Imaging of disease relevant lncRNAs by Stellaris® RNA fluorescence *in situ* hybridization (RNA FISH)

Hans E. Johansson, Arturo V. Orjalo, Jr., Sally R. Coassin
Biosearch Technologies, Inc., Petaluma, CA

Abstract

Most RNAs produced by the extensive transcription of the genome lack protein coding potential. Long non-coding RNAs (lncRNAs) have been shown to play important roles in all the central aspects of gene function including imprinting, epigenetic regulation, transcription, splicing and nuclear/cytoplasmic trafficking, translation and RNA stability. These all have wide ranging effects on cell cycle and differentiation. Direct visualization and quantification of lncRNAs in single cells now enters as an essential tool for both basic science and clinical research.

We recently streamlined both the design and synthesis of fluorescently labeled probe sets containing multiple, tiled 20-mer GC-balanced oligonucleotides for use in RNA fluorescence *in situ* hybridization (RNA FISH). In contrast to traditional collective and cell-disruptive RNA analyses (*e.g.* northern blotting and qPCR) that report relative population averages of RNA content, single molecule (sm)RNA FISH affords specific and sensitive detection and quantification of RNAs, while establishing the intracellular localization of the target lncRNAs. Probe sets with balanced binding characteristics further allow simultaneous detection of multiple RNA targets. The gentle hybridization conditions also enable immunofluorescence and smRNA FISH to be performed on the same samples.

We present here data on the expression of multiple disease relevant lncRNAs. We verify the location of and large variation in expression for RNAs such as MALAT1 to nuclear speckles, and NEAT1 to nuclear paraspeckles. In effect, such RNA FISH probe sets function as "RNA land markers", taking the place of immunofluorescence with particle specific antibodies. The subcellular location of several other lncRNAs and the intracellular variation in their expression is also presented for tumor cell lines and primary tumor tissues. RNA FISH multiplexing for lncRNAs and indicator mRNAs provides the appropriate framework to address physiologically induced changes in lncRNA expression and patterns.

Our continued efforts will shed light on how expression and localization of lncRNAs may contribute to the diseased phenotype, while adding a large selection of novel biomarkers that can be interrogated for improved diagnosis, prognosis, and therapeutic choice.

Methods

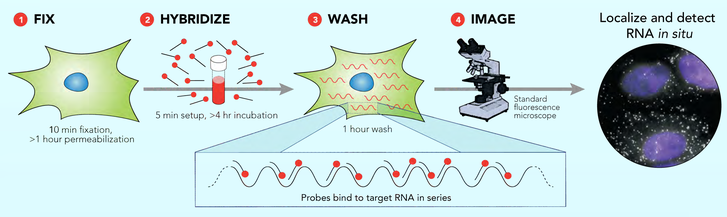

Figure 1. Stellaris RNA FISH

For each target, a mix of multiple 20-mer oligonucleotides, each labeled with a single Quasar® 570 or Quasar 670 fluorophore was designed* and synthesized. All probe sets contained ≥ 36 oligos each, unless noted. Human A549 cells grown on coverslips were 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, nuclei stained with DAPI, and imaged.

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

NEAT1

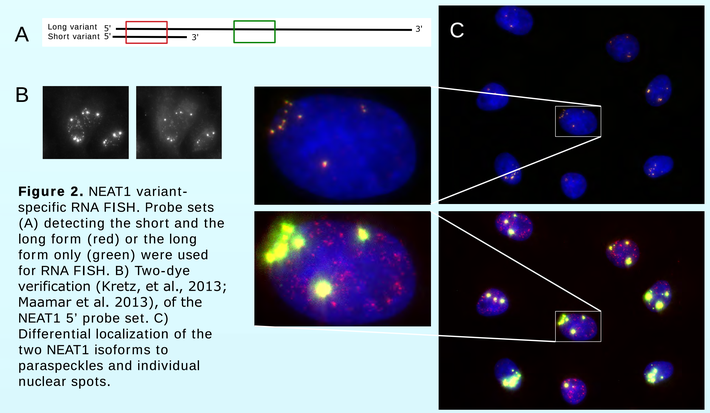

Figure 2. NEAT1 variant-specific RNA FISH. Probe sets (A) detecting the short and the long form (red) or the long form only (green) were used for RNA FISH. B) Two-dye verification (Kretz, et al., 2013; Maamar et al. 2013), of the NEAT1 5' probe set. C) Differential localization of the two NEAT1 isoforms to paraspeckles and individual nuclear spots.

MALAT1

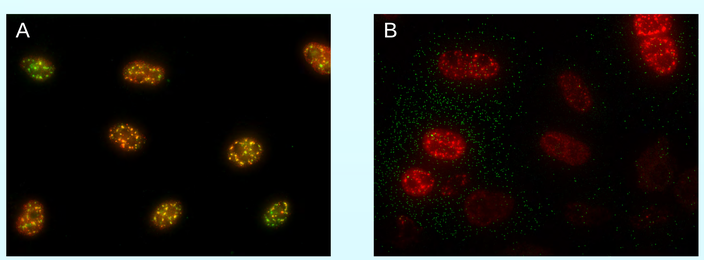

Figure 3. MALAT1 lncRNA. A) RNA FISH with a Quasar 570 labeled MALAT1 probe set (red) combined with immunofluorescence with an α-SC35 mAb (green) reveals co-localization. B) Strong co-expression of MALAT1 lncRNA (red) and mRNA for the G1 cell cycle marker CDKN1A (p21, Cip1) (green). No correlation was observed for CDK4 or CCND1.

H19, TERC, PCA3, XIST

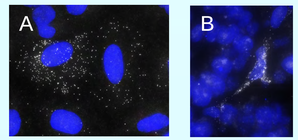

Figure 4. Cytoplasmic localization of H19 lncRNA in cultured cells (A) and frozen ovarian tissue (B). Note cell-to-cell variability.

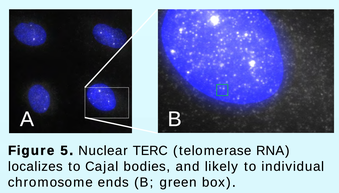

Figure 5. Nuclear TERC (telomerase RNA) localizes to Cajal bodies, and likely to individual chromosome ends (B; green box).

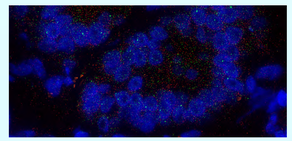

Figure 6. PCA3 lncRNA (green) and GAPDH mRNA (red) distribution in FFPE prostate tissue.

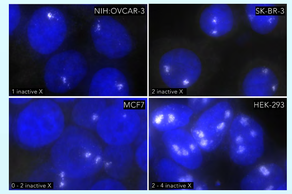

Figure 7. XIST clouds in 4 female cancer cell lines with variable X-chromosome karyotype.

References

- Batish M, Raj A, Tyagi S. (2011) Single molecule imaging of RNA *in situ*. *Methods Mol. Biol.* **714,** 3-13.
- Kretz M, Siprashvili Z, Chu C, Webster DE, Zehnder A, Qu K, Lee CS, Flockhart RJ, Groff AF, Chow J, Johnston D, Kim GE, Spitale RC, Flynn RA, Zheng GX, Aiyer S, Raj A, Rinn JL, Chang HY, Khavari PA. (2013) Control of somatic tissue differentiation by the long non-coding RNA TINCR. *Nature* **493**(7431), 231-5.
- Maamar H, Cabili MN, Rinn J, Raj A. (2013) linc-HOXA1 is a noncoding RNA that represses Hoxa1 transcription in *cis*. *Genes Dev.* **27**(11), 1260-71.
- Mao YS, Sunwoo H, Zhang B, Spector DL. (2011) Direct visualization of the co-transcriptional assembly of a nuclear body by noncoding RNAs. *Nature Cell Biol.* **13**(1), 95-101.
- Naganuma T, Nakagawa S, Tanigawa A, Sasaki YF, Goshima N, Hirose T. (2012) Alternative 3'-end processing of long noncoding RNA initiates construction of nuclear paraspeckles. *EMBO J.* **31**(20), 4020-34.
- Orjalo A Jr, Johansson HE, Ruth JL (2011) Stellaris™ fluorescence *in situ* hybridization (FISH) probes: a powerful tool for mRNA detection. *Nature Methods* **8**(10), pp. I-III.
- Raj A, Tyagi S. (2010) Detection of individual endogenous RNA transcripts *in situ* using multiple singly labeled probes. *Methods Enzymol.* **472,** 365-85.
- Sirchia SM, Tabano S, Monti L, Recalcati MP, Gariboldi M, Grati FR, Porta G, Finelli P, Radice P, Miozzo M. (2009) Misbehaviour of XIST RNA in breast cancer cells. *PLoS One.* **4**(5), e5559.
- Yang F, Bi J, Xue X, Zheng L, Zhi K, Hua J, Fang G. (2012) Up-regulated long non-coding RNA H19 contributes to proliferation of gastric cancer cells. *FEBS J.* **279**(17), 3159-65.
- Zhong FL, Batista LF, Freund A, Pech MF, Venteicher AS, Artandi SE. (2013) TPP1 OB-fold domain controls telomere maintenance by recruiting telomerase to chromosome ends. *Cell* **150**(3), 481-94.

Disclaimers

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