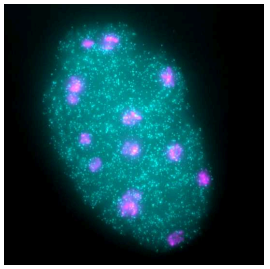
Feb 01, 2025

smFISH in C. elegans embryos



DOI
https://dx.doi.org/10.17504/protocols.io.8epv52m74v1b/v1

Laura Breimann[1]

[1]Harvard University

MDC

Laura Breimann
Harvard University

DOI: https://dx.doi.org/10.17504/protocols.io.8epv52m74v1b/v1

Protocol Citation: Laura Breimann 2025. smFISH in C. elegans embryos. **protocols.io** https://dx.doi.org/10.17504/protocols.io.8epv52m74v1b/v1

Manuscript citation:
Breimann L, Bahry E, Zouinkhi M, Kolyvanov K, Street LA, Preibisch S, Ercan S. Analysis of developmental gene expression using smFISH and *in silico* staging of *C. elegans* embryos. bioRxiv [Preprint]. 2024 May 16:2024.05.15.594414. doi: 10.1101/2024.05.15.594414. PMID: 38798598; PMCID: PMC11118362.

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

Created: January 18, 2025

Last Modified: February 01, 2025

Protocol Integer ID: 118677

Keywords: smFISH, C. elegans, embryo, microscopy, RNA, imaging, different steps of rna smfish, rna smfish, rna, embryo, embryos this protocol, mrna of the sample, smfish, mrna, target mrna

Abstract

This protocol walks through the different steps of RNA smFISH for C. elegans embryos. It discusses probe design, sample preparation, staining, mounting and imaging steps. The goal of the protocol is prepare C. elegans embryos optimally for staining, while preserving structure and mRNA of the samples. A successful staining results in low background and high SNR staining of the target mRNA.

Image Attribution

Laura Breimann

Safety warnings

Paraformaldehyde and Formamide are toxic chemicals and should only be handled in the appropriate environment, like a chemical hood.

Probe design

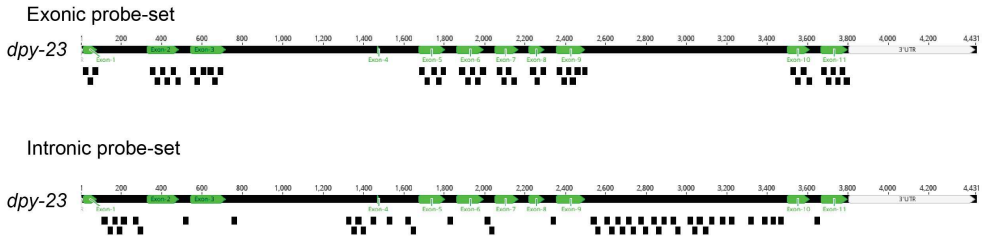
1 RNA smFISH probes can be designed with tools like Stellaris RNA FISH Probe Designer (Biosearch Technologies) https://www.biosearchtech.com/support/tools/design-software/stellaris-probe-designer or Paintshop **https://paintshop.io/**

2 Probes can be designed to target exons, introns, or both. Probes should be designed to have at least two nucleotide spaces in between neighboring probes to avoid quenching. For Stellaris Pobes, we recommend the dyes Quasar 670, CAL Fluor Red 610, or Quasar 570.

> Note
>
> Typically, far-red dyes performed better due to the autofluorescence of C. elegans embryos, especially in older stages.

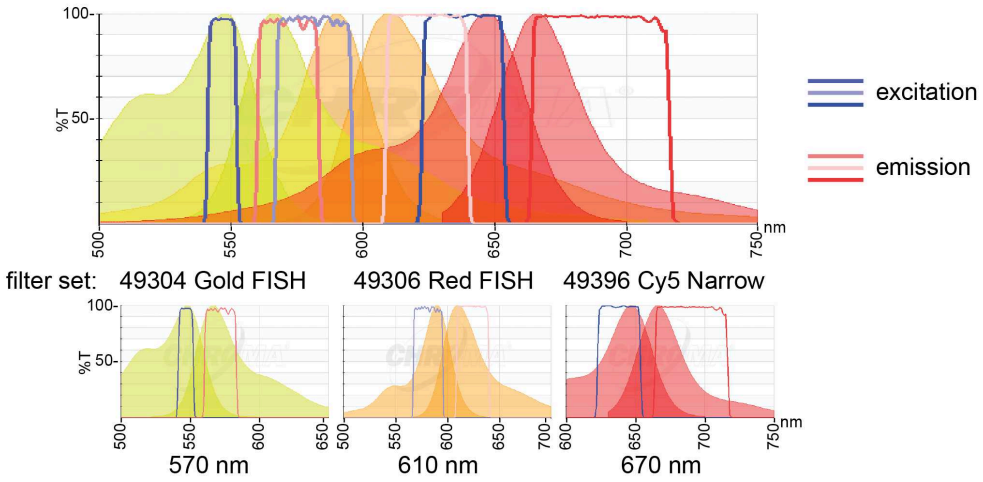
3 For exonic or intronic probe design, sequences listed at wormbase.org can be used, and splice regions are masked with "n" nucleotides to avoid probes in these areas.

4 If possible, the maximum amount of probes (48 per order) should be selected for each exonic or intronic sequence.



Example probe distribution for the gene dpy-23, showing exonic and intronic probe sets.

5 Fluorescent filter sets that work well with the dyes (Quasar 670, CAL Fluor Red 610, and Quasar 570) for a Nikon microscope are: 49304 Gold FISH, 49306 Red FISH, and 49396 Cy5 Narrow, all from Chroma.



Fluorescent spectra for the different FISH dyes with corresponding filter sets.

Preparation of worms and collection of embryos

6 Worms are synchronized by bleaching or egg-laying, and grown to the adult stage.

> **Note**
>
> At this step, having non-starved, healthy adult worms in sufficient amounts is crucial.

7 Typically, at least 5 × 6 cm plates full of worms are used to collect embryos.

8 For embryo collection, worms and older embryos were washed off plates using M9 (5.8 g Na2HPO4, 3.0 g KH2PO4, 0.5 g NaCl, 1.0 g NH4Cl, Nuclease-free water to a final volume of 1000 ml) and collected in a 35 µm nylon filter (N35R, CellMicroSieves, 35 mircon pore size, BioDesign Inc. of New York).

Nylon filter set-up for worm collection.

9 Worms are washed at least three times with H2O, then carefully transferred to a falcon tube using M9 and allowed to settle down.

10 The supernatant is removed, and 5 ml of freshly prepared bleaching solution (2.5 ml 4N NaOH, 2.5 ml 5% NaClO, 5 ml Nuclease-free water) is added to the worms.

11 The dissolving of the adult worms has to be closely observed, and after 3 minutes, the tubes are spun at 3000 g for 1 minute to collect the embryos.

Note

The solution will turn a bit yellow at this point; it is important not to let the reaction go on for too long. Inverting the tube and checking in a stereoscope at this point can help.

12 The supernatant is quickly removed, and the embryo pellet is vortexed.

Note

This step is important in order to have nicely separated embryos later.

13 Next, 10 ml of 1x PBS (with 0.05% Triton X-100) is added, and the tube is centrifuged again for 3 min at 3000 g.

14 This step is repeated twice until a clean embryo extract is left in the tube.

Note

The solution appears much more clear, with little debris, when only embryos are left.

Fixing and permeabilization

15 Embryos are resuspended in 1 ml fixation solution (freshly made, 4% paraformaldehyde (PFA) in 1xPBS (DEPC treated + autoclave + 0.05% Triton X-100)) and incubated at RT for 15 min, while rotating.

16 Next, the tube is submerged in liquid nitrogen for 1 minute to freeze and crack the embryo eggshells.

17 The tube is then transferred to a beaker with RT water to thaw. Once fully thawed, it is kept on ice for an additional 20 minutes.

18 After this incubation, the tubes are spun down at 3000 g for 3 minutes, the supernatant is removed, and the embryos are washed twice with 1 ml 1x PBS (with 0.05% Triton X-100).

19 The embryos are resuspended in 70% EtOH and kept at 4 °C for at least 24 hours. Embryos can be kept at 4 °C for at least several weeks.

smFISH staining

20 For smFISH staining of previously fixed embryos, tubes are centrifuged at 3000 g for 3 minutes, and the ethanol is carefully removed.

> Note
>
> The pellet can be loose at this step, so removing the supernatant should be done in two stages.

21 Embryos are then resuspended in 1 ml wash buffer (40 ml nuclease-free water, 5 ml deionized formamide, 5 ml 20x SSC) and vortexed.

22 Tubes are centrifuged, as above, and the supernatant is removed.

23 The embryos are then resuspended in 50 µl hybridization solution (50 µl H20 (RNAse free), 37.5 µl ethylene carbonate (EC) 5 mg/ml, 25 µl formamide (at RT), 12.5 µl SDS (dissolved), 125 µl dextran sulfate 10%), and 1 µl of each probe set (12.5 µM stock solution) is added directly to the sample.

24 Tubes are then vortexed lightly and incubated overnight at 37°C in the dark.

25 The next day, 0.5 ml of wash buffer is added, and the tubes are vortexed and centrifuged to remove the supernatant.

26 Next, 1 ml of wash buffer is added, and samples were incubated at 37°C for 30 minutes.

27 After that, tubes are centrifuged again to remove the supernatant, and the embryo pellet is vortexed before adding 1 ml wash buffer.

28 In this step, DAPI (5 ng/mL) is added to the wash buffer, and tubes were incubated at 37 °C for 30 minutes.

29 After centrifugation, the wash buffer is removed, and samples are washed once with 2x SSC.

Mounting

30 Most liquid is removed from the stained embryos in the tube in order to mount the embryos.

31 About 15 µl of dense embryo solution (in 2x SSC) is used per microscopy slide and spread onto a coverslip (#1.5, 22 × 22 mm Marienfeld, High precision).

32 The sample is left to dry for about 15 minutes, and then 15 µl of ProLong™ Diamond Antifade Mountant (Thermo Fisher) is added to the sample

33 A glass slide (SuperFrost PLUS, Thermo Scientific) is pressed gently but firmly onto the embryos in mounting media while resting on a Kimtech wipe, allowing some of the mounting media to spill out on the sides.

34 Slides are left at RT in the dark for 24 hours to cure before sealing the sides with nail polish and then stored at 4°C.

35 Images are acquired within two weeks of the sample being prepared.

Imaging smFISH embryos

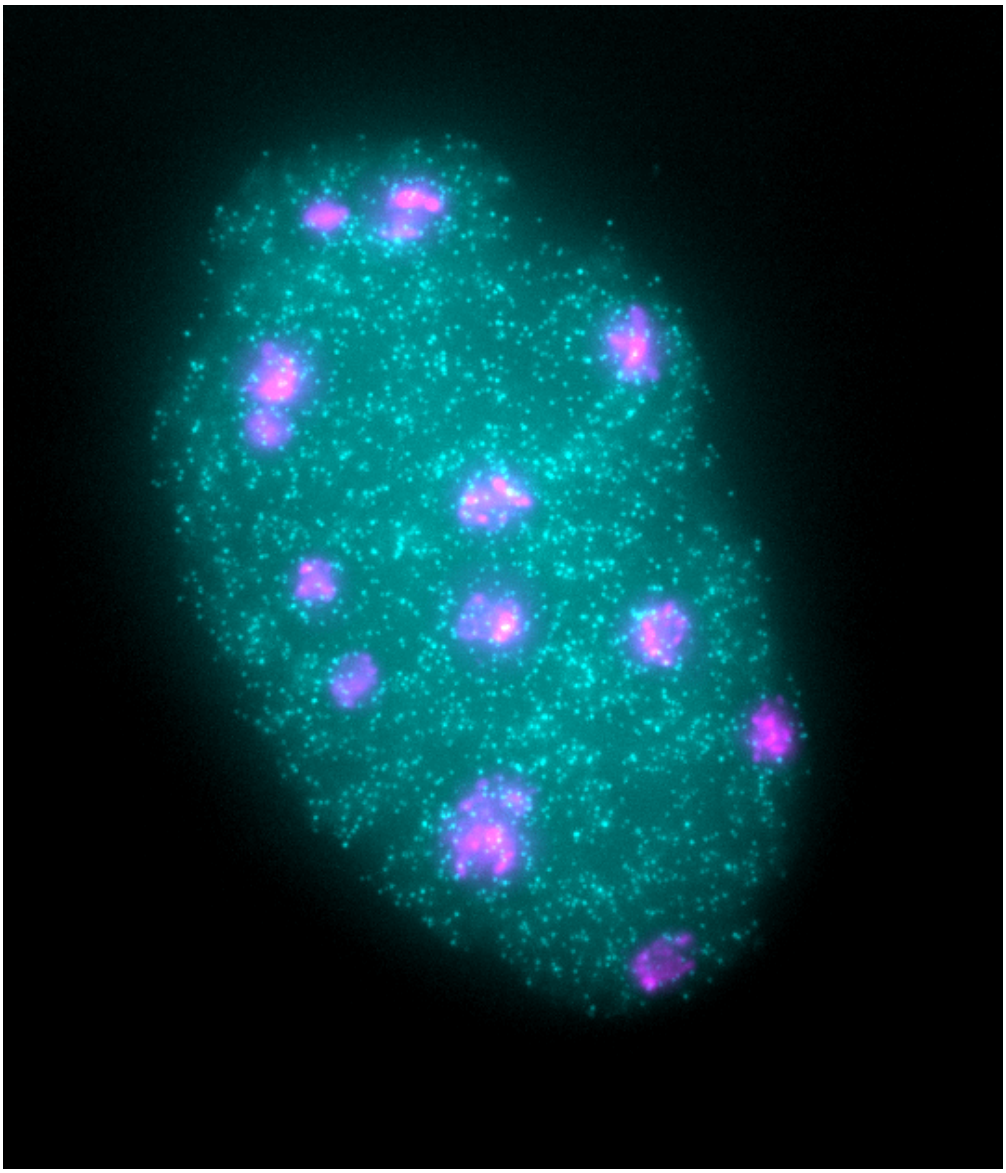
36 Several microscopy setups can be used for imaging. We had success with the following approach.
Embryos are imaged on a Nikon Ti inverted fluorescence microscope with an EMCCD camera (ANDOR, DU iXON Ultra 888), Lumen 200 Fluorescence Illumination Systems (Prior Scientific), and a 100x plan apo oil objective (NA 1.4) using appropriate filter sets (Gold FISH (Chroma 49304), Red FISH (Chroma 49306), Cy5 Narrow (Chroma 49396), GFP, DAPI).

37 Images are acquired with 90 z-stack positions with 200 nm step-width using Nikon Elements software.

38 Every field of view has 1024×1024 pixels (XY axes) and ~ 90 slices (Z), with 0.13 µm lateral and 0.2 µm axial resolution.

39 Positions of embryos are marked in the software for semi-automatic imaging.

40



Example image of a C. elegans embryo with smFISH staining.