#9908: Spatially resolved pharmacodynamics in intact neural tissue: single-section, protease-free co-detection of an oligonucleotide therapeutic, its target transcript, and target protein

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MOLECULAR INSTRUMENTS

BACKGROUND AND OBJECTIVES

Therapeutic small interfering RNAs (siRNAs) mediate post-transcriptional gene silencing through sequence-specific recognition and degradation of target mRNAs. Characterizing these therapeutics requires mapping siRNA distribution relative to target mRNA expression and protein-defined cellular compartments. Detecting siRNAs in situ remains challenging because their short length limits the sequence space available for probe binding. Conventional workflows may also evaluate therapeutic siRNAs, endogenous transcripts, and proteins using separate assays, obscuring their spatial relationships. Here, we show a protease-free HCR™ Pro RNA-ISH workflow for detecting an administered therapeutic siRNA and visualizing it alongside its endogenous target mRNA. We then combined 2-plex HCR™ Pro RNA-ISH with HCR™ Gold 2°IF to incorporate secondary antibody-based detection of a protein marker within the same tissue section.

WORKFLOW AND METHODS

The primary objective was to establish sensitive and specific spatial detection of an administered therapeutic siRNA and demonstrate its compatibility with same-section mRNA and protein detection.

The study addressed four experimental questions:

1. **Is the tissue suitable for RNA detection?**
   Ppib served as a positive control to confirm RNA integrity, accessibility, and assay performance.
2. **Is assay background sufficiently low?**
   dapB was used as a negative control to establish the nonspecific-background baseline and support interpretation of true-negative results.
3. **Is siRNA detection specific?**
   Therapeutic siRNA signal was compared between buffer-treated tissue and tissue collected four days after siRNA administration.
4. **Can the therapeutic siRNA, target mRNA, and protein be visualized together?**
   A fluorescent 2-Plex HCR™ Pro RNA-ISH assay was used to visualize the therapeutic siRNA and endogenous target mRNA within the same tissue section. HCR™ Gold 2°IF added secondary antibody-based detection of a protein marker, providing spatial context for assessing siRNA distribution relative to target-expressing and protein-defined cells and tissue regions.

2-PLEX DETECTION OF siRNA and mRNA

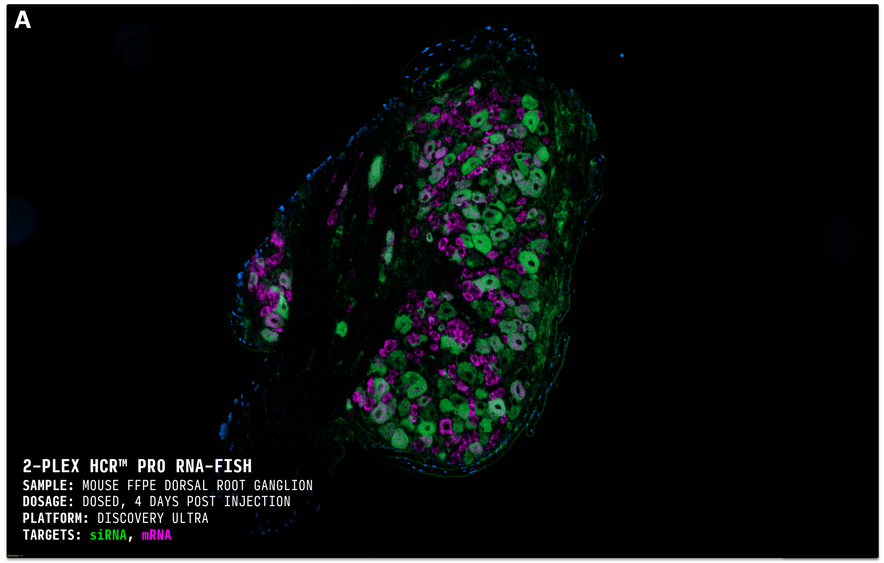


Figure A. 2-Plex HCR™ Pro RNA-FISH detection of therapeutic siRNA and endogenous target mRNA in dosed tissue. Both RNA targets were visualized within the same tissue section, demonstrating simultaneous detection of an administered siRNA and its target transcript while preserving their spatial relationship.

MULTIPLEXED siRNA, mRNA, AND PROTEIN DETECTION

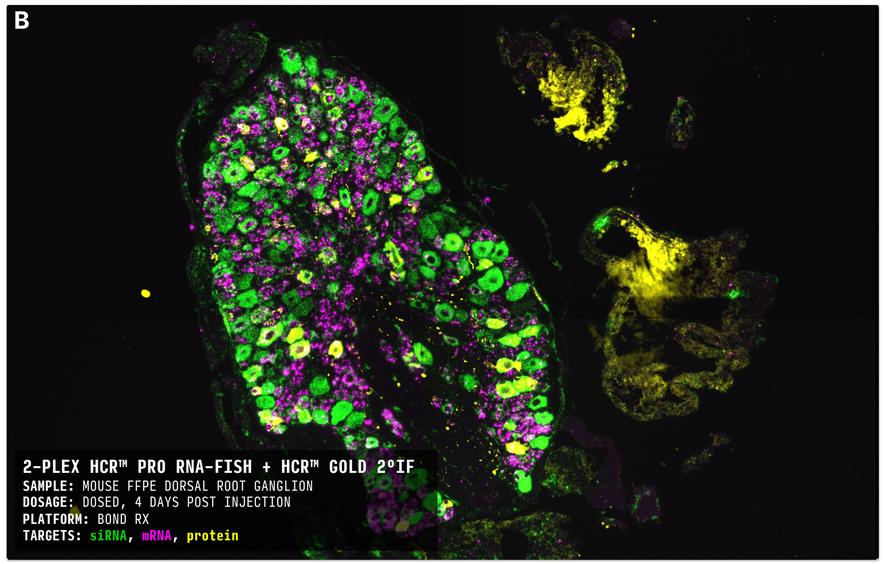
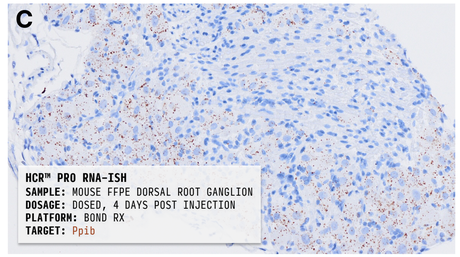


Figure B. Same-section visualization of therapeutic siRNA, endogenous target mRNA, and protein. 2-Plex HCR™ Pro RNA-ISH detected the therapeutic siRNA and its target mRNA, while HCR™ Gold 2°IF provided secondary antibody-based detection, integrating therapeutic siRNA, target mRNA, and protein context within the same tissue section. The combined assay integrates all three signals within preserved anatomical context, enabling therapeutic distribution to be evaluated relative to target expression and protein-defined cells or tissue compartments.

VALIDATION OF RNA QUALITY AND DETECTION SPECIFICITY



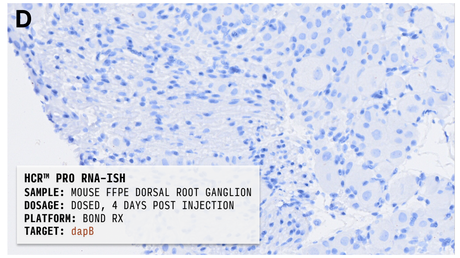
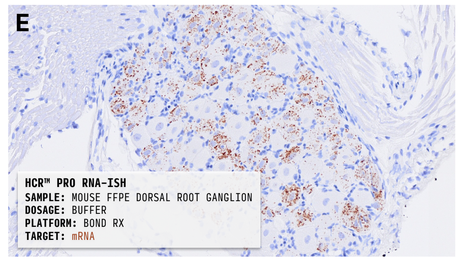
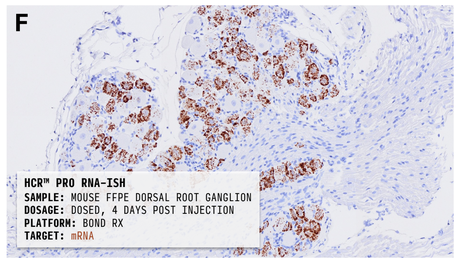
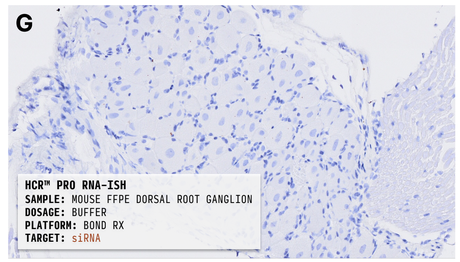


Figure C. Robust *Ppib* signal confirmed RNA integrity and assay performance in dosed tissue. **Figure D.** Absence of *dapB* signal demonstrated minimal nonspecific background, indicating that signals observed with real targets reflect specific detection rather than nonspecific probe binding.





Figures E and F. Target mRNA was detected in both the buffer-treated sample (E) and the siRNA-dosed sample (F), with visibly stronger signal in the dosed tissue. This unexpected difference highlighted the value of 2-plex imaging for determining whether the increased mRNA signal occurred in the same cells and regions containing the therapeutic siRNA or elsewhere in the tissue.



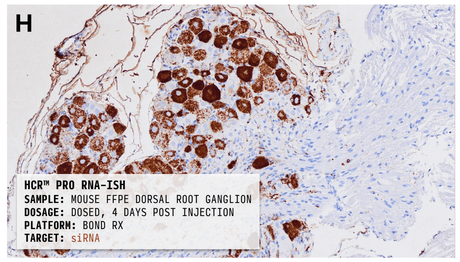


Figure G. No detectable therapeutic siRNA signal was observed in the buffer-treated control, consistent with the absence of administered siRNA. **Figure H.** Robust therapeutic siRNA signal was detected in the siRNA-dosed sample. This treatment-dependent signal supports the specificity of the siRNA probe.

CONCLUSIONS

This study addresses a key technical barrier in siRNA development by enabling specific visualization of a short therapeutic siRNA within preserved tissue. 2-Plex HCR™ Pro RNA-ISH placed therapeutic distribution and endogenous target mRNA expression within the same spatial context, eliminating the need to infer their relationship from separate assays or serial sections. Combining this RNA assay with HCR™ Gold 2°IF extended the workflow to secondary antibody-based detection, integrating therapeutic siRNA, target mRNA, and protein context within the same tissue section. This protease-free approach provides a flexible framework for investigating therapeutic biodistribution, cellular localization, target expression, and protein-defined tissue context while preserving RNA integrity and tissue architecture.

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