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Whole Cell Patch Clamp of Dispersed Human Islet Cells

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Human Islet Research Network

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Cells use exocytosis to secrete a wide variety of molecules, including proteins, hormones, and neurotransmitters. Exocytosis can be monitored at the single-cell level by using patch-clamp electrophysiology to measure changes in membrane capacitance as vesicles fuse with the cell membrane and release their content. Dispersion of pancreatic islets into single cells allows for individual characterization of electrophysiological characteristics and allows for collection of cellular content for recovery of full-length transcriptomes by use of Smart-seq2.

Described in this protocol is the dispersion of pancreatic islets into single cells followed by whole-cell patch clamp electrophysiology which includes parameters representing cell size, exocytosis, sodium channel currents, and calcium channel currents. Cells are then collected individually after recording to be processed for single-cell RNA sequencing.

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https://hpap.pmacs.upenn.edu/explore/workflow/islet-physiology-studies?protocol=10

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HPAP, HIRN, Whole Cell Patch Clamp

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References

Joan Camunas-Soler, Xiao-Qing Dai, Yan Hang, Austin Bautista, James Lyon, Kunimasa Suzuki, Seung K Kim, Stephen R Quake, Patrick E MacDonald. Pancreas patch-seq links physiologic dysfunction in diabetes to single-cell transcriptomic phenotypes. Cell Metabolism, 2020. https://doi.org/10.1016/j.cmet.2020.04.005

Equipment and recording solution

1. EPC10 patch-clamp amplifier (HEKA Instruments Inc, Germany)
2. Inverted microscope (Zeiss)
3. Motorized micromanipulator (Sutter Instrument, MP-225)
4. PatchMaster and Fitmaster Software (HEKA Instruments Inc, Germany)
5. Capillary Glass tubing with flame polished ends pipettes (Warner Instrument)
6. Extracelluar recording solution (in mM): 118 NaCl, 20 Tetraethylammonium, 5.6 KCl, 1.2 MgCl2, 2.6 CaCl2, 5 HEPES, and either 1, 5 or 10 glucose (pH 7.4 with NaOH)
7. Pipette recording solution (in mM): 125 Cs-glutamate, 10 CsCl, 10 NaCl, 1 MgCl2, 0.05 EGTA, 5 HEPES, 0.1 cAMP, and 3 Mg-ATP (pH 7.15 with CsOH).
8. Lysis buffer for collecting cells: H2O **1340 mL** , recombination RNase inhibitor **50 mL** , ERCC (1:600000) **50 mL** , 10% Triton **10 mL** , 10mM dNTP **500 mL** , 100mM dT **50 mL** , total **2000 mL** . dT is a customized oligo (IDT),

the sequence is as follows:

AAG CAG TGG TAT CAA CGC AGA GTA CTT TTT TTT TTT TTT TTT TTT TTT TTT TVN

patch-clamp amplifier
EPC10

HEKA **895273**

Inverted microscope

Zeiss **None**

Motorized micromanipulator
MP-225

Sutter Instrument **None**

PatchMaster and Fitmaster Software

HEKA **None**

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Capillary Glass tubing with flame polished ends pipettes

Warner Instrument None

Procedure

1 On the day receiving the shipped human pancreatic islets, hand-picked islets are dissociated to single cells using StemPro accutase (Gibco/Fisher, A11105-01). Plate cells in **35 mm** cell culture dishes, and culture in DMEM with 5.5 mM glucose, 10% FBS, and 100 U/mL penicillin/ streptomycin for 1-4 days.

2 Start patch clamping single cells after one overnight incubation, and continue patching cells for up to 4 days. Electrical activities are measured by using a pipette coated with sylgard (3 ~ 5 MΩ) in a heated chamber (32–35°C). Quality control is assessed by the stability of seal (>10 GΩ) and access resistance (<15 MΩ).

3 Perform electrical activity measurements in 1 minute from “break in” of cell membrane; measurement protocols include (in order): exocytosis, voltage-gated Na and Ca channel currents activated at -10 mV, and -120 mV, voltage-gated Na and Ca channel currents activated from -60 to +30 mV, steady-state inactivation of voltage-gated Na channel currents, reversal potential, hyperpolarization-activated non-selective cation currents activated at -140 mV.

4 When finishing all the measurements, use another big-tip pipette (0.2 ~ 0.5 MΩ) prefilled with **0.5 mL** lysis mix, suck the cell into the pipette, and transfer it into a **0.2 mL** PCR tube prefilled with **4 mL** lysis mix.

5 Save cells in **-80 °C** freezer before shipping out for sequencing.

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Data Analysis

6 Using the software of Fitmaster (HEKA Instruments Inc, Germany), analysis is performed on the level of recording traces.

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