



SCIENCE FOR THE BENEFIT OF HUMANITY

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A Novel Method For MicroRNA, Non-Coding RNA and mRNA Detection By *In-Situ* Hybridization

RU 910+RU1030

Technology Summary

The detection of coding and non-coding RNAs in tissue sections is important for expression analysis in molecular pathology. Alterations in gene expression are seen in many diseases, including cancer, metabolic, infectious, and several neurological diseases. *In-situ* hybridization (ISH) is one of the most common and widely used method for visualizing gene expression in specific cell types implicated in disease pathology. However, conventional ISH is not efficient for RNA detection in tissues. The principle drawbacks of conventional ISH are the release and diffusion of small- and even large-sized RNAs from thinly sliced paraffin-embedded and formalin-fixed tissues during the fixation process, mishybridization of the probes to other abundant RNAs and poor signal amplification resulting in inconsistent or false results.

Our scientists have developed a novel fixation strategy to irreversibly crosslink or immobilize different RNAs for ISH, substantially improving their retention in tissues, thereby facilitating either their direct detection using fluorescently labeled oligonucleotide probes, or for less abundant and short RNAs, such as miRNAs, they have drastically enhancing the detection using signal amplification. They employ specific oligonucleotide probe design to avoid mishybridization to unrelated highly abundant RNAs. In addition, our scientists have designed several probes for miRNA, non-coding RNAs and mRNAs quantification. As proof of concept, we have visualized miRNAs expressed at high, medium and low levels in different tissues including the heart, liver, muscle, skin, pancreas, breast and brain and cancer tissues and cell lines. This improved ISH method is a broadly-enabling advance to visualize miRNAs, non-coding RNAs and mRNAs in paraffin-embedded tissues for diagnostic and research purposes.

Application

- An efficient research tool to study and detect RNA expression patterns in biological and clinical samples.
- A diagnostic kit for visualization of RNA in pathological samples with limiting availability.
- Novel probes for enhanced detection of miRNA using signal amplification
- Novel probes for direct detection of non-coding reference RNAs of distinct length and sub-cellular localization for normalization of RNA content or integrity in archived tissues.

Advantages:

- Highly sensitive method to visualize and quantify gene expression.

Stage of Development

- Discovery

Lead Inventors

- Dr Thomas Tuschl

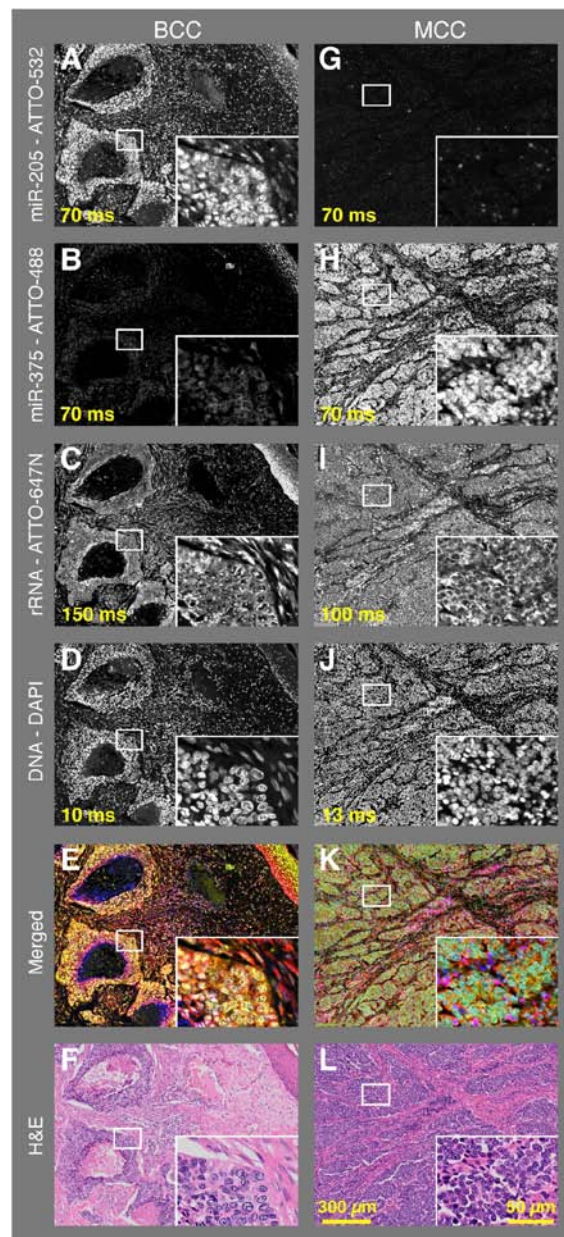
Patent Information

- US Patents 8,394,588; 9,005,893 and 9,359,636.

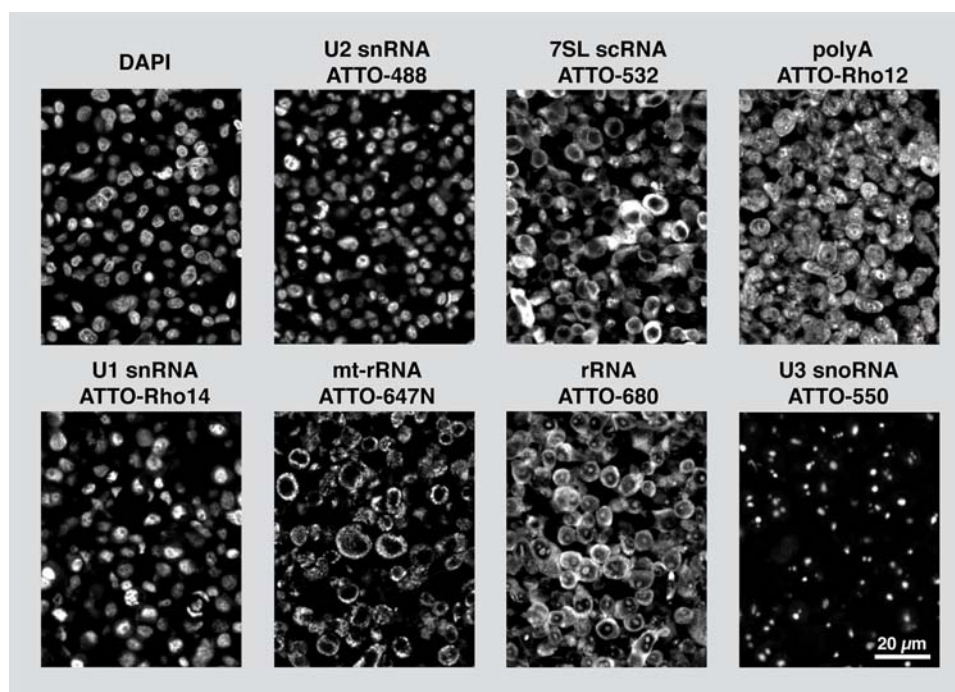
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References

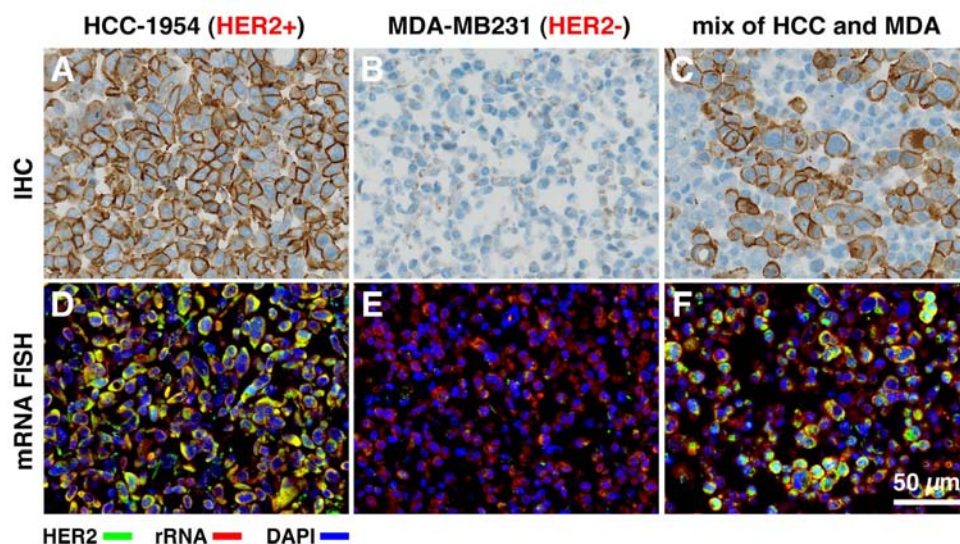
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Multicolor miRNA FISH for BCC and MCC differential diagnosis. Parallel detection of miR-205, miR-375, and 28S rRNA in FFPE BCC and MCC tissue sections (samples BCC1 and MCC1) by multicolor miRNA FISH. Images were recorded at 20X magnification. Representative areas, indicated by white rectangles, are shown at 60X magnification to illustrate signal localization. Exposure times are indicated in ms. Scale bar, 300 μ m, for insert, 50 μ m (A-L).



RNA FISH of 6 non-coding RNAs, polyA RNA and DAPI nuclear DNA staining. RNA FISH was performed using the HCC-1954 cell line. We used 12-17 nt long LNA-modified DNA probes targeting U2 snRNA, 7SL scRNA, U1 snRNA, mt-rRNA, rRNA, U3 snoRNA and polyT probes targeting polyA conjugated to ATTO-488, ATTO-532, ATTO-Rho12, ATTO-Rho 14, ATTO-647N, ATTO-680 and ATTO-550, respectively. Probes (50 nM) were hybridized overnight as above but at 50 °C. Scale bar, 20 µm.]



Comparative detection of HER2/ERBB2 in breast cancer cell lines using IHC and RNA FISH. HER2 detection was assessed in HER2 positive (HCC-1954) and HER negative (MDA-MB231) cell lines using either IHC or RNA FISH. Positive IHC (brown) and RNA FISH (green) signals were seen as expected in HER2 positive (A,D) but not HER2 negative (B,E) cells. Mixtures of cells yielded the expected mixture of signals (C,F). rRNA (red) and nuclei (blue) were visualized using rRNA probes and DAPI stain (D-F). Scale bar, 50 µm, (A-F).