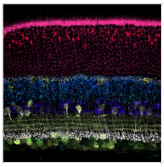

Aug 21, 2020

7: User-friendly protocol: Retina Tissue Sections RNA FISH

In 1 collection

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1 *Works for me* This protocol is published without a DOI.

Human Cell Atlas Method Development Community

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ABSTRACT

This protocol is about SABER RNA FISH with retina tissue.

Notes: This stepwise protocol corresponds to the most rapid version we have tested for 40µm sections. Experiments in the manuscript employ pretreatment steps and longer wash and development steps that we determined to be inessential in a test involving two 500 nt probes (see Fig. S3, protocol 6).

This protocol is part of the SABER-FISH collection.

EXTERNAL LINK

http://saber.fish/

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Kishi, J.Y., Lapan, S.W., Beliveau, B.J. et al. SABER amplifies FISH: enhanced multiplexed imaging of RNA and DNA in cells and tissues. Nat Methods 16, 533–544 (2019). https://doi.org/10.1038/s41592-019-0404-0

ATTACHMENTS

SABER amplifies FISH_enhanced multiplexed imaging of RNA and DNA in cells and tissues.pdf

PROTOCOL CITATION

Jocelyn Y. Kishi, Sylvain W. Lapan, Brian J Beliveau, Emma R. West, Allen Zhu, Hiroshi M. Sasaki, Sinem Saka, Yu Wang, Constance L Cepko, Peng Yin 2020. 7: User-friendly protocol: Retina Tissue Sections RNA FISH. **protocols.io** https://protocols.io/view/7-user-friendly-protocol-retina-tissue-sections-rn-bh9wj97e

MANUSCRIPT CITATION please remember to cite the following publication along with this protocol

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EXTERNAL LINK

http://saber.fish/

COLLECTIONS

SABER-FISH – Signal amplification for multiplexed fluorescence in situ hybridization assays

KEYWORDS

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PARENT PROTOCOLS

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SABER-FISH – Signal amplification for multiplexed fluorescence in situ hybridization assays

GUIDELINES

Fig. S3 demonstrates that the protocol is robust to certain variations. Tissues can be treated with methanol. Hyb1 and Hyb2 incubation time can be increased, and fluorescent oligo can be applied at a range of concentrations. To reduce viscosity for ease of solution exchange, dextran sulfate can be eliminated from Hyb2. Probes can be purified using ethanol precipitation; however, this resulted in a mild decrease in signal for unknown reasons.

Branching is performed similarly to primary probe incubation, in Hyb1 solution. Branches are applied after primary probe washes are complete, and before fluorescent detection. Branches are extended to length of ~350-500 nt and incubated for at least 5 hours (longer may be required, depending on tissue penetration) in 40% formamide Hyb solution. Hyb temperature must be adjusted depending on the branches being used. We recommend using a temperature at least 1 degree lower than the Tm of the branch with the lowest melting temperature (see Fig. S2). Note that formamide concentration can be adjusted instead of oven temperature. Another option is to extend the branch annealing portion (the 30mer) by adding an additional full or partial repeat (primer sequence + T) to match the Tm of the branch with highest Tm. If doing serial multiplexing with branching, 30mers with lower melting temperatures may be partially displaced by displacement of the fluorescent oligos. For this reason we recommend using a branch with high Tm (e.g. 27*27*27*) and extending other branches as needed to match this melting temperature.

MATERIALS

NAME	CATALOG #	VENDOR

NAME	CATALOG #	VENDOR
Tween-20	P9416	Sigma Aldrich
µ-Slide 8 Well Glass Bottom	80827	Ibidi
UltraPure™ SSC, 20X	15557044	Thermo Fisher
Pierce™ 20X Borate Buffer	28341	Thermo Fisher
Pierce™ 16% Formaldehyde (w/v), Methanol-free	28908	Thermo Fisher
PBS	10010023	Gibco - Thermo Fischer
Poly-D-lysine hydrobromide (PDL)	P7886	Sigma Aldrich
ddH2O		
Formamide Deionized	S4117	Merck Millipore
Glycerol		
Propyl gallate		
Dextran sulfate sodium salt	D8906	Sigma Aldrich

MATERIALS TEXT

Solution preaparation:

ddH2O is used for all solutions, not DEPC. We recommend using sterile plastic tubes and not reusable glassware for solutions. Formamide and formamide-containing solutions are aliquoted and stored at **-20 °C** .

Whyb (Wash hyb):

- [M]**2 X SSC**
- [M]**1 % Tween-20**
- [M]**40 % Formamide**

Example mix for 40% formamide Whyb: **1 mL 20×SSC** , **1 mL 10% Tween** , **4 mL Formamide (for 40% mix)** , **4 mL H2O** .

Hyb 1 (for primary probe hyb):

- [M]**2 X SSC**
- [M]**1 % Tween-20**
- [M]**40 % Formamide**
- [M]**10 % Dextran sulfate**

Probes are generally used at a concentration of 1µg /120 µL volume

Example Hyb1 master mix: **1 mL 20xSSC** , **1 mL 10% tween** , **4 mL Formamide** , **2 mL Dextran sulfate (50% solution)** .

Example Hyb1/Probe mix: **96 µl Hyb1 master mix** , **5 µl 200ng/ µL probe 1** , **5 µl 200ng/ µL probe 2** , **14 µl ddH2O** .

Notes: Stock of Hyb1 should be made accounting for the fact that probes, which are eluted in ddH2O, have a water volume. The example Hyb1 mix above allows up to **24 µl probe** per **96 µl Hyb mix** for a total of **120 µl** volume to add to the well. For higher multiplexing probes can also be concentrated to reduce water volume by using a SpeedVac or heat block. After addition of probe to Hyb1 mix, the solution should be mixed well by pipetting until uniform consistency is observed.

Likewise, mix well by rocking after adding dextran sulfate to the Hyb1 mix. Dextran sulfate is usually added from [M]**50 % (w/v) stock** made by dissolving powder in ddH2O. Dextran sulfate takes time to dissolve, and volume will shrink as powder dissolves. Use tick marks on the side of the tube to add water to final volume after most of the powder has dissolved.

Hyb 2 (for fluorescent detection):

- [M]**1 X PBS**
- [M]**0.2 % Tween-20**
- [M]**10 % Dextran sulfate**

Fluorescent oligos added to concentration of [M]**0.2 Micromolar (µM)** - [M]**1 Micromolar (µM)** .

Example Hyb2 master mix: **1 mL 10×PBS** , **200 µl 10% Tween-20** , **2 mL dextran sulfate** , **4.8 mL H2O** .

Example Fluor/Hyb2 mix: **96 µl Hyb2 master mix** , **2.4 µl Fluor Oligo 1 (10µM)** , **2.4 µl Fluor Oligo 2 (10µM)** , **19.2 µl ddH2O** .

Notes: Master mix aliquots are stored at **4 °C** . Side-by-side testing with two probes indicates [M]**0.2 Micromolar (µM) fluorescent oligo** is sufficient. Dextran sulfate can be excluded from the Hyb2 mixture (tested for fluorescent oligo at [M]**1 Micromolar (µM)**).

Displacement buffer:

- [M]**1 X PBS**
- [M]**50 % formamide**

Stored at **-20 °C** .

Example master mix: **1 mL 10×PBS** , **5 mL Formamide** , **4 mL H2O** .

Glycerol mounting media:

- [M]**50 % glycerol**
- [M]**1 X PBS**
- [M]**20 Milimolar (mM) Tris, pH 8**
- [M]**2.5 mg/ml propyl gallate**

Example mix: **8 mL 100% glycerol** , **1 mL 10×PBS** , **200 µl 1M Tris** , **25 mg propyl gallate**

Stored at **4 °C** .

Note: Resuspend fully and spin in centrifuge before use (3 minutes on max in table top centrifuge) to remove undissolved propyl gallate specks that fluoresce. This mounting media appears to have a shelf life of 6 months when stored properly and appears to degrade signal beyond this point.

PBSTw:

Mg/Ca-free, RNAse/DNAse free PBS (Gibco #10010-023) with [M]**0.1 % Tween-20** .

SAFETY WARNINGS

For hazard information and safety warnings, please refer to the SDS (Safety Data Sheet).

BEFORE STARTING

Prepare solutions as described in section 'Materials'.

Note: After rehydrating sections, do not allow them to dry completely at any point in the protocol. Washes should be performed such that sections do not remain devoid of solution for more than a few seconds. For washes done at elevated temperature, solutions should be prewarmed and added in or near the oven. Avoid letting samples cool during these wash steps. Volumes used are 120 µL for Hyb1 (minimal to cover tissue), and 150-200 µL for all other steps. When pipetting the hyb mixes with dextran sulfate, allow time for the viscous solution to fully accumulate in the pipette tip to avoid loss of volume.

Fixation

1 Dissect neural retinas in PBS and fix in [M]**4 % FA** (diluted from [M]**16 % FA ampule** , Thermo Fisher #28908) for **00:25:00** at **Room temperature** .

2 Embed retinas in a **1:1 (v/v) mixture** of [M]**30 % sucrose (in 1×PBS)** and OCT, frozen in ethanol bath (200 proof ethanol cooled in dry ice). Be careful to ensure that ethanol bath does not contact the embedded retina or the embedding solution.`

3 Store frozen blocks at **-80 °C** prior to sectioning.

Preparing the slide

4 

Prepare PDL using [M]**0.3 mg/ml PDL (sigma P7886)** dissolved in [M]**2 X Borate buffer** , diluted from [M]**20 X stock (Thermo Scientific, #28341)** with ddH2O.

5 Store PDL in aliquots at **-20 °C** .

6 

Apply PDL 8-well Ibidi chamber slide (Cat #80827) for at least **00:30:00** .

7 Then remove PDL.

8 Dry the slide.

9 

Wash once in **ddH2O.**

10 Dry again.

Sectioning

11 Place the PDL coated and fully dried slide inside the cryostat ahead of sectioning. Do not remove slide until all sections are in place or condensation will form that can impede adhesion.

12 Place sections inside wells using brushes, and flatten sections as much as possible to prevent folding when the slide is transferred to **Room temperature** .

13 

After removing the slide from the chamber, spin at **600 x g** for **00:03:00** (plate spinner centrifuge) 1-2 times to promote adhesion.

Hybridization

14 

Wash sections 3× **00:05:00** in PBSTw to remove OCT.

14.1 Wash sections **00:05:00** in PBSTw to remove OCT. (1/3)

14.2 Wash sections **00:05:00** in PBSTw to remove OCT. (2/3)

14.3 Wash sections **00:05:00** in PBSTw to remove OCT. (3/3)

15 

Replace PBSTw with Whyb.

16 Place in hybridization oven set to **43 °C** , minimum **00:10:00** .

In oven, set to 43°C

17 

Replace Whyb with Hyb1/Probe mixture pre-warmed to **43 °C** . Seal chamber with parafilm. Fill unused wells with Whyb to maintain humidity in the chamber sli.

18 

Incubate **16:00:00** minimum.

19

Replace Hyb1 with Whyb (quick wash to remove residual Hyb1).

20

Wash 2× **00:30:00** in Whyb.

20.1 Wash **00:30:00** in Whyb. (1/2)

20.2 Wash **00:30:00** in Whyb. (2/2)

21

Wash 2× **00:05:00** in **2 X SSCT** .

21.1 Wash **00:05:00** in **2 X SSCT** . (1/2)

21.2 Wash **00:05:00** in **2 X SSCT** . (2/2)

22

Return to **Room temperature** (*Pause point:* sample can be stored in **2 X SSCT** or **PBSTw** at **4 °C** for several weeks).

Fluorescent detection

23

Replace **2 X SSCTw** with **PBSTw** (2 washes at **Room temperature**).

24 Set oven to **37 °C** .

25 Transfer slide to **37 °C** for hybridization and subsequent wash steps.

26

Once slide is warm, remove PBSTw and add Hyb2/fluor solution (*prewarmed*).

27

Incubate at least **00:10:00** at **37 °C** .

28 

Replace Hyb2 with PBSTw (*prewarmed*, quick wash).

29 

Wash 2× **00:05:00** in PBSTw.

29.1 Wash **00:05:00** in PBSTw. (1/2)

30 

Return to **Room temperature** (*Pause point*: Samples can be stored at **4 °C** without obvious signal loss for at least 1 week).

Imaging

31 

Wash once in PBS and add mounting media until sections are covered (See below for notes on imaging).

Serial detection (complete before repeating fluorescent detection)

32 

Remove glycerol mounting media with 3 or 4 PBSTw washes (~ **00:05:00**).

32.1 Wash PBSTw. (1/4)

32.2 Wash PBSTw. (2/4)

32.3 Wash PBSTw. (3/4)

32.4 Wash PBSTw. (4/4)

33 

Wash 3× **00:05:00** in Displacement buffer at **Room temperature** .

33.1 Wash **00:05:00** in Displacement buffer at **Room temperature** . (1/3)

33.2 Wash **00:05:00** in Displacement buffer at **Room temperature** . (2/3)

33.3 Wash **00:05:00** in Displacement buffer at **Room temperature** . (3/3)

34

Wash 3× **00:02:00** in PBSTw.

34.1 Wash **00:02:00** in PBSTw. (1/3)

34.2 Wash **00:02:00** in PBSTw. (2/3)

34.3 Wash **00:02:00** in PBSTw. (3/3)