

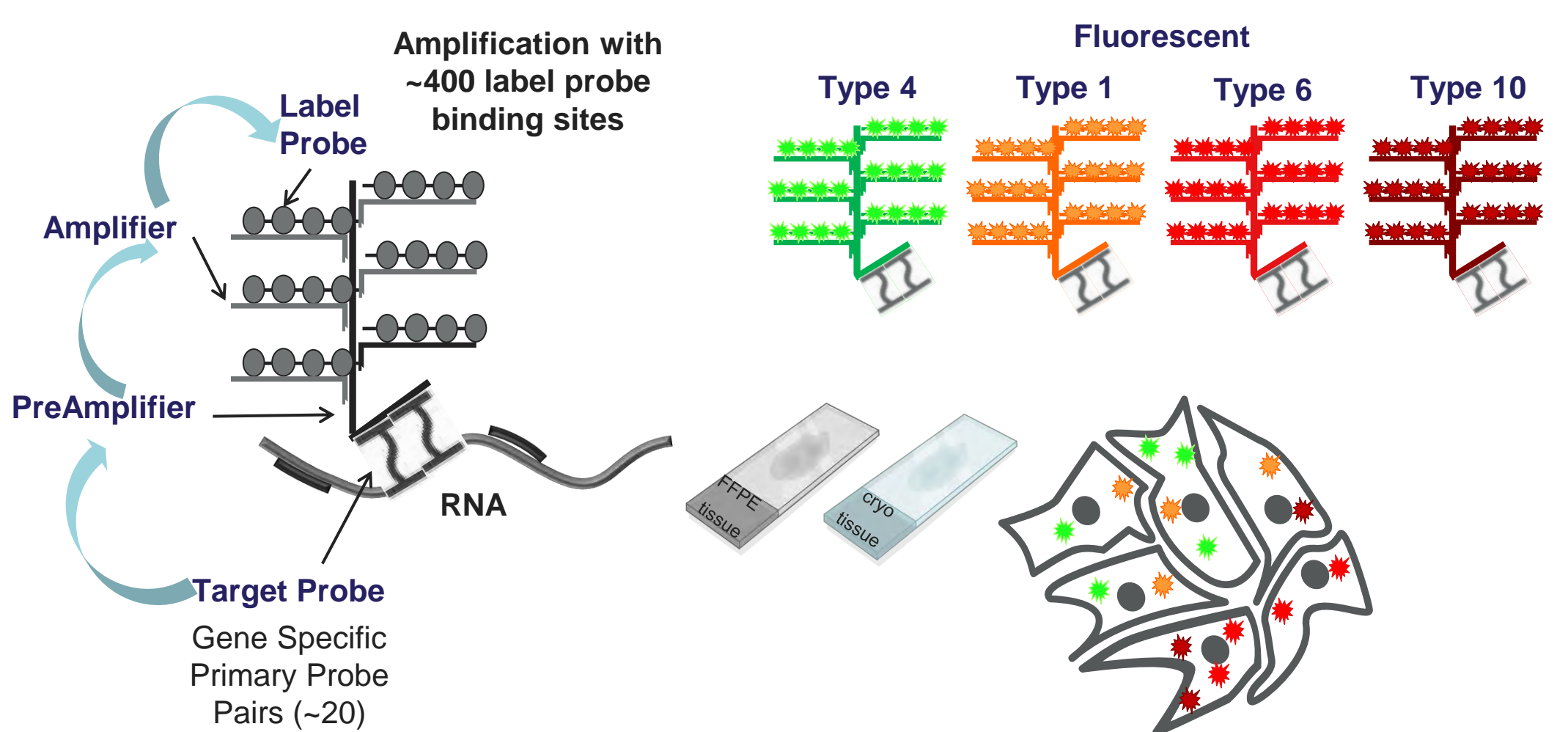
Expanding tools for multiplex mRNA imaging in spatialomics through the ViewRNA Tissue Assay Fluorescence Kit.

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Introduction

Spatialomics is a rapidly growing field as it allows researchers to gain a deeper understanding of transcriptomes and corresponding protein expression profiles in cells within complex tissue microenvironments. Thermo Scientific™ has a broad portfolio of detection reagents and modalities that allow researchers to further their understanding in this ever-evolving scientific branch. One critical technology for spatialomics is in-situ hybridization (ISH) technology, which enables direct visualization and quantitation of nucleic acid in cells with single molecule resolution. The Invitrogen™ ViewRNA ISH assays incorporate branched DNA (bDNA) technology provides tools for interrogating multiple RNA transcripts at the same time, paving the way for improved spatialomics research. This technology is a powerful tool for spatialomics, giving insight into important mechanisms within cells and tissue. With researchers increasingly using spatialomic data in fields like immuno-oncology, cancer metabolomics & single-cell analytics, scientists need the rapid and efficient detection of mRNA co-expression profiles that ViewRNA portfolio provides.

The Thermo Scientific™ ViewRNA Tissue Assay Fluorescence Kit leverages the branched DNA technology, proprietary probe set design and signal amplification technology with Alexa Fluor™ probes offering a unique, robust, and sensitive in situ hybridization assay for RNA localization in fixed tissues. With fluorescence detection using Alexa Fluor™ 488, Alexa Fluor™ 546, Alexa Fluor™ 594, Alexa Fluor™ 647, and Alexa Fluor™ 750, these kits allow multiplexed detection and imaging paving the way for improved spatialomics. This technology lays the foundation for expanded fluorescent profiles to provide scientists additional tools to probe multiple transcripts per sample, allow broader spatialomics research.



Workflow



For Research Use Only. Not for use in diagnostic procedures.

Materials and methods

Tissue

Mouse brain and human lung tissue sections were purchased from Zyagen, Inc. Cancer tissue sections were purchased from BioChain Institute Inc.

ViewRNA ISH

ISH was performed using Thermo Scientific™ ViewRNA Tissue Assay Fluorescence Kit. Target probes for specific mRNAs were designed and synthesized by Thermo Scientific™. Tissue sections were treated according to the user manual for Thermo Scientific™ ViewRNA Tissue Assay Fluorescence Kit. In brief, cryo-preserved tissue sections were fixed overnight in 4% paraformaldehyde and FFPE slides were de-paraffinized in xylene and ethanol. Then a silicon barrier was placed around the sections.

Tissues were then protease digested and fixed before the target probe set hybridization mixture was applied (40 ° C for 2 h). The signal was then amplified by applying PreAmplifier Mix solution followed by an Amplifier Mix solution and a Label Probe Mix solution. The signals for mRNA were detected as described in figure legends. After detection of ISH signals, sections were counterstained with DAPI and mounted with Invitrogen™ ProLong™ Glass Antifade Mountant.

Fluorescence imaging

Widefield fluorescence imaging was performed on an EVOS™ M7000 Imaging System and confocal fluorescence imaging was performed with a Zeiss LSM980 laser scanning microscope. Images were post-processed for visualization.

Results

We tested the ViewRNA ISH Tissue Assay using over 20 RNA targets and 8 tissue types (both normal & cancerous tissues) to demonstrate product stability as well as flexibility. Furthermore, we expanded the Alexa Fluor™ Label Probe offering to include Alexa Fluor™ 594, increasing imaging flexibility and multiplexing opportunities. The Alexa Fluor™ Label Probes produce strong signal, minimal background and off-target signal.

Detection of mRNA in formalin-fixed paraffin-embedded tissues

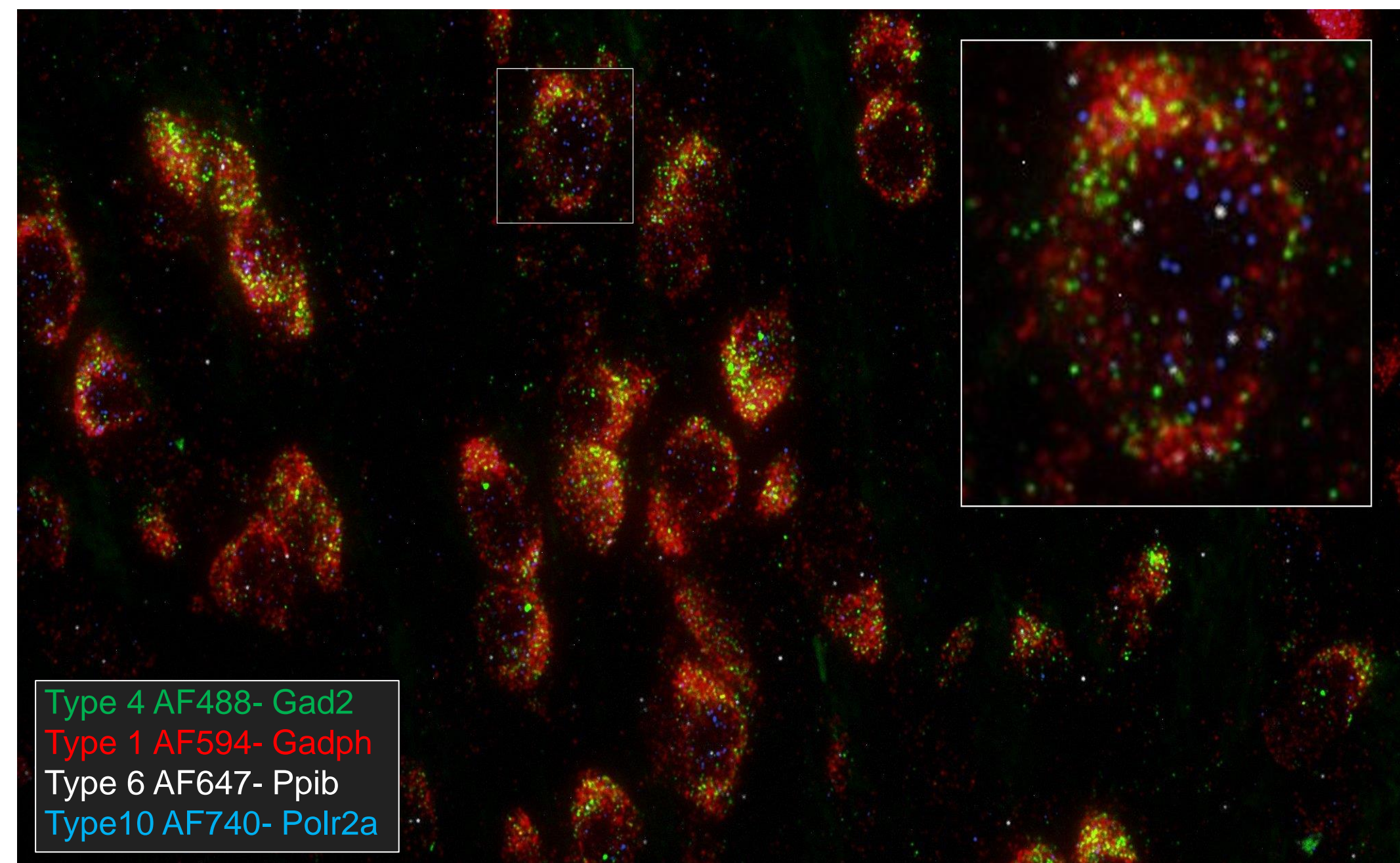


Figure 1. RNA-ISH in mouse brain tissue using ViewRNA Tissue Assay Fluorescence Kit.

Mouse brain (FFPE) tissue was processed and peptidyl-prolyl cis-trans isomerase B (Ppib), glutamate decarboxylase 2 (Gad2), RNA polymerase II subunit A (Polr2a), Gapdh mRNA was labeled using ViewRNA probe sets. These probe sets were detected using ViewRNA ISH Tissue Assay. A fluorescent image was taken using 40x objective on EVOS™ M7000 imaging system.

Detection of mRNA in cryo-preserved tissues

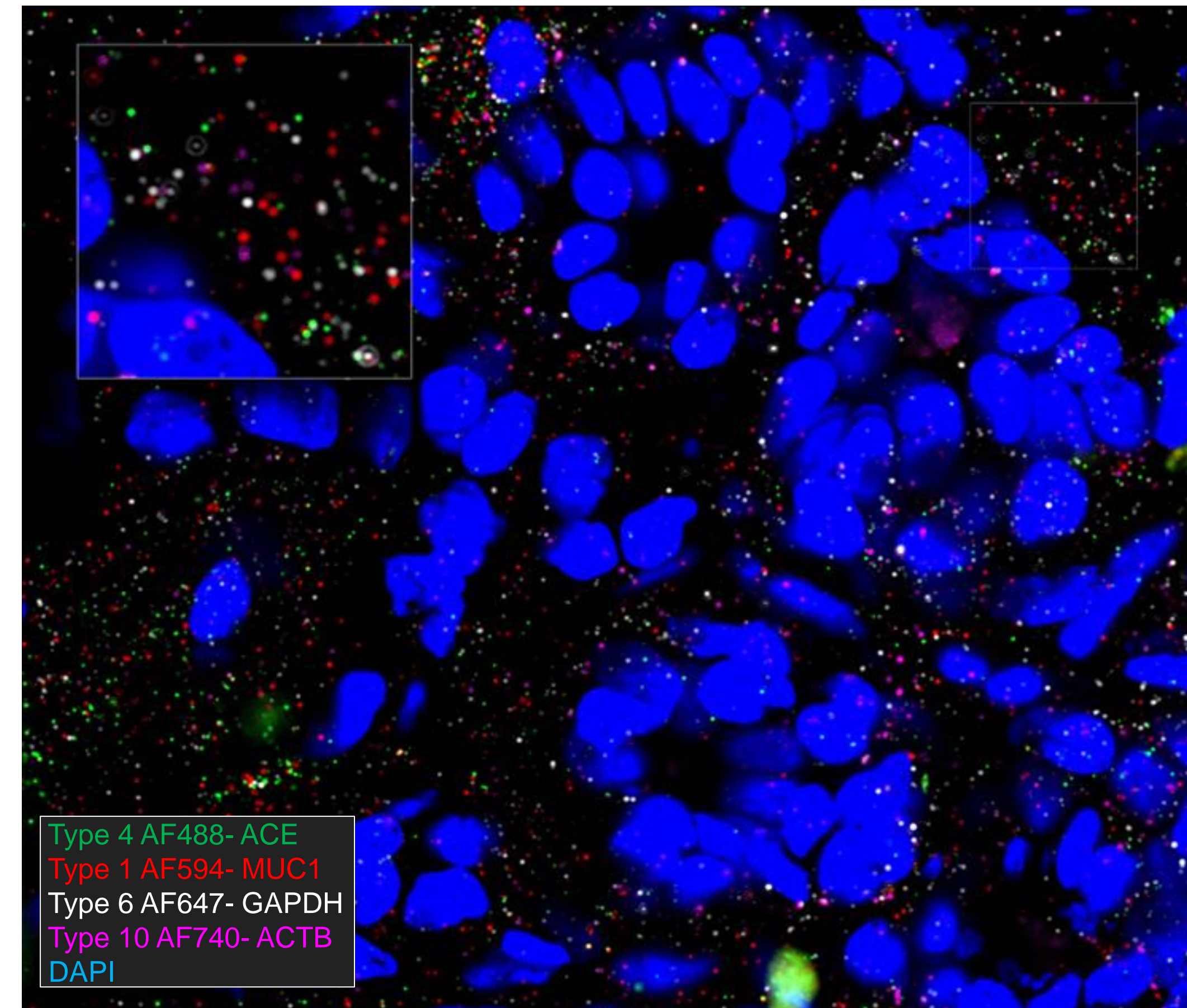


Figure 2. RNA-ISH in human lung tissue using ViewRNA Tissue Assay Fluorescence Kit.

Human lung (cryo preserved) tissue was processed and angiotensin-converting enzyme (ACE), mucin1 (MUC1), actin (ACTB) and GAPDH mRNA was labeled using ViewRNA probe sets. These probe sets were detected using ViewRNA ISH Tissue Assay. A fluorescent image was taken using a 63x objective on ZEISS LSM 980 imaging system.

Detection of mRNA in cancer tissues

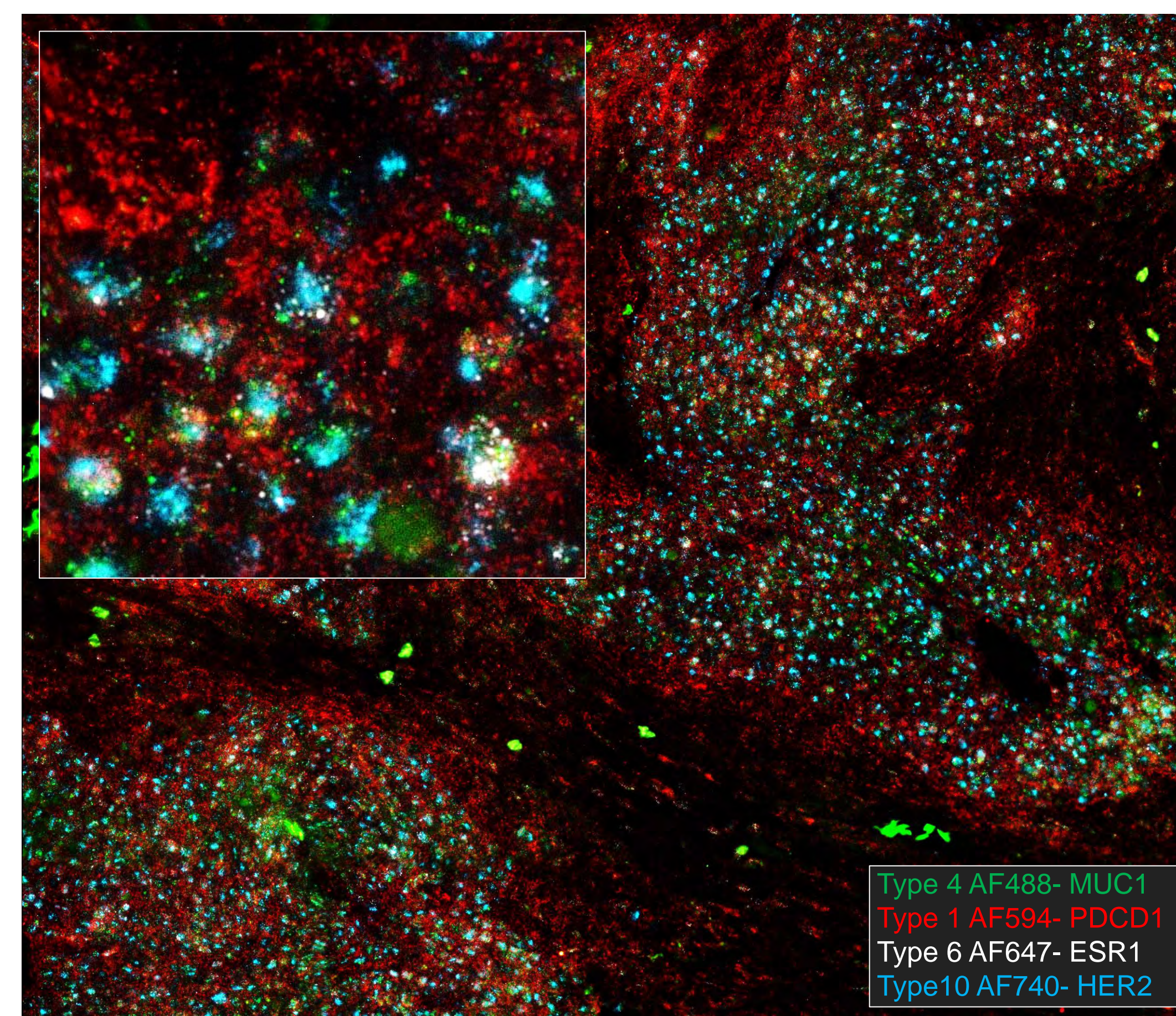


Figure 3. RNA-ISH in human breast invasive ductal carcinoma tissue using ViewRNA Tissue Assay Fluorescence Kit.

Human breast cancer (FFPE) tissue was processed and programmed cell death protein 1 (PDCD1), mucin1 (MUC1), estrogen receptor 1 (ESR1) and human epidermal growth factor receptor 2 (HER2) mRNA was labeled using ViewRNA probe sets. These probe sets were detected using ViewRNA ISH Tissue Assay. A tiled series of fluorescent images were taken using a 40x objective EVOS™ M7000 imaging system.

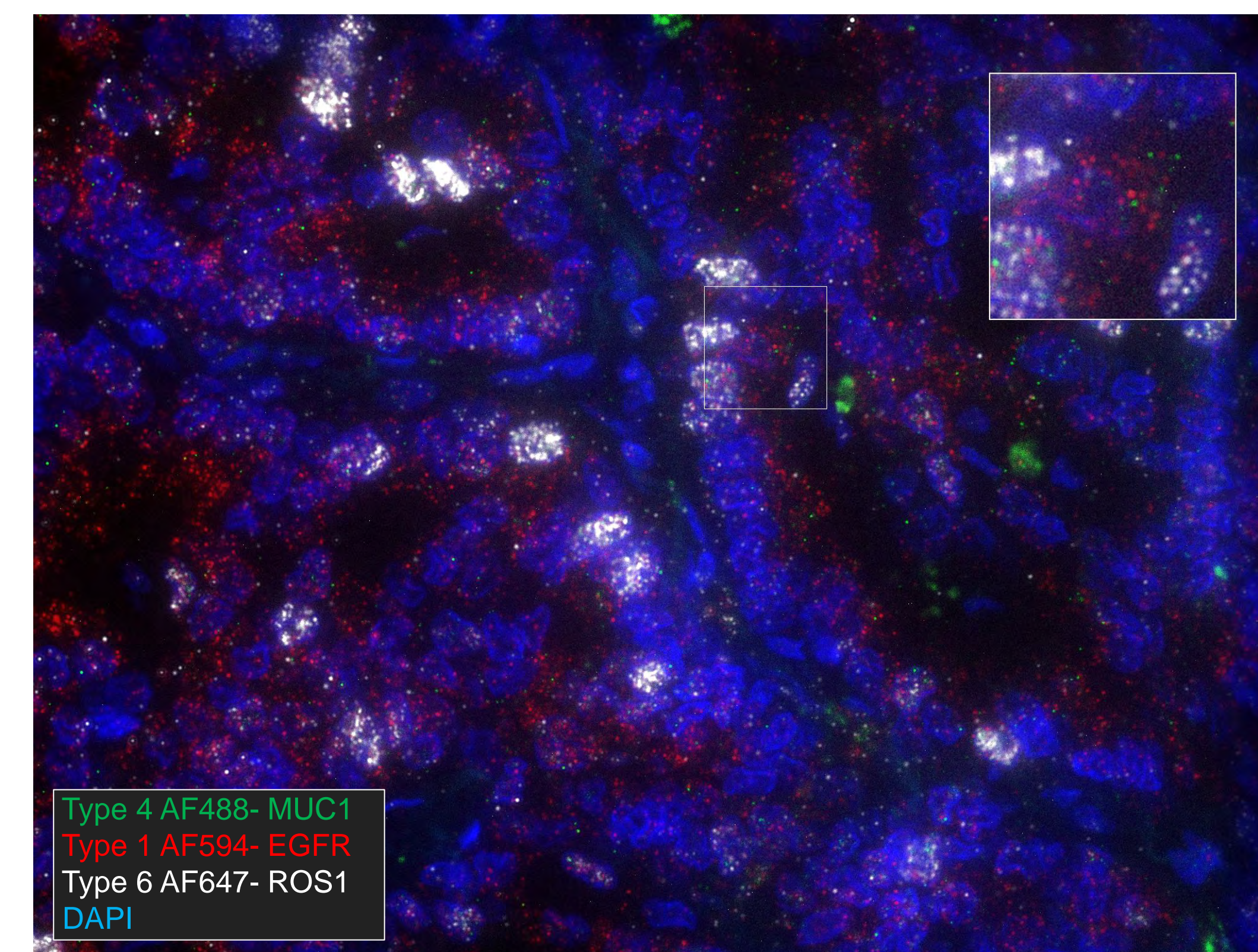


Figure 4. RNA-ISH in human lung adenocarcinoma tissue using ViewRNA Tissue Assay Fluorescence Kit.

Human lung cancer (FFPE) tissue was processed and proto-oncogene tyrosine-protein kinase ROS1 (ROS1), mucin1 (MUC1) and epidermal growth factor receptor (EGFR) mRNA was labeled using ViewRNA probe sets. These probe sets were detected using ViewRNA ISH Tissue Assay. A fluorescent image was taken using 40x objective on EVOS™ M7000 imaging system.

Conclusion

The Thermo Scientific™ ViewRNA Tissue Assay Fluorescence Kit can detect mRNA transcripts in FFPE and cryo-preserved samples, in both normal and cancerous tissues. This assay offers the foundation for expanding tools for transcriptome profiling in spatialomics studies

Catalog information, trademarks and licensing

Thermo Scientific™ ViewRNA Tissue Assay Fluorescence Kit, Type 1, 4, 6, 10 Catalog number: QVT4800

Kit Type	Label	Catalog Number
ViewRNA Type 6 Core Kit	Alexa Fluor™ 647	QVT0600C
ViewRNA Type 1 Module Kit	Alexa Fluor™ 594	QVT0694B
ViewRNA Type 1 Module Kit	Alexa Fluor™ 546	QVT4646B
ViewRNA Type 4 Module Kit	Alexa Fluor™ 488	QVT4688B
ViewRNA Type 10 Module Kit	Alexa Fluor™ 750	QVT0640B

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