2/13/20 CP

nuclei isolation from human heart tissue

be organized, diligent and keep sample and solutions cold at all times

on dry ice
flat bottom mortar and pestle, hammer and foreceps
sample-flash frozen heart tissue
scale plate

once everything is cold,
assemble scale and cover plate with weighing paper
weigh 300mg tissue
transfer tissue immediately into mortar and cover with pestle

laminar air hood

on dry ice
pulverize tissue in mortar using pestle and hammer
hammer gently, scrape off tissue stuck to pistill and hammer again 3-6x

on wet ice
transfer pulversized tissue in 6cm dish containing 4ml cell lysis buffer on ice
start timer
segregate particles and transfer into douncer A with transfer pipette
wash plate with 2ml cell lysis buffer and transfer into douncer A
dounce carefully 30x
filter through 100um mesh in 50ml Falcon tube
wash douncer A with 2ml cell lysis buffer and filter as well
keep 10ul for QC #1
transfer into douncer B
dounce 20x
filter through 40um mesh in 50ml Falcon tube
wash douncer B with 2ml cell lysis buffer and filter as well
transfer into 15ml Falcon tube
take time: should have taken 10min
spin 400g for 7min at 4C
aspirate supernatant

centrifugation

resupend pellet in 600ul homogenisation buffer (D)
add 1ml Optiprep working solution- 30% iodixanol
keep 10ul for QC #2
transfer into centrifugation tube (40ml)
underlayer carefully nuclear sample with 8ml 35% iodixanol using serological pipette
underlayer carefully both layers with 4ml 40% iodixanol
centrifuge at 8,000xg for 20min at 4C; no breaks
collect ring of nuclei at 35% -40% iodixanol interface
add same volume of nuclear buffer
spin at 500xg for 10min at 4C
aspirate carefully and resuspend in nuclear buffer
stain 5ul of sample as well as all fractions of QC with DAPI
check nuclei for complete lysis, nuclei morphology, purity and count

! during testing, collect all 3 phases of Optiprep centrifugation, add same volume of nuclear buffer and spin to check for quality and quantity of seperation of nuclei and cell debris. Adjustments may be required.

	C (work)	D (hom)	per sample
30% Optip	1	0.6	1.6
35% Optip	7	3	10
40% Optip	4	1	5
per solution	12	4.6	

fixation

resuspend nuclei in 100ul nucelar buffer
add drop wise 400ul 100% icecold (-20C) methanol to suspension and transfer into -80C

stock cell lysis buffer (store at 4C):
10 mM Tris-HCl, pH 7.4
10 mM NaCl
3 mM MgCl2

1ml cell lysis buffer-prepare fresh -9ml/sample
950 ul stock cell lysis Buffer
10ul IGEPAL CA-630
10ul 20U/ul SUPERase In RNase Inhibitor
10ul 10% BSA
10 ul 0.2M Spermine
10ul 10% Tween-20

A **OptiPrep (product stock)**

B **OptiPrep diluent (store at 4C)**
150 mM KCl
30 mM $MgCl_2$
120 mM Tris-HCl
(pH7.4)

C **working solution-prepare fresh-50% iodixanol-13.5ml/sample**
11.25ml Optiprep (A)
2.25 ml Optiprep diluent (B)
60ul 20U/ul SUPERase In RNase Inhibitor
60ul 10% BSA
60 ul 0.2M Spermine

stock homogenization buffer
0.25 M Sucrose
25 mM KCl
5mM MgCl2
20 mM Tris-HCl

D **homogenization buffer-prepare fresh-6ml/sample**
970ul stock homogenization buffer
10ul 20U/ul SUPERase In RNase Inhibitor
10ul 10% BSA
10 ul 0.2M Spermine

1ml of nuclear buffer-prepare fresh-4ml/sample
940ul stock homogenization buffer
10ul 20U/ul SUPERase In RNase Inhibitor
10ul 10% BSA
10 ul 0.2M Spermine
10ul 10% Tween-20