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Human Brain Collection and Cryopreservation

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Marc Bosse[1], Sean Bendall[1], Kausalia Vijayaragavan[1]

[1]Stanford University

Kausalia Vijayaragavan
Stanford University

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Protocol status: Working
We use this protocol and it's working

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Keywords: cryopreservation, optimized cryoprotectant, human brain aging, mechanistic insights into human brain aging, rigorous biospecimen stewardship, microglia, such as microglia, preservation of native molecular phenotype, human brain collection, quality human brain collection, cell viability, rapid stabilization of metabolic, artifactual activation of vulnerable cell type, vulnerable cell type, resolution cellular atlas

Abstract

High-quality human brain collection and cryopreservation are essential for enabling reliable single-cell and single-nucleus analyses. Postmortem interval, tissue handling, and rapid stabilization of metabolic and transcriptional states critically determine cell viability, RNA integrity, and preservation of native molecular phenotypes. Best-practice workflows, described here including standardized dissection, immediate cooling, optimized cryoprotectants, and controlled-rate freezing, is described to minimize ischemic stress, degradation, and artifactual activation of vulnerable cell types such as microglia. Consistent documentation and chain-of-custody procedures further ensure reproducibility across donors and studies. Rigorous biospecimen stewardship is therefore foundational for generating high-resolution cellular atlases and for advancing mechanistic insights into human brain aging and disease.

Brain Regions

1
- Hippocampus at the level of the LGN with adjoining Entorhinal Cortex
- Substantia Nigra
- Middle Frontal Gyrus
- Middle Temporal Gyrus
- Caudate
- Cerebellum
- Cortex (Any)

Tissue Collection from Autopsy

2
- Place 8× 10ml aliquots (50ml falcon tubes) of Harvesting buffer for tissue collection into Iglool cool box with ice pack
- Bring the tubes and box over to Autopsy room and leave in the fridge

Material and solutions

3
- Brain Tissue Harvesting Buffer (see Brain Tissue Buffers)
- CryoStor® CS10 (https://www.stemcell.com/products/cryostor-cs10.html)
- TissueVault Cryogenic Freezing Bag (TV0909) (https://www.origen.com/products/solid-tissue-cryopreservation/tissuevault-cryogenic-freezing-bag)
- Freezing Trays (Origen)
- Sterile petri dishes
- Sterile Surgical kit (forceps, scissors, blades)
- Flacon tubes (15ml and 50ml)
- Vacuum sealer
- Igool cool box
- Ice

3.1
- Thaw 1-2× 35ml aliquots (50ml falcon tubes) of Harvesting buffer for washing

4
- Prepare TC hood with all required material
- Sterilize the surgical instruments with 70% ETOH

Tissue Preparation

5m

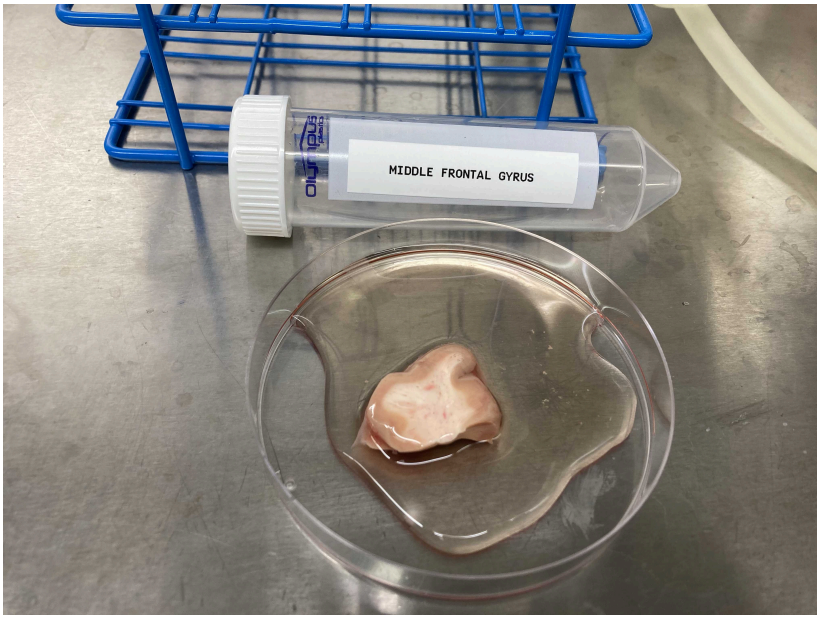
5
- Determine the weight of each brain region

	A	B	C	D
	Brain Region	**50ml Falcon with 10ml media (g)**	**tissue + Falcon**	**tissue alone**
	Hippocampus at the level of the LGN with adjoining Entorhinal Cortex	23.76	m	
	Substantia Nigra	23.76	24.88	1.1199999999999974
	Middle Frontal Gyrus	23.76	25.88	2.119999999999 9974
	Middle Temporal Gyrus	23.76	26.88	3.119999999999 9974
	Caudate	23.76	27.88	4.119999999999 997
	Cerebellum	23.76	28.88	5.119999999999 997
	Cortex (Any)	23.76	29.88	6.119999999999 997
	xxx	23.76	30.88	7.1199999999999 97

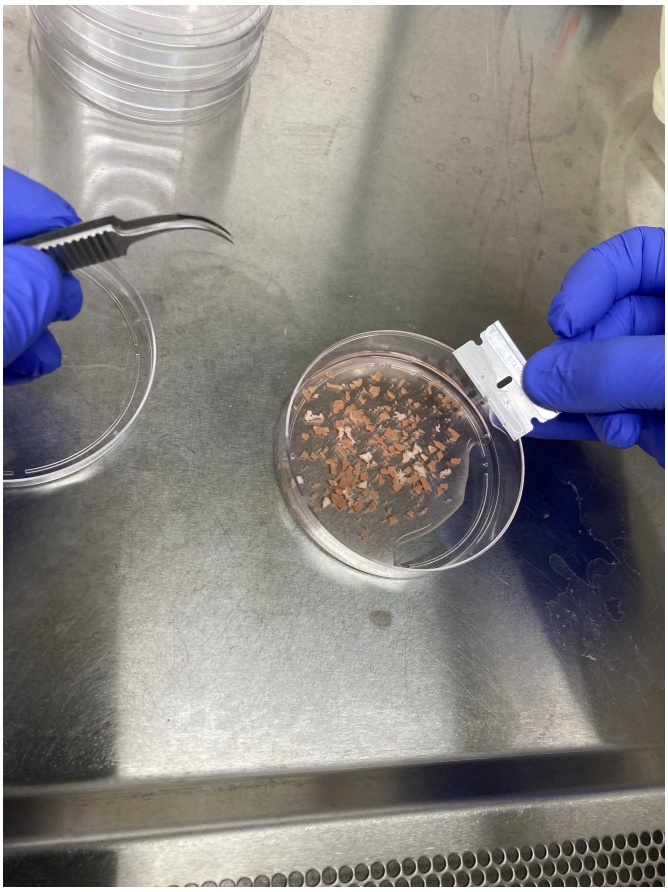
weight of each tissue

5.1
- Keep all tissue on ice until ready to process
- Process one tissue at a time

5.2
- Carefully transfer any one tissue onto, with its Harvesting Buffer, a sterile petri dish
- Document take a picture



5.3 ▪ Chop up tissue into couscous size bits



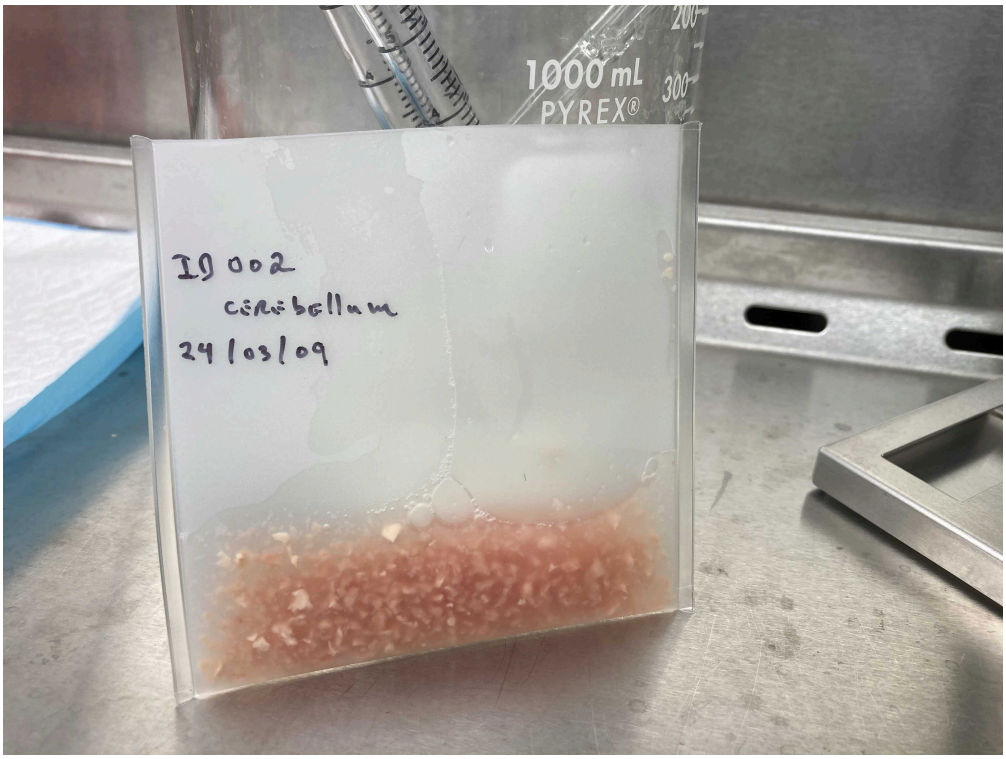
5.4
- Transfer the pieces with a wide-mouth transfer pipette to a 15ml falcon tube and keep on ice
- Continue to process all tissues

5.5
- Once all tissue has been chopped up and transfered to the falcon tubes
- Spin tubes at 200 x g, 4°C
- Remove supernatant
- Add CS10 2x according to volume of tissue

5m

	A	B	C	D
	Brain Region	**Volume of tissue (ml)**	**CS10/ml**	**CS10 (ml)**
	Hippocampus at the level of the LGN with adjoining Entorhinal Cortex	3	2	6
	Substantia Nigra	3	2	6
	Middle Frontal Gyrus	4	2	8

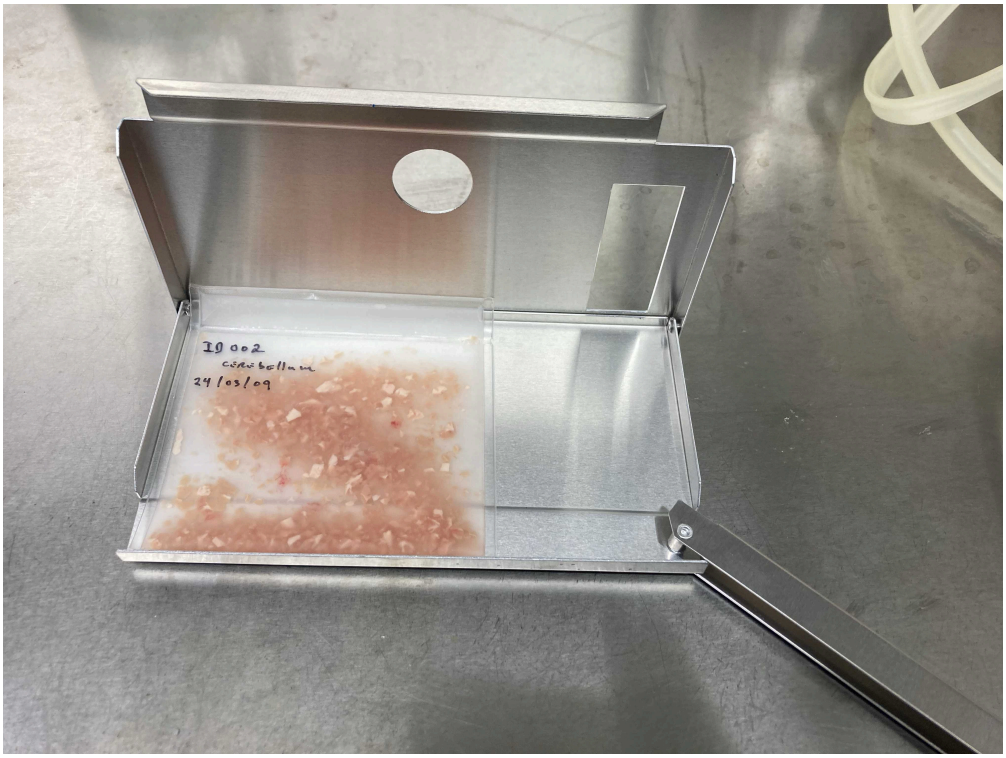
	A	B	C	D
	Middle Temporal Gyrus	5	2	10
	Caudate	2	2	4
	Cerebellum	5	2	10
	Cortex (Any)	5	2	10
	xxx			



option one

5.6
- Label TV0909 Freezing Bag with De-identified ID #, Brain region, Date
- Transfer the tissue pieces/CS10 suspension to Freezing Bag with a transfer pipette
- Vacuum seal the tissue pieces/CS10 suspension/Freezing Bag into 2 sections or 4 sections
- Place o-ring from corning coolcell onto of bag

- Wrap the whole thing in puppy-pad
- Store in -80 °C for 20min
- Unwrap from puppy-pad
- Place the tissue pieces/CS10 suspension/Freezing Bag to tray



- Place tray in Liquid nitrogen