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Intra-cardiac mouse perfusion

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Protocol status: Working

We use this protocol and it's working

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Abstract

Hnasko lab intra-cardiac mouse perfusion protocol



Prepare subject

- 1 Anesthetize with a lethal dose of pentobarbital (200 mg/kg i.p. or 0.1-0.2 ml at 10 mL/kg in 0.9% NaCl).
- 2 After 2-3min, verify that the animal is completely anesthetized by checking for absence of response to hind paws / tip of tail pinch using nails or small tweezers.
- 3 Once fully anesthetized, affix the animal to a silicone dissection pad (dissection pins through paws) in a splay position with ventral side up. Place silicone pad on a grid panel over a container for blood and PFA collection. Tilt the grid/pad slightly so that blood/PFA flows backward off the pad into the container. Work inside a chemical fume hood to prevent PFA inhalation.

Procedure

2h 7m

- 4 Cut skin transversally at the base of the thorax.
- 5 Then cut muscle transversally at the same level.
- 6 Slightly lift the thorax by pulling on the xiphoid process (white tip of the thoracic body). Do not lift too much to not damage inner organs.
- 7 Cut the diaphragm with fine scissors (Be careful to not puncture the heart or lungs at this step).
- 8 Cut the thorax laterally on both sides (Be careful to not puncture the heart or lungs at this step too), pull backward, and fix thorax to pad with a needle on the left side of the animal.
- 9 Clear heart from connective tissues.
- 10 Place butterfly needle (connected to perfusion pump – prefilled with ice cold 1X PBS) in the tip of the left ventricle (darker and longer side of the heart – prominent tip). Be careful to not puncture through the right ventricle or the left atrium by going too deep. Cut the right atrium (darker atrium) to allow blood/perfusate to escape circulation.



- 11 Clamp the butterfly needle in place using a hemostat (or hold in place and clamp in next step).
- 12 Start the pump using a flow rate of 5-6 mL /min. Perfuse with ~ 10 mL of ice-cold 1X PBS (~ 00:02:00). 2m
- 13 Blood should come out of the right atrium with a regular flow (If you see clear liquid right away, you might have punctured through the heart – can try to slightly pull back the needle, but you may have a failed perfusion). Clamp the butterfly needle in place if you did not in previous step.
- 14 When blood has been depleted (~2min) switch to ice-cold freshly made 4% paraformaldehyde (PFA) 7.4 for 00:05:00 to 00:08:00 (30-50 mL). 13m
- 15 Remove brain or other tissue of interest.
- 16 Post-fix tissue submerged in 4% ice-cold PFA for 02:00:00 , Overnight , or as appropriate for intended use. 2h