Stellaris RNA FISH

Protocol for *S. cerevisiae*

General protocol and storage

Product description

A set of Stellaris™ RNA FISH Probes is comprised of up to 48 singly labelled 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 Hybridization Buffer

Stellaris RNA FISH Hybridization 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

Protocol for *S. cerevisiae*

Reagents and equipment

Reagents and consumables:

a) TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0)
b) 37% formaldehyde solution
c) Nuclease-free water
d) Deionised formamide
e) Sorbitol powder
f) Potassium phosphate dibasic (K_2HPO_4) powder
g) Ethanol for molecular biology
h) Zymolyase
i) Stellaris RNA FISH Hybridization Buffer (LGC, Biosearch Technologies Cat# SMF-HB1-10, SMF-HB1-10)
j) Stellaris RNA FISH Wash Buffer A (Biosearch Technologies Cat# SMF-WA1-60, SMF-WA1-60)
k) Stellaris RNA FISH Wash Buffer B (Biosearch Technologies Cat# SMF-WB1-20, SMF-WB1-200)
l) 4',6-diamidino-2-phenylindole (DAPI)
m) Vectashield® Mounting Medium (Vector Laboratories Cat #H-1000)
n) CoverGrip™ Coverslip Sealant (Biotium Cat# 23005) or clear nail polish
o) 18 × 18 mm square #1 coverglass
p) RNase free consumables such as pipette tips
q) Kimwipes™
r) Superfrost Plus Microscope slides
s) 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 or sCMOS camera, ideally optimised for low-light level imaging rather than speed (13 µm pixel size or less is ideal).

Stellaris RNA FISH

Protocol for *S. cerevisiae*

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 two set volumes (1X and 10X). Please adjust accordingly.

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 to create a probe stock of 12.5 µM. *Mix well by pipetting up and down, and then vortex and centrifuge briefly.*

DesignReady or 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 to create a probe stock of 12.5 µM. *Mix well by pipetting up and down, then vortex and centrifuge briefly.*

Fixation Buffer:

Mix 1.2 M Sorbitol, 0.1 M Potassium phosphate dibasic, pH 7.5, for a final volume of 1 L stock:

- 218 g Sorbitol powder (Mw = 182.17 g)
- 17.4 g Potassium phosphate dibasic powder
- Nuclease-free water to a final volume of 1,000 mL

Hybridization Buffer (1 mL/10 mL):

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

Hybridization 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 Hybridization Buffer for all of your samples.

For a final volume of 1 mL (10 mL), mix:

- 900 µL (9 mL) Stellaris RNA FISH Hybridization Buffer
- 100 µL (1 mL) deionised formamide

Mix thoroughly by vortexing and pipetting up and down.

NOTE: Do not freeze Hybridization Buffer.

WARNING! Formamide is a teratogen that is easily absorbed through the skin and should be used in a chemical fume hood. Please consult the appropriate SDS (Safety Data Sheet) prior to use.

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

Wash Buffer A (10 mL/100 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 (100 mL), mix:

- 2 mL (20 mL) Stellaris RNA FISH 5X Wash Buffer A
- Add 7 mL (70 mL) nuclease-free water
- Add 1 mL (10 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 (880 mL) of nuclease-free water to bottle 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 hybridisation in step g below.

Mounting media:

- Vectashield Mounting Medium from Vector Laboratories.

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

Protocol for *S. cerevisiae*

NOTE: This protocol is adapted from the Raj lab protocol[3] and has not been tested at Biosearch Technologies. Currently, we only offer limited support for the use of Stellaris RNA FISH Probes on yeast such as *S. cerevisae*.

Fixation of *S. cerevisiae*

a) Grow yeast to an OD of around 0.2-0.4 in a 45 mL volume of minimal media. Do not fix yeast at OD>0.5.
b) Add 5 mL of 37% formaldehyde directly to the growth media. Mix well by inverting conical tube a few times and incubate for 45 minutes at room temperature.
c) Centrifuge at 1600 x g for 4 minutes to spin down yeast.
d) Resuspend cells in 1 mL of ice-cold Fixation buffer. Transfer to a 1.5 mL microcentrifuge tube.
e) Spin down and wash cells once more in 1 mL of ice-cold Fixation buffer.
f) Resuspend cells in 1 mL of Fixation buffer plus 2.5 µL Zymolyase. Digest at 30 °C until most of the cells turn dark when checked by a phase contrast microscope. Note: Zymolyase digestion usually takes 45-90 minutes, depending on cell concentration.

g) Centrifuge at 400 x g for 5-6 minutes to spin down yeast. Note: Do not spin faster than 1,000 x g after digestion with zymolyase. Cells may be subject to bursting and deformation due to cell wall digestion.
h) Wash cells with ice-cold Fixation buffer. Centrifuge at 400 x g for 5-6 minutes to spin down yeast. Repeat once more.
i) To permeabilise, resuspend 1 mL of 70% ethanol and store overnight at +2 to +8 °C. Cells can be stored at +2 to +8 °C in 70% ethanol up to a week before hybridisation.

Hybridisation for *S. cerevisiae*

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

To prepare the Hybridization Buffer containing probe, add 1 µL of probe stock solution to 100 µL of Hybridization 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 b.

a) Centrifuge 300 µL of fixed yeast cells in 70% ethanol at 400 x g.
b) Resuspend yeast in 100 µL of Hybridization Buffer containing probe. Mix well by pipetting up and down.
c) Incubate in the dark at 30 °C overnight.
d) Add 100 µl of Wash Buffer A (see recipe above) to the hybridised yeast cells. Centrifuge at 400 x g for 5 minutes. Aspirate carefully (pellet will be fluffy and easy to lose).
e) Resuspend yeast in 1 mL of Wash Buffer A and incubate in the dark at 30 °C for 30 minutes.
f) Centrifuge at 400 x g for 5 minutes.
g) Resuspend yeast in 1 mL of DAPI nuclear stain (Wash Buffer A consisting of 5 ng/mL DAPI) to counterstain the nuclei and incubate in the dark at 30 °C for 30 minutes.
h) Centrifuge at 400 x g for 5 minutes.
i) Resuspend yeast in 1 mL of Wash Buffer B for 2-5 minutes.
j) Centrifuge at 400 x g for 5 minutes.
k) Resuspend yeast in a small drop (approximately 15-30 µL) of Vectashield Mounting Medium.
l) Place 5-10 µL of Vectashield suspended yeast cells on a clean glass microscope slide and then place an 18 × 18 mm square #1 coverglass over the cells to evenly spread the solution.
m) Place a Kimwipe over the cover glass and apply gentle pressure over the surface of the coverglass, pressing it firmly onto the surface of the slide. While applying pressure, be careful not to move the coverglass horizontally as this could result in sheared cells. The Kimwipe will wick up excess mounting medium.
n) Seal the cover glass perimeter with CoverGrip (or clear nail polish), and allow to dry.

Proceed to imaging.

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, 877-879. doi: 10.1038/nmeth.1253
2. Femino, A.M., Fay, F.S., Fogarty, K., and Singer, R.H. Visualisation of single RNA transcripts *in situ.* Science 1998; 280, 585–590. doi: 10.1126/science.280.5363.585
3. Raj A, Tyagi S. Detection of individual endogenous RNA transcripts in situ using multiple singly labeled probes. Methods Enzymol. 2010; 472, 365-86. doi: 10.1016/S0076-6879(10)72004-8

Guidelines 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 labelled with <your dye of choice> (Catalog #, LGC, Biosearch Technologies, Petaluma, CA) were hybridised to <samples>, 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 designed with the Stellaris FISH Probe Designer:

"Custom Stellaris™ RNA FISH Probes were designed against <your RNA of interest (include accession number and nucleotides covered if relevant)> by utilising the Stellaris RNA FISH Probe Designer (LGC, Biosearch Technologies, 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 labelled with <your dye of choice> (Biosearch Technologies), 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 accession number and nucleotides covered if relevant)> and labelled with <your dye of choice>, were purchased from LGC, Biosearch Technologies (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>."

Stellaris RNA FISH

Protocol for *S. cerevisiae*

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 accession number and nucleotides covered if relevant)> by utilising the Stellaris™ RNA FISH Probe Designer (LGC, Biosearch Technologies, Petaluma, CA) available online at www.biosearchtech.com/stellarisdesigner (version #). Probes were labelled with <your dye of choice> using <insert your labellling protocol or citation of previously published labelling protocol>. The <samples> were hybridised with the <your RNA of interest> oligonucleotides (Biosearch Technologies), 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 accession number and nucleotides covered if relevant)> were purchased from LGC, Biosearch Technologies (Petaluma, CA). Probe set sequences utilised in the experiments have been previously described <cite published manuscript>. Probes were labelled with <your dye of choice> using <insert your labelling protocol or citation of previously published labelling protocol>. The <samples> were hybridised with the <your RNA of interest> oligonucleotides (Biosearch Technologies), 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>."

Technical support

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

For Research Use Only. Not for use in diagnostic procedures.

Integrated tools.
Accelerated science.

@LGCBiosearch | biosearchtech.com

BIOSEARCH™ TECHNOLOGIES
GENOMIC ANALYSIS BY LGC