Whole-cell patch clamp

The MacDonald Lab

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I. 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 $MgCl_2$, 2.6 $CaCl_2$, 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 $MgCl_2$, 0.05 EGTA, 5 HEPES, 0.1 cAMP, and 3 Mg-ATP (pH 7.15 with CsOH).
8. Lysis buffer for collecting cells: H_2O 1340 µl, recombination RNase inhibitor 50 µl, ERCC (1:600000) 50 µl, 10% Triton 10 µl, 10mM dNTP 500 µl, 100µM dT 50 µl, total 2000 µl. 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

II. 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 µl lysis mix, suck the cell into the pipette, and transfer it into a 0.2ml PCR tube prefilled with 4 µl lysis mix.
5. Save cells in -80 °C freezer before shipping out for sequencing.

III. Data analysis

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

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