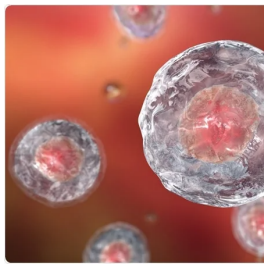
Apr 24, 2019 Version 1

Single Nuclei extraction from frozen brain V.1



DOI
dx.doi.org/10.17504/protocols.io.2a5gag6

Neurogenomics And Informatics[1]
[1]Washington University, Saint Louis

Neurodegeneration Met... Functional Genomics

Neurogenomics And Informatics
Washington University, Saint Louis

DOI: https://dx.doi.org/10.17504/protocols.io.2a5gag6

Protocol Citation: Neurogenomics And Informatics 2019. Single Nuclei extraction from frozen brain. **protocols.io** **https://dx.doi.org/10.17504/protocols.io.2a5gag6**

Manuscript citation:
A single-nuclei RNA sequencing study of Mendelian and sporadic AD in the human brain Jorge L. Del-Aguila, Zeran Li, Umber Dube, Kathie A. Mihindukulasuriya, John P Budde, Maria Victoria Fernandez, Laura Ibanez, Joseph Bradley, Fengxian Wang, Kristy Bergmann, Richard Davenport, John C. Morris, David M. Holtzman, Richard J. Perrin, Joseph Dougherty, Carlos Cruchaga, doi: https://doi.org/10.1101/593756

Protocol status: In development
We are still developing and optimizing this protocol

Created: April 24, 2019

Last Modified: May 01, 2023

Protocol Integer ID: 22589

Keywords: frozen brain, single nuclei, RNA-Seq, single nuclei extraction from frozen brain protocol, iodixanol gradient use bsl2 precaution, single nuclei extraction, iodixanol, purification of nuclei, supplemented nuclei wash buffer, frozen brain protocol, discarded supernatant, ml homogenization tube for each brain, desired dilution, ml with molecular biology grade water, purification, biohazard waste stream, dilutant, buffer for nuclei wash, extraction, other tissue byproduct, weight of brain sample, nuclei from the same sample, nuclei wash buffer to the tube, nuclei wash, nuclei wash buffer, rnasin per ml, ul of nuclei wash buffer, discard supernatant, molecular biology grade water, brain sample, dilution, top pink debris layer of myelin, brain tissue, mls of nuclei wash, volume of dilutant, mm tricine, rnase free water, mls of nuclei wash buffer, polycarbonate centrifuge tubes from beckman coulter, rnase zap, free h20 supplementation, grams tricine, sterilization

Abstract

Protocol: Purification of nuclei from human brains using an iodixanol gradient

Use BSL2 precautions. Samples are not known to be infective, but should be treated as such. Gloves, mask if outside hood, disposable gown. Every surface and reusable equipment (homogenizers, centrifuge tubes) washed with 25% bleach.

Discarded supernatants or other tissue byproducts should go into 10% bleach for sterilization.

Discarded tubes and tips go into biohazard waste stream.

Solutions:

1. Stock optiprep 60% Iodixanol [Sigma-Aldrich D1556-250mL]
2. Dilutant:
3. Final Concentrations: 900 mM KCL; 30 mM $MgCl_2$; 120 mM Tricine-KOH (pH 7.8)
4. Recipe for 50 mls

1) 22.5 ml of 2M KCL, +
2) 1.5 ml of 1M MgCl2, +
3) 6 ml of 1 M Tricine-KOH
4) brought up to 50 ml with molecular biology grade water.

1. Components

1) Tricine-KOH is 17.92 grams Tricine (Sigma T0377) and 100 mL RNAse free water for 1 M solution (pH to 7.8 with KOH as needed. Usually takes about 3-4 mL of 3M KOH).
2) 1 M MgCl2 (AM9530G) and 2M KCl (AM9460G) are commercial RNAse free stocks from Ambion.

1. Homogenization medium (need 6 ml per sample + ~ 2ml extra for 30% Iodixanol):
2. Recipe (stable at 4C) for 100 mls / 50 mls

1) .25 M Sucrose (8.56 g / 4.28g)
2) 150 mM KCL (7.5 ml / 3.75of 2M)
3) 5 mM $MgCl_2$ (.5 ml / .25 ml of 1M)
4) 20 mM Tricine-KOH (2 ml / 1 ml of 1M)
5) ddH_2O up to 100 ml /50 ml in RNAse free H20

1. **Supplementation (right before use):**

1) For 4 samples make 35 mls of homogenization buffer and supplement with:
1. 3.5 ul spermine,
2. 3.5 ul spermidine,
3. 35 ul RNASIN
4. EDTA free protease inhibitor tablet.

1.

1. Iodixanol Dilutions:
2. 50% Iodixanol

1) Recipe: 5 volumes stock (60%) optiprep + 1 volume of dilutant.
2) Scale: need 2 ml per sample tube + 3 ml per sample for 35% Iodixanol
3) Example: 4 samples add 17.5 ml stock to 3.5 ml diluant.

1. 35% Iodixanol

1) Recipe: 7 ml 50% Iodixanol + 3 ml supplemented homogenization medium.
2) Scale: need 4 ml per sample
3) For 4 samples add 5.14 mL supplemented homogenization buffer to 12 mL of 50% Iodixanol.
1. Resupsension Buffer for Nuclei Wash (new from 10x Genomics recipe)
i. 1x PBS with 1.0% BSA and 0.2 U/uL
1) Recipe:_____ commercial PBS (46-013CM 10x PBS from Corning)
2) Recipe: _____ BSA (001000162 from Jackson Immunoresearch)
3) Recipe: ___1:1000_____ RNAsin

Protocol:

1. Turn on the Beckman Allegra 64R, and Eppendorf 5430R Centrifuges so they can get to 4^{o}.
i. For Beckman put in swing-out rotor (S0410).
Make sure acceleration and deceleration are set to 0 on Beckman
2. Supplement the homogenization medium.
i. Add fresh .15mM spermine (1:10,000) (1 ul per 10 ml)
ii. Add fresh .5mM spermidine (1:10,000) (1 ul per 10 ml)
iii. Add 1 µl RNASIN per ml of homogenization medium if using for RNA
iv. Add EDTA free protease inhibitor tablet
3. Prepare working concentrations of rest of solutions.
4. Aliquot 2ml of supplemented homogenization medium per 15 ml homogenization tube for each brain. We have used 1g of tissue successfully to yield 15-20 million nuclei. We also used 2g and double that.
5. Check weight of brain sample and keep on ice in 2ml supplemented homogenization medium.
6. Homogenize the brain tissue in the 2 ml supplemented homogenization medium.
i. Be careful at all steps to treat all samples exactly equally with regard to handling, as different cell type's nuclei might be more or less vulnerable to physical destruction. All pipetting and resuspension steps should be gentle and consistent to avoid shearing and clumping of nuclei.
ii. Put tissue into 15 ml Kontes glass homogenizer tubes
iii. Homogenize 10 strokes on ice with 'A', and 10 strokes on ice with 'B' pestle.
i. Use in BSL2 space for human tissue (currently using chem fume hood, with diapers).

iv. Check to see that no large pieces have escaped homogenization.
v. Transfer pipette with 2ml strippette the homogenate equally into 2 × 2 ml siliconized snap cap microcentrifuge tubes.
vi. Add another 500ul supplemented homogenization buffer to the glass homogenizer and transfer remaining homogenate to the same microcentrofuge tubes.
i. After use with human tissue, decontaminate homogenizers with 10% bleach during a break in the protocol. Dispose of diapers, tips, etc, in BSL2 waste.
ii. Then RNASe Zap, then rinse with ethanol then ddh20 water.
7. Spin tubes at low speed (500 RCF) in the Eppendorf for 5 minutes at 4^{o}.
8. Pour off the supernatant, add 1 ml supplemented homogenization medium, and turn over or flick to resuspend.
i. Take 100 ul of supernatant and freeze at -80 if you want cytoplasmic RNA later
9. Repeat step 8.

10. While spinning you can add 4ml of 35% Iodixanol to a centrifuge tube for each sample. *Mix the solution up and down to mix again after it has been sitting*
 i. These are polycarbonate centrifuge tubes from Beckman Coulter, stored in cabinet under the Sorvall in the shared equipment room.
 ii. May need to do a quick spin of the 35% to get it to settle down if some is stuck on the walls.
11. Resuspend each pellet in 700 uls of supplemented homogenization buffer. Combine all tubes for each sample into a single 15 ml conical.
12. Add an equal volume of 50% Iodixanol to the samples in the 15 ml conicals. This will create a final 25% Iodixanol. *Mix 50% Iodixanol right before usage*
13. Pipette the samples up and down with low retention pipette tips and carefully layer the ~ 3ml-4mls of 25% Iodixanol (2 tubes) on top of the 4 mL of 35% Iodixanol using a low attachment tip (silver box). You will need to use the same volume of all samples so that tubes are balanced, so scale to your smallest sample.
 i. Layering is best done by holding the bottle almost horizontal and using a P1000 to pipette slowly into the wall of the tube slightly above the 35 % Iodixanol. It is important to make sure the layers do not mix.
14. Centrifuge the tubes at 10,000 rpm (top speed) for 30 minutes in the Beckman Allegra *Check acceleration and deceleration again*
15. Carefully remove the centrifuge tubes. Take back to BSL2 space. The nuclei should be visible as a cloudy layer in the interface of the 25%/35% Iodixanol. with a transfer pippette, remove the top pink debris layer of myelin (suck off with a transfer pipette).
16. With a low retention pipette remove the nuclei by vacuuming them up from just above the layer. Then place into a 1.5mL Eppendorf – NONSTICK TUBES (for nuclei). No more than 500 uls per tube. Usually 2 tubes per sample.
17. Add 1 ml of supplemented Nuclei Wash buffer to the tubes & mix.
18. Centrifuge at 500g's (rcf) at 4o.
19. Nuclei should be pelleted at the bottom
20. Remove & discard supernatant
21. Resuspend nuclei in about 750 mls of Nuclei Wash buffer. Bring together all nuclei from the same sample into a single tube.
22. Centrifuge at 500g's (rcf) at 4o.
23. Nuclei should be pelleted at the bottom
24. Remove & discard supernatant
25. Resuspend nuclei in about 1000 mls of Nuclei Wash buffer
26. Remove & discard supernatant
27. Resuspend nuclei in about 1000 ul of Nuclei Wash buffer.
28. Let it drip through a 40 um Nylon mesh cell strainer (#229481)to remove clumps per 10x protocol.
29. Take out 20 uls, mix 1:1 with a 1:2000 DAPI solution, and count on hemacytometer or countess and confirm lack of clumps.
30. Adjust aliquot of nuclei to desired dilution (1,200 cells per ul). Count again to confirm dilution.
31. The rest may be stored as follows: Pellet and resuspend in 100% methanol

Attachments

Protocol Purificatio...

21KB

Troubleshooting