# Stellaris® RNA fluorescence *in situ* hybridization (RNA FISH) for the detection of long non coding RNA biomarkers

#A2-44

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## ABSTRACT

Long non-coding RNAs (lncRNAs) play important roles in all the central aspects of the gene expression cascade with wide ranging effects on cell proliferation and differentiation. As such, lncRNAs are highly promising additions to many cancer biomarkers portfolios and enable more precise diagnostic staging. This, in turn, provides a clearer prognosis and treatment options of different cancers. Direct visualization and quantification of lncRNAs in single cells and in tissue are thus emerging as essential tools for both basic and clinical research. Interestingly, studies have demonstrated that transcript levels are more strongly correlated to clinical traits than the corresponding protein levels. Because RNA biomarkers have the potential to contribute to the development of targeted cancer therapies, there is also an obvious need for a robust and dependable methodology to discover and validate these RNAs.

RNA fluorescence *in situ* hybridization (RNA FISH) provides a powerful means to detect RNA biomarkers in single cells, while still maintaining tissue morphology. Significant advances in RNA FISH technology, such as the hybridization of pools of singly labeled fluorescent 20-mer oligonucleotides to the RNA target, now afford specific and sensitive multiplex detection of RNA to yield information on the RNA's distinct spatial distribution within cells and tissue.

Data is presented on the design of probe sets against multiple disease relevant pre-lncRNAs and against the introns of their precursors, as well as on the simultaneous dual-channel imaging of the pre-lncRNAs and mature lncRNAs. Because the absolute majority of post-transcriptional processing, including splicing of mRNAs and lncRNAs, occurs co-transcriptionally, the location of introns can be used as a proxy nuclear location for the encoding gene. We find mature lncRNAs to locate at or close to the site of transcription, but also well away from it. Next, we present data on emerging non-coding RNA biomarkers in breast and prostate cancer tissue and in cell lines utilizing RNA FISH.

## METHOD

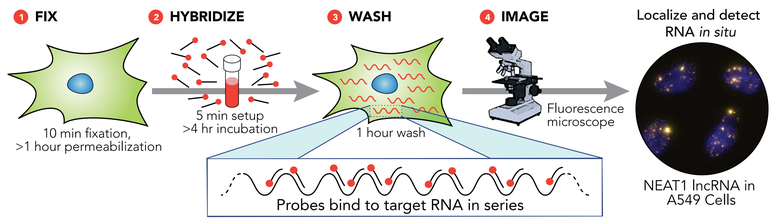



**Figure 1. Schematic of Stellaris RNA FISH assay**
For each target, a mix of multiple 20-mer oligonucleotides, each labeled with a single Quasar® 570, Quasar 670, or CAL Fluor® Red 610 fluorophore was designed* and synthesized. All probe sets contained at least 32 oligos. Adherent cells were grown on #1 cover glass and subsequently fixed and permeabilized. Hybridizations were carried out for 4 to 16 hours at 37 °C in 50 µl hybridization solution (10% dextran sulfate, 10% formamide in 2X SSC). Samples were then washed, DAPI stained, and imaged.

*Stellaris probe designer and protocols: www.biosearchtech.com/stellaris

## RNA FISH IN ADHERENT CELL LINES

MYC, PVT1, and H19 belong to a triumvirate of aggressive oncogenes.

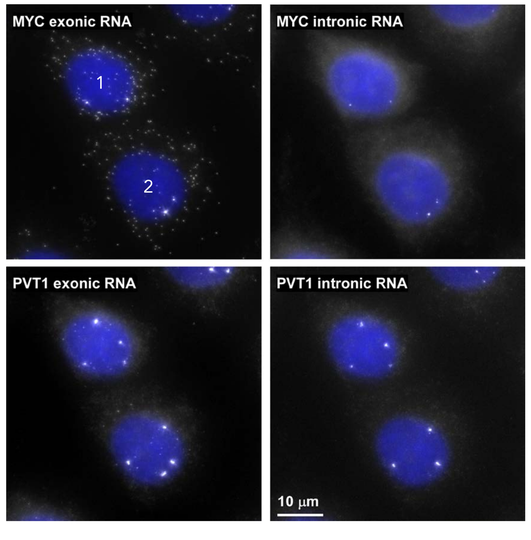



**Figure 2. Quadruplex Stellaris RNA FISH detection of MYC mRNAs and PVT1 lncRNAs.**
Fixed A549 cells were probed simultaneously for mature MYC mRNAs, MYC introns, mature PVT1 lncRNAs and PVT1 introns. Four bright nuclear foci for PVT1 introns indicates robust transcription from all four PVT1 loci, in contrast to the two bright and two weak MYC intron foci indicating variable activity at the MYC loci.

1: 123 spots
2: 135 spots

## RNA FISH IN ADHERENT CELL LINES (CONT'D)

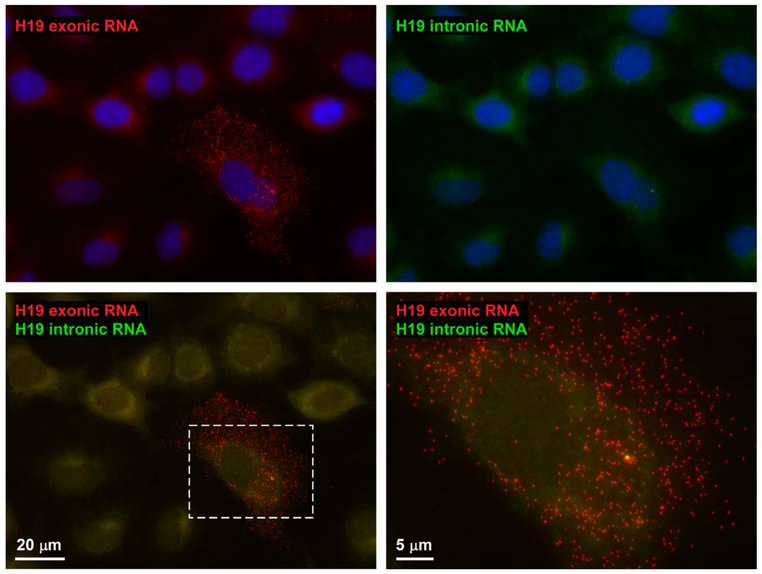
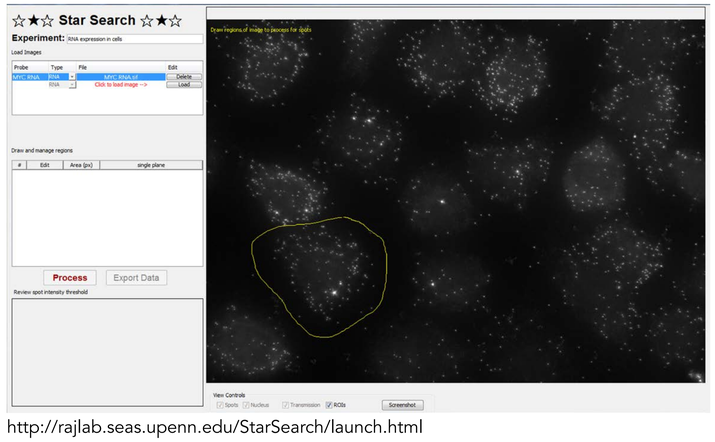



**Figure 3. Duplex Stellaris RNA FISH to detect H19 lncRNA introns and exons.**
Fixed A549 cells were probed simultaneously for mature H19 lncRNAs (pseudo colored red) and introns (pseudo colored green). A Single nuclear transcription burst is revealed by both H19 probe sets as expected for the paternally imprinted gene, and mature H19 lncRNAs consistently localized to cytoplasm, revealed as punctate spots by the exonic probe set.

RNA Spot Counting Software

A




http://rajlab.seas.upenn.edu/StarSearch/launch.html

B

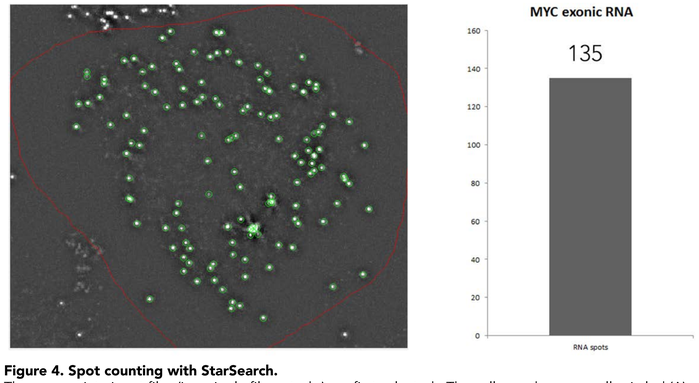



**Figure 4. Spot counting with StarSearch.**
The appropriate input files (i.e., single file z-stacks) are first selected. The cells are then manually circled (A), and the spots are identified and enumerated (B).

## RNA FISH IN TISSUE

Overexpression of PCA3 lncRNAs is a specific prostate biomarker.

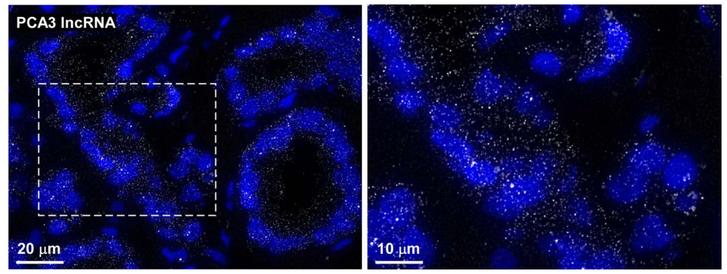



**Figure 5. PCA3 gene expression in FFPE prostate cancer tissue.**
PCA3 lncRNAs primarily localize to the cytoplasm of cells in the prostatic glands and not in the stroma.

H19 and HOTAR lncRNA gene expression in ovarian cancer tissue.

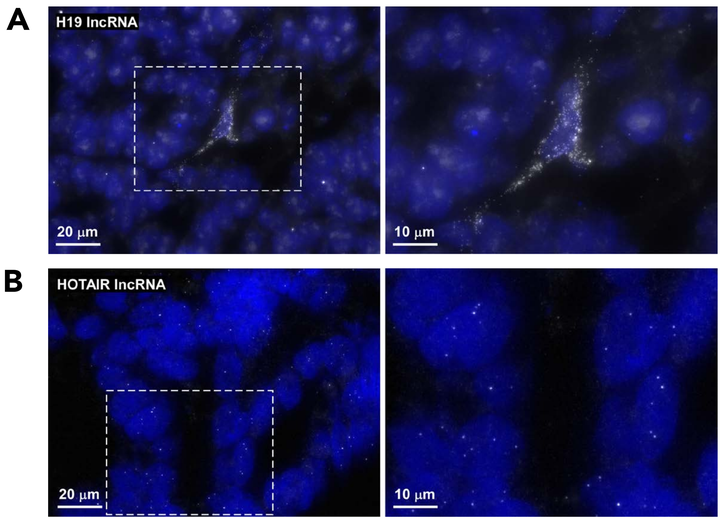



**Figure 6. H19 and HOTAIR lncRNAs in frozen human ovarian tissue.**
(A) Mature H19 lncRNAs consistently localized to the cytoplasm in human ovarian tissue samples, consistent with the work in adherent cells. (B) Conversley, HOTAIR lncRNAs are predominantly nuclear.

## CONCLUSIONS AND REFERENCES

Stellaris FISH is a method that enables detection, localization, and quantification of RNA biomarkers (both mRNA and lncRNA) at the single cell level. The results and tools presented here will contribute to advancing the current capabilities of the detection and treatment of specific cancers, as well as in the continued discovery and development of cancer drug candidates.

- Tseng YY, Moriarity BS, Gong W, Akiyama R, Tiwari A, Kawakami H, Ronning P, Reuland B, Guenther K, Beadnell TC, Essig J, Otto GM, O'Sullivan MG, Largaespada DA, Schwertfeger KL, Marahrens Y, Kawakami Y, Bagchi A. (2014). PVT1 dependence in cancer with MYC copy-number increase. Nature. 512(7512):82-6.
- Barsyte-Lovejoy D, Lau SK, Boutros PC, Khosravi F, Jurisica I, Andrulis IL, Tsao MS, Penn LZ. (2006). The c-Myc oncogene directly induces the H19 noncoding RNA by allele-specific binding to potentiate tumorigenesis. Cancer Res. 66(10):5330-7.
- de Kok JB, Verhaegh GW, Roelofs RW, Hessels D, Kiemeney LA, Aalders TW, Swinkels DW, Schalken JA. (2002). DD3(PCA3), a very sensitive and specific marker to detect prostate tumors. Cancer Res. 62(9):2695-8.
- Coassin SR, Orjalo AV, Semaan SJ, Johansson HE. (2014) Simultaneous detection of nuclear and cytoplasmic RNA variants utilizing Stellaris® fluorescence in situ hybridization in adherent cells. Methods Mol Biol. 1211:189-99.

## ACKNOWLEDGEMENTS

**Dr. Ron Cook, Peter Coassin and the Biosearch Technologies Stellaris Team**
**OPKOLab and**
**Dr. Louis Dubeau - Keck School of Medicine of USC, ovarian tissue samples**



Scan this QR Code to learn more about Stellaris RNA FISH Probes.