# Intracardial perfusion of the African turquoise killifish

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This perfusion protocol is essential for preserving tissue morphology in order to perform good quality immunohistochemical stainings. Here, we show you how we perform our perfusions on the African turquoise killifish.

This protocol was already used in the following publications:
Aging impairs the essential contributions of non-glial progenitors to neurorepair in the dorsal telencephalon of the Killifish Nothobranchius furzeri - PubMed (nih.gov)
Single-cell sequencing of the adult killifish (N. furzeri) brain identifies an atypical progenitor, glial and neuronal heterogeneity | bioRxiv

DOI

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Valerie Mariën, Jolien Van houcke, Lutgarde Arckens 2022. Intracardial perfusion of the African turquoise killifish. **protocols.io** https://dx.doi.org/10.17504/protocols.io.b2ryqd7w

protocol

Van houcke, J. et al. (2021) 'Aging impairs the essential contributions of non-glial progenitors to neurorepair in the dorsal telencephalon of the Killifish Nothobranchius furzeri', Aging Cell. Available at: https://doi.org/10.1111/acel.13464.

Perfusion, fish, killifish, N. furzeri, Nothobranchius furzeri

protocol ,



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MATERIALS
0.1 % Tricaine in reverse osmosis water (MS-222, Sigma-Aldrich)
4 % Paraformaldehyde (PFA) in PBS (Sigma-Aldrich)
70 % EtOH solution
24-well plate
2 x 10 ml Syringe (VWR)
1.5 ml Pasteur pipette cut open to use as a scoop (VWR)
BD Microlance 20 G Needles (VWR)
Dumont #5 Forceps (Fine Science Tools - Item No. 11251-20)
Fishing Net
Hematocrit Capillaries (Hirschmann - 75µl)
Leica S9i Stereomicroscope
Capillary puller (Sutter instrument)
Phosphate buffered Saline 1X (PBS) - pH 7.5 - 0.1 M
Paraffin tray
Terumo Needle Surflo Winged infusion set (21G X 3/4 - VWR)
Vannas Spring Scissors - 2.5 mm Cutting Edge (Fine Science Tools - Item No. 15000-08)
Vannas Spring Scissors - 4 mm Cutting Edge (Fine Science Tools - Item No. 15018-10)

SAFETY WARNINGS
Wear gloves, a lab coat, safety goggles, and a disposable dust mask while handling PFA. Make sure to perfuse underneath a fume hood while wearing the dust mask to protect yourself and others. Carefully handle all sharp materials, throw away needles in a needle container.

## Preparation

1h

1 Prepare 0.1% tricaine solution in reverse osmosis water.
Prepare 4% paraformaldehyde (PFA) solution in PBS.
Disinfect all dissection material with 70 % EtOH.
Cut the needle off the Terumo Needle Surflo Winged infusion set.



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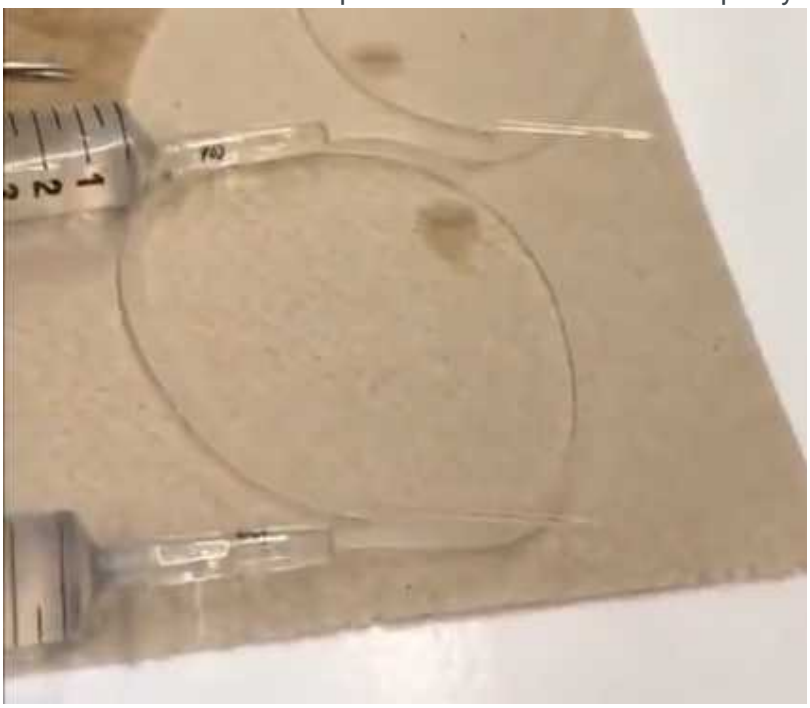
10m

2 Fill one of the 10 ml syringes with PBS and the other one with 4% PFA.
Attach the Terumo Surflo tube onto the syringe and fill the tube.
Open the tip of the capillary with forceps and attach the capillary onto the Terumo Surflo tube.
Push out the fluid until a drop is visible at the end of the capillary.



Capillary attached to Terumo Surflo, attached to 10 ml syringe.



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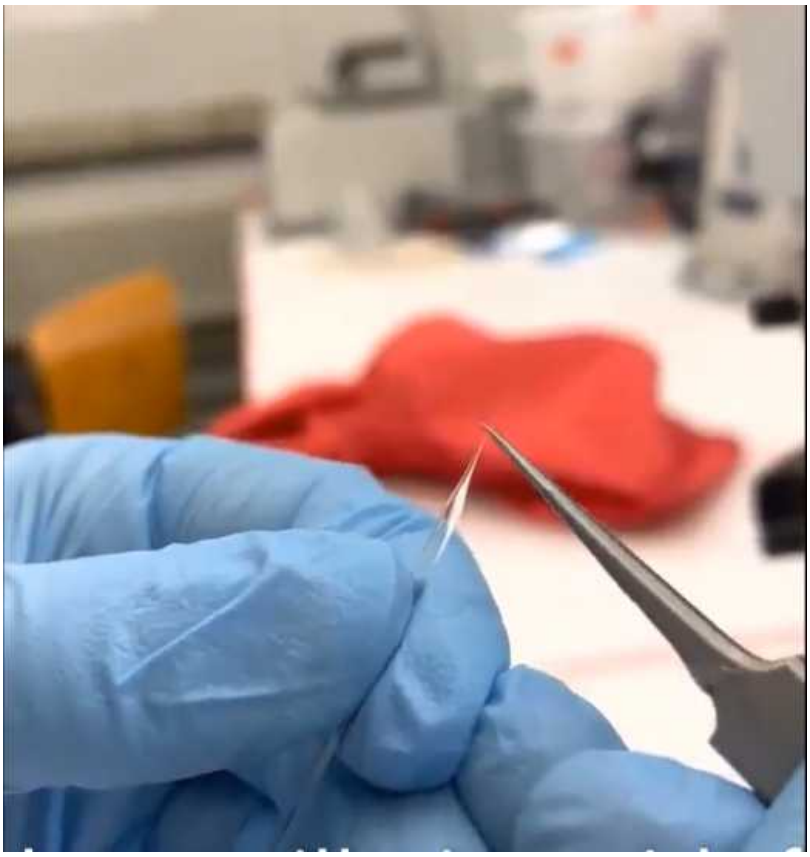

Open the capillary with forceps to create a small cavity.

3 Euthanize a fish in an overdose (0.1% ) Tricaine solution. After 10 minutes, the fish is euthanized. 10m

Start dissection 5m

4 When the fish is euthanized, place the fish on the paraffin tray with its belly pointing up. 5m
Hold the head with forceps while gently making a T-shaped incision. Start cutting from the cloaca to the left and right dorsal sides.
Be careful not to cut into any organs!
Start cutting towards the head from the opening that was just made parallel to the body.
These steps can be carried out by using the bigger scissors (Item No. 15018-10).
Pin down the loose parts on the sides with pins or needles.
Use the small scissors to cut up till the gills in order to expose the chest cavity. Also pin down the sides close to the head. The heart is now visible and contracting.



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## Start Perfusion 11m

11m

5 Cut in the atrium to allow blood to flow out.
Insert the capillary connected to the syringe containing PBS in the ventricle of the heart and gently push PBS through the heart and into the vascular system. You can see that the ventricle will swell up. The liver will become whiter as blood is flowing out the atrium of the heart.
When the liver is white, most of the blood is removed from the body (approx. 5 minutes of flushing with PBS). Then, insert the capillary connected to the syringe containing PFA into the ventricle of the heart and let the PFA flow through the vascular system for another 4-5 minutes.

6 The fish is now perfused and you can collect your tissue of interest for further processing. For example collect the brain and let it post-fix overnight in a 4% PFA solution at 4°C in a 24 well-plate.



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