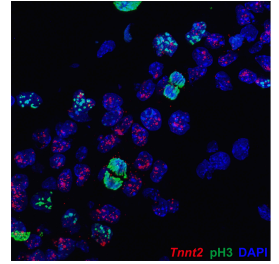


Apr 08, 2025

🌐 Optimized RNAscope Protocol for Cardiomyocyte Nuclei Identification

DOI

<https://dx.doi.org/10.17504/protocols.io.eq2ly6mbmgx9/v1>



Zhe Yu¹, Sen Zhang², Julius Bogomolovas³, Ju Chen³, Sylvia M. Evans^{1,3,4}

¹Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California San Diego, La Jolla, CA, USA;

²Department of Pharmacology & Regenerative Medicine, University of Illinois Chicago, Chicago, IL, USA;

³Department of Medicine, University of California San Diego, La Jolla, CA, USA;

⁴Department of Pharmacology, University of California San Diego, La Jolla, CA, USA



Zhe Yu

UCSD

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.eq2ly6mbmgx9/v1>

Protocol Citation: Zhe Yu, Sen Zhang, Julius Bogomolovas, Ju Chen, Sylvia M. Evans 2025. Optimized RNAscope Protocol for Cardiomyocyte Nuclei Identification. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.eq2ly6mbmgx9/v1>

Manuscript citation:

Yu, Z., Zhang, S., Bogomolovas, J. et al. Intronic RNAscope probes enable precise identification of cardiomyocyte nuclei and cell cycle activity. Commun Biol 8, 577 (2025). <https://doi.org/10.1038/s42003-025-08012-z>

Yu, Z., Zhang, L., Cattaneo, P. et al. Increasing Mononuclear Diploid Cardiomyocytes by Loss of E2F Transcription Factor 7/8 Fails to Improve Cardiac Regeneration After Infarct. Circulation 147, 183-186 (2023). <https://doi.org/10.1161/CIRCULATIONAHA.122.061018>

License: This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We use this protocol and it's working

Created: March 13, 2025

Last Modified: April 08, 2025

Protocol Integer ID: 124295

Keywords: Cardiomyocyte, RNAscope, intronic probe, Nuclei, optimized rnascope protocol for cardiomyocyte nuclei identification, cardiomyocyte nuclei identification, based intronic rnascope probe, intronic rnascope probe, intronic rnas within nuclei, nuclei in cardiac section, cardiac sarcomeric gene, isolated cardiomyocyte, intronic rna, application of cardiac sarcomeric gene, identifying cardiomyocyte, performing rnascope, optimized rnascope protocol, cm chromatin at all stage, cm specificity of common nuclear marker, cardiac science, inducing unintended cardiac phenotype, cm chromatin, common nuclear marker, unintended cardiac phenotype, chromatin, method for nuclear localization, nuclear localization, cm nuclei, cardiac section, sarcomeric protein, nuclei

Funders Acknowledgements:

National Institutes of Health, National Heart, Lung, and Blood Institute

Grant ID: R35HL144984

American Heart Association

Grant ID: 24TPA1288908

National Institute of Neurological Disorders and Stroke

Grant ID: P30NS047101

Disclaimer

This protocol is intended for research purposes only. The authors are not responsible for any unexpected outcomes or misuse of the procedure. Users should validate the protocol under their specific experimental conditions.



Abstract

Unequivocally identifying cardiomyocyte (CM) nuclei in cardiac sections is a technical challenge in the field of cardiac science. The use of antibodies to sarcomeric proteins is error-prone, the CM specificity of common nuclear markers is controversial, and utilizing genetically modified mouse models poses risk of inducing unintended cardiac phenotypes. The application of cardiac sarcomeric gene-based intronic RNAscope probes overcomes the above shortcomings. Intronic probes label intronic RNAs within nuclei and can therefore be utilized as a method for nuclear localization. Intronic RNAscope probe labeled CM nuclei that had undergone DNA replication, and remained closely associated with CM chromatin at all stages of mitosis, even with nuclear envelope breakdown. Here, we provide a step-by-step protocol for performing RNAscope staining on cryosections and isolated cardiomyocytes.

Guidelines

If you want to detect RNAs only, treat your samples with Protease III for 40 minutes at 40°C for sections or 15 minutes for CMs. For RNA and protein co-detection, treat your samples with Protease III for 20 minutes at room temperature for sections or 15 minutes for CMs. Alternatively, you can use the commercially available RNAscope™ Manual PretreatPro from ACD, treating for 40 minutes at 40°C for sections or 15 minutes for CMs.

Materials

RNAscope probes and RNAscope™ Multiplex Fluorescent Reagent Kit v2 (323100) were provided by Advanced Cell Diagnostics; TSA® Plus fluorescein detection kit (NEL741001KT), TSA® Plus Cyanine 3 (Cy3) detection kit (NEL744001KT), and TSA® Plus Cyanine 5 (Cy5) detection kit (NEL745001KT) were ordered from Akoya Biosciences. Equipment: HybEZ™ II Hybridization System from Advanced Cell Diagnostics: HybEZ oven (PN 321710/321720), a humidity control tray (PN 310012), and EZ-Batch Slide Holder (PN 321716).

Optimized RNAscope Protocol for Cryosections

1 Day 1:

- 1.1 Refix cryosections in 4% PFA/PBS at room temperature for 15 minutes, followed by a single wash with ddH₂O.
- 1.2 Incubate in 50% EtOH for 5 minutes, 70% EtOH for 5 minutes, and two washes in 100% EtOH for 5 minutes each. Allow sections to dry.
- 1.3 Treat with H₂O₂ for 10 minutes at room temperature.
- 1.4 Wash twice with ddH₂O for 2 minutes each.
- 1.5 Incubate in 100% EtOH for 3 minutes, allow to dry, and circle the sections with a pap pen.
- 1.6 Incubate with Protease III for 20 minutes at room temperature (40 minutes at 40°C if antibody staining is not required in subsequent steps).
- 1.7 Wash twice with ddH₂O for 2 minutes each.
- 1.8 Drop *Tnnt2* intronic RNAscope probe (or other C2/C3/C4 probes) onto the sections.
- 1.9 Incubate with probe for 2 hours at 40°C in hybridization oven.
- 1.10 Wash twice with 1 x wash buffer for 2 minutes each.
- 1.11 Incubate in 5 x SSC (pH5.2) at room temperature overnight.

2 Day 2:

- 2.1 Incubate with AMP1 for 30 minutes at 40°C, followed by two 2-minute washes with 1 x wash buffer.
- 2.2 Incubate with AMP2 for 30 minutes at 40°C, followed by two 2-minute washes with 1 x wash buffer.
- 2.3 Incubate with AMP3 for 15 minutes at 40°C, followed by two 2-minute washes with 1 x wash buffer.
- 2.4 Incubate with HRPC1 (or C2/C3/C4) for 15 minutes at 40°C, followed by two 2-minute washes with 1 x wash buffer.
- 2.5 Incubate with Cy3 (or FITC/Cy5)/TSA buffer (1:500) for 30 minutes at 40°C, followed by two 2-minute washes with 1 x wash buffer.
- 2.6 Incubate with HRP blocker for 15 minutes at 40°C, followed by two 2-minute washes with 1 x wash buffer.
- 2.7 Repeat step 4 to 6 if you have C2/C3/C4 probes (HRPC2/C3/C4).
- 2.8 Proceed with either the EdU assay or antibody immunostaining steps, followed by DAPI staining.

Optimized RNAscope Protocol for Isolated Cardiomyocytes (CMs)

- 3 Day 1:
 - 3.1 Incubate isolated CMs (in 100% MeOH at -20°C) in 70% MeOH/PBT (PBS containing 0.1% Tween 20) for 5 minutes, followed by 50% MeOH/PBT for 5 minutes, and two washes in PBT for 5 minutes each. After each step, centrifuge at 800 rpm for 1 minute, remove the supernatant, and resuspend the CMs.
 - 3.2 Remove the supernatant, resuspend the CMs in 200 µL Protease III and incubate for 15 minutes at room temperature (at 40°C if antibody staining is not required in subsequent steps).
 - 3.3 Add 1 mL of PBT, centrifuge at 800 rpm for 1 minute, and remove the supernatant. Wash again with 1 mL of PBT for 5 minutes, centrifuge at 800 rpm for 1 minute, and remove the

supernatant.

3.4 Add 200 μ L *Tnnt2* intronic RNAscope probe (or other C2/C3/C4 probes) and resuspend the CMs.

3.5 Incubate with probe overnight at 40°C in hybridization oven.

4 Day 2:

4.1 Add 1 mL of 1 x wash buffer, centrifuge at 800 rpm for 1 minute, and remove the supernatant. Wash again with 1 mL of 1 x wash buffer for 5 minutes, centrifuge at 800 rpm for 1 minute, and remove the supernatant. Resuspend CMs with AMP1 and incubate for 30 minutes at 40°C.

4.2 Add 1 mL of 1 x wash buffer, centrifuge at 800 rpm for 1 minute, and remove the supernatant. Wash again with 1 mL of 1 x wash buffer for 5 minutes, centrifuge at 800 rpm for 1 minute, and remove the supernatant. Incubate with AMP2 for 30 minutes at 40°C.

4.3 Add 1 mL of 1 x wash buffer, centrifuge at 800 rpm for 1 minute, and remove the supernatant. Wash again with 1 mL of 1 x wash buffer for 5 minutes, centrifuge at 800 rpm for 1 minute, and remove the supernatant. Incubate with AMP3 for 15 minutes at 40°C.

4.4 Add 1 mL of 1 x wash buffer, centrifuge at 800 rpm for 1 minute, and remove the supernatant. Wash again with 1 mL of 1 x wash buffer for 5 minutes, centrifuge at 800 rpm for 1 minute, and remove the supernatant. Incubate with HRPC1 (or C2/C3/C4) for 15 minutes at 40°C.

4.5 Add 1 mL of 1 x wash buffer, centrifuge at 800 rpm for 1 minute, and remove the supernatant. Wash again with 1 mL of 1 x wash buffer for 5 minutes, centrifuge at 800 rpm for 1 minute, and remove the supernatant. Incubate with Cy3 (or FITC/Cy5)/TSA buffer (1:500) for 30 minutes at 40°C.

4.6 Add 1 mL of 1 x wash buffer, centrifuge at 800 rpm for 1 minute, and remove the supernatant. Wash again with 1 mL of 1 x wash buffer for 5 minutes, centrifuge at 800 rpm for 1 minute, and remove the supernatant. Incubate with HRP blocker for 15 minutes at 40°C.

4.7 Repeat step 4 to 6 if you have C2/C3/C4 probes (HRPC2/C3/C4).

4.8 Proceed with either the EdU assay or antibody immunostaining steps, followed by DAPI staining.



Protocol references

Yu, Z., Zhang, S., Bogomolovas, J. *et al.* Intronic RNAscope probes enable precise identification of cardiomyocyte nuclei and cell cycle activity. *Commun Biol* **8**, 577 (2025). <https://doi.org/10.1038/s42003-025-08012-z>

Yu, Z., Zhang, L., Cattaneo, P. *et al.* Increasing Mononuclear Diploid Cardiomyocytes by Loss of E2F Transcription Factor 7/8 Fails to Improve Cardiac Regeneration After Infarct. *Circulation* **147**, 183–186 (2023). <https://doi.org/10.1161/CIRCULATIONAHA.122.061018>

Acknowledgements

Special thanks to Qingquan Zhang from the Neil Chi Lab at UCSD School of Medicine for optimizing the RNAscope protocol. We also thank Advanced Cell Diagnostics (ACD) for designing all intronic RNAscope probes and providing technical support.