

ABSTRACT

Most RNAs produced by the extensive transcription of the genome lack protein coding potential. Long non-coding RNAs (lncRNAs) play important roles in all the central aspects of the gene expression cascade, beginning in the nucleus and moving to the cytoplasm and beyond. lncRNAs have wide ranging effects on events such as the cell cycle and differentiation. Direct visualization and quantification of lncRNAs in single cells are emerging as essential tools for both basic and clinical research.

We have streamlined the process of producing probe sets for Stellaris RNA fluorescence *in situ* hybridization (RNA FISH), from design to synthesis of pools of singly labeled fluorescent 20-mer oligonucleotides. Stellaris RNA FISH affords specific and sensitive detection of the target RNA while establishing the intracellular localization of the target lncRNAs. If observed as single molecules, these discrete spots can be accurately counted. A similar T_m for all probe sets further allows for simultaneous detection of multiple RNA targets. The hybridization conditions also allow immunofluorescence and RNA FISH to be performed on the same samples.

We present here data on the design of probe sets against the introns of multiple disease relevant pre-lncRNAs, as well as the simultaneous dual-channel imaging of the intronic RNAs with the 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. We also examine the location of the mature lncRNAs in relation to NEAT1 in nuclear paraspeckles.

METHOD

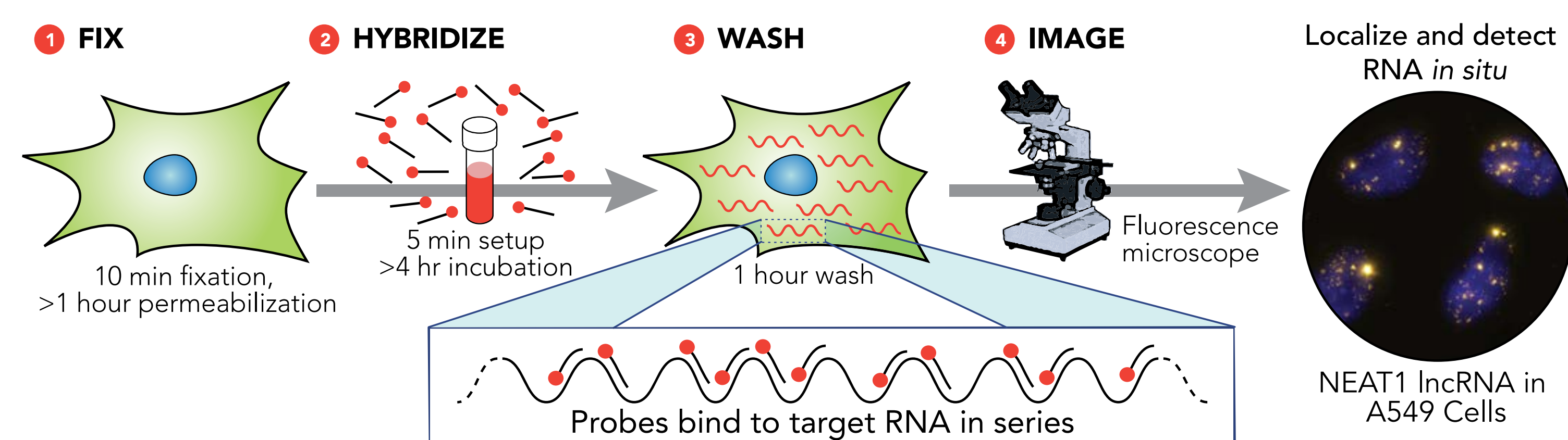


Figure 1. Schematic of Stellaris RNA FISH assay

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

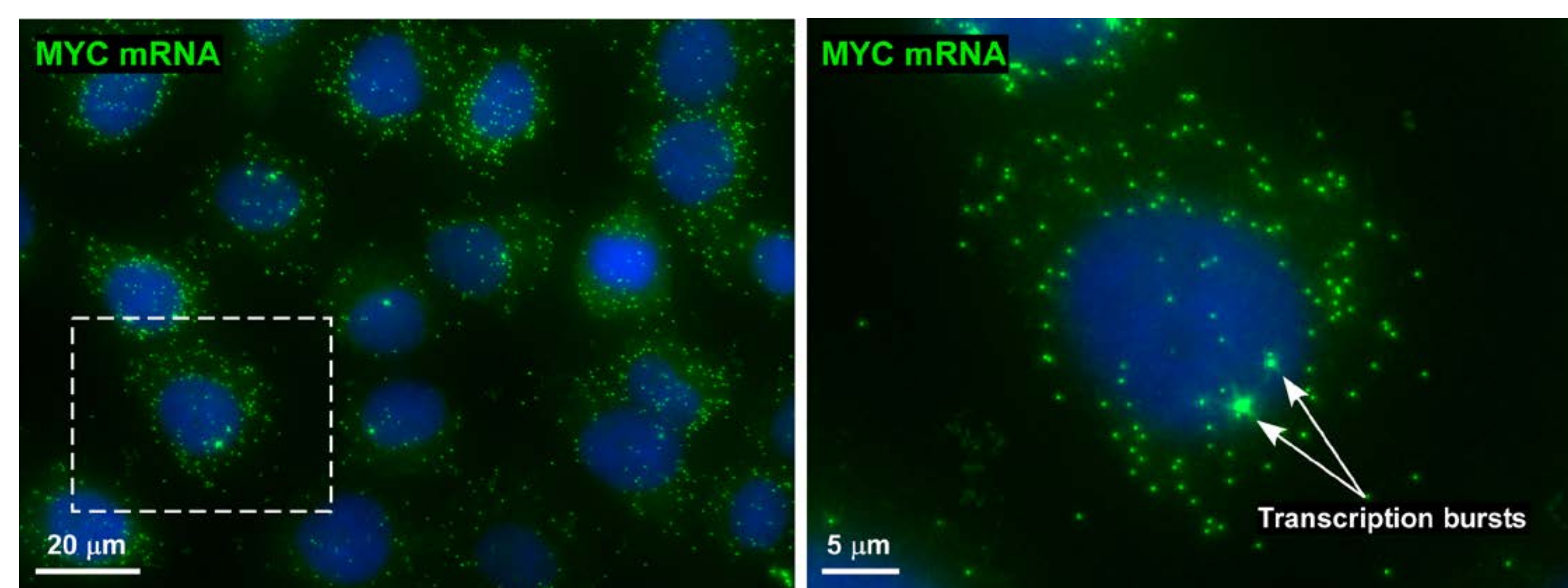


Figure 2. Stellaris RNA FISH detection of MYC mRNAs.

Fixed A549 cells were probed for mature MYC mRNAs. Two bright nuclear foci are often observed, corresponding to the buildup of nascent mRNAs (transcription bursts).

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Probes.



RNA FISH IN ADHERENT CELL LINES (CONT'D)

PVT1 lncRNAs co-increase in MYC-driven cancers.

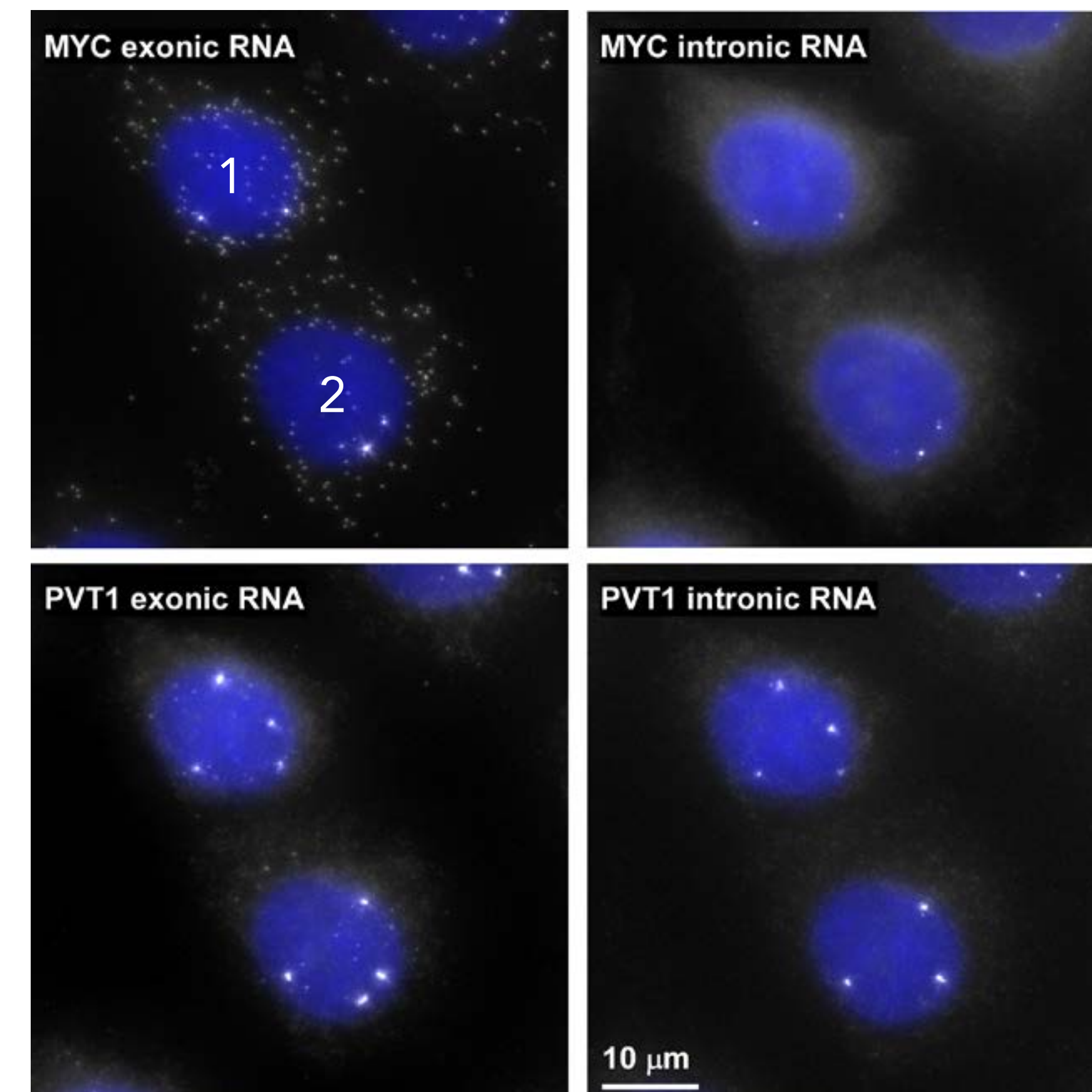


Figure 3. Quadruplex Stellaris RNA FISH detection of MYC mRNAs and PVT1 lncRNAs.

Fixed A549 cells were probed simultaneously for mature MYC mRNAs, MYC introns, mature PVT1 lncRNAs and PVT1 introns. Discrete single molecule spots and bright mature PVT1 foci co-localizing together with the PVT1 introns indicate that PVT1 functions at and away from site of synthesis. 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

PVT1 lncRNAs co-localize with NEAT1-containing paraspeckles.

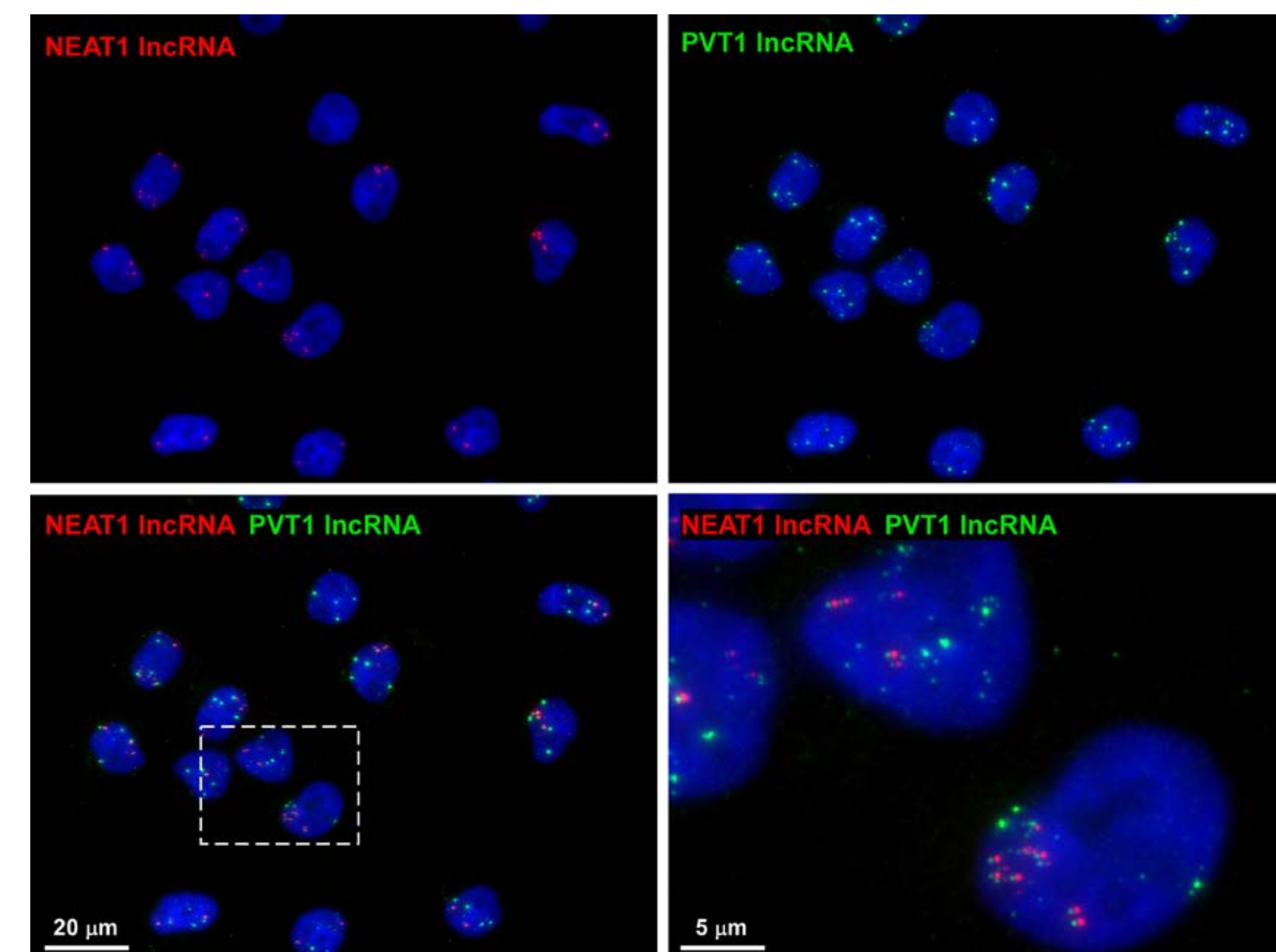


Figure 4. Stellaris RNA FISH detection of NEAT1 and PVT1 lncRNAs.

Fixed A549 cells were probed for mature NEAT1 and PVT1 lncRNAs. Co-localization of PVT1 single molecules and the paraspeckles landmarker NEAT1 is often observed, suggesting an important biological interaction and function of mature PVT1 lncRNAs.

CONCLUSIONS AND REFERENCES

In summary, the combination of RNA FISH to detect pre-lncRNA introns, also known as an iceFISH[™] assay, with imaging of mature lncRNAs provides a useful tool to gauge if the site of action of a particular lncRNA is proximal or distal to its encoding gene locus

- Tseng YY, Moriarity BS, Gong W, et al. (2014). PVT1 dependence in cancer with MYC copy-number increase. *Nature*. 512: 82-6.
- 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
- Orjalo AV and Johansson HE. (In Press) Stellaris[®] RNA fluorescence in situ hybridization for the simultaneous detection of immature and mature long non-coding RNAs in adherent cells. *Methods Mol Biol*.

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