Human Islet Procedure for Single Cell Multiome

Kaestner Lab, University of Pennsylvania
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HUMAN ISLET PROCEDURE FOR SINGLE CELL MULTIOME (v.11/29/2022 HD) Case Number: Condition: Date:

NOTE:

- Recommended starting material: 7,000 IEQs
- Estimated processing time to first stopping point: 5 hours

Description starting material: Total IEQs used:_____ Purity:_____. Islets concentration/flasks:_____ Number of flasks:_____

Date of pancreas dissociation:_____

On the Day before the procedure:

- Empty and clean the vacuum
- Empty and autoclave Biohazard trashes

On the Day of the procedure:

- Prewarm at 37C for 20min: the **0.05% trypsin** (Invitrogen #25300054, thaw ~2ml for one sample), **100% FBS** (Hyclone #SH30910003, thaw ~2ml for one sample), and **Prodo+ media** (thaw ~20ml for one sample).
- Cool down at 4C benchtop centrifuge. Clean your bench and pipettes. Spay your gloves with RNaseZap regularly.
- **Aliquot 10ml of 1X PBS w/o Ca^{2+}/Mg^{2+}** (Corning 21-031-CV) into a 15ml conical tube.
- Prepare the following buffers: **0.04% BSA/PBS buffer, Lysis buffer**, **Wash buffer** and **Diluted Nuclei buffer.** Add reagents in the order listed.
- Look at the islets under the microscope.

PROCEDURE:

1. In the tissue culture hood, **Use a prewet 10 ml serological pipette** to **gently** mix flask containing the islets and split the volume between 50 ml conical tubes (25ml of media/50ml conical tube=> 2tubes/flask).
2. Centrifuge 1 min, 1200rpm, RT. Aspirate the supernatant.
3. **Re-suspend islets in 10ml of Prodo+ Media** by gently pipetting up and down 5 times with a **prewet** 10ml serological pipette.**Transfer Islets to an untreated petri dish** and bring to Bay 12-165.
4. Use a P20 pipette set to 20ul to **pick all good looking islets** (~300 circular, large and slightly brown islets) and **transfer them to the 10ml PBS aliquot**.
   Keep the remaining islets in the petry dish in the cell incubator until the Multiome first BioA is completed.
5. Centrifuge for 2min at RT and 180g. Aspirate the supernatant.
6. **Add 1ml of warm 0.05% trypsin to the islets**. Pipette up and down 10 times with a P1000 pipette set to 1000ul. **Incubate at 37C for 9 min in the waterbath**. Pipette up and down at t=7min, 5min, 3min, 1min.
7. **Stop digestion by adding 1ml of 100% FBS to the islets**. Pipette up and down.
8. **Filter through a 40um cell strainer** (Corning #352235). Use 1ml of PBS to rinse the conical tube and pass through the filter.
9. Tranfer the 3ml of filtered cells to a new 15ml conical tube and fill it up with PBS to rinse. Centrifuge 4min at 400g at 4C. Aspirate the supernatant.
10. Resuspend in 500ul of 0.04% BSA. Keep the cell suspension on ice at all time.
11. To count the cells mix 10ul of Trypan blue (Invitrogen #T10282) with 10ul of the cell suspension. Transfer 10ul to each side of the counting slide (Invitrogen #C10283/C10312) and read at the Cell Countness II (Bay 12-160). **Note:** Cell Countness II already adjusts for the trypan dilution in the count displayed on its screen.
    Write down Cell count and viability:_____
12. Homogenize well the cell suspension with a P1000 set at 500ul and **transfer between 100,000 and 1,000,000 cells** to a 1.5ml microcentrifuge tube. Centrifuge at 300g for 5min at 4C.
13. **Remove ALL the supernatant** without disrupting the cell pellet.
14. **Add 100ul of chilled lysis buffer**. Pipette mix 10x. **Incubate for 5min**.
    During incubation thaw the ATAC buffer B (10X genomics #2000193), place the ATAC enzyme B (10X genomics #2000265) on ice. Set up the thermocycler with the transposition program containing a holding step.
15. **Add 1ml of chilled Wash buffer** to the lysed cells. Pipette mix 5x. Centrifuge at 500g for 5min at 4C. Remove supernatant and **repeat for a total of 2 washes**.
16. **Resuspend in 200ul of chilled Nuclei buffer**.
17. Count the Nuclei concentration by mixing 10ul of the nuclei suspension with 10ul of trypan blue and read at the Countess II.
    ~50% of nuclei loss is expected during the cell lysis. The nuclei shape should be round and homogenous.
    Write down Nuclei count and lysis efficacy (viability):_____
18. Adjust the nuclei buffer volume for a **concentration of 2,500 nuclei/ul**. Capture 5000 Nuclei total.
19. **Proceed immediately** with the Chromium Next GEM Single Cell Multiome ATAC + Gene Expression Guide (CG000338). 3hrs of processing time remaining at this point until the first safe stopping point at -80C.

Transposition Mix, prepare fresh **maintain on ice**		
Reagents	**How to mix**	**1 sample**
Nuclei suspension at 2,500 nuclei/ul	Pipette mix	3.22 ul
Diluted Nuclei buffer	Pipette mix and centrifuge	1.78 ul
ATAC buffer B (10X genomics #2000193)	Vortex and centrifuge	7 ul
ATAC enzyme B (10X genomics #2000265)	Pipette mix and centrifuge	3 ul

20. **Freeze down extra cells or nuclei** at the concentration of 5x10^6/ml in recovery cell culture freezing medium (Gibco #12648010). This medium contains DMSO and is toxic to the cells and nuclei. Cells/Nuclei should be placed in a cryovial (Corning #430488) at -80C in Mrs frosty immediately after resuspension. The day after, transfer vials to nitrogen task and update the N2 tank paper log for position and sample description.

Appendix: Buffer to Prepare before the procedure

0.04% BSA/PBS buffer, prepare fresh maintain on ice			
Reagents	**Stock conc.**	**Final conc.**	**5ml**
PBS without Ca^{2+}/Mg^{2+} (Corning 21-031-CV)	1X	-	4.980ml
BSA * (EMD Millipore #126593-10MG)	10%	0.04%	20ul

*Make 10% BSA/PBS stock by dissolving 1g of BSA in 10ml PBS, work in sterile condition and aliquot in 500ul fractions to store at -20C
*Note: When our stock finishes, order MACS BSA stock solution (Miltenyi Biotec #130-091-376), recommended by 10X genomics and cheaper by volume.

Lysis buffer, prepare fresh maintain on ice						
Reagents	**Stored at**	**How to mix?**	**Stock c.**	**Final c.**	**500ul (1X)**	**1ml (2X)**
Nuclease-free Water	RT	-	-	-	418.5ul	837ul
Tris-HCL pH 7.4 (Invitrogen #15568-025)	RT	Invert/vortex thoroughly	1M	10mM	5ul	10ul
NaCl (Invitrogen #AM9760G)	RT	Invert/vortex thoroughly	5M	10mM	1ul	2ul
MgCl2 (Invitrogen #AM9530G)	RT	Invert/vortex thoroughly	1M	3mM	1.5ul	3ul
Tween-20 (Roche #11332465001)	+4C #B	Invert/Pipette mix	10%	0.1%	5ul	10ul
Nonidet P40 Substitute (Roche #11332473001)	+4C #B	Invert/Pipette mix	10%	0.1%	5ul	10ul
Digitonin (Millipore #300410-250MG**)	-20C #B	Vortex, incubate at 65C	5%	0.01%v	1ul	2ul
BSA (EMD Millipore #126593-10MG)	-20C #B	Invert/Pipette mix	10%	1%	50ul	100ul
DTT (Sigma #646563)***	RT	vortex thoroughly	1M	1mM	0.5ul	1ul
RNase Inhibitor* (Roche #03335402001)	-20C #A	Pipette mix, centrifuge	40U/ul	1U/ul	12.5	25ul

*Vortex mix before adding **RNase Inhibitor**, centrifuge after having put all reagents.
** **Digitonin** (Millipore #300410-250MG**) is a non-ionic detergent used to solubilize membrane-bound proteins. It is toxic. To reconstitute at 5%: 250mg in 5ml of boiling H_2O. Heat for 15 min at 98°C. Cool and place in the refrigerator (4°C) for 3 h. Filter off any precipitate. Freeze 5% stock at -20C.
*** **DTT** is toxic. It is a reducing agent used to stabilize or denature dissulfide bonds, enzymes and other proteins.

Wash buffer, prepare fresh maintain on ice						
Reagents	**Stored at**	**How to mix**	**Stock conc.**	**Final conc.**	**2ml (1X)**	**4ml (2X)**
Nuclease-free Water	RT	-	-	-	1.698ml	3.396ml
Tris-HCL (pH7.4) (Invitrogen #15568-025)	RT	Invert/vortex thoroughly	1M	10mM	20ul	40ul
NaCl (Invitrogen #AM9760G)	RT	Invert/vortex thoroughly	5M	10mM	4ul	8ul
MgCl2 (Invitrogen #AM9530G)	RT	Invert/vortex thoroughly	1M	3mM	6ul	12ul
Tween-20 (Roche #11332465001)	+4C #B	Invert/Pipette mix	10%	0.1%	20ul	40ul
BSA (EMD Millipore #126593-10MG)	-20C #B	Invert/Pipette mix	10%	1%	200ul	400ul
DTT (Sigma #646563)	RT	vortex thoroughly	1M	1mM	2ul	4ul
RNAse inhibitor* (Roche #03335402001)	-20C #A	Pipette mix, centrifuge	40U/ul	1U/ul	50ul	100ul

*Vortex mix before adding RNase Inhibitor, centrifuge after having put all reagents.

Diluted Nuclei buffer, prepare fresh maintain on ice						
Reagents	**Stored at**	**How to mix**	**Stock conc.**	**Final conc.**	**300ul (1X)**	**600ul (2X)**
Nuclease-free water	RT		-	-	247.5ul	495ul
Nuclei buffer (10X genomics #2000207)	-20C #B	vortex thoroughly	20X	1X	15ul	30ul
DTT (Sigma #646563)	RT	vortex thoroughly	100%	10%	30ul	60ul
RNase inhibitor* (Roche #03335402001)	-20C #A	Pipette mix, centrifuge	40U/ul	1U/ul	7.5ul	15ul

*Vortex mix before adding RNase Inhibitor, centrifuge after having put all reagents.