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HCR RNA-FISH protocol for the whole-mount antenna of Drosophila

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

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

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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 Drosohpila antenna. 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**. Modifications include higher amount of probe and hairpins to boost signal, and the description of fixation conditions.

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.

https://doi.org/10.1101/2020.11.07.368720 LINK

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.

Collection and fixation of antenna

2h 45m

1 Take aliquots of fixation solution (4%PFA in PBS+3%TritonX) from the -20C and thaw on ice. Put a tube of 1X PBS in ice to cool it down.

2 Fill a dissection glass with EtOH and a second one with cold PBS.

3 Collect flies in the fly pad, transfer themto a Falcon tube an put themin ice (this maintains the flies anesthesized but reducing their exposure to CO2).

4 Before dissecting each fly, dip it quickly in EtOH (this removes the wax covering the cuticle making the fly and antenna to sink in the solutions).

5 Dissect antenna in cold PBS using forceps. Transfer the antenna using a 20ul pipet with the tip cut, to the fixation solution (keep it in ice). Usually, I dissect as many antenna as I can in 30-40 min - around 24 to 30 antenna is a good number for one in situ run.

6 After dissecting enough antenna fix for **30 min at RT** in fixation solution. 30m

Room temperature Gently shaking 00:30:00

Pre-heat an aliquot of Probe Hybridization Buffer @37C if proceeding with hybridization on the same day OR
move to Step 7

7 Wash antenna before long term storage. To avoid loosing samples during this process, use a dissection scope to remove liquid during washes. 30m

PBT 00:05:00
PBT 00:05:00
PBT 00:05:00
MeOH wash 00:05:00
MeOH wash 00:05:00
MeOH wash 00:05:00

8 Label with name of species, tissue, and date before storing in MeOH at -20 °C

In theory, tissues can be stored like this for months, we usually proceed to the HCR within a week

Day 1

1h 45m

9 For each sample/tube, transfer 550µl of Probe Hybridization Buffer (store at -20C) to a 1.5 mL tube and warm to 37 °C

10 For all next steps, washes and rinse use around 1ml unless a specific volume is mentioned. Rinse means adding the solution and removing it as soon as the antenna settle in the bottom

15m

Rehydrate samples:
MeOH 75% /PBT 00:05:00
MeOH 50% /PBT 00:05:00
MeOH 25% /PBT 00:05:00
PBT rinse

11 **Post-fix:** Incubate for 10 minutes in 4% paraformaldehyde in PBT

12 **Wash:**
PBT 00:05:00
PBT 00:05:00
PBT 00:05:00

15m

13 Pre-hybridize samples in 300 µL of Probe Hybridization Buffer for:
00:30:00 37 °C
CAUTION: probe hybridization buffer contains formamide, a hazardous material.

30m

14 In the meantime, prepare a probe solution by adding 8 µL of probe to 200µL of warm Probe Hybridization Buffer. Even when using multiple probes, maintain the final of buffer to 200µL.

15 Remove the pre-hybridization solution from the sample and add the probe solution from step 9

3m

16 Add probe mixture and Incubate samples
Overnight around 24h
37 °C

Day 2

1h 20m

17 Pre-heat an aliquot of Probe Wash Buffer (2mL per tube/sample) 2m
37 °C

18 Equilibrate an aliquot of Amplification Buffer (stored at 4C) to room temperature (350ul per sample/tube) 2m
Room temperature

19 Remove and **SAVE PROBE SOLUTION**, which can be reused at least 3 times. Save used probe solutions at -20 °C

20 Remove excess probes by washing 37 °C : 50m

400 ul pre-heated Probe Wash Buffer 00:10:00
400 ul pre-heated Probe Wash Buffer 00:10:00
400 ul pre-heated Probe Wash Buffer 00:10:00

Before the next wash, take hairpins from -20C and thaw on ice

400 ul pre-heated Probe Wash Buffer 00:10:00
400 ul pre-heated Probe Wash Buffer 00:10:00

21 In the meantime, prepare **separately** each hairpin (h1 and h2) of each amplifier. Add 5 µL / sample of the 3 µM stock of each hairpin to a **separate** PCR tube. For example, if amplifiers B1 and B2 are being used, prepare 4 PCR tubes that contain B1-h1, B1-h2, B2-h1, and B2-h2, respectively. Incubate the tubes in a thermocycler at 95 ºC for 90 sec. Immediately take them out of the machine (while it is still at 95 ºC), place them in a rack and incubate them at room temperature **in the dark** for at least 30 min. 30m
00:30:00 or longer
Room temperature

22 Wash samples Room temperature : 15m

500 µL of 5X SSCT 00:05:00
500 µL of 5X SSCT 00:05:00
500 µL of 5X SSCT 00:05:00

23 Pre-amplify each sample with 250 µL of Amplification Buffer 30m
00:30:00
Room temperature

24 In the meantime, add 5 µL of each hairpin from step 21 to 100 µL of Amplification Buffer (keep the volume of Amplification Buffer at 100 µL even if multiple hairpin sets are being used)
Room temperature

25 Remove 250 µL liquid from the samples and add the Amplification Buffer + hairpins from step 18 2m

26 Incubate in **the dark (place tubes inside a cooler and in a drawer)**
Overnight
Room temperature

Day 3 3h 15m

27 **SAVE HAIRPIN MIXTURE**. Can be reused multiple times. Save used hairpin mixtures at -20°C. 20m

28 During the next washes minimize exposure to light by keeping the tubes in a drawer or inside a cooler. 20m
Wash:

500 µL of 5X SSCT
500 µL of 5X SSCT
500 µL of 5X SSCT
500 µL of 5X SSCT 00:15:00
500 µL of 5X SSCT 00:05:00

29 Rinse with 1X Nuclease-Free PBS to remove the detergent

2m

30 Remove the PBS and add VectaShield. 2m

31 Mount on slides using circular holders. 10m

32 Proceed with imaging

Citations

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