Protocols

Bromodeoxyuridine (BrdU) Labelling

Contents

Instructions

1. Fix tissue with 4% FA for 10 min at ambient temperature.
2. Incubate with 1X PBS 3 times for 5 minutes each time to wash off fixative.
3. Incubate tissue with 2 N HCl for 30-45 minutes at 37C.
    a. For slides; place slides in Coplin jar with 2 N HCl and then incubate in a 37C oven.
    b. For cell cultures; incubate at room temperature for 15 minutes followed by an incubation at 37C for 15 minutes.
4. Rinse once quickly in Boric acid buffer and then wash for 10 minutes once in Boric acid buffer.
5. Wash with 1X PBS 3 times for 5-10 min each time.
6. Block for 60 min at room temperature with 3% BSA, 10% serum, 0.1% triton x-100 (alter blocking buffer as needed).
7. Incubate with anti-BrdU antibody overnight at 2-8 Â°C in a humidified chamber.

Notes

1. Use 10 uM for cultures and 50 mg/kg for live mice.
2. If performing co-labeling with another antibody, I recommend doing the stainings sequentially, 1st using the other antibody (through primary Ab, secondary Ab, all washes), then fixing at the end of the 1st immunostaining before starting the BrdU protocol from step 1. This will improve the quality of the non-BrdU immunostaining.

Equipment

- Humidified chamber
- Coplin jar (for slides)

Consumables and Reagents

- 4% formaldehyde (FA)prepared from paraformaldehyde (PFA)
- Boric Acid Buffer; Dissolve boric acid in water (0.31 g/50 mL). Adjust pH to 8.5 with NaOH (only a few drops required). Always prepare fresh.
- anti-BrdU antibody; Abcam Rat anti BrdU (#ab6326). Dilution factor 1:500.

References

1. This protocol was transcribed from Florian Merkle's protocol of the same name.

Cryosectioning human brain

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Decontaminating equipment exposed to fur-mites with ivermectin.

Index

Purpose

Outline the procedure for decontaminating equipment that has been exposed to fur-mites to prevent transferring the organism to non-affected animal holding rooms.

Instructions

1. Wipe down equipment with ivermectin using a spray bottle.
2. Wipe down equipment with 70% ethanol.
3. Enclose the equipment in a clean bag.
4. Wipe down the bag with ivermectin using a spray bottle
5. Wipe down the bag with 70% ethanol.
6. Dispose of all waste generated inside of the room.
7. Take the bag with equipment outside of the room.
8. Once outside of the room and depending on the type of equipment being treated; oil or lubricate any moving parts.

Notes

- Rooms found to have fur-mites are likely to be decontaminated over a period of 7 weeks using a combination of Moxidectin/Ivermectin along with Methoprene. So any equipment being relocated from a contaminated space may have trace amounts of these agents on them.

Equipment

n/a

Consumables and Reagents

- ivermectin
- 70% ethanol
- paper towels
- Oil or lubricant (depending on the specific equipment)

References

1. Procedure derived from email correspondence between UCSF LARC veterinarian Kelly Jensen and Alvarez-Buylla Lab Manager Joseph Elsbernd.
2. Ivermectin and Moxidectin Toxicology Studies http://www.inchem.org/documents/jecfa/jecmono/v36je03.htm
3. NOTE: Methoprene is not used to decontaminate equipment in this procedure, but because it is used to decontaminate rooms with fur-mites it may be found on any equipment from fur mite contaminated rooms that are undergoing treatment for the pest. Methoprene toxicology study http://www.epa.gov/opp00001/chem_search/reg_actions/reregistration/fs_PC-105401_1-Jun-01.pdf

Electron microscopy

EM Embedding Protcol

Index

Purpose

Instructions

Day 1 of 2

1. Wash:
    a. If the samples are in fixative solution...
        i. Wash with **PB 0.1 M**, 5 x 5. (If the samples are in fixative solution).
    b. If the samples are in 0.1 M PB with Azide
        i. Wash with **PB 0.1 M**, 3 x 5. (If the samples are in PB 0.1 M-Azide)
2. **NOTE**: In the case of adult mouse brain slices, no more than 5 slices per vial.
3. Remove the last wash and add the **2% osmium** solution (in PB 0.1M). Keep at room temperature and in the dark for 1.5-2 hours on an orbital shaker in a fume hood. If you do not have a shaker, you can strike the vials occasionally (every 30 min). If the osmium solution begins to present as a red wine color, remove it immediately.
    a. **NOTE:** You can prepare the araldite in this step
    b. **NOTE:** If we need to observe some structures or labeling macroscopically after araldite inclusion (for example, Pre-embeding immunos), we will use 1% osmium solution + 7% glucose in PB 0.1M (only during 30 min), in order to obtain lighter slices.
4. Wash with Milli-Q water water**,** 3 x 5 min (Fridge; 2-8C). No more than 15 min.
5. Dehydrate: (Fridge; 2-8C)
    a. 30% alcohol solution**:** 1 wash x 5 min
    b. 50% alcohol solution**:** 1 wash x 5 min
    c. 70% alcohol solution**:** 1 wash x10 min
6. **NOTE:** To prevent the hydration of the samples, wash quickly.
7. Wash with **2% uranyl acetate** in 70% alcohol solution (2h and 30min, Fridge).
8. Dehydrate: (Fridge)
    a. **70º** alcohol solution**:** 2 washes x 5'

    b. **96º** alcohol solution**:** 2 washes x 10’
    c. **100º** alcohol solution**:** 2 washes x 10’
    d. **100º** alcohol solution**:** 1 wash x 10’ (new bottle or 200% alcohol bottle)
    e. NOTE: To prevent the hydration of the samples, wash quickly.
9. Wash with propyleneoxide solution (at room temperature), filling the vial.
    a. 2 washes for 10 minutes
    b. NOTE: use latex gloves, propyleneoxide eats plastic.
10. The sample should be transferred from the propylene oxide solution to the aluminum foil mold with araldite (you can use brush or forceps to transfer). This is a critical step, the samples must be transferred very quickly and they should be immersed immediately in the resin.
    a. NOTE: You can clean the brush with the propylene oxide solution.
11. Place the aluminum foil molds in a plastic petri dish and leave them in the orbital(?) shaker overnight.

Day 2 of 2

1. Get the samples into blocks (between acetate sheets, 200 mm slices, or in silicone molds).
    a. For the 200 micron of adult brains use the acetate sheets
    b. For the E14 embryo brains, use the silicone molds. For cutting, it is very important that the zone of interest will be placed on the straight orientaton.
2. Polymerization; leave the sheets or silicone molds with samples in the 70C incubation chamber for 3 days.

Notes

Equipment

1. Gloves
    a. Propylene oxide is only compatible with certain materials. Use butyl gloves or another compatible material
2. Fume hood
3. Analytical weigh balance
4. Fridge
5. timer

Consumables and Reagents

1. Reagents to purchase
    a. Uranyl acetate (s)
    b. Propylene oxide (l)
    c. 96% ethanol (l)
    d. Araldite by Durkupan
        i. Components AM, B, C, D
    e. Glucose (s)
2. Reagents to prepare
    a. 0.1 M Phosphate buffer (PB)
        i. 1:1 dilution, 0.2 M PB in water
        ii. (0.2 M PB -> solution 1:4, Nah2PO4 0.2M - Nas2HPO4 0.2M)
    b. 2% Osmium in 0.1 M PB (0.5 mL/vial; 1 mL)
        i. Prepare inside of a fume hood
        ii. 0.50 mL 4% osmium (stock)
        iii. 0.50 mL 0.2 M PB
    c. 1% Osmium + 7% Glucose in 0.1 M PB (0.5 mL/vial; 1 mL)
        i. Prepare inside of a fume hood
        ii. 0.25 mL 4% osmium (stock)
        iii. 70 mg glucose

iv. 0.50 mL 0.2 M PB
v. 0.25 mL H2O

d. 30% Ethanol solution
i. 300 mL 96% ethanol
ii. 660 mL Milli-Q water or equivalent
iii. Store and use solution at 2-8C

e. 50% Ethanol solution
i. 500 mL 96% ethanol
ii. 460 mL Milli-Q water or equivalent
iii. Store and use solution at 2-8C

f. 70% ethanol
i. 700 mL 96% ethanol
ii. 260 mL Milli-Q water or equivalent
iii. Store and use solution at 2-8C

g. 2% Uranyl acetate in 70% ethanol solution (1 mL/vial)(1 mL)
i. Prepare in a fume hood and wear a respirator
ii. 20 mg uranyl acetate
iii. 1 mL 70% ethanol
iv. Use a sonicator to dissolve the uranyl acetate. The solution is completely dissolved when it is transparent. Do not heat the solution.

3. Milli-Q water

References

1. Protocol translated from Spanish to English by Arantxa.

Semithin Sectioning

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Purpose

Instructions

Notes

Equipment

Consumables and Reagents

1. Milli-Q water or equivalent
2. Reagents to prepare
    a. Toluidine Blue
        i. 100 mL Milli-Q Water
        ii. 1 gram toluidine blue (Panreac 251176.1606)
        iii. 1 gram Sodium borate (Panreac 143052.1211)
        iv. Dissolve the toluidine blue solution and then add the borate. Shake for 24 hours before using the solution.
    b. Microscopes slices with gelatin
        i. Dissolve 0.14 grams Chromium (Sigma; 24-336-1) in 100 mL of Milli-Q water using shaking and heating.
        ii. When the solution reaches 60-70C, add 0.45 grams of gelatin (Sigma; G-2500). When the solution reaches ambient temperature, add 1.1 grams of sodium azide (Sigma; S8032).
        iii. Working solution is 1:10
    c. Reynolds staining
        i. 1 gram Sodium hydroxide
        ii. 1.33 gram Lead nitrate
        iii. 2.14 grams sodium citrate
        iv. Boil water in a flask. Once the water is boiling turn off the heat and cover with cotton (is this to remove Co2 from the water). Allow the water to cool. Dissolve lead nitrate in about 30 mL H2O.
        v. Add the sodium citrate. Shake the solution vigorously. The solution will become white. Leti t stand in the darkness for about 1 hour. You should see two phaes (one transparent and one white), if you do not, shake and wait again.
        vi. Weight the sodium hydroxid eand dissolve it in 25 mL H2O. Cover it with cotton.
        vii. Gently add 8 mL of sodium hydroxide to the leade citrate solution and bring the volume to 50 mL/
        viii. Store the solution in the dark and at 2-8C.

References

Ependymal flow assay and high-speed live imaging of ciliary beating

Contents

Purpose

Instructions

Ependymal flow assay and high-speed live imaging of ciliary beating

Wholemounts of the lateral wall of the lateral ventricle were freshly dissected as described previously (Mirzadeh et al., 2010a). For the ependymal flow assay, a glass micropipette filled with fluorescent polystyrene latex microbeads (2 µm, Polysciences) attached to an MO-10 micromanipulator (NARISHIGE) was lowered onto the wholemount, where microbeads were deposited onto the ventricular surface. We recorded the movement of microbeads using a Leica MZFLIII fluorescent dissection microscope and Retiga 2000R high-speed digital camera (QImaging) plugged into OpenLab imaging software (Improvision) at 10 frames per second (fps).

For high-speed imaging of ciliary beating, the wholemount preparations were incubated with rat anti-CD24 antibody conjugated with PE in Neurobasal medium (Gibco) supplemented with B-27 serum-free supplement (Gibco), glutamine and antibiotics for 20 min at RT, rinsed with

L-15 medium (Gibco), and placed in a glass bottomed dish (BD Falcon). 1-2% low melting point agarose (invitrogen) and Neurobasal medium with the supplements were placed on the wholemounts. Ciliary beating was recorded with 15 msec exposure time at 61 fps for 200 frames at RT using a Leica DMI600 B microscope, HCX PL APO 100x oil-immersion lens (NA 1.44, Leica), Rolera EM-C2 high-speed camera (QImaging) and Metamorph software.

Notes

Equipment

Consumables and Reagents

References

1. Method performed by Shinya Ohata

Equipment Maintenance

Cleaning and care of sliding microtome blade assembly

Purpose

To clean and protect the blade assembly for the sliding microtome.

Instructions

1. Remove the top-plate (3 bolts, 2 springs, 1 plate) with the screwdriver.
2. Clean the blade holder and top-plate parts with greater than or equal to 70% ethanol.
3. Wipe down the top-plate and blade holder with a small amount of oil (this will displace water from the plaques and pits and protect all of the metal. A exteremly light, nearly nonexistent layer of oil is the target quantity.
4. Leave everything disassembled so that all parts can properly dry.

Notes

Equipment

Consumables and Reagents

- Greater than or equal to 70% ethanol.
- Oil
-

References

1. Leica Sliding Microtome (SM 2010 R)

How to clean the Microm Cryostat

Index

Purpose

From use and time the Microm Cryostat will build up with ice, OCT compound, and tissue fragments. The buildup of this materials will impair the function of the cryostat and so it is important to clean it regularly – once every 6 months (give or take time for frequency of use).

Instructions

Refer to the manual whenever necessary.

1. Remove the plug on the drain hose and place the hose end into a bucket that can hold around 1 gallon of fluid.
2. Set the temperature to 5°C and allow the unit to equilibrate overnight.
3. Turn off the cryostat and unplug it from the wall.
4. Disassemble the cutting and mounting stage.
    a. Details needed
5. Disassemble the mechanical stage.
    a. Details needed
6. Reassemble the cryostat
    a. Details needed
7. Plug the cryostat back in and power it on. Permit the system to equilibrate to 5°C
8. Decrease the temperature to the target temperature and give the Cryostat time to reach the temperature before use.

Notes

1. Reassembly note:
    a. When reattaching the hand crank to the central mechanism, each must be attached in opposition to each other. Both pieces are weighted and they should be attached opposite each other so that the weights cancel each other out.

Equipment

- Microm Cryostat
- Sonicator (optional)

Consumables and Reagents

- 100% Ethanol
- Low temperature grease
- Low temperature oil
- Lint-free cleaning sheets (Kim-Wipes)
- Tergazyme (optional, for use with sonicator)

References

Generating circular plots of ciliary orientation with Oriana4

Contents

Purpose

Instructions

1. Measure angle of deviation from the expected direction of orientation using the measure function of ImageJ: open image, rotate image so that expected direction of orientation is towards the right edge of the image. Using the line tool, draw a line to indicate the direction of the object (basal feet or patch). Using the measure function, measure the angle of the line and the right edge of the image.
2. Copy angle measurements into Excel. Convert angles from (-180 to 180) to (0 to 360).
3. Copy angle measurements into Oriana: File: New, select Angles, select Separate; select Circular data only; select the number of columns, save. Analyses: Stats, select Mean Angle, Length of Vector, Standard Deviation.
4. Copy stats into Excel. Copy rows representing Mean Angle and Length of Vector, paste into columns using Paste Special: Transpose.
5. Copy columns into Oriana: File: New, select Angles, select Separate; select Circular data only; select two columns, save. Graphs: Circular-linear, select Arrows and change parameters to create graphs according to your needs.
6. Export graph as a bitmap.

Notes

Equipment

Consumables and Reagents

References

How to connect to the lab's server

Index

Purpose

To connect a computer to the lab data server either through an operating system file browser or the Synology web-based file browser.

PC access (via UCSF network)

1. Press and hold the 'Windows Key and 'R" at the same time.
2. Type: //AAB_NAS
3. Enter your Username / Password when prompted.
4. Bookmark or Favorite this folder or any subfolder for quicker access.

Mac Instructions (via UCSF network)

1. Open 'Finder'
2. Click "Go"
3. Click 'Connect to Server'
4. Type: smb://AAB_NAS
5. Enter your username / password when prompted.
6. Bookmark/Favorite this folder or any subfolder for quicker access.

Web-based instructions (Mac+PC)

NOTE: The web-based connection will permit you to access the server anywhere with an internet connection. Speeds will be slow if running through the internet and not the local network.

1. Open a web-browser
2. Navigate to: http://quickconnect.to/alb
3. Enter your username / password when prompted.

Notes

- If you do not have UCSF network access, use the web-based instructions.

Equipment

- PC or Mac computer connected to the UCSF network or internet.

References

- Data Server

How to return purchased items via UCSF distribution

1. Here's how to place a "Return to Preferred Vendor".
2. Log on with your User ID and Password at https://clsdsb2b.ucsf.edu/.
3. Click on Service Requests
4. Click on Return to Vendor
5. Click on "Preferred Vendor"
6. Click on "Continue"
7. Select Fisher Scientific
8. Fill in the quantity field
9. Fill in the description
10. Fill out the "Common Information Page" with location, DPA/Fund (this is a
11. no charge p/u though)
12. Click on "Continue"
13. You'll receive a *5-digit return number"*
14. Write that number on a slip of paper and tape it securely to the package
15. Our automated warehouse management system will assign that pick-up to your

16. route, and if you get it in before 2:00 PM today, someone should be coming
17. around the following business day to pick that package up.

FedEx pickup times and locations

FedEx pickup sites and times

1. Starbucks building (2nd floor)
    a. 5pm
2. Cell culture facility (10floor HSW)
    a. ~230pm
3. Nursing building (6th floor, across from elevator (in office))
    a. 4pm sharp

Hybridoma Fusion Protocol

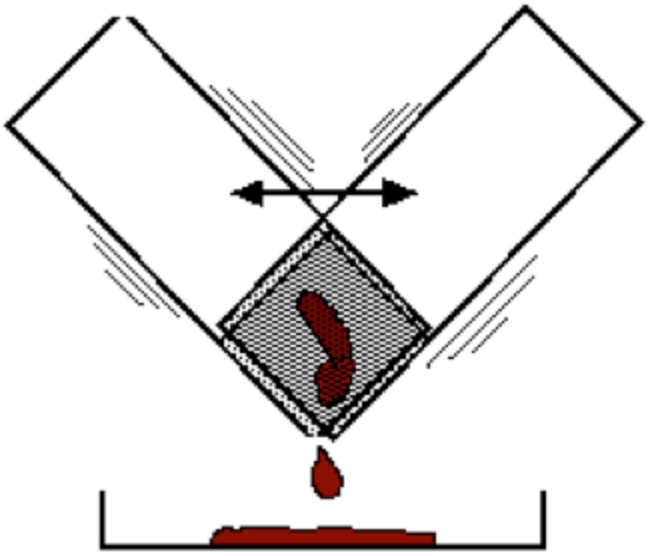
Index

Purpose

To generate a hybridomas which express monoclonal antibodies.

Instructions

1. Euthanize the boosted mouse via CO_2 inhalation.
2. Test mouse reflexes via tail or toe pinch to ensure death, and place mouse into a beaker containing 70% ethanol. Change your gloves.
3. Move the mouse to the dissection BSC.
4. Cut the skin at the point of the xiphoid process and with both hands pull the skin back to remove the skin from the spleen area.
5. Cut up through the ribcage on both sides, and across the diaphragm to expose the mouse heart. Insert a needle and syringe into the left ventricle to take the heart bleed. You may need to move the needle back and forward slightly. Alternatively, before cutting the ribcage, locate the heart around the fourth rib, and take heart bleed through ribcage.
6. Open the peritoneal cavity by cutting down the midline of the mouse body, and then make two perpendicular cuts either side of the spleen (the spleen is on the left hand side of the mouse, just below the liver). Peel the peritoneal flap back to expose the spleen and change tools. Grab the fat below the spleen, lift up, and cut the fat below the tweezers. DO NOT touch the body of spleen with the tweezers and be very careful not to nick/pierce the spleen.
7. Weigh the spleen in a tared 10cm petri dish and take a photograph against a metric ruler.
8. Place the spleen directly into a pre-warmed 50mL aliquot of DMEM. Wash the spleen by pipetting medium up and down at least 10 times.
9. Aspirate medium and wash 3 more times with 25mL of medium.
10. Add 10mL of medium to the tube containing the spleen, and carefully pour into a 10cm petri dish.
11. Use two frosted glass slides to tease the spleen. DO NOT touch the frosted area with your hands. Firstly remove any excess fat, make a small nick in the spleen and gently rub the spleen between the slides (see picture). Occasionally put the spleen back into the dish to wash the spleen, and repeat until the casing has turned white indicating that few cells remain in the casing.
    a. (Alternative methods can be used dependent on individual preference and reliably successful outcome).



b.

12. Wash both sides of each slide with fresh DMEM (15mL to wash 2 slides).
13. Transfer cells to a fresh 50mL tube. Leave as much fat and casing behind as possible.
14. Wash the 10cm petri dish with 10mL of medium and transfer to 50mL tube containing cells.
15. Pipette up and down at least 15 times to break up any cell clumps. Set in a rack and wait 10 minutes (DO NOT MOVE the tube containing spleen cells).
16. During the 10 min incubation
    a. Clean up the dissection hood
    b. Spin Heart Bleed for 5 min at 16000G. Put supernatant into a fresh tube and dilute 1:10 with PBS and azide)
    c. Set-up the hemocytometer
    d. Change gloves
    e. Collect SP20 cells into conical tubes (2x15cm dishes per mouse)
17. Following the 10 min incubation, remove the spleen cell containing media and pipette into a fresh 50mL tube, being sure to leave any debris that have accumulated in the bottom of the tube behind. Be careful to leave behind all debris, while minimizing spleen cell loss.
18. Centrifuge spleen and SP2/0 cells by 400xg for 6min. During this time, prepare for counting cells.
    a. For spleen cells: Add 1.9mL DMEM to 1x15mL tube
    b. For SP20 cells: Add 1mL DMEM to 1x15mL tube
19. Aspirate DMEM from SP20 cells and tap the tubes to loosen the pellet. Wash again with 25mL DMEM and centrifuge at 400xg for 6min.
20. Aspirate media from spleen cells and tap to loosen the pellet. Re-suspend in 5mL DMEM. Mix gently but thoroughly. Add 100uL to the corresponding 15mL falcon (containing 1.9mL media). Mix well. Remove 100uL of diluted cells and diluted 1:5 with trypan blue.
21. Count spleen cells. On average, an immunized mouse has 2-3x10^8 spleen cells. Use the fusion count sheet to determine how many SP20 cells are needed if the spleen cell to SP20 ratio is 8:1.
22. Warm H.A.T media
23. After the spin, aspirate the DMEM from the SP20 cells. Tap to loosen the pellet. Add 10mL DMEM to each tube and combine so you have 20mL of SP20 cells in a single tube. Mix thoroughly. Remove 500uL and add to the corresponding 15mL tube (containing 1mL DMEM). Warm PEG in the 37-degree water bath.
24. Remove 100uL of diluted SP20 cells (in 1mL) and dilute 1:5 with Trypan blue. Count SP20 cells. Calculate volume of SP20 cell suspension required. Add the appropriate volume to the spleen cells and mix thoroughly. Spin at 400xg for 6min.
    a. Prepare PEG 1500: Add 1mL DMEM to 4mL vial of 50% PEG 1500 and mix (40% final concentration).
    b. Prepare the balance for the next centrifuge spins (1 balance for 50mL tube containing 3mL, 1 balance for 50mL tube containing 13mL)
    c. Label 96 well plates
25. Aspirate media from SP20+spleen cell pellet (as dry as possible). Tap the pellet. Add 2mL of PEG at a rate of 1mL/minute while turning the tube constantly. You may pipette up and down to re-suspend, but do so GENTLY and a maximum of twice.
26. Centrifuge at 400xg for 6min.
27. Add 10mL DMEM (without aspiration) at a rate of 2mL/min. You may pipette up and down GENTLY and no more then twice.
28. Centrifuge at **200xg for 5min.**
29. During the spin measure out HAT media (150mL per # of spleens fused)
30. Aspirate supernatant from cell pellet. Tap to loosen the pellet. Re-suspend in 25mL HAT media thoroughly by pipetting up and down gently. Transfer cell suspension into remaining HAT media.
31. Wash tube with 10mL of H.A.T media and add to cell suspension.
32. Rock back and forth to ensure even suspension.
33. Transfer into reagent reservoir.
34. Fill each well on the 96 well plates with 150uL of cell suspension. Leave the four corners (A1, A12, H1, H12) empty. These spaces can be used for controls during the screening.
35. Put plates into the 37°C incubator.
36. 5 days following, feed cells with 100uL HAT medium.

Notes

Equipment

- 10x96 well plates per mouse
- Pipet Aid*
- Pipettemen (P1000, P200, P20)
- Clean and autoclaved tools for spleen dissection*
- 2x2 autoclaved frosted slides**
- 1x 10cm Petri dish*
- 1x 10cm Petri dish**
- 4x50mL conical tubes (6x for 2 mice)**
- 2x15mL conical tubes**
- 1x1.5mL microcentrifuge tubes*
- 1x1mL syringe and 25g needle*
- Timer
- Lab absorbent paper soaked with 70% ethanol*
- Stereological pipettes (at least 10x25mL, 5x10mL and 2x5mL)**
- Pipette tips (P200 and P1000)**
- 300mL of 70% ethanol in a 500mL beaker
- Ziploc bag for the mouse carcass*
- Fusion cell counting sheet
- Nitrile gloves
- Polyethylene Glycol (PEG) 1500 (Roche #783 641) – 4mL vial
- 1x sterile reservoir boat**
- 250 or 500mL sterile filter
- 6x50mL DMEM aliquots pre-warmed to 37 degree.
- 250-500mL HAT media (prepared fresh on the day of the fusion).
- Matrix equalizer multichannel pipette and sterile tips
- 2x15cm dish of confluent SP2/0
- Hemocytometer
- Cell counter
- Trypan Blue
- Balance
- Metric Ruler
- Camera

*Place under UV lights in dissection BSC for 20mins prior to beginning the fusion.

** Place under UV lights in the fusion BSC for 20mins prior to the beginning of the fusion.

Consumables and Reagents

H.A.T media -250mL

DMEM -11965	140 mL
FC-1	50 mL
NEAA	2.5 mL
Pen Strep	2.5 mL
Macrophage conditioned media	25 mL
NCTC-109 21340	25 mL
H.A.T (50x) H0262	5 mL

References

1. Protocol by Ashleigh Evans (August 13th 2012) , NeuroMab UC DAVIS

Immunohistochemistry

- DAB stained vibratome-cut Sections
- Immunohistochemistry of Mouse or Human Brain
- Immunohistochemistry Staining Procedure Using the Perkin-Elmer TSA kit with Human Tissue

DAB stained vibratome-cut Sections

Contents

Purpose

This protocol is for staining, with DAB, free floating 50 um vibratome-cut sections from formaldehyde perfused adult mouse brains.

Instructions

1. Quench endogenous peroxidase activity with 1% H_2O_2 in 1X Phosphate buffered saline (PBS) for 2 hours at ambient(room) temperature.
2. Wash 3 times in 1X PBS for 5 minutes each time.
3. Block in blocking solution for 1 hour at ambient temperature.
4. Incubate with desired primary antibody at appropriate dilution in blocking solution for 24+ hours at 2-8 °C. Avoid using recycled antibody stored in sodium azide (NaN_3).
5. Rinse 3 times in 1X PBS. Once for >1 hour on an orbital shaker and then rinse twice with PBS.
6. Incubate with appropriate biotinylated secondary antibody in blocking solution overnight at 2-8 Â°C.
7. Rinse 3 times in 1X PBS. Once for >1 hour on an orbital shaker and then rinse twice with PBS.
8. While sections are rinsing prepare ABC solution (just prior to use).
9. Incubate in ABC solution for 30 minutes at room temperature.
10. Rinse 3 times in PBS and prepare DAB solution.
11. Rinse 2x in 0.1M Tris-HCl, pH 7.6.
12. Incubate for 5 minutes in DAB solution.
13. Incubate as needed (end point determined by monitoring) in active DAB solution in the dark.
14. Stop reaction by rinsing 3 times in 1X PBS. Store in 1X PBS at 2-8 Â°C if necessary.
15. Mount sections on SuperFrost Plus slides
16. Process for hematoxylin or dehydrate in alcohol series for cover slipping with histoclear and neutral mounting medium.

Notes

- For GFP visualization, Florian increases the detergent concentration and incubates in primary for up to 3 days.

Equipment

- Orbital shaker
- 2-8 °C fridge

Consumables and Reagents

- 1X Phosphate buffered saline (PBS)
- Superfrost Plus slides / Fisher Scientific
- Vectastain kit
- ABC solution (10 mL)
    - Combine
        - 4 drops of Reagent A
        - 4 drops of reagent B
        - 10 mL 1X PBS.
    - Incubate in the dark for 30 minutes at room temperature before use.
- DAB solution (20 mL); 20 mL 0.1M Tris-HCl pH 7.6, 6 mg DAB powder. Mix by sonication for 15 minutes. Optional: Add 4 mL Nickel solution for dark stain.
- Active DAB solution (10 mL); 10 mL DAB solution, 3 uL 30% H2O2 (0.01% final). Make immediately before use with fresh H2O2.
- H_2O_2
- Blocking solution; 8 mL normal goat serum (or species-appropriate serum), 2 g bovine serum albumin (2% final), 1 mL 10% Triton X-100 in 1X PBS (0.1% final). Dilute to 100 mL with 1X PBS and then sterile filter with a 0.2 um membrane. Note: Florian sometimes increase the triton x-100 to 0.5% final.
- Primary antibody
- Biotinylated secondary antibody

References

This protocol was transcribed from Florian Merkleâ€™s protocol of the same name. The protocol was also modified by Rebecca Ihrie.

Immunohistochemistry of Mouse or Human Brain

Index

Purpose

To selectively probe for antigens of interest in order to visualize their location and intensity within a piece of tissue, often done in parallel to discern the relation between multiple probed antigens with their environment.

Protocol Variants

There are many factors which govern how well an antibody staining procedure will work and as such there are many different protocol variants. Below are variants that are or have been used in our lab.

Variant 1

1. Postfix for 3 hours at 2-8 °C in 4% formaldehyde (FA).
2. Block in blocking solution for at least 1 hour at ambient temperature.
3. Incubate overnight at 2-8 °C with antibody diluted in blocking solution.
4. Wash for 2-3 hours at ambient temperature in Washing solution. Change washing solution every 15-20 minutes.
5. Incubate secondary antibody (diluted in blocking solution, ~1/1000) for 45 - 60 min at ambient temperature. Alternatively; incubate

overnight at 2-8 °C (1/5000 dilution).
6. Wash for 2-3 hours at ambient temperature in Washing solution. Change washing solution every 15-20 minutes.
7. Coverslip

Variant 2

1. Start with 30 um thick mouse sections on slides.
2. Wash 3X with 0.1 M phosphate buffered sale (PBS) for 10 min each time
3. Perform antigen retrieval by boiling samples in a microwave for 15 min in 10 mM citrate buffer (pH 6) containing 10 mM Sodium Citrate and (v/v) 0.1% Tween-20.
4. Allow slides to cool to room temperature
5. Wash 3X with PBS for 10 min each time.
6. Block tissue for 90 min at ambient temperature in blocking buffer (10% donkey serum, 0.1% Triton X-100, 0.2% gelatin (v/v)).
7. Incubate overnight (>16 hours) at ambient temperature with (v/v) 2% donkey serum, 0.02% Triton X-100, (w/v) 0.04% gelatin, and primary antibodies.
8. Wash 3X with PBS for 10 min each time.
9. Incubate 1-2 hours at ambient temperature in 2% donkey serum, 0.02% Triton X-100, (w/v) 0.04% gelatin
10. Wash 3X with PBS for 10 min each time.
11. Wick-dry slides and coverslip with Mowiol.

Variant 3 - Julien's IHC Protocol

1. Postfix for 3 hours at 4°C in PFA 4%: I did this just because Shawn said that not postfixing for too long might help the SST staining. I need to double check, but I am pretty convinced that even much longer post fixation time length (overnight) resulted in a pretty good staining in Paris. Despite short postfix length, SST staining is kind of carppy anyway.
2. Blocking: TBS, 10%NGS, 1% Triton at least for an hour at RT.
3. 1st antibody: overnight at 4°C. 1/1000 for GABA diluted in the blocking solution.
4. Intensive washing for 2-3 hours at RT in TBS 1% Triton, renewing the washing solution every 15-20 mins.
5. 2nd antiboy: 45mins-1h @ RT, diluted in blocking solution. I guess I used the donkey anti-rabbit-561 (1/1000) antibody for the GABA staining. Staining are usually nicer when the secondary antibody is used overnight at 4°C (1/5000 in that case).
6. Intensive washing for 2-3 hours in TBS 1% Triton, renewing the washing solution every 15-20 mins.

Variant 4 - Human Immunohistochemistry staining procedure using the Perkin-Elmer TSA kit

The following variant is much more in depth and unique and so it was given its own protocol page.

Immunohistochemistry Staining Procedure Using the Perkin-Elmer TSA kit with Human Tissue)

Variant 5 - IHC for 12 micron thick mouse sections (CKT)

Warm the slides to ambient by storing them covered at ambient.
Wash slides in PBS for 10-15 minutes to remove OCT.
Wick dry slides and encircle tissue using a PAP pen.
Fix the slides in 4% PFA for 15 minutes.
This gives better staining and makes tissue stronger, but you need to rinse well as it will inhibit ab binding).
Alternative:
3 hours @ 2-8C in 4% FA
Wash slides in PBS for 10 min 3 times
Incubate the slides with blocking solution (PBS containing 0.1% triton x-100 and 5% donkey or goat serum) for 30-120 minutes.
Incubate the slides with primary antibody in blocking solution overnight at 2-8C (or 2 hr at ambient).
Optional:
Place a coverslip or plastic sheet cut to the size of a coverslip onto the slide to prevent/reduce drying out.
Wash the slides in PBS containing 0.1% TX-100 for 10 minutes twice
Wash the slides in PBS for 10 min.
Incubate with secondary antibody in blocking solution for 2 hr at ambient (or overnight at 2-8C).
Alternative:
1/5000 dilution and stained overnight at 2-8C
Julien S. experienced better staining this way.
Wash the slides in PBS containing 0.1% Triton X-100 for 10 min twice
Wash the slides in PBS containing 100 ng/mL DAPI for 10 min (can combine with 2' but need to use diluted form)
Wash the slides in PBS for 10 min.
Mount the sections after rinsing with water using water based mounting.

Epitope Specific Notes

NeuN and GFAP

1. CKT says NeuN and GFAP probably dont need Ag retrieval.

Chibby1 staining

Talk to Shinya Ohata for details.

Chibby1 staining from Dr. Camille Boutin

Dissection

PFA4% TX100 0.1% 15min r.t.

Wash PBS 1X 0.1%TX100 3x10min w/ agitation

Scalp

Block PBS BSA 3% 1h w/ agitation

AbI in PBS-BSA3% over night RT w/agitation

Chibby (8-2) Mouse monoclonal IgG2a Santa Cruz sc-101551 (lot n° C0909)

1/500 (maybe try 1/250)

GTU-88(Gamma Tubulin-centrosome Marker) Mouse Monoclonal

IgG1 Abcam ab11316 1/500

Wash PBS 1X 0.1%TX100 3x10min w/ agitation

AbII in PBS 1h RT w/ agitation

Alexa Fluor 488 Goat anti Mouse Monoclonal IgG2a Invitrogen A21131 1/800

Alexa fluor 568 Goat anti Mouse Ig G1 (g1) Invitrogen A21124 1/800

DAP1 1/1000

Wash PBS 1X 0.1%TX100 3x10min w/ agitation

slide +Mowiol +coverslip

Fixation Length

1. To stain for VIP, brains must be postfixed for no longer than 20min. Daniel Vogt from the Rubenstein lab recommends timing the entire PFA perfusion/postfixing at 20min. Caroline did side-by-side staining comparing 2hr postfix and 20min postfix on adult visual cortex and confirmed that only the 20min postfixed tissue has VIP signal.
2. Somatostatin is also sensitive to fixation length. The rabbit antibody from Swant performs better on lightly fixed tissue. The goat antibody from Santa Cruz is less sensitive to fixation.

Equipment

1. Refrigerator
2. Timer
3. Orbital shaker
4. 2-8 °C chamber
5. Coplin jars (or equivalent)

Consumables and Reagents

1. 4% formaldehyde (FA) prepared from paraformaldehyde (PFA)
2. Tris buffered saline (TBS)(50 mM TRIS, 0.9% NaCl, pH 7.4)
3. Blocking solution

    a. Variations
        i. TBS with 10% NGS and 1% Triton X-100)
        ii. 5% serum, 0.1% TX in PBS
        iii. 10% serum, 1% TX in PBS
4. Washing solution (TBS with 1% triton x-100)
5. 1X Phosphate buffered saline (PBS)
6. Phosphate buffered sale with triton x-100 (PBST), pH 7.4 (0.1% Triton X-100)
7. Antibodies
8. DAPI
9. PAP pen
10. Mountant
    a. Aqua Polymount
    b. Mowiol

References

Immunohistochemistry Staining Procedure Using the Perkin-Elmer TSA kit with Human Tissue

Index

Purpose

To probe for and amplify the signal of antigens of interest in human tissues using fluorescent molecules.

Instructions

DAY 1

1. Rehydrate slides in TNT for 10 minutes.
2. Incubate with 1% H2O2 in 1X PBS for 30-40 minutes.
3. Wash 3 times with TNT for 10 min each time.
4. Block with TNB for 60 minutes
5. Incubate with primary antibodies in TNB overnight. [Example; Rabbit anti doublecortin (1:200)]

DAY 2

1. Wash 3 times with TNT for 10 min each time.
2. Incubate with biotinylated secondary antibody for 150 minutes (2.5 hours). [Example; biotin-SP Goat anti Rabbit 1:500, DAPI 1:5000]
3. Wash 3 times with TNT for 10 min each time.
4. Incubate with Strepavidin conjugated to horse radish peroxidase (HRP) in TNB for 30 minutes
5. Wash 3 times with TNT for 5 min each time.
6. Incubate with Tyramide in amplification diluent solution for 4-7 minutes. [Example; Fluorescein 1:50]
7. Wash 2 times with TNT for 5 min each time.
8. Wash 6 times with TNT for 10 min each time.
9. Fix the sample with 4% formaldehyde (FA) for 15 minutes.
10. Wash 3 times with TNT for 10 min each time.
11. Incubate with 1-2% H2O2 in 1X PBS for 30-60 minutes.
12. Wash 3 times with TNT for 10 min each time.
13. Block with TNB for 30 minutes.
14. Incubate with the second primary antibody in TNB overnight. [Example; Chicken anti aGFAP (1:250)]

DAY 3

1. Wash 3 times with TNT for 10 min each time.
2. Incubate with biotinylated secondary antibody and/or fluorphore conjugated antibody for 150 minutes (2.5 hours). [Example; biotin-SP donkey anti chicken 1:500, AlexaFluor 647 Goat anti Chicken 1:750]
3. Wash 3 times with TNT for 10 min each time.
4. Incubate with Strepavidin conjugated to horse radish peroxidase in TNB for 30 minutes
5. Wash 3 times with TNT for 5 min each time.
6. Incubate with Tyramide in amplification diluent solution for 4 minutes. [Example; Cy3 1:100]
7. Wash 4X with TNT?
8. Wash 2 times with TNT for 5 min each time.
9. Wash 6 times with TNT for 10 min each time.
10. Coverslip

Notes

- Depending on the target antigen, antigen retrieval might be needed. This is done typically with 10mM Sodium citrate at 95C for 10 minutes. Often done as the first step on Day 1
- Works for tissue up to 30 um thick and mounted on Superfrost Plus slides (Fisher Sci).
- Protocol is executed on tissue that was embedded in OCT, stored at -80 Â°C, cryosectioned at -20 Â°C, and then stored at -80 Â°C.
- When using the tyramide system, only one tyramide targeted antibody can be used at a time and so this protocol is repeated as many times as there are tyramide system targets. During the second round of tyramide treatment a fluophore conjugated antibody can be used along with the tyramide treatment (2 targets via different tyramide products and 1 target via a standard fluorescent antibody).
- Flurophore Tyramine Working Solution Concentrations:
    - Fluorescein + 0.6 mL DMSO - Diluted 1:50 with amplification diluent
    - Cy3 + 0.3 mL H2O - Diluted 1:100 with amplification diluent
    - Cy5 + 0.3 mL H2O - Diluted 1:50 with amplification diluent
- Quick rinses between washes can help reduce background if that is a problem
- To clean slide before coverlipping you can airdry them for several minutes and rinse with ddH2O before coverlip

Equipment

- Light-proof rack to store slides

Consumables and Reagents

- TNB Buffer 0.5% blocking powder (Perkin Elmer) in 0.1 M Tris (pH 7.5) and 0.15 M NaCl. For 400 mL; 40 mL of 1M Tris, 12 mL of 5M NaCl, 2g blocking powder. Prepare the buffer without the blocking powder (including pHâ€™ing), transfer the solution to a bottle, and heat it to 55-60 Â°C for 1-2 hours. Next add the blocking powder and stir for 1-2 hours until dissolved (reheat if necessary). Aliquot into 50 mL conical tubes and store at -20 Â°C.
- TNT Buffer; 1X phosphate buffered saline (PBS) + 0.05% Tween 20 (or Triton X-100). Tris buffered saline (TBS) can be substituted in place of PBS.
- Alternatives to the Kit
    - TSA Amplification Diluent – Comes with the kits but it is extensively cheaper to make yourself.
    - Tyramide Amplification and Synthesis – This is the do-it-yourself alternative to buying the kit from Perkin-Elmer.
- TSA Kit Components
    - PerkinElmer / SAT704B001EA / Cyanine 3 Tyramide Reagent Pack
    - PerkinElmer / SAT701B001EA / TSA fluorescein & amplification diluent kit 5 fluo tubes/ 2x amp diluent
    - PerkinElmer / FP1020 / Blocking powder
    - PerkinElmer / NEL75000 / strep-HRP
    - PerkinElmer / NEF710001EA / Antifluorescein-HRP Conjugate

References

1. Protocol originated from Perkin Elmer TSA Renaissance kit.
2. Protocol modified by Mercedes Paredes and Thuhien Nguyen of Alvarez-Buylla lab.
3. Protocol edited by Joseph Elsbernd (28APR2014)
4. For other immunohistochemistry protocols, go here: Immunohistochemistry of Mouse or Human Brain

TSA Amplification Diluent

Index

Purpose

To prepare a cheaper alternative to the amplification diluent sold by Perkin-Elmer. This diluent is used in the following protocols; Immunohistochemistry Staining Procedure Using the Perkin-Elmer TSA kit with Human Tissue and Tyramide Amplification and Synthesis

Instructions

Amplifcation Diluent (0.1 M Borate, pH 8.5 with 0.003% H2O2)

1. Dissolve 6.18 g/L boric acid in ~90% final volume Milli-Q water or equivalent.
2. Adjust pH to 8.5 using NaOH.
3. Dilute to the final volume with Milli-Q water.
4. Add H_2O_2 to final concentration of 0.003%.

Notes

1. Amplification Diluent can be stored at 4°C for several months. I suggest that you make the borate buffer stock and then add hydrogen peroxide to a smaller volume (100 ml) for daily use.

Consumables and Reagents

1. Boric acid
2. Sodium hydroxide
3. Milli-Q Water
4. 30% stabilized hydrogen peroxide (SIGMA #H1009)

References

1. Protocol was obtained by Shawn Sorrells from ?.

Tyramide Amplification and Synthesis

Index

Purpose

To generate and amplify tyramide compounds for use in fluorescent labeling of tissue mounted on slides. This method is tremendously cheaper than purchasing the kit from Perkin-Elmer.

Instructions

Synthesis of tyramide compounds

1. Prepare succinimidyl ester (46.6 mg (FITC)/0.3 ml) and tyramine solutions (14.6 mg/0.3 ml) in DMSO and mix them together at a 1:1 ratio in a light proof bottle at room temperature overnight.
2. This tyramine stock can be used at a 1:50,000 dilution. NOTE: for other tyramine compounds calculate the molarity to match that of the FITC, siccinimidyl ester shown above.
3. The tyramide compounds are stable for years when stored at 4°C.

Amplification

1. Dilute the tyramide compound in amplification diluent to 1:50,000 (or 1:150 for DuPont NEN) and add directly to slides after antibody incubations etc...
2. Incubate for 10 minutes at room temperature and stop the reaction by washing in PBS.
3. DAPI stain and mount with gelvatol.

Notes

1. This protocol provides the sensitivity of enzymatic detection (HRP) in immunohistochemical procedures with the versatility and convenience of fluorescence detection. Tyramide amplification was first developed at DuPont NEN by Litt's group (Bobrow et al. 1989, J. Imm. Meth., 125: 279-285; Bobrow et al. 1991, J. Imm. Meth. 137: 103-112) and has since been made into an off-the-shelf kit called "TSA" for tyramide signal amplification. I have included here the use of the TSA kit as well the the synthesis of the tyramide reagents and the recipe for the reaction buffer. Synthesis of these reagents in house is not only simple but can potentially save a lot of money. For approximately $300.00 a single TSA kit can be purchased for one fluorochrome that is enough for approximately 75-100 slides. For that same amount of money, reagents can be purchased to stain over 1,000,000 slides using two different fluorochromes.
2. This protocol is an alternative to the Perkin-Elmer TSA kit used in the Immunohistochemistry Staining Procedure Using the Perkin-Elmer TSA kit with Human Tissue protocol.

Equipment

Consumables and Reagents

1. TSA Amplification Diluent
2. Tyramine HCl (5 g)
    a. Sigma-Aldrich #T9,035-2
3. 5-(and-6)carboxyfluorescein, succinimidyl ester (100 mg)
    a. Molecular Probes #C-1311
4. Texas Red succinimidyl ester is available from Molecular Probes
5. Cy-3 succinimidyl ester is available from Amersham
6. Biotin
    a. Calbiochem
7. succinimidyl ester
    a. Calbiochem

References

1. Protocol obtained by Shawn Sorrells from ?

Intracerebral Virus Delivery in Mouse Embryos

Index

Purpose

Instructions

Timed pregnant mice with embryos at different gestational ages are anesthetized with isofluorane (1-3%) using an anesthetic vaporizer apparatus. The abdomen is wet-shaved and analgesia administered (subcutaneous injection of buprenorphine (0.05-0.1mg/kg)), followed by a local injection of lidocaine (7-8mg/kg) at the incision site. A 2 cm midline laparotomy is performed. The uterine horn is carefully taken out and one side is selected for injection. A sharp glass micropipette (diameter < 50 microns) is inserted through the uterus into the cerebral ventricles of healthy embryo. One microliter of replication-incompetent virus is injected. In order to confirm the placement of the micropipette, Fast Green dye will be added to the viral or plasmid solution. After the abdomen is opened, the surgery up to this point is limited to one hour to optimize survival of the mother and the embryos. The uterine horn is returned to the abdomen, and the incision is closed with a 6-0 silk suture. The animal is placed on a heated pad and monitored until recovery. Upon recovery, buprenorphine is given via intraperitoneal injection in a single dose at 4-12 hours after surgery. These analgesics will be administered every 8-12 hours as needed thereafter. The embryos are then carried to term and pups allowed to survive from 1 day to adulthood before euthanasia. After the appropriate number of days, the animals are deeply anesthetized (see below) and transcardially perfused.

Notes

Equipment

Consumables and Reagents

References

Isolation of Medial Ganglionic Eminence (MGE)

Index

Purpose

Instructions

1. Sacrifice pregnant mouse.
2. Perform laparotomy
    a. Completely remove uterus from mother
        i. Leave uterus intact because its easier to keep track of all of the embryos

ii. Put uterus into a dish of Leibovit'z L-15 media.
1. Dish is sitting upon ice
3. Remove pups from uterus and transfer heads of embryos into a second dish.
4. Dish is sitting upon ice
5. Transfer a head, one at a time, to a third dish filled with chilled media.
6. Place third dish under a dissection scope
7. Remove skin and skull from the head of an embryo
8. Separate hind and forebrain, isolate the forebrain.
9. Cut forebrain in half along midline to get two hemispheres.
10. Dissect out the MGE from each hemisphere and place each into the same tube, filled with x uL of media and kept on ice.
    a. The tube is surrounded by aluminum foil, it is said to help with cell viability

Notes

Equipment

1. Forceps; fine, curved
    a. Vendor: FST
    b. Catalog: 5/45
2. Scissors
3. Surgical knife
    a. Disposable, but it is reusable
4. Ice bucket
5. Anesthesia machine
6. Dissection microscope

Consumables and Reagents

1. Dish; 10 cm, plastic
2. Dish; 6 cm, plastic
3. Leibovit'z L-15 media without NaHCO3
4. Pregnant mouse

References

Microscope

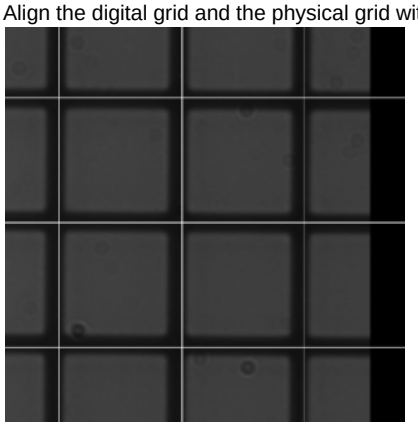
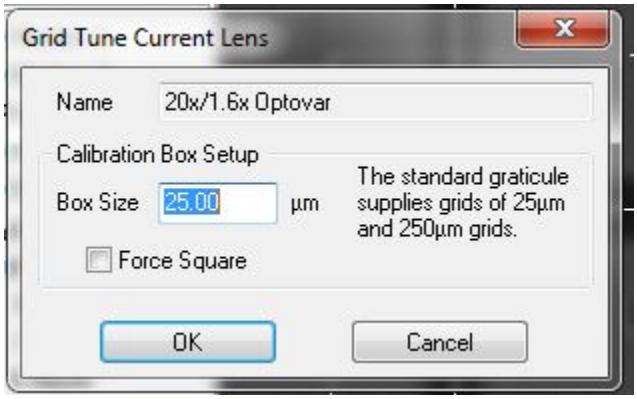
Aligning and calibrating the Axiovert 200M camera with automatic stage and software.

Index

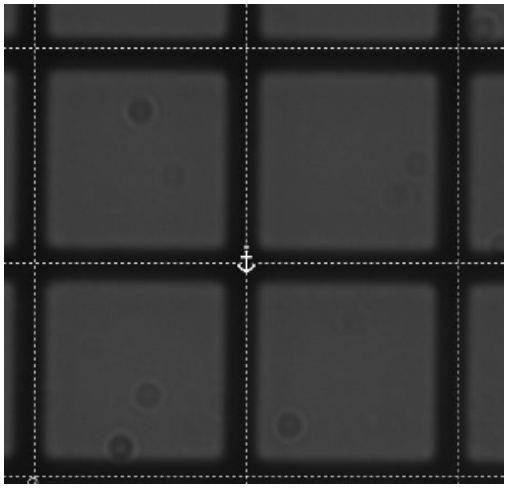
Purpose

Whenever the camera or stage have been altered in some way they must each be calibrated in-order to take tiling images – single still images work fine without it.

Instructions

1. The Camera
    a. The camera screws onto the mount in a typical fashion.
    b. The camera's orientation is secured by three 3mm-head hexagonal screws near the camera port (closer to the microscope than the camera) – these are what hold the camera steady. 2 of the 3 screws need to be loosened in order to rotate the camera.
    c. When the hexagonal screws are loose the camera can be rotated LEFT, RIGHT, FORWARD, and BACKWARD. The amount of forward and backward movement is very small but significant.
    d. With Neurolucida, focus on the calibration slide. Start at a low magnification and view the 250 micron grid.
    e. Click the 'Display grid' button. 
    f. Align the digital grid and the physical grid with eachother by positioning the camera and tightening the hexagonal screws.
    g. 
    h. Tightening the screws will distort your alignment. You will likely need to try this method repeatedly. Try compensating for the alteration of screw tightening.
2. The Stage and Software
    a. The stage and software need to be calibrated at each objective. Repeat this procedure for each objective.
        i. For <10X use the 250 micron grid.
        ii. For >10X use the 25 micron grid.
    b. Within Neurolucida click 'Tools'
    c. Click 'Grid Tune Current Lens'
    d. 

    e. Choose your box size (dependent upon which size grid you are using) and then click 'ok'.
    f. Click and drag the anchor symbol to the upper left of the screen and align it at the intersection of a horizontal and vertical line.

i. 

g. Move the cursor over the dotted portion of the virtual grid. The cursor will turn into a double arrow. Click and drag to align the dotted line into the black lines.
h. Align the vertical lines first and then the horizontal. You will need to click and drag the dotted line from many different points to make the alignment acceptable across the whole image.
i. Once complete, right click the screen and click 'Finish Calibraiton Current Lens"
j. Repeat this procedure for each objective.

Notes

Equipment

1. Zeiss Axiovert 200M Inverted Fluorescent Microscope 200M
2. Neurolucida
3. Calibration Slide (250 um squares and 25 um squares)

References

1. Axiovert200M_Manual.pdf

Laser Dissection Microscopy and PCR

Index

Purpose

Instructions

Notes

Equipment

Consumables and Reagents

1. Platinum Pfx DNA Polymerase
    a. 2500U / 100 uL / $302.44
    b. Invitrogen 11708-021
2. Taq DNA Polymerase
    a. 500U / 100 uL / $197.67
    b. Invitrogen 18038-042
3. 100 mM dNTP Set
    a. 4x250 uL / $218.50
    b. Dilute to 10 mM. For 2000 reactions
4. 8-strip optically clear flat caps
    a. 125 per box / $30
    b. E&K 490028
5. Framestar break-away 96-well plate
    a. 50 per case / $246
    b. E&K / EK-75098
6. Multiply ustrip Pro 8 PCR tubes
    a. 120 per box
    b. E&K / 72991102
7. Adhesive PCR foil seal
    a. 100 per case / $109
    b. E&K / EK-75113
8. PEN-membrane slides 2 micron
    a. 50 per box / $289
    b. JH Technologies / 11505158

References

Molecular Biology

Protocols

Amplification and purification of DNA polymerase

Index

Purpose

To cheaply produce Thermus Aquaticus(Taq) of Pyrococcus furiosus(Pfu) DNA polymerase for use in polymerase chain reaction.

	Error Rate	Speed
Pfu DNA Pol.	1 in 1,300,000 bp	8.3 bp / sec
Taq DNA Pol	1 in 9,000 bp	100 bp / sec

Instructions

1. 500 µl of overnight culture to 1 litre of LB broth with ampicillin (80 mg/L)

1. Grow at 37Â°C for 11 hours to an OD600 of approx. 0..6 to 0.8

2. Add IPTG to a concentration of 125 mg/L/ 0.5 mM). Grow overnight for between 12-16 hours.
3. Carry out following procedures on ice
4. Harvest cells by centrifugation and wash in 100 ml per litre of original culture volume of buffer A
5. Recover cells by centrifugation and resuspend in 50 ml per litre of original culture volume of pre-lysis buffer (buffer A plus 4 mg/ml lysozyme)
6. After 15 minutes at room temperature, add an equal volume of lysis buffer
7. Incubate lysis mixture in 200 ml aliquots in pyrex flasks at 75 Â°C for 1 hour
8. Transfer lysis mixture to plastic bottles for centrifugation at 15,000 rpm for 10 minutes at 4Â°C, transfer clarified lysate to a clean pyrex flask
9. Recover Taq polymerase from the clarified lysate by adding 30 g of powdered (NH4)2SO4 per 100 ml of lysate while stirring rapidly at room temperature
10. Centrifuge solution at 15000 rpm for 10 minutes and harvest the protein precipitate (both in pellets and as surface precipitate)
11. Resuspend in 20 ml of A buffer per 100 ml of original cleared lysate
12. Dialyze resuspended protein against 2 changes of at least 12 hours each of storage buffer at 4Â°C
13. After dialysis, dilute resulting protein 1:1 with sterilized storage buffer and stored at -70Â°C

Dialysis tube treatment:

1. 1. Wearing gloves, cut the dialysis membrane in to the desired length (You need to do calculation. For the Fisher dialysis we have, 3.5 cm is enough for 20ml, allow an additional 2-4 cm on both ends for clamps, so totally 6 cm will do).
2. 2. Soak the membrane in ddH2O for 15 min at room temperature.
3. 3. Put the membrane into a large volum (500 ml-1L)10 mM sodium bicarbonate and heat up, while stirring, to 80 Â°C.
4. 4. Transfer the membrane into 10mM EDTA and soak for 30 min at room temperature.
5. 5. Replace sulotion with ddH2O and stir for 30 min at 80 Â°C.
6. 6. Allow membrane to cool down, transfer it into 20-50% ethanol and store in refrigerator (Donâ€™t freeze. Never let the membrane dry!)
7. 7. Before use, wash the membrane inside and out with ddH2O completely and soak it in dialysis buffer (the storage buffer in this case).
8. 8. Secure clamp on one end of the membrane. Fill with ddH2O or dialysis buffer then seal the other end to check the integrity of the tubing and clamps. Now the tubing is ready for use.
9. 9. Load smple into the treated tubing. Check the integrity of the tubing and clamps again. Immerse dialysis tubing ina beaker or flask containing a large volume (usually 100-1000-fold that of the sample) of dialysis buffer (Storage buffer in this case) and dialysis for several hours with gentle stirring.

Notes

1. Protocol was provided by Shawn Sorrells to and then edited by Joseph Elsbernd. Protocol is originally from Pluthero (1993). Some details were taken from a DIYbio forum (https://groups.google.com/forum/#!topic/diybio/Hx0Iqge46-w). Some details were supplied by unknown persons.
2. The protocol was written with Taq in mind, Pfu can be grown and purified the same way without changes.
3. E.coli strain INVlalphaF' transformed with the pTaq plasmid (Taq gene expressed under control of the tac promoter) or pET16B.Pfu plasmid.
4. Something to try/improve overall upon for next time. For my tubing I only soaked it in dd water water with mild agitation for ~2 hours.
5. Resulting average yields are in excess of 106 units of Taq polymerase per litre of starting culture.

Equipment

1. Shaker

Consumables and Reagents

1. Plasmid containing DNA polymerase gene from Taq or Pfu
2. LB Amp Plates
3. LB amp broth
4. Dialysis Tubing
5. Buffer A
    a. 50 mM Tris-HCl pH 7.9
    b. 50 mM dextrose
    c. 1 mM EDTA
    d. molecular biology grade, take care to avoid contamination with biological material or metal ions
6. Lysis buffer
    a. 10 mM Tris-HCl pH 7.9
    b. 50 mM KC1
    c. 1 mM EDTA
    d. 1 mM PMSF (can be substituted with protease inhibitor tablets)
    e. 0.5 % Tween 20
    f. 0.5 % Nonidet P40 (or Triton X-100)
7. Storage buffer

a. 50 mM Tris-HCl pH 7.9
b. 50 mM KCl
c. 0.1 mM EDTA
d. 1 mM DTT
e. 0.5 mM PMSF (can be substituted with protease inhibitor tablets)
f. 50% glycerol

References

1. Pluthero FG. Rapid purification of high-activity Taq DNA polymerase. Nucleic Acids Res [Internet]. 1993 Oct 11 [cited 2014 Mar 27];21(20):4850â€“1. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=331521&tool=pmcentrez&rendertype=abstract PMID: 8233838
2. See Comparison of homemade Taq DNA polymerase and purchased DNA Pol for details on how well this protocol works.

E. Coli transformation

Index

Purpose

To insert a plasmid into E. Coli.

Instructions

1. Warm LB plates and recovery media in a 37C incubator for at least 30 minutes.
2. Chill empty micro-centrifuge tubes on ice and thaw competent Dh5 Alpha cells onice.
3. Gently mix cells with a pipette and aliquot 50uL of cells into each chilled tubes.
4. Pipet 1- to 50ng of plasmid DNA directly into each tube that contains competent cells.
5. Incubate on ice for 30 minutes
6. Heat shock cells at 42C for 45 seconds
7. While near a flame, add 950uL of recovery medium to each tube.
8. Incubate tubes for 60 minutes at 37C whiles haking.
9. For each tube, transfer 150uL of transformed cells to an LB plate.
10. Use a cell spreader to evenly distribute the transformed cells across the plate.
11. Spin the remaining cells in a centrifuge for 1 minute to pellet the cells.
12. Remove 700uL of supernatant and then resuspend the pelleted cells in the remaining media (approximately 150uL).
13. Foreachtube,platetheremaining150uLofcellsolutionontoanLBplate.
14. Incubate the plates at 37C overnight. Make sure the plates are inverted.
15. For each tube, two LB plates should have been made.

Notes

Equipment

1. Centrifuge
2. Ice-bucket
3. 37C Incubator
4. Pipettes and tips (10uL, 100uL, 1000uL)

Consumables and Reagents

1. E. Coli Dh5 Alpha (competent cells)
2. Recovery media (LB broth or SOC media)
3. LB plates with appropriate antibiotic
4. Plasmid DNA
5. 1.5mL sterile micro-centrifuge tubes

References

Outgoing lab member checklist

- [ ] Update contact information
  - [ ] Personal email
  - [ ] Phone
  - [ ] Contact address
- [ ] Record destination, goal, or plans after leaving
- [ ] Record the title of their research project
- [ ] Notebooks and data
  - [ ] Obtain all physical notebooks, logbooks, data, and papers. Place inside ziploc-style bag.
  - [ ] Obtain copies of all electronic notebooks, logbooks, data, and papers.
  - [ ] Ensure physical and electronic items are labeled
  - [ ] File all things not in active use away
- [ ] Ask for feedback on
  - [ ] Overall experience
  - [ ] Fellow lab members
  - [ ] Any issues
  - [ ] Good things
  - [ ] Suggestions
- [ ] Archive all training materials
- [ ] Have lab member update protocols on the Wiki.
- [ ] Record who the point of contact is for their old things after they leave (ex: Mercedes handles Nader's stuff).

Post-perfusion care of mouse brain tissue

Index

Purpose

To properly prepare the mouse brain tissue for later use.

Instructions

1. Immediately after dissecting out the brain from the perfused animal place the brain into 4% PFA and store at 2-8C.
    a. The amount of time the tissue drop fixes for is dependent upon the proteins of interest. Some proteins are damaged by long fixations and others are not accessible without long fixations
    b. Dropfix tissue for at least two hours
2. Inside of a fume hood, carefully pour away the 4% PFA solution.
    a. Be careful not to pour out the brain along with the solution
    b. It is recommended that one always pour off solutions into a tube or beaker and NOT the waste container. If a brain falls into a tube it is of little consequence, but if the brain falls into a waste container it is ruined.
3. Rinse the brain. Pour a few mL's of 1X PBS into the tube and immediately pour it off.
4. Wash the brains with 1X PBS 3 times for 10 minutes each time on a rocker platform at ambient temperature.
5. Add 1X PBS to the tube and agitate it for 10 minutes on a rocker platform.

Notes

Equipment

1. Rocker platform
2. timer

Consumables and Reagents

1. 1X PBS
    a. Use if the brains are going to be sectioned on vibratome.
2. 1X PBS w/Azide
    a. Use if the brains are to be stored medium to long term.
    b. If the brains are going to be frozen during or before sectioning (sliding microtome / cryostat) you must cryo-protect the brains before freezing them.
3. 30% sucrose in 1x PBS?
    a. ?

References

Reagents and Stock Solutions

- 10X Phosphate Buffered Saline (PBS), 1 liter
- 1M Tris Buffer
- 1X Phosphate buffered saline (PBS)
- 20% Triton X-100
- 293 Cell Media
- 4% formaldehyde (FA) prepared from powdered paraformaldehyde (PFA)
- Bovine Lacto Transfer Technique Optimizer (BLOTTO)
- Sucrose in 0.1 M Phosphate buffer
- TNB Blocking Solution

1M Tris Buffer

Index

Purpose

Instructions

1. Choose a target pH
2. Combine and completely dissolve the corresponding quantities of conjugate acid and conjugate base in 100 mL water.
3. pH the solution to the exact desired pH.
4. Optional: Filter

pH	TRIS HCl (g)	TRIS base (g)
7.2	14.04	1.34
7.3	13.70	1.60

7.4	13.22	1.94
7.5	12.70	2.36
7.6	12.12	2.78
7.7	11.44	3.32
7.8	10.64	3.94
7.9	9.76	4.60
8.0	8.88	5.30
8.1	8.04	5.94
8.2	7.08	6.68
8.3	6.14	7.40
8.4	5.28	8.06
8.5	4.42	8.72
8.6	3.66	9.30
8.7	3.00	9.80
8.8	2.46	10.26
8.9	1.92	10.64
9.0	1.52	10.94

Notes

Equipment

Consumables and Reagents

References

1X Phosphate buffered saline (PBS)

1X PBS is made in 10 liter batches from a commercially bought 10X stock solution.

Manufacturer	Teknova
Product name	10X PBS Solution
Product Number	P0496

1X solution composition:

- 0.135 M Sodium chloride
- 2.7 mM Potassium chloride
- 4.3 mM Sodium phosphate
- 1.4 mM Potassium phosphate
- pH 7.4

10X solution composition:

- 1.35 M Sodium chloride
- 27 mM Potassium chloride
- 43 mM Sodium phosphate
- 14 mM Potassium phosphate

4% formaldehyde (FA) prepared from powdered paraformaldehyde (PFA)

Contents

Instructions

1. Heat 450 mL of distilled water to 50-60 Â°C on a hot stirring plate within the fume hood. It is best if the glassware used is dedicated to this task.
2. Add 40 g of PFA (Sigma / P6148, reagent grade, crystalline) to the water.
3. Begin adding, drop-wise, 5 N NaOH to the solution and stop once the pH of the solution is approximately 7.0 or the PFA has completely dissociated into FA. Do not exceed pH 7.2. See list below for the approximate number of drops to use (~125-150 uL of 5 N NaOH). The solution may become more acidic as the PFA dissolves, add more NaOH if necessary.
    a. Table:

No. of Drops	pH
0	5.0-5.5
2	6.0-6.5
3	6.5-7.0
4	7.0-7.5
5	7.5-8.0

4. Add 500 mL of 0.2 M Phosphate buffer (pH 7.4).
5. Wait for solution to reach room temperature.
6. If necessary, adjust the pH of the solution to 7.40.
7. Dilute to the final volume (1 L)
8. Filter solution through a 0.22 um membrane (Corning / 430758) and store at 2-8Â°C in the dark. The solution is good for a few days at 2-8C and for a month at -20C.

Notes

1. If the temperature of the water ever exceeds 60 Â°C, the preparation must be restarted because the heat will cause the PFA to breakdown.
2. Do not exceed pH 7.2 â€" if you do, the preparation must be restarted.
3. Nomenclature
    a. Thus, when paraformaldehyde actually becomes a fixative, it is no longer paraformaldehyde by chemistry or fixation capacity. Rather, it is formaldehyde in water without methanol or any other stabilizer. There is, therefore, no such chemical as paraformaldehyde fixative. Without heat and an alkaline environment, paraformaldehyde in water is simply paraformaldehyde suspension or solution with little fixation capacity. And when paraformaldehyde is heated at a slightly alkaline pH, paraformaldehyde no longer exists. It is converted to formaldehyde, which in water constitutes formalin or formaldehyde solution with cross-linking capacity.[1]

4. Thresholds
    a. Never exceed 65C â€" protocol says do not exceed 60C because temperature adjustment can be slow and its better to target low to avoid crossing the 65C threshold. Never reach or exceed pH 7.4. Above this point the PFA breaks down into molecules that are bad for tissue fixation.
5. Fixation Length
    a. Fixation length has a huge effect on whether an antibody recognizes an epitope. Sensitive proteins need a 5 min fix, some only 15 minutes, and others need over an hour. Epitopes which are exclusive to these fixation length extremes may not be able to be simultaneously labeled.
6. Dedicated Materials
    a. It is recommended to use equipment dedicated to FA prep when possible (EX: stirbar, thermometer, pH electrode).

Equipment

- Heat/stir plate
- Thermometer
- pH meter or pH paper
- stir bar
- Filter apparatus

Consumables and Reagents

- Paraformaldehyde (30525-89-4)
- 0.2 M Phosphate buffer (pH 7.4)[pH 7.4=81% Na2HPO4, 19% NaH2PO4]
- 5 M Sodium hydroxide (NaOH)
- double distilled water (or equivalent)
- filter membrane

References

1. Manoonkitiwongsa et. al. 2002, Proper Nomenclature of formaldehyde and paraformaldehyde fixatives for histochemistry PDF / PUB MED

10X Phosphate Buffered Saline (PBS), 1 liter

Index

Purpose

Instructions

KH2PO4 (M.W. = 136.09) 2.3 g

Na2HPO4 (M.W. = 141.96) 7.4 g

NaCl (M.W. = 58.44) 87.7 g

Add the above reagents to 90% final volume and stir until dissolved. Adjust pH to 7.4 and then dilute to the final volume with distilled water. Mix well before bottling.

For perfusions:

Dilute the 10X stock to 1X and check that the pH is 7.4. Filter the 1X solution through a Whatman #2 filter. Chill the solution to 2-8 C.

Notes

Equipment

Consumables and Reagents

References

20% Triton X-100

Index

Purpose

Instructions

	100 mL	200 mL
Triton X-100	20 mL	40 mL

Warm the triton in a water bath to make it less viscous. Add the triton to 70% total volume distilled water and dissolve at room temperature. Dilute to the final volume with distilled water and mix well before bottling.

Notes

Equipment

Consumables and Reagents

References

293 Cell Media

Index

Purpose

Instructions

1. Perform all work inside of a biological safety cabinet for sterility.
2. Combine the reagents in the quantities specified in the table below and mix well.

Reagent	Volume (mL)
1X Dexter's modified eagle media (DMEM)	440
Fetal bovine serum	50
Sodium pyruvate	5
Penicillin / Streptomycin	5
Total	**500**

3. Filter solution through a sterile 0.22um membrane.
4. Store the media at 2-8C. Warm the media to 37C before use.

Notes

Equipment

1. Stirplate and stirbar
2. Biological safety cabinet
3. Pipet-Man and serological pipettes
4. mixing vessel (sterile 500mL bottle or sterile 600mL beaker)
5. 500mL graduated cylinder or 250mL graduated cylinder

Consumables and Reagents

1. 1X DMEM (Dexter's Modified Eagle Media High Glucose with Glutamax, stored at 2-8C)
2. Fetal bovine serum (Heat inactivated Fetal Bovine Serum, Vendor: Invitrogen Product Number: 1600-044, stored at -20C) or equivalent
3. Sodium pyruvate (100mM / 100x, stored at 2-8C)
4. Penicillin / Streptomycin (100 units/mL, Product Number: VMBMS GB130, stored at -20C)

References

Bovine Lacto Transfer Technique Optimizer (BLOTTO)

Index

Purpose

For blocking membranes for use in western blotting.

Instructions

Notes:

- It will take at least 30 minutes of stirring to dissolve the milk powder.

	1 liter	2 liters	4 liters
1M TRIS, pH 8	20 mL	40 mL	80 mL
3M NaCl	50 mL	100 mL	200 mL
Non-fat dry milk powder	40 g	80 g	160 g

Combine the ingredients in an appropriate sized beaker in 60% total volume distilled water and stir until dissolved. Dilute to the final volume with distilled water and mix well before bottling.

Notes

Equipment

Consumables and Reagents

References

Sucrose in 0.1 M Phosphate buffer

Index

Purpose

Instructions

20% Sucrose

	100 mL	200 mL	300 mL
Sucrose	20 g	40 g	60 g
0.2 M PB, pH 7.4	50 mL	100 mL	150 mL

Dissolve the sucrose in the of 0.2 M PB, pH 7.4. Dilute to the final volume with distilled water and mix well before bottling.

Alternate preparation:

Dissolve and dilute the sucrose in 0.1 M PB, pH 7.4 instead of buffer and distilled water.

30% Sucrose

	100 mL	200 mL	300 mL
Sucrose	30 g	60 g	90 g
0.2 M PB, pH 7.4	50 mL	100 mL	150 mL

Dissolve the sucrose in the of 0.2 M PB, pH 7.4. Dilute to the final volume with distilled water and mix well before bottling.

Alternate preparation:

Dissolve and dilute the sucrose in 0.1 M PB, pH 7.4 instead of buffer and distilled water.

Notes

Equipment

Consumables and Reagents

References

TNB Blocking Solution

Contents

Purpose

To prepare a solution which is used to prevent non-specific binding of antibodies.

Instructions

1. Combine 40 mL of 1 M TRIS buffer, pH 7.5 and 12 mL of 5 M sodium chloride.
2. Dilute to ~ 300 mL with Milli-Q water or equivalent and mix well.
3. Adjust pH to 7.50.
4. Dilute to 400 mL with Milli-Q water or equivalent and mix well.
5. Transfer solution into a bottle (including the stir bar).
6. Heat to approximately 55C in a water bath.
7. Aliquot out 10-30 mL of solution.
8. Add 2 g Perkin-Elmer Blocking Powder Reagent to the bottle of solution.
9. Use all of the 10-30 mL of solution aliquoted in the previous step to rinse all of the powder into the solution.
10. Stir at ambient for about 1.5 hours.
11. Aliquot into nine 50 mL tubes and store at -20 °C until needed.

Notes

- It is easy to mistakenly dilute the solution to 500 mL instead of 400 mL. If this is a familiar plight, use a piece of lab tape to mark the 400 mL line to help remind oneself to avoid this mistake.

Equipment

Consumables and Reagents

1. Milli-Q Water or equivalent
2. Perkin-Elmer Blocking Powder Reagent
3. 5M Sodium Chloride (NaCl)
4. 1M Tris Buffer, pH 7.5

References

SDS-PAGE

- SDS-PAGE Gel Buffers
- SDS-PAGE Reducing sample buffer (RSB)
- SDS-PAGE resolving gel recipe
- SDS-PAGE Running Buffer
- SDS-PAGE stacking gel recipe

SDS-PAGE Gel Buffers

Index

Purpose

Instructions

Resolving Gel Buffer, pH 8.9, 125 mL

1 M HCl – 48mL (Alternative; Use 4.1 mL if using 11.6 M HCl)

TRIS base -- 36.3 g

Add HCl to ~40 mL of distilled water and stir until dissolved. Add TRIS and stir until dissolved. Adjust pH to 8.9 if necessary. Dilute to the final volume with distilled water and stir until dissolved.

Stacking Gel Buffer, pH 6.9, 100 mL

1 M Phosphoric Acid – 25.6 mL (Alternative; Use 1.74 mL if using 14.7 M phosphoric acid)

TRIS base 5.7 g

Add Phosphoric acid to ~50 mL of distilled water and stir until dissolved. Add TRIS and stir until dissolved. Adjust pH to 6.9 if necessary. Dilute to the final volume with distilled water and stir until dissolved.

Notes

Equipment

Consumables and Reagents

References

SDS-PAGE Reducing sample buffer (RSB)

Index

Purpose

Instructions

	1X stock (mL)	2X stock (mL)	
Stacking gel buffer	5	10	
Glycerol	4	8	
1% Bromophenol blue, pH 8.0	0.8	1.6	
10% SDS	8	16	
Mercaptoethanol	2	4	
Distilled water	20.2	0.4	

Combine ingredients and mix well.

Notes

Equipment

Consumables and Reagents

References

SDS-PAGE resolving gel recipe

Total volume (mL)	Percent gel (%)	Distilled H 2O	30% Acrylamide Stock (mL)	Resolving Gel Buffer (mL)	10% SDS (uL)	TEMED	10% Ammonium persulfate (uL)
10	5	6.3	1.7	1.9	100	5	100
10	6	6	2	1.9	100	5	100
10	7	5.9	2.1	1.9	100	5	100
10	7.5	5.5	2.5	1.9	100	5	100
10	9	5	3	1.9	100	5	100
10	10	4.6	3.4	1.9	100	5	100
10	12	4	4	1.9	100	5	100
10	15	3	5	1.9	100	5	100
20	5	12.6	3.4	3.8	200	10	200
20	6	12	4	3.8	200	10	200
20	7	11.8	4.2	3.8	200	10	200
20	7.5	11	5	3.8	200	10	200
20	9	10	6	3.8	200	10	200
20	10	9.2	6.8	3.8	200	10	200
20	12	8	8	3.8	200	10	200
20	15	6	10	3.8	200	10	200
30	5	19	5	5.7	300	15	300

30	6	18	6	5.7	300	15	300
30	7	17	7	5.7	300	15	300
30	7.5	16.5	7.5	5.7	300	15	300
30	9	15	9	5.7	300	15	300
30	10	14	10	5.7	300	15	300
30	12	12	12	5.7	300	15	300
30	15	9	15	5.7	300	15	300

Notes

References

SDS-PAGE Running Buffer

Index

Purpose

Instructions

5X Running buffer

	2 liter	4 liter	20 liter
TRIS base	60 g	120 g	600 g
Glycine	288 g	576 g	28880 g

Dissolve the chemicals in 60% final volume and stir until dissolved. Dilute to the final volume and stir until dissolved.

1X Running buffer

5X Electrode buffer	400 mL
10% SDS	20 mL
Distilled water	1580 mL

Combine electrode buffer and SDS solution with 60% final volume and mix well. Dilute to the final volume and and stir until dissolved.

Notes

Equipment

Consumables and Reagents

References

SDS-PAGE stacking gel recipe

Index

Total volume (mL)	Percent gel (%)	Distilled H 2O	30% Acrylamide Stock (mL)	Resolving Gel Buffer (mL)	10% SDS (uL)	TEMED	10% Ammonium persulfate (uL)
5	4	3.5	0.66	0.625	50	2.5	50
10	4	7	1.33	1.25	100	5	100
15	4	10.5	2	1.875	150	7.5	150

Notes

References

Stereotaxic injection of vectors or molecules into the mouse brain

Index

Purpose

To introduce vectors and molecules into specific regions of the brain in a repeatable fashion.

Instructions

1. Prepare surgical theater
2. Prepare vector injection needle.
    a. Put blunt end of injection needle into mineral oil and allow needle to fill via capillary action.
        i. keep needle vertical (pointy side up)
        ii. Fill needle ½ to ¾ of the way
    b. Dip the tip of the plunger into silicone lube.
    c. Insert the plunger into the blunt end of the needle.
    d. Remove all air from needle.
    e. Put pointy side down
    f. Position the needle into the stereotaxic rig.
3. Weigh mouse and calculate the quantities of Avertin and Buprenorphine required.
4. Anesthetize mouse with Avertin.
    a. Ensure mouse is fully under by using toe pinch technique on both feet.
5. Administer buprenorphine
    a. Buprenorphine and avertin can be administered simulatenously.
6. Administer lidocaine to the area where surgery is to be performed (surgical area)
7. Shave away the hair from the surgical area
8. Mount the mouse to the stereotaxic rig with the ear bars.
9. Apply eye lube.
10. Clean the surgical area with alcohol
11. Apply betadine / iodine solution to surgical area
12. Monitor breathing rate of mouse to ensure it is amply anesthetized but not dead
13. Make an incision in between the two ears by pulling up on the skin with forceps and making a small cut (along the midline) with scissors.
14. Lengthen the incision along the animal's midline enough to expose the top of the skull.
15. Hold the tissue apart using ear clips.
16. Use a sterile cotton tipped applicator and rub the top of the skull to remove any connective tissue that would be in the way.
17. Use the surgical microscope and position the injection needle at bregma
    a. Zero the x, y, and z coordinates to bregma
18. Move the injection needle to the x, y position of the future injection.
19. Mark the position on the skull.
20. Move the injection needle out of the way.
21. Use a rotary tool to drill through the skull
    a. Important: Do not push through too quickly as once you get through the skull the drill bit may slip and stab/drill the brain.
    b. Important: Use a sterile cotton tipped applicator and clean the drilled area of blood and skull debris.
22. Draw up your virus/toxin/molecule into the needle.
23. Once again, zero the x, y, and z coordinates to the bergma.
24. Approach the injection site with the injection needle
25. Lower the needle tip to the brain surface, zero the z-axis to this position.
26. Slowly enter the brain with the needle.
27. Slowly inject the vector into the brain
28. Wait two minutes. While waiting, monitor breathing rate of mouse to ensure it is amply anesthetized but not dead
29. Slowly remove the needle from the brain and move it so that it is not in the way of the surgical area.
30. Close up the surgical area using cyanoacrylate (superglue).
    a. Do not put superglue into the hole in the skull.

31. Ensure the mouse is placed somewhere warm and wait for it to awaken.
32. Observe the mouses behavior and check for any warning signs.

Notes

Equipment

1. Stereotaxic apparatus
2. Surgical instruments; sterile
    a. Forceps, curved
        i. Two pairs
            1. Fine
            2. Course
    b. Scissors
    c. Ear clips
        i. To hold tissue apart
    d. Rotary tool and bits
        i. MICRO-DRILL BURR SET
            1. Manufacturer = Cell point scientific
            2. Part number = 60-1000
            3. Tip diameters in kit = 0.6, 0.8, 0.10, 0.12, 1.60 mm
            4. Cost = $26 USD
3. Heat source for anesthetized rodents
4. Recovery cage
5. Dissection microscope
    a. Stereo
6. Balance/scale

Consumables and Reagents

1. Superglue
    a. For the closure of skin
2. Personal protective equipment appropriate for the procedure
3. Surgical documentation / forms
4. Used or applied externally of mouse
    a. 70% ethanol or isopropanol
    b. Betadine / iodine solution
    c. Mouse eye lubricant
        i. For keeping mouse eyes moist.
5. Silicone lubricant
6. Used or applied internally of mouse
    a. 1 mL insulin syringe
    b. 3/10 mL insulin syringe
    c. Injection needle and plunger
        i. Needles are hand pulled and honed by lab members
7. Lidocaine or Bupivicaine
    a. For local anesthesia
8. Avertin
    a. For systemic anesthesia
9. Buprenorphine
    a. Analgesic
10. Mouse
11. Cotton-tipped applicator; sterile
12. Sterile drapes
13. Parafilm
    a. < 4 cm squared
14. Vector / toxin / molecule
    a. ex: virus, tamoxifen.

References

1. Joseph Elsbernd was taught by Cheuk Ka Tong and Cristina Guinto and JE wrote these details down (June 2013).

Tamoxifen Gavaging Protocol

Contents

Purpose

For the administration of tamoxifen to rodents to induce gene expression.

Instructions

1. Heat 5+ ml corn oil in scintillation vial (Falcon tube ok too) at 42Â°C for 30 minutes. Take out tamoxifen vial and let equilibrate at RT.
2. Weigh out 100 mg tamoxifen, protect from light.
3. add to 5 ml hot corn oil, invert a few times, and place in sonicator.
4. Sonicate for 30-45â€™ or until fully dissolved, checking and inverting periodically. Remove foil before placing in sonication bath!
5. Store at 4Â°C wrapped in foil. Sonicate for 5â€™ before each use. Good for one month although it may start to precipitate before that â€“ once tamoxifen precipitates it should not be used further.
6. Gavaging: use Popper disposable gavaging needles. Hold the animal as if for IP injection but head up. Enter the side of the animals mouth (avoids tip being bitten off) and proceed back along the roof of the mouth. Then rotate syringe up pushing the mouse's head back to insert needle down fully into stomach (goes in about halfway down the length of the needle, perhaps more). DOES NOT REQUIRE FORCE, when done properly the animal should be relatively calm. Avoid blocking the windpipe since this will cause agitation. This procedure can also be done under isofluorane anesthesia (for example, on mice with miniosmotic pumps which cannot be scruffed easily).

Notes

1. Tamoxifen is a carcinogen. Avoid body contact and avoid ingestion.
2. For embryonic gavaging of Cre-ERT2 lines, I typically administer 4 mg per 30 g pregnant mouse. However the early embryonic times (prior to E10.5) can be sensitive so I typically give 3 mg or less. Adults can tolerate much higher doses (10 mg). Postnatal survival can be a bit of a problem sometimes so you may try adding bedding or nesting material and putting a 'do-not'disturb' sign on the cage to minimize stress on the mother. Adding an extra female to assist with the domestic duties may also help.

Equipment

1. Sonicator
2. Ear protection (against sound) for use when near a running solicitor
3. Isoflurane machine (optional)

Consumables and Reagents

1. Corn Oil / Sigma / CAT#: C-8267
2. Tamoxifen / Sigma / CAT#: T-5648; see Note [A]
3. Scintillation vial or â€˜Falconâ€™ tube.
4. Gavaging Needles / Fine Science Tools / CAT#: 18061-20
5. Aluminum foil
6. Syringe, disposable

References

1. Revised 5/17/2010 by Rebecca Ihrie of the Alvarez-Buylla lab.
2. The protocol was adapted from procedures provided by the Joyner and Fishell labs.

Transcardial perfusion (mouse)

Index

Purpose

Instructions

Mice are anesthetized and euthanized by an overdose of Avertin (700 mg/kg injected IP) and then checked for lack of a pedal reflex before the procedure begins. An initial incision perpendicular to the midline is made into the peritoneal cavity. Cuts are then made rostrally along the ribcage on each side of the animal to enter the thoracic cavity. Next, the diaphragm is cut away to allow the rib cage to be retracted. A perfusion needle is inserted into the left ventricle of the exposed heart and the right atrium is then cut. Between 15 and 30 mL of 0.9% saline will be pumped through the animal followed by approximately 30 mL of 4% formaldehyde in phosphate buffered saline (PBS). When perfusion is completed, the animal is decapitated and it's brain removed from the skull for further processing.

Notes

Equipment

Consumables and Reagents

References

Vibratome Sectioning

Index

Purpose

1. Vibratomes are used over the other options when cutting samples that can not be frozen (such as live tissue sections) or when trying to avoid the background that is caused by freezing tissue.
2. Vibratomes can not cut thin sections as nicely as the others. Vibratome's lower limit is around 50 microns.

Instructions

1. In brief:
    a. Setup vibratome tray.
    b. Secure brain to metal stage using superglue
    c. Drizzle a layer of 3% agarose over the brain, ensuring it gets into any crevices. Cool.
    d. Secure metal stage into vibratome tray.
    e. Submerge brain with 1X PBS
    f. Begin cutting and collecting brains

Notes

Equipment

1. Vibratome
    a. AAB Lab has a Leica.
2. Vibratome tray
3. Blade
    a. Vibratome blade or ½ of a shaving razor blade
4. Metal stage
    a. Brain is glued to this, then secured into the tray.
5. Forceps
6. Small paint brush
7. Lightsource
8. Small tray
    a. example = lid of a pipette box
    b. For pouring brains out of the tubes they are stored in.

Consumables and Reagents

1. 3% Agarose
    a. Melt in microwave or waterbath
    b. If hot enough to hurt back of your hand, do not use.
2. Superglue
3. Brain(s)
4. 1X PBS
5. 1X PBS with 0.1% azide
6. 24-well plate
7. Transfer pipette

References

Viral Injection Surgery

Index

Purpose

Instructions

Blurb taken from section 12 Vertebrate Animals of our NS grant competitive renewal.

Mice are anesthetized before any surgical incision is made. Adult mice are weighed and anesthesia is induced by an intraperitoneal (IP) injection of tribromoethanol (Avertin, 125-400 mg/kg) and monitored by toe pinch and eyelid reflex. Hair will be shaved with an electric shaver at the incision site. The head is then held through the ears and snout in a stereotaxic apparatus and the skin above the skull is wiped with 70% ethanol. Using sterilized instruments and aseptic technique, the skin is cut along the midline in the rostrocaudal direction to expose the skull. A small window is cut through the skull with a drill. Virus injections are performed with a glass micropipette polished to have a 50 m diameter at the tip. Nanoliter volumes are injected using a hydraulic microdrive. Similar methods of stereotaxic injection are used to deliver retrograde tracers. The skin is sutured and the animal is allowed to recover in a warm box (30ºC). The time for surgery will be minimized to ensure rapid recovery. Mice typically recover within one hour after surgery and by 2 hours are eating and drinking. Following institutional recommendations, post-operative analgesics (Buprenorphine) will be used on adult mice. Newborn mice used for virus injections are anesthetized by hypothermia on ice for 1-2 minutes. The pups are then taped onto a head mold and injected using aseptic technique through the skin and skull with a glass micropipette (50 m diameter at the tip). After 2 minutes, the pipette is retracted and the pups are returned to their mother. Recovery occurs within 1-2 minutes of returning the mice to their cage.

For AraC brain infusions, adult mice will be anesthetized and immobilized on a stereotaxic apparatus, as described above. An AraC solution (2% diluted in 0.9% saline) or saline alone will be infused onto the surface of the brain using a mini-osmotic pump (Alzet, Cupertino, CA, model 1007D; flow rate 0.5 l/hour, 7 day pump). Cannulas will be implanted onto the surface of the brain at anterior 0 mm, lateral 1.1 mm relative to Bregma. This treatment produces no lesion to the ventricular cavities or to the SVZ. Buprenorphine will be injected to minimize post-surgery pain. After 6 days of infusion, mice will be re-anesthetized to remove the pump and allowed to survive for various time intervals, as described in our aims.

Notes

Equipment

Consumables and Reagents

References

Wholemount Staining and Ependymal Flow

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About

Our lab along with the Journal of Visualized Experiments (JOVE) created a video to demonstrate how to perform whole mount dissections of the mouse brain.

Link to the Video

http://www.jove.com/video/1938/the-subventricular-zone-en-face-wholemount-staining-and-ependymal-flow

PDF with details

jove-protocol-1938-the-subventricular-zone-en-face-wholemount-staining-and-ependymal-flow.pdf

References

1. Mirzadeh Z, Doetsch F, Sawamoto K, Wichterle H, Alvarez-Buylla A. The subventricular zone en-face: wholemount staining and ependymal flow. Journal of visualized experiments: JoVE [Internet]. 2010 Jan [cited 2014 Apr 18];(39). Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3144601&tool=pmcentrez&rendertype=abstract PMID: 20461052