PREPARATION OF PRECISION CUT MURINE LUNG SLICES (PCLS)

Created: Janine Strehmel
Adapted from: Dr. Preethi Jayanth and Prof. Luke J. Janssen
Date: 2018
Bowdish Lab, McMaster University
Hamilton, ON, Canada
www.bowdish.ca

NOTES

- This protocol describes the isolation of mouse lungs and the processing in order to obtain *precision cut lung slices* (PCLS) which can be used to study respiratory immune responses

EQUIPMENT

Medium, buffers, solutions:

- 500 mL DMEM (Culture medium):
  - 10 % FBS (50 mL 100 %)
  - 2 mM L-Glutamine (5 mL 200 mM)
  - 5 ml Penicillin (100U/mL) & Streptomycin (100 µg/ml)
  - 2.5 ug/mL Amphotericin B (5 mL of 250 ng/mL stock solution)

- 0.2 M HEPES (pH 7.4)
  - Dilute 1 M HEPES (pH 7.4) in ddH_2O
  - Store at 4 °C

- HBSS buffer (10x HBSS, GIBCO #14065-056; No phenol red or sodium carbonate)
  - **2xHBSS (200 mL)**
    → 40 ml 10x HBSS +140 ml water+ 20 ml 0.2 M HEPES (pH 7.4)
  - **1xHBSS (500 mL)**
    → 50 ml 10x HBSS+400 ml water+ 50 ml 0.2 M HEPES (pH 7.4)
    → adjust the pH to 7.2
    → Filter sterilize through 0.22 µm pore filter

- Low melting agarose solution (UltraPure™ Low Melting Point Agarose; Thermo Fisher # 16520050)
  - Dissolve 4 g of Agarose in 100 ml water (4 % w/v) in microwave
  - Mix 4 % agarose with 2x HBSS at a ratio of 1:1 to obtain 2% agarose
  - Keep warm at 42 °C
  - Store at 4 °C for usage on multiple days

Materials for dissection of mouse lungs:

- Mice
- Isoflurane
- Gloves
- Blue diapers

- Tweezers
- Scissors
- 70 % Ethanol
- String
- Sterile gauze
- Needles to pin animal down (18 gauge)
- Styrofoam to pin animals down
- 1x PBS (for optional lung perfusion)
- 5 ml syringes (for optional lung perfusion)
- 1 ml syringes (Infiltration of agarose into lungs)
- 18 Gauge Blunt Fill Needle (38.1mm (1½") (VWR #CABD305180) – agarose infiltration
- 25 Gauge needles (Lung perfusion)
- 2 % low melting agarose
- Hot plate
- Ice (to keep lungs cool)
- Falcon tubes containing 1x HBBS (Storage of lungs until cutting)
- Biohazard mice disposal bags

Materials for cutting and culturing lung slices:

- Compresstome / Microtome
- Sterile Petri dish
- Sterile Scalpel
- Sterile 24-well plates
- Ice cold 1x HBSS
- 5 mL or 10 mL pipettes
- Pipette aid
- Glue
- Hot plate
- 2 % low melting agarose
- 3 mL plastic transfer pipettes
- Ice (Storage of lung slices until cultivation)
- Brush
- DMEM (Cultivation of PCLSs)

PROTOCOL

1. **Set up:**
    - Place DMEM into 37 °C water bath to warm up
    - Place 1x HBSS on ice
    - Dissolve 4 % or 2 % low melting agarose in microwave
    - Fill 5 mL syringes with 5 mL 1x PBS and connect with capped 25 G needle
    - Place one 50 mL tube containing approximately 10 – 20 mL 1x HBSS for each lung on ice
    - Keep melted agarose warm on hot plate, while stirring

2. **Lung preparation:**

Euthanize mice with isoflurane

- put mice in anaesthesia chamber (oxygen level 2, open isoflurane (level 4)
- Put mouse nose into nose cone to avoid wake up of the mice
- Spray front of the mouse with 70 % EtOH
- Open body
  → cut the aorta (mouse dies) and stop bleeding with gauze
  → Puncture and cut away diaphragm to stop breathing of mice

Perfusion of lungs

- Expose lungs and heart as much as possible by removing surrounding tissue and rip cage (be careful not to puncture / disconnect lungs and heart)
- Stick needle, connected to syringe that contains 5 mL 1x PBS, into right ventricle of heart
- Inject slowly 5 mL of 1x PBS until lungs turn light pink/white
- lungs get white

Agarose infiltration

- Carefully cut away fur and skin around trachea (jaw level) and ensure that you soak up any excess blood because you don't want blood in the airways.
- Locate the trachea and carefully remove covering skin from trachea
- Clear blood away from around trachea with gauze
- Feed suture string under trachea with the help of small tweezer (Do not puncture trachea)
- Make a small incision close to jaw, big enough for a 18 G blunt needle, using scissors
- Stick 18 G blunt-ended needle in trachea (not too far, keep it before the branching point) and then tie a knot around the needle with the suture
- Mix 4 % fluid agarose 1:1 with 2xHBSS solution thoroughly and keep at 42 °C or directly use pre-made 2 % agarose
- Check with the thermometer that it is no more than 40 °C before injecting the solution
- Fill 1 mL syringe with 2 % melted agarose in 1x HBSS solution and connect carefully to blunt ended needle
- Inject 1.3 mL of agarose slowly into the lungs without damaging (watch lung inflation)
- Following the injection of agarose, inject 0.2 mL of air using the same 1 ml syringe
- Place mouse with needle and syringe still in trachea on ice (4 °C), cover lungs with gauze and saturate gauze with chilled 1x HBSS
- Following 15 min on ice remove syringe and needle, tie the black string tight
- Remove lungs from mouse by cutting all connections and place lung in a 50 mL tube containing ice cold 1x HBSS until slicing

3. **Lung Slicing with Compresstome VF200-0Z**

Compresstome set up:

- Ethanol off everything
- Wipe down with water
- Cool down chilling block for 15 min at -20 °C
- Snap blade in half and mount blade with 5 uL of glue onto blade holder
  →Make sure that old glue residues are removed completely before mounting blade, otherwise the blade won't be plane, change blade for every experiment
- Insert specimen tube surrounded by metal tube into buffer tray until little knob and fix micrometer

- Connect blade holder and blade to Compresstome with the blade facing the specimen tube, slightly fix it with Allen wrench
- Bring blade down until it is half way in front of the tube
- Adjust blade by aligning it very close to the tube, but make sure it doesn't touch it, and tighten blade holder

Agarose embedding

- Dissolve 1 low melting agarose tablet in 25 ml H2O (for 2 % agarose) by swirling (see guide)
  → Heat agarose for 30 to 40 sec in microwave and keep it liquid at 42 - 53 °C on stirring plate
- **OR:** Use already melted and warm 2 % agarose
- Separate lobes of lung using a 22-sized scalpel and take the bigger lobes for slicing
- Cut lung lobe in an appropriate sized piece, that has a flat bottom and fits onto specimen tube
- Tap bottom of the piece on Kimwipe to remove excess liquid before mounting
- Remove metal part of specimen tube
- Put a drop of glue in petri dish and use 5 µL to mount the tissue onto the flat side of the specimen tube
- Put specimen tube into the metal ring and elongate with transparent ring
- Fill the transparent ring with 2 % melted agarose using a disposable Pasteur pipette until tissue is covered
- Make sure that there are no air bubbles, remove if needed
- Slowly pull down the specimen tube (white part) until tissue in agarose is completely in the metal tube
- Disconnect transparent ring from metal ring
- Solidify the agarose by putting the ice-cold chilling block around the specimen tube for a few minutes
- Make sure the white specimen tube doesn't fall out of the ring

Slicing the lung

- Put specimen tube into appropriate spot of Compresstome and fix micrometer
- Pour ice cold 1xHBSS into the buffer tray until specimen tube is covered (at least half of it)
- Compresstome settings: Advance: 2.5 - 3 Oscillation: 5
- Set the desired slice thickness (small marks = 10 µm; large marks = 50 µm) – usually used 300 µm
- Cut slices, carefully remove surrounding agarose using tweezers and brush
- Place the PCLSs in wells of a 24-well plate containing approximately 1 mL ice cold 1x HBSS (2 slices per well) – keep on ice until plate is full

4. Cultivation of PCLSs

- Transfer PCLSs into 24-well plates containing 1 mL/well DMEM (10 % FBS, 2 mL L-glutamine, Pen/Strep and AmphothericinB)
- Incubate at 37 °C, 5 % CO_2
- Change medium with a pipette every hour for the first 3 hours after putting lungs in culture medium
- Change medium every 24 hours – 48 h thereafter
- PCLSs can be cultured and used for functional assay for 1 to 2 weeks after slicing
- **From 1 big lung lobe approximately 24 PCLSs (300 µm thick) can be obtained**

POSSIBLE IMMUNOASSAYS

- ELISA following specific treatments
- Viability testing
- Gene expression using qPCR
- Antimicrobial properties