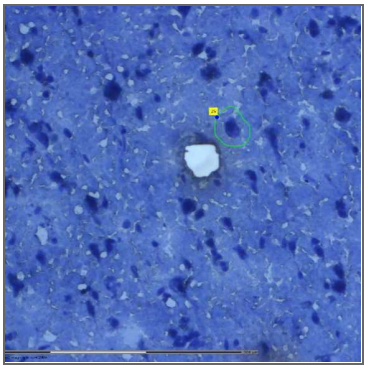

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Single-cell Whole Genome Amplification (scWGA) of human frozen post-mortem brain samples isolated by Laser Capture Microdissection (LCM)

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ABSTRACT

This protocol uses Laser Capture Microdissection (LCM) technology on human post-mortem brain tissue slides with rapid Giemsa staining to isolate single nuclei for Single-cell Whole Genome Amplification (scWGA) in order to do low coverage (<1x) single-cell whole genome sequencing to detect mega-base somatic Copy Number Variations (CNVs).

MANUSCRIPT CITATION:
REFERENCES:
Part of this protocol was adapted from the publication: Keinath MC, Timoshevskiy VA, Timoshevskaya NY, Tsonis PA, Voss SR, Smith JJ. Initial characterization of the large genome of the salamander Ambystoma mexicanum using shotgun and laser capture chromosome sequencing. Sci Rep. 2015 Nov 10;5:16413. doi: 10.1038/srep16413. PMID: 26553646; PMCID: PMC4639759

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We use this protocol and it's working

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ATTACHMENTS

LCM_for_scWGS_protocol_final.pdf

MATERIALS

Materials:

- OCT Mounting media (VWR 361603E)
- Cryochem Cryostat disinfectant (Solmedia REA023)
- PEN Membrane Slide (4 µm) glass (Leica 11600288)
- Collection tubes
  - o For Leica LCM: 0.2 mL MaxyClear thin-walled flat cap Maximum Recovery PCR tube (Axygen AXY2050)
  - o For Zeiss LCM: AdhesiveCap 200 opaque (Zeiss 415190-9181-000)
- Microtome blades compatible with the cryostat to be used (e.g., Feather S35 type)
- Giemsa (Sigma #1092040100)
- Buffer pH 6.8 (Merck #111374): Dissolve 1 tablet in 1000 mL in Water and store @ Room temperature

> **Note**
>
> **Note**: This Buffer is stable for up to 4 weeks.

- Ultra-Pure DNase/RNase-Free Distilled Water (Thermo Fisher 10977049)
- 70% EtOH for surface cleaning
- DNA AWAY Surface Decontaminant (Thermo Scientific 7010PK)
- Cleaning wipes (e.g., Conti Washcloth Dry Brosch Direct PH5959)
- brush, pencil, slide jars, slide box
- PicoPlex (Takara R300671, R300672, R300673)

Equipment:

- Cryostat (we use Bright Instruments OTF6000)
- Laser Capture Microdissection (we have used Leica LMD7000 and Zeiss Microbeam PALM Laser Capture Microscope system)
- UV source (UV stratalinker or hood with a UV lamp)

Section 1: Tissue cryo-sectioning

1 UV pre-treat PEN slides and pre-label them with a pencil prior use.

Note

Note: Use a PCR or cell culture hood with UV lab and UV the slides for 30 min. If available, a UV stratalinker can be used.

2 Clean and UV the cryostat prior use and set the temperature approx. @ -20 °C.

3 Place a new blade on cryostat.

Note

Caution! Be careful because the blade is sharp.

Note: Change blade when cutting samples from different donors or brain regions to avoid contamination.

4 Transfer human post-mortem frozen tissue chunks with dry-ice to the cryostat.

5 Embed the tissue in OCT embedding medium.

Note

Note: Use minimal amounts OCT, as it will affect the cryo-sectioning process.

6 Mount the cryo-block onto the specimen clamp.

7 Trim the tissue to get a plane surface.

8 Cut thick sections of tissue (14 mm) and immediately place the cryo-sectioned tissue onto the pre-labelled PEN membrane glass slides.

9 Keep the slides in a box on dry ice during the until all cryo-sectioning procedure is completed for the slides of interest.

10 Use slides immediately or store the slides @ -80 °C in tightly sealed containers.

Section 2: Giemsa staining

11 Prepare Giemsa working solution: 10 Giemsa (stock) + 90 mL Buffer pH 6.8, Mix well and 10m to stand for 00:10:00 @ Room temperature.

Note

Caution! Work with Giemsa under chemical hood and store it in a glass bottle.

12 Thaw slides gradually, e.g., from @ -80 °C → 00:02:00 @ -20 °C → 00:02:00 @ 4 °C → 00:02:00 @ Room temperature. 6m

13 Immerse slides in a jar containing ice-cold Methanol for 00:05:00 @ Room temperature. 5m

14 Quick (00:00:05) air-dry the slides. 5s

Note

Note: If needed, at this stage remove excess OCT with a brush.

15 Immerse slides in a jar containing Giemsa working solution for 00:02:30 @ Room temperature 2m 30s

16 Rinse slides with DNase free water.

17 Air-dry the slides for at least 00:15:00 @ Room temperature . 15m

18 Proceed directly to LCM.

Note

Note: Stained slides can be stored in the fridge and used later for cutting but single-cell whole genome amplification efficiency may be reduced.

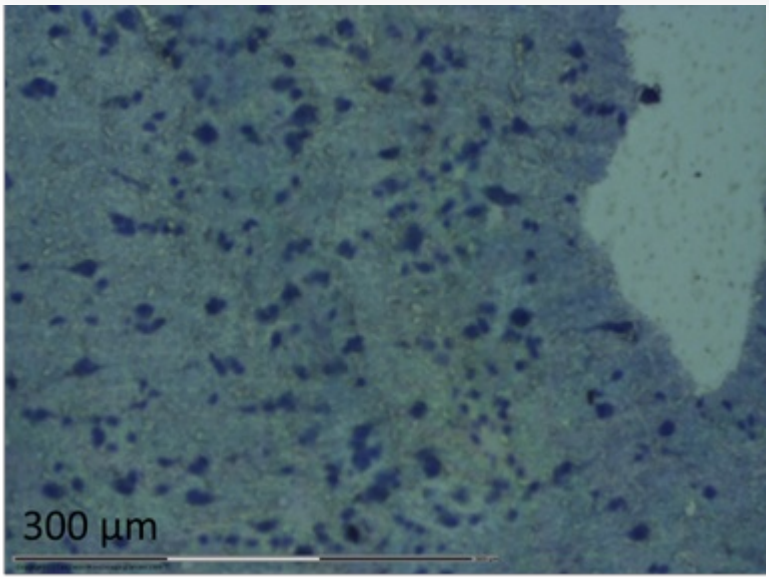

Image 1: Example of human cingulate cortex tissue image on a PEN slide stained with

Giemsa.

Section 3: Laser Capture Microdissection (LCM)

19 Prepare fresh Chromatin Digestion Buffer ([M] 1 millimolar (mM) EDTA, [M] 20 millimolar (mM) TRIS pH pH 8.0 , 0.2 mg/mL Proteinase K, 0.001% Triton X, in nuclease free water) as on Keinath et al 2015.

20 UV collecting tubes for at least 30 min prior to use.

21 Clean the LCM with 70% EtOH and DNase Away prior use.

22 Capture single nuclei using the LCM guidelines.

Note

We have used 2 different LCM technologies and cells were cut in 20x magnification while keeping laser power to a minimum. It is advisable to take images prior and after cutting of the slide, and also on the cap after cutting for each single cell.

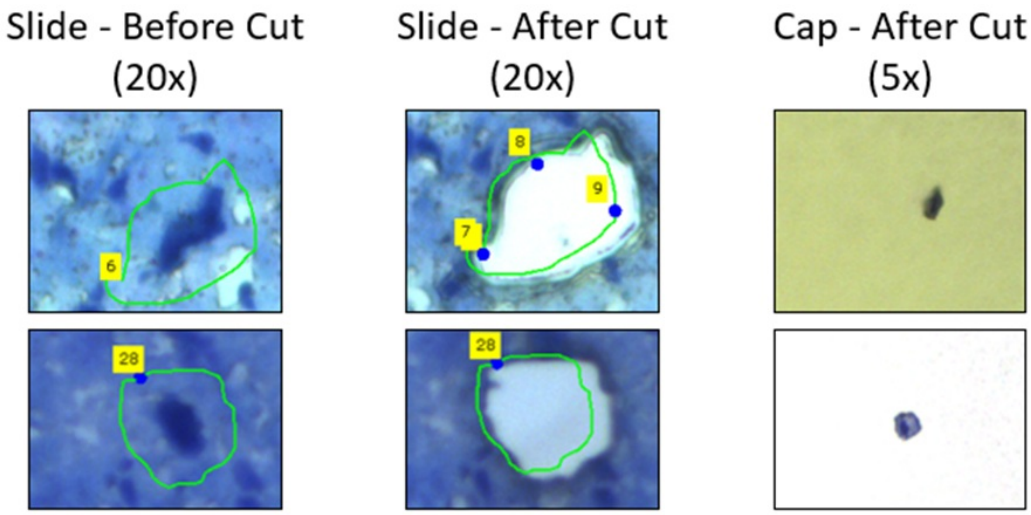

Image 2: Examples of a single nuclei prior cut and after cut on the slide, as well as after cut

on the cap.

Note

Note 1: To minimize contamination, cut closely to the nuclei of interest.

Note 2: Use at least one negative control, which could be an empty collection tube that had been placed on the collector, but additional controls could include:

- Collection tube with a micro-dissected area of the slide that does not contain a nucleus.
- An Empty collection tube not opened outside PCR hood.
- Collection tube with a micro-dissected area of the slide which contains a nucleus that is only partly cut.

STEP CASE

Option A 10 steps

Leica Laser Capture Microscope system LMD system

23 Observe the nucleus on the cap in the Leica LCM microscope.

24 Add 10 µL of Chromatin Digestion Buffer on the cap and close the tube carefully.

25 Vortex tubs for at least 00:00:05. 5s

26 Spin down for 00:05:00 @ Room temperature and place On ice until further use. 5m

27 Incubate tubes @ 55 °C in an oven O/N.

Section 4: Single-cell Whole Genome Amplification (scWGA) 14m

28

Note

Note: We found PicoPlex amplification with typical PicoPlex lysis steps for LCM samples was suboptimal, hence our lysis is more extensive.

After O/N incubation @ 55 °C in an oven O/N:

28.1 Quick spin.

28.2 Heat treat: @ 75 °C for 00:10:00 → @ 95 °C for 00:04:00 → @ 4 °C hold. 14m

28.3 Quick spin and transfer samples On ice.

28.4 Follow PicoPlex protocol steps skipping the lysis step.