

FEB 07, 2024

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External link:

https://doi.org/10.1371/journal.pg en.1010802

Protocol Citation: Amanda A. G. Ferreira, Bogdan Sieriebriennikov, Hunter Whitbeck 2024. HCR RNA-FISH protocol for the whole-mount brains of Drosophila melanogaster. **protocols.io**

https://protocols.io/view/hcr-rnafish-protocol-for-the-wholemount-brains-o-cx26xqhe

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Protocol status: In development An alternative version of dx.doi.org/10.17504/protocols.io. bzh5p386 aiming to use less reagent.

HCR RNA-FISH protocol for the whole-mount brains of Drosophila melanogaster

Forked from HCR RNA-FISH protocol for the whole-mount brains of Drosophila and other insects

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ABSTRACT

This is a protocol to perform RNA fluorescent in situ hybridization (RNA-FISH) using hybridization chain reaction (HCR) on whole-mount samples of the brains of the fly *Drosophila melanogaster* and other insects, e.g. the jumping ant *Harpegnathos saltator*. Probes and HCR reagents are purchased from Molecular Instruments. This protocol is loosely based on the "generic sample in solution" protocol published by Molecular Instruments. Our modifications include the description of fixation conditions, counterstaining by Hoechst, and altered washes. Additionally, we use larger concentrations of probes and hairpins following the protocol described by Younger, Herre et al. 2020. We have successfully employed this protocol to stain insect brains with up to 4 different probe sets simultaneously (hairpins conjugated with Alexa Fluor 488, 546, 496, and 647).

CITATION

Meg A. Younger, Margaret Herre, Alison R. Ehrlich, Zhongyan Gong, Zachary N. Gilbert, Saher Rahiel, Benjamin J. Matthews, Leslie B. Vosshall (2020). Non-canonical odor coding ensures unbreakable mosquito attraction to humans. bioRxiv.

LINK

https://doi.org/10.1101/2020.11.07.368720



Created: Aug 01, 2023

Last Modified: Feb 07, 2024

PROTOCOL integer ID: 85822

Keywords: brain, drosophila, ant, hcr. in situ. larva. rna fish

GUIDELINES

WORKING PRACTICES:

Prepare all buffers using nuclease-free water. Use filter tips and nuclease-free tubes. If using spot plates, pre-clean them first with household bleach diluted 1:10 in water and then with 70% ethanol. Wear gloves and adhere to other practices aimed at minimizing RNA degradation in the sample. Working in a clean bench is not required if other RNAsefree practice are followed.

PROBE DESIGN:

We select the target sequence or isoform of the gene of interest and let Molecular Instruments design the probes. For genes with multiple isoforms, either target the isoform that includes as many as possible constitutive exons and as few as possible alternatively spliced exons, or the isoform that has the highest RNA-seg coverage (assessed visually in IGV) if RNA-seq data are available. Aim for the highest number of probes in a set, ideally 40, although we have successfully performed experiments with probe sets containing <20 probes.

AMPLIFIER CHOICE:

We routinely perform multiplexed stainings with up to 4 different probe sets and a Hoechst counterstain. We use amplifiers conjugated with Alexa Fluor 488, 546, 594, and 647. We are able to detect clearly distinguishable signals with minimal bleed-through on our confocal microscope (Leica SP8). However, be aware that simultaneously using fluorophores with partially overlapping spectra (e.g. AF 546 and 594) requires setting narrower detection ranges, which reduces the amount of signal detected.

MATERIALS

REAGENTS TO PURCHASE:

- Nuclease-Free Water (not DEPC-Treated) **Thermo Fisher**Scientific Catalog #AM9937
- 20X PBS (Phosphate Buffered Saline) pH 7.4 growcells.com Catalog #MRGF-6396
- Schneider's Drosophila Medium Thermo Fisher Catalog #21720024
- Paraformaldehyde, 16% (wt/vol) Electron Microscopy Sciences Catalog #15710

Safety information

Paraformaldehyde is toxic, consult the SDS sheet for proper handling instructions

Note

Avoid long-term storage of the paraformaldehyde solution after opening the ampoule

- 20 10% Tween 20 BIO-RAD Catalog #1662404
- 20X SSC Quality Biological Catalog #351-003-131
- SlowFade™ Gold Antifade Mountant Invitrogen Thermo Fisher Catalog #S36936

or any other antifade mountant

- Hoechst 33258, Pentahydrate (bis-Benzimide), 100 mg **Thermo**Fisher Catalog #H1398
- dissolve in DMSO to 5 mg / mL, aliquot and store at -20 °C
- Methanol Fisher Scientific Catalog #A412-4
- X HCR Probe Hybridization Buffer Molecular Instruments
- X HCR Probe Wash Buffer Molecular Instruments

Safety information

Hybridization and Wash Buffers contain formamide, consult the SDS sheet for proper handling instructions

X HCR Amplification Buffer Molecular Instruments

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Buffer to prepare:

Note

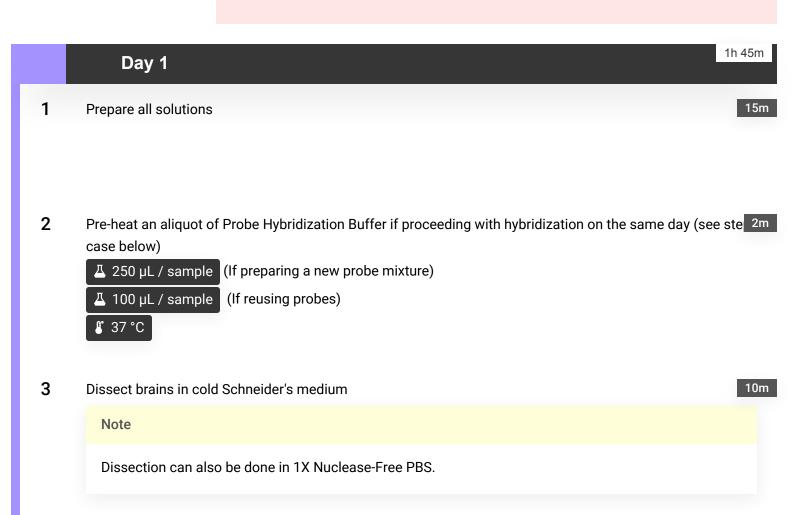
Prepare fresh using nuclease-free water, store at 4 °C if required after the 1st day of the protocol.

- 1% PBTx (1X PBS with 1% v/v Triton X-100 and 1mM glycine)
- 5X SSCT (5X SSC with 0.1% v/v Tween-20)
- 1X PBS

Fix brains in 800 µL of 4% PFA / Schneider's medium

SAFETY WARNINGS

This protocol uses solutions of paraformaldehyde and formamide, which are highly toxic chemicals. Consult the SDS sheets of the reagents used in this protocol for proper handling instructions.



20m



Note

For fixation and all subsequent steps, samples can be placed either in Eppendorf tubes or in wells of a spot plate (e.g. Pyrex spot plate with 9 depressions, Catalog #CLS722085). Tubes are incubated on a nutator and plates are incubated on an orbital (horizontal) shaker.

Finse 3x with 150 μL of 1% PBTx

3m

Room temperature

6 Wash 3x 15 min with 150 μL of 1% PBTx

45m

- **(?)** 00:15:00
- **(:)** 00:15:00
- 00:15:00
- Room temperature
- (5) 24 rpm Nutator or (5) 60 rpm Orbital shaker

7 Pre-hybridize samples by incubating them with 100 μL of warm Probe Hybridization Buffer from step 2

10m



(5) 00:10:00 can be extended to 30 min

37 °C 37 °

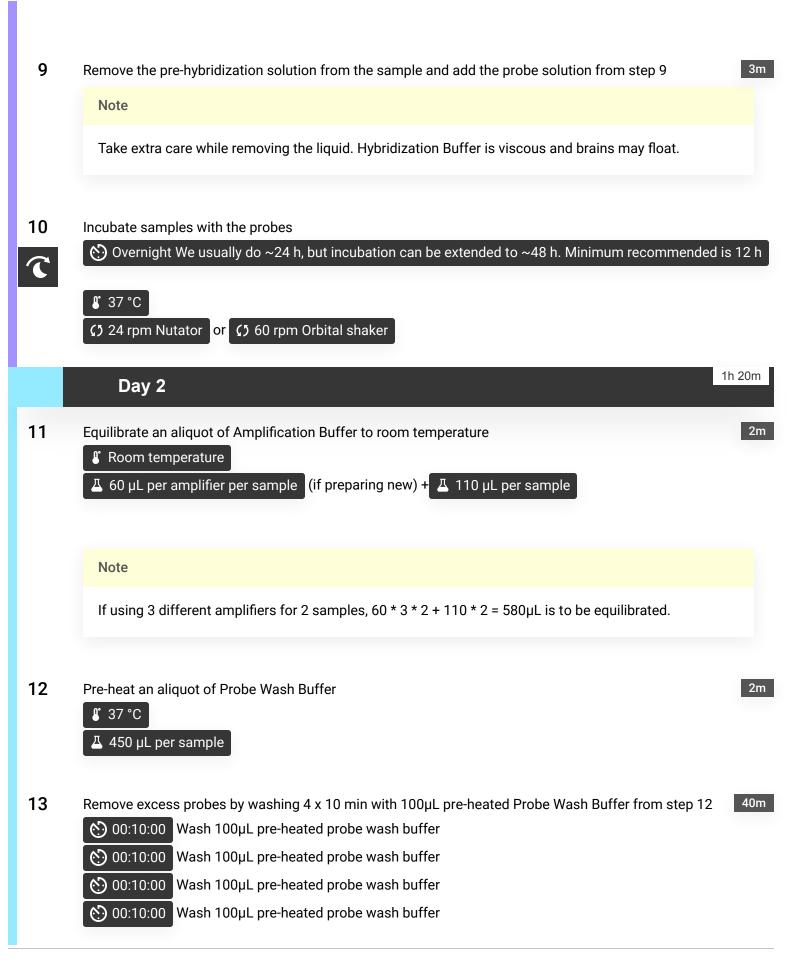
Note

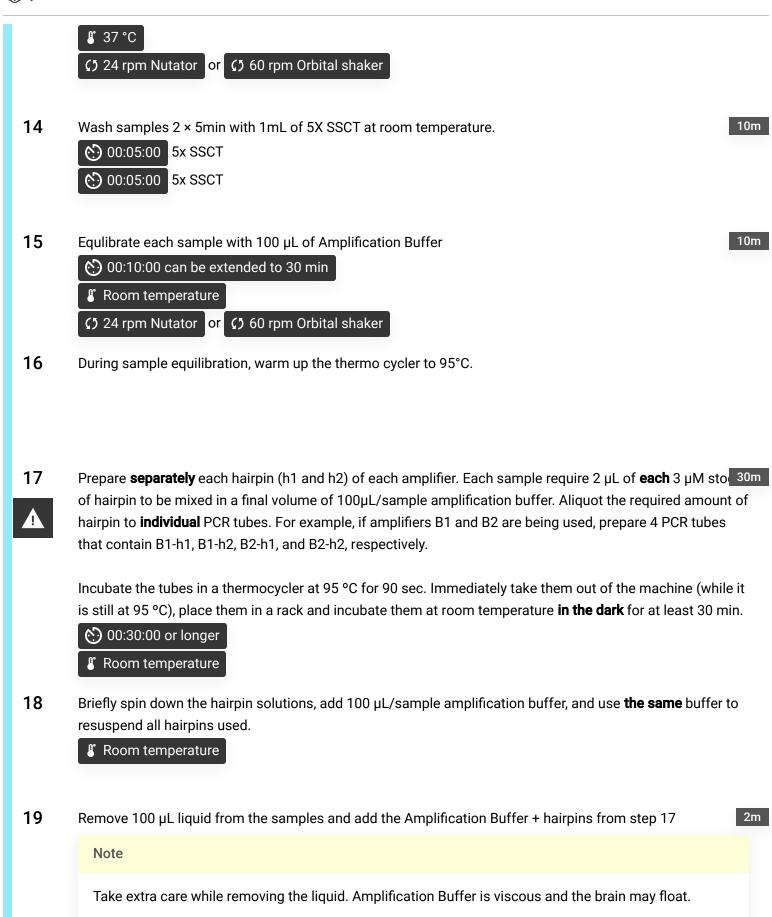
Hybridization buffer is viscous, and the brains might be stuck on the wall. Be careful not to remove them or let them dry out by accident.

In the meantime, prepare a 8nM probe solution by adding 0.4 pmol of each probe mixture (e.g. $0.4 \mu L$ of 1 μM stock) to the warm Probe Hybridization Buffer for the total volume of 100 μL .

 Δ 0.4 µL 1µM probe per sample

 Δ 100 µL Warm probe hybridization buffer per sample





If using DAPI: incubate samples with 150 μ L of SSCT + DAPI (1:1000 of the 5 mg/mL stock - final

concentration 10 µg/mL) for 1 h

02:00:00

