Sep 18, 2024

Transcardial Perfusion in Mice for **S**ynaptic **E**valuation and **QU**antification by **I**maging **N**anostructure (SEQUIN) Analysis

Forked from Transcardial Perfusion in Mouse

DOI
dx.doi.org/10.17504/protocols.io.36wgqnk85gk5/v1

Saroj Kumar Sah[1], Thomas Biederer[1]

[1]Yale University

Saroj Kumar Sah
Yale University

OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.36wgqnk85gk5/v1

Protocol Citation: Saroj Kumar Sah, Thomas Biederer 2024. Transcardial Perfusion in Mice for Synaptic Evaluation and QUantification by Imaging Nanostructure (SEQUIN) Analysis. **protocols.io https://dx.doi.org/10.17504/protocols.io.36wgqnk85gk5/v1**

Protocol status: Working
We use this protocol and it's working

Created: September 14, 2024

Last Modified: September 18, 2024

Protocol Integer ID: 107618

Keywords: Transcardial Perfusion, Perfusion Solutions, SEQUIN

Funders Acknowledgement:
Aligning Science Across Parkinson's
Grant ID: ASAP-020616

Abstract

This protocol details the method for transcardial perfusion in mice for **S**ynaptic **E**valuation and **QU**antification by **I**maging **N**anostructure (SEQUIN) Analysis. This transcardial perfusion can also be used for immunohistochemical analysis of p-Syn staining along with synaptic proteins.

Guidelines

A record of mouse anesthesia and surgery procedures, drugs used and any leftovers should be maintained, and any leftovers should be stored/disposed of according to the Animal Care and Use Committee (IACUC) and Environmental Health & Safety guidelines.

Materials

Materials Needed:

- stack Styrofoam
- Labmat (to absorb any Paraformaldehyde/buffer spills)
- 27G needle
- Perfusion pump
- 1ml 1cc Syringe

Chemicals:
4% PFA in 1x PBS
1x PBS
NaOH
HCL

Drugs:
Ketamine
Xylaxine
Normal Saline
70% Ethanol

Safety warnings

Paraformaldehyde is toxic, so precautions should be taken to avoid direct contact or inhalation of paraformaldehyde powder or solution.

Ethics statement

All animal procedures undertaken in this method were approved by the Yale University Institutional Animal Care & Use Committee and complied with the National Institutes of Health guidelines for the care and use of laboratory animals.

Before start

Before starting perfusion, a lab mat should be placed inside the chemical hood to absorb any spills. An anesthetic drug cocktail should be prepared based on the desired concentration and volume sufficient for the number of mice.

Transcardial Perfusion

1 Make a mixture of ketamine (100 mg/kg) and xylazine (10 mg/kg), sufficient to anesthetize all mice.

2 Administer anesthesia to one mouse via intraperitoneal injection and place it in a clean cage until the anesthesia takes effect. Perform a firm toe pinch to ensure the mouse does not react, confirming the absence of pain sensation before proceeding.

3 Place the mouse, abdomen up, on a stack of Styrofoam and make it immovable by pinching the toes with a 27-gauge needle through the Styrofoam. Spray the abdomen with 70% ethanol. Using forceps, grasp the skin below the ribcage and cut upward with scissors, cutting through the ribcage to expose the heart.

> **Note**
>
> Avoid blood vessels and organs. Diaphragm should carefully be cut circumferentially.

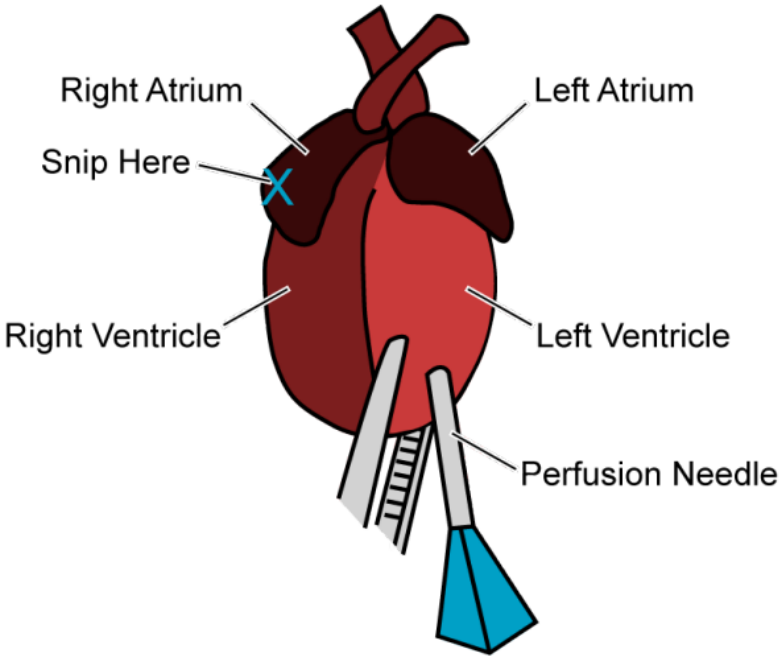
4 Remove pericardium and peripheral fat to expose heart. Hold back ribs with hemostats.

5 Insert a blunted 27G needle tip into the left ventricle and inject ice-cold 1x phosphate-buffered saline (PBS) for 00:04:00, followed by ice-cold 4% paraformaldehyde (PFA) in PBS pH 7.6 for 00:04:00 at a rate of 6 mL/min using a peristaltic pump. 8m

> **Note**
>
> Liver should lose pigment and effluent should be dark blood then become more PBS as perfusion continues.

6 After perfusion is complete, carefully remove brain using scissors and transfer to 4% PFA. Store the brain in 4% PFA at 4 °C for 12 to 18 h. Rinse the fixed brains in 1x PBS and cut coronally into 40 to 50 µm-thick slices using a vibratome (Leica VT1000 S) for SEQUIN.

Perfusion Schematic



Perfusion Solutions

7 Weigh PFA under the hood.

8 Heat 1x PBS, transfer PFA into the 1x PBS, and stir (inside chemical hood) to make 4% PFA.

9 Let dissolve the PFA around 60 °C (use the thermometer). Do not let the temperature exceed 65 °C (remove from hot plate temporarily if necessary).

10 Add NaOH to make the PFA dissolved (it should make the PFA clear). Let the PFA cool down to Room temperature . Add HCL to make the pH 7.6 .

11 Filter the 4% PFA into a bottle/w lid.

12 Store the PFA covered at 4 °C and use it for perfusion within 12 h.