a sterile pipette to take a representative aliquot ~100 μl (containing ~100 islets). Transfer to an eppendorf tube, and add 900 μl diluted dithizone solution to this, incubate for 15 min at 37 °C.

3. Rinse islets with PBS twice, count islets and estimate islet purity under a microscope. Extrapolate islet number to the total islet count based on the total culture volume. Transfer the islets to an eppendorf tube, spin, remove supernatant and snap freeze. This islet aliquot can be used for DNA purification.

Note that this quantitation of islet numbers is only intended to provide an extra verification step. The number of islets can differ substantially from the initial more accurate quantitation that is performed after isolation due to several reasons, including islet aggregation or cell death.

III. Snap Freezing islets for RNA, protein, and DNA purification

1. After culture, collect islets in the center of the culture dish by very slow constant anticlockwise swirling of the dish. Use a pipette to remove as much debris and non-islet material as possible

2. Transfer islets to nuclease-free eppendorf tubes (~1,000 islets per tube). Spin at 500g for 1-3 min in a benchtop centrifuge (Eppendorf Centrifuge 5415R) at room temperature.

3. Remove most supernatant. Add PBS with protease inhibitor cocktail, spin again at 800 rpm (100g) for 1 min, remove as much supernatant as possible, and snap freeze in liquid nitrogen or dry-ice ethanol bath. Store at -80 ºC.

IV. Fixation of the islets for ChIP and FAIRE experiments

1. After culture, collect islets in the center of the culture dish by very slow constant anticlockwise swirling of the dish. Use a pipette to remove as much debris and non-islet material as possible.

2. Transfer islets from two or more culture dishes to 50 ml Falcon tubes. Spin at 500g for 1 min in a benchtop centrifuge (Eppendorf 5804 R) at room temperature.

3. Remove most supernatant, and transfer all islets into a single new 15 ml tube.

4. Rinse islets with 10 ml PBS and spin at 500g for 1 min. Remove the supernatant. Repeat this twice.

Do not at any point expose islets to unnecessary physical stress, such as inverting or vigourous shaking of tubes. Pipetting fixed islets will cause significant loss of the sample due to adherence to plastic surface.

5. Remove supernatant and add PBS up to 10 ml.

6. Add 625 µl of fresh 16% formaldehyde (R1026 Agar Scientific, final concentration 1%) and fix cells shaking gently for 10 min at room temperature.

These fixation conditions are adequate for most ChIP assays and FAIRE, but need to be optimized for certain antibodies.

7. Stop fixation by adding 641.9 µl 2 M Glycine (final concentration 125 mM) and incubate for 5 minutes shaking gently at RT.

8. Centrifuge islets at 500g for 1 minute at room temperature.

9. Remove the supernatant, resuspend cells in 1 ml ice-cold PBS with protease inhibitor cocktail. Centrifuge at 500g for 1 min at 4˚C. Repeat this step twice.

10. Remove the supernatant, snap-freeze fixed islets in liquid nitrogen or dry ice ethanol, and store the samples at -80˚C or liquid nitrogen.

Reagents and Solutions:

Dithizone stock solution
Add 10 mg dithizone to 2 ml dimethyl sulfoxide (DMSO).
Store the solution at -20˚C.

RPMI1640
heat-inactivated FCS
penicillin/streptomycin
Hanks
PBS
16% Formaldehyde (R1026 Agar Scientific)
2 M Glycine
Protease inhibitor cocktail
Dry ice
Ethanol