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Advancing Nucleic Acid Technology℠

Detection of Long Non-coding RNAs by Stellaris™ FISH Probes

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Agenda

- Background of lncRNA mechanisms of gene regulation
- Methodology and application of Stellaris FISH
- Examples of lncRNAs as visualized by Stellaris FISH

Long non-coding RNAs (lncRNAs)

Eukaryotic genomes transcribe up to 90% of the genomic DNA

- ONLY 1 – 2% of these transcripts encode for protein
- Vast majority = non-coding RNAs (ncRNAs)

Largest subset of ncRNAs = long non-coding RNA (lncRNA) (>200 nt in length)

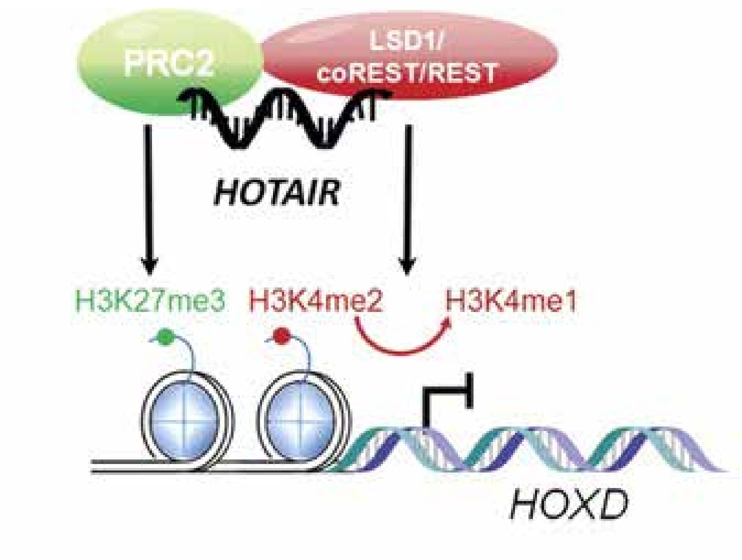
Functionally implicated in embryonic stem cell pluripotency, cell cycle regulation, and diseases (e.g,. cancer).

LncRNA function and activity remain poorly understood.

How do lncRNAs regulate gene expression?

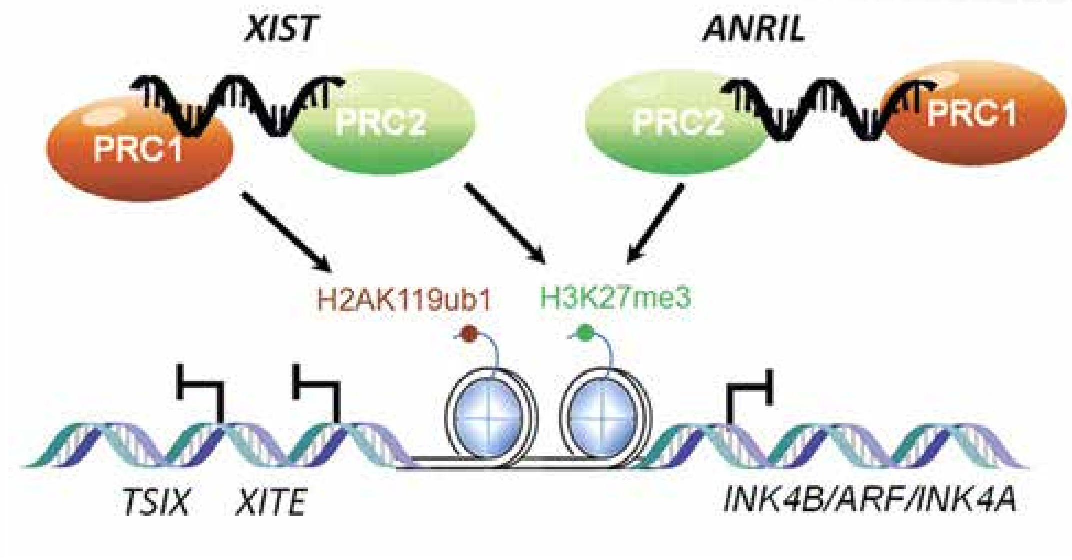
1. Epigenetic silencing
2. Transcriptional regulation
3. Post-transcriptional regulation

Epigenetic silencing



In *trans*

HOTAIR: HOX transcript antisense RNA

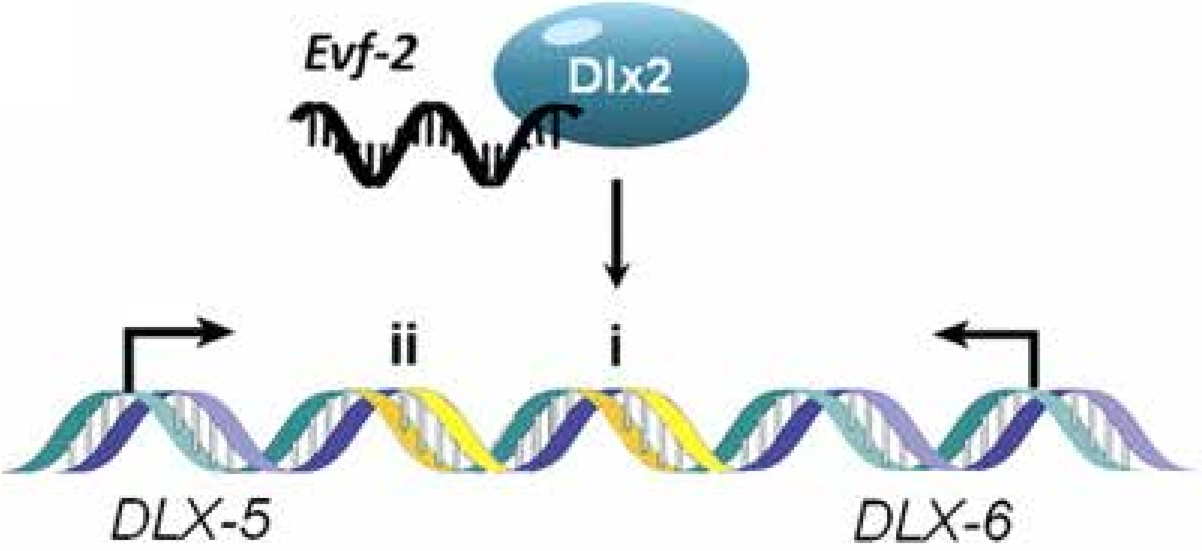


In *cis*

XIST: X (inactive)-specific transcript
ANRIL: antisense noncoding RNA
in the INK4 locus

(Kaikkonen et al., Cardiovasc Res, 2011)

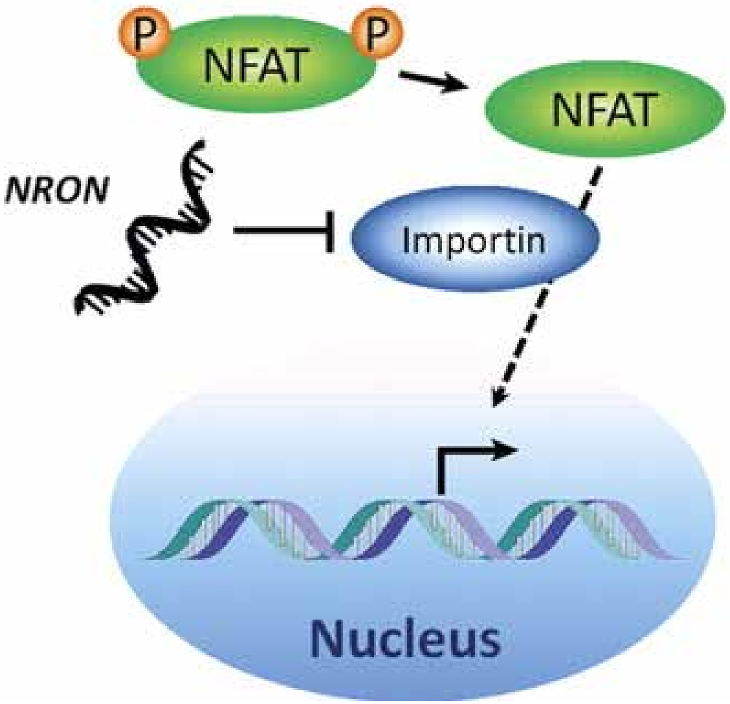
Transcriptional regulation



DLX6-AS1 (aka Evf-2): distal-less homeobox 6 antisense RNA 1

(Kaikkonen et al., Cardiovasc Res, 2011)

Post-transcriptional regulation



(Kaikkonen et al., Cardiovasc Res, 2011)

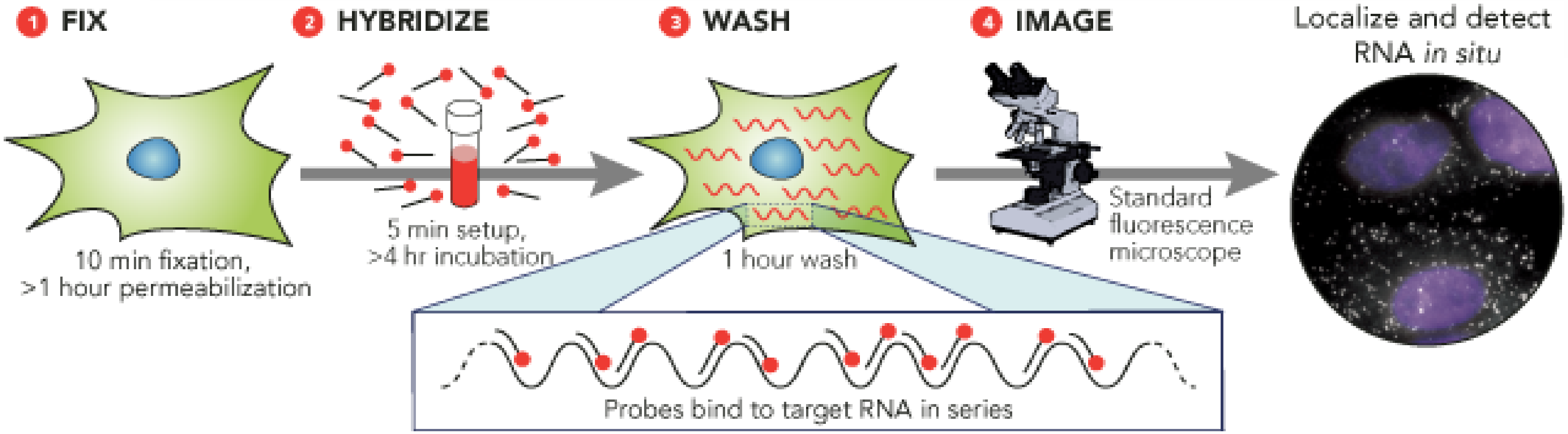
NRON: non-protein coding repressor of nuclear activated T cells (NFAT)

What is Stellaris™ FISH?

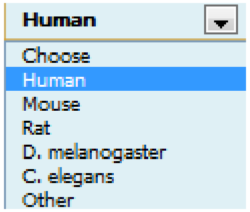
Stellaris FISH

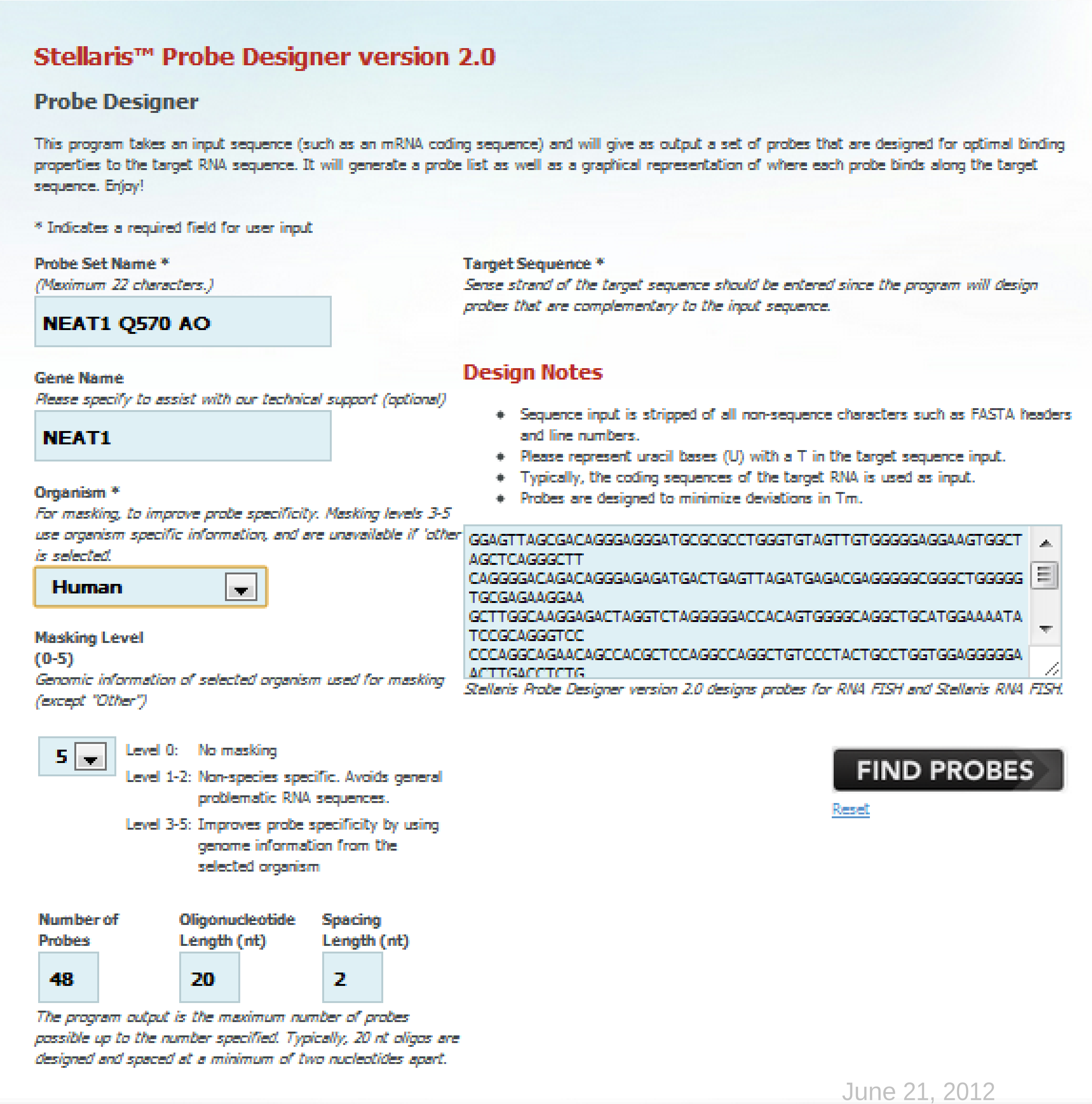
An RNA detection method that enables detection, localization, and quantification of individual RNA molecules at the cellular level.

Stellaris FISH protocol



Stellaris Probe Designer





Stellaris™ Probe Designer version 2.0

Probe Designer

This program takes an input sequence (such as an mRNA coding sequence) and will give as output a set of probes that are designed for optimal binding properties to the target RNA sequence. It will generate a probe list as well as a graphical representation of where each probe binds along the target sequence. Enjoy!

* Indicates a required field for user input

Probe Set Name *
(Maximum 22 characters.)
NEAT1 Q570 AO

Gene Name
Please specify to assist with our technical support (optional)
NEAT1

Organism *
For masking, to improve probe specificity. Masking levels 3-5 use organism specific information, and are unavailable if 'other' is selected.
Human

Masking Level
(0-5)
Genomic information of selected organism used for masking (except "Other")
5

Level 0: No masking
Level 1-2: Non-species specific. Avoids general problematic RNA sequences.
Level 3-5: Improves probe specificity by using genome information from the selected organism

Number of Probes	Oligonucleotide Length (nt)	Spacing Length (nt)
48	20	2

The program output is the maximum number of probes possible up to the number specified. Typically, 20 nt oligos are designed and spaced at a minimum of two nucleotides apart.

Target Sequence *
Sense strand of the target sequence should be entered since the program will design probes that are complementary to the input sequence.

Design Notes

- Sequence input is stripped of all non-sequence characters such as FASTA headers and line numbers.
- Please represent uracil bases (U) with a T in the target sequence input.
- Typically, the coding sequences of the target RNA is used as input.
- Probes are designed to minimize deviations in Tm.

```
GGAGTTAGCGACAGGGAGGGATGCGCGCCTGGGTGTAGTTGTGGGGGAGGAAGTGGCT
AGCTCAGGGCTT
CAGGGGACAGACAGGGAGAGATGACTGAGTTAGATGAGACGAGGGGGCGGGCTGGGGG
TGCGAGAAGGAA
GCTTGGCAAGGAGACTAGGTCTAGGGGGACCACAGTGGGGCAGGCTGCATGGAAAATA
TCCGCAGGGTCC
CCCAGGCAGAACAGCCACGCTCCAGGCCAGGCTGTCCCTACTGCCTGGTGGAGGGGGA
ACTTGACCTCTG
```

Stellaris Probe Designer version 2.0 designs probes for RNA FISH and Stellaris RNA FISH.

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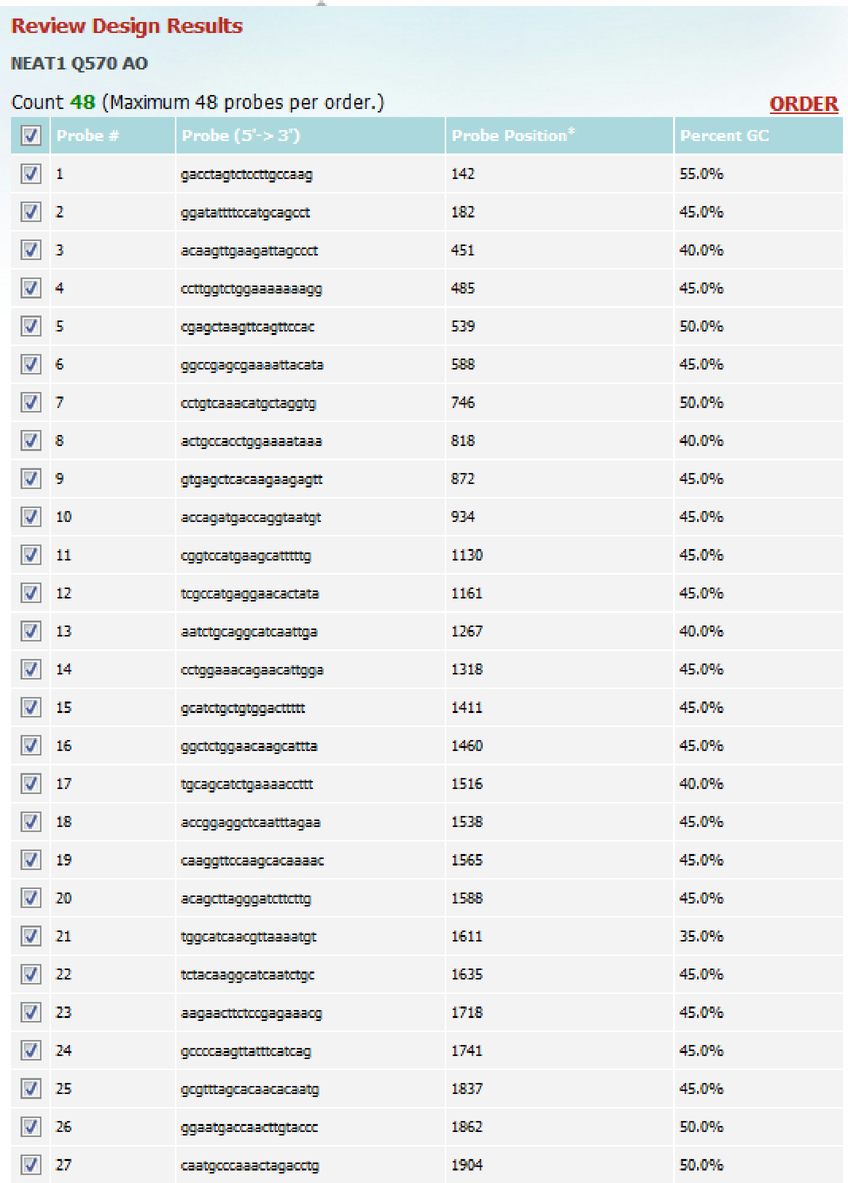
Stellaris Probe Designer



Review Design Results

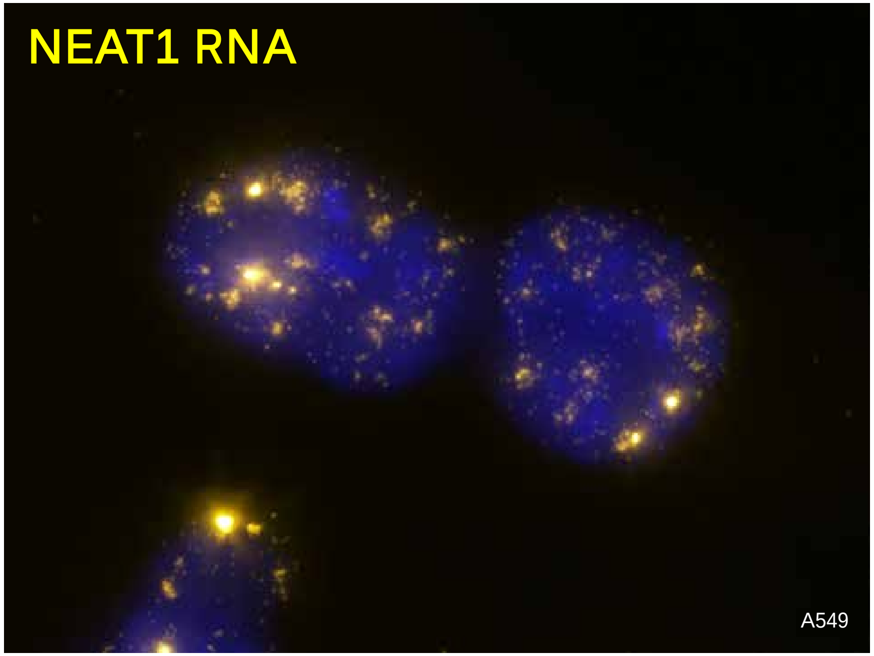
NEAT1 Q570 AO

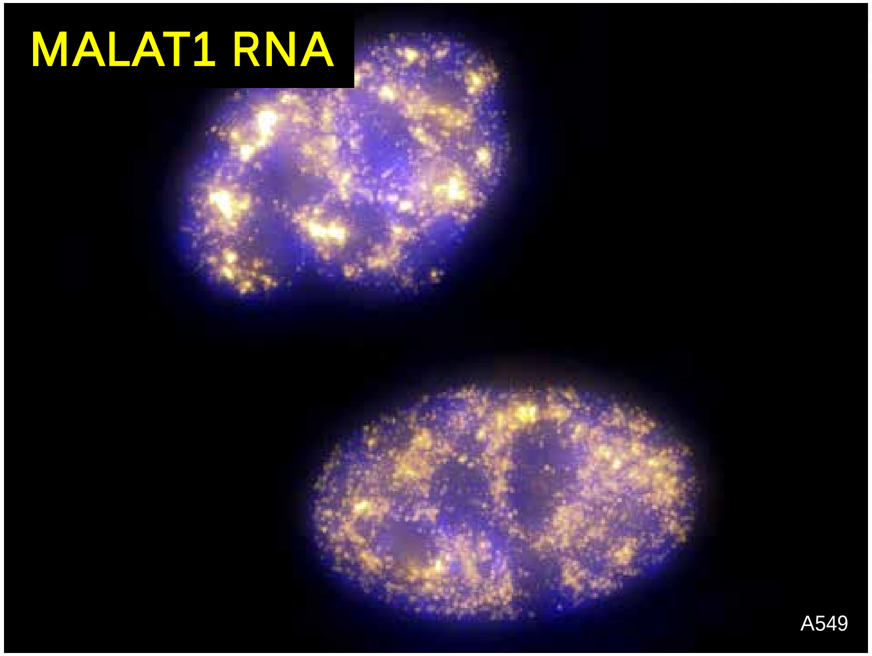
Count **48** (Maximum 48 probes per order.) **ORDER**

☑	Probe #	Probe (5'-> 3')	Probe Position*	Percent GC
☑	1	gacctagtctccttgccaag	142	55.0%
☑	2	ggatattttccatgcagcct	182	45.0%
☑	3	acaagttgaagattagccct	451	40.0%
☑	4	ccttggtctggaaaaaaagg	485	45.0%
☑	5	cgagctaagttcagttccac	539	50.0%
☑	6	ggccgagcgaaaattacata	588	45.0%
☑	7	cctgtcaaacatgctaggtg	746	50.0%
☑	8	actgccacctggaaaataaa	818	40.0%
☑	9	gtgagctcacaagaagagtt	872	45.0%
☑	10	accagatgaccaggtaatgt	934	45.0%
☑	11	cggtccatgaagcatttttg	1130	45.0%
☑	12	tcgccatgaggaacactata	1161	45.0%
☑	13	aatctgcaggcatcaattga	1267	40.0%
☑	14	cctggaaacagaacattgga	1318	45.0%
☑	15	gcatctgctgtggactttttt	1411	45.0%
☑	16	ggctctggaacaagcattta	1460	45.0%
☑	17	tgcagcatctgaaaaccttt	1516	40.0%
☑	18	accggaggctcaatttagaa	1538	45.0%
☑	19	caaggttccaagcacaaaac	1565	45.0%
☑	20	acagcttagggatcttcttg	1588	45.0%
☑	21	tggcatcaacgttaaaatgt	1611	35.0%
☑	22	tctacaaggcatcaatctgc	1635	45.0%
☑	23	aagaacttctccgagaaacg	1718	45.0%
☑	24	gccccaagttatttcatcag	1741	45.0%
☑	25	gcgtttagcacaacacaatg	1837	45.0%
☑	26	ggaatgaccaacttgtaccc	1862	50.0%
☑	27	caatgcccaaactagacctg	1904	50.0%

```
ggagttagcgacagggagggatgcgcgcctgggtgtagttgtgggggaggaagtggctag


ctcagggcttcaggggacagacagggagagatgactgagttagatgagacgagggggcgg


gctgggggtgcgagaaggaagcttggcaaggagactaggtctaggggaccacagtgggg
                     gaaccgttcctctgatccag
                     Probe # 1

caggctgcatggaaaatatccgcagggtcccccaggcagaacagccacgctccaggccag
 tccgacgtaccttttatagg
 Probe # 2

gctgtccctactgcctggtggagggggaacttgacctctgggagggcgccgctcttgcat


agctgagcgagcccgggtgcgctggtctgtgtggaaggaggaaggcagggagaggtagaa


ggggtggaggagtcaggaggaataggccgcagcagccctggaaatgatcaggaaggcagg


cagtgggtgcagggctgcaggagggccgggagggctaatcttcaacttgtccatgccagc
                              tcccgattagaagttgaaca
                              Probe # 3

agcccctttttttccagaccaagggctgtgaacccgcctggggatgaggcctggtcttgt
    ggaaaaaaaggtctggttcc                                      ca
    Probe # 4                                                 Pr

ggaactgaacttagctcgacggggctgaccgctctggcccagggtggtatgtaattttcg
ccttgacttgaatcgagc                             atacattaaaagc
obe # 5                                        Probe # 6

ctcggcctgggacggggcccaggccgggcccagcctggtggagcgtccaggtctgggtgc
gagccgg


gaagccaggcccctgggcggaggtgaggggtggtctgaggagtgatgtggagttaaggcg


ccatcctcaccggtgactggtgcggcacctagcatgtttgacaggcggggactgcgaggc
                         gtggatcgtacaaactgtcc
                         Probe # 7

acgctgctcgggtgttggggacaacattgaccaacgctttattttccaggtggcagtgct
                                     aaataaaaggtccaccgtca
                                     Probe # 8

ccttttggacttttctctaggtttggcgctaaactcttcttgtgagctcactccacccct
                               ttgagaagaacactcgagtg
                               Probe # 9

tcttcctccctttaacttatccattcacttaaaacattacctggtcatctggtaagcccg
                                 tgtaatggaccagtagacca
                                 Probe # 10
```

lncRNAs NEAT1 and MALAT1

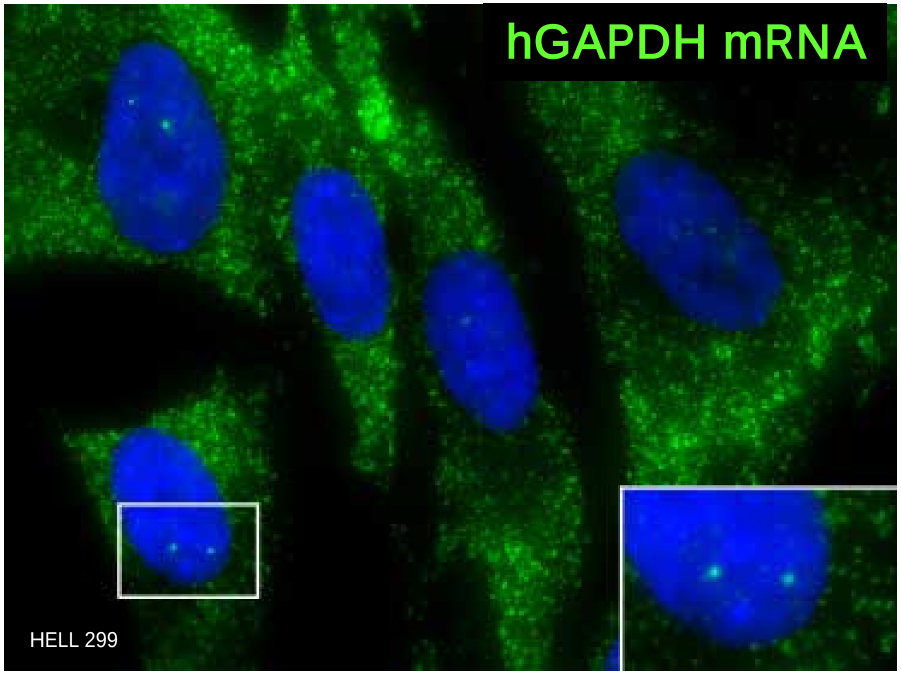


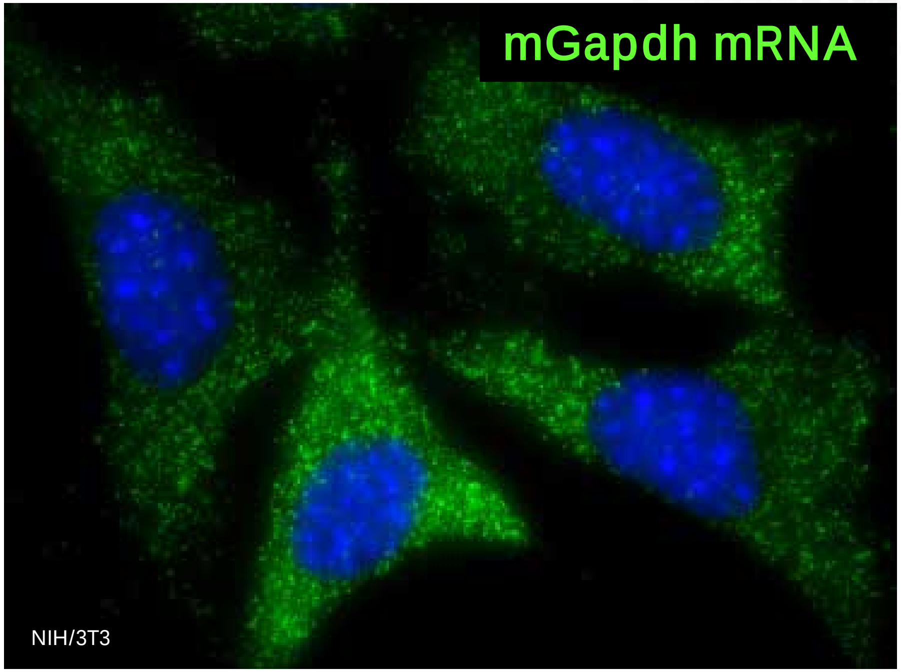


NEAT1: nuclear paraspeckle assembly transcript 1
MALAT1: metastasis associated lung adenocarcinoma transcript 1

A549: human lung adenocarcinoma cell line

RNA **Detection**

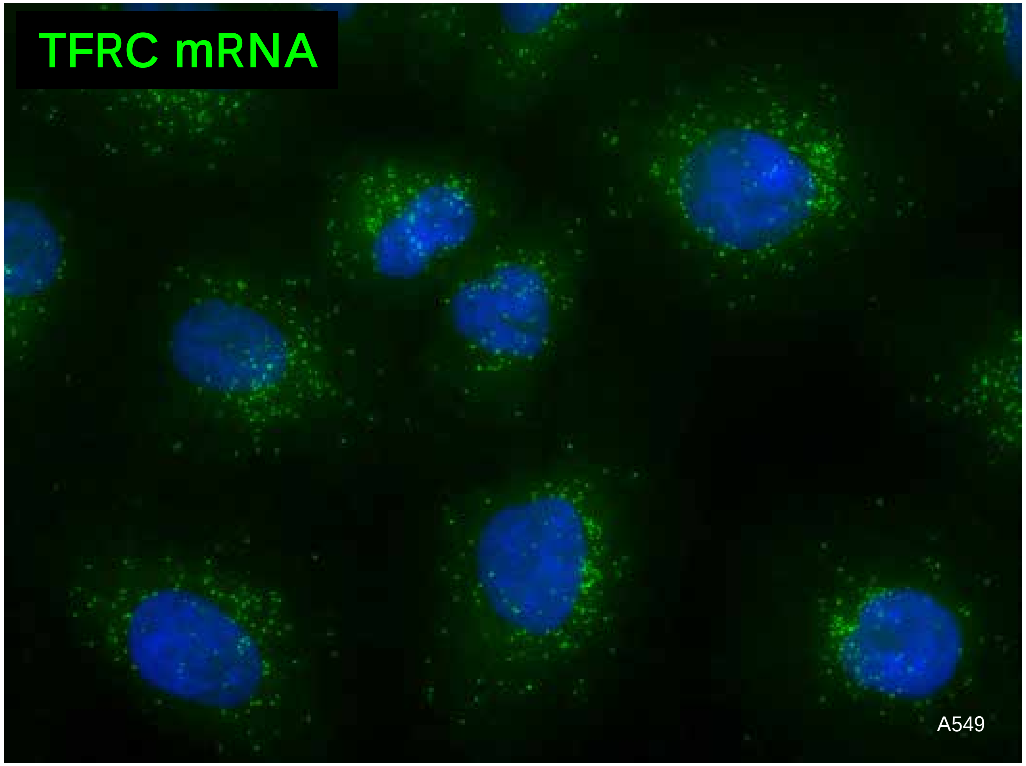


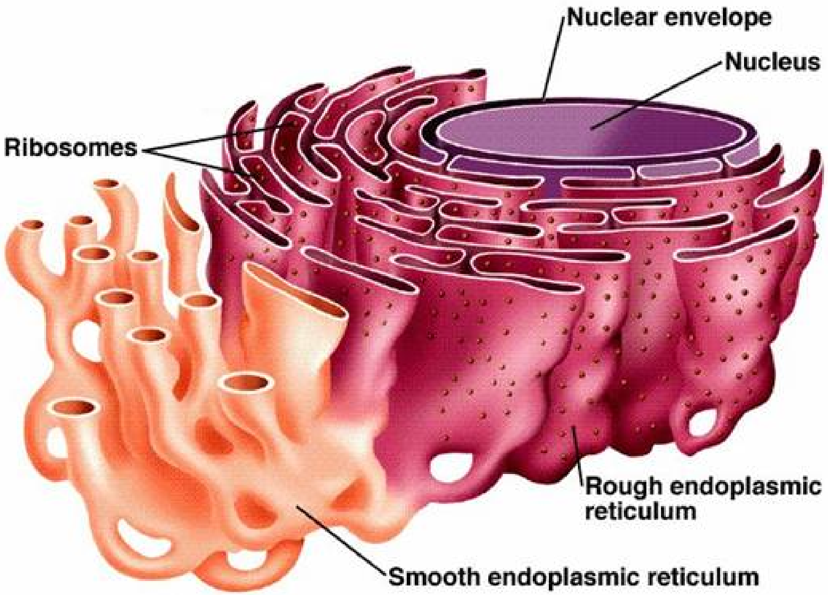


GAPDH: glyceraldehyde-3-phosphate dehydrogenase

HEL 299: normal human lung fibroblasts
NIH/3T3: mouse embryonic fibroblast cell line

RNA Localization

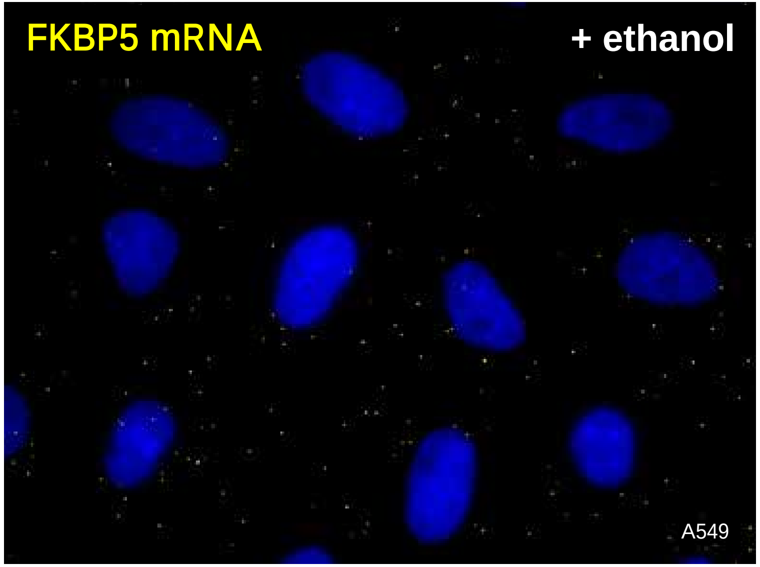


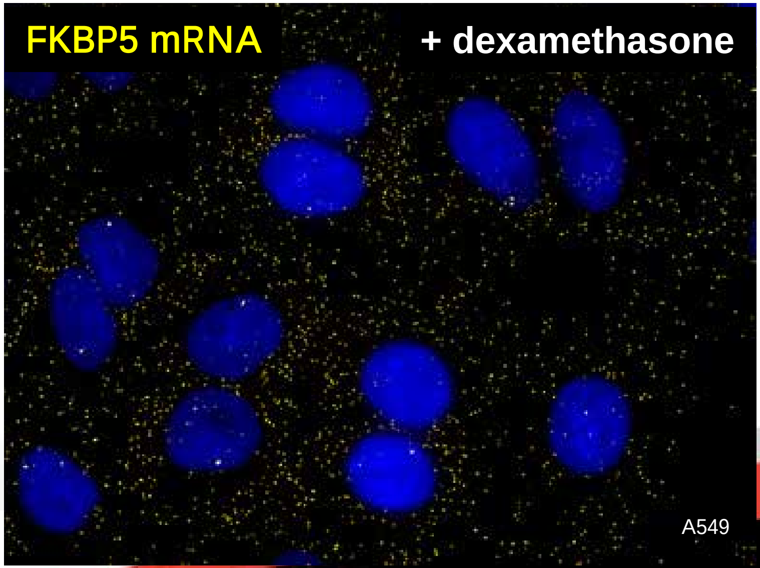


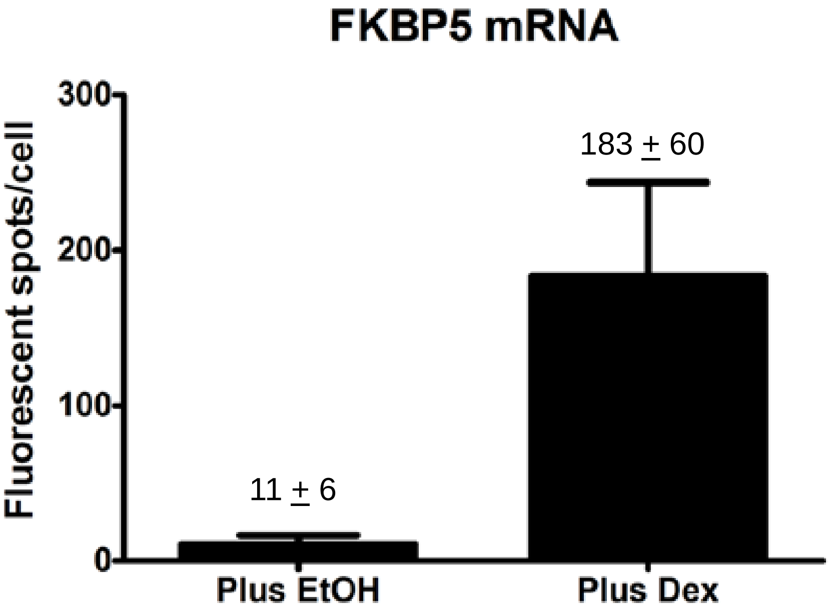
http://jaewoneportfolio.blogspot.com/2011/09/boat-speech-endoplasmic-reticulum.html

TFRC: transferrin receptor

RNA Quantification

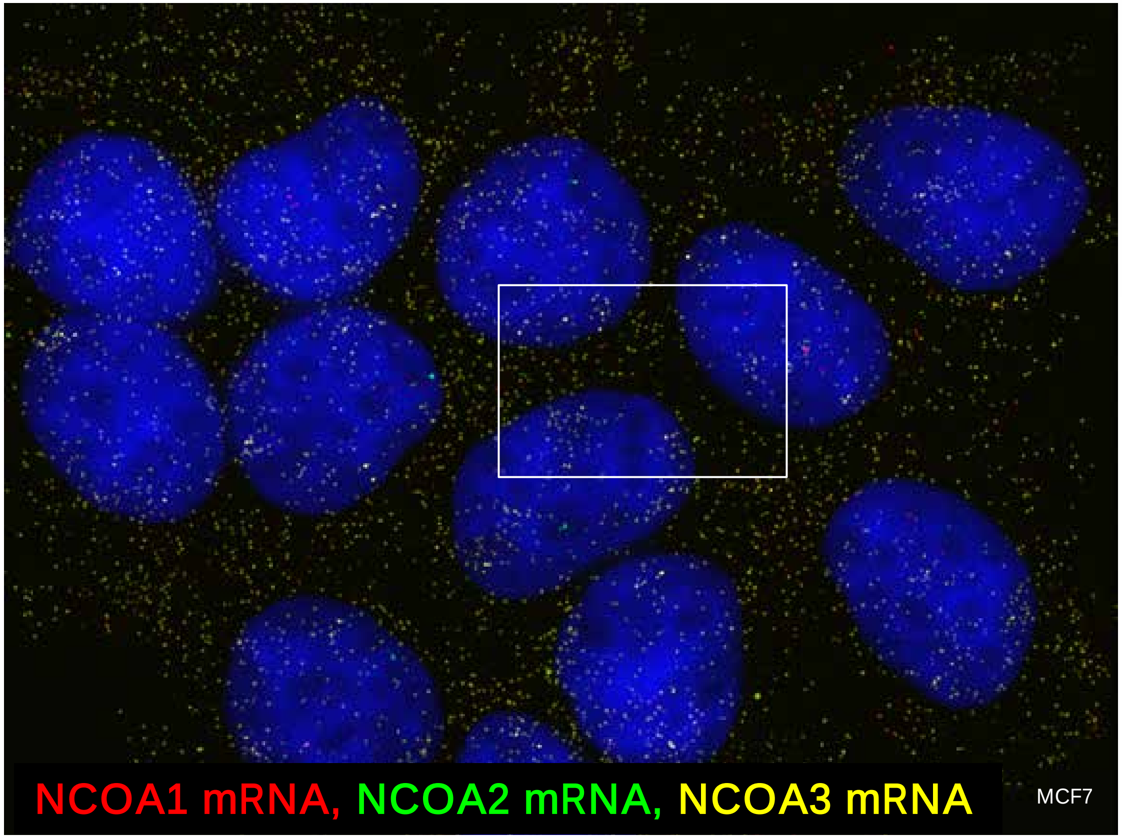


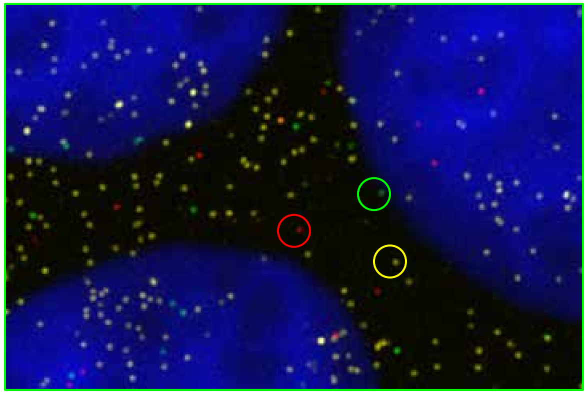




FKBP5: FK506 binding protein 5

Multiplexing with Stellaris FISH probes





MCF7: human breast adenocarcinoma cell line

NCOA: nuclear coactivator

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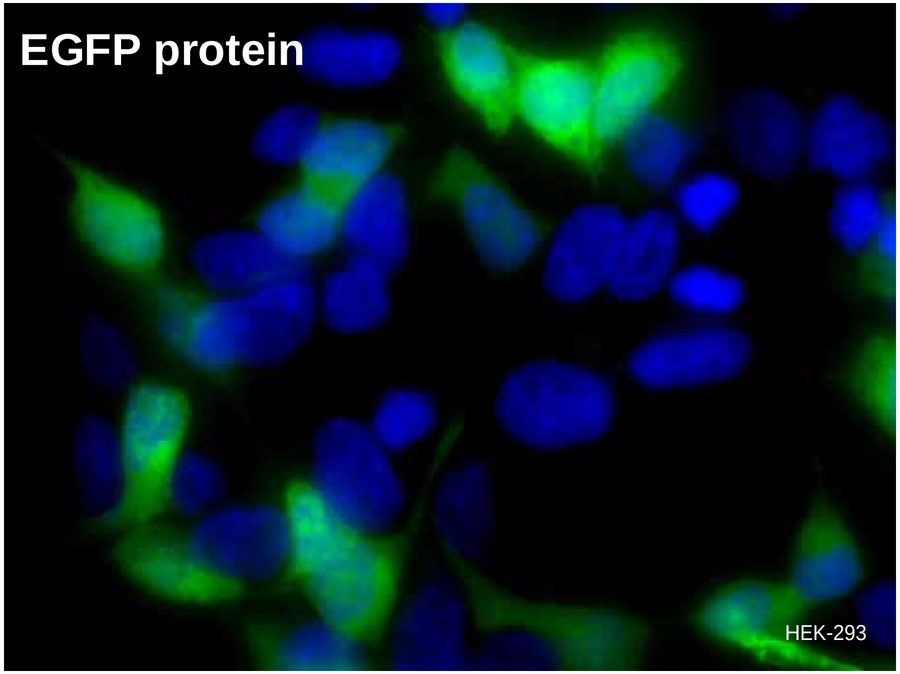
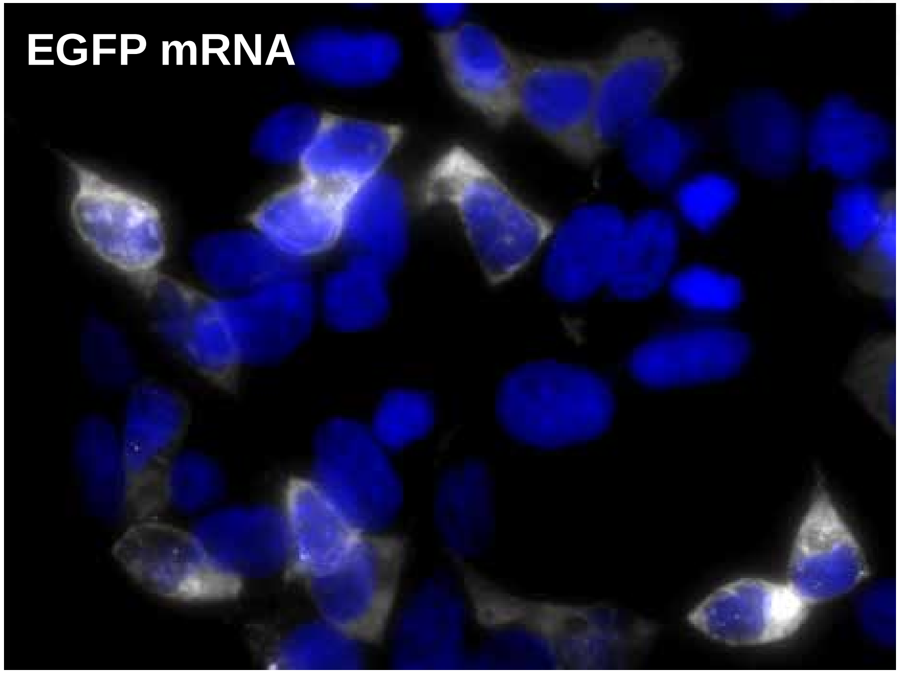
Compatible with Fluorescent Reporters

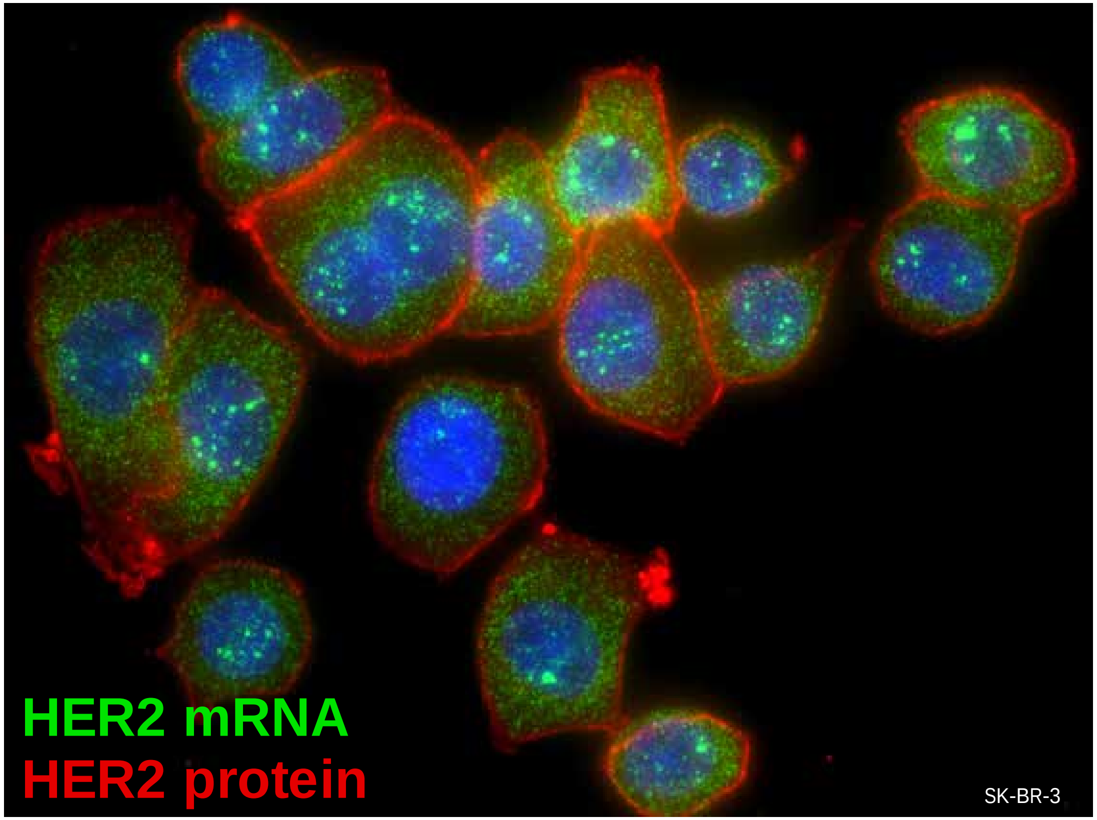

EGFP protein


EGFP mRNA

EGFP: enhanced green fluorescent protein

HEK-293: human embryonic kidney cell line

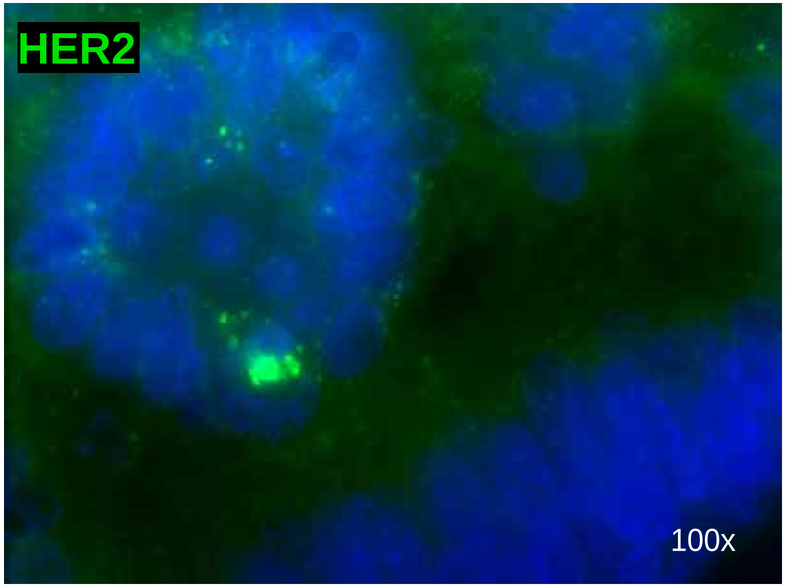
Stellaris FISH + Immunofluorescence



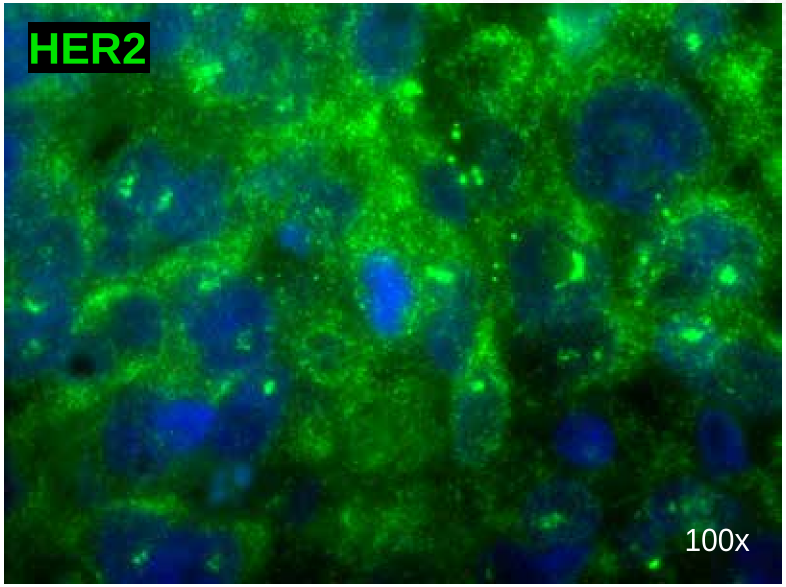
HER2: Human Epidermal Growth Factor Receptor 2

SK-BR-3: human breast adenocarcinoma cell line

Stellaris FISH in frozen and FFPE tissue



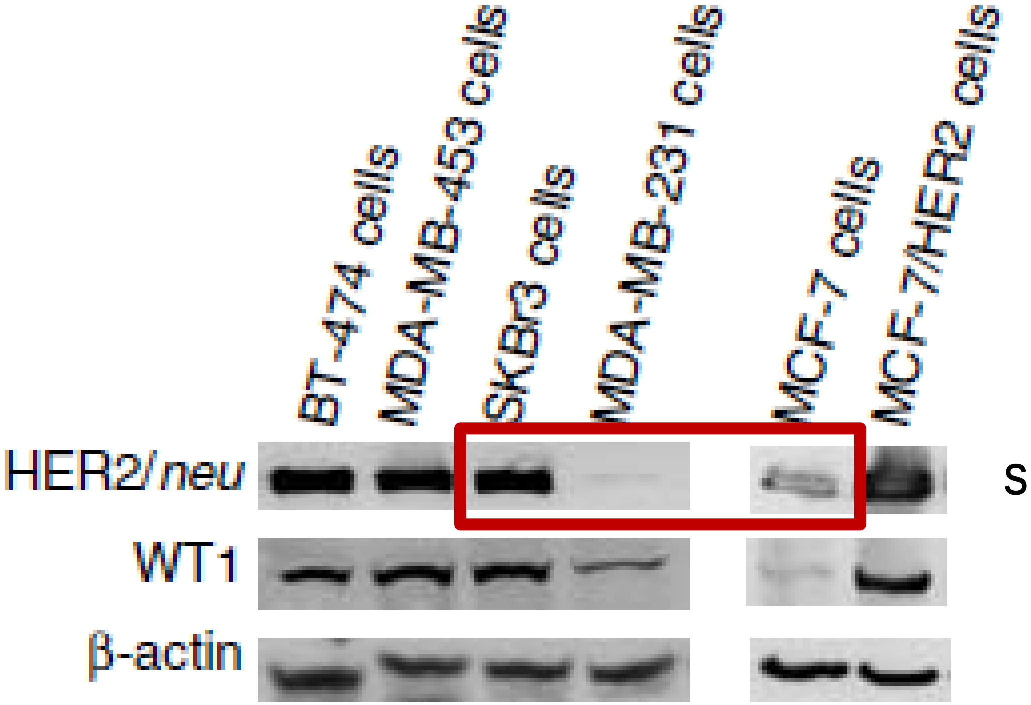
Female Caucasian
Right breast mastectomy
Invasive ductal carcinoma
HER2 negative
Surgically removed in 2011
Age 69



Female Caucasian
Left breast mastectomy
Invasive ductal carcinoma
HER2 positive
Surgically removed in 2011
Age 80

Is Stellaris FISH data comparable to the data from published literature?

HER2 protein

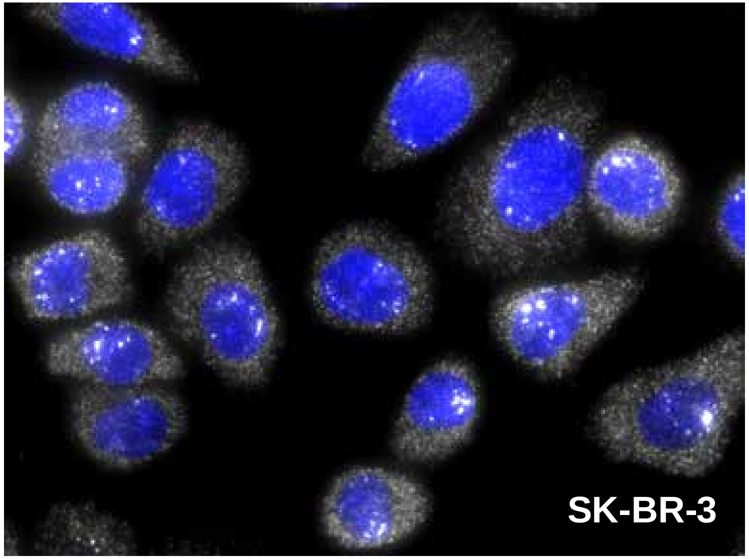


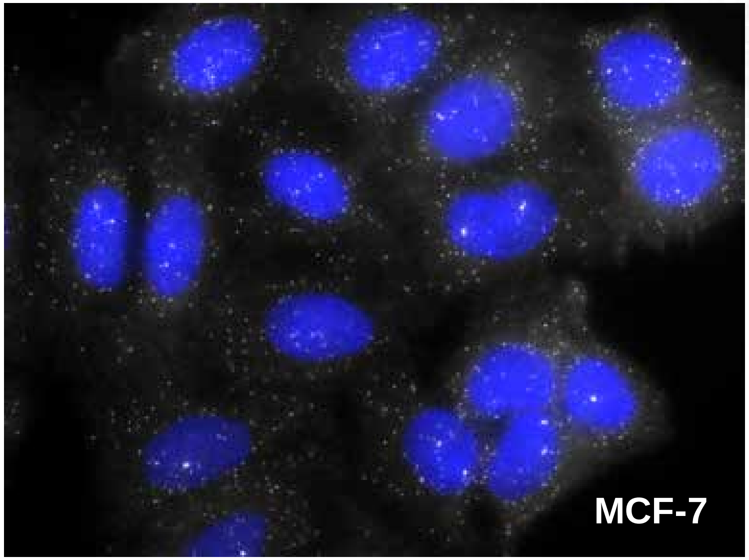
SK-BR-3 > MCF-7 > MDA-MB-231

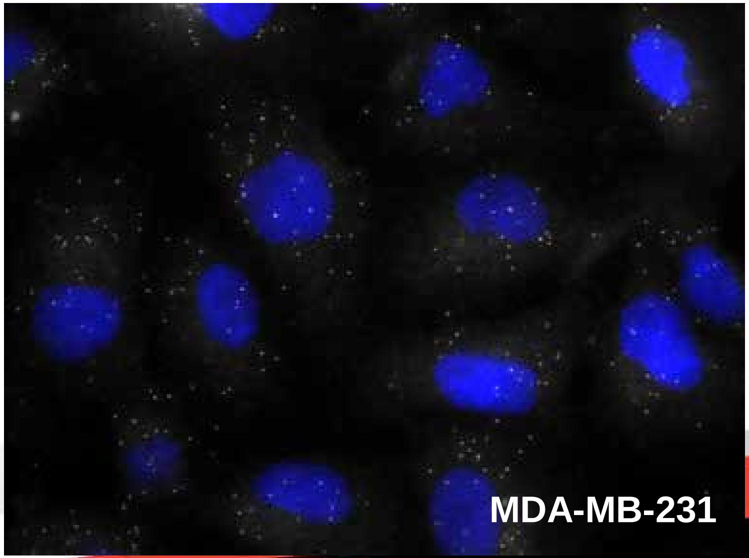
(Tuna et al., 2005, Oncogene)

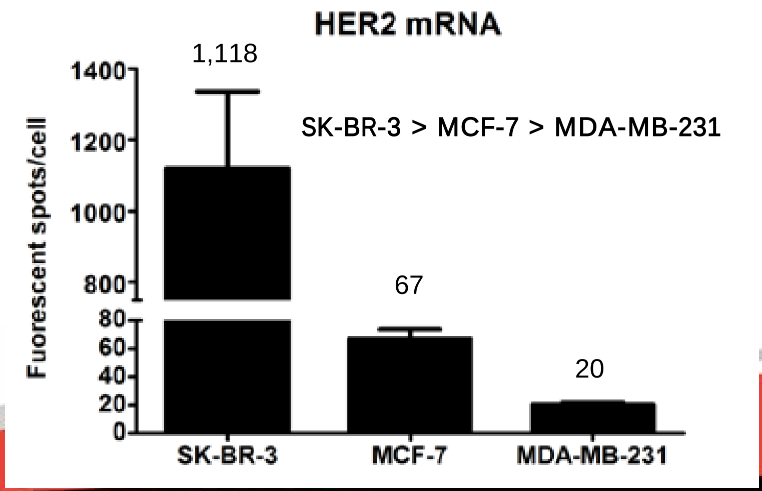
SK-BR-3, MCF7, MDA-MB-231: human breast adenocarcinoma cell lines

HER2 mRNA





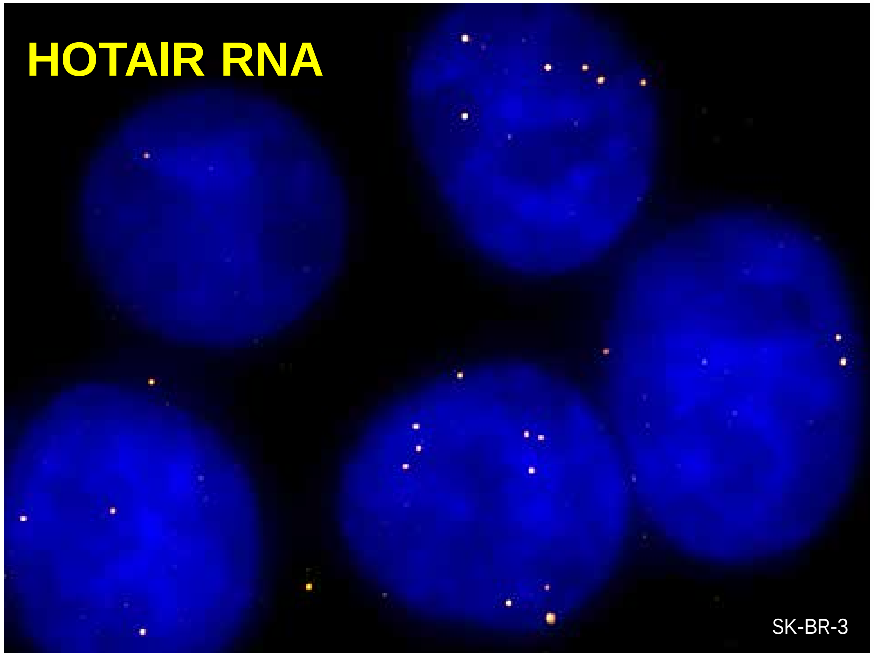


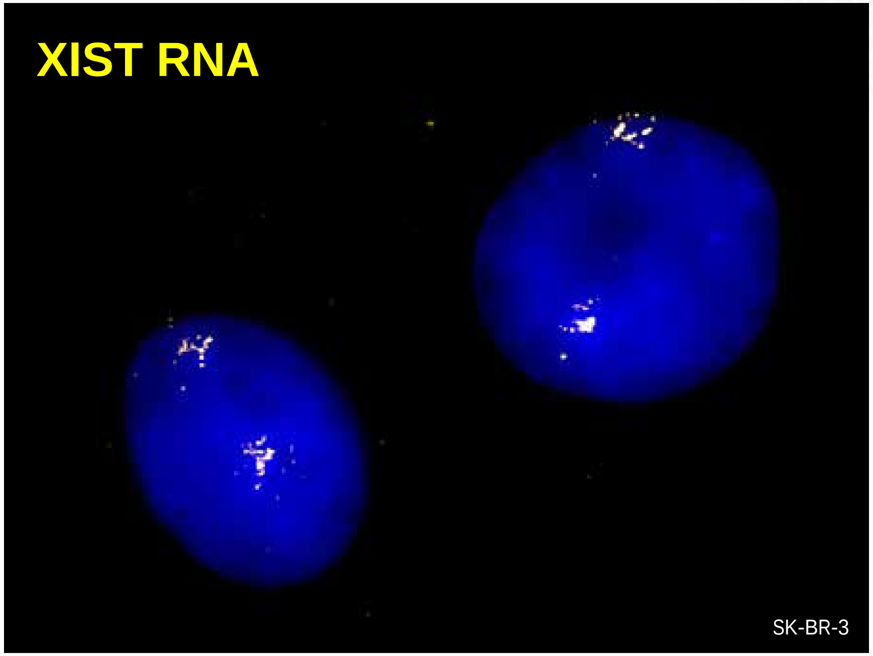


SK-BR-3 > MCF-7 > MDA-MB-231

Stellaris FISH and lncRNA detection?

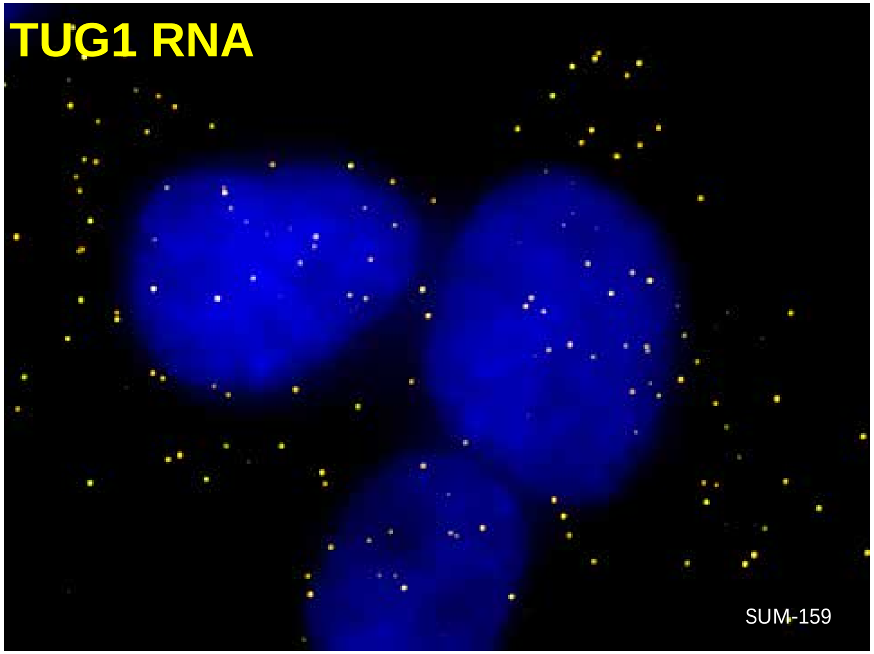
lncRNAs HOTAIR and XIST

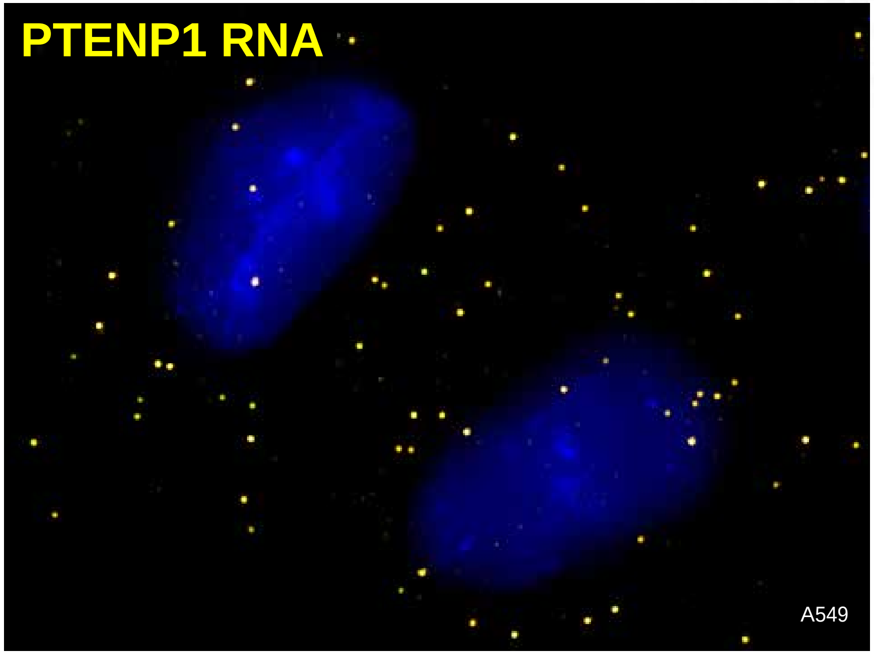




HOTAIR: HOX transcript antisense RNA
XIST: X (inactive)-specific transcript

lncRNAs TUG1 and PTENP1

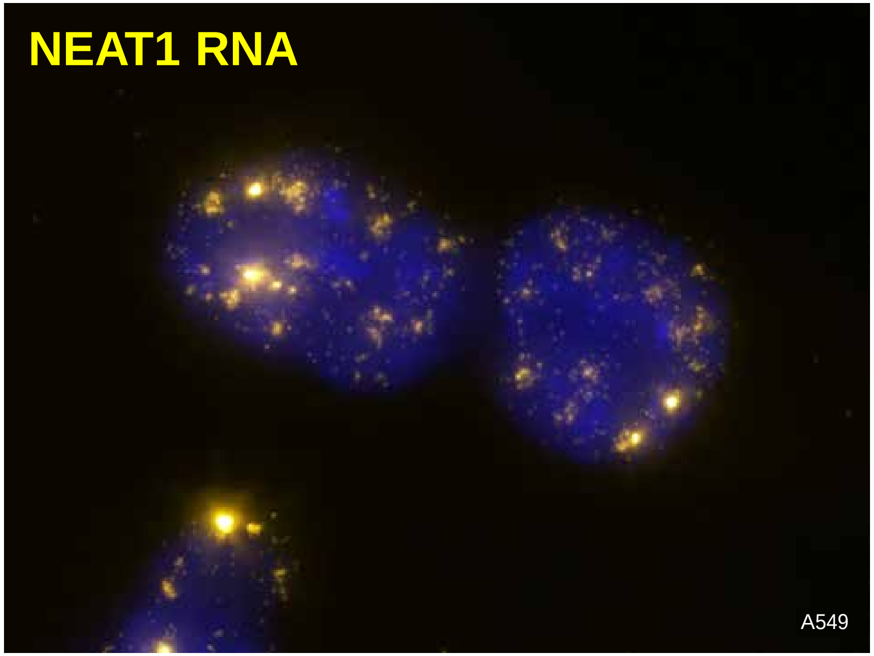


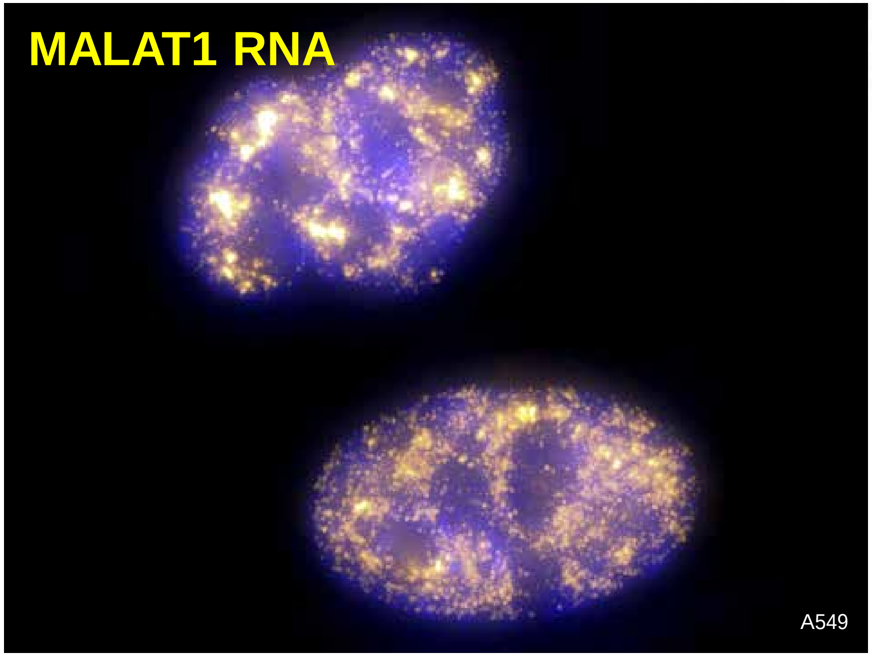


TUG1: taurine upregulated 1
PTENP1: phosphatase and tensin homolog pseudogene 1

SUM-159: human breast cancer cell line

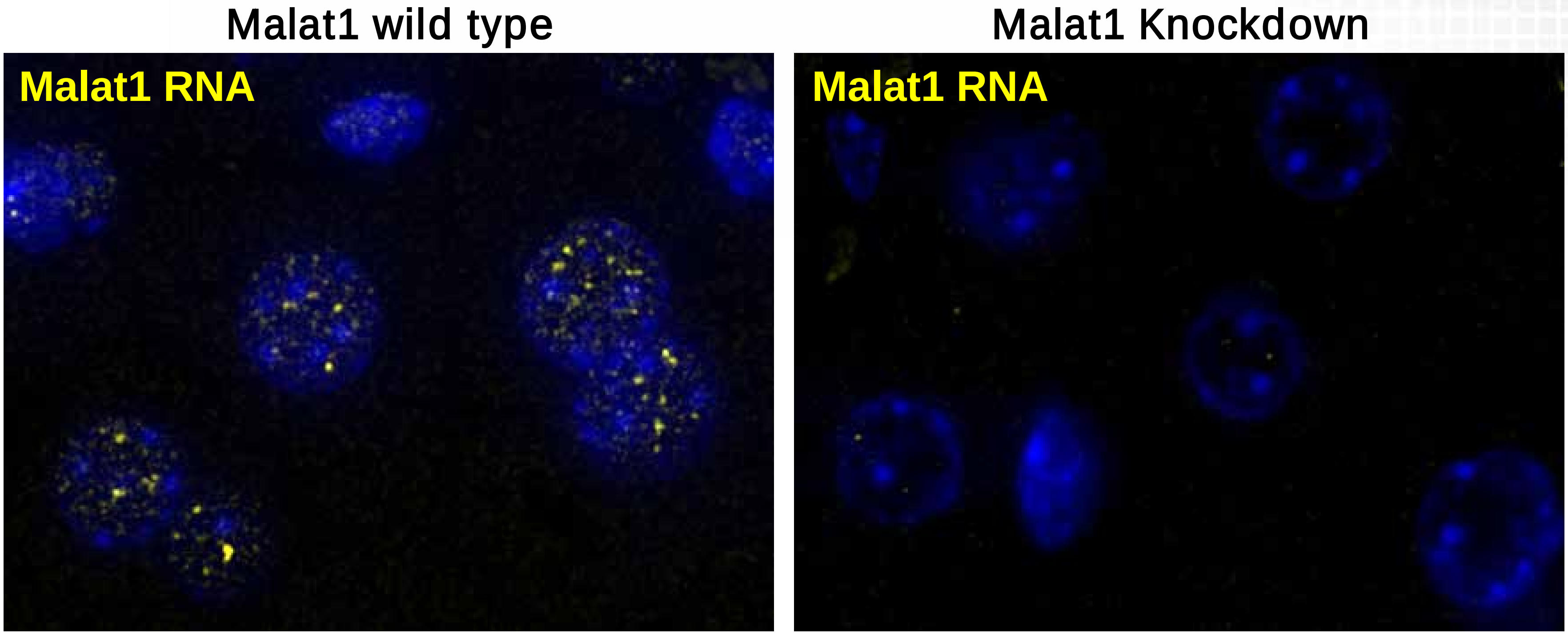
lncRNAs NEAT1 and MALAT1





NEAT1: nuclear paraspeckle assembly transcript 1
MALAT1: metastasis associated lung adenocarcinoma transcript 1

lncRNAs *in vivo*



Malat1 wild type

Malat1 Knockdown

FFPE mouse liver tissue

Summary

- We can easily visualize and quantify RNAs *in situ* with Stellaris FISH probes.
- Multiplexing is merely dependent on microscope filter sets, which are readily available.
- Stellaris FISH is compatible with immunofluorescence and fluorescent proteins.
- Stellaris FISH probes work in frozen and FFPE tissue.

No other tool exists to look at lncRNAs!

Important links

- Stellaris RNA FISH

http://www.biosearchtech.com/stellaris

- Stellaris FISH probe designer

http://www.biosearchtech.com/stellarisdesigner/

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Acknowledgements

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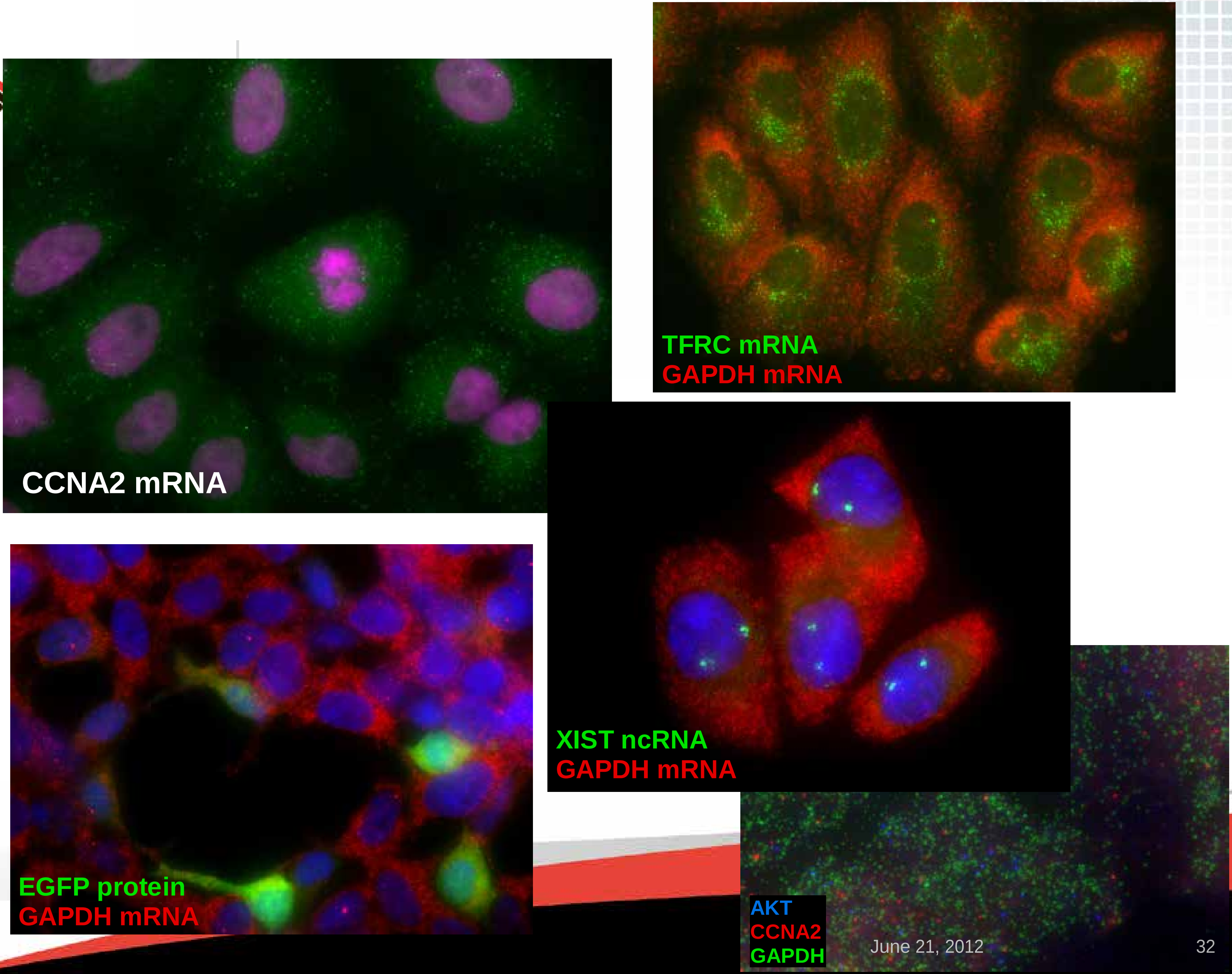
TFRC mRNA
GAPDH mRNA
CCNA2 mRNA
XIST ncRNA
GAPDH mRNA
EGFP protein
GAPDH mRNA
AKT
CCNA2
GAPDH