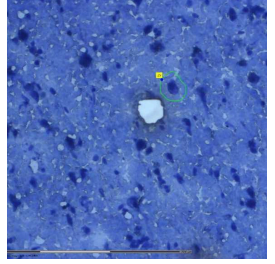




Dec 07, 2023

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



DOI

dx.doi.org/10.17504/protocols.io.5qpvorjw7v4o/v1

Ester Kalef-Ezra^{1,2}, Amy Bowes^{3,4}, Christos Proukakis^{1,2}

¹Department of Clinical and Movement Neurosciences, UCL Queen Square Institute of Neurology, London, UK;

²Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD, 20815;

³Cancer Genomics Group, The Francis Crick Institute, London, UK;

⁴Sarcoma Biology and Genomics Group, UCL Cancer Institute, London, UK

University College London



Ester Kalef-Ezra

University College London

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.5qpvorjw7v4o/v1>



Protocol Citation: Ester Kalef-Ezra, Amy Bowes, Christos Proukakis 2023. Single-cell Whole Genome Amplification (scWGA) of human frozen post-mortem brain samples isolated by Laser Capture Microdissection (LCM). **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.5qpvorjw7v4o/v1>

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

License: This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We use this protocol and it's working

Created: June 01, 2023

Last Modified: May 31, 2024

Protocol Integer ID: 82740

Keywords: Giemsa, Laser Capture Microdissection, LCM, Single-cell, Whole Genome Amplification, WGA, brain, ASAPCRN, cell whole genome amplification, whole genome sequencing, cell whole genome, whole genome, laser capture microdissection, brain sample, genome, single nuclei, lcm

Funders Acknowledgements:

Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD, 20815

Grant ID: 000430

Disclaimer

DISCLAIMER – FOR INFORMATIONAL PURPOSES ONLY; USE AT YOUR OWN RISK

The protocol content here is for informational purposes only and does not constitute legal, medical, clinical, or safety advice, or otherwise; content added to **protocols.io** is not peer reviewed and may not have undergone a formal approval of any kind. Information presented in this protocol should not substitute for independent professional judgment, advice, diagnosis, or treatment. Any action you take or refrain from taking using or relying upon the information presented here is strictly at your own risk. You agree that neither the Company nor any of the authors, contributors, administrators, or anyone else associated with **protocols.io**, can be held responsible for your use of the information contained in or linked to this protocol or any of our Sites/Apps and Services.



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).

Attachments



LCM_for_scWGS_protoc.







1.2MB



Materials

Materials:

- OCT Mounting media (VWR 361603E)
- Cryochem Cryostat disinfectant (Solmedia REA023)
- PEN Membrane Slide ( 4 μm) glass (Leica 11600288)
- Collection tubes
 - For Leica LCM:  0.2 mL MaxyClear thin-walled flat cap Maximum Recovery PCR tube (Axygen AXY2050)
 - 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)

Troubleshooting

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 leave 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 .

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

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

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 .

- 16 Rinse slides with DNase free water.

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

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.

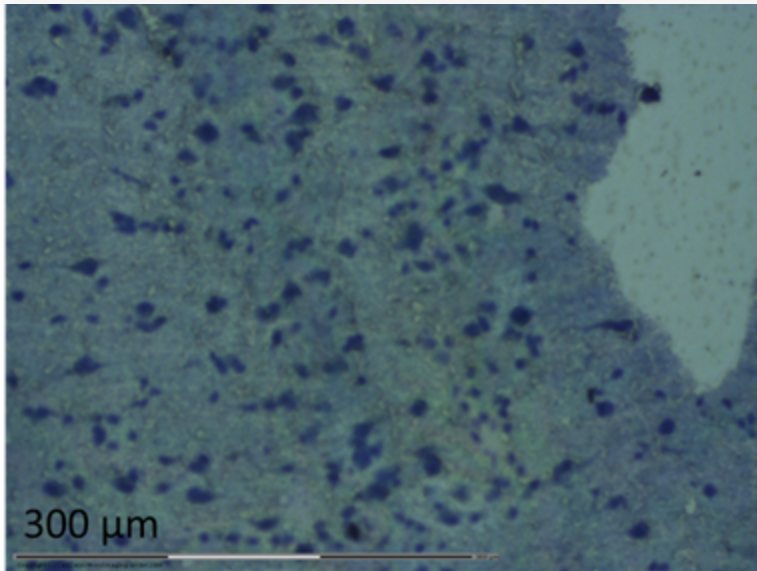


Image 1: Example of human cingulate cortex tissue image on a PEN slide stained with Giemsa.

Section 3: Laser Capture Microdissection (LCM)

19 Prepare fresh Chromatin Digestion Buffer ([M] 1 millimolar (mM) EDTA, [M] 20 millimolar (mM) TRIS pH 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.

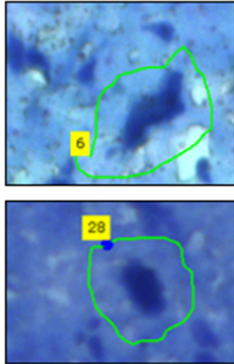
21 Clean the LCM with 70% EtOH and DNase Away prior use.

22 Capture single nuclei using the LCM guidelines.

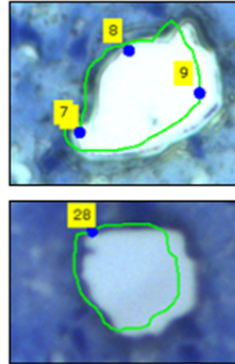
Note

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.

Slide - Before Cut
(20x)



Slide - After Cut
(20x)



Cap - After Cut
(5x)

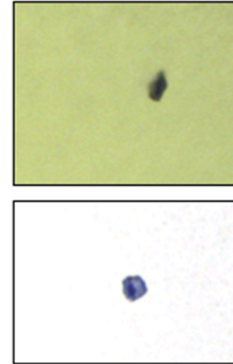


Image 2: Examples of a single nuclei prior cut and after cut on the slide, as well as after cut on the cap.

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 tubes 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 $^{\circ}\text{C}$ 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 $^{\circ}\text{C}$ in an oven O/N:

28.1 Quick spin.

28.2 Heat treat: @  75 $^{\circ}\text{C}$ for  00:10:00 → @  95 $^{\circ}\text{C}$ for  00:04:00 → @  4 $^{\circ}\text{C}$ hold.

14m



28.3 Quick spin and transfer samples  On ice .

28.4 Follow PicoPlex protocol steps skipping the lysis step.