Abstract #354

Parallel 10-plex imaging of RNA and protein targets with HCR™ Gold RNA-FISH

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ABSTRACT

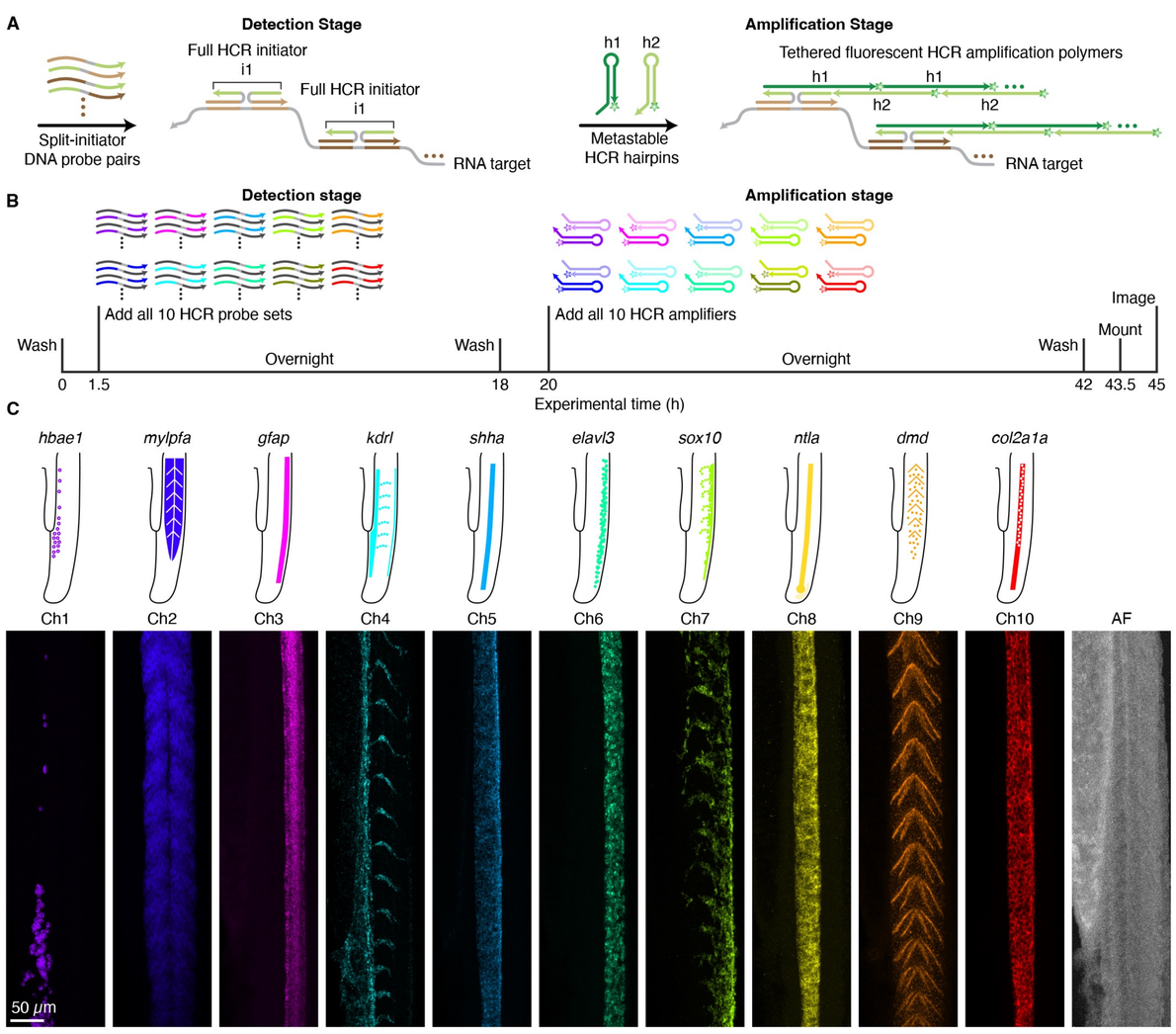
HCR™ RNA-FISH is a fundamental tool for developmental biologists, empowering researchers with high performance, multiplex, quantitative imaging of RNA expression in any sample type, including highly autofluorescent whole-mount embryos and FFPE tissue sections. The ability to map spatial organization of RNA and protein molecules within the same sample is critical for advancing our understanding of key developmental changes like organogenesis, thus providing deeper insights into developmental disorders. Molecular Instruments® (MI) supports a vast community of developmental researchers worldwide, engaged in studying over 500 diverse species. MI is excited to announce the immediate availability of ready-to-use up to 10-plex assays that can be seamlessly adaptable to any sample type of interest to the scientist. Additionally, we also present an affordable, scalable, and automated platform capable of performing HCR™ assays to streamline high-throughput workflows.

ENGINEERING GOALS

HCR™ v3.0 RNA-FISH has been widely used across over 500 diverse species. However, a primary challenge researchers face with RNA-FISH methods is the ability to perform multiplexing for more than four targets due to the difficulty in differentiating signals from fluorophores with overlapping spectra. Here, we present HCR™ Gold RNA-FISH, a new product line that features several generational improvements, including:

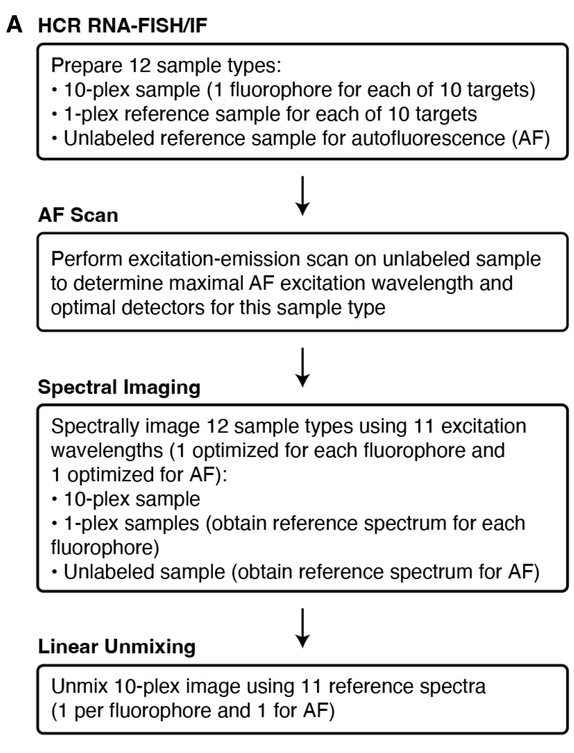
- Catalog availability of up to 10-plex imaging of RNA/protein targets
- Optimized protocols for automated staining on InSituPro
- Improved performance from refined probe design & manufacturing

10-PLEX HCR™ GOLD RNA-FISH

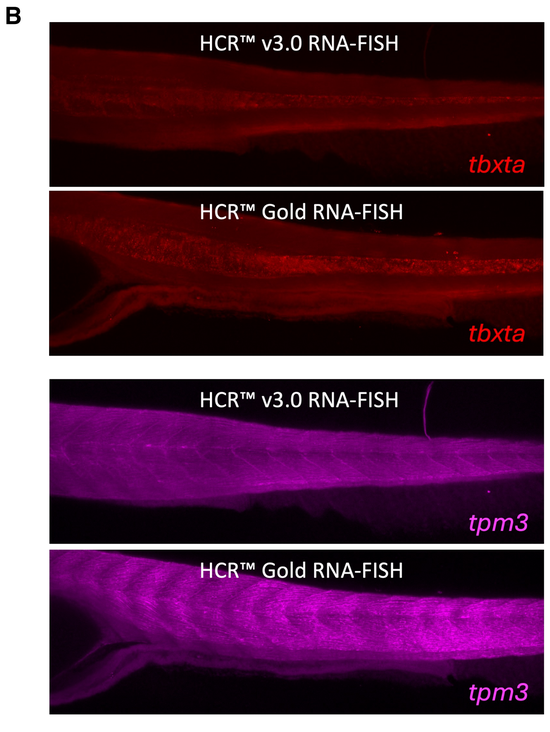


(A): Two-stage HCR™ Gold RNA-FISH protocol. Detection stage: split-initiator DNA probe pairs bind to RNA targets to colocalize full HCR initiator i1; wash. Amplification stage: colocalized full initiator i1 triggers self-assembly of fluorophore-labeled HCR hairpins into tethered fluorescent amplification polymers; wash. (B): 10-plex HCR RNA-FISH timeline. During the detection stage, all ten targets are detected in parallel. During the amplification stage, signal amplification is performed for all ten targets in parallel. (C): Top: Expression atlas for ten RNA targets in the tail of a zebrafish embryo (lateral view). Bottom: Linearly unmixed channels from a 10-plex confocal image including autofluorescence (AF) as an 11th channel; maximum intensity z-projections; 0.57×0.57×4.0 μm pixels. Embryo fixed at 27 hpf. Adapted from Schulte et al. (3)

10-plex HCR™ Gold RNA-FISH/IF Spectral Imaging Workflow

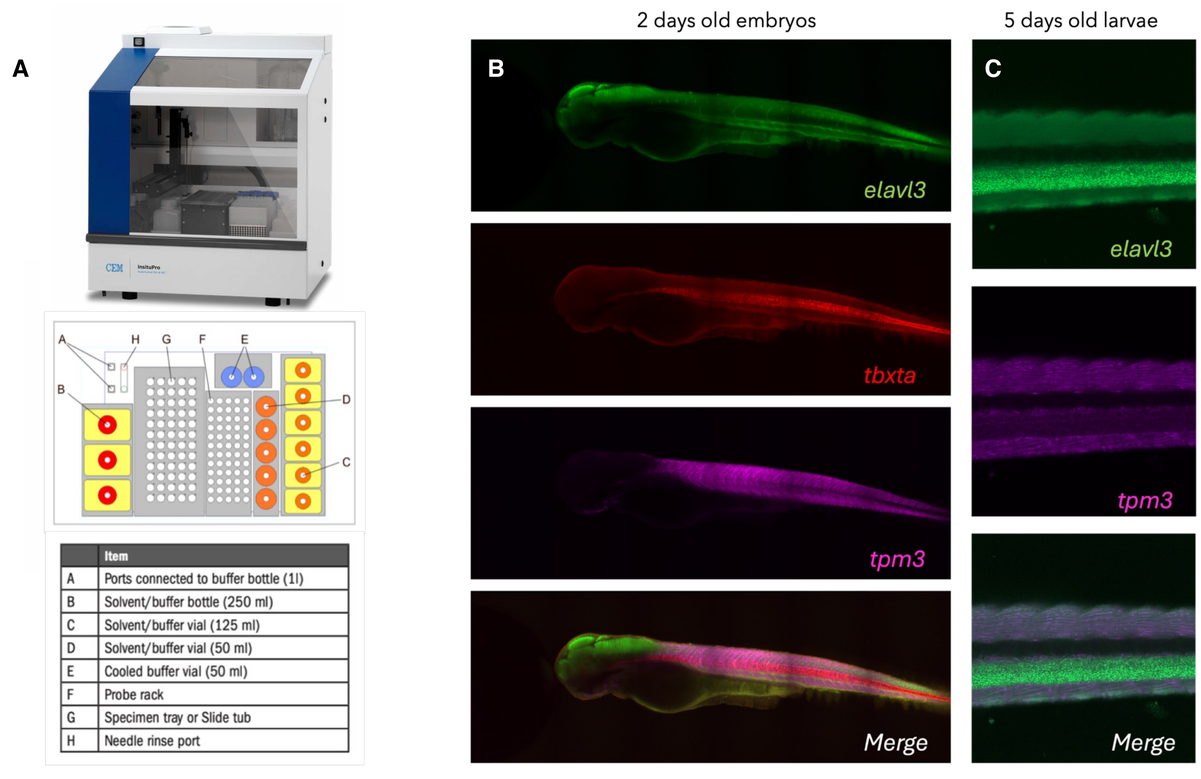


Comparison of HCR™ v3.0 RNA-FISH versus HCR™ Gold RNA-FISH Performance



(A): Workflow for performing a 10-plex HCR™ Gold RNA-FISH experiment with spectral imaging and linear unmixing. (B) and (C): Comparison between HCR™ v3.0 RNA-FISH and HCR™ Gold RNA-FISH probes for two targets (*tbxta*, red, and *tpm3*, magenta). Similar laser parameters were used to ensure that HCR™ v3.0 RNA-FISH signals were not saturated. Maximum intensity z-projections are shown in each panel.

AUTOMATED HCR™ GOLD RNA-FISH



(A): The InSituPro features a compact (top panel) system for automating all repetitive washing and incubation steps for HCR™ assays. (B) and (C): 2 dpf zebrafish embryos and 5 dpf zebrafish larvae for different targets. Samples were transferred to InsituPro at the beginning of the assay, retrieved after the last step, mounted and imaged. Maximum intensity z-projections are shown in each panel.

REFERENCES

1. Choi et al., *Development* (2018)
2. Schwarzkopf et al., *Development* (2021)
3. Schulte et al., *Development* (2024)