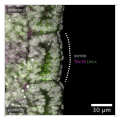

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Hybridization chain reaction (HCR) protocol for tails of mouse embryos

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1 Works for me dx.doi.org/10.17504/protocols.io.7pyhmpw

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ABSTRACT

This protocol is for the in situ hybridization chain reaction (HCR) of intact tails (somites and presomitic mesoderm, PSM) of mouse embryos. This is adapted from the in situ HCR v 3.0 protocol for whole-mount mouse embryos available in the website of Molecular Instruments (for details, please follow this link).

References

- Choi, H., Schwarzkopf, M., Fornace, M., Acharya, A., Artavanis, G., & Stegmaier, J. et al. (2018). Third-generationin situhybridization chain reaction: multiplexed, quantitative, sensitive, versatile, robust. *Development*, *145* (12), dev165753. doi: 10.1242/dev.165753
- Choi, H., Calvert, C., Husain, N., Huss, D., Barsi, J., & Deverman, B. et al. (2016). Mapping a multiplexed zoo of mRNA expression. *Development*, *143* (19), 3632-3637. doi: 10.1242/dev.140137

Day 1: Recovery and fixation of samples

1 Recover samples, wash quickly with cold PBS, and fix in 4% formaldehyde (**1.08 ml** 37% formaldehyde diluted to **10 ml** with PBS). Keep in formaldehyde overnight (around **16:00:00**) at **4 °C** in the cold room, with gentle shaking/rolling.

Formaldehyde is toxic. Operate under a fume hood and wear appropriate hand, body, and eye protection.

To reduce autofluorescence, it is recommended to use fresh fixative and PBS with no divalent cations (like Ca^{2+} and Mg^{2+}).

Day 2: Dehydration of samples

2 Wash samples with PBST (0.1% Tween20 in 1x PBS).

- Wash 1/2 **00:05:00**
- Wash 2/2 **00:05:00**

3 Dehydrate samples (one sample per tube) with a series of graded methanol : PBST (PBS + 0.1% Tween) washes.

- 25% methanol : 75% PBST **00:05:00**
- 50% methanol : 50% PBST **00:05:00**
- 75% methanol : 25% PBST **00:05:00**
- 100% methanol **00:05:00**
- 100% methanol **00:05:00**

4 Incubate samples at **-20 °C** overnight (around **16:00:00**) or until use.

Day 3: Rehydration of samples and HCR detection stage

5 Rehydrate samples (one sample per PCR tube, capacity = **200 µl** max) with a series of graded methanol : PBST (PBS + 0.1% Tween) washes.

- 100% methanol **00:05:00**
- 75% methanol : 25% PBST **00:05:00**
- 50% methanol : 50% PBST **00:05:00**
- 25% methanol : 75% PBST **00:05:00**
- 100% PBST **00:05:00**
- 100% PBST **00:05:00**

6 Treat samples with **10 ug/mL** proteinase K (Merck, CAS # 38450-01-6) for **00:05:00** at **Room temperature** .

7 Wash samples with PBST.

- Wash 1/2 **00:05:00**
- Wash 2/2 **00:05:00**

8 Fix samples in 4% formaldehyde (2.16 ml 37% formaldehyde diluted to 20 ml with PBST) for 00:20:00 at Room temperature .

Pre-warm probe hybridization (PH) buffer at 37 °C for later use.

Formaldehyde is toxic. Operate under a fume hood and wear appropriate hand, body, and eye protection.

Probe hybridization (PH) buffer contains formamide, which is toxic.

To reduce autofluorescence, it is recommended to use fresh fixative and PBS with no divalent cations (like Ca^{2+} and Mg^{2+}).

9 Wash samples with PBST.

- Wash 1/3 00:05:00
- Wash 2/3 00:05:00
- Wash 3/3 00:05:00

10 Wash samples with PH buffer for 00:05:00 .

Probe hybridization (PH) buffer contains formamide, which is toxic.

11 Put fresh PH buffer to the tubes and incubate for 00:30:00 at 37 °C .

Meanwhile, prepare probe solution by adding 2 pmol (2 µl of [M]1 Micromolar (µM) stock) of odd probe mixture and 2 pmol of even probe mixture to every 500 µl of pre-warmed PHP buffer (from Step 8).

Probe hybridization (PH) buffer contains formamide, which is toxic.

12 Remove PH buffer and add the probe solution. Incubate overnight (around 16:00:00) at 37 °C .

Day 4: HCR amplification stage

13 Pre-warm probe wash buffer at **37 °C** , and equilibriate amplification buffer to **Room temperature** .

Probe wash (PH) buffer contains formamide, which is toxic.

14 In separate tubes, for every **500 µl** hairpin mixture, prepare 30 pmol (**15 µl** of **2 Micromolar (µM)** stock or **10 µl** of **3 Micromolar (µM)** stock) of hairpin h1 (tube 1) and 30 pmol of hairpin h2 (tube 2). Snap-cool hairpins by heating the tubes at **95 °C** for **00:01:30** and cool to **Room temperature** in the dark for at least **00:30:00** .

15 Wash samples with probe wash buffer at **37 °C** .

- Wash 1/4 **00:15:00**
- Wash 2/4 **00:15:00**
- Wash 3/4 **00:15:00**
- Wash 4/4 **00:15:00**

Probe wash (PH) buffer contains formamide, which is toxic.

16 Wash samples with 5x SSCT (0.1% Tween20) at **Room temperature** .

- Wash 1/2 **00:05:00**
- Wash 2/2 **00:05:00**

To prepare **500 ml** of 5x SSCT, dilute **125 ml** of 20x SSC and **0.5 ml** Tween20 to **500 ml** with distilled H_2O.

17 Wash samples with pre-equilibriated amplification buffer (Step 13) for **00:05:00** at **Room temperature** .

Meanwhile, prepare the hairpin mixture (30 pmol of each hairpin in **500 µl** mixture) by mixing the snap-cooled hairpins h1 and h2 (Step 14) to amplification buffer at **Room temperature** .

18 Remove the amplification buffer from the sample tubes, and add the hairpin mixture. Incubate overnight (around **16:00:00**) in the dark at **Room temperature** .

For dHCR imaging, amplify for a shorter period of time to ensure single-molecule dots are diffraction-limited.

Day 5: Washing, nuclear staining, and imaging

19 Wash samples with 5x SSCT for **00:30:00** in the dark at **Room temperature** .

20 Stain nuclei with 1:1000 DAPI (**5 ug/mL** : **20 µl** of **5 mg/mL** DAPI diluted to **20 ml** with 5x SSCT) in the dark at **Room temperature** .

- Wash + DAPI 1/3 **00:30:00**
- Wash + DAPI 2/3 **00:30:00**
- Wash + DAPI 3/3 **00:30:00**

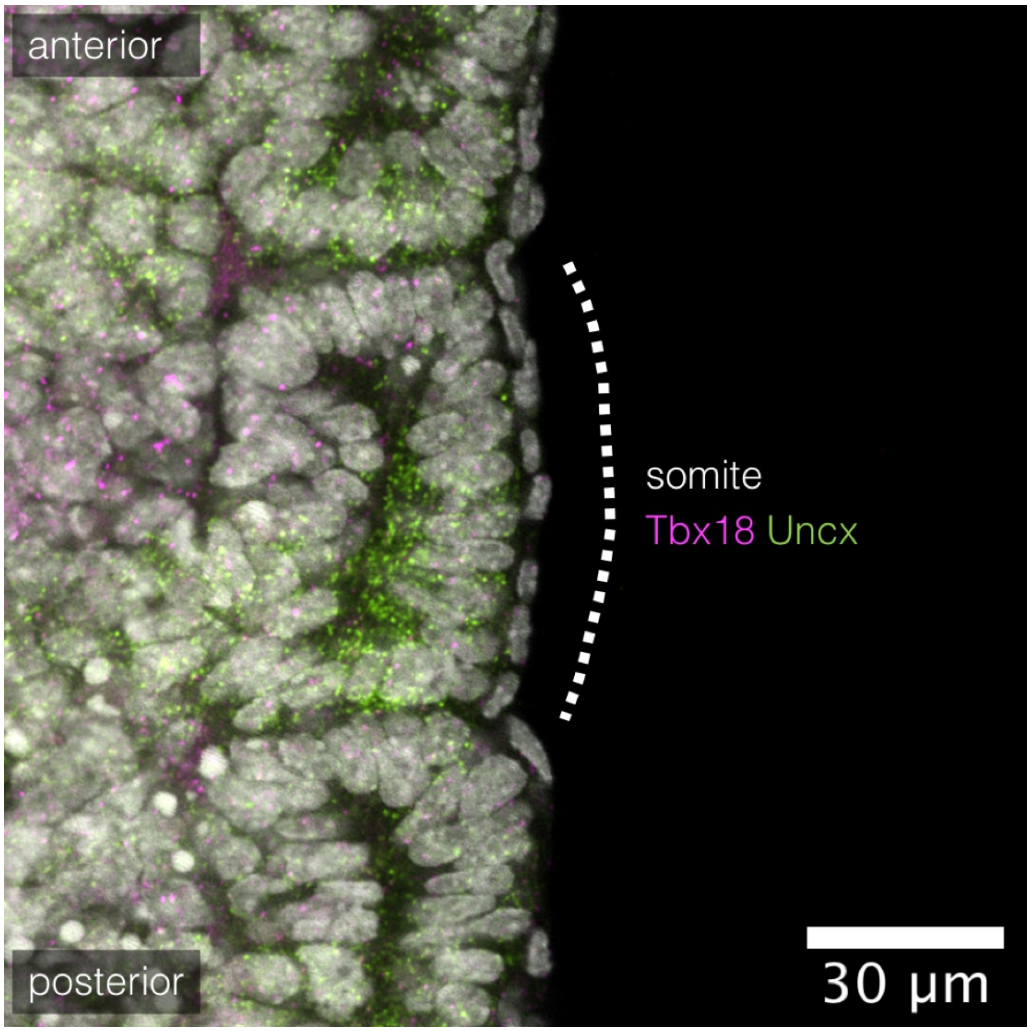
21 Wash samples with 5x SSCT in the dark at **Room temperature**

- Wash 1/2 **00:30:00**
- Wash 2/2 **00:30:00**

22 Samples are now ready for imaging.

Prior to imaging, the samples could be cleared using the fructose-glycerol clearing solution described in Dekkers et al., 2019. Incubate samples in clearing solution in the dark at **4 °C** for at least **02:00:00** .

To prepare the fructose-glycerol clearing solution, dissolve **29.72 g** fructose in **33 ml** glycerol and **7 ml** water on a magnetic stirrer (start the dissolution at least a day before intended day of clearing because it will take a while for fructose to dissolve). The clearing solution can be stored in the dark at **4 °C** for at most 1 month. This solution can be used to mount the sample on microscope slide/cover glass. Take note that the clearing is solution has high viscosity.



Tbx18 and Uncx marking the two halves (anterior and posterior, respectively) of somites in tail of a mouse embryo. Transcripts were visualized using HCR version 3.0. Nuclei were stained using DAPI.