Stellaris RNA FISH

Protocol for C. elegans

General protocol and storage

Product description

A set of Stellaris™ RNA FISH Probes is comprised of up to 48 singly labeled oligonucleotides designed to selectively bind to targeted transcripts. Stellaris RNA FISH Probes bound to target RNA produce fluorescent signals that permit detection of single RNA molecules as diffraction-limited spots by conventional fluorescence microscopy.

Storage guidelines

Stellaris RNA FISH Probes

Stellaris RNA FISH Probes are shipped dry and can be stored at +2 to +8 °C in this state. Dissolved probe mix should be subjected to a minimum number of freeze-thaw cycles. For daily and short-term use of dissolved probe mix, storage at +2 to +8 °C in the dark for up to a month is recommended. For storage lasting longer than a month, we recommend aliquoting and freezing probes in the dark at -15 to -30 °C.

Stellaris RNA FISH Hybridisation Buffer

Stellaris RNA FISH Hybridisation Buffer should be stored at +2 to +8 °C for short-term and long-term use.

Stellaris RNA FISH Wash Buffer A and Wash Buffer B

Stellaris RNA FISH Wash Buffers A and B should be stored at room temperature for short-term and long-term use.

BIOSEARCH™ TECHNOLOGIES

GENOMIC ANALYSIS BY LGC

Stellaris RNA FISH

General protocol and storage

Reagents and equipment

Reagents and consumables:

a) M9 buffer (1.0 L H_2O, 5.8 g Na_2HPO_4, 3.0 g KH_2PO_4, 0.5 g NaCl, 1.0 g NH_4Cl)
b) Bleaching solution (40 mL of H_20, 7.2 mL 5N NaOH, 4.5 mL 6% Sodium Hypochlorite)
c) TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0)
d) 37% Formaldehyde Solution
e) 10X Phosphate Buffered Saline (PBS), RNase-free
f) Nuclease-free water
g) Deionised Formamide
h) Ethanol for molecular biology
i) Stellaris RNA FISH Hybridisation Buffer (Biosearch Technologies Cat# SMF-HB1-10)
j) Stellaris RNA FISH Wash Buffer A (Biosearch Technologies Cat# SMF-WA1-60)
k) Stellaris RNA FISH Wash Buffer B (Biosearch Technologies Cat# SMF-WB1-20)
l) 4',6-diamidino-2-phenylindole (DAPI)
m) Vectashield® Mounting Medium (Vector Laboratories Cat #H-1000)
n) 2-well chambered coverglass
o) 18 x 18 mm square #1 coverglass
p) RNase free consumables such as pipette tips
q) Humidified chamber (or equivalent): 150 mm tissue culture plate; bottom lined evenly with a flat water-saturated paper towel and a single layer of Parafilm® placed on top of the paper towel
r) 37 °C laboratory oven

Microscope:

a) Wide-field fluorescence microscope (e.g., Nikon Eclipse Ti or equivalent). We provide limited support for confocal applications.
b) A high numerical aperture (>1.3) and 60-100x oil-immersion objective.
c) Strong light source, such as a mercury or metal-halide lamp (newer LED-based light sources may also be sufficient).
d) Filter sets appropriate for the fluorophores.
e) Standard cooled CCD camera, ideally optimised for low-light level imaging rather than speed (13 µm pixel size or less is ideal).

Preparation of reagents

NOTE: When performing Stellaris RNA FISH, it is imperative to limit RNA degradation. Please ensure that all consumables and reagents are RNase-free. Recipes below are for set volumes. Please adjust accordingly.

Stellaris RNA FISH

General protocol and storage

Reconstituting the dried probe stock:

ShipReady Probe Set (1 nmol):

- A ShipReady probe set can provide up to 80 hybridisations.
- Re-dissolve the dried oligonucleotide probe blend in 80 µL of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) to create a probe stock of 12.5 µM.
- Mix well by pipetting up and down, and then vortex and centrifuge briefly.

DesignReady and Custom Probe Set (5 nmol):

- A DesignReady or custom probe set can provide up to 400 hybridisations.
- Re-dissolve the dried oligonucleotide probe blend in 400 µL of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) to create a probe stock of 12.5 µM.
- *Mix well by pipetting up and down*, and then vortex and centrifuge briefly.

M9 Buffer:

For a final volume of 1 L, mix:

- 1.0 L H_2O
- 5.8 g Na_2HPO_4
- 3.0 g KH_2PO_4
- 0.5 g NaCl
- 1.0 g NH_4Cl

Fixation Buffer:

Final composition is 3.7% (vol./vol.) formaldehyde in 1X PBS

For a final volume of 10 mL, mix:

- 1 mL 37% Formaldehyde solution
- 1 mL 10X Phosphate Buffered Saline (PBS), RNase-free
- 8 mL Nuclease-free water

Hybridisation Buffer:

Final composition is 10% (vol./vol.) formamide in Hybridisation Buffer

Hybridisation Buffer should be mixed fresh for each experiment:

- Due to viscosity of the solution, we recommend accounting for a 10% final volume excess in order to have enough Hybridisation Buffer for all of your samples.

For a final volume of 1 mL, mix:

- 900 µL Stellaris RNA FISH Hybridisation Buffer (Biosearch Technologies Cat# SMF-HB1-10)
- 100 µL Deionised Formamide

Note: Do not freeze Hybridisation Buffer.

WARNING! Formamide is a teratogen that is easily absorbed through the skin and should be used in a chemical fume hood.

WARNING! Be sure to let the formamide warm to room temperature before opening the bottle.

Wash Buffer A (10 mL):

Final composition is 10% (vol./vol.) formamide in 1X Wash Buffer A

Mix and dilute Wash Buffer A fresh for each experiment:

For a final volume of 10 mL, mix:

- 2 mL Stellaris RNA FISH Wash Buffer A (Biosearch Technologies Cat# SMF-WA1-60)
- Add 7 mL Nuclease-free water
- Add 1 mL Deionised Formamide
- Mix well by vortexing gently

Wash Buffer B:

Add Nuclease-free water to Wash Buffer B bottle upon first use.

- Add 88 mL of Nuclease-free water to bottle (Biosearch Technologies Cat# SMF-WB1-20) before use. Mix thoroughly.

Nuclear stain for use after hybridisation:

- 4',6-diamidino-2-phenylindole (DAPI) prepared in Wash Buffer A (see above) at 5 ng/mL. This solution is to be used in Step J of the Hybridisation for *C. elegans* using chambered coverglass section.

Mounting media:

- Vectashield Mounting Medium from Vector Laboratories (#H-1000).

Note: For best results, samples mounted with Vectashield Mounting Medium should be imaged the same day.

Protocol for C. elegans

Note: This protocol is adapted from the Raj lab protocol[3] and has not been tested at Biosearch Technologies. Currently, we can only offer limited support for the use of Stellaris probes on C. elegans.

Fixation of C. elegans embryos

a) To a plate of gravid hermaphrodites add 5 mL M9 buffer (1.0 L H_2O, 5.8 g Na_2HPO_4, 3.0 g KH_2PO_4, 0.5 g NaCl, 1.0 g NH_4Cl) and swirl to release worms from surface. Move worms to a 15 mL conical centrifuge tube.

b) Distilled or deionised water may be used instead of M9 in this and subsequent steps.

c) Spin down and add bleaching solution (40 mL of H_20, 7.2 mL

5N NaOH, 4.5 mL 6% Sodium Hypochlorite).

d) Vortex for roughly 4-8 minutes until worms disappear and only embryos remain.
e) Spin down and aspirate supernatant, then wash twice with M9 buffer.
f) Resuspend in 1 mL of fixation buffer, transfer to a microcentrifuge tube, and incubate at room temperature for 15 minutes.
g) Vortex and then immediately submerge tube in liquid nitrogen for 1 minute to freeze crack the embryos' eggshells.
h) Thaw in water at room temperature.
i) Once thawed, vortex and place on ice for 20 minutes.
j) Spin down and aspirate supernatant, then wash twice with 1 mL of 1X PBS.
k) To permeabilise, add 1 mL of 70% ethanol and store overnight at +2 to +8 °C. Embryos can be stored for up to one week between fixation and Hybridisation.

Fixation of C. elegans larvae

a) Grow larvae in a plate seeded with OP50.
b) Add 5 mL M9 buffer (1.0 L H_2O, 5.8 g Na_2HPO_4, 3.0 g KH_2PO_4, 0.5 g NaCl, 1.0 g NH_4Cl) and swirl in plate to release worms from surface, then move worms to a 15 mL conical centrifuge tube.
c) Distilled or deionised water may be used instead of M9 in this and subsequent steps.
d) Spin down worms and aspirate off the supernatant.
e) Wash with 5 mL M9 buffer.
f) Spin down worms and aspirate off the supernatant.
g) Add 1 mL fixation buffer, transfer to microcentrifuge tube, and incubate at room temperature for 45 minutes
h) Spin down and aspirate supernatant, then wash twice with 1 mL of 1X PBS.
i) To permeabilise, add 1 mL of 70% ethanol and store overnight at +2 to +8 °C. Larvae can be stored for up to one week between fixation and Hybridisation.

Hybridisation for C. elegans using chambered coverglass

If frozen before using, warm the reconstituted probe solution to room temperature. Mix well by vortexing, then centrifuge briefly.

To prepare the Hybridisation Buffer containing probe, add 1 µL of probe stock solution to 100 µL of Hybridisation Buffer, and then vortex and centrifuge (enough for one coverglass). This creates a working probe solution of 125 nM. This solution will be used on step d.

a) Centrifuge the fixed embryos or larvae to pellet and aspirate 70% ethanol.
b) Add 1 mL of Wash Buffer A (see recipe above), and incubate at room temperature for 2–5 minutes.
c) Assemble humidified chamber: 150 mm tissue culture plate; place a single water-saturated paper towel alongside the inner chamber edge. This chamber will help prevent evaporation of the probe solution from under the coverglass.

d) Spin down and aspirate Wash Buffer A, and dispense 100 µL of the Hybridisation Buffer containing probe onto the *C. elegans* embryos or larvae and transfer to the chambered coverglass.
e) Gently place a clean 18 x 18 mm #1 coverglass over the embryos or larvae within the chambered coverglass.
f) Place the chambered coverglass into the humidified chamber. Cover the humidified chamber with the lid and seal with Parafilm.
g) Incubate in the dark at 37 °C for at least 4 hours. (Incubation can be continued up to 16 hours).
h) Add 1 mL of Wash Buffer A to the chambered coverglass. Use forceps to carefully remove the 18 x 18 mm coverglass so as not to disturb the cells underneath.
i) Incubate in the dark at 37 °C for 30 minutes.
j) Aspirate Wash Buffer A, and then add 1 mL of DAPI nuclear stain (Wash Buffer A consisting of 5 ng/mL DAPI) to counterstain the nuclei.
k) Incubate in the dark at 37 °C for 30 minutes.
l) Aspirate the DAPI staining buffer, and then add 1 mL of Wash Buffer B. Incubate at room temperature for 2-5 minutes.
m) Add a small drop (approximately 15-30 µL) of Vectashield Mounting Medium onto the *C. elegans* embryos or larvae within the chambered coverglass. Place a clean 18 x 18 mm #1 coverglass over the cells to evenly spread the mounting medium. Alternatively, GLOX anti-fade may be used if necessary.

Proceed to imaging.

Stellaris RNA FISH

References and citation

References

1. Raj, A., van den Bogaard, P., Rifkin S.A., van Oudenaarden, A., and Tyagi, S. Imaging individual mRNA molecules using multiple singly labeled probes. *Nat. Methods*. 2008; 5: 877–9.
2. Femino, A.M., Fay, F.S., Fogarty, K., and Singer, R.H. Visualisation of single RNA transcripts *in situ*. *Science* 1998; 280: 585–90.
3. Raj A, Tyagi S. Detection of individual endogenous RNA transcripts in situ using multiple singly labeled probes. Methods in Enzymology 2010; 472: 365–86.

Guidelines for citing the use of Stellaris RNA FISH Probes and methods in scientific publications

Please acknowledge the use of Stellaris RNA FISH Probes and/or protocols in the experimental **Materials and Methods** or **Methods** section of your manuscript. *Refer to the following examples as guidelines for proper citation of the Stellaris RNA FISH probe sets and/or protocols:*

Citing catalogued probe sets:

"Stellaris RNA FISH Probes recognising <catalogued gene set name> and labeled with Quasar™ 570 dye (Catalog #, Biosearch Technologies, Inc., Petaluma, CA) were hybridised to <samples>, following the manufacturer's instructions available online at www.biosearchtech.com/stellarisprotocols (access date). Briefly, <describe any deviations from the published protocol or a short summary of what was actually performed>."

Citing custom probe sets designed with the Stellaris RNA FISH Probe Designer:

"Custom Stellaris FISH Probes were designed against <your RNA of interest (include NM# and nucleotides covered if relevant)> by utilising the Stellaris RNA FISH Probe Designer (Biosearch Technologies, Inc., Petaluma, CA) available online at www.biosearchtech.com/stellarisdesigner (version #). The <samples> were hybridised with the <your RNA of interest> Stellaris RNA FISH Probe set labeled with <your dye of choice> (Biosearch Technologies, Inc.), following the manufacturer's instructions available online at www.biosearchtech.com/stellarisprotocols. Briefly, <describe any deviations from the published protocol or a short summary of what was actually performed>."

Citing custom probe sets utilising previously published sequences:

"Custom Stellaris RNA FISH Probes recognising <your RNA of interest (include NM# and nucleotides covered if relevant)> and labeled with <your dye of choice>, were purchased from Biosearch Technologies, Inc. (Petaluma, CA). Probe set sequences utilised in the experiments have been previously described <cite published manuscript>. The <samples> were hybridised with the <your RNA of interest> Stellaris RNA FISH Probe set, following the manufacturer's instructions available online at www.biosearchtech.com/stellarisprotocols. Briefly, <describe any deviations from the published protocol or a short summary of what was actually performed>."

Citing 3' Amine Oligos in plates used for Stellaris RNA FISH designed with the Stellaris FISH Probe Designer:

"Custom 3' amine oligos in plates were designed against <your RNA of interest (include NM# and nucleotides covered if relevant)> by utilising the Stellaris RNA FISH Probe Designer (Biosearch

Stellaris RNA FISH

References and citation

Technologies, Inc., Petaluma, CA) available online at www.biosearchtech.com/stellarisdesigner (version #). Probes were labeled with <your dye of choice> using <insert your labeling protocol or citation of previously published labeling protocol>. The <samples> were hybridised with the <your RNA of interest> oligonucleotides (Biosearch Technologies, Inc.), following the manufacturer's instructions available online at www.biosearchtech.com/stellarisprotocols. Briefly, <describe any deviations from the published protocol or a short summary of what was actually performed>."

Citing 3' Amine Oligos in plates used for Stellaris RNA FISH using previously published sequences:

"Custom 3' amine oligos in plates recognising <your RNA of interest (include NM# and nucleotides covered if relevant)> were purchased from Biosearch Technologies, Inc. (Petaluma, CA). Probe set sequences utilised in the experiments have been previously described <cite published manuscript>. Probes were labeled with <your dye of choice> using <insert your labeling protocol or citation of previously published labeling protocol>. The <samples> were hybridised with the <your RNA of interest> oligonucleotides (Biosearch Technologies, Inc.), following the manufacturer's instructions available online at www.biosearchtech.com/stellarisprotocols (access date). Briefly, <describe any deviations from the published protocol or a short summary of what was actually performed>."

Technical support

If you require additional information or technical assistance please feel free to email our Technical Support Team at: techsupport@lgcgroup.com.

Integrated tools.
Accelerated science.

@LGCBiosearch | biosearchtech.com

BIOSEARCH™ TECHNOLOGIES
GENOMIC ANALYSIS BY LGC