Mouse Post-bleomycin Cell Transplantation Protocol – Kotton Laboratory

INTRO
This protocol describes the methods to transplant cells post bleomycin injury in order to achieve alveolar engraftment. This protocol was published in Cell Stem Cell as part of Herriges et al., 2023 (https://www.cell.com/cell-stem-cell/fulltext/S1934-5909(23)00283-7)

REAGENTS

- Donor cells of interest in 100ul of FACS buffer (2% FBS in PBS) or media
- Syngeneic or NSG (https://www.jax.org/strain/005557) mice
- Fenestrated forceps (ROBOZ, RS-5160) remove the screw
- Isoflurane
- Isoflurane chamber with oxygen
- Bleomycin (Sigma Aldrich, B8416) dissolved in sterile 0.9% NaCl at 1.5units/ml (Aliquots of 500ul stored at -20°C)*
- Stage for suspending mice**

PROTOCOL

1. Lung Injury
   a. Thaw an aliquot of bleomycin and dilute it with an equal amount of sterile 0.9% NaCl. Vortex to mix thoroughly. Using a p200 pipette prepare 20ul/(g mouse weight) of the diluted bleomycin.
   b. Place the recipient mouse in an isoflurane chamber with 3% O₂ and 3% Isoflurane. Once the mouse has stopped moving about the chamber, reposition the mouse on its side in the chamber so that it is easier to monitor respiration.
   c. Keep the mouse in the chamber until it is display agonal breathing (deep gasping that moves the whole chest) at a rate of 20 breaths/minute.
   d. Quickly remove the mouse from the chamber and place hang it on the stage as pictured below. The mouse should be hanging by its upper incisors with its back to the stage.
   e. Using the fenestrated forceps in your off hand, grab the mouse’s tongue and pull it up and towards you such that the lower jaw is fully open towards you.
   f. While holding the tongue out of the mouth, use the p200 pipette administer the bleomycin to the opened mouth of the mouse, aiming for the back of the throat. Once you are done pipetting, continue holding the tongue out and use your main hand to lightly cover the mouse’s nose. You should initially see the liquid pool in the mouth, but it will be pulled in as the mouse’s agonal breathing continues. Liquid should disappear after 3-4 breaths. Once the liquid is down, you can let go of the tongue, but continue to plug the nose for another 30 seconds. You should hear popping noises from the mouse as liquid is taken into the lungs. Leave the mouse on the stage until it starts to awaken from the anesthesia, at which point you can put it back into its cage for recovery.

2. Cell transplantation (3 days post injury for NSG mice, 10 days post injury for immunocompetent mice)
   a. Digest and/or sort purify donor cells of interest prior to transplantation.
b. Resuspend your cells as a single cell suspension in their standard media and incubate them for 2-3 hours at 37°C.
c. Count cells and resuspend desired cell counts in 100ul of FACS buffer or media.
d. Deliver the cells orally, using the same protocol described under the above Lung Injury section

**COMMENTS**

*: There is a lot of batch-to-batch variability with bleomycin. The concentrations described here are a good starting point for any batch, but you may find you will need to increase or decrease the concentrations/volumes you use. 10-20% weight loss by 10 days post injury is promising, but not necessary for a good injury. Ultimately, histological assessment of the injury and fate of transplanted cells is the best way to determine if you are getting insufficient or excessive injury. In addition to altering dosage, I have seen some batches for which transplantation at 7 days post injury is more effective.

**: This stage can be purchased from Kent Scientific (https://www.kentscientific.com/products/intubation-stands/). Alternatively, a propped up Styrofoam board with suture looped around two needles can provide a more affordable and flexible option. See picture shown to the right.
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