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

Arturo Orjalo, Jr.
June 21, 2012
Agenda

- Background of IncRNA mechanisms of gene regulation
- Methodology and application of Stellaris FISH
- Examples of IncRNAs as visualized by Stellaris FISH
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 IncRNAs 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)
DLX6-AS1 (aka Evf-2): distal-less homeobox 6 antisense RNA 1

(Kaikkonen et al., Cardiovasc Res, 2011)
Post-transcriptional regulation

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

(Kaikkonen et al., Cardiovasc Res, 2011)
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

1. **FIX**
   - 10 min fixation, >1 hour permeabilization

2. **HYBRIDIZE**
   - 5 min setup, >4 hr incubation

3. **WASH**
   - 1 hour wash

4. **IMAGE**
   - Standard fluorescence microscope
   - Localize and detect RNA in situ

Probes bind to target RNA in series
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

48

Oligonucleotide Length (nt)

20

Spacing Length (nt)

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.

FIND PROBES

June 21, 2012
# Stellaris Probe Designer

## Review Design Results

**NEAT1 Q570 AG**

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IncRNAs NEAT1 and MALAT1

NEAT1 RNA

MALAT1 RNA

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
TFRC: transferrin receptor

RNA Quantification

FKBP5 mRNA + ethanol

FKBP5 mRNA + dexamethasone

FKBP5: FK506 binding protein 5

A549
NCOA: nuclear coactivator

NCOA1 mRNA, NCOA2 mRNA, NCOA3 mRNA

MCF7: human breast adenocarcinoma cell line
Compatible with Fluorescent Reporters

EGFP: enhanced green fluorescent protein
HEK-293: human embryonic kidney cell line
Stellaris FISH + Immunofluorescence

HER2 mRNA
HER2 protein

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 IncRNA detection?
IncRNAs HOTAIR and XIST

HOTAIR: HO X transcript antisense RNA
XIST: X (inactive)-specific transcript
IncRNAs TUG1 and PTENP1

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

SUM-159: human breast cancer cell line
NEAT1 RNA

MALAT1 RNA

NEAT1: nuclear paraspeckle assembly transcript 1
MALAT1: metastasis associated lung adenocarcinoma transcript 1
**IncRNAs in vivo**

**Malat1 wild type**

**Malat1 Knockdown**

**Malat1 RNA**

FFPE mouse liver tissue
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 IncRNAs!
Important links

- Stellaris RNA FISH
  http://www.biosearchtech.com/stellaris

- Stellaris FISH probe designer
  http://www.biosearchtech.com/stellarisdesigner/

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Acknowledgements

Biosearch Technologies
R&D
Technical Support
Marketing

UPenn
Arjun Raj

UMDNJ
Sanjay Tyagi